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Full-length article

Altered ATP-sensitive potassium channels may underscore obesity-triggered increase in blood pressure[1]

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Key words

ATP-sensitive potassium channel; obesity-hypertension; electrophysiology; aorta; mesenteric artery

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Abstract

Aim: To determine whether ATP-sensitive potassium channels are altered in VSMC from arotas and mesenteric arteries of obese rat, and their association with obesity-triggered increase in blood pressure. **Methods:** Obesity was induced by 24 weeks of high-fat diet feeding in male Sprague–Dawley rats. Control rats were fed with standard laboratory rat chow. Blood pressure and body weight of these rats were measured every 4 weeks. At the end of 24 weeks, K_{ATP} channel-mediated relaxation responses in the aortas and mesenteric arteries, K_{ATP} channel current, and gene expression were examined, respectively. **Results:** Blood pressure and body weight were increased in rats fed with high-fat diet. K_{ATP} channel-mediated relaxation responses, currents, and K_{ATP} expression in VSMC of both aortas and mesenteric arteries were inhibited in these rats. **Conclusion:** Altered ATP-sensitive potassium channels in obese rats may underscore obesity-triggered increase in blood pressure.

Introduction

Obesity is recognized as a significant contributor to persistently elevated arterial blood pressure. Risk estimates indicate that at least two-thirds of the prevalence of hypertension can be directly attributed to obesity[1]. From intervention studies, it is estimated that a weight loss of 1 kg produces a 1 mmHg reduction in systolic blood pressure and a 0.5 mmHg reduction in diastolic blood pressure[2]. However, the mechanisms that underlie these observations are not understood fully. Increased sympathetic nerve activity, Na^+ and volume retention, insulin resistance and hyperinsulinemia, renal abnormalities, and more recently, abnormal adipokines secreted by adipocytes, have been implicated in obesity-related hypertension[3].

Altered ion channels in membranes are an important pathomechanism of hypertension. ATP-sensitive potassium (K_{ATP}) channels in vascular smooth muscle cells (VSMC) contribute to the maintenance of resting membrane potential and local blood flow, which is critical for the regulation of blood pressure[4]. The removal of the resting vasodilator contribution of the inward rectifier Kir6.1 in transgenic mice leads to hypertension[5]. It was found that inflammatory mediators altered K_{ATP} channel function and expression in colonic smooth muscle cells[6]. Adipose tissue is recognized as a critical endocrine organ and secretes inflammatory mediators[7]. In addition, a recent report found that periadventitial fat releases vasoactive factors which may regulate arterial relaxation through ion channel[8]. Adipose tissue is altered by obesity, so it is reasonable to postulate that obesity may have an effect on K_{ATP} channels, which may contribute to hypertension in obese individuals. However, to our knowledge, no direct study has yet been conducted on obese animals to address whether high-fat, diet-induced obesity relates to K_{ATP} channel function and expression in the vascular system. The objective of the present study was to directly examine the effect of high-fat, diet-induced obesity on blood pressure and K_{ATP} channel function and expression in VSMC in rats.

Materials and methods

Animals and materials The investigation was in compliance with the Guide for the Care and Use of Laboratory

Animals published by the US National Institutes of Health (NIH publication No 85-23, revised 1996). Male Sprague–Dawley rats (n=36, age: 5 weeks, weight: 140±5 g) were obtained from the Medical Laboratory Animal Center of Xi'an Jiaotong University School of Medicine (Xi'an, China) and maintained in standardized conditions. Eighteen rats (control group) were fed with standard laboratory rat chow, and 18 rats were fed a high-fat diet (40.12% fat including 2% cholesterol, 20.72% protein, and 39.16% carbohydrates) to induce obesity (obese group). Tail-cuff blood pressure measurements of the rats were recorded in the conscious state with a computer-driven data acquisition system (ABI, USA)[9]. The recorded blood pressure was the average of 3 readings. At the end of 24 weeks, all of the rats were killed after 12 h of fasting. The aorta, mesenteric artery, and other tissues were then rapidly separated and either used or immediately frozen in liquid nitrogen and then stored at –80 °C until analysis. In the endothelium-denuded experiments, the arterial endothelium was denuded by perfusion of the vessel for 10 s with 0.1% Triton X-100. Serum total cholesterol, triglyceride free fatty acids (FFA), and glucose concentrations were measured by CHODPAP, GPO-PAP, the colorimetric method, and glucose oxidase method, respectively.

