# Preventive and Therapeutic Effects of Large-Dose Nicotinamide Injections on Diabetes Associated with Insulitis

## An Observation in Nonobese Diabetic (NOD) Mice

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**SUMMARY**

**This experiment was undertaken to explore a novel method of therapy for insulin-dependent diabetes mellitus (IDDM), using nonobese diabetic (NOD) mice that had symptoms and histologic changes similar to those of human IDDM patients. We examined preventive and therapeutic effects of large-dose nicotinamide administration on diabetes in NOD mice. Eighteen young female NOD mice without glycosuria were randomly divided into two groups; nine received subcutaneous nicotinamide (0.5 mg/g body wt) injections every day and the other nine were maintained as a control group and not injected. After 40 days, all of the mice given nicotinamide showed almost normal glucose tolerance and only mild insulitis on histologic study. On the other hand, marked glycosuria and severe insulitis were observed in six of the nine mice not injected. Four of six NOD mice given nicotinamide from the day of the first occurrence of marked glycosuria displayed a disappearance of glycosuria and an improvement in glucose tolerance during the therapy; however, urine sugar became negative in only one of six mice that received nicotinamide from 1 to 2 wk after the onset of marked glycosuria. These results indicate that nicotinamide has preventive and therapeutic effects on diabetes in NOD mice, and suggest the reversibility of B-cell damage, at least at a very early stage of IDDM. DIABETES *31*:749–753, September 1982.**

Mononuclear cell infiltration in the islets of Langerhans, for which von Meyenburg[1] coined the name "insulitis," has often been observed in autopsy pancreata of recent-onset insulin-dependent diabetes mellitus (IDDM).[2] Nonobese diabetic (NOD) mice,[3,4] a recently discovered animal model for human IDDM, are characterized by the presence of insulitis, i.e., marked mononuclear cell infiltration surrounding and/or invading the islets causing extreme reduction in the number of islet cells. Insulitis occurs spontaneously from the age of 5 wk in female NOD mice; they abruptly exhibit glycosuria, persistent hyperglycemia, ketonuria, and rapid weight loss at around 120 days of age. Cumulative incidence of diabetes up to the age of 210 days is about 80% in female NOD mice, but less than 20% in male NOD mice. It has been recently reported that multiple, subdiabetogenic dose injections of streptozotocin (STZ) induce progressive insulitis.[5] From a histologic point of view, IDDM patients, NOD mice, and STZ-induced diabetic animals have marked similarities in lesions of the islets.

STZ reportedly depresses islet nicotinamide adenine dinucleotide (NAD) content,[6,7] possibly through the augmentation of islet nuclear poly(ADP-ribose) synthetase activity.[8] Administration of nicotinamide to animals rapidly increases intracellular NAD concentrations in many tissues.[9] It has been shown that nicotinamide not only prevents diabetes induced with a single injection of large-dose STZ,[10–13] but also delays the onset of STZ-induced insulitis.[14] Nicotinamide is a precursor for new NAD synthesis and an inhibitor of poly(ADP-ribose) synthetase[15] and other ADP-ribosyltransferases.[16]

In this article we conclude that the occurrence of diabetes in NOD mice is substantially inhibited with large-dose nicotinamide treatment. As far as we know, this is the first article showing the preventive effects of large-dose nicotinamide injections on spontaneously occurring IDDM.

### MATERIALS AND METHODS

**Animals.** Female NOD mice at the 45th generation of inbreeding were supplied by the Shionogi Research Laboratory in Japan. Female ICR mice, serving as normal controls, were purchased from Clea Japan, Inc. All of the mice were housed in stainless-steel cages and provided standard mouse chow and water ad libitum. Urine samples from the

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NOD mice were obtained once daily, every afternoon, and urine sugar was determined using Tes-Tape (Eli Lilly and Co., Indianapolis, Indiana). Onset of overt diabetes was defined as the day of the first appearance of marked glycosuria (3+ or more).

