# Superoxide Dismustase Mimetic Tempol Decreases Blood Pressure by Increasing Renal Medullary Blood Flow in Hyperinsulinemic-Hypertensive Rats

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**Insulin resistance and compensatory hyperinsulinemia often coexist in hypertensive patients, which may play a role in the development of hypertension. Because medullary blood flow (MBF), which is strongly influenced by the nitric oxide (NO) system, is thought to be an important component of blood pressure and sodium balance, we focused particularly on MBF in fructose-induced hypertensive rats. Moreover, it has been reported that the increased reactive oxygen species (ROS) in the kidney may contribute to the development of hypertension. Our study was thus designed to test the hypotheses that MBF is diminished in fructose-hypertensive rats (FFR) and that administration of tempol, a membrane-permeable mimetic of superoxide dismutase (SOD), decreases mean arterial pressure (MAP) by increasing MBF. Male Sprague-Dawley rats (180 to 200 g) were divided into 6 groups: control untreated (C, n = 5), control tempol-treated (in drinking water) (CT, n = 4), control L-arginine–treated (in drinking water) (CA, n = 6), fructose-fed untreated (F, n = 7), fructose-fed tempol-treated (FT, n = 7), and fructose-fed L-arginine–treated rats (in drinking water) (FA, n = 6). MAP and 24-hour urine samples were measured weekly over a 4-week test period. Changes in MBF, cortical blood flow (CBF), and renal blood flow (RBF) were determined by implanted optical fiber-, laser- and pulse-Doppler flow measurement techniques 4 weeks after starting the diet. Fructose feeding resulted in hyperinsulinemia, significantly elevated MAP, decreased MBF without changes in RBF or CBF, and decreased sodium excretion in the F group compared to the C group. Administration of tempol significantly decreased MAP and plasma insulin in contrast to increased MBF and sodium excretion in the FT group compared to those in the F group. Results indicated that MBF played an important role in the development of hypertension in the F group. Impairment of renal medullary NO systems may induce sustained elevation of blood pressure and retention of sodium in fructose-fed rats. The decrease in MAP with an increase of MBF in the FT group is consistent with the hypothesis that tempol increases the level of NO available to influence mechanisms involved in the control of MBF.**


INSULIN RESISTANCE and compensatory hyperinsulinemia often coexist in hypertensive patients, and the accumulation of these factors increases the risk of cardiovascular diseases.[1-3] Recently, insulin resistance and hyperinsulinemia have emerged as common causes of these diseases in the clinical field, and hyperinsulinemia may play a role in the development of hypertension.[4,5] Cowley pointed out that renal medullary circulation plays an important role in sodium balance and the development of hypertension.[6,7] Nakanishi et al previously demonstrated in a laser Doppler study that chronic nitric oxide (NO) blockades induced a sustained decrease in medullary blood flow (MBF) without a change in cortical blood flow (CBF), resulting in the retention of sodium and development of hypertension.[8] NO activity within the medulla is the primary characterized factor in the maintenance of MBF and plays an important role in the pressure-natriuresis response.[9-13] Because MBF, which is strongly influenced by the NO system, is thought to be an important component of blood pressure and sodium balance, we focused on MBF in fructose-hypertensive rats (FFR), hypertension being secondary in the development of insulin resistance.[14] In addition, the increased reactive oxygen species (ROS) in the kidney may contribute to the development of hypertension.[13,15-17] Specifically, ROS are elevated in FFR.[18,19] It was also found that tempol (4-hydroxy-2,2,6,6,-tetramethyl piperidinoxyl), being a chemical superoxide dismutase (SOD) mimetic, enhances vasodilator mechanisms of MBF, possibly by interacting with the NO system.[20] Taken together, we suggest that tempol, by scavenging superoxide in the renal medulla, increases MBF through a NO-mediated mechanism in FFR. This study was designed to test the hypothesis that if MBF is decreased in FFR, then tempol will decrease MAP by increasing MBF and sodium excretion.

