

SUPPLEMENTAL MATERIALS

Alteration in Fasting Glucose after Prolonged Treatment with a Thiazide Diuretic

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Supplementary Methods

PEAR and PEAR-2 Study Designs and Populations

Details of the PEAR study, which investigated genetic influences of hydrochlorothiazide (HCTZ), atenolol, and their combination on BP and AMEs are previously published.¹⁹ Briefly, participants age 17 through 65 years with mild to moderate essential hypertension but without a history of heart disease or diabetes underwent a 3-8 week antihypertensive drug washout period. Participants were then randomized to receive HCTZ 12.5 mg (Arm 1) or atenolol 50 mg (Arm 2) daily followed by dose titration to HCTZ 25 mg or atenolol 100 mg daily for 6-9 weeks. (Figure 1A) The other agent was then added, with similar dose titration for 6-9 weeks of combination treatment for a total study duration of approximately 18 weeks. Changes in FG and other lab measures after nine weeks of HCTZ treatment were determined during PEAR following both HCTZ monotherapy (Arm 1) and HCTZ add-on therapy to atenolol (Arm 2). In Arm 1, change in FG during HCTZ monotherapy was defined as the difference in FG from the baseline visit to the end of HCTZ monotherapy (ΔFG_1). (Figure 1A) In Arm 2, change in FG during HCTZ add-on therapy was defined as the difference in FG from the start of HCTZ add-on therapy to the end of the trial (ΔFG_2).

PEAR-2 similarly investigated genetic influences on BP and AMEs after administration of the thiazide-like diuretic chlorthalidone and the beta blocker metoprolol. PEAR-2 was a sequential rather than a randomized study and all participants underwent a 3-4 week washout of antihypertensive medication. Participants were then treated with metoprolol 50 mg immediate release twice daily for two weeks, with dose titration to metoprolol 100 mg twice daily (if BP was greater than

120/70) for at least six weeks. (Figure 1B) Participants then underwent another 3-4 week washout period, followed by chlorthalidone 15 mg once daily monotherapy for two weeks with dose titration to chlorthalidone 25 mg once daily for at least six weeks. Inclusion and exclusion criteria were identical to PEAR. Change in FG and other lab measures after approximately eight weeks of chlorthalidone was defined as the difference in FG from the start of chlorthalidone monotherapy, to the end of the trial (ΔFG_3). (Figure 1B)

In PEAR and PEAR-2, biological samples were collected in the fasting state. Glucose, insulin, total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, potassium (serum and urine) and uric acid were acquired at the beginning and end of each treatment.¹⁹ Plasma levels of glucose, lipids, and uric acid were determined using an Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Plasma insulin was measured using the Access Ultrasensitive Insulin Immunoassay system (Beckman Instruments, Brea, CA). Race/ethnicity was self-described by study patient. All participants enrolled in both PEAR and PEAR-2 provided voluntary, written informed consent, and the institutional review boards (IRBs) of participating study centers approved the study protocols. PEAR and PEAR-2 are registered at ClinicalTrials.gov (NCT00246519 and NCT01203852 respectively).

PEAR Follow-Up Study Design and Population

The PEAR Follow-Up Study consisted of a single study visit, for which participants were asked to be in the fasting state, not having consumed food or beverages other than water within eight hours prior to the visit. The study visit included collection of a

medical history and detailed medication history, designed to assess the dose and duration of therapy of thiazide diuretics and other antihypertensive medications that might alter metabolic status. Antihypertensive treatment was also confirmed using most recent clinic notes. Antihypertensive treatment other than thiazide diuretics was not included in antihypertensive categorizations for statistical analysis if participants had been prescribed the medication for less than 6 months at the time of the study visit or the medication had been discontinued for 30 days or more. The interview also included an adherence assessment for antihypertensive treatment. Data were collected for other medications that alter metabolic status, including statins or other lipid-lowering agents, alpha adrenergic agonists, tricyclic antidepressants, corticosteroids, anti-diabetic and glucose-lowering medications, birth control, and potassium supplementation. A social history including weekly alcohol consumption and cigarette smoking status was obtained.

Anthropomorphic measurements acquired during the visit included height, weight, and waist and hip circumference. Three BP measurements were acquired using an automated sphygmomanometer and averaged for follow-up SBP and DBP values. A baseline blood draw was obtained to acquire whole blood FG, plasma insulin, HbA1c, a lipid panel, uric acid, and serum potassium. Each participant then drank a 75 gram glucose solution (Azer Scientific, Morgantown, PA) and whole blood glucose measurements were acquired at one hour and two hour time points after ingestion of the solution. PEAR Follow-Up Study visits were performed at the UF general clinical research center (GCRC) or UF Department of Community Health and Family Medicine Clinics.

