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Research Article

Effects of Hyperinsulinemia on Blood Pressure in High-Fat Diet Fed Rats

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Abstract. *Objective.* To determine the mechanisms of hyperinsulinemia-induced elevation in blood pressure in rats. *Methods.* Male Sprague-Dawley rats were divided into normal diet (normal control) and high-fat diet group. After 36 weeks of feeding, high-fat diet group was further randomized into high-fat diet control group and streptozocin treatment group. Plasma insulin, endothelin-1(ET-1), norepinephrine (NE), aldosterone, and angiotensin II levels were measured. AT_1 receptor, ET-1, and ET_A receptor mRNA expression in the aorta was evaluated by real-time PCR. *Results.* After 9 months, systolic blood pressure (SBP) in high-fat diet group was higher than in the normal control group (155.4 ± 1.6 vs.132.1 ± 5.3 mmHg, $P < 0.01$). The levels of plasma insulin in high-fat diet group were higher than in normal control group (116.63 ± 12.31μIU/mL versus 29.10 ± 4.92μIU/mL, $P < 0.01$). High-fat diet group also exhibited higher plasma levels of ET-1, NE, aldosterone, and angiotensin II,and a lower 24 h urinary sodium excretion than the normal control group ($P < 0.05$). The expression of AT_1 receptor, ET-1 and ET_A receptors in the aorta in the high-fat diet group was greater than in the normal control group ($P < 0.05$). Streptozocin treatment reduced SBP by an average of 20.9 ± 3.3 mmHg ($P < 0.05$), and reduced insulin level from 110.63 ± 14.86 μIU/mL to 39.45 ± 6.59 μIU/mL ($P < 0.01$). The streptozocin group also showed a higher level of urine sodium excretion, and a lower level of plasma ET-1 and NE than in the high-fat diet control group ($P < 0.05$). *Conclusions.* Hyperinsulinemia following high-fat diet is associated with an elevation in blood pressure. Sodium retention, increased plasma endothelin-1 and noradrenaline, as well as activation of renin-angiotensin system may all contribute to the blood pressure elevation.
Keywords: hyperinsulinemia; hypertension; endothelin; sodium; angiotensin

1. Introduction

The association between hyperinsulinemia, insulin resistance, and essential hypertension has led to the theory that insulin may play a role in the pathogenesis of this disorder [1–3]. The pro-hypertensive action of insulin may be related to several mechanisms, such as an increase in sodium reabsorption in the renal tubules, stimulation of renin-angiotensin-aldosterone pathway, or the impairment of natriuretic pathways (e.g., the renal dopamine system). It is possible that insulin may increase the blood pressure of some subjects but not others. Data from several studies

have suggested that the activation of the sympathetic nervous system and sodium retention may play roles in insulin-induced increases in blood pressure. These two mechanisms have also been reported to be involved in salt-overload hypertension which suggests that hyperinsulinemia may affect salt sensitivity [4–8].

Various studies have reported on the mechanism of hypertension in diabetic patients with an emphasis on hyperinsulinemia and insulin resistance [4, 9–12]. Although hyperinsulinemia and insulin resistance may affect blood pressure via sodium retention or the activation of the sympathetic nervous system, the independent contribution of hyperinsulinemia or insulin resistance in the elevation of blood pressure has not been established [4, 6, 9–12]. The purpose of the present study was to determine the role of hyperinsulinemia and insulin resistance in the development of hypertension.

2. Materials and Methods

2.1. Animal Models. 36 male Sprague-Dawley (SD) rats (12 weeks of age, weight 280–320 g) were fed a normal (66.5% carbohydrate, 10.2% fat, 23.3% protein) or high-fat diet (66.5% fat, 10.2% carbohydrate, 23.3% protein). All animals had free access to their diet and drinking water. Housing was at a constant temperature of 21 ± 1°C in a 12 h light/dark cycle. All animal care and experimental procedures were in accordance with the guidelines of the Animal Care Committee of the China-Japan Friendship Hospital of China.

