Eldecalcitol, an active vitamin D analog, for type 2 diabetes prevention in prediabetes (DPVD)

SUPPLEMENTARY APPENDIX

Table of Contents

DPVD Study Personnel

Principal Investigator

Tetsuya Kawahara, MD PhD (Kokura Medical Association Health Testing Center)

Co-Principal Investigator

Yoshiya Tanaka, MD PhD (University of Occupational Environmental Health)

Trial Steering Committee

Gen Suzuki, MD PhD (International University of Health and Welfare Clinic)

Tetsuya Kawahara, MD PhD (Kokura Medical Association Health Testing Center)

Shoichi Mizuno, PhD (National Cancer Center Hospital)

Tetsuya Inazu, MD PhD (Ritsumeikan University)

Fumiyoshi Kasagi, PhD (Radiation Effects Association)

Takuya Uno, MD (Kokura Medical Association)

Ryuichiro Imawatari, MD (Kokura Medical Association)

Hideo Nishimura, PhD (Translational Research Center for Medical Innovation)

Yosuke Okada, MD PhD (University of Occupational Environmental Health)

Yoshiya Tanaka, MD PhD (University of Occupational Environmental Health)

Advisors

Masanori Fukushima, MD PhD (Translational Research Center for Medical Innovation)

Eiji Nakatani, PhD (Translational Research Center for Medical Innovation)

Hideaki Kaneda, PhD (Translational Research Center for Medical Innovation)

Naoki Kunugita, MD PhD (National Institute of Public Health)

Masayuki Yokota, PhD (Pharmaceuticals and Medical Devices Agency)

Data and Safety Monitoring Board

Tetsuya Inazu, MD PhD (Ritsumeikan University)

Hideo Nishimura, PhD (Translational Research Center for Medical Innovation)

Biostatisticians

Shoichi Mizuno, PhD (National Cancer Center Hospital East)

Fumiyoshi Kasagi, PhD (Radiation Effects Association)

Investigators

Yosuke Okada, MD PhD (University of Occupational Environmental Health)

Chie Kawahara, MD (University of Occupational Environmental Health)

Tatsuro Takano, MD (Fujisawa City Hospital)

Laboratory Team

Shizuyo Sugino, Yasuhisa Satake, Yukiko Honjo, Satoko Yumae, Takeru Ohshita, Masamichi Suzuki, Shuji Saitou, Setsuko Taki, Ryota Shimizu and Haruko Yumiya

Field Team

Etsuko Matsuda, Atsuko Kanada, Keiko Akahoshi, Eriko Matsushita, Hidenori Niwa, Kenji Shiratsuchi, Motokazu Sano, Akitoshi Futatsuishi, Hirotaka Iwasaki, Koji Shirahama, Chinatsu Yamagami, Eriko Inuki, Shigesato Mochiyama, Satoshi Honjo, Noboru Takamura, Takeshi Ishimura, Takayuki Shinohara, Seiji Ono, Taishi Murayama, Chiharu Kodama, Keiko Tajimi, Taro Ishibashi, Norifumi Kawakami, Yurie Gohda, Taiji Kume, Ritsuko Satoh, Takuya Honda, Fujiko Kimura, Yoshinori Tango, Keiji Kitamura, Makoto Kawaguchi, Takao Yamada, Eri Murata, Kimitoshi Ohmura, Kei Nakata, Toru Kannawa, Chisato Osugi, Toshihiro Okunaga, Chika Goto, Sakurako Tonegawa, Yuriko Tokunaga and Souta Hirayama

Ouma Naika Clinic, Adachi Uno Naika Clinic, Nakamura Naika Clinic, Kitakyushu Municipal Medical Center, Tahara Clinic, Nitta Orthopedics Clinic, Matsumura Naika Clinic, Ohkubo Medical Clinic, Nishimura Urology clinic, Obstetrics and gynecology Tsuda clinic, Kido Naika Clinic, JCHO Kyushu Hospital, Fujita Dermatology Clinic, Kirigaoka Tsuda Hospital, Kasho Clinic, Iwamoto Naika Clinic, Yoshinaga Clinic, Kokura Memorial Hospital, Iwamoto Naika Clinic, Yoshizawa Clinic, Nonaka Naika Clinic, Saiseikai Yahata General Hospital, Inokuchi Naika Clinic, Kuroki Hiro Clinic, Kyushu Rosai Hospital, Imawatari Cardiology Clinic, Tohya Clinic, Touwa Hospital, Maeyama Orthopedics Clinic, Sugimoto Clinic, and Tobata Kyoritsu Hospital.

