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Pioglitazone Attenuates Diet-Induced Hypertension in Rats

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Consumption of diets rich in fats or sugars is correlated with the onset of insulin resistance and hypertension in rats. In the present study, rats were fed diets that induce hypertension; 50% of the rats were also treated with pioglitazone, a thiazolidinedione derivative that sensitizes target tissues to insulin and decreases plasma insulin concentration in insulin-resistant animals. Pioglitazone treatment prevented the development of hypertension and reduced plasma insulin concentration by 70% and 37% in rats fed a high-fat or glucose diet, respectively ($P < .05$ compared with rats fed the same diet without pioglitazone). In rats fed a control diet, neither insulin nor blood pressure (BP) was affected by pioglitazone treatment. The effect of pioglitazone on insulin and BP could not be attributed to a reduction in body weight, since pioglitazone increased the weight gain of rats fed the high-fat or glucose diet. These findings suggest that in rats fed a diet high in fat or glucose, treatment with pioglitazone maintains plasma insulin concentration and BP at control levels, regardless of body weight.

HYPERTENSION is associated with elevated plasma insulin concentration and/or resistance to one or more of the actions of insulin.[1-6] However, a causal role has not been established for hyperinsulinemia/insulin resistance in the pathogenesis of hypertension. Environmental factors, particularly diet, may contribute to the development and persistence of insulin resistance. Consumption of diets rich in sugars or fats is associated with the development of insulin resistance,[7-12] and in some instances this effect is independent of changes in body weight.[13,14] Consumption of diets rich in sugars or fats is also correlated with the onset of hypertension in rats.[15-20]

In the present study, rats were fed diets that induce hypertension, and half of the rats were also treated with pioglitazone. Pioglitazone is a thiazolidinedione derivative that sensitizes target tissues to insulin without stimulating endogenous insulin secretion and without causing hypoglycemia.[21-23] Pioglitazone and related compounds enhance insulin-mediated glucose transport and metabolism.[21-22] They are thought to act by augmenting early postbinding steps in the insulin signal-transduction system, and they do not affect glucose transport/metabolism in the absence of insulin.[21-22] These agents do not produce hypoglycemia in normal or insulin-resistant animals, presumably because plasma insulin concentration declines.[22]

Pioglitazone was used in these experiments because it is known to enhance insulin sensitivity and reduce plasma insulin concentrations in insulin-resistant animal models.[21-24] Our objective was to test the following hypotheses: (1) plasma insulin concentration and blood pressure (BP) are correlated; (2) rats consuming a diet high in fat or glucose have a higher plasma insulin concentration and higher BP than rats fed a control diet; and (3) pioglitazone treatment reduces both plasma insulin concentration and BP in rats fed a diet high in fat or glucose.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed individually in stainless steel cages in a room maintained at 20° ± 2°C with a 12-hour light-dark cycle. At 9 weeks of age, rats were divided into six groups (nine rats per group) and offered one of three semisynthetic diets (Table 1). Of the three diets, the control diet most closely resembled the composition of typical stock diet. Diets were formulated to provide equal quantities of protein, vitamins, and minerals. Because caloric density of the high-fat diet is much higher than that of the glucose and control diets, rats typically consumed a smaller weight of the high-fat diet.[18] To provide approximately equal quantities of protein, vitamins, and minerals to all animals, each kilogram of the high-fat diet contained a greater quantity of protein, vitamins, and minerals

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Table 1. Composition of Experimental Diets

Component	High-Fat	Glucose	Control
Caloric density (kcal/g)	6.10	4.14	4.18
Methionine	4.0	3.0	3.0
AIN-76 Vitamin Mix	13.5	10.0	10.0
AIN-76 Mineral Mix*	47.3	35.0	35.0
Choline chloride	2.7	2.0	2.0
Cellulose	67.5	50.0	50.0
Caseint	317.0	210.0	224.0
Corn starch	151.0	0	625.0
Glucose	0	642.0	0
Corn oil	73.0	48.0	51.0
Lard	324.0	0	0
Protein	21.5	21.5	21.5
Carbohydrate	12.0	66.4	66.4
Fat	66.5	12.1	12.1

NOTE. Values for protein, carbohydrate, and fat are % of energy. All other values are measured in g/kg. AIN-76 Vitamin Mix and AIN-76 Mineral Mix from ICN Nutritional Biochemicals, Cleveland, OH.

*Because of differences in caloric density and anticipated food intake, high-fat diet contained 3.49 mg NaCl and 7.03 mg elemental calcium per gram, whereas glucose and control diets contained 2.58 mg NaCl and 5.20 mg elemental calcium per gram.

