Impaired dopamine D₁ receptor-mediated vasorelaxation of mesenteric arteries in obese Zucker rats

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Abstract

Background: Obesity plays an important role in the pathogenesis of hypertension. Renal dopamine D₁-like receptor-mediated diuresis and natriuresis are impaired in the obese Zucker rat, an obesity-related hypertensive rat model. The role of arterial D₁ receptors in the hypertension of obese Zucker rats is not clear.

Methods: Plasma glucose and insulin concentrations and blood pressure were measured. The vasodilatory response of isolated mesenteric arteries was evaluated using a small vessel myograph. The expression and phosphorylation of D₁ receptors were quantified by co-immunoprecipitation and immunoblotting. To determine the effect of hyperinsulinemia and hyperglycemia on the function of the arterial D₁ receptor, we studied obese Zucker rats (six to eight-weeks old) fed (6 weeks) vehicle or rosiglitazone, an insulin sensitizer (10 mg/kg per day) and lean Zucker rats (eight to ten-weeks old), fed high-fat diet to induce hyperinsulinemia or injected intraperitoneally with streptozotocin (STZ) to induce hyperglycemia.

Results: In obese Zucker rats, the vasorelaxant effect of D₁-like receptors was impaired that could be ascribed to decreased arterial D₁ receptor expression and increased D₁ receptor phosphorylation. In these obese rats, rosiglitazone normalized the arterial D₁ receptor expression and phosphorylation and improved the D₁-like receptor-mediated vasorelaxation. We also found that D₁ receptor-dependent vasorelaxation was decreased in lean Zucker rats with hyperinsulinemia or hyperglycemia but the D₁ receptor dysfunction was greater in the former than in the latter group. The ability of insulin and glucose to decrease D₁ receptor expression and increase its phosphorylation were confirmed in studies of rat aortic smooth muscle cells.

Conclusions: Both hyperinsulinemia and hyperglycemia caused D₁ receptor dysfunction by decreasing arterial D₁ receptor expression and increasing D₁ receptor phosphorylation. Impaired D₁ receptor-mediated vasorelaxation is involved in the pathogenesis of obesity-related hypertension.

Keywords: Dopamine D₁ receptor, Vasorelaxation, Hyperinsulinemia, Hyperglycemia, Obesity-related hypertension, Obese Zucker rats

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**Background**

There is an increasing incidence of metabolic syndrome (MS) which is characterized by abdominal obesity, hypertriglyceridemia, low serum high density lipoprotein cholesterol, elevated blood pressure, and elevated fasting plasma glucose [1]. The metabolic syndrome can cause or intensify cardiovascular, as well as renal disease [1,2]. One of the possible etiologies of the metabolic syndrome is insulin resistance associated with hyperinsulinemia [1-4]. The stimulatory effect of insulin on sympathetic drive [5], vascular smooth muscle growth [6] and sodium and water retention [7] and its inhibitory effect on prostacyclin synthesis [8] have been suggested to be involved in the pathogenesis of obesity-related hypertension.

Dopamine, a well recognized neurotransmitter in the central nervous system, is also an important modulator of renal and adrenal function, sodium balance, and blood pressure. Dopamine receptors are classified into two subfamilies: the D1-like receptor subfamily includes the D1 and the D5 receptors, while the D2, D3 and D4 receptors belong to the D2-like receptor subfamily. Dysfunction of the renal dopaminergic system is implicated in the pathogenesis and/or maintenance of hypertension [9,10].

The obese Zucker rat is a model of metabolic syndrome characterized by hyperinsulinemia, hyperglycemia, and hypertension. Dopamine has been implicated in the development of obesity, caused, in part, by increased food intake due to decreased dopaminergic function, specifically the D2 receptor, in the central nervous system [11,12]. D2 receptor mediated-natriuresis and diuresis are also impaired in obese Zucker rats [13,14]. Although the kidney is important in the long-term regulation of blood pressure [15], hypertension is also accompanied by increased vascular resistance [16]. Dopamine receptors are expressed in resistance vessels. In the rat mesenteric artery, D1 and D2 receptors are expressed in the tunica media while D2-like receptors are expressed mainly at the adventitia-tunica media transitional zone. The increase in blood pressure with aging has been related to a decrease in the expression of arterial D1, D2, and D5 receptor [17]. However, the role of the arterial D1 receptor in the hypertension of obese Zucker rats is not clear. Our current study tested the hypothesis that the vasorelaxant effect of the D1 receptor is impaired in obese Zucker rats.

**Methods**

**Animal experiments**

Male obese Zucker rats and age-matched male lean Zucker rats were housed in plastic cages and fed normal rodent chow and tap water. Twelve obese Zucker rats (six to eight-weeks old) were randomly divided into two groups: 1) one group consisted of obese rats treated (6 weeks) with oral rosiglitazone maleate (10 mg/kg, suspended in 1% carboxy methyl cellulose in distilled water); and 2) the other group consisted of obese control rats treated with the vehicle (1%) carboxy methyl cellulose). Eighteen lean Zucker rats (eight to ten-weeks old) were randomly divided into three groups: 1) control rats fed normal diet and treated with vehicle; 2) rats fed control diet and treated with STZ (single intraperitoneal injection, 65 mg/kg); and 3) rats fed high-fat diet (with 50% fat-derived calories) for one month.

All experiments were approved by the Daping Hospital Animal Use and Care Committee and all procedures were approved by the Experimental Animals Committee of Daping Hospital.