## MATERIALS AND METHODS

Experiments were performed on 8-week-old male Sprague-Dawley rats (Charles River Inc, Yokohama, Japan) with body weights in the range of 180 to 200 g. The rats were housed in the Animal Resource Center at Toho University School of Medicine, and they were divided into 6 groups: control untreated (C, n = 5), control tempol-treated (1 mmol/L tempol mixed with drinking water) (CT, n = 4), control L-arginine–treated (1 mmol/L L-arginine mixed with drinking water) (CA, n = 6), fructose-fed (containing 60% fructose, 5% fat, and 20% protein; Oriental Yeast Co, Osaka, Japan) untreated (F, n = 7), fructose-fed tempol-treated (FT, n = 7), and fructose-fed L-arginine–treated (in drinking water) (FA, n = 6). At weekly intervals over a 4-week test period, weight was recorded and MAP measured in all conscious rats using the indirect tail-cuff method (BP-98A; Softron, Tokyo, Japan) on a 37°C preheated cloth jacket for about 10 minutes. The averages of 3 such recordings were taken as the individual mean arterial blood pressure (MAP) and individual heart rate. At weeks 0, 2, and 4, 24-hour urine collections were taken for each rat using metabolic cages for concentration of sodium. Rats were studied for 4 weeks. Plasma glucose and plasma insulin were measured at weeks 0 and 4.

At the end of week 4, all rats were anesthetized with inactin (100 mg/kg intraperitoneally) and placed on a heated surgical table to maintain a body temperature of 37°C. Cannulas were placed in the femoral arteries for measurement of arterial blood pressure and collection of blood and in the jugular vein for infusion of solution. Surgical

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**Table 1. Baseline Characteristics of the Six Groups**

| | C | CT | CA | F | FA | FT |
|---|---|---|---|---|---|---|
| Mean arterial pressure (mm Hg) | 98 ± 5.1 | 98.5 ± 2.68 | 102.7 ± 1.71 | 108.1 ± 7.5 | 104 ± 3.4 | 112.3 ± 7.18 |
| Plasma glucose (mg/dL) | 132 ± 11 | 130.7 ± 14.3 | 128.4 ± 14.6 | 125.1 ± 9.7 | 138 ± 11 | 132.7 ± 13.4 |
| Plasma insulin (μg/mL) | 6.4 ± 1.7 | 6.5 ± 2.08 | 6.5 ± 1.60 | 7.4 ± 3.3 | 5.7 ± 0.94 | 7.7 ± 2.3 |
| Urinary sodium excretion (mEq/d) | 0.93 ± 0.17 | 1.1 ± 0.08 | 0.88 ± 0.13 | 0.99 ± 0.16 | 0.91 ± 0.24 | 0.92 ± 0.09 |
| Urinary volume (mL/d) | 7.33 ± 2.16 | 10.0 ± 1.23 | 6.8 ± 1.92 | 8.04 ± 4.54 | 7.33 ± 2.58 | 6.15 ± 1.11 |
| Urinary sodium concentration (mEq/L) | 137 ± 51.3 | 111.4 ± 15.2 | 135 ± 27.3 | 149.7 ± 64.8 | 134.3 ± 48.0 | 152.1 ± 31.1 |
| Body weight (g) | 181.2 ± 5.74 | 170 ± 4.08 | 181 ± 3.60 | 169 ± 3.45 | 171.1 ± 5.74 | 165 ± 5.0 |

NOTE. Values are expressed as means ± SD.

Abbreviations: C, control; CT, control tempol-treated; CA, control L-arginine–treated; F, fructose-fed; FT, fructose-fed, tempol-treated; FA, fructose-fed, L-arginine–treated; FT, fructose-fed, tempol-treated.

fluid losses were replaced by continuous intravenous infusion of 2% bovine serum albumin (Sigma) in a 0.9% sodium chloride solution at 1 mL/h/100 g body weight through the experiment. The left kidney was exposed through a midline incision, isolated, and placed in a holder.