Ethical Approval and Trial Registration

The study was approved by the Institutional Review Boards at Kokura Medical Association, University of Occupational Health, and Fujisawa City Hospital, Japan (IRB reference number 250510 and 13060904-2), and registered with University Hospital Medical Information Network (UMIN) Clinical Trial Registry (UMIN 000010758).

Eligibility Criteria for Participation in the DPVD Study

Diagnostic criteria of impaired glucose tolerance

Fasting plasma glucose level of < 126 mg/dL [7.0 mmol/L], a 2-hour plasma glucose level of 140 to 199 mg/dL [7.8 to 11.0 mmol/L] during a 75-g oral glucose tolerance test, and glycated hemoglobin (HbA1c) level of < 6.5% [48 mmol/L]). Additionally, he/she should not be medicated with any antidiabetic drug.

Inclusion criteria

1. Men and women aged ≥ 30 years old.
2. Individuals diagnosed with impaired glucose tolerance.
3. Serum calcium level (corrected value) < 11.0 mg/dL.

Exclusion criteria

1. Individuals who have participated in other clinical trials.
2. Individuals who have been treated with active vitamin D, vitamin D supplement, and/or calcium preparation within the preceding 3-month period.
3. Individuals who have already been diagnosed with type 1 or 2 diabetes.
4. Individuals in whom drug treatment for pre-diabetes has been initiated.
5. Individuals who are pregnant or have severe diseases, such as renal insufficiency (serum creatinine > 1.5 mg /dL), hepatic insufficiency, psychosis, collagen diseases, heart diseases, and/or cerebrovascular diseases.
6. A sub-investigator may preclude participant involvement in the study based on screening and assessment of the participants' condition.

Statistical Analyses

Subjects for analyses of effectiveness and safety

The intention-to-treat population, comprising all participants who undergo randomization and receive at least one dose of the study drug, will be analyzed for effectiveness and safety. In this analysis plan, the data set is called the full analysis set (FAS). The per protocol set (PPS), comprising all participants who undergo randomization and adhere to the inclusion and exclusion criteria, is defined as the secondary population for sensitivity analysis. A sensitivity analysis to assess non-informative censoring was conducted as an evaluation of the robustness of the primary analysis result.

Imputation method for missing data

The last observation carried forward (LOCF) method was originally used for participants who do not complete the follow-up period. However, we amended it to multiple imputation (MI) method for time-trends analyses.

Handling of discontinuation data

In FAS, all values that are measured before discontinuation will be analyzed, regardless of the point of discontinuation. In PPS, discontinuation within 3 months from treatment initiation is considered as a missing case. If the discontinuation occurs after 3 months, the value will be analyzed.

Handling non-adherence to treatment protocol

In FAS, regardless of the period of non-compliance to the assigned treatment, practical data will be used for analyses. In PPS, practical data will be denoted as missing data during the non-compliance period.

Handling participant use of prohibited medicine during the follow-up period

In FAS, regardless of the prohibited treatment period, practical data will be used for analyses. In PPS, practical data will be denoted as missing data during the prohibited treatment period.

Data obtained by alternate methods and/or conditions

When summating information in each specified 3-month period, data beyond the accepted observation date or data obtained by other methods or conditions are denoted as missing data. Investigators, sub-investigators, and statisticians will collectively determine whether or not data are acceptable for statistical analysis and implement remedial measures as required. Unlocking will be performed after fixing all data.

Amendments of secondary endpoints

The secondary endpoints (in the UMIN clinical trial registry on May 19, 2013)

#1. The improvement ratio from impaired glucose tolerance to normoglycemia.

#2. The change in homeostasis model assessment of insulin resistance (HOMA-R).

#3. The change in body weight and body mass index (BMI).

#4. Influence of hypertension, hyperlipidemia, obesity, fasting plasma glucose, 2-h plasma glucose, 25-hydroxy vitamin D, smoking, HOMA-R, and insulinogenic index on the incidence of type2 diabetes.

The secondary endpoints (in the protocol on January 20, 2014, and in the BMJ Open on July 2016.)

#1. The number of participants who achieve normoglycemia at 48, 96, and 144 weeks.

#2. Hazard ratios (HRs) and 95% confidence intervals (CIs) of type 2 diabetes onset in each subgroup at baseline: age, sex, presence or absence of hypertension, dyslipidemia, obesity, family history of diabetes, fasting plasma glucose, 2-h plasma glucose, 25-hydroxy vitamin D, HOMA-R, and insulinogenic index.