2.2. Experimental Design. 36 Male SD rats were divided into two groups: the normal diet group ($n = 7$) and the high-fat diet fed group ($n = 29$). After 36 weeks of feeding, high-fat diet rats were further randomized into control group named as metabolic syndrome group (MS group, $n = 6$) and streptozocin group (STZ group, $n = 23$) injected with streptozocin 15 mg/kg for 3 weeks by intraperitioneal injection to induce to DM and eliminate hyperinsulinemia as well).In the process of modeling 5 rats died. We devided the STZ group into HI group (use insulin glargine 2–4 U/every day hypodermic injection for 3 weeks, $n = 10$) and DS group (the same amount of normal saline hypodermic injection for 3 weeks, $n = 8$). To research the relationship between hyperinsulinmia/insulin resistance and hypertension.

2.3. Biochemical Measurements. Plasma insulin, endothelin-1 (ET-1), norepinephrine(NE), aldosterone and angiotensin II levels were measured using radioimmunoassay method. 24 h urinary sodium was also measured.

2.4. Hyperinsulinemic-Euglycemic Clamp. The hyperinsulinemic-euglycemic clamp technique was performed as previously described [13]. In brief, experiments were performed in rats fasted for 8–10 h with free access to drinking water. The hyperinsulinemic clamp (12 mU/kg/min of human insulin. Novo Nordisk A/S, Bagsværd, Denmark) was performed constantly. The glucose concentrations were clamped at euglycemic levels (5 mmol/l) by infusion of 20% glucose, and the plasma glucose concentration was determined with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA, USA). The glucose infusion rate (GIR) was used to evaluate insulin sensitivity.

2.5. Blood Pressure Measurement. The onset and development of hypertension were assessed with the tail-cuff method with a Narco Biosystems Electro-Sphygnomanometer after the rats were warmed at 35°C for 5 minutes, while under slight restraint. Blood pressure was measured under conscious conditions at the beginning of the experiment, and every two weeks of during the experiment. The average of 5 pressure readings was recorded for each measurement.

2.6. Isolation of Total RNA and Real Time-PCR. Total RNA was isolated from the endothelial cells using TRIzol reagent (Gibco BRL, Invitrogen Corporation, Carlsbad, CA, USA). cDNA synthesis was performed using a ReverTraAce-α^{TM} Kit (Toyobo Co. Ltd., Osaka, Japan) according to the manufacturer's instructions in a total volume of 20 μL, under conditions of 42°C for 20 min, 99°C for 5 min, and 4°C for 5 min. Quantitative real time-PCR amplification was performed using SYBR Green PCR Master Mix (Toyobo Co. Ltd.) in a total volume of 50 μL according to the manufacturer's instructions. The reaction conditions were 40 cycles of 95°C for 1 min, 95°C for 15 s, 55–60°C for 15s, and 72°C for 45 s, and the amplification was carried out using an ABI 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The sequences of the primers used were previously described [14]. The primers were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. (Shanghai, China), as Table 1.

2.7. Statistical Analysis. All values are presented as mean ± SD. Statistical significance of differences among groups was evaluated by one-way ANOVA. All tests were performed using the SigmaStat statistical program (SPSS, Chicago, IL, USA). Significance was determined by P values < 0.05.

3. Results

3.1. Plasma Insulin, Endothelin-1 (ET-1), Norepinephrine (NE), Aldosterone, Angiotensin II. As shown in Table 2 2.6, the levels of plasma insulin in high-fat diet group were higher than normal control group ($P < 0.01$). After injection of streptozocin, there was a significant reduction in insulin levels (110.63 ± 14.86 μ IU/mL versus 39.45 ± 6.59 μIU/mL, $P < 0.01$).

Table 1

GAPDH	The sense primer: 5′-CATGCCAACGCCCTCTTCGA-3′ The antisense primer: 5′-TGTCCCCGTTCTCATCCTGCAC-3′
AT_1R	The sense primer: 5′-TATCACAGTGTGCGCGTTTCA-3′ The antisense primer: 5′-TGGTAAGGCCCAGCCCTAT-3′
ET-1	The sense primer: 5′-TCCCGTGATCTTCTCTCTGC-3′ The antisense primer: 5′-GGCTCTGTAGTCAATGTGCT-3′
ET_AR	the sense primer: 5′-CGCCACTCTCCTAAGAATC-3′ The antisense primer: 5′-ATCCAAAGAGCCACCAGTC-3′

Table 2: Plasma angiotensin II (AngII), aldosterone (ALD), noradrenaline (NE) and endothelin-1 (ET-1) and levels.

