

# Aldosterone Antagonism Attenuates Obesity-Induced Hypertension and Glomerular Hyperfiltration

Rogério B. de Paula, Alexandre A. da Silva, John E. Hall

**Abstract**—This study examined the importance of aldosterone (ALDO) in mediating changes in renal function and increased mean arterial pressure (MAP) during the development of dietary-induced obesity in chronically instrumented dogs. Mean arterial pressure, heart rate (HR), and cardiac output (CO) were recorded 24 hours per day in lean dogs ( $n=7$ ) before and after administration of an ALDO antagonist, eplerenone (EP) (10 mg/kg twice daily), for 10 days. After 10 days of EP treatment, the dogs ( $n=7$ ) were given a supplement of cooked beef fat for 5 weeks while EP was continued. An untreated group ( $n=6$ ) was fed a high fat diet for 5 weeks and used as control (C). In lean dogs, EP decreased MAP from  $89\pm 4$  to  $84\pm 4$  mm Hg and glomerular filtration rate from  $67.4\pm 6.8$  to  $53.2\pm 4.9$  mL/min while inducing a small negative  $\text{Na}^+$  balance ( $-42\pm 12$  mEq). Plasma renin activity increased from  $0.4\pm 0.1$  to  $2.7\pm 0.7$  ng AI/mL per hour and plasma  $\text{K}^+$  increased from  $4.8\pm 0.1$  to  $6.1\pm 0.3$  mEq/L. After 5 weeks of a high fat diet, body weight increased 45% to 53% in EP and C obese dogs. In C dogs, MAP increased by  $16\pm 3$  mm Hg, compared with only  $7\pm 1$  mm Hg in EP dogs. Compared with untreated dogs, the EP dogs had smaller increases in CO ( $18\pm 4.6\%$  versus  $43\pm 1.5\%$ ), HR ( $33\pm 5\%$  versus  $60\pm 3\%$ ), glomerular filtration rate ( $19\pm 5\%$  versus  $38\pm 6\%$ ), and cumulative  $\text{Na}^+$  balance ( $138\pm 35$  mEq versus  $472\pm 110$  mEq) after 5 weeks of a high fat diet. Thus, EP markedly attenuated glomerular hyperfiltration, sodium retention, and hypertension associated with chronic dietary-induced obesity. These observations indicate that ALDO plays an important role in the pathogenesis of obesity hypertension. (*Hypertension*. 2004; 43:41-47.)

**Key Words:** blood pressure ■ renin-angiotensin system ■ kidney ■ sodium ■ potassium ■ glomerular filtration rate ■ cardiac output ■ obesity

The importance of obesity as a cause of hypertension is widely recognized, with experimental studies showing that excess weight gain raises blood pressure, clinical studies demonstrating that weight loss lowers blood pressure in most hypertensive patients, and population studies showing that overweight and obesity are major risk factors for development of hypertension.<sup>1-5</sup> Also, most patients with hypertension are overweight, and evidence from epidemiological studies suggests that 65% to 75% of the risk for human essential hypertension can be directly attributed to excess weight.<sup>4,5</sup>

Although the importance of obesity as a cause of hypertension is well established, the mechanisms that link excessive weight gain with increased blood pressure are not as well understood. Previous studies suggest that obesity impairs renal-pressure natriuresis as the result of increased tubular sodium reabsorption.<sup>5-8</sup> These abnormalities of renal function may be due in part to activation of the renin-angiotensin-aldosterone system (RAAS).

Studies in experimental animals and humans have shown that obesity activates most components of the RAAS.<sup>9,10</sup> A significant role for angiotensin II (Ang II) in stimulating renal

sodium reabsorption and in contributing to obesity hypertension is supported by the finding that treatment of obese dogs and humans with an Ang II receptor antagonist or an ACE inhibitor attenuates sodium retention and volume expansion as well as increased arterial pressure.<sup>11,12</sup> However, the importance of aldosterone in contributing to sodium retention and hypertension in obesity has, to our knowledge, not been previously reported. Therefore, the primary goal of the present study was to determine whether blockade of the actions of aldosterone, through the use of the specific antagonist eplerenone, attenuates or prevents the sodium retention and hypertension associated with development of obesity in dogs fed a high fat diet. Previous studies suggest that this model of dietary-induced obesity closely mimics the neurohumoral and hemodynamic changes observed in obese humans.<sup>10,14,15</sup>

## Methods

Experiments were conducted in chronically instrumented mongrel dogs ( $n=13$ ) that were conditioned before the study. All experimental protocols were approved by the Institutional Animal Care and Use

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Committee of the University of Mississippi Medical Center and were performed according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and the guidelines of the Animal Welfare Act.