Pinacidil-induced relaxation responses in aortas and mesenteric arteries The responses of the arterial segments to pinacidil were measured with a myograph system[10,11]. Briefly, the fresh vessels were then cut into 1 or 2 mm-long cylindrical segments. Then the segments were placed in temperature-controlled (37 °C) tissue baths containing Kreb's buffer solution, and were mounted on 2 L-shaped preparations, one of which was connected to a force displacement transducer for recording of the isometric tension. The solution was continuously gassed with 5% CO_2 in O_2. After stabilization for 1 h, the contractile capacity of each vessel segment was examined by exposure to a K^+-rich (60 mmol/L) buffer Kreb's solution in which NaCl was exchanged for an equimolar concentration of KCl. When 2 reproducible contractions had been achieved, the vessels were used for further experiments. At the end of this stabilization period, the rings were preconstricted with 1×10^{-6} mol/L phenylephrine (PE), and the responses to a range of concentrations of pinacidil (1×10^{-10}–1×10^{-3} mol/L) were determined. E_{max} represented the maximal relaxation induced by pinacidil (1×10^{-10}–1×10^{-3} mol/L) in preconstricted arterial rings. EC_{50} values represented the concentration of pinacidil that produced half the maximal relaxation. Concentration–response curves were plotted from the data.

Cell dissociation VSMC were dissociated from fresh endothelium-denuded segments according to methods modified from previous reports[12]. Briefly, the obtained segments were transferred to a low Ca^{2+} buffer solution that contained (in mmol/L) 127 NaCl, 5.9 KCl, 1.2 $MgCl_2$, 0.008 $CaCl_2$, 12 glucose, and 10 HEPES (pH 7.4/NaOH). The segments were cut into small pieces and then left to stand for 20 min at room temperature. After that, tissue pieces were digested with 4 mg/mL papain, 1.25 mg/mL bovine serum albumin, and 2 mg/mL dithiothreitol in 2 mL low Ca^{2+} dissolution solution at 37 °C for approximately 12–15 min. The digested tissue was washed with a low Ca^{2+} solution 3 times, and single smooth muscle cells were obtained by gentle agitation of the tissue pieces through a fire polished Pasteur pipette.

Patch clamp Whole-cell currents were recorded according to the whole-cell patch-clamp technique[13] in a 25 °C temperature-controlled bath. All recordings were performed using an Axopatch 200B patch-clamp amplifier (Axon Instruments, USA); and micropipettes with a resistance of 3–5 MΩ when filled with recording solutions. Membrane currents were filtered at 5 kHz, digitized using a Digidata 1320A interface (Axon Instruments, Foster City, CA, USA), and analyzed using pCLAMP software (Axon Instruments, Union City, CA, USA). The whole-cell current was generated by clamp pulses from a holding potential of 0 mV to voltages ranging from -60 to +60 mV in 10 mV steps. Isolated smooth muscle cells were placed in a recording chamber on the stage of an inverted microscope with an extracellular bath solution that contained (in mmol/L) 134 NaCl, 6 KCl, 1.2 $MgCl_2$, 0.1 $CaCl_2$, 10 HEPES, and 10 glucose (pH 7.4/NaOH). The cells were superfused with a 140 mmol/L high K^+ bath solution, made by substituting NaCl with KCl in the above solution, at 1–2 mL/min, and solution exchanges were complete within 30–60 s. Whole-cell K_{ATP} currents were recorded with a pipette solution that contained (in mmol/L) 140 KCl, 10 HEPES, 10 EGTA, 1.2 $MgCl_2$, 1 $CaCl_2$, 0.1 Na_2ATP, 1 Mg ADP, 0.1 Na_2GTP (pH 7.4/ KOH). In total, 100 nmol/L iberiotoxin was present in the bath solution to further block the BK_{Ca} channel activities. Seal resistance was >10 GΩ, and access resistance was below 10 MΩ. The whole-cell current could be augmented by the application of 10 μmol/L pinacidil (Sigma–Aldrich, St Louis, MO, USA) and inhibited in the presence of 10 μmol/L glibenclamide (Sigma–Aldrich, USA). The glibenclamide-sensitive current was interpreted as the K_{ATP} current. The recorded current was normalized by cell capacitance to obtain the whole-cell current density.