#3. HRs and 95% CIs of type 2 diabetes development after adjusting for confounding factors: age, sex, presence or absence of hypertension, dyslipidemia, obesity, family history of diabetes, fasting plasma glucose, 2-h plasma glucose, 25-hydroxy vitamin D, HOMA-R, and insulinogenic index.

#4. HRs of the incidence of adverse events.

The secondary endpoints (in the protocol on August 17, 2016)

#1. Improving ratio from impaired glucose tolerance to normoglycemia at 1, 2, and 3 years.

#2. Hazard ratios and 95% confidence intervals (CI) of type 2 diabetes onset in each subgroup at baseline: age (≥ / <65 years), sex (male/female), presence or absence of hypertension (systolic ≥ 140 mmHg and/or diastolic ≥90 mmHg), obesity (BMI ≥ / <25 kg/m^2), family history of diabetes, FPG (≥ / <110 mg/dl), 2h-PG (≥ / <170 mg/dl), 25(OH)D_3 (≥ / <20 ng/ml), HOMA-R (~1.6 / 1.61–2.49 / 2.5~), and Insulinogenic Index (≥ / <0.4)

#3. Hazard ratios and 95% CI of type 2 diabetes development after adjusting for treatment group (eldecalcitol or placebo) and each confounding factor: age, sex (male/female), presence or absence of hypertension (systolic ≥140 mmHg and/or diastolic ≥90 mmHg), BMI, family history of diabetes (yes / no), HbA1c, fasting plasma glucose, 2-h plasma glucose, 25-hyrdoxy vitamin D, HOMA-R, and/or insulinogenic index.

#4. Hazard ratios of the incidence of adverse events: active vitamin D group vs. control group

The secondary endpoints (post submission to the BMJ)

#2. Hazard ratios and 95% confidence intervals (CI) of type 2 diabetes onset in each subgroup at baseline: age, systolic blood pressure, BMI, HbA1c, FPG, fasting IRI, 2h-PG, 25(OH)D_3, HOMA-R, HOMA-β, and Insulinogenic Index. Analysis was conducted by using continuous variables instead of dividing them into two groups. This was removed from the secondary endpoints and changed to a sensitivity analysis.

Table A. Baseline characteristics - Taking drugs that may impact glucose and bone metabolism

	Eldecalcitol group (n=630)	Placebo group (n=626)	P value
Calcium blocker	8	9	N.S.
Angiotensin II Receptor Blocker	6	4	N.S.
Angiotensin Converting Enzyme Inhibitor	3	2	N.S.
β blocker	4	5	N.S.
Statin	5	3	N.S.
Fibrate	2	4	N.S.
Ezetimibe	2	1	N.S.

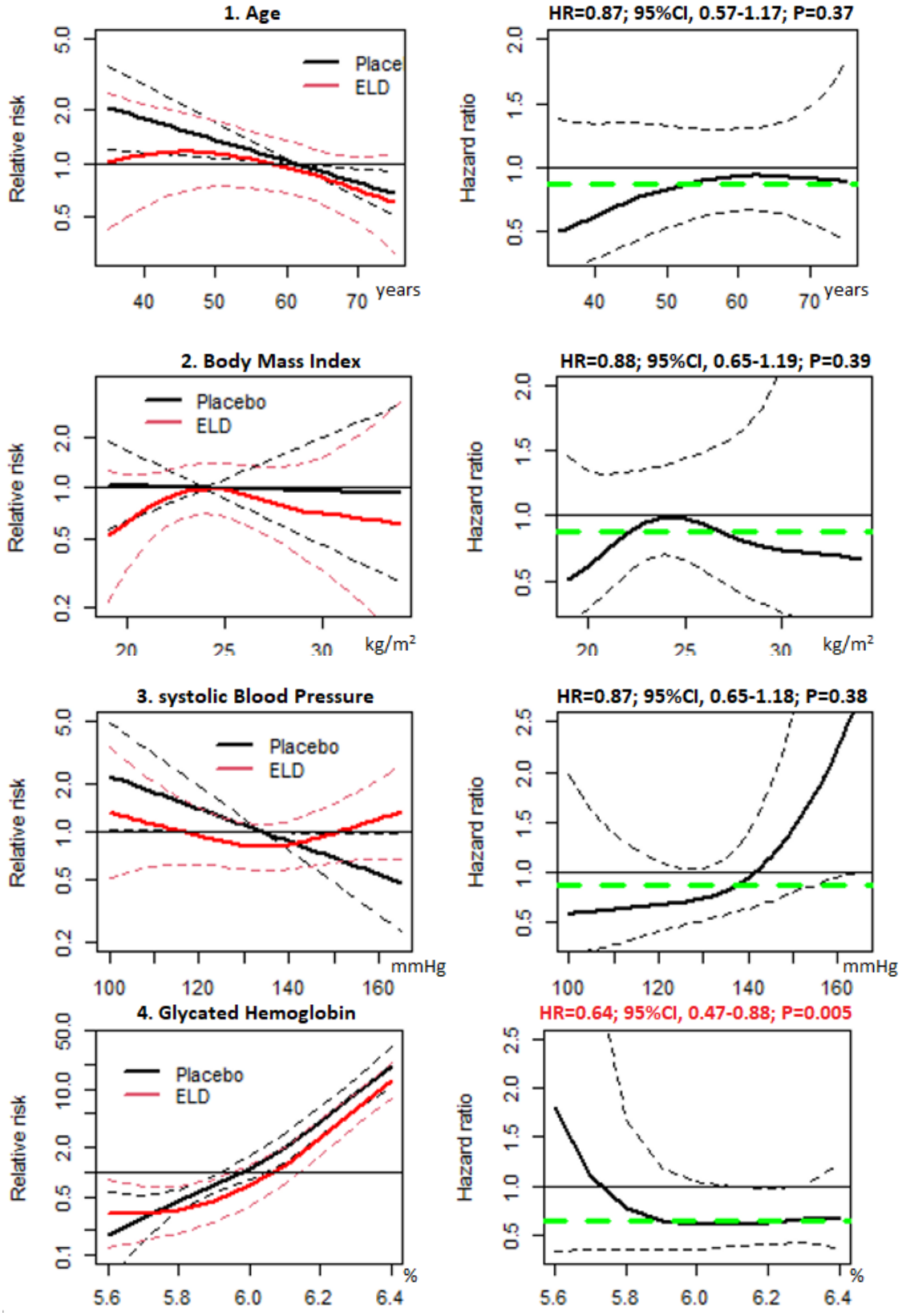
Patients with mild hypertension and/or dyslipidemia participated in the current study; therefore, the number of participants taking medications was described.

