embedded in paraffin. Staining of de-waxed, paraffin-embedded tissue was performed according to previously published protocols (26, 27). For immunostaining of tissue nonspecific alkaline phosphatase (TNAPase), paraffin sections were incubated with rabbit serum to TNAPase and viewed using diaminobenzidine substrate by light microscopic observation. For tartrate-resistant acid phosphatase (TRAPase) staining, specimens were rinsed with PBS and incubated in a mixture of 8 mg of naphthol AS-BI phosphate (Sigma), 70 mg red violet LB salt (Sigma), and 50 mM L(+)-tartaric acid (0.76 g; Nacalai Tesque, Kyoto, Japan).

Osteoclast Assay. For resorption pit assay (28, 29), aliquots of crude osteoclast preparations were plated on dentine slices and cultured in αMEM (Sigma) supplemented with 10 % fetal bovine serum with or without GIP (Peptide Institute, Inc., Osaka, Japan). Resorption pits on the dentine slices were visualized by staining with Mayer's hematoxyline solution.

Osteoblast Assay. Osteoblasts grown from the calvarium of 2-day-old mice were collected by treatment of collagen gel cultures with collagenase (30). A human osteoblastic cell-line, Saos-2 cells (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), and primary mouse osteoblasts were cultured in McCoy's 5A modified medium (GIBCO-BRL, Grand Island, NY) and αMEM, respectively, supplemented with 10 % fetal bovine serum. Cells were treated with the indicated concentrations of human GIP and human PTH (Peptide Institute, Inc.) for 1 hour, and incubated for an additional 6 hours in the presence or absence of 50 μM etoposide (Nipponkayaku, Tokyo, Japan). The pyknotic fragmented nuclei typical of apoptotic cells were viewed (31) using Hoechst 33342 (Sigma) and propidium iodide (PI) (Sigma) in an inverted fluorescence microscope with UV excitation at 340-380 nm (Axiovert 135, CarlZeiss, Oberkochen, Germany). Apoptotic cells were enumerated by Trypan blue (GIBCO-BRL) staining (22).

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FOOTNOTE

Abbreviations: GIP, gastric inhibitory polypeptide; GIPR, GIP receptor; PTH, parathyroid hormone; TNAPase, tissue nonspecific alkaline phosphatase; TRAPase, tartrate-resistant acid phosphatase; PI, propidium iodide; [cAMP]i, intracellular cAMP concentration

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Figure 1 Effects of GIP on endochondral ossification

(A) Growth of *GIPR*$^{+/+}$ and *GIP* $^{-/-}$ mice at 10 weeks of age.

(B) Soft X-ray analyses of *GIPR*$^{+/+}$ and *GIPR*$^{-/-}$ mice

Figure 2 Effects of GIP on bone trabeculae

Histological analyses of tibiae from *GIPR*$^{+/+}$ mice and *GIPR*$^{-/-}$ mice. The tibiae of 8-week-old male mice were examined. Original Magnification: A, ×40; B, ×100.

Figure 3 Dysregulation of bone remodeling in *GIPR*$^{-/-}$ mice

Immunostaining of tissue nonspecific alkaline phosphatase (TNAPase) (A) and tartrate-resistant acid phosphatase (TRAPase) staining (B) of tibiae from *GIPR*$^{+/+}$ and *GIPR*$^{-/-}$ mice are shown.

Figure 4 Effects of GIP on calcium homeostasis *in vivo*

(A) Urinary elimination of deoxypyridinoline crosslinks from *GIPR*$^{+/+}$ (open column) and *GIPR*$^{-/-}$ (filled column) mice. Values are means ± SE. *, $P < 0.05$ for *GIPR*$^{+/+}$ mice vs. *GIPR*$^{-/-}$ mice.

(B) Plasma calcium concentration of *GIPR*$^{+/+}$ and *GIPR*$^{-/-}$ mice before (open) and after (filled) meal was shown. Values are means ± SE. *, $P < 0.05$ vs. fasting.

Figure 5 Effects of GIP on pit-forming activity of osteoclasts *in vitro*

The crude osteoclast preparation was cultured on dentine slices in the presence or absence of GIP at 100 μM, using calcitonin as the positive control. (A) The resorption pits formed on the slices were stained with Mayer's hematoxylin solution for observation by light microscope (upper), or scanned by electron microscope (lower).

