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Pioglitazone treatment enhances the sympathetic nervous system response to oral carbohydrate load in obese individuals with metabolic syndrome

Nora E. Straznicky[a], Mariee T. Grima[a], Carolina I. Sari[a], Nina Eikelis[a], Gavin W. Lambert[a], Paul J. Nestel[b], Katrina Richards[a], John B. Dixon[a], Markus P. Schlaich[c], Elisabeth A. Lambert[a]

Laboratories of [a]Human Neurotransmitters, [b]Cardiovascular Nutrition and [c]Neurovascular Hypertension & Kidney Disease, Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, AUSTRALIA

Author affiliations also include the Faculty of Medicine, Nursing and Health Sciences (GWL, MPS) and the Departments of Physiology (EAL, MPS) and Primary Health Care (JBD), Monash University, and the Department of Physiology, University of Melbourne (EAL), Melbourne, Victoria, AUSTRALIA

Correspondence to: Dr Nora E. Straznicky, Baker IDI Heart & Diabetes Institute, P.O. Box 6492, St Kilda Road Central, Melbourne, Victoria 8008, AUSTRALIA

Email: Nora.Straznicky@bakeridi.edu.au Fax: 61 3 8532 1100 Tel: 61 3 8532 1371

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Abbreviations: ANOVA, analysis of variance; $AUC_{0\text{-}120}$, area under the curve during oral glucose tolerance test; CSF, cerebrospinal fluid; HOMA-IR, homeostasis model assessment-insulin resistance; ISI, insulin sensitivity index; M, steady state glucose utilization during euglycemic hyperinsulinemic clamp expressed as mg per kg fat free mass per minute; MetS, metabolic syndrome; OGTT, oral glucose tolerance test; PIO, pioglitazone; SNS, sympathetic nervous system

ABSTRACT

Context: Insulin resistance is associated with blunted sympathetic nervous system (SNS) response to carbohydrate ingestion which may contribute to postprandial hypotension and impaired body weight homeostasis.

Objective: This study was conducted to examine the effects of pharmacological insulin sensitization on whole-body norepinephrine kinetics during a standard 75-g oral glucose tolerance test (OGTT) in obese, insulin resistant subjects with metabolic syndrome.

Methods: Un-medicated individuals (n=42, mean age 56 $\pm$ 0.8 yrs, body mass index 34 $\pm$ 0.6 kg/m^2) were randomized to 12-weeks pioglitazone (PIO, 15 mg for 6 weeks, then 30 mg daily) or placebo using a double-blind, parallel group design. Whole-body norepinephrine kinetics (arterial norepinephrine concentration, calculated spillover and clearance rates), spontaneous cardiac baroreflex sensitivity, heart rate and blood pressure were measured at times 0, 30, 60, 90 and 120 minutes during OGTT. Insulin sensitivity was assessed by euglycemic hyperinsulinemic clamp (M) and Matsuda index.

Results: PIO increased clamp derived glucose utilization by 35% ($P<0.001$) and there were concurrent reductions in inflammatory status and plasma triglycerides ($P<0.05$). Fasting norepinephrine kinetic parameters were unaltered. PIO treatment was associated with lower plasma insulin incursions, greater reduction in diastolic blood pressure and enhanced baroreflex sensitivity during OGTT (P all <0.05). The overall norepinephrine spillover response ($AUC_{0\text{-}120}$) increased significantly in the PIO group (group x time interaction, P=0.04), with greatest increment at 30 minutes post-glucose (101 $\pm$ 38 ng/min at baseline versus 241 $\pm$ 48 ng/min post treatment, P=0.04) and correlated with percent improvement in M.

Conclusions: PIO enhances the early postprandial SNS response to carbohydrate ingestion.

Keywords: pioglitazone, sympathetic nervous system, norepinephrine, insulin resistance, metabolic syndrome

1. INTRODUCTION

The postprandial rise in sympathetic nervous system (SNS) activity fulfils two key physiological functions. First, it protects against postprandial hypotension induced by splanchnic vasodilation and second, it modulates facultative thermogenesis, a component of the thermic effect of food that accounts for 3-4% of daily caloric expenditure [1,2]. In the context of carbohydrate ingestion, sympathoactivation is primarily mediated by the increase in endogenous plasma insulin concentration via a central neural action of insulin involving various signalling pathways, following transit across the blood-brain-barrier [3,4]. Further contributing mechanisms include reflex SNS activation due to insulin induced vasodilation and gastrointestinal factors such as stomach distension [5]. Our group and others have previously demonstrated blunted sympathetic neural outflow to a standard oral glucose tolerance test (OGTT) in overweight and obese insulin resistant versus matched insulin sensitive subjects [6,7]. This concurs with emerging evidence that insulin-resistant states are accompanied by reduced cerebrospinal fluid (CSF) insulin concentrations, attenuated transport of insulin across the blood-brain-barrier and altered central insulin signalling [8-10]. Weight loss, the first-line treatment for the metabolic syndrome (MetS), reverses blunted SNS responses to glucose ingestion in insulin-resistant subjects [11] however there is a paucity of data concerning the effects of pharmacological insulin-sensitization in this regard.

Pioglitazone (PIO) is a nuclear peroxisome proliferator-activated receptor-γ agonist that enhances hepatic, skeletal muscle and adipose tissue insulin sensitivity by influencing the expression of genes involved in glucose and lipid metabolism. PIO has been shown to decrease resting muscle sympathetic nerve activity and enhance arterial baroreflex sensitivity in patients with diabetes [12]. In the present study we examined the effects of 12-weeks PIO treatment on the dynamic SNS response to OGTT in a group of obese, insulin resistant subjects with MetS. The technique of norepinephrine kinetics was used to estimate the concurrent processes of whole-body norepinephrine spillover into and clearance from the central plasma compartment. We

hypothesised that PIO would enhance the sympathetic response to OGTT and that this would relate to improvements in insulin sensitivity and baroreflex function.