**Blood pressure and plasma glucose and insulin measurements**

After an overnight fast, obese and lean Zucker rats were anesthetized with pentobarbital (Nembutal) with an initial dose of 60 mg/kg, followed by constant infusion at 40 mg/kg/hr [18]. Following a tracheotomy, the left carotid artery was catheterized with polyethylene-50 tubing for blood pressure monitoring. Blood pressure and heart rate were allowed to stabilize for 10 minutes [19] before accepting the blood pressure and heart rate observed as baseline. The abdomen was opened to expose the abdominal aorta and blood samples were collected from the aorta in EDTA-coated tubes for measurement of plasma glucose and insulin, and then the rats were sacrificed by an overdose of pentobarbital (100 mg/kg body wt). Plasma glucose concentrations were determined by using Accu-Chek Advantage glucose monitoring system. Plasma insulin levels were measured by a rat insulin 96-well plate assay (Millipore Co., St Charles, MO).

**Preparation and study of small resistance arteries**

Each rat was anesthetized with sodium pentobarbital (60 mg/kg). The entire mesenteric bed was removed carefully and placed in ice-cold physiological salt solution (PSS) containing (mM): NaCl 119, KCl 4.7, CaCl₂·H₂O 2.5, MgSO₄·H₂O 1.7, NaH₂CO₃ 25, KH₂PO₄ 1.18, EDTA 0.027, and glucose 5.5, adjusted to pH 7.4. The mesenteric artery was carefully and quickly dissected from the surrounding fat and connective tissues. Third-order branches of the superior mesenteric artery (resting arterial diameter: 200 ± 20 μm) were cut into rings approximately 2 mm in length, and mounted on 40 μm stainless-steel wires in an isometric Mulvany-Halpern small-vessel myograph (model 91 M610, J.P. Trading, Science Park, Aarhus, Denmark) [20]. One wire was attached to a force transducer and the other to a micrometer so that wall tension can be measured at a predetermined internal diameter. The rings were maintained in PSS at 37°C and continuously bubbled with oxygen (95%) and carbon dioxide (5%) (Carbogen) [21]. The dissecting procedures were performed with extreme care to protect the endothelium from inadvertent damage, proved by a normal response to acetylcholine (Ach) (10⁻⁵ Ach-induced relaxation >80% of basal values of arteries.
preconstricted with phenylephrine HCl [PHE, 10^{-5}M]). In some vessels, the endothelium was removed by pulling a hair along the inside of the vessel; successful denudation of the endothelium was confirmed by the absence of relaxation with Ach (10^{-5}M) [22]. Following mounting, the arterial ring was equilibrated in PSS for 1 hr at 37°C at a wall tension of 0.1 mN/mm. Based on preliminary data from >100 vessels, we confirmed that a normalized circumference (L0) = 0.9 L100 resulted in maximal active force development. The vessels were studied at L0 in all subsequent protocols. After determining the response to Ach, as indicated above, the vessels were rinsed three times with fresh PSS and allowed to recover to baseline for 15 minutes. In the first set of experiments, the rings were contracted with phenylephrine HCl (10^{-5}M) and high-potassium PSS (KPSS, 125 mM) to obtain the maximal response. After obtaining the maximal response to PHE (10^{-5}M), the response curves to fenoldopam, a D1 receptor agonist [9,10], were measured by a cumulative concentration-dependent protocol (10^{-9} to 10^{-3}M). Response to every single concentration of fenoldopam was observed for 2 minutes. To test the vasorelaxant specific effects of D1 receptors, the arteries were incubated with the D1-like receptor antagonist SCH23390 (10^{-7}M) for 30 minutes before fenoldopam treatment. In order to determine specificity of the vasodilatory effect of D1 receptor stimulation, we studied the vasodilation induced by sodium nitroprusside (SNP, 10^{-10} to 10^{-4}M) in PHE-preconstricted mesenteric arteries.

Cell and sample preparation
Embryonic thoracic aortic smooth muscle cells from normotensive Berlin–Druckrey IX rats (A10, ATCC, Hercules, CA) were cultured at 37°C in 95% air/5% CO2 atmosphere in Dulbecco’s Modified Eagle’s Medium. A10 cells (80% confluence) and mesenteric arteries from Zucker rats were flash frozen by liquid nitrogen and homogenized in ice-cold lysis buffer (5 ml/gm tissue) (20 mM Tris-HCl, pH 7.4; 2 mM EDTA, pH 8.0; 2 mM EGTA; 100 mM NaCl; 10 μg/ml leupeptin; 10 μg/ml apro tinin; 2 mM phenylmethylsulfonyl fluoride; 1% NP-40), sonicated, kept on ice for 1 hr, and centrifuged at 16,000 g for 30 minutes. All samples were stored at -70°C until use.

Immunoblotting
After boiling the homogenates in sample buffer (35 mmol/L Tris-HCl, pH 6.8, 4% SDS, 9.3% dithiothreitol, 0.01% bromophenol blue, 30% glycerol) at 95°C for 5 min, 100 μg of protein were separated by SDS-PAGE (10% polyacrylamide), and then electroblotted onto nitrocellulose membranes (Bio-Rad). The blots were blocked overnight with 5% nonfat dry milk in phosphate buffered saline with Tween 20 (PBST) (0.05% Tween 20 in 10 mmol/l phosphate buffered (isotonic) saline) at 4°C with constant shaking, then incubated with polyclonal rabbit anti-rat D1 receptor antibodies (1:400 dilution; Millipore) overnight in the cold-room at 4°C. The membranes were then further incubated with infrared- labeled secondary antibodies (donkey anti-rabbit IRDye 800, Li-Cor Biosciences, Lincoln, NE) added to bind to the primary antibody at room temperature for 1 hr. The membranes were washed three times with PBST. The bound complex was detected using the Odyssey Infrared Imaging System (Li-Cor Biosciences). The images were analyzed using the Odyssey Application Software to obtain the integrated intensities.