**Plasma glucose.** Blood samples were taken from the retroorbital sinus with hematocrit tubes under light ether anesthesia. Plasma glucose was analyzed by the glucose-oxidase technique, using a Beckman Glucose Analyzer (Beckman Instruments Inc., Fullerton, California). Intraperitoneal glucose tolerance test (IPGTT) was performed after a 6–9-h fast. Blood samples were taken at 0, 30, and 60 min after glucose (1 mg/g body wt) injection.

**Histologic examinations.** The pancreata were quickly removed under ether anesthesia and fixed in Bouin's solution. Paraffin-embedded sections were stained by hematoxylin and eosin, aldehyde fuchsin, and Grimelius' method.

**Studies on the prevention of diabetes with large-dose nicotinamide injections.** Eighteen female NOD mice, aged 92 ± 10 days (mean ± SD), without glycosuria from six groups of littermates were divided into two groups by apportioning the littermates as evenly as possible. The control group consisted of nine mice (aged 94 ± 11 days, weighing 24.7 ± 3.6 g); the treatment group also consisted of nine mice (aged 91 ± 8 days, weighing 24.6 ± 2.3 g). The control group mice were maintained without treatment. Nicotinamide, in a 5% aqueous solution and at a dose of 0.5 mg/g body wt, was injected subcutaneously into the treatment group mice every day. Nicotinamide injections were continued for at least 40 days (mean 72 days, range 40–109 days).

**Studies on the treatment of diabetes with large-dose nicotinamide injections.** Nicotinamide at a dose of 0.5 mg/g body wt/day was injected into 12 NOD mice after the onset of overt diabetes. Six of the mice (aged 119 ± 23 days, weighing 24.0 ± 3.4 g) received nicotinamide injections from the day of the first appearance of marked glycosuria, and the other six (aged 209 ± 89 days, weighing 27.3 ± 3.9 g) were given the same injections starting 1–2 wk after the appearance of marked glycosuria. These 12 mice, unless they died, were injected with nicotinamide for at least 40 days.

**Effect of nicotinamide on glucose tolerance in normal mice.** IPGTT was performed on 14 ICR mice (aged 20 wk, weighing 25.5 ± 2.6 g) to determine the normal range of glucose tolerance. Five of the mice were given nicotinamide (0.5 mg/g body wt) for 2 wk after the first IPGTT. IPGTT was carried out again on the nicotinamide-injected mice on the day after the last injection to determine the effect of nicotinamide on glucose tolerance in normal mice.

## RESULTS

**Prevention of diabetes with large-dose nicotinamide injections.** As shown in Table 1, six of the control group mice (67%) became diabetic during the 40 days of observation. This result closely agrees with previously reported findings.[4] On the contrary, none of the treatment group mice developed diabetes during the 40-day period of nicotinamide injection. No significant differences in body weight were observed between the animals in the treatment group and the aglycosuric mice of the control group during the 40-day nicotinamide treatment. The preventive effect of nicotinamide on diabetes was statistically significant ($\chi^2 = 6.25$, $P < 0.02$). The effect of nicotinamide injection was analyzed using the glucose-loading test; IPGTT performed on the nine mice of the treatment group on the day after the last injection revealed glucose levels at 0, 30, and 60 min to be 145 ± 28, 217 ± 74, and 192 ± 74 mg/dl (mean ± SD), respectively (Figure 1, left, solid circles). Although urine sugar of five of the treatment group mice was followed for 70 days after discontinuing the nicotinamide treatment, no glycosuria was observed (the other four mice were killed for histologic examinations after the 40 days of injections). In these five mice, the glucose concentrations at 0, 30, and 60 min on IPGTT were 148 ± 27, 221 ± 68, and 181 ± 64 mg/dl, respectively, on the day after the last injection, and 140 ± 23, 237 ± 38, and 182 ± 29 mg/dl at 0, 30, and 60 min, respectively, 70 days after discontinuance (Figure 1, left, open circles). IPGTT was also performed on the control group mice at the same time as on the treatment group mice. Plasma glucose levels in IPGTT in the two surviving diabetic mice of the control group at 0, 30, and 60 min were 645, 668, and 625 mg/dl and 498, 641, and 632 mg/dl, respectively (Figure 1, right).