2. MATERIALS and METHODS

2.1 Subjects

Forty-two un-medicated, non-smoking, obese subjects, aged 45 to 65 years, were studied. Inclusion criteria were: fulfilment of the harmonized MetS definition [13]; HOMA of insulin resistance (HOMA-IR) >2.5 and a stable body weight ($\pm$ 1kg) in the previous 6 months. Exclusion criteria included a history of secondary hypertension, cardiovascular, cerebrovascular, renal, liver, or thyroid disease. Supine clinic blood pressure was measured as the average of 5 readings after 5 minutes rest (Dinamap, Model 1846SX, Critikon Inc, Tampa, FL, USA). The study was approved by the Alfred Hospital Human Research Ethics Committee. Informed, written consent was obtained from each participant. Findings regarding resting SNS activity have been previously reported [14].

2.2 Study design

The study utilized a randomized, double-blind, parallel group comparison of PIO (15 mg for first 6 weeks and then 30 mg daily, Actos, Eli Lilly Australia Pty Ltd) versus matched placebo. Stratified randomization by gender and hypertensive status in blocks of four, was used to ensure equal distribution of these factors. PIO 15 mg tablets were dispensed in opaque gelatine capsules. Placebo treatment comprised lactose powder dispensed in identical gelatine capsules. The capsules were prepared and dispensed by the Alfred Hospital Pharmacy Department. Subjects consumed one capsule daily (in the morning with breakfast) for the first 6 weeks and then increased to two capsules daily for the next 6 weeks, according to their allocated treatment. The study randomization and attrition flow chart is presented in Supplemental Figure 1.

Investigations were performed in a quiet research room (temperature 22°C) in a supine position, at baseline and after 12-weeks treatment. Subjects attended at 8 am after an overnight fast,

having abstained from alcohol and heavy exercise for 36 hours, caffeine for 18 hours and having consumed their allocated medication at 0630 hours.

2.3 Norepinephrine kinetics

After a priming intravenous bolus of 1.81 μCi of 1-[ring-2,5,6-^{3}H]-norepinephrine (Perkin-Elmer, Waltham, MA, US; specific activity, 10-30μCi/mmol), a constant infusion was commenced at 0.18 μCi·min^{-1}[14]. Fasting steady state brachial arterial blood samples were obtained 30 minutes after commencement of the infusion. Subjects then ingested 75-g glucose (Glucaid, Fronine PTY, LTD, Taren Point, NSW 2229, Australia) with further arterial blood sampling at 30, 60, 90 and 120 min for catecholamine determination. Calculations comprised:

$$\text{Norepinephrine spillover (ng/min)} = \frac{\text{plasma norepinephrine (pg/ml) x clearance (ml/min)}}{1000}$$

$$\text{Norepinephrine clearance (L/min)} = \frac{[^{3}\text{H}]\text{-norepinephrine infusion rate (dpm/min)}}{[^{3}\text{H}]\text{-norepinephrine plasma concentration (dpm/ml) x 1000}}$$

2.4 Spontaneous cardiac baroreflex function

Spontaneous cardiac baroreflex sensitivity was assessed by the sequence method. The slope of the regression line between cardiac interval and intra-arterial systolic blood pressure was calculated for each validated sequence whereby systolic blood pressure either increased or decreased for three consecutive heartbeats. Individual slopes were averaged over 15 minutes at time 0 and over 5 minutes at 30, 60, 90 and 120 minutes post glucose ingestion.

2.5 Metabolic parameters

Insulin sensitivity was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR), the Matsuda insulin sensitivity index (ISI)- derived from glucose and insulin perturbations during the OGTT [15], and on a separate morning, by euglycemic hyperinsulinemic clamp using a primed constant infusion of 40 mU·m^{2}·min^{-1} (Actrapid 100 IU/ml Novo Nordisk, Gentofte, Denmark)[14].

2.6 Statistical Analysis

Data are expressed as the means $\pm$ SEM. Dynamic changes in norepinephrine kinetics, cardiovascular and metabolic variables during the OGTT were analysed by 2-way repeat measures ANOVA, with the Holm-Sidak test for multiple pairwise comparisons. Non-parametric data were log transformed. Areas under the plasma concentration-time curve ($AUC_{0\text{-}120}$) were calculated by the trapezoidal rule. Associations between variables were evaluated by univariate correlation analysis. Statistical significance was accepted at a two-sided P value $\leq$ 0.05 (SigmaStat 3.5 for Windows, Systat Software, San Jose, CA).

3. RESULTS

3.1 Subjects

Baseline demographic, anthropometric and metabolic characteristics were similar in PIO and placebo groups, with no statistically significant differences in any parameter (Table 1). Capsule counts indicated >99% compliance. Improvement in insulin sensitivity after PIO treatment was evidenced by a 35% increase in clamp derived glucose utilization (M) and similar changes in HOMA-IR and Matsuda ISI (P all<0.001, Table 1).

3.2 Cardiovascular responses during OGTT

Figure 1 summarises dynamic sympathetic and cardiovascular responses during the OGTT within PIO treated subjects. The insulin $AUC_{0\text{-}120}$ was significantly lower and the reduction in diastolic blood pressure during OGTT was augmented after 12-weeks PIO treatment (Figure 1A and 1E). The increments in systolic blood pressure and heart rate were not significantly altered. Cardiac baroreflex sensitivity decreased significantly after glucose ingestion in both treatment phases (time effect, P<0.001), representing vagal withdrawal. However, at week 12, there was a greater and earlier recovery in baroreflex sensitivity as evidenced by a higher 90-minute post-glucose value versus baseline (P=0.046, Figure 1F). In the placebo group there were no significant alterations in plasma insulin levels, blood pressure or heart rate responses during OGTT, however baroreflex

sensitivity at 30 minutes post-glucose was higher at week 12 compared to baseline (P=0.02, Supplemental Figure 2F). Figure 2 shows comparative data at week 12 in PIO and placebo treated subjects. Compared to placebo, the initial baroreflex unloading at 30 minutes, was greater following PIO treatment (-4.2 $\pm$ 1.9 versus 0.2 $\pm$ 1.2 msec/mmHg, P=0.05, Figure 2D).