Determination of basal D1 receptor phosphorylation by co-immunoprecipitation
Equal amounts of lysates (1.0 mg protein/ml supernatant from A10 cells or mesenteric artery) were incubated with polyclonal antiphosphoserine antibody (HKS3, New Territories, HK) (2.5 μg/ml) for 1 hr and protein-G agarose at 4°C for 12 hr. The immunoprecipitates were pelleted and washed four times with lysis buffer. The pellets were suspended in sample buffer, boiled for 10 min, and subjected to immunoblotting with polyclonal affinity-purified rabbit anti-rat D1 receptor antibody. In order to determine the specificity of the bands, normal rabbit IgG (negative control) and D1 receptor antibody (positive control) were used as the immunoprecipitants [23]. The bound complexes were detected using the Odyssey Infrared Imaging System (Li-Cor Biosciences). The images were analyzed using the Odyssey Application Software to obtain the integrated intensities.

Materials
Fenoldopam, rosiglitazone maleate, STZ, mitogen-activated protein kinase (MAPK) inhibitor PD98059, Ach, SNP, PHE, and D1 receptor antagonist SCH23390 were obtained from Sigma–Aldrich (St. Louis, MO). Rabbit anti-rat D1 receptor polyclonal antibodies and rat insulin 96 well plate assay kit were obtained from Millipore Corporation (St Charles, MO); and anti-phosphoserine antibodies were obtained from Abcam Ltd (New Territories, Hong Kong, China).

Statistical analysis
The data are expressed as mean ± SEM. Comparison within groups was made by repeated measures ANOVA (or paired t-test when only 2 groups were compared), and comparison among groups (or t-test when only 2 groups were compared) was made by factorial ANOVA with Holm-Sidak test. A value of $P < 0.05$ was considered significant.

Results
D1 receptor-mediated vasorelaxant effect is impaired in mesenteric arteries from obese Zucker rats
Consistent with previous reports [24], several variables, including body weight, and fasting plasma glucose and insulin
concentrations and blood pressure, were higher in obese than lean Zucker rats (Table 1). Fenoldopam, the D1 receptor agonist, (10⁻⁷M to 10⁻⁵M) induced a concentration-dependent vasorelaxation in the third-order mesenteric arteries from lean Zucker rats (Figure 1A). The absence of endothelium did not affect the fenoldopam-induced vasorelaxation, indicating that the fenoldopam-induced vasorelaxation was endothelium-independent (Figure 1B). The vasorelaxant effect of fenoldopam was via a D₁-like receptor because a D₁-

| Table 1 General and biochemical parameters of lean and obese Zucker rats (12-14 weeks old) |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Lean control | Obese control | Obese+ROG | Lean+HFD | Lean+STZ |
| Body weight (g) | 273.83±5.37 | 439.64±7.34* | 425.72±8.26 | 305.80±3.77* | 222.79±3.38* |
| Plasma glucose (mmol/L) | 5.86±0.17 | 8.88±0.48* | 5.94±0.27* # | 6.22±0.15 | >33.00* |
| Plasma insulin (ng/ml) | 0.79±0.02 | 7.94±1.04* | 2.97±0.44* # | 2.19±0.32* | 0.46±0.06* |
| Mean blood pressure | 118.91±1.85 | 138.26±3.62* | 124.85±2.51* | 120.59±2.39 | 129.66±1.51* |

Values are means ±SE; n=6. *P < 0.05 vs. lean control rats; #P < 0.05 vs. obese control rats. Obese rats were treated with rosiglitazone (ROG): 10mg/kg/day for 6 weeks; Lean rats were treated with high fat diet (HFD) and streptozotocin (STZ) for 4 weeks.

![Figure 1](image-url)
like receptor antagonist, SCH23390 \((10^{-7}\text{M})\), blocked the fenoldopam-induced vasorelaxation (Figure 1C), although SCH23390, by itself, was without any effect. The impaired vasorelaxant effect of fenoldopam was not a generalized phenomenon, because sodium nitroprusside \((10^{-10} \text{ to } 10^{-4}\text{M})\)-mediated vasodilation was not impaired in obese Zucker rats, consistent with previous reports \([25,26]\) (Figure 1D).

To determine whether or not the arterial D1 receptor is involved in the pathogenesis of obesity-related hypertension, we studied the D1 receptor function in third-order mesenteric arteries from obese Zucker rats. We found that the fenoldopam-induced vasorelaxation was lost in obese Zucker rats in the presence (Figure 1A) or absence (Figure 1B) of the endothelium. The dysfunction of renal D1 receptor in hypertension \([27]\) and obese Zucker rats \([28,29]\) has been reported to be caused by basal hyperphosphorylation of the renal D1 receptor. To determine whether or not this phenomenon also exists in third order mesenteric arteries of obese Zucker rats, D1 receptor expression and phosphorylation was studied by immunoblotting and immunoprecipitation. We found that D1 receptor expression was lower (Figure 2A), while basal D1 receptor phosphorylation (Figure 2B) in third order mesenteric arteries was higher in obese than lean Zucker rats.