TABLE 1
Preventive effect of large-dose nicotinamide injections on diabetes in NOD mice

| Group | Number of mice | Number of mice in which diabetes developed |
|---|---|---|
| Prevention | 9 | 0 |
| Control | 9 | 6* |

$\chi^2 = 6.25$, $0.01 < P < 0.02$.
* One more mouse became diabetic at the age of 154 days.

On histologic examination of the diabetic mice in the control group, pancreatic islets were small, decreased in number, accompanied by marked infiltration of mononuclear cells in and around the islets, and devoid of normal islet architecture (Figure 2A). Aldehyde fuchsin stain and Grimelius stain revealed that A-cells were relatively well preserved compared with B-cells. On the contrary, islets in the mice killed immediately after the nicotinamide treatment showed no marked decrease in number; the architecture of the tissue was well preserved and inflammatory cells were scanty and limited to the periphery of the islets (Figure 2B). Aldehyde fuchsin stain and Grimelius stain revealed that neither A-cells nor B-cells had decreased in number. However, insulitis was found to have progressed to a certain extent in the mice killed 70 days after the discontinuation of the treatment, although no glycosuria was observed.

**Treatment of diabetes with large-dose nicotinamide injections.** Six mice were given nicotinamide injections from the day of the first appearance of marked glycosuria. Four of the six showed marked reductions in urine sugar within 1 wk and became aglycosuric within 3 wk. The other two continued to excrete glucose in spite of the treatment (Table 2). After nicotinamide injections had been given for 40 days, IPGTT was done on three of the above four mice no longer having glycosuria, and showed mildly impaired tolerance (112 ± 32, 300 ± 38, and 219 ± 47 mg/dl at 0, 30, and 60 min, respectively, Figure 3). IPGTT performed again 70

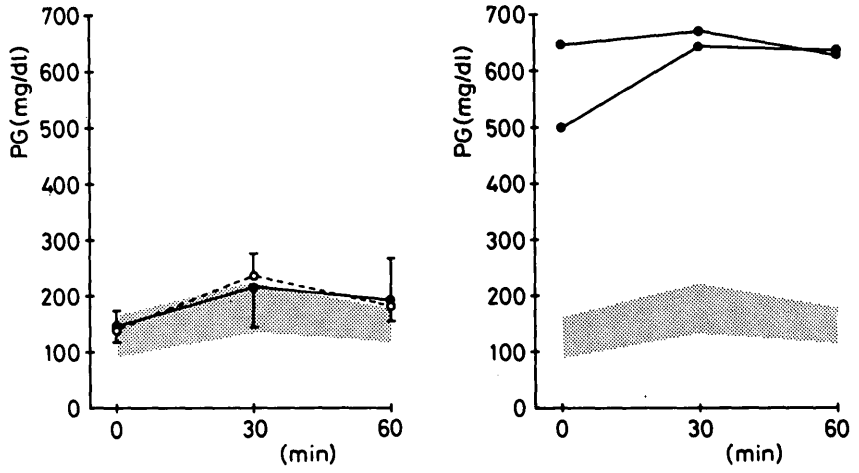


**FIGURE 1. Left panel shows IPGTT in female NOD mice after preventive treatment with large-dose nicotinamide injections (mean ± SD). ●—●: IPGTT performed on the day after the last injection (N = 9). ○---○: IPGTT performed 70 days after discontinuation of the injections (N = 5). Right panel represents IPGTT in the two surviving diabetic mice of the control group. Shaded areas represent glucose tolerance of normal ICR mice (N = 14, mean ± SD).**

days after the cessation of therapy also showed mildly impaired glucose tolerance (131 ± 17, 228 ± 78, and 226 ± 75 mg/dl at 0, 30, and 60 min, respectively, Figure 3). During these 70 days urine sugar was invariably negative. However, histologic examinations 70 days after the discontinuation of therapy revealed that insulitis had progressed, resulting in a considerable reduction in the number of islet cells.