(B) The number of resorption pits' was calculated.

Figure 6 Effects of GIP on preventing apoptosis of osteoblasts *in vitro*

(A) Saos-2 cells, a human osteoblastic cell line, were stimulated with the indicated concentrations of human GIP and human PTH for 30 minutes, and cAMP levels were measured. **, $P < 0.01$ vs. control.

(B) Saos-2 cells were cultured for 6 hours with 50 μM etoposide in the absence or presence of preincubation for 1 hour with 100 nM GIP. The pyknotic fragmented nuclei typical of apoptotic cells viewed using Hoechst 33342 fluorescent dye. Original Magnification: ×100. Insets: percentage of cells undergoing apoptosis.

(C) Saos-2 cells were cultured for 1 hour in the indicated concentration of GIP or PTH and then incubated for an additional 6 hours in the absence (open) or presence of 50 μM etoposide (filled). Apoptotic cells were enumerated by trypan blue staining. Values are indicated as means ± SE. *, $P < 0.05$; **, $P < 0.01$ vs. etoposide alone.

(D) Primary mouse osteobalsts were cultured for 1 hour with or without GIP and then incubated for an additional 6 hours in the absence (open) or presence of 50 μM etoposide (filled). Apoptotic cells were enumerated by trypan blue staining. Values are indicated as means ± SE. **, $P < 0.01$ vs. etoposide alone.

Figure 7 Schematic model of cyclic (GIP) and persistent (PTH) activation of osteoblasts in bone remodeling.

GIP induces a cyclic increase of the intracellular cAMP concentration ([cAMP]i) in osteoblasts that induces bone formation, calcium from the blood calcium pool depositing on bone. Endogeneous PTH induces a persistent increase of [cAMP]i in osteoblasts that induces bone resorption, calcium from bone releasing into the blood calcium pool.

Table 1. Bone histomorphometry of tibia of *GIPR* $^{+/+}$ and *GIPR* $^{-/-}$ mice.

		GIPR $^{+/+}$	***GIPR*** $^{-/-}$
Bone Volume / Tissue Volume	BV/TV (%)	11.0 ± 1.7	9.5 ± 1.0
Trabecular Thickness	Tb. Th (μm)	31.7 ± 2.1	30.2 ± 1.5
Trabecular Separation	Tb. Sp (μm)	287.3 ± 36.6	299.0 ± 20.2
Eroded Surface / Bone Surface	ES/BS (%)	31.6 ± 2.9	34.7 ± 2.0
Osteoclast Surface	Oc. S/BS (%)	12.8 ± 2.0	13.1 ± 1.2
Osteoclast Number	N. Oc/TV (/mm^2)	19.3 ± 2.5	25.0 ± 1.8*
Osteoclast Number	N. Oc/BS (/mm)	3.2 ± 0.6	4.1 ± 0.3
Multinuclear Osteoclast Number	N. Mu. Oc	8.1 ± 1.2	11.6 ± 0.9*
Multinuclear Osteoclast Number	N. Mu. Oc/TV (/mm^2)	12.2 ± 1.8	18.1 ± 1.2**
Multinuclear Osteoclast Number	N. Mu. Oc/BS (/mm)	2.1 ± 0.4	2.9 ± 0.1*
Osteoblast Surface	Ob. S/BS (%)	39.2 ± 4.1	38.4 ± 2.7
Mineral Apposition Rate	MAR (μm/day)	2.8 ± 0.2	2.3 ± 0.1*
Ajusted Apposition Rate	Aj. AR (μm/day)	2.5 ± 0.2	1.8 ± 0.1*
Osteoid Maturation Rate	Omt (day)	1.4 ± 0.1	1.8 ± 0.1*
Mineralizing Lag Time	Mlt (day)	1.5 ± 0.2	2.2 ± 0.2*
Bone Formation Rate	BFR/BV (%/year)	2914.7 ± 261.0	2311.3 ± 120.1*
Bone Formation Rate	BFR/TV (%/year)	273.0 ± 31.3	206.9 ± 20.6
Bone Formation Rate	BFR/BS (mm^3/mm^2/year)	0.43 ± 0.02	0.34 ± 0.02**

* Significantly different from *GIPR* $^{+/+}$, $P<0.05$.