3.3 Sympathetic nervous responses during OGTT

Due to difficulties with arterial line placement in 2 subjects, paired norepinephrine kinetic data were available for 21 placebo and 19 PIO subjects. Fasting arterial norepinephrine concentration and calculated norepinephrine spillover and clearance rates were unaltered in both groups (Figures 1 and S2). However, the increment in norepinephrine clearance and spillover rates at 30 minutes post glucose (Figure 1C and 1D) and the overall $AUC_{0\text{-}120}$ for norepinehrine spillover rate (Table 1) increased significantly in PIO treated but not placebo treated subjects (group x time interaction, P=0.04). Absolute change in norepinephrine spillover at 30 minutes relative to time 0, averaged 101 $\pm$ 38 ng/min at baseline versus 241 $\pm$ 48 ng/min at week 12 (P=0.04) in the PIO group. At week 12, the relative increment in arterial norepinephrine at 60 minutes post glucose was greater in PIO versus placebo group (31 $\pm$ 8% and 13 $\pm$ 5% respectively, P=0.05, Figure 2A).

3.4 Correlation analyses

Change in resting baroreflex sensitivity following PIO treatment correlated positively with changes in Matsuda ISI (r=0.53, P=0.02) and inversely with changes in HOMA-IR (r=-0.56, P=0.02) and 2-hour glucose (r=-0.60, P=0.008). Change in the norepinephrine spillover $AUC_{0\text{-}120}$ was positively associated with percent change in M value in the pooled data set (r=0.38, P=0.02).

DISCUSSION

This study sought to examine the impact of pharmacological insulin sensitization on the sympathetic nervous response to oral carbohydrate ingestion in obese individuals with MetS. The rationale was based on previous scientific evidence that peripheral insulin resistance coexists with

central insulin resistance and confers blunted sympathetic neural responses to glucose ingestion [6-10]. Our findings demonstrated an enhancement in arterial norepinephrine and norepinephrine spillover rate in PIO treated subjects at 30 and 60 minutes post-glucose, which then returned to baseline values from 90 minutes onward. Changes in cardiac baroreflex sensitivity mirrored those of sympathetic parameters, with greater initial baroreflex unloading at 30 minutes and then enhanced baroreflex sensitivity from 90 minutes onward in the PIO group.

Several mechanisms may have contributed to the early enhancement in SNS response in the PIO group. A greater vasodilatory response to endogenous hyperinsulinemia was evidenced by the augmented reduction in diastolic blood pressure during the OGTT, consistent with previous reports of improved nitric oxide mediated vasodilation after PIO treatment [16]. Improvement in endothelial dysfunction may in turn translate to enhanced baroreflex sensitivity, with greater vagal withdrawal during vasodilation [17]. Our findings are consistent with those of Yokoe *et al.* showing that 12-weeks treatment with PIO 15 mg per day improved stimulated arterial baroreflex sensitivity in patients with type 2 diabetes [12]. It is also possible that improvements in peripheral insulin sensitivity enhanced insulin's central actions to facilitate sympathetic outflow and to increase the gain of baroreflex control of heart rate [18,19]. Decreased CSF to plasma insulin ratio, impaired transendothelial insulin transport across the blood-brain-barrier, and blunted brain insulin signalling are recognised features of obesity and relate to the level of peripheral insulin resistance [8-10]. In a rat model of type 2 diabetes, PIO ameliorated intracerebral insulin resistance without increasing CSF insulin levels [20].

The strengths of our study include its methodological rigour. There are however, some limitations. Firstly, we did not measure post-glucose energy expenditure to gauge the thermogenic impact of elevations in norepinephrine spillover. Secondly, it is possible that fluid retention or other pleitropic effects of PIO may have influenced measured parameters. In conclusion, our findings support the notion that pharmacological insulin-sensitization with PIO may positively impact on the postprandial sympathetic response.

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Author Contributions: NES, PJN and EAL conceived the study. NES, MTG, CIS, PJN, MPS and EAL collected clinical data. NE, KR and GWL performed laboratory assays. NES performed data analysis and wrote the manuscript. All authors read and had final approval of the manuscript.

REFERENCES

[1] Fagius J, Berne C. Increase in muscle nerve sympathetic activity in humans after food intake. Clin Sci 1994;86:159-167.

[2] Schwartz RS, Jaeger LF, Silberstein S, Veith RC. Sympathetic nervous system activity and the thermic effect of feeding in man. Int J Obes 1987;11:141-9.

[3] Rahmouni K, Morgan DA, Morgan GM, Liu X, Sigmund CD, Mark Al, Haynes WG. Hypothalamic PI3K and MAPK differentially mediate regional sympathetic activation to insulin. J Clin Invest 2004;114:652-658.

[4] Ward KR, Bardgett JF, Wolfgang L, Stocker SD. Sympathetic response to insulin is mediated by melanocortin 3/4 receptors in the hypothalamic paraventricular nucleus. Hypertension 2011;57:435-441.

[5] Rossi P, Andriesse GI, Oey PL, Wieneke GH, Roelofs JM, Akkermans LM. Stomach distension increases efferent muscle sympathetic nerve activity and blood pressure in healthy humans. J Neurol Sci 1998;161:148-55.