**Roles of hyperinsulinemia and hyperglycemia in the D1 receptor vascular dysfunction in obese Zucker rats**

The pathogenesis of obesity is complex. To determine whether or not high plasma levels of glucose and insulin are involved in the obesity-related hypertension, the obese Zucker rats were treated with the insulin sensitizer \([30]\), rosiglitazone \((10 \text{ mg/kg oral, daily})\). We found that rosiglitazone restored the impaired fenoldopam-mediated vasorelaxation in obese Zucker rats (Figure 3A), increased the decreased D1 receptor expression (Figure 3B), and decreased the increased phosphorylation (Figure 3C) of D1 receptor in mesenteric arteries from obese Zucker rats consistent with those reported in the kidney \([28]\).

Consistent with other reports \([28,30]\), our current study found that rosiglitazone reduced plasma insulin and glucose levels and blood pressure (Table 1). Therefore, it was difficult to determine whether hyperinsulinemia, hyperglycemia, or both led to the dysfunction of the arterial D1 receptor. To overcome this dilemma, we used the hyperinsulinemic and hyperglycemic lean Zucker rat models. Lean Zucker rats fattened by a high-fat diet for one month developed hyperinsulinemia but had normal blood pressure, similar to other reports \([31,32]\). To establish a hyperglycemia model, lean Zucker rats were intraperitoneally injected with a single dose of STZ \((65 \text{ mg/kg})\) \([33]\); the STZ-treated lean Zucker rats would be expected to develop slightly high blood pressure and hyperglycemia but with low plasma insulin levels (Table 1), as described by others \([34]\). We found that both hyperinsulinemic (Figure 4A) and hyperglycemic (Figure 4B) lean Zucker rats had impaired D1 receptor-mediated vasorelaxation, accompanied by decreased D1 receptor expression (Figure 4C) and increased D1 receptor phosphorylation (Figure 4D), but the dysfunction of the D1 receptor was greater in the hyperinsulinemia than hyperglycemia.

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**Figure 2** D1 receptor expression and phosphorylation in mesenteric arteries from lean and obese Zucker rats. (A): D1 receptor expression was determined by immunoblotting. (B): Serine-phosphorylated D1 receptor in rat mesenteric arteries was determined by co-immunoprecipitation and immunoblotting. Normal rabbit IgG (negative control) or D1 receptor antibody (positive control) was used as the immunoprecipitant. D1 receptor serine phosphorylation was normalized by D1 receptor protein. \(*P < 0.05\) vs. lean rats, \(n = 6\).
model, indicating that insulin plays a more important role than hyperglycemia in the impairment of arterial D1 receptor-mediated relaxation (Figures 4A-D).

Effect of insulin and glucose on D1 receptor expression and phosphorylation in A10 cells

To further confirm the in vivo results, the effects of high insulin and high glucose concentrations on D1 receptor expression and phosphorylation were studied in A10 cells, a rat thoracic aorta-derived smooth muscle cell line. Treatment with insulin (Figures 5A and 5B) or high glucose (Figures 5C and 5D) decreased D1 receptor expression and increased D1 receptor phosphorylation. In the presence of a MAP kinase inhibitor, PD98059 (10⁻⁶ M), the effects of insulin on D1 receptor expression and phosphorylation were blocked, indicating that MAP kinase is involved in the signaling pathway (Figures 6A and 6B).

Discussion

Role of hyperinsulinemia in obesity-related hypertension

Obesity is a well-known risk factor for hypertension [15,35]. Indeed, risk estimates according to the Framingham study show that roughly 80% of essential hypertension in men and 65% in women can be directly attributed to obesity [36]. It is accepted that insulin resistance is epidemiologically linked with hypertension.
The compensatory hyperinsulinemia that occurs with insulin resistance increases renal sodium reabsorption and sympathetic activity and leads to elevated arterial pressure [39]. Insulin resistance and compensatory hyperinsulinemia impair the production of nitric oxide and favor the production of vasoconstrictors [40-42]. Our current study found hyperinsulinemia, hyperglycemia, and elevated blood pressure in obese Zucker rats that were reduced by rosiglitazone treatment similar to previous reports [43,44]. We also found that lean Zucker rats fed a high-fat diet had hyperinsulinemia and STZ-treated lean Zucker rats were hyperglycemic and hypoinsulinemic, similar to other reports [31,45].

Impairment of D₁ receptor-mediated vasodilation is involved in obesity-related hypertension

Dopamine has been reported as an important modulator of sodium balance, renal and adrenal function, and blood pressure and is relevant to the pathogenesis and/or maintenance of hypertension. In humans with essential hypertension and rodents with genetic hypertension (SHRs and Dahl salt-sensitive rats), D₁-like receptor agonist-mediated natriuretic and diuretic responses are impaired [46,47]. The ability of D₁-like receptors to stimulate adenylyl cyclase activity in renal arteries is also impaired in SHRs [48] and we have also reported an impaired ability of D₁ receptors to dilate the mesenteric arteries of SHRs [49]. Other studies have shown that the D₁ receptor is hyperphosphorylated and uncoupled from G protein subunits, leading to the D₁ receptor dysfunction in SHRs, which precedes the onset of hypertension and co-segregates with the hypertensive phenotype [10,50]. Thus, D₁ receptor dysfunction is a primary defect in the hypertension of SHRs. Lokhandwala et al have found that obese Zucker rats have defective renal