** Significantly different from *GIPR* $^{+/+}$, $P<0.01$.

Figure 1

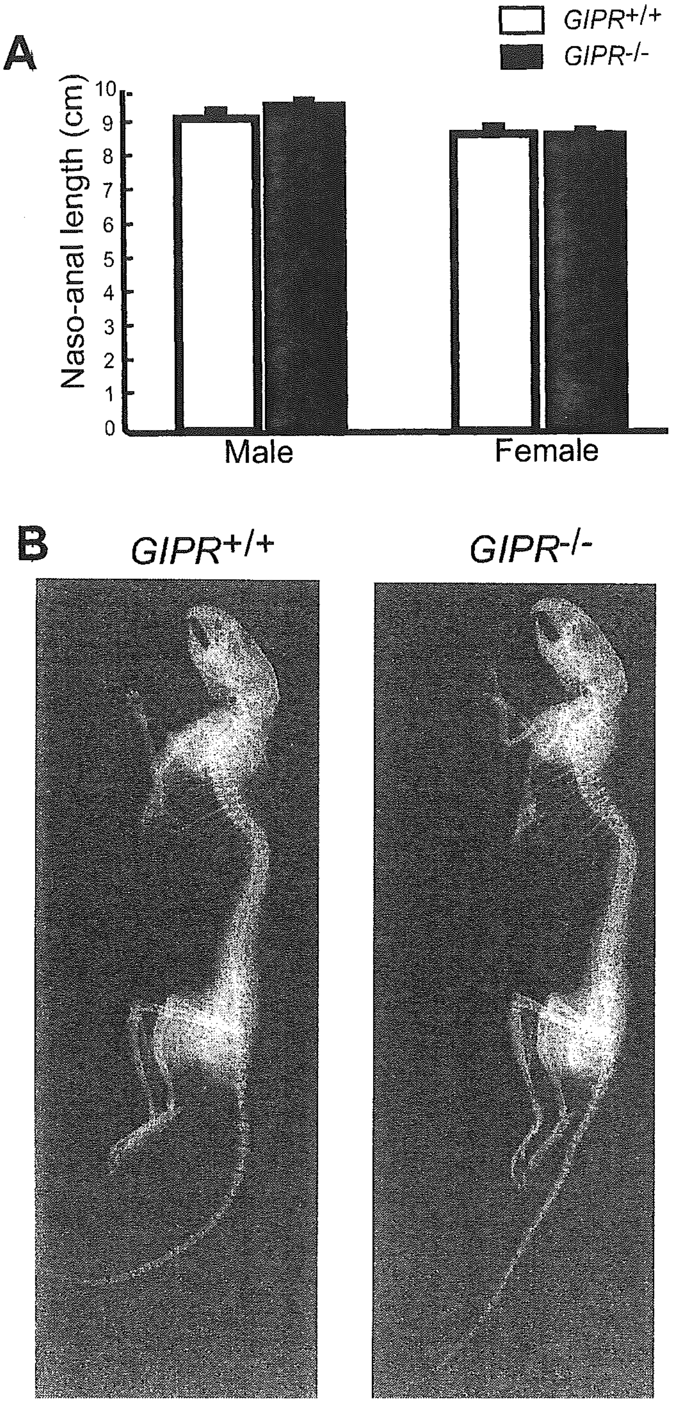

A
$GIPR^{+/+}$
$GIPR^{-/-}$
Naso-anal length (cm)
10
9
8
7
6
5
4
3
2
1
0
Male
Female
B
$GIPR^{+/+}$
$GIPR^{-/-}$