Group	AngII (pg/mL)	ALD (pg/mL)	NE (pg/mL)	ET-1(pmol/l)
Control	89.2 ± 12.2	958.9±49.2	104.5±19.7	1.56±0.07
HFDgroup	200.5±23.1*	1348.6±42.4*	318.4±61.4*	2.28±0.13*
Compared with ND group, *$P < 0.05$.				

Arterial plasma ET-1, NE, aldosterone, and angiotensin II were measured after 36 weeks of observation (Table 2 2.6). Plasma ET-1, NE, aldosterone and angiotensin II levels in the high-fat diet group were higher than in the normal control group ($P < 0.05$).

ET-1 and NE levels in the streptozocin group were lower than in the normal control group (ET-1: 1.59 ± 0.08 pmol/L versus 2.36 ± 0.14 pmol/L, $P < 0.05$; NE: 122.2 ± 27.1 pg/mL versus 326.8 ± 66.4 pg/mL, $P < 0.05$). There was no significant difference between angiotensin II and aldosterone levels in the two groups.

After injection insulin for 3 weeks, ET-1 and NE levels in HI group are higher than in DS group, but there is no difference in ALD and AngII between HI group and DS group.($P > 0.05$) (Table 2 2.6).

3.2. Hyperinsulinemic-Euglycemic Clamp. After 9 months of high fat diet feeding, the glucose infusion rate (GIR) in the high-fat diet group was lower than in the normal control group (12.67 ± 1.2 mg/kg/min versus 7.18 ± 0.7 mg/kg/min ($P < 0.05$). There was no significant difference in glucose infusion rate between the normal control group and streptozocin group (7.18 ± 0.7 mg/kg/min versus 7.41 ± 0.9 mg/kg/min, $P > 0.05$). After injecting insulin for 3 weeks, there is no difference in GIR between HI group and DS group. ($P > 0.05$) (Table 2 2.6).

3.3. Fasting Insulin. At the end of 24weeks, the fasting insulin in MS group is three times higher than in the normal control group (119.63 ± 12.31 μIU/mL versus 29.50 ± 4.92 μIU/mL, $P < 0.01$). There is significant difference between DM group and before injection streptozocin (32.45 ± 6.59 μIU/mL versus 119.63 ± 14.86 μIU/mL, $P < 0.01$). The fasting insulin is obviously higher in HI group than in DS group (120.20 ± 20.57 μIU/mL versus 38.86 ± 7.82 μIU/mL, $P < 0.01$).

3.4. Blood Pressure and Heart Rate. At the end of 9 month study, the systolic blood pressure (SBP) in the high-fat diet group was higher than in the normal control group (155.4 ± 1.6 versus 132.1 ± 5.3 mmHg, $P < 0.01$). After injection of streptozocin, there was a significant reduction in SBP compare with MS group (20.9 ± 3.3 mmHg, $P < 0.05$). But there is no difference in heart rate between two groups (DM group*versus* 369.3 ± 20.1 bpm, 376.2 ± 12.5 bpm, $P > 0.05$). SBP in HI group is higher than in DS group. Heart rate is also higher in HI group. Heart rate is also elevated in HI group (Table 2 2.6).

3.5. 24 h Urinary Sodium. The high-fat diet group rats exhibited a lower 24 h urinary sodium than normal control group (0.29 ± 0.03 mmol/24 h versus 0.74 ± 0.08 mmol/24 h, $P < 0.05$). Streptozocin group exhibited higher 24 h urinary sodium than MS group (0.57±0.09 mmol/24 h versus 0.22 ± 0.04 mmol/24 h, $P < 0.05$). 24 h urinary sodium in HI group is lower than DS group ($P < 0.05$) (Table 2 2.6).

3.6. AT_1 Receptor, ET-1, ET_A Receptor mRNA Expression. AT_1 receptor, ET-1, ET_A receptor mRNA expressions in the aorta in the high-fat diet group were greater than in the control group ($P < 0.05$). Streptozocin administration decreased ET-1, ET_A receptor mRNA expression in STZ group was lower than in the high-fat diet group, but there was no significant difference in AT_1 receptor expression in the two groups ($P > 0.05$).