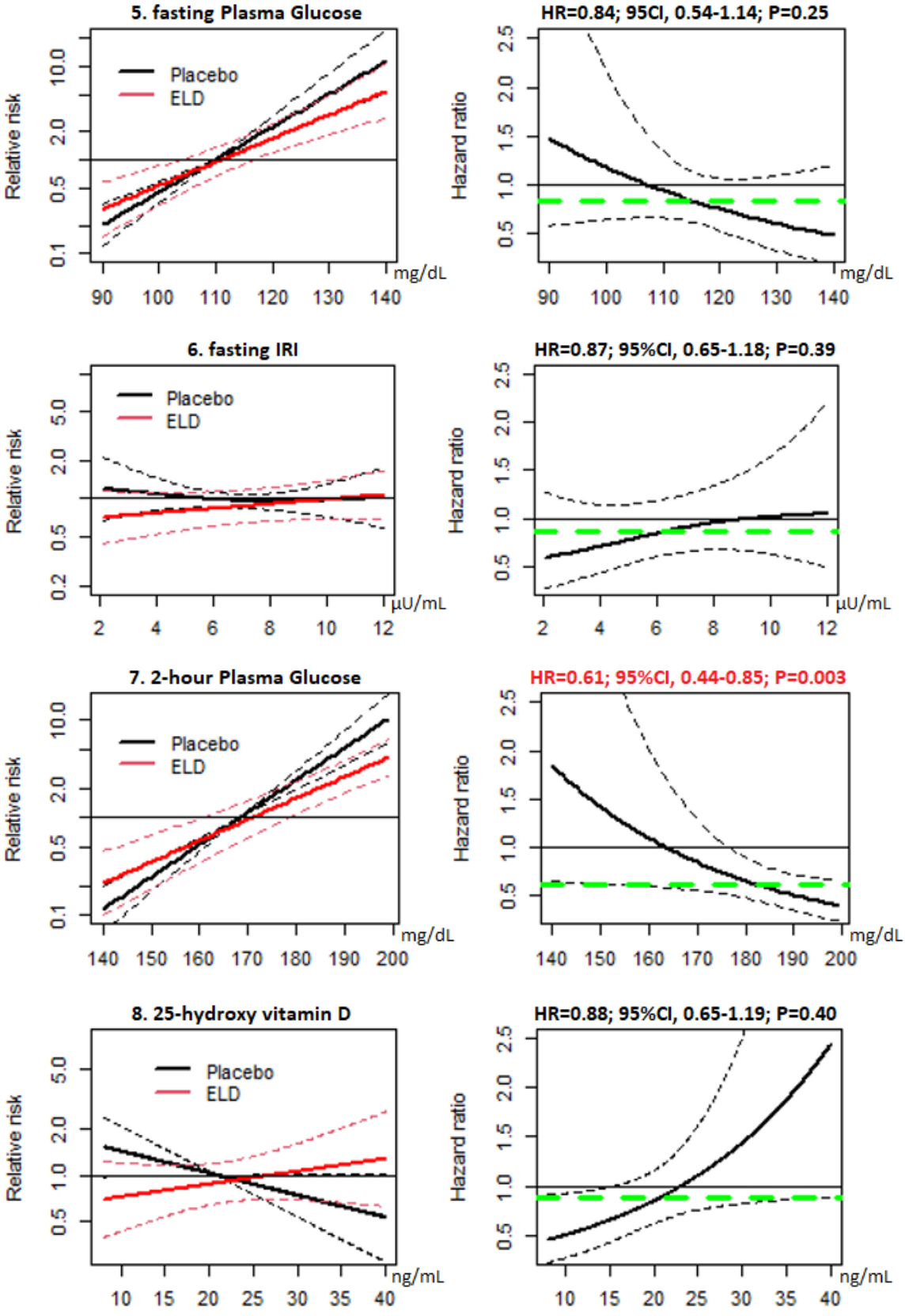
Table B. Distributions of parameters related to glucose metabolism between intention to treat and complete population