RT–PCR The Kir6.1 is inwardly rectifying subunit of the major K_{ATP} channels in VSMC. Total RNA

was respectively extracted from approximately 3–5 mg endothelium-denuded aortic or mesenteric arterial specimens by using an RNeasy fibrous tissue mini kit (QIAGEN GmbH, Hilden, Germany). RT–PCR were performed and optimized, according to standard protocols (TaKaRA RNA RCP kit, TaKaRa, Dalian). The PCR primers for Kir6.1 were: sense, 5'-CCGCAGCAGC GACACTTT-3' and antisense, 5'-CAGACATGCAGGC CAACT-3', amplifying a 285 bp product. For the control PCR experiments for the housekeeping gene β-actin, the PCR primers were: sense, 5'-GAGGGAAATCGTGCGTGAC-3' and antisense, 5'-CTGGAAGGTGGACAGTGAG-3', amplifying a 445 bp product.

Western blot The protein expression of the K_{ATP} channels was tested by Western blotting, which was performed as described previously[14]. Briefly, the endothelium-denuded arterial specimens were homogenized in lysis buffer (including 50 mmol/L Tris). After centrifugation, the supernatant was collected for the immunoblotting analysis. A total of 10 μg denatured protein was fractionated by electrophoresis. The proteins were then transferred onto nitrocellulose membranes (Hybond-ECL, Amersham). The membranes were probed with polyclonal rabbit anti-Kir6.1 (1:300 dilution; Alomone Labs, Jerusalem, Israel), followed by the infrared, fluorescent-labeled secondary antibodies (antirabbit; Rockland Immunochemicals, USA). The blots were detected with the Odyssey infrared imaging system (LI-COR Biosciences, Inc), and β-actin was used as the loading control.

Statistics The results were expressed as mean±SEM. Using SPSS 13.0 software (SPSS, Chicago, IL, USA), the independent *t*-test was performed for testing the differences between the 2 groups, and paired-samples *t*-test was used in 1 group. Differences were considered statistically significant at $P<0.05$.

Results

Blood pressure and body weight At the end of 24 weeks of high-fat diet treatment, the rats had higher MAP and more weight than the control rats ($P<0.05$, Figure 1A, 1B). The serum cholesterol, triglyceride FFA, and glucose concentrations (Table 1) increased in the obese group compared with the control rats ($P<0.05$).

Pinacidil-induced relaxation In aortic rings with endothelium, pinacidil induced the concentration-dependent relaxation in the 2 rat groups. However, the responsiveness was reduced in the obese group (Figure 2A). The EC_{50} to pinacidil was higher, and E_{max} was lower in the obese rats compared to the control rats ($P<0.01$). Glibenclamide (1×10^{-5} mol/L) abolished the pinacidil-induced relaxation on the arteries. As shown in Figure 2A and Table 2, similar results were observed in endothelium-denuded aortic rings. There was little difference between the EC_{50} values or E_{max} values with endothelium-intact or endothelium-denuded preparations.

The pinacidil-induced relaxation on mesenteric arterial rings decreased in the obese rats. The EC_{50} values were higher, and the E_{max} values were lower in obese rats than those of the control rats ($P<0.01$). Glibenclamide also abolished the pinacidil-induced relaxation on mesenteric arterial rings. In endothelium-denuded mesenteric arterial

Table 1. Plasma assay of rats fed a high-fat diet or standard rat chow. n=15–18 each group. Mean±SEM. $^bP<0.05$ *vs* control group.