[6] Straznicky NE, Lambert GW, Masuo K, Dawood T, Eikelis N, Nestel PJ, McGrane MT, Mariani JA, Socratous F, Chopra R, Esler MD, Schlaich MP, Lambert EA. Blunted sympathetic neural response to oral glucose in obese subjects with the insulin-resistant metabolic syndrome. Am J Clin Nutr 2009;89:27-36.

[7] Fagius J, Ellerfelt K, Lithell H, Berne C. Increase in muscle nerve sympathetic activity after glucose intake is blunted in the elderly. Clin Auton Res 1996; 6:195-203.

[8] Kern W, Benedict C, Schultes B, Plor F, Moser A, Born J, Fehm HL, Hallschmid M. Low cerebrospinal fluid insulin levels in obese humans. Diabetologia 2006;49:2790-2792.

[9] Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Schwartz MW. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. Diabetes 2000;49:1525-1533.

[10] Tschritter O, Preissl H, Hennige AM, Stumvoll M, Porubska K, Frost R, Marx H, Klosel B, Lutzenberger W, Birbaumer N, Haring H-U, Fritsche A. The cerebrocortical response to hyperinsulinemia is reduced in overweight humans: A magnetoencephalographic study. PNAS 2006;103:12103-12108.

[11] Straznicky NE, Lambert GW, McGrane MT, Masuo K, Dawood T, Nestel PJ, Eikelis N, Schlaich MP, Esler MD, Socratous F, Chopra R, Lambert EA. Weight loss may reverse blunted sympathetic neural responsiveness to glucose ingestion in obese subjects with metabolic syndrome. Diabetes 2009;58:1126-1132.

[12] Yokoe H, Yuasa F, Yuyama R, Murakawa K, Miyasaka Y, Yoshida S, Tsujimoto S, Sugiura T, Iwasaka T. Effect of pioglitazone on arterial baroreflex sensitivity and sympathetic nerve activity in patients with acute myocardial infarction and type 2 diabetes mellitus. J Cardiovasc Pharmacol 2012;59:563-569.

[13] Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James PT, Loria CM, Smith SC Jr. Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung and Blood Institute; American Heart Association; World Heart Federation;

International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640-1645.

[14] Straznicky NE, Grima MT, Sari CI, Eikelis N, Lambert GW, Nestel PJ, Karapanagiotidis S, Wong S, Richards K, Marusic P, Dixon JB, Schlaich MP, Lambert EA. A randomized controlled trial of the effects of pioglitazone treatment on sympathetic nervous system activity and cardiovascular function in obese subjects with metabolic syndrome. J Clin Endocrinol Metab 2014; 99:E1701-1707.

[15] Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing. Diabetes Care 1999;22:1462-1470.

[16] Rizza S, Cardellini M, O Porzio, Pecchioli C, Savo A, Cardolini I, Senese N, Lauro D, Sbraccia P, Lauro R, Federici M Pioglitazone improves endothelial and adipose tissue dysfunction in pre-diabetic CAD subjects. Atherosclerosis 2011;215:180-183.

[17] Van De Borne P, Hausberg M, Hoffman RP, Mark AL, Anderson EA. Hyperinsulinemia produces cardiac vagal withdrawal and nonuniform sympathetic activation in normal subjects. Am J Physiol Regul Integr Comp Physiol 1999;276:178-183.

[18] Berne C, Fagius J, Pollare T, Hjemdahl P. The sympathetic response to euglycaemic hyperinsulinaemia. Evidence from microelectrode nerve recordings in healthy subjects. Diabetologia 1992;35:873-879.

[19] Pricher MP, Freeman KL, Brooks VL. Insulin in the brain increases gain of baroreflex control of heart rate and lumbar sympathetic nerve activity. Hypertension 2008;51:514-520

[20] Hu SH, Jiang T, Yang SS, Yang YY. Pioglitazone ameliorates intracerebral insulin resistance and Tau-protein hyperphosphorylation in rats with type 2 diabetes. Exp Clin Endocrinol Diabetes 2013;121:220-224.