On the other hand, urine sugar disappeared in only one of the six mice given nicotinamide from 1 to 2 wk after the onset of marked glycosuria (Table 2). The other five continued to have marked glycosuria. One month after the start of the injections, IPGTT was done on the mouse that had become aglycosuric during the treatment and on two of the five mice with marked glycosuria in spite of treatment. The mice with glycosuria showed severe glucose intolerance (664, 885, and 631 mg/dl and 645, 677, and 628 mg/dl at 0, 30, and 60 min, respectively, Figure 4, left). Although the aglycosuric mouse showed moderately impaired glucose tolerance after 1 mo of nicotinamide therapy, its tolerance gradually improved as injections were continued (Figure 4, right).

**Glucose tolerance in normal mice before and after nicotinamide treatment.** Glucose tolerance examined by means of intraperitoneal glucose load in normal mice did not change significantly after 2 wk of nicotinamide injections. Plasma glucose levels at 0, 30, and 60 min were 124 ± 24, 148 ± 21, and 150 ± 16 mg/dl, respectively, before treatment and 131 ± 3, 164 ± 19, and 155 ± 23 mg/dl, respectively, after treatment.

## DISCUSSION

In this experiment using NOD mice, it was shown that large-dose nicotinamide injections, if started before the onset of diabetes, completely prevent the occurrence of glycosuria, and that injections started immediately after the onset of glycosuria have a significant therapeutic effect on diabetes.

In female NOD mice, insulitis occurs around the age of 5 wk; the onset of glycosuria occurs around the age of 120 days.[4] Therefore, mild insulitis was already present when preventive treatment was started at the age of 91 ± 8 days. Large-dose nicotinamide injections prevented further degeneration of the islets and preserved insulin-secreting capacity. Seventy days after treatment discontinuation, insulitis had progressed to some extent; the number of islets seemed to be reduced, but glucose tolerance was still normal.

In the treatment group, urine sugar disappeared in four of the six mice receiving nicotinamide from the day of the first appearance of marked glycosuria. When the treatment was started 1–2 wk after the onset of overt diabetes, however,

**FIGURE 2. Degenerated islet with mononuclear cell infiltration in a diabetic control group mouse (A: hematoxylin and eosin). In the mice preventively injected with nicotinamide and killed immediately after discontinuation of the injections, mild infiltration of mononuclear cells was seen in the periphery of the islets (B: hematoxylin and eosin).**