Figure 2

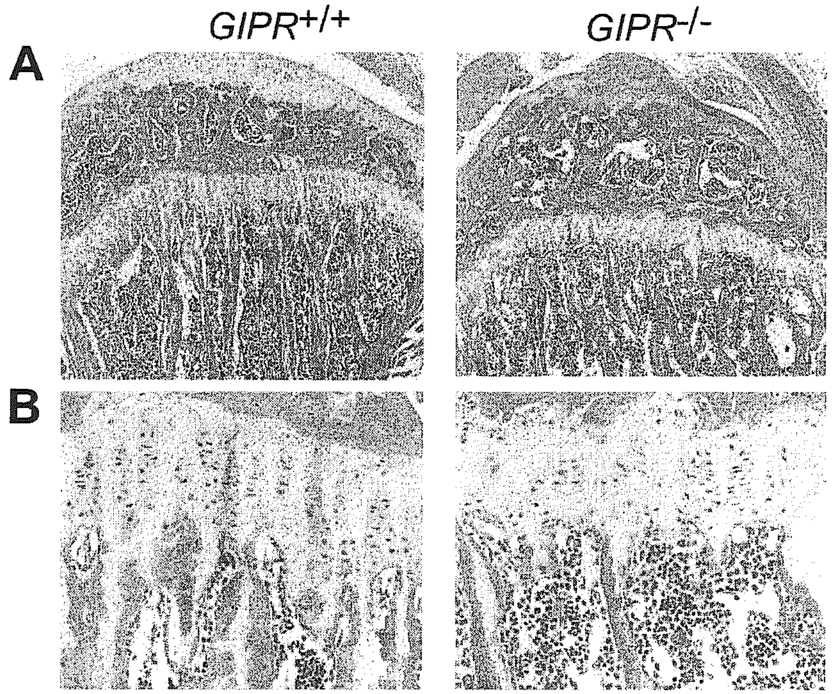


Figure 3

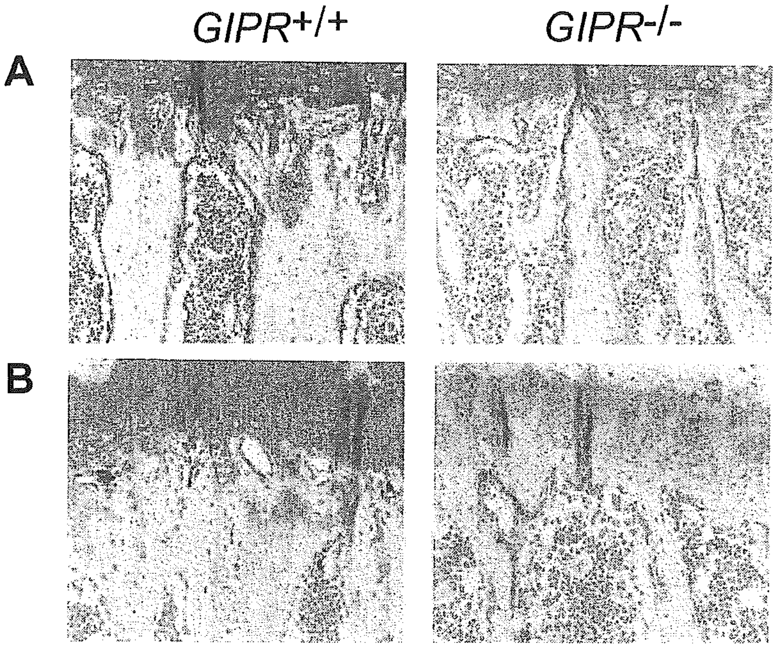