†Casein (ICN Nutritional Biochemicals) was vitamin-free.

than the glucose or control diet (Table 1). Animals were allowed ad libitum access to food and water.

Half of the rats in each diet group received pioglitazone 20 mg/kg/d (Upjohn, Kalamazoo, MI) mixed in the diet. Body weight and food intake were monitored throughout the study. Gross energy content of the diet was determined by adiabatic calorimetry (Parr Instrument, Moline, IL).

Systolic BP was measured on conscious restrained animals using a photoelectric sensor (IITC, Woodland Hills, CA) and tail-cuff sphygmomanometer between 8 and 11 AM as described previously.[18,19] Pressures recorded during three to five successive inflation-deflation cycles were averaged to obtain a single weekly BP reading.

After 7 weeks of treatment, a random plasma sample was obtained via the tail vein under light ether anesthesia. Glucose level was measured by the glucose oxidase method (YSI 2300 Glucose Analyzer; YSI, Yellow Springs, OH). Insulin level was measured by a radioimmunoassay kit using rat insulin as the standard (Incstar, Stillwater, MN).

Data are presented as the mean ± SD. Multiple comparisons were made by ANOVA (diet × drug), followed by the Student-Neuman-Keuls test for individual differences.

RESULTS

Characteristics of experimental animals after 7 weeks of treatment are listed in Table 2. Compared with rats fed the control diet, rats fed the high-fat diet consumed more energy, gained more weight, and had higher plasma insulin levels. Plasma insulin levels were slightly but not significantly elevated in glucose-fed rats. However, plasma glucose levels were higher in rats fed the glucose diet than in rats fed the control diet.

Pioglitazone treatment reduced plasma insulin levels in rats fed the high-fat or glucose diet, but did not affect insulin levels of rats fed the control diet (Table 2). In rats treated with pioglitazone, random-sample plasma insulin concentration did not differ among diet groups. Pioglitazone treatment had no effect on energy intake or body weight of rats fed the control diet. Pioglitazone increased weight gain of rats fed the high-fat or glucose diet.

After 7 weeks of treatment, BP was 173 ± 6, 167 ± 8, and 159 ± 7 mm Hg (mean ± SD) in rats fed the high-fat, glucose, and control diets, respectively ($P < .05$ for high-fat or glucose *v* control). Pioglitazone treatment did not affect BP of rats fed the control diet. In pioglitazone-treated rats, BP remained at control levels in all diet groups (Fig 1). The relationship between systolic BP and random-sample plasma insulin during week 7 of treatment is depicted in Fig 2. In rats not treated with pioglitazone, BP was positively correlated with plasma insulin ($P < .001$). The relationship between systolic BP and body weight during week 7 of treatment is depicted in Fig 3. In rats not treated with pioglitazone, BP was positively correlated with body weight ($P < .05$).

To determine whether pioglitazone would ameliorate existing hypertension, the same diet treatments were continued during weeks 8 to 14. Rats that had not received pioglitazone during weeks 0 to 7 were given pioglitazone (20 mg/kg) during weeks 8 to 14. BP was recorded during week 14 of the study. Pioglitazone treatment reduced systolic BP from 173 ± 6 mm Hg (week 7) to 152 ± 11 (week 14) in rats fed the high-fat diet ($P < .05$). Pioglitazone treatment reduced systolic BP from 167 ± 8 mm Hg (week 7) to 149 ± 14 (week 14) in rats fed the glucose diet ($P < .05$). Pioglitazone treatment had no significant effect on BP of rats fed the control diet (159 ± 7 mm Hg during week 7 *v* 148 ± 14 during week 14).

Table 2. Effects of Diet and Pioglitazone Treatment

	High-Fat		Glucose		Control	
Parameter	+	−	+	−	+	−
Energy intake (kcal)	4,694 ± 291*	4,645 ± 247*	4,632 ± 243*†	4,363 ± 148	4,264 ± 156	4,286 ± 271
Body weight (g)	400 ± 24*†	377 ± 20*	372 ± 28	349 ± 17	364 ± 15	350 ± 23
Weight gain (g)	209 ± 19*†	189 ± 19*	185 ± 19*†	163 ± 18	174 ± 13	163 ± 21
Glucose (mmol/L)	10.4 ± 1.1	11.4 ± 1.2	10.3 ± 1.4†	12.3 ± 1.9*	10.8 ± 1.4	11.1 ± 1.1
Insulin (pmol/L)	228 ± 48†	748 ± 239*	283 ± 129†	444 ± 145*	219 ± 128	343 ± 180

NOTE. Mean ± SD for 9 rats per group. Absence and presence of pioglitazone treatment are indicated by − and +, respectively. Body weight and plasma glucose and insulin levels were measured during week 7 of diet treatment with or without pioglitazone (20 mg/kg/d). Energy intake represents the cumulative energy intake during 7 weeks of treatment.

