Supplementary Materials

Hypothalamic AMP-activated protein kinase regulates biphasic insulin secretion from pancreatic β cells during fasting and in type 2 diabetes

Shinji Kume, Motoyuki Kondo, Shiro Maeda, Yoshihiko Nishio, Tsuyoshi Yanagimachi, Yukihiro Fujita, Masakazu Haneda, Keiko Kondo, Akihiro Sekine, Shin-ich Araki, Hisazumi Araki, Masami Chin-Kanasaki, Satoshi Ugi, Daisuke Koya, Sawako Kitahara, Kiyosumi Maeda, Atsunori Kashiwagi, Takashi Uzu and Hiroshi Maegawa

Figure S1

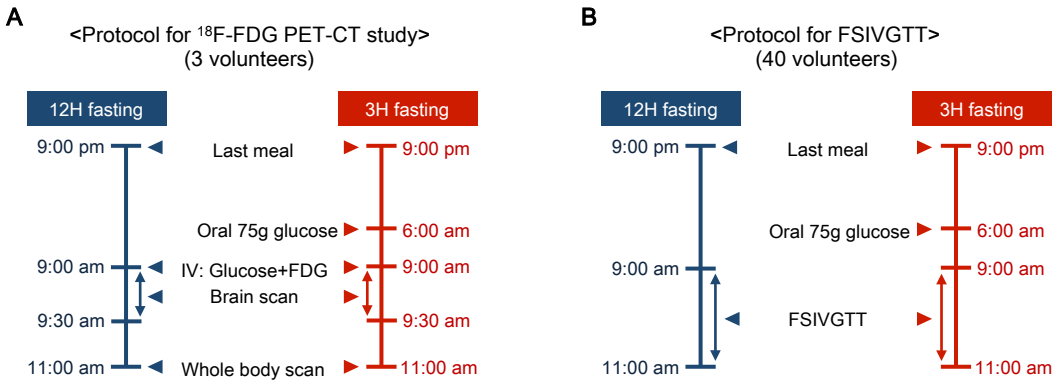

Figure S1. Experimental protocols for human PET-CT and IVGTT studies.
(A) Protocol design for the ^{18}F-FDG PET-CT study under 3- or 12-h fasting conditions to examine the effects of different fasting periods on the brain and whole-body glucose distributions after an acute glucose injection. (B) Protocol design for the frequent-sampling intravenous glucose tolerance test (FSIVGTT) under 12-h and 3-h fasting conditions.

Figure S2

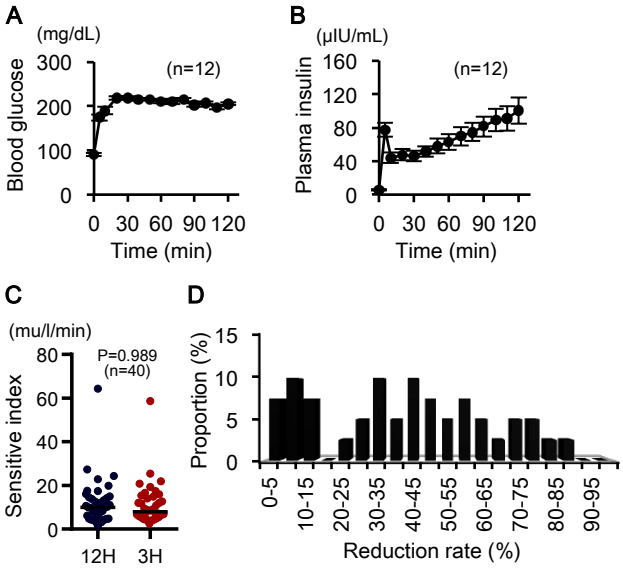

Figure S2. Different fasting periods altered first-phase GSIS in healthy volunteers.
(A and B) Changes in the blood glucose (A) and plasma insulin levels (B) during a hyperglycemic (HG) clamp study in 12 healthy volunteers. (C) Plots of the insulin sensitive index determined with the MINMOD model in the 12-h and 3-h fasting IVGTT in 40 healthy subjects. (D) Proportional distribution of the reduced rate of fasting-induced suppression of first-phase GSIS. $P<0.05$ indicates statistical significance.