Variables Used in Univariate and Stepwise Linear Regressions

Laboratory measures included in univariate models for each phenotype were baseline and short term changes in FG, LDL, HDL, and total cholesterol, triglycerides, HOMA, plasma insulin, serum uric acid, and serum potassium. Independent variables for univariate regressions also included BMI, age, gender, race, baseline systolic and diastolic BP, heart rate, smoking status at follow-up, alcohol consumption at follow-up, waist circumference, abdominal obesity (defined as waist circumference ≥ 88.9 centimeters for females or ≥ 101.6 centimeters for males), previous CV disease, family history of T2D (in a first degree relative), treatment with beta blockers, ACEIs, ARBs, statins, other lipid-lowering agents, alpha adrenergic agonists, selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), corticosteroids, anti-diabetic medications, birth control, and potassium supplementation, and duration of thiazide diuretic therapy.

Table S1. Variables associated with FG at follow-up

Independent variable	Parameter estimate (β) ^a	p value ^b
Univariate associations ($p \leq 0.20$)		
Baseline FG, mg/dL	0.30	0.09
Duration of thiazide treatment, months	0.18	0.12
Beta blocker treatment	7.78	0.06
ACEI treatment	-7.30	0.08
Chlorthalidone treatment	7.73	0.10
Family history of T2D ^c	7.43	0.07
BMI, kg/m ²	0.48	0.20
Baseline HDL, mg/dL	-0.22	0.17
Baseline DBP, mmHg	0.37	0.12
Stepwise Results ($R^2=0.22$)		
Family history of T2D [†]	9.31	0.03
Chlorthalidone treatment	9.59	0.05

FG indicates fasting glucose; mg/dL, milligrams per deciliter; ACEI, angiotensin I converting enzyme inhibitor; T2D, type 2 diabetes; BMI, body mass index; HDL, high density lipoprotein; DBP, diastolic blood pressure.

^aUnits for parameter estimates for fasting glucose changes are mg/dL. ^bp values determined using linear regression excluding participants with anti-diabetic treatment.

^cHistory of type 2 diabetes in a first degree relative

Table S2. Significant Associations after Stepwise Regressions for Change in Lab Values during Long-Term Thiazide Treatment

Independent variable	Parameter estimate (β) ^a	p value ^b
HOMA ($R^2=0.19$)		
Duration of thiazide treatment, months	0.06	0.009
Insulin ($R^2=0.59$)		
Baseline insulin, $\mu\text{U/mL}$	-0.73	<0.0001
Race (white)	-8.42	0.01
Triglycerides ($R^2=0.45$)		
Baseline triglycerides, mg/dL	-0.46	<0.0001
LDL ($R^2=0.31$)		
Baseline LDL, mg/dL	-0.47	0.003
Baseline DBP, mmHg	1.23	0.04
HDL ($R^2=0.79$)		
Beta blocker treatment	-6.40	0.0001
Change in HDL during short-term thiazide therapy, mg/dL	0.58	0.0003
Total Cholesterol ($R^2=0.19$)		
Baseline total cholesterol, mg/dL	-0.39	0.005
Serum potassium ($R^2=0.51$)		
Baseline serum potassium, mEq/L	-0.77	<0.0001
Current smoker	0.57	0.008
Baseline HDL, mg/dL	0.01	0.03

DBP, diastolic blood pressure; HOMA indicates homeostatic model assessment; LDL, low density lipoprotein; mEq/L, milliequivalents per liter; mg/dL, milligrams per deciliter; mmHg, millimeters of mercury; $\mu\text{U/mL}$, microunits per milliliter.

^aUnits for parameter estimates for fasting glucose changes are mg/dL.

^bP values and R^2 determined using linear regression excluding participants with anti-diabetic treatment.

Table S3. Fasting glucose levels at baseline and at follow-up by drug treatment status

Drug treatment ^a	FG at baseline ^b	FG at follow-up ^b	p value ^c
Type of thiazide			
HCTZ (n=30)	90 (12)	92 (12)	0.29
Chlorthalidone (n=10)	93(12)	100 (14)	
Beta blocker			
Beta blocker treated (n=17)	90 (13)	98 (12)	0.05
No beta blocker (n=23)	92 (12)	91 (13)	
ACEI			
ACEI treated (n=18)	90 (14)	90 (10)	0.18
No ACEI (n=22)	92 (10)	97 (15)	
Statin			
Statin treated (n=9)	87 (17)	95 (13)	0.30
No statin (n=31)	92 (11)	94 (13)	

FG indicates fasting glucose; HCTZ, hydrochlorothiazide; ACEI, angiotensin I converting enzyme inhibitor.

^aExcludes individuals treated with anti-diabetic drugs with the exception of the anti-diabetic drug heading

^bUnits for fasting glucose measurements are mg/dL.