Surgical procedures were performed under isoflurane anesthesia under aseptic conditions. Arterial and venous catheters were implanted for a continuous monitoring of arterial pressure, blood sampling, and intravenous infusions, as previously described.<sup>10</sup> An electromagnetic flow probe was chronically implanted on the ascending aorta for continuous measurements of cardiac output.<sup>10</sup> After surgery, the dogs were permitted to recover, antibiotics were administered daily, and rectal temperatures were monitored to ensure that the dogs were afebrile throughout the studies.

After a 10- to 14-day recovery period, the dogs were placed in individual metabolic cages in a temperature-controlled room with a 12-hour light/dark cycle and fitted with harnesses containing pressure transducers for arterial pressure measurements, as previously described.<sup>10</sup> Analog signals were sampled at a rate of 400 samples per second in bursts of 12 seconds each minute, 24 hours per day, digitized, and processed by the computer to determine systolic, diastolic, and mean arterial pressures and heart rates. Cardiac output signals were monitored with an electromagnetic flowmeter (Zepeda Instruments) 24 hours per day, 400 samples per second, in bursts of 12 seconds each minute. Aortic flow signals were processed to determine stroke volume, cardiac output, and peak aortic flow, and total peripheral resistance was computed on a beat-by-beat basis by use of the cardiac output and arterial pressure signals. The averages of arterial pressure, heart rate, cardiac output, and total peripheral resistance for each day were calculated from values recorded over an 18-hour period between 1:00 PM and 7:00 AM. All routine care of the dogs, including feeding and cleaning of cages, and studies of renal function and blood sampling were conducted between 7:00 AM and 1:00 PM.

Each day, the dogs were fed a standard kennel ration of 2 cans of a sodium-deficient diet (H/D, Hills Pet Products) that provided  $\approx 7$  mmol of sodium and 65 mmol of potassium per day and were given 5 mL of a vitamin syrup (VAL Syrup, Fort Dodge Labs). Total sodium intake, including sodium in the food, was held constant throughout the study at approximately 76 mmol/d by continuous intravenous infusion of 450 mL/d of sterile isotonic saline through one of the femoral vein catheters.

## Experimental Protocols

After the dogs were placed in metabolic cages and intravenous infusions were started, a 2-week control period was begun and the dogs were trained to lie quietly for collection of blood samples and studies of renal function. After at least 1 week of stable control measurements, the aldosterone antagonist eplerenone was administered orally, 10 mg/kg twice daily, for 10 days to assess the cardiovascular and renal actions of aldosterone blockade before starting the high fat diet ( $n=7$ ). This dose of eplerenone has been shown to effectively block the actions of aldosterone in dogs.<sup>16</sup> After 10 days of eplerenone treatment, the dogs were given a supplement of cooked beef fat (0.5 to 0.9 kg/d) for 5 weeks while eplerenone treatment was continued and measurements of systemic hemodynamics, renal function, and blood sampling were conducted.

In a second group of dogs ( $n=6$ ), the same protocol was followed as described above, except that the dogs were not given eplerenone. This group served as a control for the effects of the high fat diet on arterial pressure and renal function.

## Analytical Procedures

Glomerular filtration rate (GFR) was determined from clearance of <sup>125</sup>I-iothalamate (Glofil, Questcor Pharmaceuticals), as previously described.<sup>17</sup> Plasma and urine sodium and potassium concentrations were determined with ion selective electrodes (Nova Biomedical). Plasma chloride concentration was measured by coulometric titration (Buchler chloridometer, Haake Buchler Instruments). Filtered load of electrolytes was determined from measurements of GFR and plasma electrolytes, and total renal reabsorption of electrolytes was calculated from the difference between filtered load and urinary excretion. Plasma renin activity (PRA) and plasma concentrations of

aldosterone and insulin were determined by radioimmunoassay, as previously described.<sup>13</sup> Plasma concentration of glucose was measured with the glucose oxidation method (Beckman glucose analyzer 2), and plasma protein concentration was measured with a refractometer.

## Statistical Analysis

All results are presented as mean  $\pm$  SEM. Control data obtained before starting the high fat diet were compared with experimental data for the same dogs after the high fat diet by ANOVA and Dunnett *t* tests for multiple comparisons where appropriate.<sup>18,19</sup> A 2-way ANOVA followed by a Bonferroni test was used to analyze differences between eplerenone-treated and untreated dogs during the high fat diet.<sup>18</sup> Statistical significance was accepted at a value of  $P<0.05$ .

## Results

### Hemodynamic, Renal, and Hormonal Effects of Eplerenone in Lean Dogs

Eplerenone administration for 10 days to lean dogs slightly decreased mean arterial pressure (MAP) from  $89 \pm 4$  to  $84 \pm 4$  mm Hg (Figure 1). Cardiac output decreased from  $3.2 \pm 0.2$  to  $2.6 \pm 0.2$  L/min and mean values for total peripheral resistance (TPR) increased slightly but not significantly, from  $29.2 \pm 3.3$  to  $35.5 \pm 4.2$  mm Hg/L/min during 10 days of eplerenone administration in lean dogs. Eplerenone also increased heart rate by  $\approx 5$  bpm in lean dogs.