Figure S3

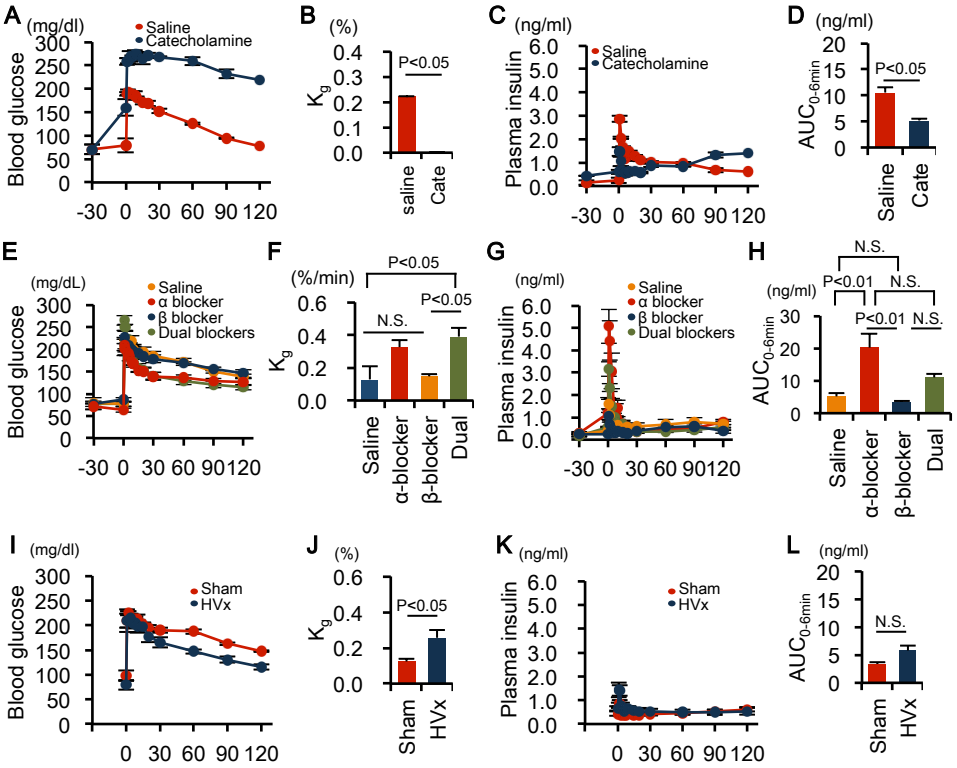

Figure S3. Role of autonomic nerve in fasting-dependent decrease in First-phase GSIS in rats.
(A–D) Changes in the glucose levels (A), glucose kinetics (B), insulin levels (C) and the AUC of the insulin levels (D) were measured during the dual IVGTT in 24-h-fasted healthy rats that were intravenously infused with saline or adrenaline and noradrenaline (n = 4 per group) 30 min prior to the IVGTT. (E–H) Changes in the glucose levels (E), glucose kinetics (F), insulin levels (G) and the AUC of the insulin levels (H) were measured during the dual IVGTT in 72-h-fasted healthy rats that were intravenously pre-infused with saline, α blocker (phentolamine), β blocker (propranolol), or both α and β blockers (n = 4, 5, 4, 5 per group) for 30 min prior to the IVGTT. (I-L) Changes in the glucose levels (I), glucose kinetics (J), insulin levels (K) and the AUC of the insulin levels (L) were measured during the dual IVGTT in 72 h fasted healthy rats that received a sham operation or hepatic vagotomy (HVx) (n = 4, 5 per group, respectively.) $P<0.05$ indicates statistical significance. N.S. indicates no statistical significance. All values are presented as the means ± SEM.

Figure S4

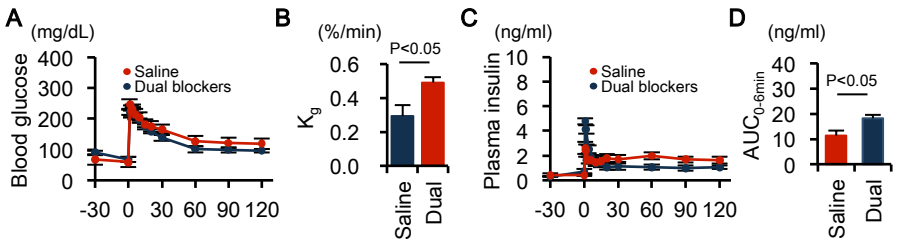

Figure S4. Role of autonomic nerve in diabetes-induced inhibition of first-phase GSIS in rats.
(A–D) Changes in the glucose levels (A), glucose kinetics (B), insulin levels (C) and the AUCs of the insulin levels (D) were measured during the IVGTT in the 24-h fasted OLETF rats that had been intravenously pre-infused with saline or both α and β blockers (n = 6, 5 per group) for 30 min prior to the IVGTT. $P<0.05$ indicates statistical significance. All values are presented as the means ± SEM.