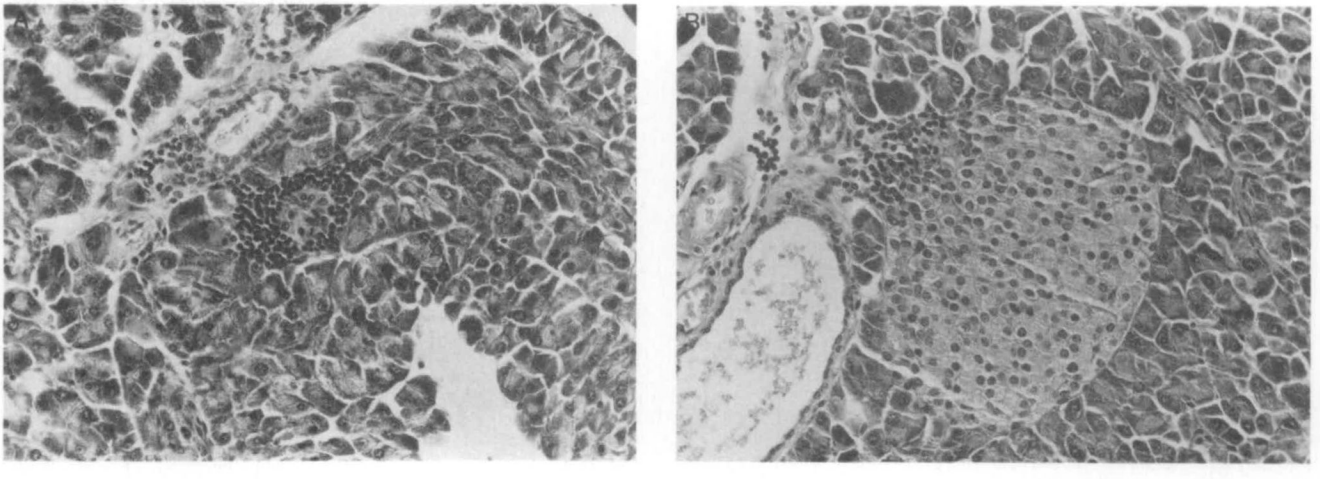

TABLE 2
Therapeutic effect of large-dose nicotinamide injections on diabetes in NOD mice

| Initiation of nicotinamide injection | Number of mice | Number of mice in which glycosuria disappeared |
|---|---|---|
| On the day of the first occurrence of marked glycosuria | 6 | 4 |
| 1–2 wk after the first occurrence of marked glycosuria | 6 | 1 |

urine sugar remained strongly positive in five of the six mice; glycosuria disappeared and glucose tolerance ameliorated in only one mouse. IPGTT performed three times on this mouse showed that glucose tolerance gradually improved during long-term therapy. Once glycosuria had disappeared during the 40 days of nicotinamide therapy, no relapses were observed for as long as 70 days after its discontinuation. However, maintenance or intermittent treatment with nicotinamide might be required to protect islets from degeneration in both prophylaxis and therapy for NOD mice.

Zawalich et al.[17] showed that nicotinamide potentiated glucose-induced insulin secretion from perifused rat islets, although nicotinamide per se had no effect on insulin secretion. Trus et al.[18] demonstrated that glucose and acetylcholine increased the concentration of NADH in rat islets, and Watkins et al.[19] described how NADH stimulated insulin release from isolated toadfish granules. Very recently, it was demonstrated that nicotinamide blocked hormone-induced receptor loss.[20,21] However, the effects of nicotinamide on diabetes in NOD mice are not due solely to potentiation of glucose-induced insulin secretion and/or prevention of insulin-induced receptor loss since the effects of nicotinamide persist for a long period after discontinuation of treatment and the injections do not alter glucose tolerance in normal ICR mice.

The results of this experiment suggest that alterations in NAD metabolism in the islets are responsible not only for

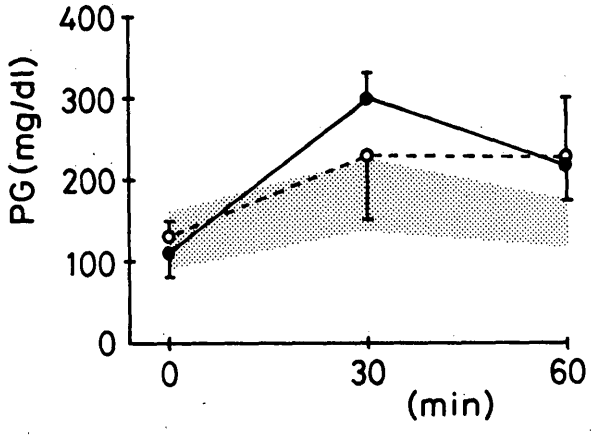


**FIGURE 3. IPGTT in female NOD mice that became aglycosuric during nicotinamide injections beginning on the day of the first occurrence of marked glycosuria (N = 4, mean ± SD). ●—●: IPGTT performed on the 40th day of nicotinamide injection. ○---○: IPGTT performed on the 70th day after discontinuation of the injections. Shaded areas represent glucose tolerance of normal ICR mice (mean ± SD).**

STZ-induced diabetes but also for diabetes in NOD mice. In STZ-induced destructive islets[8] and certain virus-infected cells,[22] the activity of poly(ADP-ribose) synthetase, which reduces intracellular NAD content, was reported to be markedly elevated. In addition, some cytotoxic agents were demonstrated as catalyzing an ADP- ribosylation of cellular proteins, which could be inhibited with nicotinamide.[16,23] Therefore, nicotinamide may increase intracellular NAD content not only by serving as a precursor in NAD synthesis, but also by possibly reducing poly- and/or mono-ADP-ribosylation.

