Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: DeFronzo RA, Tripathy D, Schwenke DC, et al. Pioglitazone for diabetes prevention in impaired glucose tolerance. N Engl J Med 2011;364:1104-15.

Supplemental Table 1. Inclusion/Exclusion Criteria

1. Inclusion Criteria

- Men and women
- All ethnic groups
- ≥ 18 years of age
- IGT with a FPG = 95-125 mg/dl plus at least one additional high risk characteristic (see text). IGT is defined as a two hour plasma glucose concentration = 140-199 mg/dl during a single 75 gram OGTT.
- BMI ≥ 25 kg/m^2 (no upper limit) (BMI > 22 kg/m^2 for Asian Americans)

Plus one other risk factor for diabetes:

(i) ≥ 1 component of metabolic syndrome, (ii) family history of T2DM in ≥ 1 first degree relative; (iii) history of gestational diabetes mellitus; (iv) polycystic ovarian syndrome; (v) minority ethnic background.

2. Exclusion Criteria

- Subjects with diabetes mellitus: FPG ≥ 126 mg/dl or 2-hour plasma glucose ≥ 200 mg/dl during OGTT.
- Subjects previously treated with a thiazolidinedione (ever) or metformin (within one year prior to randomization)
- Subjects previously treated with a sulfonylurea, a meglitinide, an alpha glucosidase inhibitor for more than one week within the last year, or within the 3 months prior to randomization
- Subjects previously treated with insulin (other than during pregnancy) for more than one week within the last year or within the 3 months prior randomization.
- Medical conditions likely to limit life span and/or increase risk of intervention
    - Cardiovascular disease

- Hospitalization for treatment of heart disease or stroke in past 6 months
- New York Heart Association Functional Class > 2
- Left bundle branch block or third degree AV block
- Aortic stenosis
- Systolic blood pressure > 180 mmHg or diastolic blood pressure > 105 mmHg; subjects can be re-screened after treatment of their hypertension
- Renal disease (creatine ≥ 1.6 mg/dl for men or ≥ 1.5 mg/dl for women, or urine protein ≥ 2+)
- Anemia (hematocrit < 33% in men and < 30% in women); if the hematocrit increases above these levels at a later date, they can be included in the study
- Hepatitis, based on history and/or serum ALT greater than 2.5 times the upper limit of normal
- Other gastrointestinal disease (pancreatitis, inflammatory bowel disease)
- Recent or significant abdominal surgery
- Pulmonary disease with dependence on oxygen or daily use of bronchodilators
- Chronic infection (e.g., HIV, active tuberculosis)

- Conditions or behaviors likely to affect conduct of the trial
  - Unwilling to accept treatment assignment by randomization

- Participation in another intervention research project that might interfere with completion of the study
- Weight loss of > 10% in past 6 months for any reason except postpartum weight loss
- Currently pregnant or within 3 months postpartum
- Currently nursing or within 6 weeks of having completed nursing
- Pregnancy anticipated during the course of the trial
- Unwilling to undergo pregnancy testing or report possible pregnancy promptly
- Unwilling to take precautions to avoid pregnancy if potentially fertile

- Major psychiatric disorders
- Excessive alcohol intake, either acute or chronic
- Medications and medical conditions likely to confound the assessment for diabetes, including:

  Thiazide diuretics at a dose greater than 25 mg/day

  Non-cardioselective beta-blockers (individuals receiving treatment with a statin or fenofibrate will not be excluded as long as the dose has been stable for 3 months prior to randomization)

  Glucocorticoids, systemic

  Prescription weight-loss or weight-gain medications
- Thyroid disease, suboptimally treated as indicated by abnormal serum thyroid-stimulating hormone
- Other endocrine disorders (e.g. Cushing's syndrome, acromegaly)
- Fasting plasma triglyceride > 400 mg/dl, despite treatment

- Individuals with a history of bladder cancer
- Individuals with hematuria at screening. However, subjects with hematuria may be randomized if the cause of the hematuria is found, treated, and thought unlikely to recur.

Supplemental Table 2. Grading scale for edema

(1) Absent

(2) Mild (<0.62 cm)

(3) Moderate (0.62-1.24 cm)

(4) Moderately Severe (1.25-1.87 cm)

(5) Severe (1.88-2.54 cm)

(6) Very Severe (>2.54 cm)

Supplemental Table 3. Number and type of adverse events concerning the cardiovascular system in the pioglitazone-treated and placebo-treated groups (events were not adjudicated).