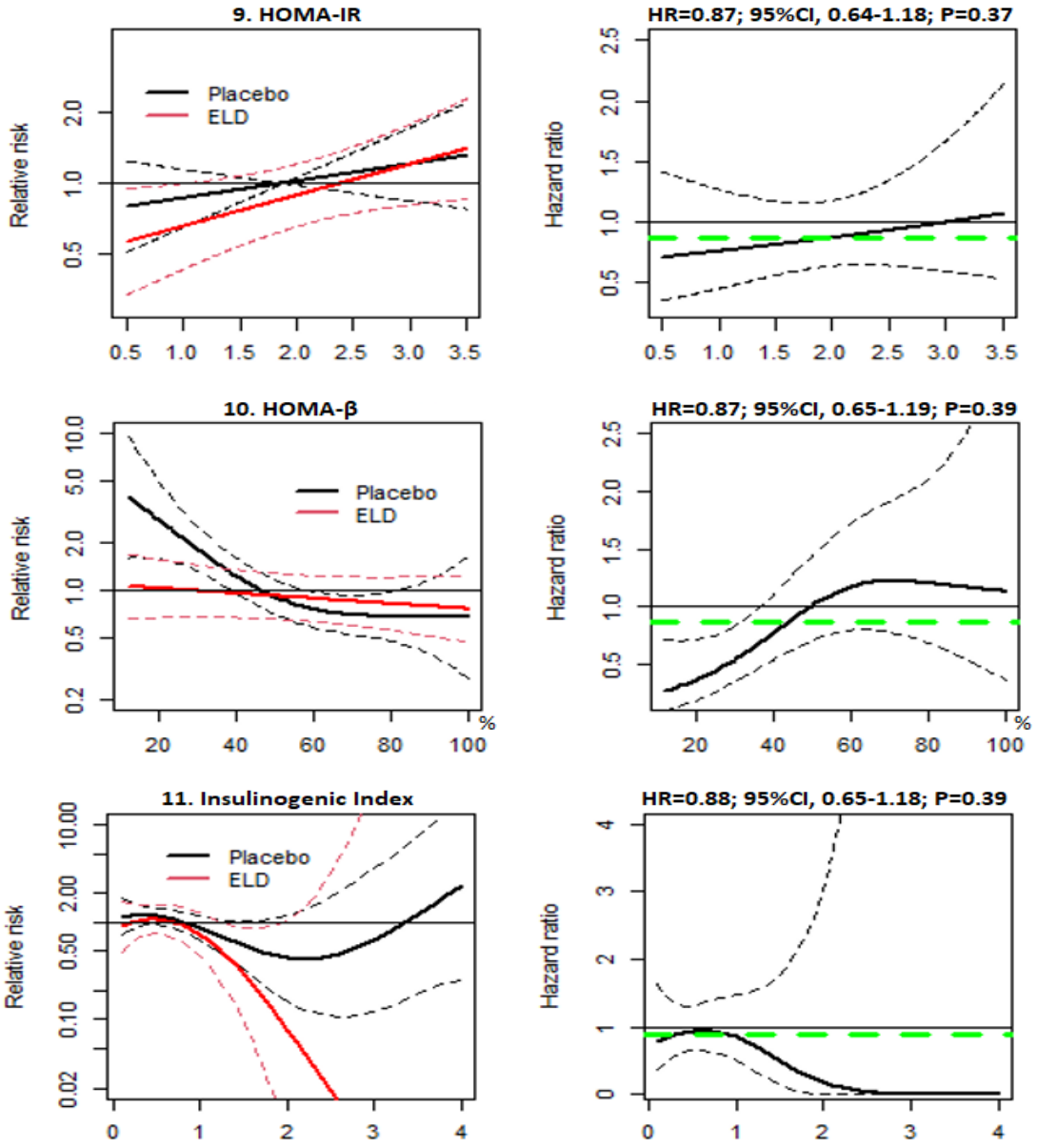
		BMI	HbA1c	0-PG	30-PG	2h-PG
Intention to treat population	**Eldecalcitol (n=630)**	24.1±2.7	5.9±0.2	110.0±9.5	177.4±19.8	168.9±20.1
	Placebo (n=626)	24.5±1.8	6.0±0.2	109.8±8.9	175.3±20.2	168.0±15.0
Complete population	**Eldecalcitol (n=598)**	24.2±2.5	5.9±0.2	109.5±9.7	176.9±20.1	168.8±18.9
	Placebo (n=594)	24.4±2.0	5.9±0.2	109.1±8.6	175.4±20.0	168.1±17.1