Figure 4

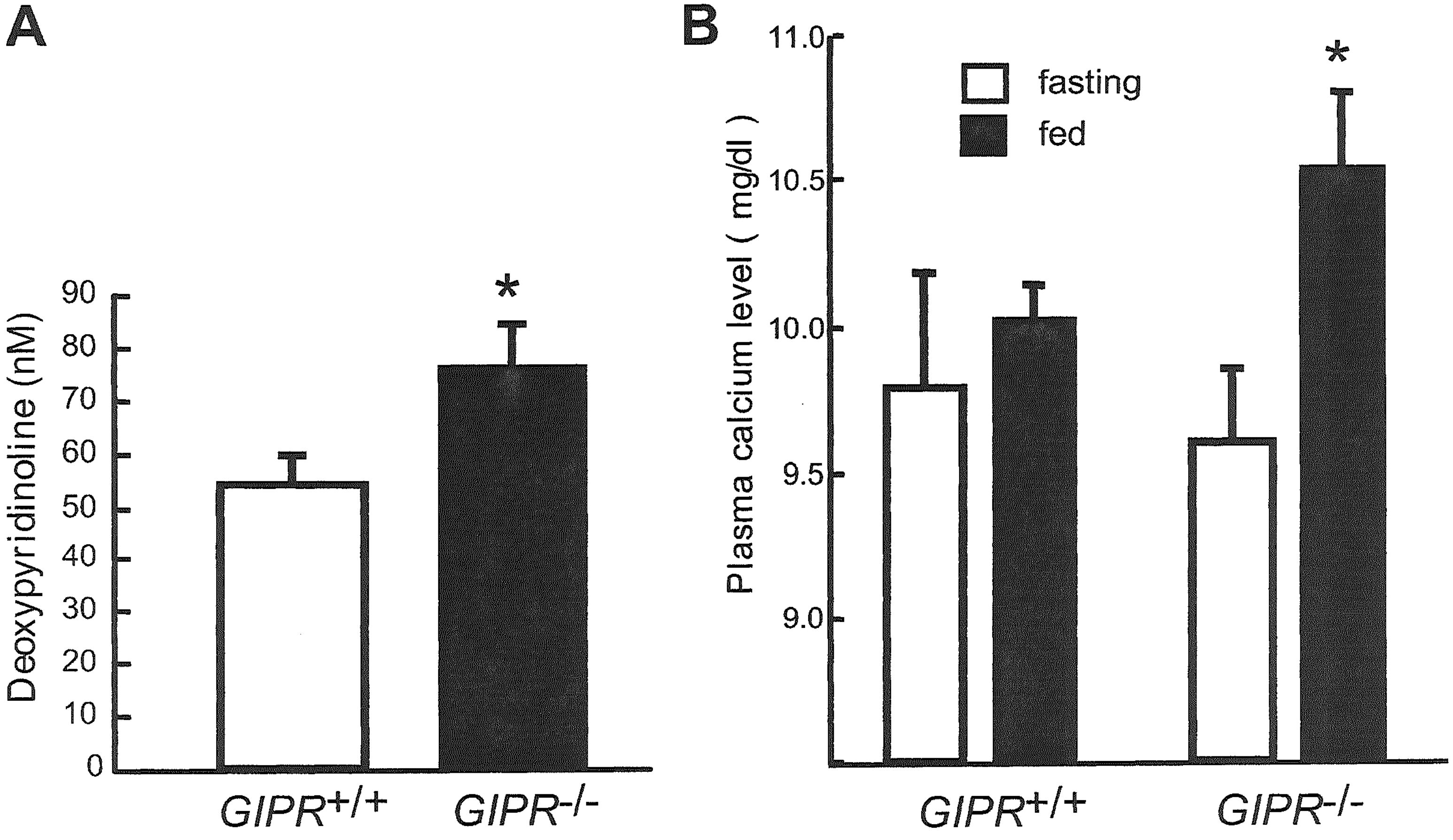
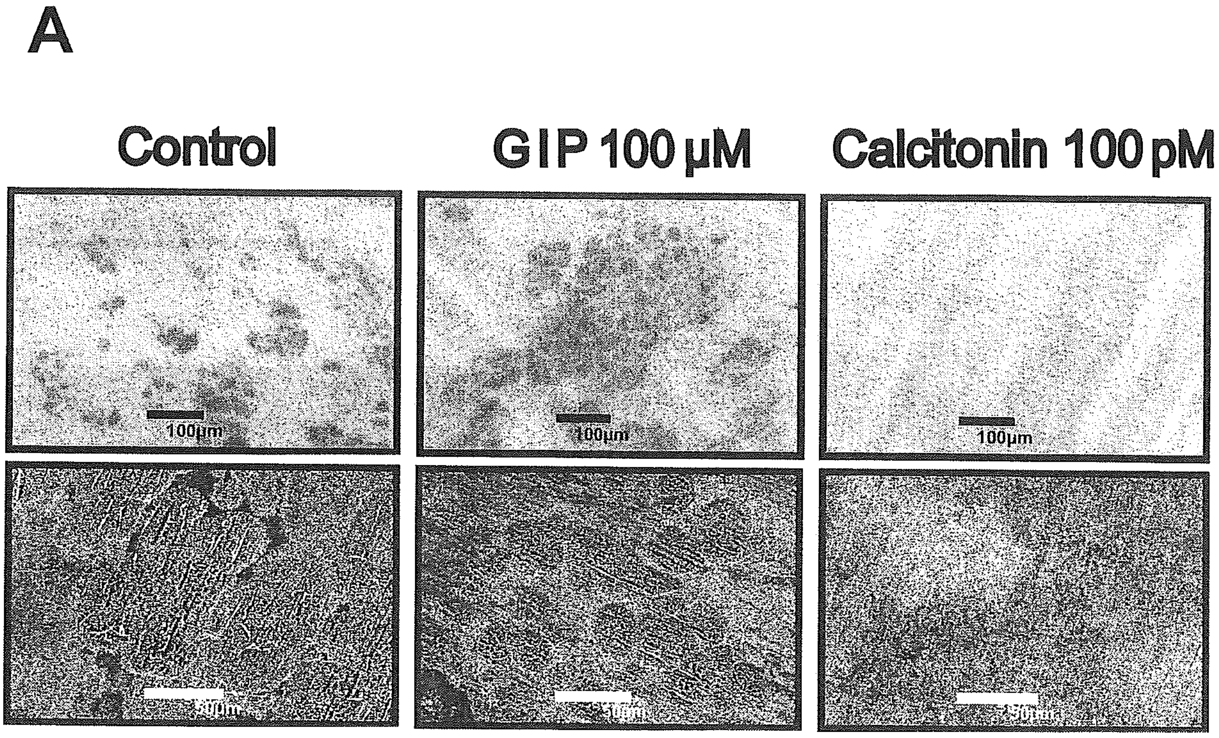