Group	Serum triglycerides (mmol/L)	Serum total cholesterol (mmol/L)	FFA (μmol/L)	Glucose (mmol/L)
Control	1.1±0.09	1.7±0.15	650.4±50.7	5.82±0.32
Obese	1.9±0.22^b	2.5±40^b	948.4±81.5^b	7.01±0.4

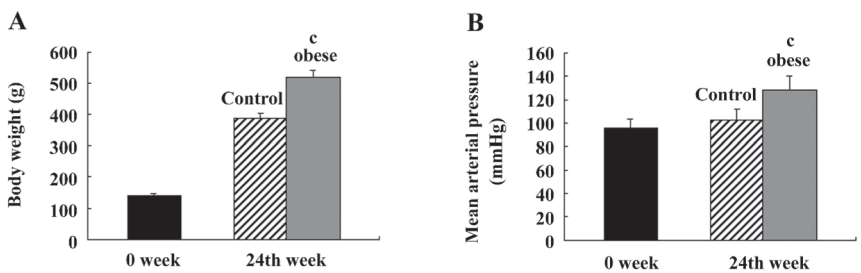

Figure 1. General feature of rats fed with high-fat diet or standard chow. (A) body weight; (B) Mean arterial pressure. n=18 each group. Mean±SEM. $^cP<0.01$ *vs* control group.