BMI, body mass index; HbA1c, glycated hemoglobin; 0-PG, 0-minute plasma glucose; 30-PG, 30-minute plasma glucose; 2-h PG, 2-hour plasma glucose.

Fig A. Subgroup analysis of continuous variables using splines




5. fasting Plasma Glucose
HR=0.84; 95CI, 0.54-1.14; P=0.25
Placebo
ELD
Relative risk
Hazard ratio
mg/dL
6. fasting IRI
HR=0.87; 95%CI, 0.65-1.18; P=0.39
µU/mL
7. 2-hour Plasma Glucose
HR=0.61; 95%CI, 0.44-0.85; P=0.003
8. 25-hydroxy vitamin D
HR=0.88; 95%CI, 0.65-1.19; P=0.40
ng/mL

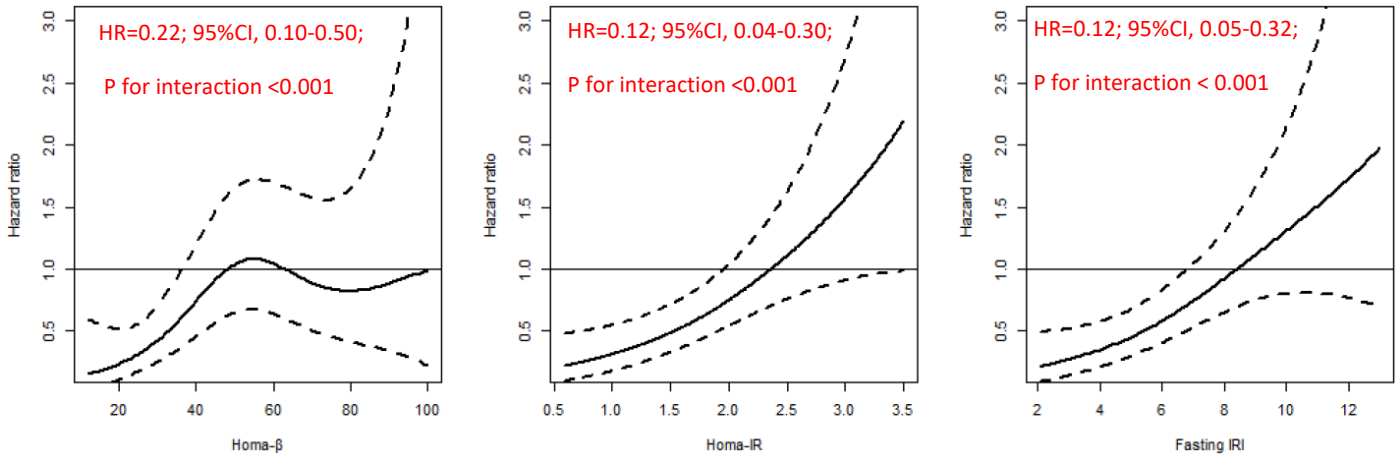


ELD, eldecalcitol; fasting IRI, fasting immunoreactive insulin; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of beta-cell function.

Left figures: The red and black lines represent continuous relative risks to subjects with median value in the placebo group estimated for the eldecalcitol and placebo groups, respectively. The red and black dotted lines represent 95% CI of them, respectively.