Table 1: Clinical variables at baseline and after 12 weeks of treatment

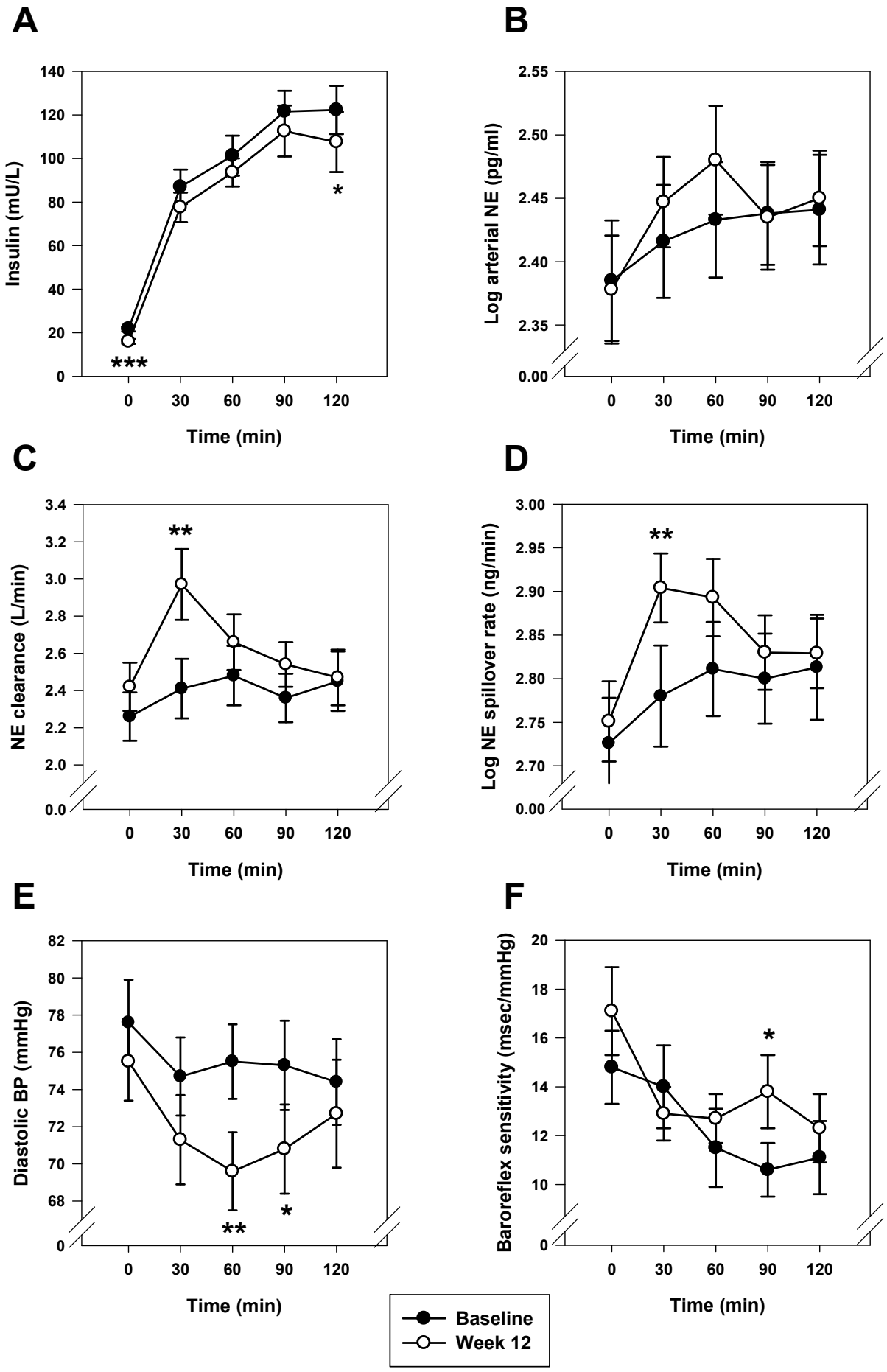
	Placebo (n=21)		Pioglitazone (n=21)		Time Effect (P)	Group effect (P)	Time x Group interaction (P)
	Baseline	Week 12	Baseline	Week 12			
Age (yrs)	56 ± 1		57 ± 1		…	0.27	…
Gender (M/F)	10/11		12/9		…	0.76	…
Weight (kg)	97.9 ± 4.0	97.6 ± 4.0	103.1 ± 4.0	103.8 ± 4.1	0.46	0.32	0.10
Body mass index (kg/m^2)	33.1 ± 0.8	33.1 ± 0.8	34.8 ± 1.0	35.0 ± 1.0	0.50	0.17	0.13
Waist circumference (cm)	106.4 ± 2.3	106.2 ± 2.2	109.9 ± 3.2	109.1 ± 3.1	0.15	0.42	0.37
HDL-cholesterol (mmol/L)	1.20 ± 0.06	1.14 ± 0.05	1.22 ± 0.07	1.23 ± 0.07	0.21	0.60	0.13
Triglycerides (mmol/L)	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.2	1.4 ± 0.1*	0.03	0.99	0.27
hs-CRP (mg/L)	3.3 ± 0.6	3.3 ± 0.7	3.1 ± 0.5	1.8 ± 0.3**†	0.04	0.22	0.04
Fasting glucose (mmol/L)	5.8 ± 0.1	5.8 ± 0.1	6.0 ± 0.1	5.6 ± 0.1***	<0.001	0.79	0.005
Fasting insulin (mU/L)	21.2 ± 1.7	21.2 ± 1.6	21.5 ± 1.0	16.0 ± 1.0***‡	<0.001	0.22	<0.001
HOMA-IR	5.4 ± 0.4	5.4 ± 0.4	5.8 ± 0.3	3.9 ± 0.3***‡	<0.001	0.22	<0.001
Matsuda ISI	1.91 ± 0.13	1.90 ± 0.12	1.72 ± 0.10	2.31 ± 0.17***†	<0.001	0.54	<0.001
Insulin $AUC_{0\text{-}120}$ ($mU/L \cdot min^{-1}$)	12100 ± 1441	11542 ± 1207	11458 ± 827	10369 ± 812**	0.009	0.78	0.09
M ($mg \cdot kg\ FFM \cdot min^{-1}$)	9.1 ± 0.7	10.0 ± 0.9	9.5 ± 0.7	12.7 ± 0.9***†	<0.001	0.14	0.03
Systolic BP (mmHg)	129 ± 3	128 ± 4	132 ± 3	129 ± 3	0.19	0.70	0.55
Diastolic BP (mmHg)	73 ± 2	74 ± 2	75 ± 2	73 ± 2	0.84	0.84	0.14
Heart rate (bpm)	63 ± 2	61 ± 1**	62 ± 2	60 ± 2*	<0.001	0.56	0.73
NE spillover $AUC_{0\text{-}120}$ ($ng/min \cdot min^{-1} \times 10^3$)	71.3 ± 7.4	67.0 ± 6.0	85.6 ± 10.3	94.4 ± 9.2*†	0.37	0.06	0.04