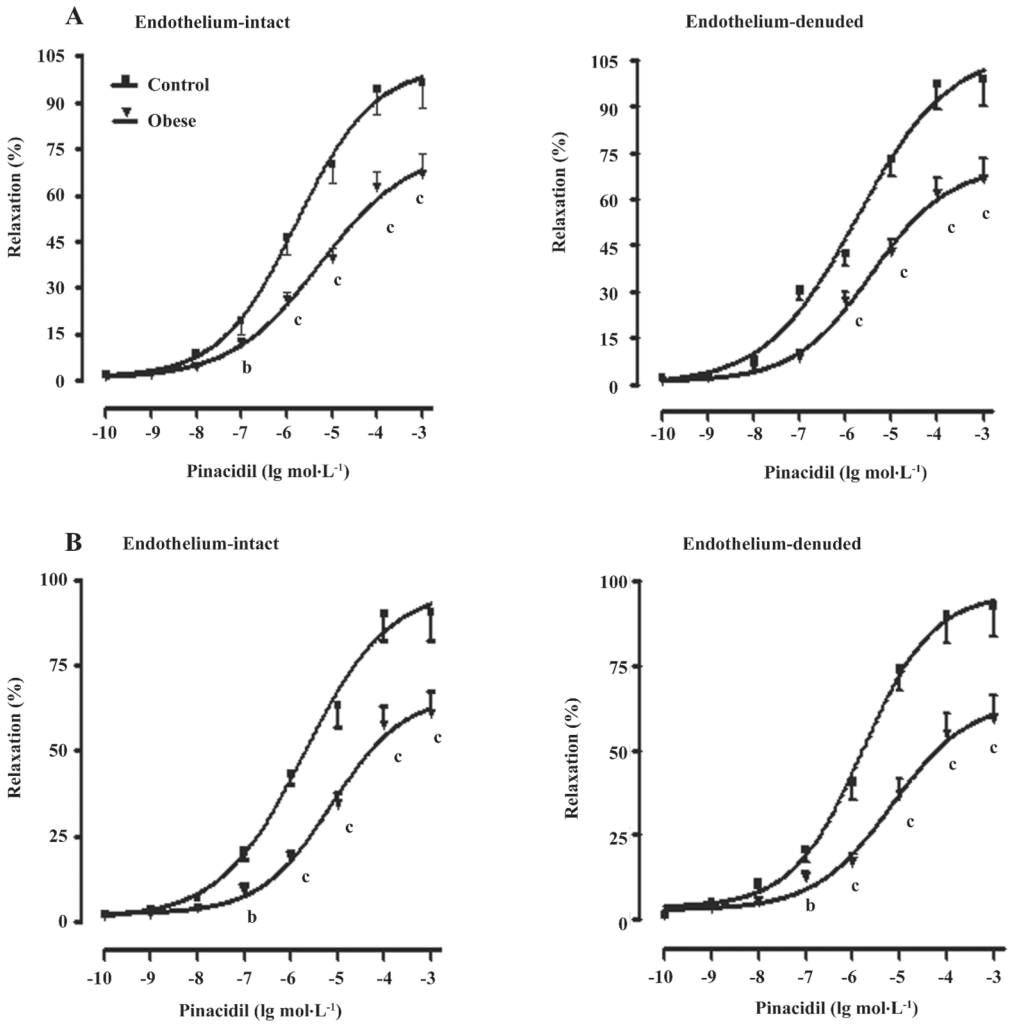

Figure 2. Effect of obesity on response curves for pinacidil on aortic (A) and mesentery arterial rings (B) of rats. Arterial segments were preconstricted with 1×10^{-6} mol/L PE. n=8–10 each group. Values are mean±SEM and expressed as a percentage of the preconstruction. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control group.

rings, the results were similar (Figure 2B), and the EC_{50} values or E_{max} values showed little difference from those in the endothelium-intact rings (Table 2).

***I_{KATP}* channel current** The current density (pA/pF) of I_{KATP} increased with command potential (Figure 3C). Under a high K^+ solution, steady currents were obtained, and the K_{ATP} channel currents were –5.75±0.68 pA/pF (n=15) and –2.43±0.25 pA/pF (n=15; Figure 3D) at –60 mV in the cells from the control and obese groups, respectively (P<0.01 between the 2 groups). They were further augmented by perfusion with 10 μmol/L pinacidil in this symmetrical K^+ gradient and were inhibited by 10 μmol/L glibenclamide (Figure 3A). The K_{ATP} channel currents in the presence of 10 μmol/L pinacidil were –20.45±2.81 pA/pF and –10.92±1.57 pA/pF, respectively (P<0.01 between 2 two groups; Figure 3B, 3D). Five of 15 aortic smooth muscle cells in the obese group did not show a statistically increased current in the presence of pinacidil.

A similar procedure was applied to smooth muscle cells from the rat mesenteric arteries of the 2 groups for the assessment of I_{KATP}. Comparable results were obtained (Figure 3). The K_{ATP} channel currents recorded at –60 mV in a high K^+ solution were –4.64±0.59 pA/pF (n=16) and –2.01±0.22 pA/pF (n=14) in the cells of the control and obese groups, respectively. The current amplitude of the control was significantly higher than that of the obese group (P<0.01). The pinacidil-evoked K_{ATP} currents were –17.65±2.11 pA/pF and –9.58±1.22 pA/pF in the 2 groups,

Table 2. Effect of a high-fat diet on pinacidil-induced responses in PE-preconstricted arteries. n=8–10. Mean±SEM. ^bP<0.05 *vs* control group.

	Aorta		Mesenteric artery	
	Control	Obese	Control	Obese
Endothelium intact				
EC_{50} (μmol/L)	1.58±0.25	3.51±0.25^b	1.85±0.26	7.8±0.4^b
E_{max} (%)	99.8±8.6	65.5±6.7^b	92.8±8.2	63.5±6.1^b
Endothelium denuded				
EC_{50} (μmol/L)	1.69±0.30	4.28±0.36^b	1.89±0.28	8.2±0.56^b
E_{max} (%)	98.5±8.3	67.6±6.4^b	90.5±7.8	61.7±6.2^b

EC_{50} values are increased in obese rats. P indicates there is significant difference of E_{max} between the groups (P<0.05), but not between endothelium-intact and endothelium-denuded arterial rings.

respectively (P<0.01 between the 2 groups; Figure 3D). Consistent with our observation from the aortic cells, 4 of 12 mesenteric smooth muscle cells from the obese group did not display further statistical increase in current after the addition of pinacidil, which was similar to deoxycorticosterone acetate–salt hypertensive rats[15]. The data suggested that K_{ATP} channel activity in VSMC was inhibited by a high-fat diet.

mRNA expression of K_{ATP} channel A predicted DNA product for Kir6.1 was observed in all of the studied samples. As determined by semiquantitative RT–PCR, the Kir6.1 transcript in the aorta was decreased in obese rats (P<0.05). In the mesenteric artery, the results were similar to those of the aorta (Figure 4).

