Clonidine Prevents Insulin Resistance and Hypertension in Obese Dogs

Albert P. Rocchini, Hui Z. Mao, Keshava Babu, Paul Marker, Albert J. Rocchini

Abstract—The role that the central sympathetic nervous system plays in the development of obesity hypertension and insulin was evaluated by feeding dogs a high fat diet with or without clonidine treatment. Thirteen adult mongrel dogs were chronically instrumented and randomly assigned to receive either a high fat diet and no clonidine (n=6) or a high fat diet plus clonidine (n=7), 0.3 mg BID. Blood pressure, heart rate, plasma insulin, and electrolytes were measured daily. Insulin resistance was assessed with a multiple-dose euglycemic clamp (1, 2, and 30 mU · kg⁻¹ · min⁻¹) before and after 1, 3, and 6 weeks of the high fat diet. Clonidine prevented the hypertension, tachycardia, and insulin resistance associated with feeding dogs the high fat diet but did not affect weight gain. The present study suggests that the central sympathetic nervous system plays a critical role in the development of both insulin resistance and hypertension associated with feeding dogs a high fat diet. (Hypertension. 1999;33[part II]:548-553.)

Key Words ■ hypertension, arterial ■ obesity ■ insulin resistance ■ clonidine ■ sympathetic nervous system ■ cardiac output

The mechanism involved in the pathogenesis of the increased blood pressure in obesity is incompletely understood. Studies in our laboratory1,2 and by others3 suggest that insulin resistance may be the link between obesity and hypertension. However, other observations suggest that the relation between insulin and obesity-induced hypertension is not so straightforward. The San Antonio Heart Study showed that hyperinsulinemia is more common in Mexican Americans than in white non-Hispanics, yet the prevalence of hypertension is high in the latter group.4 Hall et al5 failed to observe an increase in blood pressure when normal dogs were given a chronic infusion of insulin with or without norepinephrine.

We believe that an alternate hypothesis to explain the pathogenesis of obesity-induced hypertension is that chronic central sympathetic nervous system activation links insulin resistance and hypertension. Sowers et al6 observed that borderline hypertensive obese subjects had higher norepinephrine levels than did nonobese normotensive control subjects, that their blood pressure correlated with norepinephrine levels, and that weight loss was accompanied by a fall in blood pressure that correlated with a decrease in serum norepinephrine. Hall et al7 suggested that combined α- and β-adrenergic blockade reduced arterial pressure to a much greater extent in obese than in normal dogs. Fasting or caloric deprivation reduces sympathetic activity and overfeeding stimulates sympathetic activity.8

Diebert and DeFronzo9 demonstrated that impair both peripheral and hepatic resistance to the action of insulin. Jamerson et al10 demonstrated that a reflex increase in sympathetic tone in normotensive individuals can lead to acute insulin resistance in the forearm.

Thus, it is possible that central activation of the sympathetic nervous system is the physiological link that connects excess dietary intake to insulin resistance and hypertension. Through the feeding of fat with or without clonidine to dogs, the present study was designed to evaluate the role that the central sympathetic system plays in the development of hypertension and insulin resistance.

Methods

Thirteen adult mongrel dogs (6 males and 7 females) were trained to stand quietly in a padded sling and were surgically instrumented with 1 ascending aortic and 2 right atrial catheters. The dogs recovered for 3 weeks before baseline measurements were made. Dogs were randomly assigned to either a high-fat-diet, no-clonidine group (n=6) that received the control diet (1 can of dog food [Ken-L-Ration]) for 2 weeks followed by 6 weeks of a high fat diet consisting of 0.8 kg of cooked beef fat in addition to the regular diet2 or a high-fat-diet, clonidine group (n=7) that received 0.3 mg clonidine PO BID initiated 1 week before the start of the high fat diet and continued with the fat diet for an additional 6 weeks. (Figure 1) All dogs received vitamin supplements (VAL Syrup; Fort Dodge Laboratories) and antibiotics throughout the entire study. Dogs were housed in air-conditioned cages and fed between 1:00 and 3:00 PM each day. Blood pressure, heart rate, and body weight were measured daily. Cardiac output, plasma glucose, and insulin were measured twice a week. All measurements were made between 8:00 and 11:00 AM and before the daily feeding. All the procedures in this study were in accordance with the University of Michigan's policy for the care and use of animals.