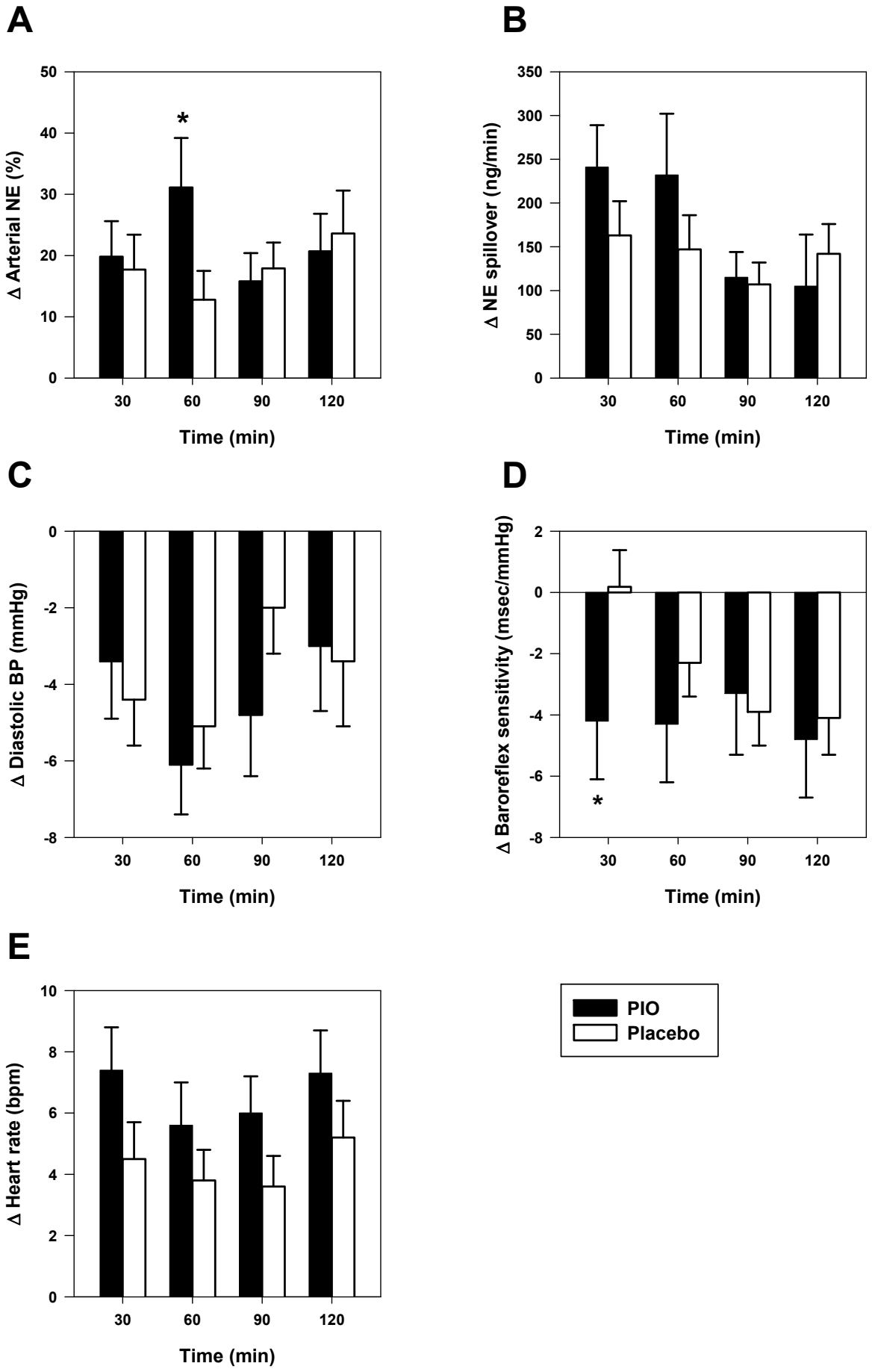
Values are mean ± SEM. $^{*}P<0.05$; $^{**}P<0.01$ and $^{***}P<0.001$ versus baseline. $^{\dagger}P<0.05$ and $^{\ddagger}P<0.01$ versus placebo group.

$AUC_{0\text{-}120}$, area under the curve during oral glucose tolerance test; BP, blood pressure; *hs*-CRP, high sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; NE, norepinephrine.

Figure 1: Norepinephrine kinetic and selected cardiometabolic parameters during oral glucose tolerance test at baseline and week 12 in pioglitazone treated subjects. **A.** Plasma insulin concentration: time effect, P<0.001; treatment effect, P=0.001; time x treatment interaction, P=0.02. **B.** Arterial norepinephrine (NE) concentration: time effect, P<0.001. **C.** Norepinephrine plasma clearance: time effect, P=0.04. **D.** Whole-body norepinephrine spillover rate: time effect, P<0.001; time x treatment interaction, P=0.03. **E.** Diastolic blood pressure: time effect, P=0.006; treatment effect, P=0.02. **F.** Cardiac baroreflex sensitivity: time effect, P<0.001. Values are the mean $\pm$ SEM. *P<0.05 and **P<0.01 versus baseline.

Figure 2: Changes in sympathetic nervous and cardiovascular parameters during oral glucose tolerance test in PIO and placebo groups at week 12. Change represents increment or decrement versus time 0. **A.** Arterial norepinehrine (NE). **B.** Whole-body norepinephrine (NE) spillover. **C.** Cardiac baroreflex sensitivity. **D.** Diastolic blood pressure. **E.** Heart rate. *P$\leq$ 0.05 versus placebo.