	Number of Persons	
	Pioglitazone	**Placebo**
Atypical chest pain	1	4
Cardiac arrhythmia	5	2
Carotid endarterectomy	0	2
Coronary artery bypass/revascularization	2	6
Coronary artery disease without revascularization	2	1
New or worsening angina	6	4
New or worsening CHF	1	1
Nonfatal MI	2	1
Peripheral vascular disease with claudication or revascularization	6	0
TIA	1	1
Malignant hypertension	0	1
Total	26	23

Supplemental Table 4. Number and type of fractures in the pioglitazone-treated and placebo-treated groups.

	Number of Fractures	
	Pioglitazone	**Placebo**
Right collarbone	1	0
Wrist	3	0
Hand	0	1
Fibula	1	1
Tibia	1	0
Ankle	0	3
Foot	2	2
Toe	1	1
Total	9	8

Fractures were not adjudicated

Supplemental Methods

Baseline blood tests included CBC, chemistry panel, TSH, T4, urinalysis, and microalbumin to creatinine ratio. Systolic/diastolic blood pressure were measured with automated Dinamap Pro 100 (GE Medical Systems, Milwaukee,WI) following 5 minutes of reclining. Body weight (nearest 0.1 kg on digital scale) (Health-O-Meter, Bridgeview, IL) and height (nearest 0.1 cm) were recorded. Waist circumference was measured using Gulick II Tape Measure (Gays Mills, WI) at midpoint between highest point at iliac crest and lowest part of costal margin in mid-axillary line. Laboratory determinations were performed in Central Laboratory (Texas Diabetes Institute, San Antonio). Plasma glucose was measured with glucose oxidase (Glucose Oxidase Analyzer, Beckman, Fullerton, CA) and HbA_{1c} by ion-exchange HPLC (Bayer DCA 2000, Leverkusen, Germany). Plasma cholesterol and triglycerides were measured using CHOD-DAOS method (WAKO, Richmond, VA) and enzymatic assay (Stanbio Lab, Boerne, TX). HDL cholesterol was measured using CHOD-DAOS method. LDL cholesterol was calculated by Friedewald equation.

Carotid intima-media thickness

High-resolution B-mode carotid artery ultrasound was used to image the far wall of the right distal common carotid artery under a standardized protocol across 7 centers (1,2). Center sonographers were uniformly trained and ultrasound images read blinded to treatment at the University of Southern California Atherosclerosis Research Unit Core Imaging and Reading Center (3). For acquisition of images, subjects were placed in the supine position with the head rotated to the left at 45 degrees. As described previously (1-5), the jugular vein and carotid artery were located in the transverse view with the former stacked above the latter. Maintaining the 2 vessels in the stacked position, the transducer was rotated 90 degrees around the central line of the transverse view to obtain a longitudinal view of the 2 vessels with the jugular vein stacked above the carotid artery. Images contained internal anatomic landmarks for reproduction of transducer angle. Each participant's baseline image was used as a guide for

longitudinal image acquisition. For each participant, the depth of field, gain, power and all other instrumentation settings established at baseline were used for longitudinal image acquisition. Images were recorded with the electrocardiogram tracing. Carotid artery wall thickness was measured by automated computerized edge detection using an in-house developed software package (4,5). Carotid intima-media thickness was the average of 70-100 individual measurements between the intima-lumen and the media-adventitia interfaces along a 1-cm length just distal to the carotid artery bulb.

Frequently sampled intravenous glucose tolerance test

Within 3-10 days after the OGTT, subjects at 4 centers were asked to return for a FSIVGTT (6), which was performed at 0800 h following an overnight fast (after 2000 h). A catheter was inserted into an antecubital vein and 3 baseline arterialized blood samples (heated box to 70°C) were obtained. At time zero glucose (300 mg/kg) was given as a smooth intravenous bolus over one minute. Insulin (0.03 units/kg) was given as an intravenous bolus 20 minutes after the start of the glucose injection. Over the 240 minutes following glucose ingestion, 22 blood samples were drawn at 2,3,4,5,6,8,10,14,19,22,24,27,30,40,50,70,90, 120,150,180,210, and 240 minutes for determination of plasma glucose and insulin concentrations.

During the FSIVGTT, first phase insulin secretion was calculated as the increment in plasma insulin concentration (AUC) above baseline from 0-10 minutes and as the peak increment in plasma insulin concentration (minus baseline) during the 0-10 minute time period. Indices of insulin sensitivity (SI) and glucose effectiveness (SG) were determined by minimal model analysis of insulin and glucose concentrations during the FSIVGTT as previously described (6).

References:

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