Figure 4 D₁ receptor expression and function in lean Zucker rats with hyperinsulinemia or hyperglycemia. Lean Zucker rats were fed high-fat diet (HFD) to induce hyperinsulinemia or intraperitoneally injected with streptomycin (STZ) to induce hyperglycemia. The mesenteric arteries preconstricted with PHE from those rats were treated with varying concentrations of fenoldopam (Fen, 10⁻⁹-10⁻⁵M) (A and B) (*P < 0.05, vs. lean Zucker rats, n = 8). D₁ receptor expression was determined by immunoblotting (C) and D₁ receptor phosphorylation (D) was determined by co-immunoprecipitation and immunoblotting. D₁ receptor serine phosphorylation was normalized by D₁ receptor protein (*P < 0.05, vs. obese rats, n = 6).
D₁ receptor function associated with a decrease in D₁ receptor expression and ability to inhibit Na, K-ATPase and Na, H-exchanger activities in renal proximal tubules [13,28,30]. These authors suggested that the renal dopaminergic dysfunction in obese Zucker rats is acquired and not inherited [14] and could be ameliorated by inhibition of reactive oxygen species production by tempol and insulin sensitization by rosiglitazone [28,29]. Because increased peripheral vascular resistance and altered vascular reactivity are distinctive features of essential hypertension [16], we tested the hypothesis that the hypertension of obese Zucker rats may be caused by an impairment of D₁ receptor-mediated vasodilation. This was indeed the case as the D₁ receptor-mediated vasorelaxation was impaired in obese Zucker rats. This dysfunction of the D₁ receptor could be ascribed to the decreased D₁ receptor expression and increased D₁ receptor phosphorylation because the rosiglitazone-mediated amelioration of the impaired D₁ receptor relaxant effect was associated with an increase in D₁ receptor expression and a decrease in D₁ receptor phosphorylation. Rosiglitazone, a PPARγ agonist, has many effects on the cardiovascular system independent of its insulin sensitizing effects, including anti-inflammation and direct stimulation of NO production [51]. Whether or not such mechanisms were involved in the rosiglitazone-mediated improvement of the vasodilatory effect of the D₁ receptor in the current report needs to be determined in a future study.

Figure 5 Effect of insulin and glucose on D₁ receptor expression and phosphorylation in A10 cells. A10 cells were treated with varying concentrations of insulin (A and B) or glucose (C and D) for 24 hrs. D₁ receptor expression was determined by immunoblotting. A10 cells were treated with insulin (10⁻⁷ M) and glucose (35 mM) for 24 hrs. D₁ receptor expression and phosphorylation were determined by co-immunoprecipitation or immunoblotting. D₁ receptor serine phosphorylation was normalized by D₁ receptor protein (*P < 0.05, vs. control, n = 3).
Role of hyperinsulinemia or hyperglycemia in the dysfunction of arterial D1 receptor in obese Zucker rats
Obese Zucker rats have increased plasma insulin and fasting glucose levels [24,52,53]. As stated above, treatment of obese Zucker rats with rosiglitazone reduced plasma insulin and glucose levels and improved the vasorelaxant effect of fenoldopam that was associated with an increase in the vascular expression of D1 receptor and a decrease in vascular serine-phosphorylated D1 receptor. Thus, hyperinsulinemia and hyperglycemia may be involved in the defective fenoldopam-mediated relaxation in small mesenteric arteries isolated from obese Zucker rats. In order to identify the mechanisms that caused the impaired D1 receptor vasodilatory function in obese Zucker rats, we used two rat models: high fat diet-induced hyperinsulinemia and STZ-induced type I diabetes. The former rat model is characterized by insulin resistance and hyperinsulinemia [31,32] while the latter rat model is characterized by hyperglycemia and low plasma insulin levels [33,34]. After the establishment of hyperinsulinemia or hyperglycemia, mesenteric arteries had an impaired fenoldopam-mediated relaxation that was more evident in the high fat diet-fed hyperinsulinemic rats than STZ-induced type I diabetic rats. Therefore, both hyperinsulinemia and hyperglycemia are involved in the dysfunction of the arterial D1 receptor, but hyperinsulinemia plays a more important role than hyperglycemia in this phenomenon.

The ability of increased insulin and glucose concentrations to decrease D1 receptor expression and increase D1 receptor phosphorylation in vivo was confirmed in vitro in A10 cells. Treatment of A10 cells with insulin decreased D1 receptor expression and increased D1 receptor phosphorylation, consistent with the findings in renal proximal tubule cells [54,55]. Mitogen-activated protein kinase (MAPK) is activated in cardiovascular diseases such as diabetes and hypertension [56,57]. The up-regulation of MAPK reduces renal D1 receptor affinity and G-protein coupling in obese rats [58]. The current studies also showed that blockade of MAPK reversed both the decreased D1 receptor expression and increased D1 receptor phosphorylation caused by high insulin in A10 cells.

Limitations
Besides of the role of hyperinsulinemia and hyperglycemia in the D1-receptor mediated arterial dysfunction, adiponectin, as an important adipose tissue-derived factor, might have some effect on dopamine function. For example, monosodium glutamate induces obesity in rodents markedly decreases adiponectin levels [59] and compromises dopaminergic systems [60]. Moreover, dopamine stimulates adiponectin release [61], and antidiabetic treatment in Zucker diabetic fatty rats inhibits the development of hypo-adiponectinemia in mesenteric resistance arteries, but is not able to improve adiponectin induced vasodilation [62]. Whether or not the plasma adiponectin would affect arterial dopamine receptor function needs to be determined in the future study.
Conclusions
In summary, this study shows that both hyperinsulinemia and hyperglycemia impair vascular D1 receptor function that is associated with decreased D1 receptor expression and increased D1 receptor phosphorylation. Besides, hyperinsulinemia plays a more important role than hyperglycemia in the dysfunction of the arterial D1 receptor in obese Zucker rats. Impaired D1 receptor-mediated vasorelaxation is involved in the pathogenesis of obesity-related hypertension.