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to determine whether a significant change in the parameters occurred over time. Within each group, a repeated measures ANOVA was performed for each variable to determine whether a significant change in the variable occurred over time. A 2-factor ANOVA for repeated measures was then performed for each variable to assess differences between the dogs fed the high fat diet and no clonidine and the dogs fed clonidine plus the high fat diet.

Results

Euglycemic Clamp Data

During the euglycemic clamp studies, the steady-state blood glucose concentration in all dogs averaged \( \sim 5.2 \text{ mmol/L} \) and did not differ from the fasting concentration at any insulin infusion rate. The coefficient of variation of glucose level at each insulin plateau was \(<5\%\).

To characterize the ability of clonidine treatment to alter the insulin-mediated glucose uptake relation that occurs in dogs fed a high fat diet, we measured insulin-mediated glucose uptake dose-response curves before and after the high fat diet. Basal rates of whole body glucose uptake (hepatic glucose production) were measured on 4 dogs in the clonidine group and 4 dogs in the no-clonidine group. The high fat diet did not significantly change basal rates of whole body glucose uptake in either group of dogs \((24.6 \pm 1.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})\) at week 0 versus \(22.8 \pm 0.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) at week 6 in the no-clonidine group and \(26.8 \pm 2.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) at week 0 versus \(25.9 \pm 1.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) at week 6 in the clonidine group). Throughout the study, the \(1-\text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) insulin dose completely suppressed hepatic glucose output in both groups of dogs. During increasing insulin dose rates, whole body glucose uptake increased in a sigmoidal fashion (Figure 2). In the no-clonidine group, the high fat diet was associated with a shift in the glucose uptake curve to the right, and the rate of maximal whole body glucose uptake was significantly decreased \((P<0.001)\) (Table 2). Compared with the control, pre–high fat period, the insulin concentration expected to produce a half-maximal response in glucose uptake (insulin ED\(_{50}\) dose) was 50% \((P<0.01)\) higher at 1 week and 85% \((P<0.001)\) higher at 6 weeks of the fat diet. In contrast to the no-clonidine group, the clonidine-treated group did not experience any change in the glucose uptake curve during the 6 weeks of the high fat diet. After 6 weeks

Minneapolis, Northwester University, and University of Michigan guidelines on animal experimentation.

Laboratory Measurements

Arterial pressure was measured with a pressure transducer mounted at the level of the heart, and the analog signals were sent to a computer to be analyzed. The computer calculated the average systolic, diastolic, and mean blood pressures and heart rate (over a 15- to 30-minute period). Cardiac output was measured with Cardiogreen dye (Waters Instruments).

Insulin resistance was assessed with a multiple insulin dose euglycemic hyperinsulinemic clamp. The multiple insulin infusion euglycemic clamps were performed twice before starting the high fat diet and at 1, 3, and 6 weeks of the high fat diet. Arterial samples were obtained to determine basal glucose, insulin, and potassium levels, and cardiac output was measured. A constant infusion of insulin was administered at 3 insulin infusion rates \((1, 2, \text{ and } 30 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})\). Concomitant with the insulin, an intravenous infusion of 20% glucose was administered with a variable infusion syringe pump to maintain euglycemia. To prevent severe hypokalemia, K\(_2\)HPO\(_4\) was infused. Blood pressure and heart rate were continuously monitored throughout the clamp procedure. During the last 30 minutes of the insulin infusion, arterial blood was sampled for glucose, insulin, and potassium levels, and cardiac output was measured as the average of 2 determinations.

In 4 no-clonidine fat–fed dogs and 4 clonidine plus fat–fed dogs, basal glucose turnover was measured (at weeks 0, 1, 3, and 6) using D-\(3^\text{H}\)glucose. The rate of hepatic glucose production was calculated, assuming steady-state conditions, using the Steele equation. These values of basal glucose production were used in the calculation of the insulin dose-response curves.

Analytic Methods

The serum glucose concentration was measured in duplicate with the glucose oxidase method using a glucose analyzer (model A23; Yellow Springs Instruments). Serum insulin was measured with double-antibody radioimmunoassay (ICN Biomedical). Plasma electrolytes were measured with flame photometry. Blood for the determination of plasma glucose specific activity was collected in sodium fluoride–treated tubes and immediately spun, and the supernatant was removed and stored at \(-20^\circ\text{C}\).