Expression of K_{ATP} channel protein To confirm the result of the gene expression determined by RT–PCR, Western blotting was performed. All protein abundances were normalized to those of the loading control, β-actin. The immunoblotting analysis showed that high-fat feeding decreased the Kir6.1 protein expression in both the aorta and mesenteric artery (P<0.05; Figure 5). Double bands (a band around 45 kDa and a band at 45–50 kDa), were observed in some control samples.

Discussion

Our current study demonstrated that obesity increased blood pressure, down-regulated K_{ATP} expression, and decreased K_{ATP} channel-mediated relaxation responses and currents in both aortas and mesenteric arteries from rats. High-fat diet could induce obesity and consequently increase hypertension in rats. The elevation of blood pressure observed in our study was noted in other high-fat diet studies[9,16].

The activity of K_{ATP} channels in VSMC was important for modulating blood pressure. The opening of arterial K_{ATP} channels causes membrane hyperpolarization, a decrease in Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels, and vasorelaxation[17,18]. Growth hormones may decrease blood pressure *via* increased K_{ATP} channel mRNA in VSMC[19]. These data clearly show that inhibited K_{ATP} channels increase blood pressure. In this study, we observed a consistent decrease in K_{ATP} channel function and expression in the aortas and mesenteric arteries of obese rats. The pinacidil-induced relaxation responses in arterial rings decreased in obese rats and were reversed by glibenclamide, a specific inhibitor of K_{ATP} channel activity, confirming that the pinacidil-induced responses were specific to its action on K_{ATP} channels. The removal of the endothelium did not affect the pinacidil-induced relaxation responses in the 2 groups, which supported the notion that the activation of K_{ATP} channels in VSMC may account for most of the modulation of vascular tone[20]. Electrophysiological results confirmed that K_{ATP} channel current had been decreased in obese rats. The reduced K_{ATP} channel mRNA level was also shown by results of RT–PCR.

Our results are not contradictory to some relevant findings in other cells. High extracellular glucose was found to down-regulate K_{ATP} channel expression in cultured pancreatic β cells[21] and neurons[22]. Recently, 3T3-L1 adipocytes were reported to down-regulate Kir6.2 expression in MIN6 insulin-secreting cells in a co-culture system[23]. The increased blood pressure in obese rats provided further evidence that the impaired K_{ATP} channels constitute a possible mechanism by which obesity increases blood pressure.

The precise mechanism of impaired K_{ATP} channels in obese rats is not clear and possibly associated with pathways as follows. Beside that the adipokines and high extracellular glucose, FFA could inhibit K_{ATP} channels by increasing cytosolic Ca^{2+}-independent PKC activity[24]. NO produced by endothelium, which may phosphorylate K_{ATP} channels *via* cGMP signal way[25], was inhibited by high-fat diet[26]. Beside that, some of those signal ways, such as PKC and adipokines, also affect other ion channels in membrane. For example, reactive oxygen species (ROS) were found to induce dysfunction of Ca^{2+}-activated (BK_{Ca}) and voltage-dependent (Kv) potassium channels in rat cerebral artery[27]. PKC also regulates Kv1.2[28] and BK_{Ca} channels in VSMC[29]. Furthermore, it was reported that oxidized LDL-(oxLDL) enhances L-type Ca^{2+} currents in