Abbreviations
STZ: Streptomyces; HFD: High-fat diet; MS: Metabolic syndrome; PSS: Physiological salt solution; Ach: Acetylcholine; PHE: Phenylephrine HCL; KPSS: High-potassium PSS; SNP: Sodium nitroprusside; MAPK: Mitogen-activated protein kinase; ROG: Rosiglitazone; Fen: Fenoldopam; SCH: SCH23390; M: mol/L.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
JIF, TH, HYW performed most of the experiments and analyzed the data and wrote the manuscript. ZW performed some experiments and write the cover letter. YKL reviewed and edited the manuscript. XIC and VC performed some experiments and contributed to the discussion. Laureano D. Asico and Pedro A. Jose edited the manuscript and contributed to the discussion. CCZ and LZ designed the experiments and wrote and edited the manuscript. All authors read and approved the final manuscript.

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References
1. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C: American Heart Association; National Heart, Lung, and Blood Institute. Definition of obesity related to definition. Circulation 2004, 109(3):433–438.
2. Singh AK, Kari JA: Metabolic syndrome and chronic kidney disease. Curr Opin Nephrol Hypertens 2013, 22(2):198–203.
3. Vonbank A, Saely CH, Rein P, Drexel H: Insulin resistance is significantly associated with the metabolic syndrome, but not with sonographically proven peripheral arterial disease. Cardiovasc Diabetol 2013, 12:106.
4. Saely CH, Azcel S, Marte T, Langer P, Hoefle G, Drexel H: The metabolic syndrome, insulin resistance, and cardiovascular risk in diabetic and nondiabetic patients. J Clin Endocrinol Metab 2005, 90(10):5698–5703.
5. Alvarez GE, Barlow SD, Ballard TP, Davy KP: Sympathetic neural activation in visceral obesity. Circulation 2002, 106(20):2533–2536.
6. Frisbee JC, Hollander JM, Brock RW, Yu HG, Boegehold MA: Integration of skeletal muscle resistance arteriolar reactivity for perfusion responses in the metabolic syndrome. Am J Physiol Regul Integr Comp Physiol 2009, 296(6):R1771–R1782.
7. Ter Maaten JC, Voorburg A, Heine RJ, Ter Wee PM, Donker AJ, Gans RO: Renal handling of urate and sodium during acute physiological hyperinsulinemia in healthy subjects. Clin Sci (Lond) 1997, 92(5):51–58.
8. Richelsen B, Borglum JD, Sorensen SS: Biosynthetic capacity and regulatory aspects of prostaglandin E2 formation in adipocytes. Mol Cell Endocrinol 1992, 85(3):233–237.
9. Zeng C, Jose PA: Dopamine receptors: important antihypertensive counterbalance against hypertensive factors. Hypertension 2011, 57(1):11–17.
10. Zeng C, Felder RA, Jose PA: A new approach for treatment of hypertension: modifying D1 dopamine receptor function. Cardiovasc Hematol Agents Med Chem 2006, 4(4):369–377.
11. Baladi MG, Daws LC, France CP: You are what you eat: influence of type and amount of food consumed on central dopamine systems and the behavioral effects of direct- and indirect-acting dopamine receptor agonists. Neuropsychopharmacology 2012, 37(1):76–86.
12. Davis LM, Michaelides M, Cheskin LJ, Morlan TH, Aja S, Watkins PA, Pei Z, Contoreggi C, McCulloch K, Hope B, Wang GJ, Volkow ND, Thoskos CP: Bromocriptine administration reduces hyperphagia and adiposity and differentially affects dopamine D1 receptor and transporter binding in leptin-receptor-deficient Zucker rats and rats with diet-induced obesity. Neuroendocrinology 2009, 89(2):152–162.
13. Lokhandwala MF, Hussain T: Defective renal dopamine D1-like receptor signal transduction in obese hypertensive rats. Acta Physiol Scand 2000, 168(1):251–255.
14. Banday AA, Hussain T, Lokhandwala MF: Renal dopamine D1 receptor dysfunction is acquired and not inherited in obese Zucker rats. Am J Physiol Renal Physiol 2004, 287(1):F109–F116.
15. Hall JE, Granger JP, do Camo JM, da Silva AA, Dubinon J, George E, Hamza S, Speed J, Hall ME: Hypertension: physiology and pathophysiology. Can J Physiol 2012, 94(4):2393–2442.
16. Schifflin EL, Touyz RM: From bedside to bench to bedside: role of renin-angiotensin-aldosterone system in remodeling of resistance arteries in hypertension. Am J Physiol Heart Circ Physiol 2004, 287(1):H435–H446.
17. Ricci A, Amenta F, Bronzetti E, Felici L, Hussain T, Lokhandwala MF: Age-related changes of dopamine receptor protein immunoreactivity in the rat mesenteric vascular tree. Mech Ageing Dev 2002, 123(5):537–546.
18. Charbit AR, Ackerman S, Goadsby PJ: Comparison of the effects of central and peripheral dopamine receptor activation on evoked firing in the trigemino-cerebrovascular complex. J Pharmacol Exp Ther 2009, 331(2):752–763.
19. McCoy CE, Douglas FL, Goldberg UJ: Selective antagonism of the hypertensive effects of dopamine agonists in spontaneously hypertensive rats. Hypertension 1986, 8(4):298–302.
20. Muhiray ML, Halpern W: Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res 1971, 29(1):19–36.
21. Lobato NS, Figueira PP, Phakol R, Giachini FR, Ergul A, Carvalho MH, Webb RC, Tostes RC, Fortes ZB: Reduced endothelium-dependent relaxation to anandamide in mesenteric arteries from young obese Zucker rats. PLoS One 2013, 8(3):e63449.
22. Wang Y, Han Y, Yang J, Wang Z, Liu L, Wang W: Relaxant effect of all-trans-retinoic acid via NO-cGMP pathway and calcium-activated potassium channels in rat mesenteric artery. Am J Physiol Heart Circ Physiol 2013, 304(1):H51–H57.
23. Chen Y, Asico LD, Zheng S, Villar VA, He D, Zhou L, Zeng C, Jose PA: Gastrin and D1 dopamine receptor interact to induce natriuresis and diuresis. Hypertension 2009, 53(5):927–933.
24. Muhammad AB, Lokhandwala MF, Banday AA: Exercise reduces oxidative stress but does not alleviate hyperinsulinemia or renal dopamine D1 receptor dysfunction in obese rats. Am J Physiol Renal Physiol 2011, 300(1):F10–F104.
25. Mingorance C, del Pozo Gonzalez M, Dolores Herrera M, de Sotomayor Alvarez M: Oral supplementation of propionyl-L-carnitine reduces body weight and hyperinsulinemia in obese Zucker rats. Br J Nutr 2009, 102(8):1145–1153.
26. Zhou W, Wang XL, Kaduce TL, Spector AA, Lee HC: Impaired arachidonic acid-mediated dilatation of small mesenteric arteries in Zucker diabetic fatty rats. Am J Physiol Heart Circ Physiol 2004, 287(1):H109–H115.
27. Py A, Asico LD, Luo Y, Andrews P, Elsen GE, Hopfer U, D1: dopamine receptor hyperphosphorylation in renal proximal tubules in hypertension. Kidney Int 2006, 70(6):1072–1079.
28. Trivedi M, Lokhandwala MF: Rosiglitazone restores renal D1A receptor-Gs protein coupling by reducing receptor hyperphosphorylation in obese rats. Am J Physiol Renal Physiol 2005, 289(3):F298–F304.
29. Banday AA, Marwaha A, Talam LS, Lokhandwala MF: Tempol reduces oxidative stress, improves insulin sensitivity, decreases renal dopamine D1 receptor hyperphosphorylation, and restores D1 receptor-G-protein coupling and function in obese Zucker rats. Diabetes 2005, 54(7):2119–2226.