Statistical Analysis

All values are mean\(\pm\)SEM. Weekly blood pressures, heart rates, and body weights were determined by averaging the daily values, and cardiac output and plasma glucose and insulin levels were determined by averaging the 2 values obtained each week. The dose-response curves for whole body glucose uptake versus insulin were fitted to a 4-parameter logistic equation using a least-squares mean iterative routine (ALLFIT)\(^{12}\) as follows: \(Y = \{(A - D)/(1 + (I/ED_{50})^B)\} + D\), where \(A\) is the expected maximal response, \(D\) is the expected minimal response, \(I\) is the insulin concentration, \(ED_{50}\) is the insulin concentration with expected response halfway between \(A\) and \(D\), and \(B\) is the slope factor. After obtaining parameters \(A\), \(D\), \(B\), and \(ED_{50}\) for the fat and no clonidine and the fat plus clonidine groups, separate analyses were performed to test whether parameters were similar between the groups. A repeated measures analysis was used in weight was associated with a significant increase in arterial pressure \((P<0.001)\), heart rate \((P<0.001)\), and cardiac output \((P<0.01)\). The clonidine plus fat–fed dogs experienced no change in these hemodynamic parameters (Table 1). Plasma glucose did not significantly increase in either group with feeding of the high fat diet; however, we observed only in the group that did not receive clonidine a significant increase in fasting serum insulin concentration \((86 \pm 10\) to \(208 \pm 21\) pmol/L, \(P<0.001)\).

Figure 1. Schematic representation of the study design of the project.
of the high fat diet, the clonidine group had both maximal glucose uptake and the insulin concentration expected to produce a half-maximal response in glucose uptake (insulin ED50 dose), which were unchanged from values obtained in this control period. (Table 2) In addition, 1 week of clonidine treatment without the high fat diet (week −1 versus week 0) also did not change the insulin-mediated glucose uptake curve. Finally, despite the infusion of the same amount of insulin per kilogram of weight in both groups of dogs, only the group of dogs that did not receive clonidine experienced, when fed the high fat diet, an increase over time in the plateau insulin values during the multiple-dose clamps (Table 2).

In the no-clonidine group, the high fat diet was associated with an increase in resting cardiac output ($P<0.01$), whereas in the clonidine-treated group, the high fat diet resulted in no significant increase in cardiac output ($P>0.1$) (Table 1). In both groups of dogs, before starting the high fat diet, euglycemic hyperinsulinemia caused a dose-dependent increase in cardiac output (Figure 3). In the no-clonidine group, feeding the high fat diet resulted in rightward shift of the effect of insulin to increase cardiac output and a reduced maximal insulin-stimulated cardiac output ($P<0.05$) (Table 2). After 6 weeks of the fat diet, euglycemic hyperinsulinemia caused virtually no increase in cardiac output. However, in the clonidine group, the high fat diet did not change the ability of insulin to increase cardiac output. After 6 weeks of the high fat diet, euglycemic hyperinsulinemia in clonidine-treated dogs still resulted in a 37% increase in cardiac output.

In both groups of dogs, before starting the high fat diet, the euglycemic clamp resulted in a $6\pm2$ mm Hg decrease in arterial pressure and a $7\pm3$ bpm increase in rate. In the no-clonidine group, after 6 weeks of a high fat diet, euglycemic hyperinsulinemia did not cause either a decrease in arterial pressure or an increase in heart rate; however, in the clonidine group, the high fat diet euglycemic hyperinsulinemia was still associated with a $5\pm2$ mm Hg decrease in pressure and a $5\pm3$ increase in heart rate.

**Discussion**

The present study demonstrates that clonidine, an antihypertensive agent that lowers blood pressure through stimulation of central $\alpha_2$-receptors and, to a lesser degree, the I$_1$ imidazole receptors, prevented both the hypertension and insulin resistance associated with weight gain in the dog. Our finding that clonidine prevented hypertension in the obese dog model is consist with the report of Hall et al. They demonstrated that
TABLE 2. Maximal and ED\textsubscript{50} Values for Insulin-Mediated Glucose Uptake, Basal Maximal and ED\textsubscript{50} Values for Cardiac Output, and Plateau Insulin Concentrations in High-Fat Clonidine-Fed and High Fat Non–Clonidine-Fed Dogs