Right figures: The black lines represent continuous hazard ratios (rate ratios of red spline to black spline in left figure), and dotted lines represent the 95% CI. The dashed green lines represent overall hazard ratios calculated using multivariable fractional polynomial Cox regression analyses.

Fig B. Interaction analysis of continuous variables adjusted for HbA1c and 2hr. plasma glucose

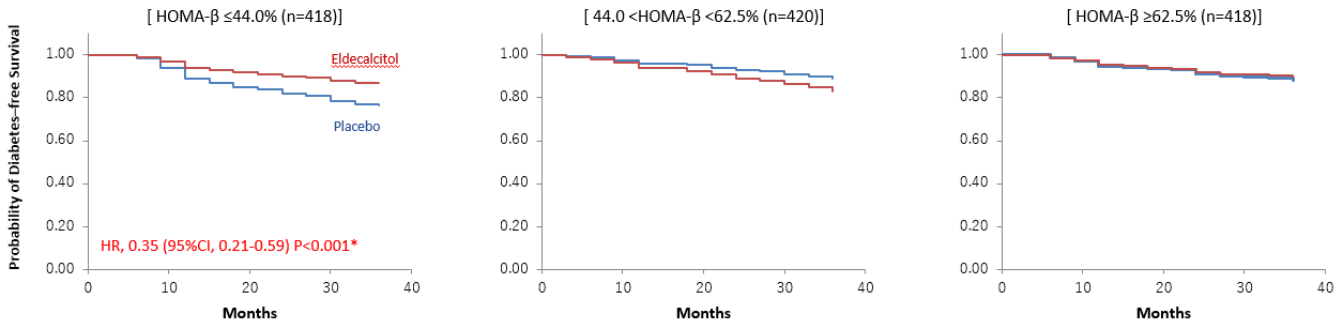


HOMA-β, homeostasis model assessment of beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; Fasting IRI, fasting immunoreactive insulin.

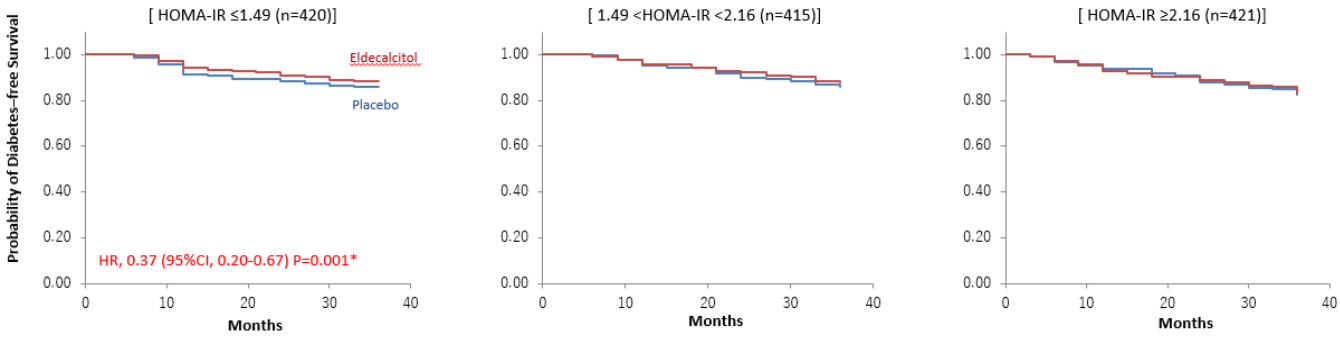
The black lines represent continuous hazard ratios after adjusting for glycated hemoglobin and 2-hour plasma glucose levels at baseline, and dotted lines represent the 95% CI. Overall hazard ratios of the eldecalcitol treatment are shown in red letters that are calculated by multivariable fractional polynomial Cox regression models with an interaction term between eldecalcitol treatment and indicated covariable, i.e., HOMA-β, HOMA-IR, or fasting IRI.

Fig C. Kaplan-Meier curves of interactions divided into tertiles and hazard ratios using multivariable fractional polynomial Cox regression analysis