An ultrasonic transit-time flow probe was placed around the left renal artery for the measurement of renal blood flow (RBF). Signals were transmitted to a transit-time flow meter (PDV-20; Crystal Biotech, Northborough, MA). This device measures absolute RBF in milliliters per minute.

For the measurement of changes in CBF and MBF in rats, two 500-μm diameter optical fibers were implanted in the left kidney and exteriorized for blood flow measurement by laser-Doppler flowmetry. Two pieces of single-mode optical fiber were cut into 25-cm lengths, and one end of each fiber was gently heated and shaped into a 1-cm radius bend to allow for stabilization of the fibers when implanted into the dorsal pole of the left kidney. A small 1-cm diameter piece of latex was anchored to the fiber with epoxy adhesive and was used to anchor the implanted fiber to the surface of the kidney using a surgical adhesive. The fibers were then sheathed with polyethylene tubing for protection. The fibers were implanted in the renal cortex and medulla by inserting them directly into the kidney tissue through a small hole made in the renal capsule with a 26-gauge needle. The fiber tips were inserted 2 mm beneath the surface of the renal cortex to measure the net flux of red blood cells in the renal cortex and 5 mm deep to monitor changes in the outer medulla. After completion of the study, the kidneys were removed for morphologic examination and to determine precisely the tip placement of the optical fibers. Animals with incorrectly placed fibers or extensive renal damage were excluded from the study.

### *Statistical Analysis*

Values are given as the means ± SD. One-way repeated-measures analysis of variance (ANOVA) was performed for each group followed by Duncan's multiple-range test for significance. $P$ values less than .05 were considered to be significant.

## RESULTS

Table 1 shows the baseline characteristics of the 6 groups, which did not differ among the groups. Table 2 shows characteristics of the 6 groups after 4 weeks. The plasma insulin concentration was increased in the F group compared with C group (32.4 ± 19.9 *v* 7.42 ± 3.35 μU/mL, repectively, $P <$ .05) The rats in the F group exhibited hyperinsulinemia, whereas those in the C group did not (plasma insulin, 32.4 ± 19.9 *v* 6.2 ± 1.0 μU/mL, respectively, $P <$ .05). Treatment with tempol in the FT group prevented the development of hyperinsulinemia (10.6 ± 4.4 μU/mL, $P <$ .05) compared with the F group. The rats in the F group exhibited hypertension, whereas those in the C group did not (MAP, 127.9 ± 2.4 *v* 100.8 ± 4.9 mm Hg, respectively, $P <$ .01). The FT group showed a significant decrease in MAP (103 ± 3.3 mm Hg, $P <$ .01) compared with the F group. The mean weekly sodium intake was not significant different among the 6 groups. The rats in the F group showed a decrease in sodium excretion compared with the C group (0.45 ± 0.06 *v* 1.10 ± 0.21 mEq/d, respectively, $P <$ .01). The FT group showed a significant increase in sodium excretion (0.73 ± 0.08 mEq/d, $P <$ .01) compared with the F group. Figures 1 through 3 show MBF, RBF, and CBF, respectively. In the F group there was a decrease in MBF (0.42 ± 0.1*v* 0.66 ± 0.1 V in controls, $P <$ .05) without changes in RBF or CBF. Administration of tempol significantly increased MBF (0.58 ± 0.08 V, $P <$ .05) in the FT group compared to values in the F group.