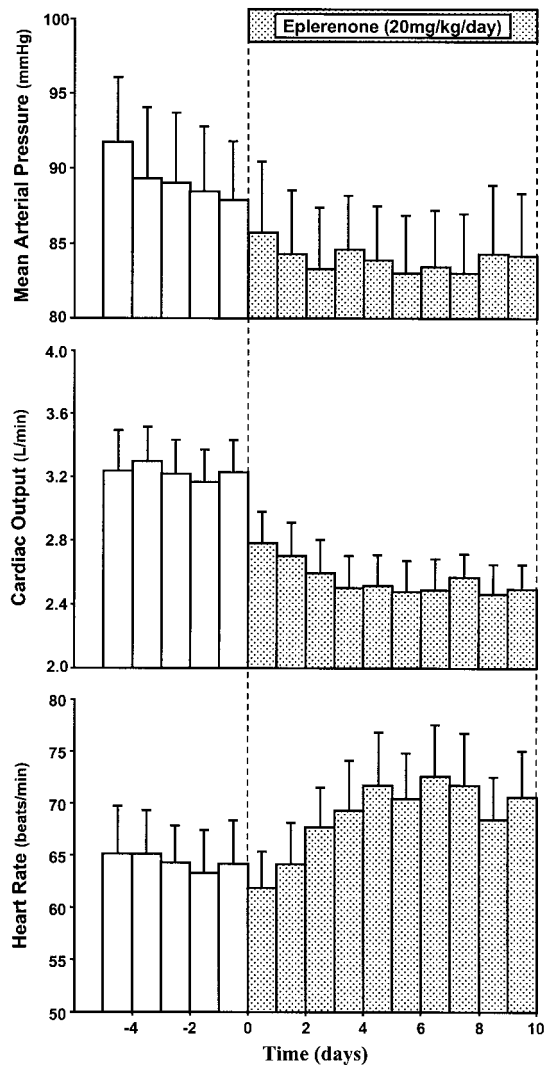
Eplerenone significantly increased sodium excretion during the first 2 days of administration and induced a negative sodium balance of  $-42 \pm 12$  mmol after 10 days. Total renal tubular sodium reabsorption and GFR decreased 20% to 23% below control levels after 10 days of eplerenone, and fractional sodium excretion increased significantly (Figure 2).

Plasma sodium concentration decreased and plasma potassium concentration increased significantly during eplerenone administration in lean dogs (Table 1). Plasma chloride concentration was not significantly altered. Plasma insulin and glucose concentrations did not change significantly during eplerenone treatment. PRA increased from  $0.4 \pm 0.1$  to  $2.5 \pm 0.6$  ng AI/mL per hour and plasma aldosterone concentration increased from  $3.0 \pm 0.6$  to  $43.9 \pm 3.8$  ng/dL after 10 days of eplerenone treatment in lean dogs. Eplerenone treatment did not significantly alter body weight, which averaged  $23.5 \pm 0.7$  kg during control and  $23.9 \pm 0.7$  after 10 days of eplerenone.

### Hemodynamic Effects of Eplerenone During Development of Obesity Hypertension

The high fat diet caused similar increases in body weight in both groups of dogs. In untreated dogs, body weight increased from  $21.3 \pm 0.7$  to  $34.6 \pm 1.0$  kg, and in the eplerenone-treated group, body weight increased from  $23.9 \pm 0.7$  to  $34.3 \pm 0.9$  kg.

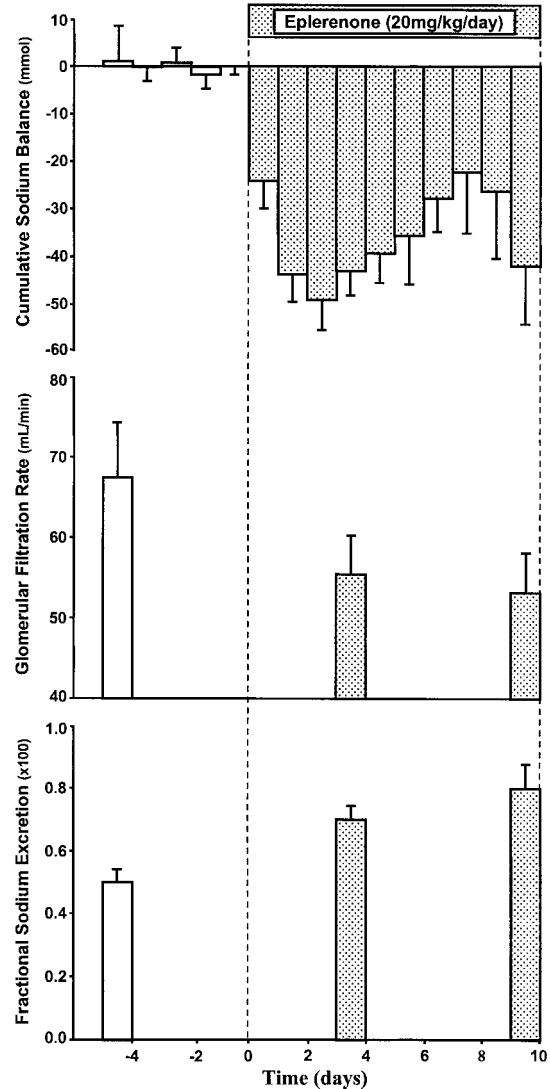
After 5 weeks of the high fat diet, MAP increased by  $16 \pm 3$  mm Hg in untreated dogs compared with  $7 \pm 1$  mm Hg in eplerenone treated dogs (Figure 3). Thus, eplerenone attenuated  $\approx 55\%$  of the rise in arterial pressure associated with the high fat diet. Eplerenone also attenuated the rise in cardiac output associated with a high fat diet. In untreated dogs, cardiac output rose 43% to 68% during weeks 3 to 5 of a high fat diet, compared with a 10% to 17% increase during