*$P < .05$ *v* rats fed control diet without pioglitazone.

†$P < .05$ *v* rats fed the same diet without pioglitazone.

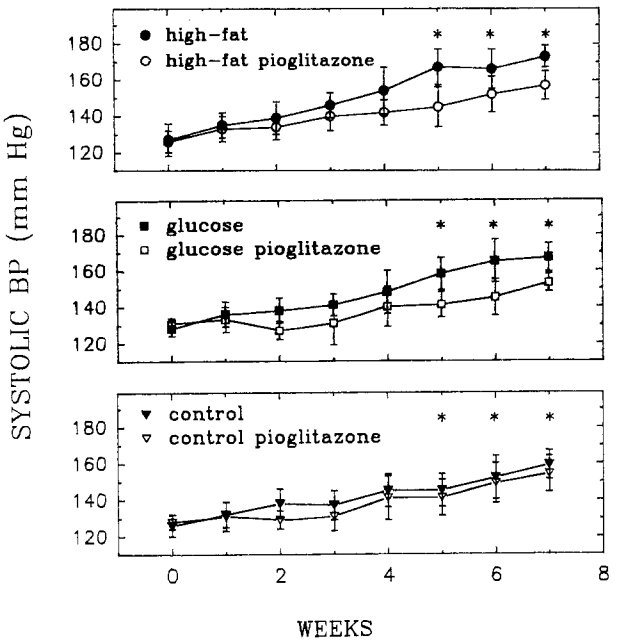

Fig 1. Effects of pioglitazone treatment on systolic BP of rats consuming a high-fat, glucose, or control diet. Mean ± SD for 9 rats per group. *Difference ($P < .05$) between pioglitazone-treated and untreated rats consuming the same diet. In rats not treated with pioglitazone, systolic BP was higher ($P < .05$) during weeks 5, 6, and 7 in rats fed the high-fat or glucose diet than in rats fed the control diet. Systolic BP of rats treated with pioglitazone did not differ among diet treatment groups.

DISCUSSION

The results of this study are in agreement with previous findings[18,19]: in rats not treated with pioglitazone, systolic BP was higher during weeks 5, 6, and 7 in rats consuming the high-fat or glucose diet versus the control diet. In the current experiments, initial BP in all groups was slightly less than that reported in previous studies.[18,19] This may be explained by the fact that BP measurements were begun at an earlier age in the current studies.

Pioglitazone treatment reduced insulin levels and prevented the development of hypertension in rats fed the high-fat or glucose diet. Pioglitazone treatment also reversed existing hypertension in rats fed the high-fat or glucose diet. In rats fed the control diet, neither plasma insulin nor BP was affected by pioglitazone treatment.

The basis for the antihypertensive effect of pioglitazone is not understood. Insulin-induced vasodilation is impaired in states of insulin resistance, and this abnormality may lead to hypertension.[24] Pioglitazone may reduce BP by ameliorating insulin resistance, thereby enhancing insulin-induced vasodilation and/or reducing vascular contractility.[24,25] In the current study, pioglitazone did not decrease BP of normotensive animals consuming the control diet, suggesting that it does not exert nonspecific hypotensive effects.

It has been demonstrated previously that rats fed the high-fat diet have a greater energy intake, weigh more, and have a higher carcass energy density (an index of obesity) than rats fed the control diet.[18,19] Although rats fed the glucose diet exhibit a slightly greater energy intake than rats fed the control diet, neither body weight nor carcass energy density differs between rats fed the glucose and control diet.[18] Energy intake and carcass energy density were not measured in the current study. However, in rats not treated with pioglitazone, those fed the high-fat diet weighed more than those fed the glucose or control diet, consistent with the results of previous studies.[18,19]

The effect of pioglitazone on insulin and BP could not be attributed to a reduction in body weight. Pioglitazone had no effect on energy intake or body weight of rats fed the control diet. Pioglitazone increased weight gain of rats fed the high-fat or glucose diet. Pioglitazone-treated rats fed the high-fat or glucose diet maintained BP at control levels despite an increased weight gain. An increase in body weight in rodents treated with pioglitazone and related compounds has been reported by others, and may be the result of enhanced insulin effects on adipose tissue lipogenesis.[21,23,26]