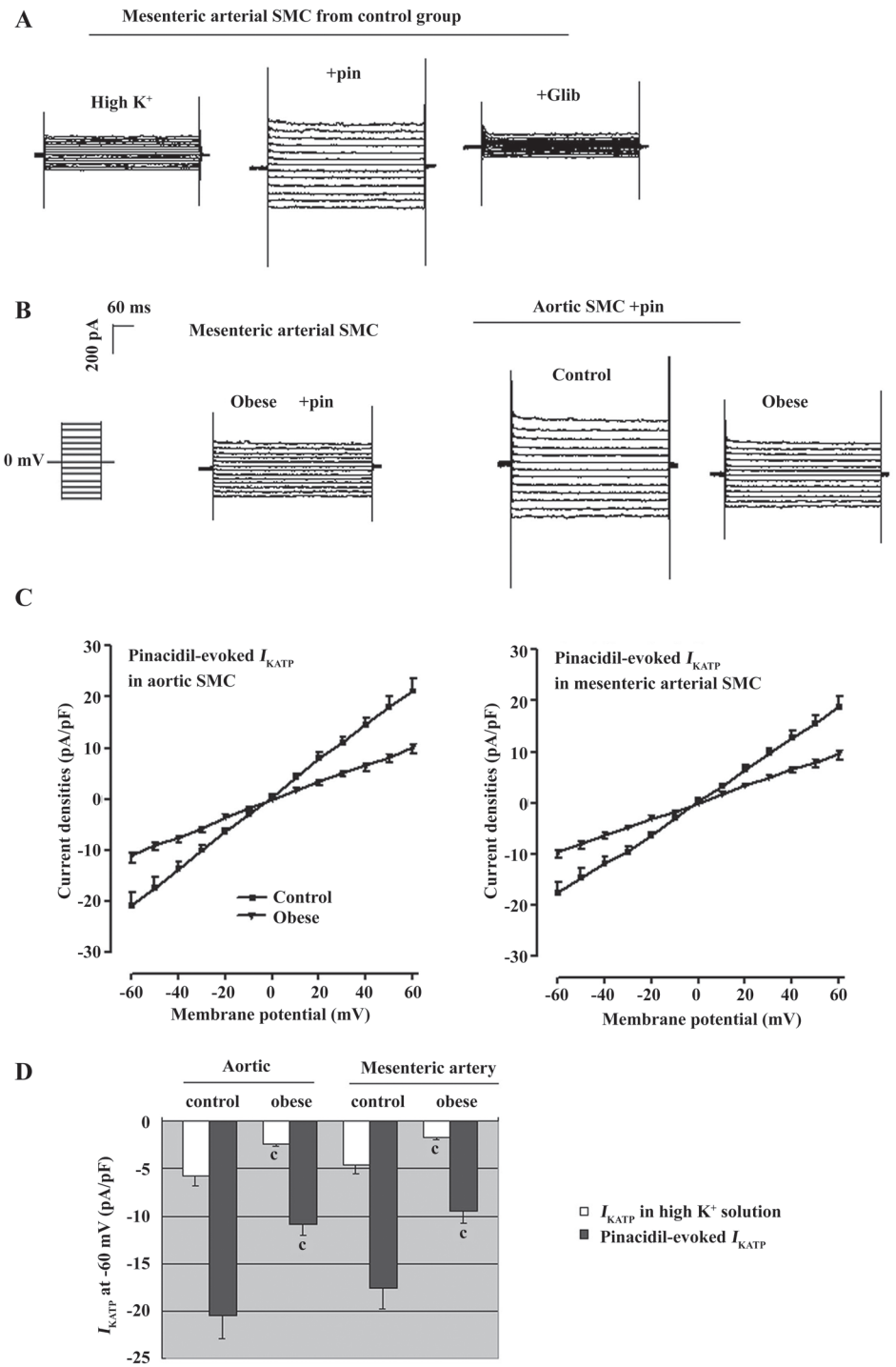

Figure 3. Effect of obesity on I_{KATP} in VSMC of aortas and mesenteric arteries of rats. Whole-cell currents were elicited in the presence of symmetrical 140 mmol/L K^+ from a holding potential of 0 mV to testing pulses of –60 to +60 mV in 10 mV increments. (A) recordings from a mesenteric arterial cell of the control group; current could be augmented by 10 μmol/L pinacidil (pin) and inhibited by 10 μmol/L glibenclamide (Glib); (B) whole-cell current traces from VSMC of control and obese rats in the presence of 10 μmol/L pinacidil; (C) *I–V* relationship of the pinacidil-evoked K_{ATP} channel currents (Glib sensitive); (D) comparison of peak K_{ATP} current densities (Glib sensitive) at –60 mV potential. n=14–16 tested cells from 7 tested animals. $^cP<0.01$ *vs* control group. *P* indicates that K_{ATP} channel currents decreased in VSMC of both aortas and mesenteric arteries of obese rats.