**Figure 1.** Mean arterial pressure (mm Hg), cardiac output (L/min), and heart rate (bpm) in lean dogs ( $n=7$ ) before and after eplerenone treatment (10 mg/kg twice daily). Mean arterial pressure and heart rate were measured 18 hours each day. Values represent mean  $\pm$  SEM.

the same period in eplerenone-treated dogs. Likewise, eplerenone attenuated the tachycardia associated with 5 weeks of the high fat diet. In untreated dogs, heart rate increased by 35 to 45 bpm, compared with an increase of 20 to 25 bpm in eplerenone-treated dogs during the high fat diet.

The sodium and water retention normally associated with 5 weeks of a high fat diet was markedly attenuated by eplerenone treatment. In untreated dogs, cumulative sodium balance increased by  $466 \pm 98$  mmol, compared with only  $135 \pm 35$  mmol in eplerenone-treated dogs (Figure 4). Eplerenone also blunted the increase in renal tubular reabsorption and glomerular hyperfiltration associated with 5 weeks of a high fat diet. In control untreated dogs, GFR increased from  $68.7 \pm 2.9$  to  $94.6 \pm 6.5$  mL/min after 5 weeks of a high fat diet. In eplerenone-treated dogs, GFR increased from  $54.7 \pm 5.0$  mL/min to only  $69.0 \pm 6.7$  mL/min after 5 weeks of a high fat diet.



**Figure 2.** Cumulative sodium balance (mEq), glomerular filtration rate (mL/min), and fractional sodium excretion ( $\times 100$ ) in lean dogs ( $n=7$ ) before and after eplerenone treatment (10 mg/kg twice daily). Values represent mean  $\pm$  SEM.

There were no major changes in plasma sodium, chloride, or potassium concentrations in untreated dogs fed a high fat diet (Table 2). However, in eplerenone-treated dogs, plasma potassium concentration was significantly higher than in untreated dogs during the control period as well as during 5 weeks of the high fat diet. Plasma insulin concentration increased significantly in both groups of dogs during the high fat diet, but there were no major changes in plasma glucose concentration in either group during the high fat diet.

Plasma renin activity increased significantly in untreated dogs, from  $0.4 \pm 0.2$  to  $0.9 \pm 0.2$  ng AI/mL per hour after 5 weeks of a high fat diet. In eplerenone-treated dogs, PRA was already elevated, compared with untreated dogs, before starting the high fat diet and increased further from  $2.5 \pm 0.6$  to  $8.3 \pm 1.6$  ng AI/mL per hour during the high fat diet (Table 2). Plasma aldosterone concentration also increased in control untreated dogs, from  $3.1 \pm 0.7$  to  $5.8 \pm 1.8$  ng/dL, after 5 weeks of a high fat diet. As expected, eplerenone treatment

**TABLE 1. Plasma Hormones, Glucose, and Electrolytes for Lean Dogs (n=7) Before and After Eplerenone Treatment**

Treatment	PRA, ng/mL/h	ALDO, mg/dL	Insulin, $\mu$ U/mL	Glucose, mg/dL	Plasma Na <sup>+</sup> , mEq/L	Plasma Cl <sup>-</sup> , mEq/L	Plasma K <sup>+</sup> , mEq/L
Control	0.4 $\pm$ 0.1	3.0 $\pm$ 0.6	18.6 $\pm$ 5.2	110 $\pm$ 4	146.9 $\pm$ 0.5	117 $\pm$ 1	4.8 $\pm$ 0.1
Eplerenone treatment							
Day 4	2.8 $\pm$ 0.8*	67.5 $\pm$ 7.1*	26.5 $\pm$ 5.6	116 $\pm$ 3	143.0 $\pm$ 0.6*	115 $\pm$ 1	5.9 $\pm$ 0.3*
Day 10	2.5 $\pm$ 0.6*	43.9 $\pm$ 3.8*	16.1 $\pm$ 6.0	111 $\pm$ 4	143.7 $\pm$ 0.5*	115 $\pm$ 1	6.2 $\pm$ 0.3*

PRA indicates plasma renin activity; ALDO, aldosterone; Na<sup>+</sup>, sodium; Cl<sup>-</sup>, chloride; K<sup>+</sup>, potassium.