A
Deoxypyridinoline (nM)
90
80
70
60
50
40
30
20
10
0
*
GIPR+/+
GIPR-/-
B
Plasma calcium level (mg/dl)
11.0
10.5
10.0
9.5
9.0
fasting
fed
*
GIPR+/+
GIPR-/-

Figure 5


A
Control
GIP 100 μM
Calcitonin 100 pM
100μm
100μm
100μm

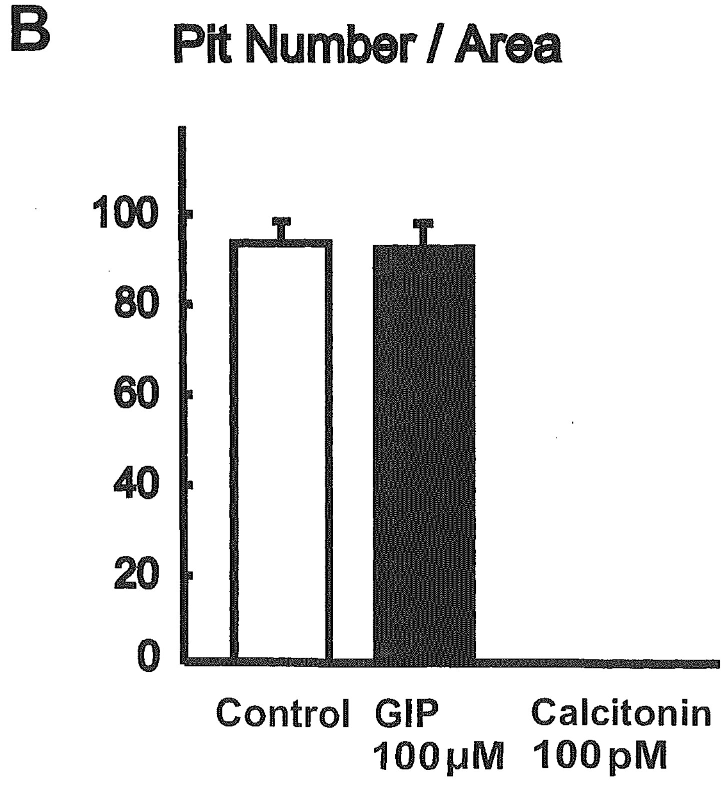
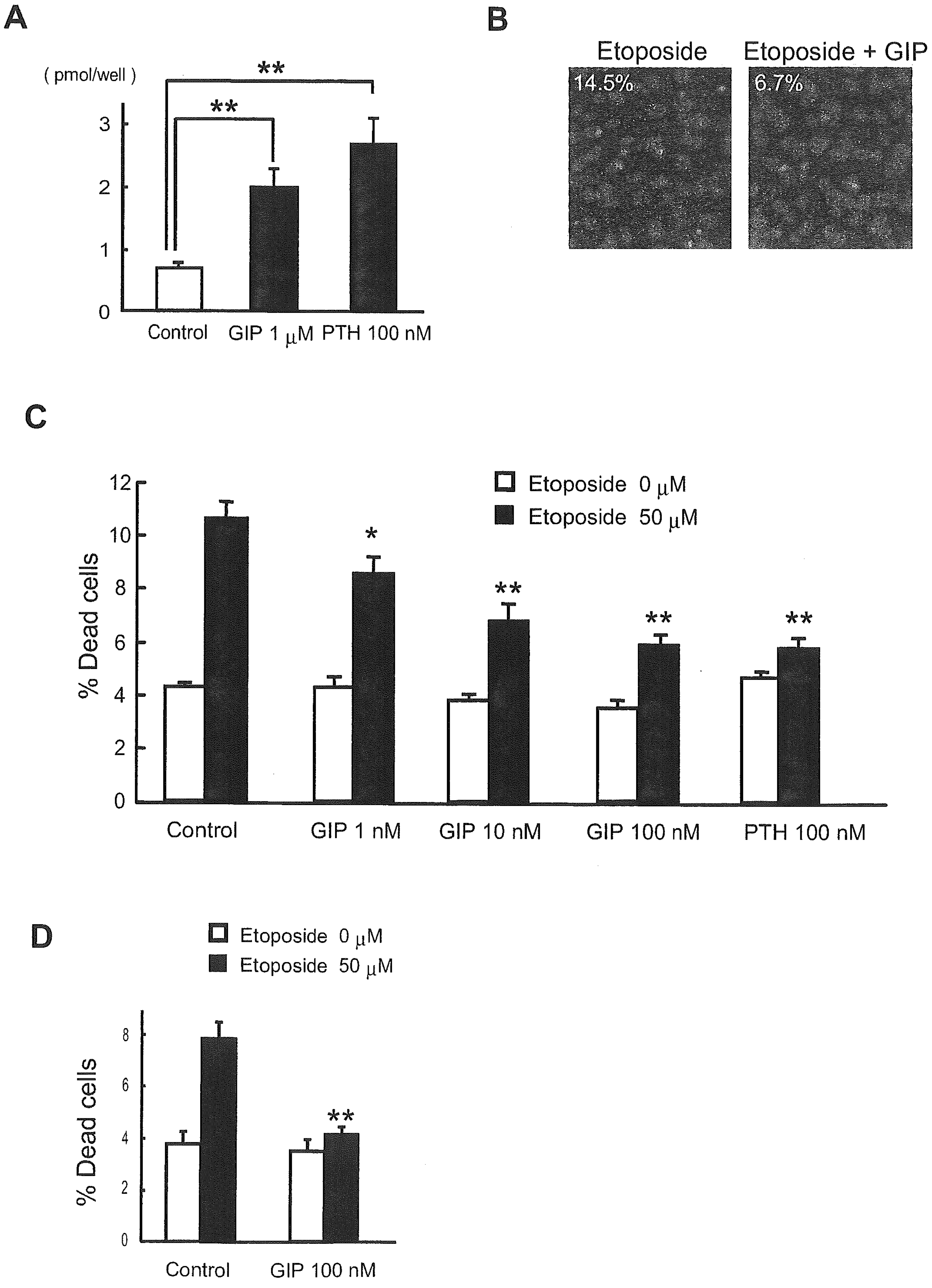
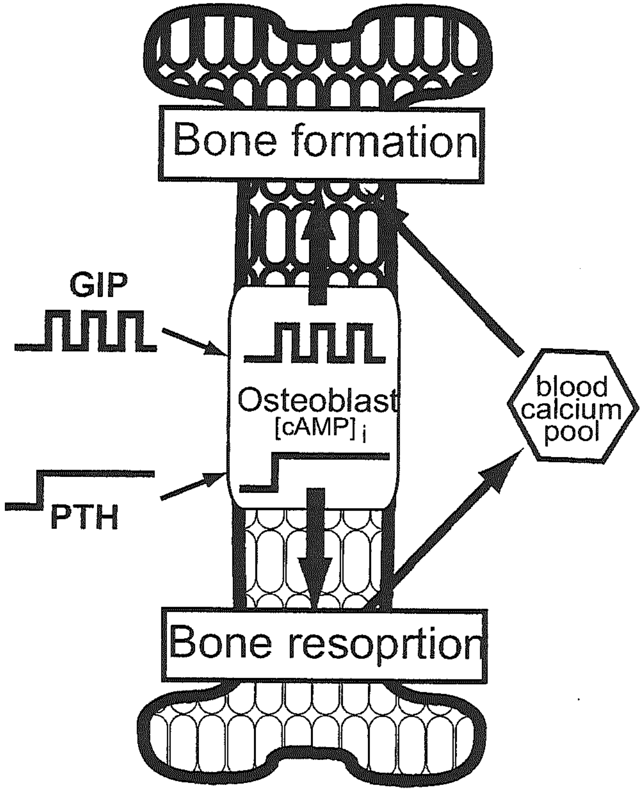