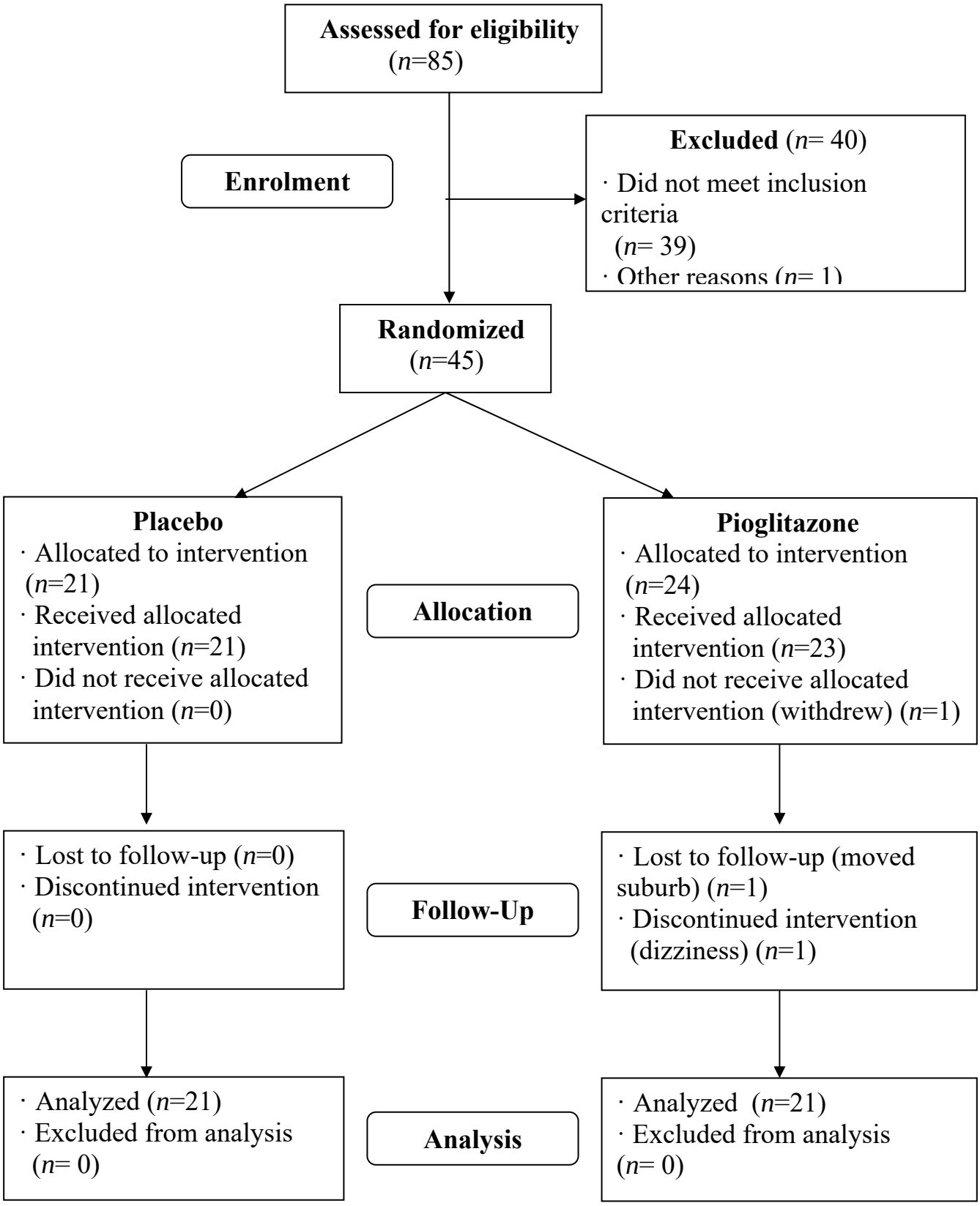

A
Insulin (mU/L)
140
120
100
80
60
40
20
0
***
*
0
30
60
90
120
Time (min)
B
Log arterial NE (pg/ml)
2.55
2.50
2.45
2.40
2.35
0.00
0
30
60
90
120
Time (min)
C
NE clearance (L/min)
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
0.0
**
0
30
60
90
120
Time (min)
D
Log NE spillover rate (ng/min)
3.00
2.95
2.90
2.85
2.80
2.75
2.70
0.00
**
0
30
60
90
120
Time (min)
E
Diastolic BP (mmHg)
82
80
78
76
74
72
70
68
0
**
*
0
30
60
90
120
Time (min)
F
Baroreflex sensitivity (msec/mmHg)
20
18
16
14
12
10
0
*
0
30
60
90
120
Time (min)
Baseline
Week 12