\* $P < 0.05$  compared with control period.

markedly elevated baseline plasma aldosterone concentration. However, aldosterone concentration increased even further during the high fat diet, from 44 $\pm$ 4 to 71 $\pm$ 9 ng/dL after 5 weeks.

### Discussion

An important new finding of this study is that aldosterone antagonism with eplerenone markedly attenuated sodium retention, hypertension, and glomerular hyperfiltration associated with the development of obesity in dogs fed a high fat diet. These observations suggest that aldosterone plays a significant role in mediating obesity-induced changes in renal function and hypertension.

Previous studies suggest that dogs fed a high fat diet exhibit changes in renal function, systemic hemodynamics, and hormonal and metabolic changes very similar to those observed in obese humans.<sup>5,6,10,14</sup> As shown in the present study, dogs fed a high fat diet have increases in heart rate, cardiac output, and blood pressure, marked sodium retention, mild activation of the RAAS, and hyperinsulinemia. Other studies have shown that obese dogs exhibit insulin resistance, glucose intolerance, and other metabolic changes that are characteristic of obese humans.<sup>6,10,14</sup> Thus, obese dogs fed a high fat diet may provide a very useful model for studying mechanisms of obesity-induced hypertension in humans.

### Aldosterone Antagonism in Lean Dogs

Aldosterone antagonism caused modest but significant sodium and water loss and decreases in cardiac output and arterial pressure even in lean dogs. This is perhaps not surprising, since aldosterone normally contributes to renal tubular reabsorption of sodium and water. In fact, after aldosterone antagonism with eplerenone, renal tubular sodium reabsorption was reduced by  $\approx 20\%$ . This reduction in tubular reabsorption was counterbalanced by a comparable decrease in GFR that along with increases in PRA and presumably Ang II formation, probably helped to restore sodium balance in the face of impaired action of aldosterone on the renal tubules.

The reduction in cardiac output and a slight increase in total peripheral vascular resistance observed after aldosterone antagonism probably reflects reduced blood flow in peripheral tissues including the kidneys, where GFR was reduced by  $\approx 20\%$ . One potential mechanism for these hemodynamic changes is inhibition of Na<sup>+</sup>-K<sup>+</sup> ATPase and subsequent reductions in energy expenditure by peripheral tissues. Aldosterone is known to stimulate Na<sup>+</sup>-K<sup>+</sup> ATPase activity, and

blockade of this effect could contribute to decreased energy utilization, which in turn would tend to decrease blood flow and raise peripheral vascular resistance.

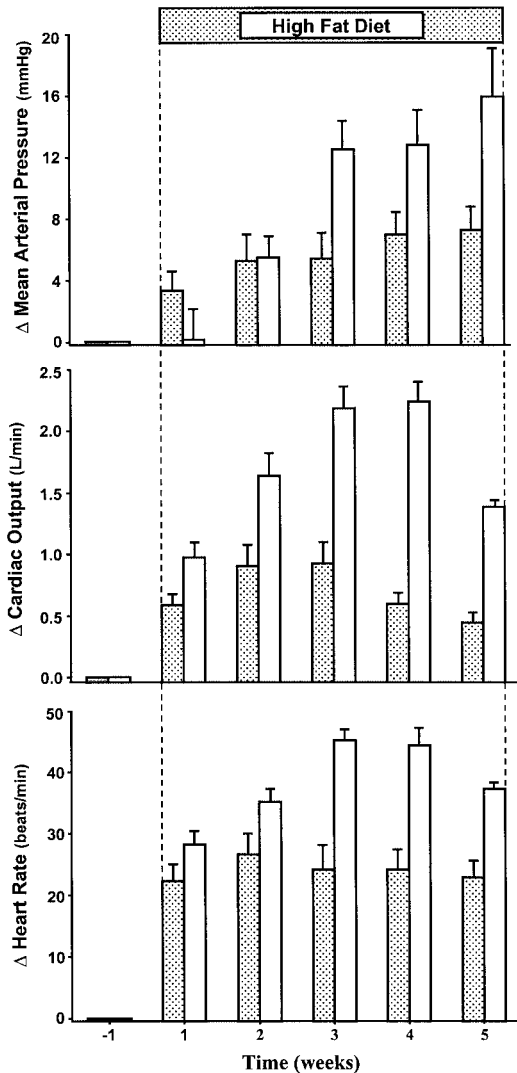
Aldosterone antagonism with eplerenone also caused significant increases in plasma potassium concentration. Previous clinical studies have not generally found large increases in plasma potassium concentration after chronic treatment with eplerenone.<sup>20–24</sup> However, the dose of eplerenone used in our experiments was higher than for clinical studies because our goal was to block, as effectively as possible, aldosterone receptors. Since increases in plasma aldosterone reduce plasma potassium by shifting potassium from the extracellular fluid into the cells and by stimulating renal tubular potassium secretion,<sup>25</sup> hyperkalemia might be predicted to be a consequence of effective aldosterone antagonism, as observed in the present study. It is important to emphasize, however, that despite the hyperkalemia caused by aldosterone antagonism, we observed no indications of arrhythmia or other untoward cardiovascular effects.