Measurement of NAD content in islets of NOD mice is now in progress in our laboratory. The relationship between the degenerative process through the immune mechanism and alterations of NAD concentration in the islets is of particular interest.

The therapeutic effect of nicotinamide in NOD mice suggests that B-cell damage might be reversible, at least in the very early stages of human IDDM. In fact, occurrence of a remission stage called the "honeymoon period" has been observed in at least some IDDM patients. Therefore, the

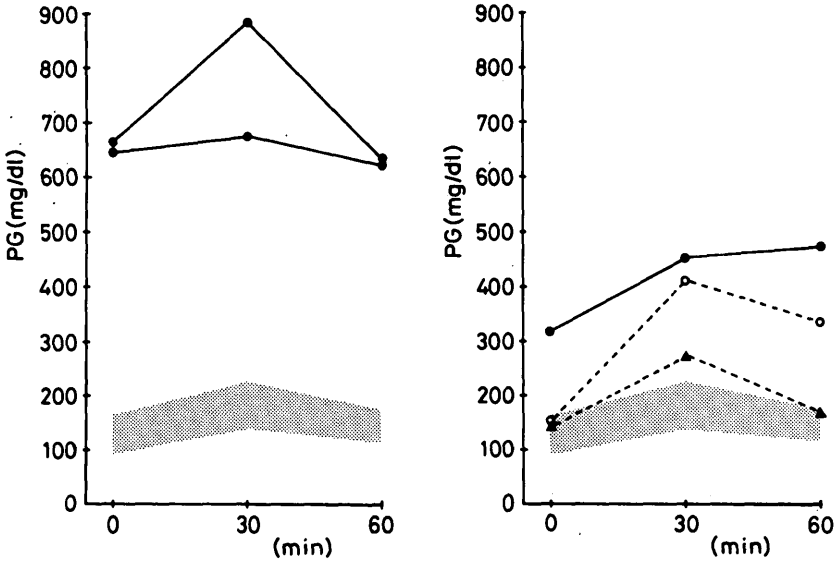


**FIGURE 4. IPGTT for female NOD mice injected with nicotinamide from 1 to 2 wk after the first occurrence of marked glycosuria. Two of the three surviving mice showed highly impaired glucose tolerance in spite of nicotinamide injections for 1 mo (left panel). Glucose tolerance of another mouse gradually improved during 4 mo of nicotinamide injections (right panel). ●—●: after 1 mo. ○---○: after 2 mo. ▲---▲: after 4 mo. Shaded areas represent glucose tolerance of normal ICR mice (mean ± SD).**

possibility of preventing islet degeneration and restoring the B-cell function in human IDDM using nicotinamide or related substances, particularly in a preketosis-prone stage or in the remission period, still remains.

It was demonstrated that STZ[24] and alloxan[25] frequently induced insulinoma when combined with nicotinamide. STZ[26] and alloxan[27] have B-cell oncogenicity even when administered without nicotinamide. On the other hand, the occurrence of insulinoma in diabetic patients is extremely rare; less than 10 case reports have been published,[28,29] and none of the subjects were well documented IDDM cases. Therefore, various environmental factors related to the pathogenesis of IDDM do not seem to have B-cell oncogenicity. However, we are continuing observation of nicotinamide-treated NOD mice to examine the probability of insulinoma occurrence. Since doses of nicotinamide reported to prevent STZ-induced diabetes were between 0.25 and 0.9 mg/g body wt,[10–13] we set the dose at 0.5 mg/g body wt in this experiment. A minimum effective dose of nicotinamide in NOD mice is also under investigation.

In conclusion, large-dose nicotinamide injections proved to have both preventive and therapeutic effects on diabetes in NOD mice. These results suggest reversibility and curability of B-cell damage, at least at a very early stage. Whether these findings will be applicable to the treatment of human IDDM remains to be established.

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