30. Trivedi M, Manwaha A, Lokhandwala M: Rosiglitazone restores G-protein coupling, recruitment, and function of renal dopamine D1 receptor in obese Zucker rats. Hypertension 2004, 43(2):376–382.

31. Ebenezer PJ, Mariapan N, Elks CM, Haque M, Francis J: Dien-induced renal changes in Zucker rats are ameliorated by the superoxide dismutase mimetic TEMPOL. Obesity (Silver Spring) 2009, 17(1):944–2002.

32. Hennissen EJ, Teachey MW, Lindborg KA, Diethl CJ, Bence AE: The high-fat-fed lean Zucker rat: a spontaneous isocaloric model of fat-induced insulin resistance associated with muscle GSK-3 overactivity. Am J Physiol Regul Integr Comp Physiol 2008, 294(6):R1813–R1821.

33. Marwaha A, Banday AA, Lokhandwala MF: Reduced renal dopamine D1 receptor function in streptozotocin-induced diabetic rats. Am J Physiol Renal Physiol 2004, 286(5):F451–F457.

34. Varu VN, Ahanchi SS, Hogg ME, Bhikhapurwala HA, Chen A, Popowich DA, Vavva AK, Martinez J, Liang Q, Sawedra JE, Hrabie JA, Kiefer KL, Kibbe MR: Insulin enhances the effect of nitric oxide at inhibiting neointimal hyperplasia in a rat model of type 1 diabetes. Am J Physiol Heart Circ Physiol 2010, 299(3):H772–H779.

35. Landsberg L, Aronne LJ, Beilin LJ, Burke V, Igel LI, Lloyd-Jones D: Insulin enhances the effect of nitric oxide at inhibiting neointimal hyperplasia in a rat model of type 1 diabetes. Am J Physiol Heart Circ Physiol 2010, 299(3):H772–H779.

36. Garrison RJ, Kannel WB, Stokes J 3rd, Castelli WP: Incidence and precursors of hypertension in young adults: the Framingham Offspring Study. Prev Med 1987, 16(2):235–251.

37. Sun P, Wu Y, He Q, He K: Fasting insulin concentrations and incidence of hypertension, stroke, and coronary heart disease: a meta-analysis of prospective cohort studies. Am J Clin Nutr 2003, 89(6):1543–1554.

38. Ruderman NB, Carling D, Prentki M, Caccione JM: AMPK, insulin resistance, and the metabolic syndrome. J Clin Invest 2013, 123(7):2764–2772.

39. Tiwari S, Razi S, Eblebarger CA: Insulin’s impact on renal sodium transport and blood pressure, insulin and diabetes. Am J Physiol Renal Physiol 2007, 293(4):F974–F984.

40. Wilcox CS: Effects of tempol and redox-cycling nitroxides in models of oxidative stress. Pharmacol Ther 2010, 126(1):119–145.