After 3 weeks' administration of insulin glargine, ET-1, ETA receptor mRNA expressions in HI group is higher than DS group ($P < 0.05$). AT1 mRNA is similar in two groups ($P > 0.05$) (Table 2 2.6).

Table 3: Statistics between HI group and DS group.

	HI (n = 10)	DS (n = 8)
FINS(μIU/mL)	120.20±20.57 **	38.86±7.82
SBP (mmHg)	138.5±4.0*	156.0±2.4
HR (bpm)	411.9±12.5*	360.6±10.5
AngII (pg/mL)	182.6.2±29.8	189.7±22.4
ALD (pg/mL)	1280.9±68.9	1366.6±55.5
NE (pg/mL)	232.3±52.8*	115.6±21.7
ET-1(pg/mL)	2.23±0.19*	1.58±0.09
GIR (mg/kg/min)	7.03±1.0	6.93±1.0
Urinary sodium (mmol/24 h)	0.33±0.05*	0.62±0.07
FINS: Fasting insulin; SBP: systolic blood pressure; HR: heart rate; AngII: angiotensin II; ALD: aldosterone; NE: noradrenaline; ET-1: endothelin-1; GIR: glucose infusion rate.		

4. Discussion

The present study demonstrated that hyperinsulinemia was associated with an increase in systolic arterial pressure, suggesting a role of insulin in diabetes-associated hypertension.

Many investigators have speculated that hyperinsulinemia contributes to the elevated blood pressure [15–20]. Insulin might elevate blood pressure via renal, neural, and/or secondary hormonal mechanisms [6, 21, 22]. In rats, hyperinsulinemia in the absence of hypoglycemia caused an increase in blood pressure that is associated with a shift in renal pressure natriuresis curve [23]. Chronic insulin administration elevates blood pressure in rats [24]. The mechanisms by which insulin increases blood pressure are not entirely clear. Insulin decreases urinary sodium excretion, and the withdrawal of insulin in humans with diabetes results in diuresis and natriuresis, whereas readministration of insulin causes sodium retention, suggesting a possible role for insulin in promoting sodium reabsorption by the kidneys [25, 26]. This is further supported by the finding that in mammalian proximal tubules, more than 60% of sodium absorption is mediated by Na-K-ATPase and Na/H exchanger, and insulin is known to stimulate both of these transporters [27]. In the present study, the effect of insulin on sodium retention was investigated. We found that in high-fat diet rats where there is a lower level of insulin resistance, there was a reduced level of sodium excretion in the urine, and higher level of systolic blood pressure. In streptozocin treated rats, there was a higher level of 24 h urinary sodium excretion, and a lower level of systolic blood pressure. These results suggest that hyperinsulinemia may elevate blood pressure through sodium retention.

ET-1 may play a role in the insulin-induced increase in blood pressure. The role of ET-1 in hypertension and vascular diseases was well observed by Iglarz and Schiffrin [28, 29]. Several lines of evidence suggest that insulin may increase ET-1 release both *in vitro* [30] and *in vivo* [31]. It is possible that hyperinsulinemia may continuously stimulate ET-1 release, which may cause an increase in blood pressure. Long-term glucose administration and hyperinsulinemia combined with cyclooxygenase-2 inhibition can significantly increase mean arterial blood pressure in dogs [32]. In human studies, subjects with syndrome X showed higher ET-1levels and hypertension than normal subjects and subjects with insulinoma [33]. In the present study, Plasma ET-1, NE, aldosterone and angiotensin II levels in the high-fat diet group were higher than in the normal control group, whereas, ET-1 and NE levels in the streptozocin group were lower than in the normal control group. These results suggest ET-1, NE and activation of renin-angiotensin system co-contributed to the elevation of blood pressure following high-fat diet.

In addition, expression of AT_1, ET-1 and ET_A receptor mRNA in the aorta in the high-fat diet group was greater than in the control group. STZ administration decreased ET-1, ET_A receptor mRNA expression. These results indicate that high-fat diet was also associated with an increased vascular response to ET-1 stimulation to the blood vessels through increase in the endothelial receptors.

In summary, the present study demonstrates that the hyperinsulinemia following high-fat diet leads to blood pressure elevation. The blood pressure elevation may be due to sodium retention, increase plasma level of endothelin-1 and noradrenaline.

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