**Table 2. Characteristics of the Six Groups After 4 Weeks**

| | C | CT | CA | F | FA | FT |
|---|---|---|---|---|---|---|
| Mean arterial pressure (mm Hg) | 100.8 ± 4.96 | 98.45 ± 8.42 | 97.8 ± 6.17 | 127.4 ± 4.15* | 105.1 ± 3.1 | 103.7 ± 3.35 |
| Plasma glucose (mg/dL) | 136 ± 9.9 | 138.7 ± 12.2 | 136.3 ± 9.9 | 136.8 ± 14.5 | 128.3 ± 14.3 | 140.5 ± 14.0 |
| Plasma insulin (μg/mL) | 6.4 ± 1.7 | 5.0 ± 0.81 | 6.16 ± 0.96 | 32.4 ± 19.9† | 10.4 ± 7.1§ | 10.6 ± 4.4§ |
| Urinary sodium excretion (mEq/d) | 0.93 ± 0.17 | 1.22 ± 0.04 | 1.1 ± 0.22 | 0.45 ± 0.06* | 0.71 ± 0.26‡ | 0.73 ± 0.08‡ |
| Urinary volume (mL/d) | 6.67 ± 1.86 | 8.4 ± 1.16 | 7.6 ± 1.51 | 8.21 ± 3.5 | 7.67 ± 1.63 | 6.52 ± 1.18 |
| Urinary sodium concentration (mEq/L) | 175 ± 54.3 | 148.5 ± 22.9 | 114 ± 12.1 | 63.5 ± 24.6 | 95.8 ± 39.6 | 115.2 ± 25.7 |
| Body weight (g) | 331.0 ± 8.16 | 311.2 ± 8.53 | 294.8 ± 7.19 | 298.5 ± 20.9 | 321.6 ± 8.16 | 301.4 ± 26.7 |

NOTE. Values are expressed as means ± SD.

*$P <$ .01 *v* C group, †$P <$ .05 *v* C group.

‡$P <$ .01 *v* F group, §$P <$ .05 *v* F group.

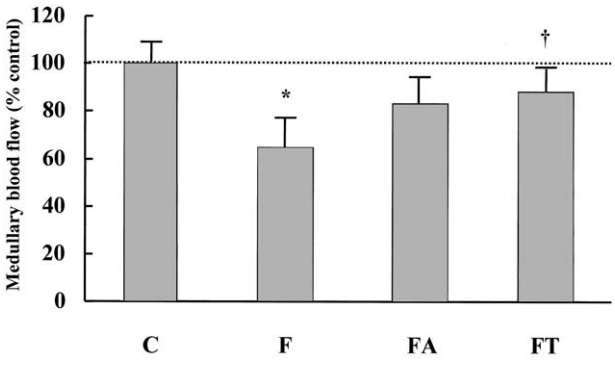


**Fig 1. Bar graph showing MBF in the control and fructose-fed rats treated with L-arginine and tempol for 4 weeks. Rats in the F group showed a decrease in MBF compared to the C group. Treatment of F rats with tempol significantly increased MBF. Values are means ± SD. *$P < .05$ *v* C group. †$P < .05$ *v* F group.**

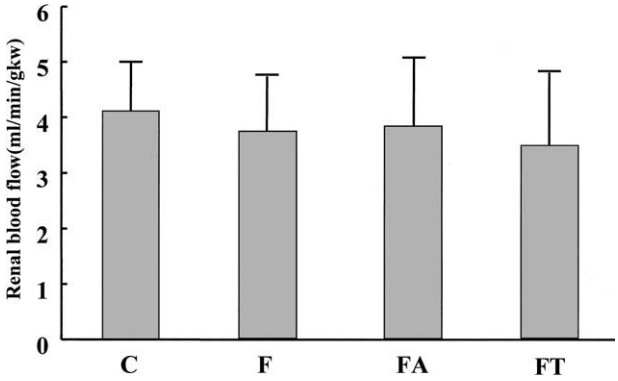


**Fig 3. Bar graph showing RBF in the control and fructose-fed rats treated with L-arginine and tempol for 4 weeks. Tempol and L-arginine did not cause any change in RBF. Values are means ± SD.**