41. Kashyap SR, Roman LJ, Lamont J, Masters BS, Bajaj M, Saura-Durand S, Belfort R, Berra R, Kellogg DR, Jr, Liu Y, DeFrancesca RA: Insulin resistance is associated with impaired nitric oxide synthase activity in skeletal muscle of type 2 diabetic subjects. J Clin Endocrinol Metab 2005, 90(2):1100–1105.

42. Hsieh WA, Lyon CJ, Quinnies MJ: Insulin resistance and the endothelium. Am J Med 2004, 117(3):109–117.

43. de Arriarena Aleixandre A, Miguel Castro M: Experimental rat models to study the metabolic syndrome. Br J Nutr 2009, 102(9):1246–1253.

44. Umranı DN, Banday AA, Hussain T, Lokhandwala MF: Rosiglitazone treatment restores renal dopamine receptor function in obese Zucker rats. Hypertension 2002, 40(6):880–885.

45. Tiwari S, Halagiappu VR, Razi S, Hu X, Eblebarger CA: Reduced expression of insulin receptors in the kidneys of insulin-resistant rats. J Am Soc Nephrol 2007, 18(10):2661–2671.

46. Ladines CA, Zeng C, Asco LD, Sun X, Poccihari F, Semenaro C, Pisegna J, Wank S, Yamauchi I, Eisner GM, Jose PA: Impaired renal D1 (1)-like and D2 (2)-like dopamine receptor interaction in the spontaneously hypertensive rat. Am J Physiol Regul Integr Comp Physiol 2001, 281(4):R1071–R1078.

47. Nishi A, Eklöf AC, Bertorello AM, Aperia A: Dopamine regulation of renal Na+, K+–ATPase activity is lacking in Dahl salt-sensitive rats. Hypertension 1993, 21(6 Pt 1):767–771.

48. De Vries PA, de Zeeuw D, de Jong PE, Nissen G: The abnormal renal vasodilator response to D1-like receptor stimulation in conscious SHR can be normalized by AT1 blockade. J Cardiovasc Pharmacol 2004, 44(5):571–576.

49. Zeng C, Wang D, Asco LD, Welch WJ, Wilcox CS, Hopfer U, Eisner GM, Felder RA, Jose PA: Aberrant D1 and D2 dopamine receptor transregulation in hypertension. Hypertension 2004, 43(3):654–660.

50. Albrecht FE, Drago J, Felder RA, Printz MP, Eisner GM, Robillard JE, Sibley DR, Westphal HJ, Jose PA: Role of the D3 receptor in the pathogenesis of genetic hypertension. J Clin Invest 1996, 97(10):2283–2288.

51. Balakumar P, Kothuria S: Submaximal PPARy activation and endothelial dysfunction: new perspectives for the management of cardiovascular disorders. Br J Pharmacol 2012, 166(7):1981–1992.

52. Wu SQ, Hopfer RL, McNeill JR, Wilson TW, Gopalakrishnan V: Altered paracrine effect of endothelin in blood vessels of the hypertensive, insulin resistant obese Zucker rat. Cardiovasc Res 2000, 46(6):994–1000.

53. Walker AB, Chattington PD, Buckingham RE, Williams G: The thiazolidinedione rosiglitazone (BRL-49653) lowers blood pressure and protects against impairment of endothelial function in Zucker fatty rats. Diabetes 1999, 48(7):1448–1453.

54. Banday AA, Fazili FR, Lokhandwala MF: Insulin causes renal dopamine D1 receptor desensitization via GRC2-mediated receptor phosphorylation involving phosphatidylinositol 3-kinase and protein kinase C. Am J Physiol Renal Physiol 2007, 293(3):F877–F884.

55. Banday AA, Ashgar M, Hussain T, Lokhandwala MF: Dopamine-mediated inhibition of renal Na, K-ATPase is reduced by insulin. Hypertension 2003, 41(6):1353–1358.

56. Yoshizumi M, Tsuchiya K, Tomaki T: Signal transduction of reactive oxygen species and mitogen-activated protein kinases in cardiovascular disease. J Med Invest 2001, 48(11–12).

57. Nishi A, Eklöf AC, Bertorello AM, Aperia A: Impaired renal dopamine D1 receptor function in streptozotocin-induced diabetic rats. Diabetes 2005, 54(7):2119–2226.

58. Banday AA, Fazili FR, Marwaha A, Lokhandwala MF: Mitogen-activated protein kinase upregulation reduces renal D1 receptor affinity and G-protein coupling in obese rats. Kidney Int 2007, 71(5):397–406.

59. Savcheniuk OA, Virchenko ON, Falalyeyeva TM, Beregova TV, Babenko LP, Lazarenko UM, Demchenko OM, Bukhov RV, Spivak MV: The efficacy of probiotics for monosodium glutamate-induced obesity: dietology concern and opportunities for prevention. PEPMA J 2014, 5(12).

60. Lebagusmi NA, Lehmenn AM, Machado UF, Okamoto MM, Marksii MS, Pinto GH, Schaan BD: GLUT4 content decreases along with insulin resistance and high levels of inflammatory markers in rats with metabolic syndrome. Cardiovasc Diabetol 2012, 11:100.

61. Borcherding DC, Hugo ER, Edelman G, De Silva A, Richtand NW, Loftus J, Endemann DH: Antidiabetic treatment restores adiponectin serum levels and APPL1 expression, but does not improve adiponectin-induced vasodilation and endothelial dysfunction in Zucker diabetic fatty rats. Cardiovasc Diabetol 2013, 12:46.

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