### Aldosterone Antagonism Attenuates Obesity-Induced Hypertension

Aldosterone antagonism not only lowered blood pressure slightly in lean dogs but also markedly attenuated the rise in arterial pressure that normally accompanies a long-term high fat diet. These observations suggest that aldosterone plays a significant role in the development of obesity hypertension in dogs. This finding is somewhat surprising in view of the fact that obesity caused only modest increases in plasma aldosterone concentration in the present study and in our previous studies.<sup>10</sup> However, even small increases in plasma aldosterone concentration may contribute to increases in arterial pressure when accompanied by marked sodium retention and volume expansion as occurred during the high fat diet in the present study. Obesity may also be associated with increased sensitivity to the effects of aldosterone as the result of volume expansion, increased mineralocorticoid receptor sensitivity, or enhanced postreceptor signaling. Future studies are needed to address these possibilities.

The precise mechanisms by which aldosterone contributes to obesity hypertension are not completely clear but appear to be due, at least in part, to sodium and water retention. After antagonism of aldosterone, the marked sodium retention that normally accompanies a high fat diet was greatly attenuated as the result of inhibition of renal tubular sodium reabsorption. This attenuation of sodium reabsorption by aldosterone antagonism is especially impressive, considering the fact that

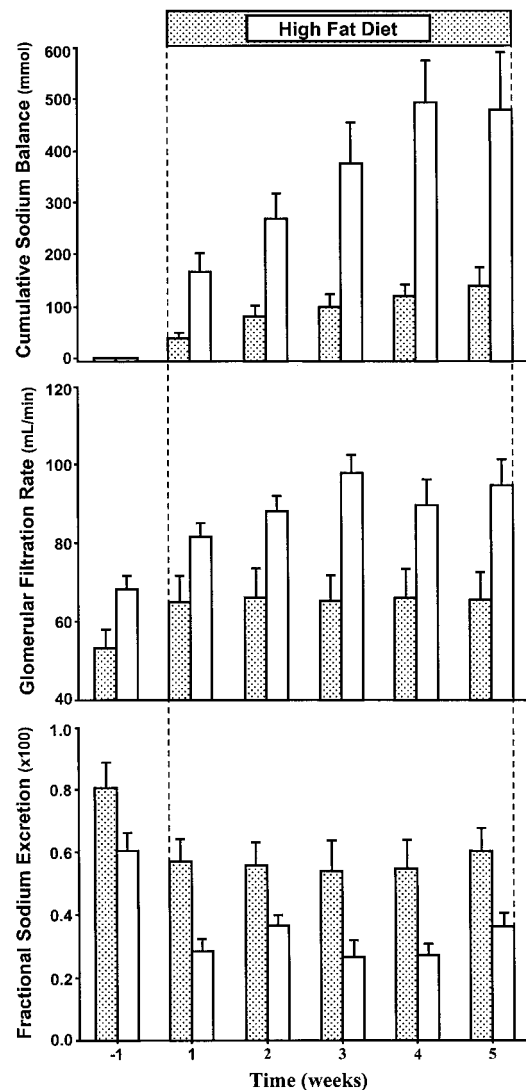




**Figure 3.** Changes ( $\Delta$ ) in mean arterial pressure (mm Hg), cardiac output (L/min), and heart rate (bpm) in control untreated dogs ( $n=6$ ) and eplerenone-treated (10 mg/kg twice daily,  $n=7$ ) dogs. Control values represent average for 5 control days preceding the high fat diet in each group. Mean arterial pressure averaged  $84 \pm 5$  and  $84 \pm 4$  mm Hg, cardiac output averaged  $3.3 \pm 0.1$  and  $2.6 \pm 0.2$  L/min, and heart rate averaged  $62 \pm 2$  and  $71 \pm 5$  bpm for untreated and eplerenone-treated dogs, respectively, during the control period before starting the high fat diet. Values represent mean  $\pm$  SEM.

PRA, and presumably Ang II formation, was severalfold higher in eplerenone-treated dogs compared with untreated dogs during the development of obesity. Since Ang II also has powerful direct effects to stimulate renal tubular sodium reabsorption,<sup>26</sup> the reduction in total sodium reabsorption despite very high PRA suggests a powerful role for aldosterone in stimulating sodium reabsorption during the development of obesity-induced hypertension.