B
Pit Number / Area
100
80
60
40
20
0
Control
GIP 100 μM
Calcitonin 100 pM

Figure 6



A
(pmol/well)
**
**
3
2
1
0
Control
GIP 1 μM
PTH 100 nM
B
Etoposide
Etoposide + GIP
14.5%
6.7%
C
Etoposide 0 μM
Etoposide 50 μM
12
10
8
6
4
2
0
% Dead cells
*
**
**
**
Control
GIP 1 nM
GIP 10 nM
GIP 100 nM
PTH 100 nM
D
Etoposide 0 μM
Etoposide 50 μM
8
6
4
2
0
% Dead cells
**
Control
GIP 100 nM

Figure 7


Bone formation
GIP
Osteoblast
[cAMP]$_i$
PTH
blood calcium pool
Bone resoprtion

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DIABETES RESEARCH AND CLINICAL PRACTICE

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A single transplantation of the islets can produce glycemic stability and reduction of basal insulin requirement

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Abstract

We investigated glycemic stability and insulin requirement 1 month after a single transplantation of the islets from non-heart-beating donors or a living donor. Overall blood glucose levels decreased immediately after transplantation. The M-value and mean amplitude of glycemic excursions (MAGE) decreased significantly from 53.0 (range, 8.9–91.0) to 4.2 (0.6–8.8, $P < 0.05$) and from 8.5 mM (4.8–11.7) to 3.3 mM (2.0–4.5, $P < 0.05$), respectively. The values after transplantation were lower than the first quartile of 102 type 2 diabetic control patients. The estimated $\mathrm{HbA_{1c}}$ level decreased significantly from 7.9% (5.7–10.9) to 5.4% (4.7–5.9, $P < 0.05$). The supplement of basal insulin decreased 43% from 0.31 units/kg/day (0.16–0.37) to 0.18 units/kg/day (0–0.22, $P < 0.05$), while that of stimulated insulin did not decrease significantly, from 0.28 units/kg/day (0.13–0.51) to 0.21 units/kg/day (0–0.41). Thus, only one islet transplantation can be sufficient to attain metabolic stability, probably by effective supply of basal insulin secretion, sufficient to avoid life-threatening severe hypoglycemia and prevent or delay the progress of secondary complications of diabetes by decreasing the $\mathrm{HbA_{1c}}$ level.

Keywords: A single transplantation of the islets; Non-heart-beating donor; Living donor; Metabolic stability

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1. Introduction

Diabetes mellitus (DM) is a clinically and genetically heterogeneous group of disorders classified mainly into type 1 and type 2 diabetes. Type 1 diabetes is caused by β-cell destruction that often results in their complete loss and insulin-dependent diabetes mellitus

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