^cp value for paired t-test for difference between groups in change in FG between baseline and follow-up

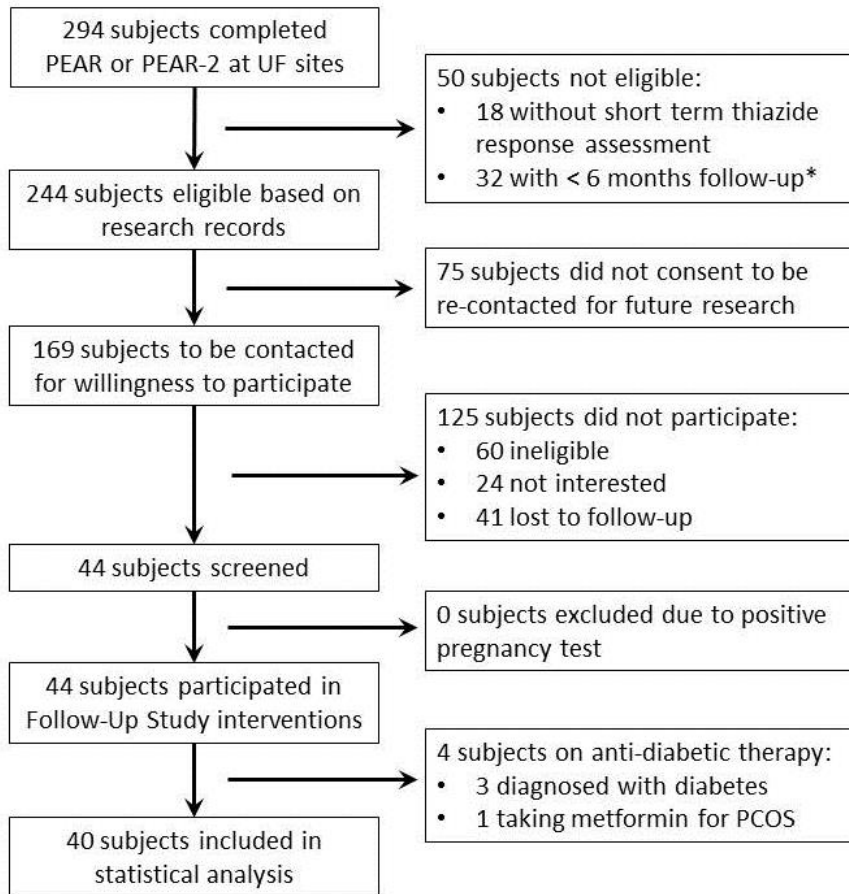


Figure S1. Progression of subjects for PEAR Follow-Up Study enrollment and analysis. UF indicates University of Florida; PCOS, polycystic ovary syndrome.*Less than six months of follow-up time prior to PEAR Follow-Up Study enrollment

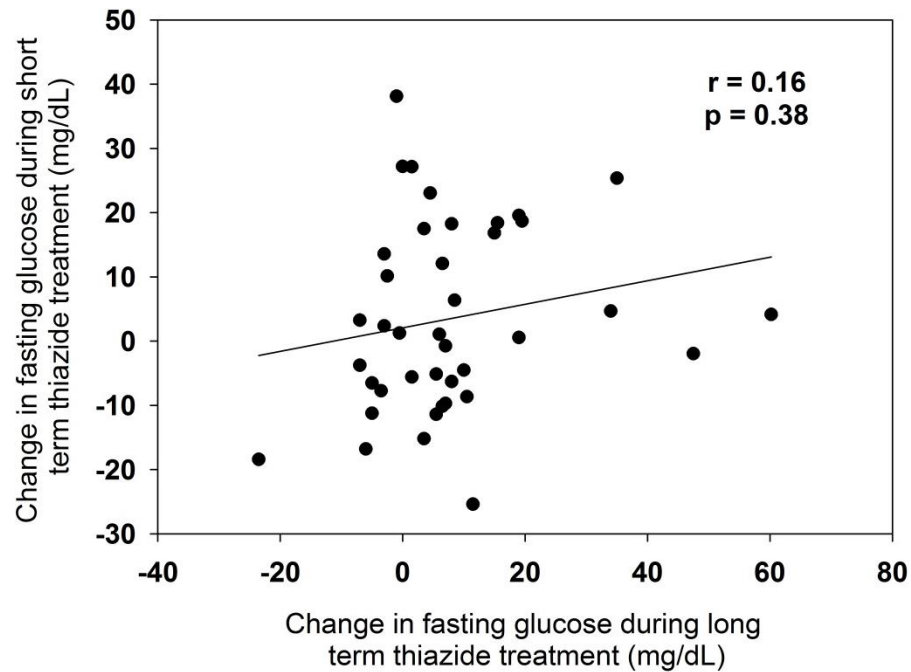


Figure S2. Change in fasting plasma glucose during short term *versus* long term thiazide diuretic treatment. P value and r calculated using Spearman partial correlation adjusted for baseline fasting glucose.