The increases in cardiac output and heart rate that accompanied obesity-induced hypertension were also substantially attenuated by aldosterone antagonism. Whether this was related to reductions in blood flow in peripheral tissues and decreases in venous return and cardiac filling or to other factors that influenced heart rate and cardiac output, such as



**Figure 4.** Changes in cumulative sodium balance (mmol), glomerular filtration rate (mL/min), and fractional sodium excretion ( $\times 100$ ) in control dogs ( $n=6$ ) and eplerenone-treated (10 mg/kg twice daily,  $n=7$ ) dogs. Control values for cumulative sodium balance represent average for 5 control days preceding the high fat diet in each group. Values represent mean  $\pm$  SEM.

sympathetic activity, was not examined in the present study. Aldosterone has been suggested to stimulate sympathetic activity through direct CNS actions,<sup>27</sup> and previous studies indicate that sympathetic nervous activation plays a major role in contributing to sodium retention and hypertension in obesity.<sup>5,14,28</sup> However, The importance of interactions between aldosterone and sympathetic activity in contributing to obesity-induced hypertension are unknown.

Insulin resistance and hyperinsulinemia have also been suggested to contribute to obesity-induced hypertension. However, we found no evidence in the present study that aldosterone antagonism significantly influenced fasting insulin or glucose concentrations.

**TABLE 2. Plasma Hormones, Glucose, and Electrolytes for Control Untreated (n=6) and Eplerenone-Treated (n=7) Dogs Before and After Dietary-Induced Obesity by a High-Fat Diet**

Experimental Group	PRA, ng/mL/h	ALDO, mg/dL	Insulin, $\mu$ U/mL	Glucose, mg/dL	Plasma Na <sup>+</sup> , mEq/L	Plasma Cl <sup>-</sup> , mEq/L	Plasma K <sup>+</sup> , mEq/L
Control, untreated	0.4 $\pm$ 0.2	3.1 $\pm$ 0.7	25.6 $\pm$ 3.2	114 $\pm$ 5	146.9 $\pm$ 1.2	115 $\pm$ 1	4.3 $\pm$ 0.2
High fat, untreated							
Week 1	1.0 $\pm$ 0.2	5.3 $\pm$ 0.6	62.3 $\pm$ 7.9*	110 $\pm$ 3	148.0 $\pm$ 0.3	117 $\pm$ 1	4.3 $\pm$ 0.1
Week 2	1.0 $\pm$ 0.2	5.2 $\pm$ 0.6	52.5 $\pm$ 10.5*	104 $\pm$ 4	148.9 $\pm$ 0.4	116 $\pm$ 1	4.3 $\pm$ 0.1
Week 3	1.0 $\pm$ 0.2	4.8 $\pm$ 0.6	50.9 $\pm$ 9.3*	101 $\pm$ 4	149.5 $\pm$ 0.4	115 $\pm$ 1	4.1 $\pm$ 0.1
Week 4	0.6 $\pm$ 0.1	4.6 $\pm$ 0.9	62.9 $\pm$ 17.9*	107 $\pm$ 3	148.7 $\pm$ 0.5	115 $\pm$ 1	4.1 $\pm$ 0.1
Week 5	0.9 $\pm$ 0.2	5.8 $\pm$ 1.8	45.8 $\pm$ 5.3*	109 $\pm$ 1	148.2 $\pm$ 0.9	113 $\pm$ 1	3.9 $\pm$ 0.1
Control, eplerenone treatment	2.5 $\pm$ 0.6	43.9 $\pm$ 3.8†	16.1 $\pm$ 6.0	111 $\pm$ 4	143.7 $\pm$ 0.5	115 $\pm$ 1	6.2 $\pm$ 0.3†
High fat, eplerenone treatment							
Week 1	9.4 $\pm$ 0.8*†	53.5 $\pm$ 6.9†	29.3 $\pm$ 3.7†	111 $\pm$ 4	145.6 $\pm$ 1.3	115 $\pm$ 0.3	5.2 $\pm$ 0.2*†
Week 2	9.1 $\pm$ 1.4*†	55.9 $\pm$ 7.2†	26.9 $\pm$ 4.2	111 $\pm$ 3	143.6 $\pm$ 0.9†	114 $\pm$ 1	5.7 $\pm$ 0.3†
Week 3	9.1 $\pm$ 1.5*†	60.6 $\pm$ 6.0†	34.8 $\pm$ 5.3*	114 $\pm$ 3	142.9 $\pm$ 1.1†	114 $\pm$ 1	5.7 $\pm$ 0.3†
Week 4	10.6 $\pm$ 1.5*†	65.2 $\pm$ 3.4†	46.6 $\pm$ 6.2*	109 $\pm$ 4	143.1 $\pm$ 1.0†	114 $\pm$ 1	5.4 $\pm$ 0.2*†
Week 5	8.3 $\pm$ 1.6*†	70.9 $\pm$ 9.3*†	48.5 $\pm$ 6.8*	123 $\pm$ 2	144.6 $\pm$ 1.5	113 $\pm$ 0.5	5.4 $\pm$ 0.2*†

\* $P < 0.05$  compared with control period; † $P < 0.05$  eplerenone-treated compared with untreated dogs.