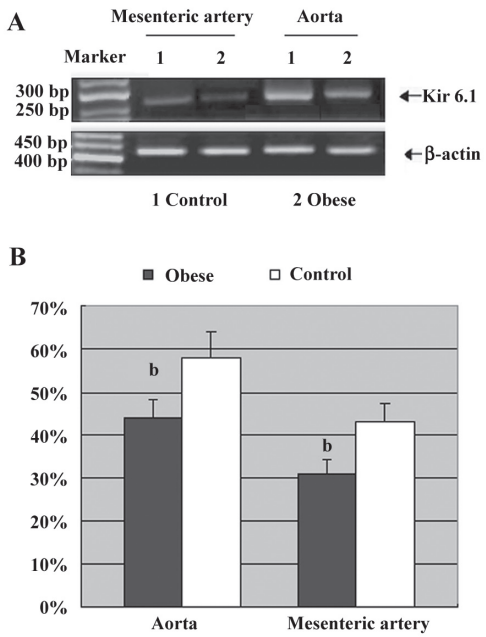

Figure 4. Effect of obesity on mRNA expression of K_{ATP} channels in aortic and mesenteric arterial smooth muscles of rats. (A) image of agarose gel; (B) densitometric analysis of Kir6.1 mRNA relative to β-actin. n=10–12 animals examined. Mean±SEM. $^bP<0.05$ *vs* control group.

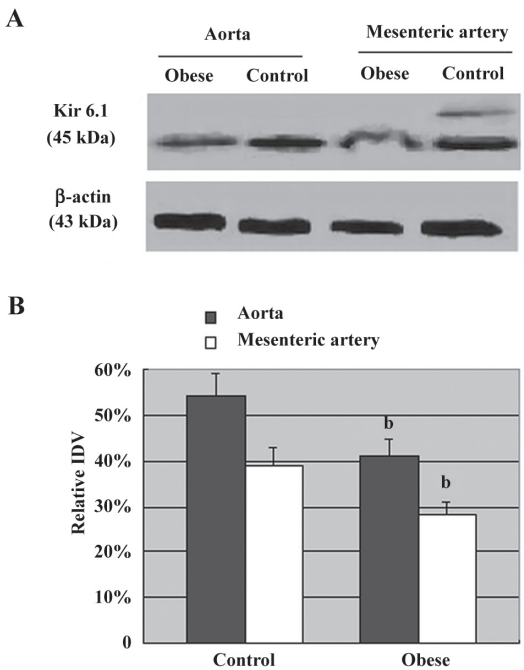

Figure 5. Effect of obesity on expression of K_{ATP} channel protein in aortic and mesenteric arterial smooth muscles of rats. (A) intensity of Kir6.1 subunit bands. (B) relative integrated density values (IDV) of Kir6.1 subunit expression to β-actin in the 2 groups. n=10–12 animals examined. Mean±SEM. $^bP<0.05$ *vs* control group.

rat ventricular myocytes[30]. So, high-fat diet may also have impacts on other ion channels in VSMC and those impacts may also contribute to hypertension in obese individual. Certainly, further investigations are needed to determine this presumption.

On the other hand, a recent report suggested high fat diet impaired mitochondrial function and biogenesis in myocardium[31]. K_{ATP} channel plays a key role in mitochondrial function. Combined our finding, it was implied that high fat diet-induced changes in K_{ATP} channel may play a role in the compromised mitochondrial biogenesis following high fat diet feeding. This hypothesis is interesting and should be further scrutinized by more studies.

In conclusion, K_{ATP} channels in both the rat aorta and mesenteric artery were altered in obese rats. Such alteration may underscore obesity-triggered increase in blood pressure.

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Author contribution

Ai-qun MA and Hong-yan TIAN designed research; Li-hong FAN performed research; Hong-yan TIAN, Zhi HU, Yong-xiao CAO, and Jian-hua HUO contributed analytic reagents and tools; Hong-yan TIAN and Li-hong FAN analyzed data; Li-hong FAN and Hong-yan TIAN wrote the paper.

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