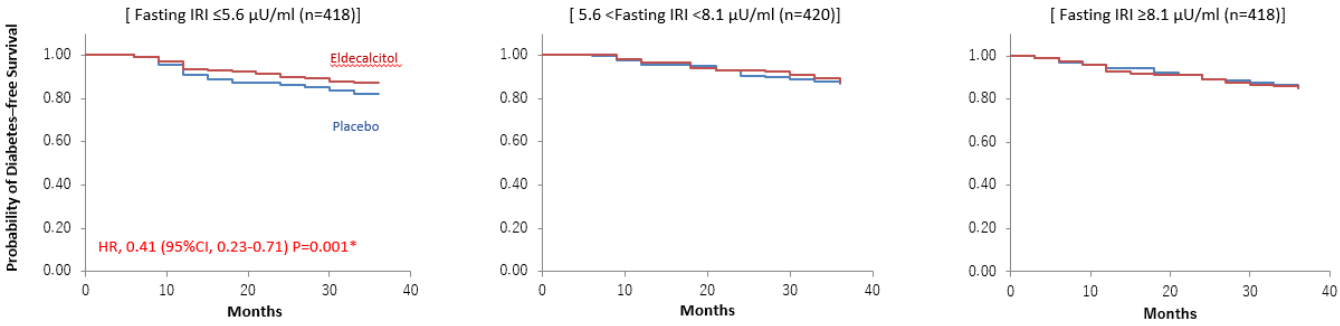
C-1



C-2



C-3

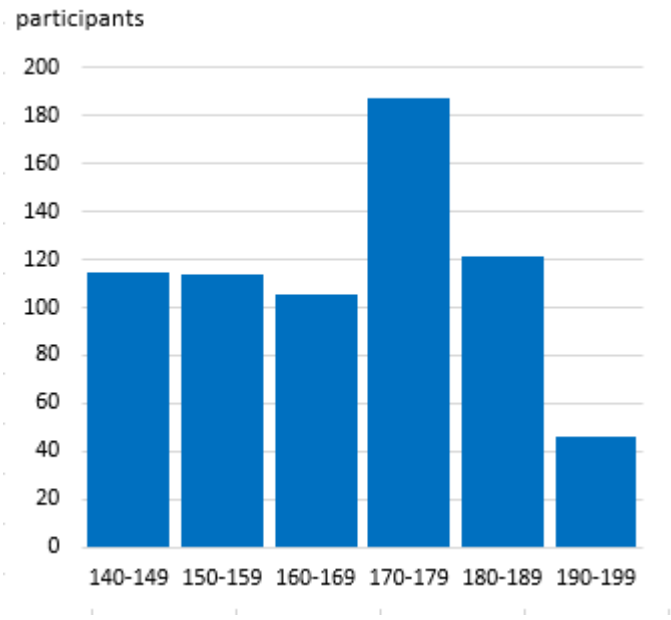


HOMA-β, homeostasis model assessment of beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; Fasting IRI, fasting immunoreactive insulin.

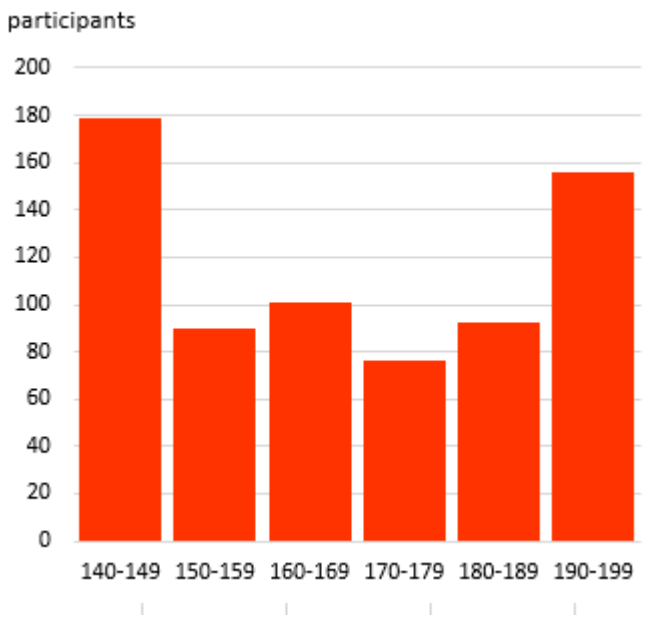
Participants were divided into 3 subpopulations at 33.3- and 66.6-percentiles of HOMA-β, HOMA-IR, and fasting IRI, and Kaplan-Meier curves were depicted without adjusting for confounders. In addition, hazard ratios were analyzed using multivariable fractional polynomials Cox regression.

3-A: HOMA-β, divided into ≤ 44.0%, 44.0</<62.5%, and ≥62.5% groups
3-B: HOMA-IR, divided into ≤ 1.49, 1.49</<2.16, and ≥2.16 groups
3-C: Fasting IRI, divided into ≤ 5.6 μU/ml, 5.6</<8.1 μU/ml, and ≥8.1 μU/ml groups

Fig D. Participants distribution in 2-hour plasma glucose levels at baseline



Placebo group



Eldecalcitol group