### Aldosterone Antagonism Attenuates Glomerular Hyperfiltration in Obesity

Another important observation of the present study is that eplerenone treatment markedly attenuated the glomerular hyperfiltration associated with obesity. Even in lean animals, aldosterone antagonism decreased GFR by  $\approx 20\%$ . We previously reported that chronic aldosterone infusion, at rates that raised plasma concentrations to about 5 to 6 times normal, increased GFR by  $\approx 20\%$ . The mechanisms by which hyperaldosteronism increases GFR and aldosterone antagonism lowers GFR are poorly understood but are unlikely to be related solely to changes in arterial pressure. We have previously shown, for example, that servo-control of renal perfusion pressure does not prevent glomerular hyperfiltration during chronic aldosterone infusion.<sup>29</sup> It is also unlikely that these changes in GFR can be explained entirely by changes in extracellular fluid volume, since aldosterone antagonism in lean dogs caused a relatively modest loss of sodium ( $< 50$  mmol) while decreasing GFR by  $\approx 20\%$ .

One possible explanation for the effects of aldosterone on GFR is altered tubuloglomerular feedback (TGF) sensitivity. If aldosterone excess reduced TGF sensitivity, this would increase GFR and distal sodium chloride delivery. Conversely, aldosterone antagonism could increase TGF sensitivity, thereby decreasing GFR and distal sodium chloride delivery. Previous acute studies support the possibility that mineralocorticoids may attenuate TGF sensitivity.<sup>30</sup> However, the importance of this mechanism for long-term GFR regulation has, to our knowledge, not been previously assessed. Regardless of the mechanisms involved, a renal vasodilator effect of aldosterone could have important adaptive value, since stimulation of sodium reabsorption in the collecting tubules and collecting ducts by aldosterone would necessitate an increase in sodium chloride delivery to these tubular segments to maintain sodium balance. Conversely, inhibition of sodium reabsorption in distal nephron segments

by aldosterone blockade would require decreased delivery of sodium chloride to these tubular segments, possibly through decreased GFR, to achieve sodium balance. However, the precise mechanisms by which aldosterone influences chronic GFR regulation are poorly understood and remain a topic for further investigation.

Despite the adaptive value of glomerular hyperfiltration in offsetting the renal sodium retaining action of aldosterone, this effect could, in the long term, contribute to obesity-induced renal injury. The combination of increased arterial pressure, renal vasodilation, and marked glomerular hyperfiltration in obesity may initiate a complex cascade of biochemical and histological changes that eventually lead to glomerular injury.<sup>5,31,32</sup> We have previously reported that even after only 7 to 9 weeks of a high fat diet, there is expansion of Bowman's capsule, increased glomerular cell proliferation, thickening of the mesangial matrix and Bowman's capsule membranes, and indications of increased glomerular TGF- $\beta$  expression.<sup>32</sup> Considerable evidence suggests that these early structural changes in obesity may be the precursors of more severe renal injury.<sup>31,32</sup> To the extent that glomerular hyperfiltration initiates or contributes to glomerular injury, prevention of increased GFR may attenuate renal injury associated with obesity. The fact that aldosterone antagonism markedly attenuates the glomerular hyperfiltration associated with obesity may have important implications for renal protection. Although there are no studies to our knowledge that have tested this concept directly in obese subjects, previous studies in various experimental models of hypertension have provided evidence that aldosterone antagonism attenuates renal injury.<sup>33</sup> Further studies are needed to determine the potential protective effects of aldosterone antagonism on the kidneys in obesity.

### Perspectives

Antagonism of aldosterone markedly attenuates sodium retention, hypertension, and glomerular hyperfiltration associ-

ated with the development of obesity caused by high dietary fat intake. Moreover, this protection against sodium retention and hypertension occurs despite marked increases in PRA, suggesting a powerful role for aldosterone in mediating changes in renal function and arterial pressure associated with obesity. Further studies are needed to determine whether aldosterone antagonism is even more effective in treating obesity-induced hypertension when combined with blockade of the RAS with an ACE inhibitor or an angiotensin receptor antagonist. The observation that blockade of aldosterone also prevented the glomerular hyperfiltration normally associated with obesity points toward another potential mechanism by which aldosterone antagonism may protect against the development of chronic renal disease. Since obesity is a common cause of human essential hypertension and may be a major risk factor for chronic renal disease,<sup>5,31</sup> results from our studies suggest another potential target for treating obese hypertensive subjects who are resistant to the usual therapeutic approaches.

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