## DISCUSSION

An interesting finding in the present study was that MBF and sodium excretion were significantly decreased without changes in RBF or CBF in the development of hypertension in FFR. Although to demonstrate pressure-natriuresis hypertension requires study of salt excretion at 2 levels of pressure, which was not performed in the present study, the hypertension in FFR could, at least in part, show impaired pressure-natriuresis hypertension with the decreased MBF. The administration of L-arginine also blunted the reduced MBF and the development of hypertension in FFR. It seems reasonable to suppose that an impaired NO system could contribute to reduced MBF and sodium excretion, which would result in the development of hypertension in FFR, although the antihypertensive effect of L-arginine and tempol could be the correction of the hyperinsulinemic state. A novel finding in the present study is that the tempol treatment in FFR significantly increased the MBF, which resulted in increased sodium excretion and decreased MAP. Although the actual mechanism of the effect of tempol was not determined in the present study, our observations suggest that SOD can prevent the development of hypertension in this model of hypertension with increased MBF. Recently, it was reported that ROS may participate in the control of vascular tone, and the interaction of superoxide and NO has been considered to be an important mechanism in the regulation of arterial blood pressure.[13,15-17,21] Shinozaki et al reported that the endothelial tetrahydrobiopterin (BH4) level was significantly reduced, and produced an imbalance of NO and superoxide in FFR. The mechanism by which BH4 decreases superoxide release from endothelial nitric oxide (eNOS) and the effect of uncoupling in oxidized BH4 metabolites may play novel roles in the development of hypertension in a state of insulin resistance.[22] Since superoxide scavenges NO to form perioxynitrite ($ONOO^-$), a short-lived and less potent vasorelaxant than NO, tempol could reduce superoxide and increase NO bioavailability. Insulin resistance can be a pathogenic factor for increased vascular resistance. Although we made no observation of the effects of BH4 or $ONOO^-$ in the present study, it seems reasonable to suppose that the imbalance of NO and superoxide may reduce MBF, which causes sodium retention and the development of hypertension. This observation is in agreement with a recent report that the renal outer medulla is a major region for superoxide production and hence tempol increased MBF and water/sodium excretion.[21] The medullary circulation may be strongly influenced by ROS. Taken together, we can say with fair certainty that increased oxidant stress such as the state of insulin resistance in the renal medulla may lead to a reduction of the MBF and sodium excretion and the development of hypertension. The increase in MBF induced by tempol may be at least in part the antihypertensive effect of insulin resistance. The effect of tempol could be stronger than than that of L-arginine in FFR.

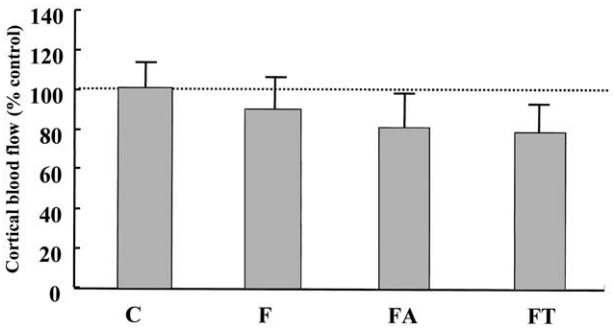


**Fig 2. Bar graph showing CBF in the control and fructose-fed rats treated with L-arginine and tempol for 4 weeks. Tempol and L-arginine did not cause any change in CBF. Values are means ± SD.**

More recently, Chen reported that SOD mimetic increased the formation of $H_2O_2$, which constricted the renal medullary vessels.[23] The interaction of $H_2O_2$ may be important in regulating MBF and sodium/water excretion under exaggerated oxidative stress. Makino et al also reported that the long-term renal medullary interstitial infusion of tempol alone failed to prevent the production and development of sustained hypertension induced by SOD inhibition.[24] Since tempol was administered orally, rather than directly into the renal medulla, and could not produce a relatively high concentration of $H_2O_2$ in the renal medulla in the present study (although we have no

definite information on $H_2O_2$ levels), tempol induced chronic increases in the MBF in FFR.

In summary, the present study demonstrated that FFR, characterized by insulin resistance and hypertension, exhibit impaired pressure-natriuresis and hypertension. MBF plays an important role in the development of hypertension in FFR. Furthermore, tempol treatment is able to prevent the development of hypertension with increased MBF. The increases in MBF induced by tempol may be due at least in part to the antihypertensive effect of insulin resistance.

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