Figure S5

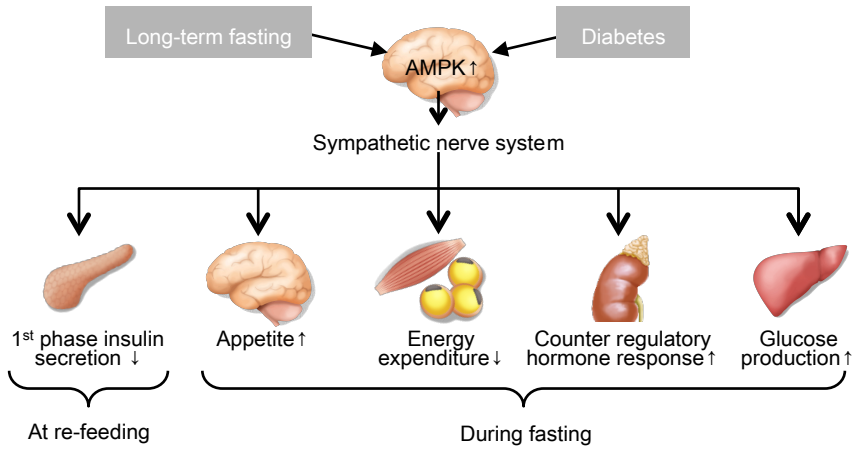

Figure S5. Physiological roles of hypothalamic AMPK in combatting starvation; its relation to diabetes. Hypothalamic AMPK senses long-term fasting and activates the sympathetic nervous system, resulting in the suppression of fast-phase insulin secretion at refeeding. Previous studies have shown that the system also upregulates appetite, counter-regulatory responses to hypoglycemia and hepatic glucose production but decreases energy expenditure in the peripheral tissues during fasting. This starvation mechanism may be involved in the pathogenesis of early type 2 diabetes.

Table S1

(N = 40)	Mean ± SEM
Male (number / %)	25 / 62.5
Female (number / %)	15 /37.5
Age (years old)	31.5 ± 6.60
Body weight (kg)	62.4 ± 12.4
Body mass index (kg/m2)	22.1 ± 2.90
Fasting blood glucose (mg/dl)	80.6 ± 7.10
Hemoglobin A1c (NGSP; %)	5.42 ± 0.21
Fasting plasma insulin (μIU/mL)	5.42 ± 2.10
HOMA-R	1.10 ± 0.43
HOMA-b	98.6 ± 40.5
Fasting free fatty acid (mEq/L)	0.53 ± 0.26
Glucagon (12-h) (pg/mL)	75.8 ± 19.2
Glucagon (3-h) (pg/mL)	77.3 ± 16.1
Active GLP-1 (12-h) (pmol/L)	9.96 ± 3.92
Active GLP-1 (3-h) (pmol/L)	11.2 ± 3.75
Active GIP (12-h) (pmol/L)	6.92 ± 3.16
Active GIP (3-h) (pmol/L)	9.70 ± 8.60
Systolic blood pressure (mmHg)	117.1 ± 12.20

Table S1. Characteristics of the 40 healthy human subjects who underwent IVGTTs.
Clinical characteristics of the 40 healthy human subjects who underwent the 3-h and 12-h fasting IVGTTs. HOMA: homeostasis model assessment, GLP-1: glucagon-like peptide-1, GIP: glucose-dependent insulinotropic polypeptide.

Table S2

Genetic risk score (17 SNPs)	
Gene name	SNP
KCNQ1	rs2237892
TCFL2	rs7903146
CDKAL1	rs7556992
HHEX	rs1111875
SLC30A8	rs13266634
KCNJ11	rs5219
CDKN2A/B	rs10811661
IGF2BP2	rs1470579
WFS1	rs10010131
MTNR1B	rs1387153
HNF1B	rs7501939
UBE2E2	rs9812056
C2CD4A/B	rs7172432
DGKB-TMEM195	rs2191349
KCNQ1	rs231362
HNF4A	rs4812829
GLIS3	rs7041847

Table S2. List of the gene names and SNPs information used in this study.
The listed SNPs were used to analyze the relationships between the genetic risk scores and insulin secretion-related parameters calculated from the data in the IVGTT (as shown in Table 1). All SNPs are related to both the insulin secretion capacity and the prevalence of type 2 diabetes. SNP, single nucleotide polymorphism.

Table S3

	LETO	Sham-OLETF	PNx-OLETF
Body weight (g)	478 ± 11.1	635.7 ± 12.1*	618.6 ± 12.1*
Diet intake (g)	24.1 ± 0.70	29.5 ± 0.97*	29.5 ±1.12*
Fasting blood glucose (mg/dl)	90.2 ± 3.9	165.3 ± 6.3*	124.3 ± 8.6*†
Fasting insulin (ng/ml)	0.209 ± 0.04	1.29 ± 0.34*	1.43 ± 0.38*
Free fatty acids (mg/dl)	0.96 ± 0.09	1.86 ± 0.23*	1.67 ± 0.12*
Triglyceride (mg/dl)	80.8 ± 3.03	78.0 ± 2.57	81.8 ± 2.45
Total cholesterol (mg/dl)	54.2 ± 5.67	265.2 ± 28.3*	227.4 ± 22.1*

Table S3. Characteristics of the 32-wk-old LETO, sham-OLETF and PNx-OLETF rats.
*P<0.05 vs. LETO. †P<0.05 vs. Sham-OLETF. All values are presented as the means ± SEM.

Legends for Movie S1

Movie S1. Movies showing whole-body ^{18}F-FDG-PET-CT images.
(A–C) Whole-body ^{18}F-FDG-PET-CT images from three independent healthy human subjects at 120 min after the injection of glucose and ^{18}F-FDG under 12-h or 3-h fasting conditions. Left and right movies in each movie file were obtained from the studies after 12-h and 3-h, respectively.