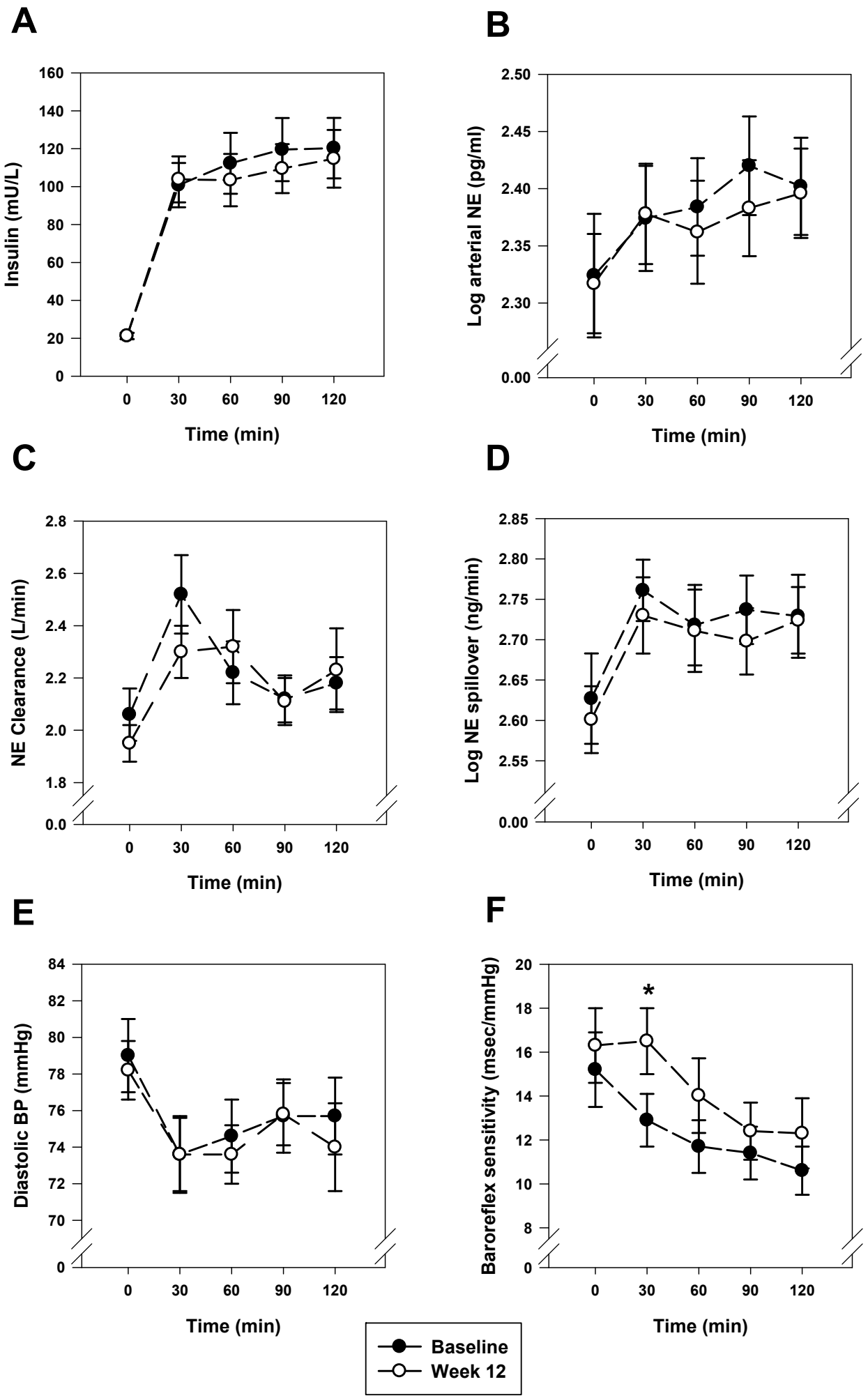

A
Δ Arterial NE (%)
50
40
30
20
10
0
*
30
60
90
120
Time (min)
B
Δ NE spillover (ng/min)
350
300
250
200
150
100
50
0
30
60
90
120
Time (min)
C
Δ Diastolic BP (mmHg)
0
-2
-4
-6
-8
30
60
90
120
Time (min)
D
Δ Baroreflex sensitivity (msec/mmHg)
2
0
-2
-4
-6
-8
*
30
60
90
120
Time (min)
E
Δ Heart rate (bpm)
10
8
6
4
2
0
30
60
90
120
Time (min)
PIO
Placebo

SUPPLEMENTARY MATERIAL

Figure S1: Randomization and attrition flow chart. Eighty-five individuals were screened for eligibility and 45 were randomized. One subject (PIO group) withdrew consent after baseline testing (dizziness after euglycemic clamp). One subject withdrew after experiencing dizziness, following a single dose of allocated treatment (PIO group). One subject (PIO group) was lost to follow-up (moved suburb). Forty-two subjects completed the study protocol.

Figure S2: Norepinephrine kinetic and selected cardiometabolic parameters during oral glucose tolerance test at baseline and week 12 in placebo treated subjects. **A.** Plasma insulin concentration: time effect, $P<0.001$. **B.** Arterial norepinephrine (NE) concentration: time effect, $P<0.001$. **C.** Norepinephrine plasma clearance: time effect, $P<0.001$. **D.** Whole-body norepinephrine spillover rate: time effect, $P<0.001$. **E.** Diastolic blood pressure: time effect, $P<0.001$. **F.** Cardiac baroreflex sensitivity: time effect, $P<0.001$; treatment effect $P=0.08$). There were no significant time x treatment interactions. Values are the mean $\pm$ SEM. $^{*}P<0.05$ versus baseline.


Assessed for eligibility
(n=85)
Enrolment
Excluded (n= 40)
· Did not meet inclusion criteria
(n= 39)
· Other reasons (n= 1)
Randomized
(n=45)
Placebo
· Allocated to intervention (n=21)
· Received allocated intervention (n=21)
· Did not receive allocated intervention (n=0)
Allocation
Pioglitazone
· Allocated to intervention (n=24)
· Received allocated intervention (n=23)
· Did not receive allocated intervention (withdrew) (n=1)
· Lost to follow-up (n=0)
· Discontinued intervention (n=0)
Follow-Up
· Lost to follow-up (moved suburb) (n=1)
· Discontinued intervention (dizziness) (n=1)
· Analyzed (n=21)
· Excluded from analysis (n= 0)
Analysis
· Analyzed (n=21)
· Excluded from analysis (n= 0)


A
Insulin (mU/L)
160
140
120
100
80
60
40
20
0
0
30
60
90
120
Time (min)
B
Log arterial NE (pg/ml)
2.50
2.45
2.40
2.35
2.30
0.00
0
30
60
90
120
Time (min)
C
NE Clearance (L/min)
2.8
2.6
2.4
2.2
2.0
1.8
0.0
0
30
60
90
120
Time (min)
D
Log NE spillover (ng/min)
2.85
2.80
2.75
2.70
2.65
2.60
2.55
0.00
0
30
60
90
120
Time (min)
E
Diastolic BP (mmHg)
84
82
80
78
76
74
72
70
0
0
30
60
90
120
Time (min)
F
Baroreflex sensitivity (msec/mmHg)
20
18
16
14
12
10
8
0
*
0
30
60
90
120
Time (min)
Baseline
Week 12