In rats not treated with pioglitazone, BP was positively correlated with random-sample plasma insulin concentration and body weight. In contrast, no correlation was noted between random-sample plasma insulin concentration and BP in pioglitazone-treated rats, perhaps because pioglitazone treatment decreased both insulin concentration and

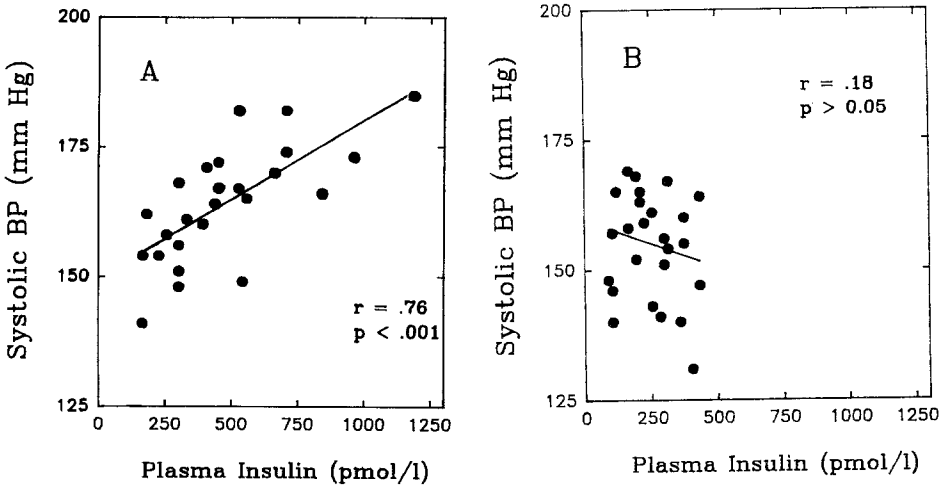

Fig 2. Relationship between systolic BP and random-sample plasma insulin during week 7 of treatment. (A) Rats not treated with pioglitazone; (B) pioglitazone-treated rats.

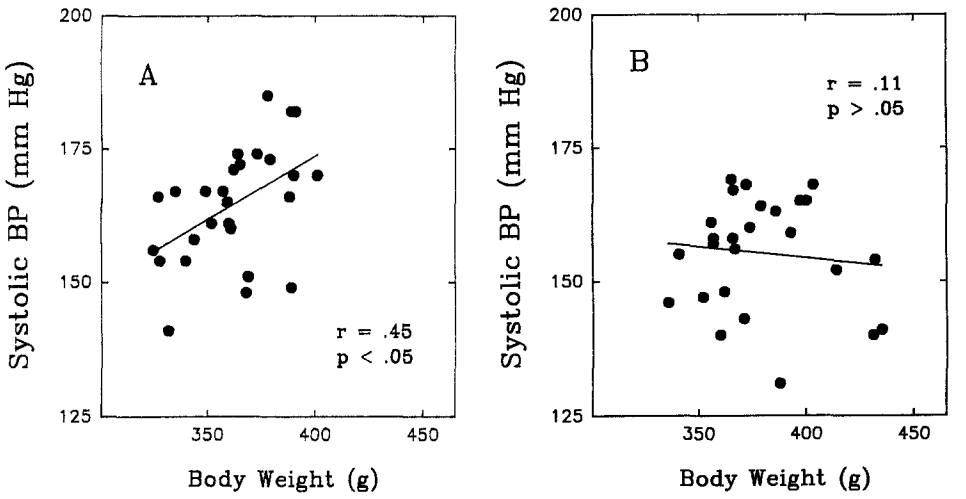

Fig 3. Relationship between systolic BP and body weight during week 7 of treatment. (A) Rats not treated with pioglitazone; (B) pioglitazone-treated rats.

BP of rats fed the high-fat or glucose diet to levels that did not differ from those of rats fed the control diet. Likewise, no correlation was noted between body weight and BP in pioglitazone-treated rats, perhaps because pioglitazone treatment decreased BP, yet increased weight gain of rats fed the high-fat or glucose diet.

In summary, the present study demonstrates that in rats fed a diet high in fat or glucose, pioglitazone treatment reduces random-sample plasma insulin concentration and BP to control levels. However, pioglitazone treatment does not affect insulin or BP in normotensive rats fed a control diet. Additional studies are needed to elucidate the mechanism(s) mediating the antihypertensive effects of pioglitazone and the role of insulin resistance/hyperinsulinemia in the pathogenesis of hypertension.

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