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 3</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole body glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uptake, (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>104±15</td>
<td>104±19</td>
<td>79±14</td>
<td>99±18†</td>
</tr>
<tr>
<td>ED\textsubscript{50}</td>
<td>695±24</td>
<td>673±21</td>
<td>1048±32</td>
<td>676±29‡</td>
</tr>
<tr>
<td><strong>Cardiac output, L/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>4.9±3</td>
<td>4.9±3</td>
<td>4.6±0.3</td>
<td>4.7±0.3</td>
</tr>
<tr>
<td>Basal</td>
<td>3.2±0.4</td>
<td>3.3±0.4</td>
<td>3.5±0.3</td>
<td>3.3±0.5</td>
</tr>
<tr>
<td>ED\textsubscript{50}</td>
<td>425±25</td>
<td>435±26</td>
<td>763±21</td>
<td>440±17‡</td>
</tr>
<tr>
<td>Plateau insulin concentration, pmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mU \cdot kg\textsuperscript{-1} \cdot 1 min\textsuperscript{-1}</td>
<td>359±28</td>
<td>358±28</td>
<td>579±25†‡</td>
<td>365±21</td>
</tr>
<tr>
<td>2 mU \cdot kg\textsuperscript{-1} \cdot 1 min\textsuperscript{-1}</td>
<td>727±57</td>
<td>701±43</td>
<td>886±64†‡</td>
<td>721±45</td>
</tr>
<tr>
<td>3 mU \cdot kg\textsuperscript{-1} \cdot 1 min\textsuperscript{-1}</td>
<td>16277±94</td>
<td>16257±89</td>
<td>17240±98†‡</td>
<td>16298±89</td>
</tr>
</tbody>
</table>

Maximum indicates whole body glucose uptake and cardiac output at maximally effective insulin concentrations; ED\textsubscript{50}, whole body glucose uptake and cardiac output at the insulin concentration (pmol/L) at which half-maximum is achieved; and Basal, resting values of cardiac output just before starting the euglycemic hyperinsulinemic clamps.

All values of glucose uptake and cardiac output are derived from curve-fitting analysis (ALLFIT) using a four-parameter logistic equation on the basis of group mean values.

*\(P<0.05\), †\(P<0.01\), and ‡\(P<0.001\), clonidine group vs no-clonidine group.

combined \(\alpha\)– and \(\beta\)-adrenergic blockade reduced arterial pressure to a much greater extent in obese than in normal dogs. We believe the mechanism whereby clonidine treatment prevented the increase in blood pressure associated with weight gain is due to central inhibition of the peripheral sympathetic nervous system. Because we did not directly measure sympathetic activity, further studies are necessary to directly prove that clonidine inhibited peripheral sympathetic activity. The failure of weight gain to induce tachycardia in the clonidine-treated dogs is consistent with the known central nervous system action of clonidine. In addition, the failure of clonidine-treated dogs to increase their cardiac output in association with weight gain is consistent with a reduced degree of renal salt and water retention. Kassab et al have shown that bilateral renal denervation prevents the hypertension and sodium retention associated with obesity in the dog. We believe that clonidine treatment could have prevented the high fat diet–induced activation of the renal efferent sympathetic nerves. Further studies that measure in detail the sodium and fluid balance in clonidine-treated dogs fed a high fat diet will be necessary to more directly answer whether clonidine does prevent sodium and fluid retention in obese dogs.

Perhaps the more important and new finding in the present study is that clonidine also prevented the insulin resistance associated with obesity in the dog. Using the multiple insulin dose euglycemic clamp, we demonstrated that clonidine prevents the reduced sensitivity and responsiveness of the insulin-mediated glucose uptake dose-response curve that is associated with weight gain (Figure 2, Table 2). It has been speculated that insulin resistance (ie, resistance to the ability of insulin to stimulate glucose uptake) is the common metabolic abnormality connecting obesity, hypertension, and increased sympathetic nervous system activity. This hypothesis is supported by numerous reports that document a relation between insulin resistance and hypertension. However, the present study supports the concept, originally proposed by Julius et al, that central nervous system–induced sympathetic activation, not insulin resistance or...
hyperinsulinemia, is the metabolic link that connects obesity to hypertension.

Our finding that clonidine treatment prevented the insulin resistance associated with weight gain in the dog is consistent with other reports. Giugliano et al\textsuperscript{10} reported that in 20 hypertensive patients with non–insulin-dependent diabetes mellitus, clonidine treatment was associated with an improvement in insulin sensitivity in peripheral tissues. Using the glucose clamp, these investigators found that clonidine significantly improved overall glucose metabolism and that this improvement was accompanied by increases in both oxidative and nonoxidative glucose metabolism. Other central acting antihypertensive drugs also have been reported to improve insulin resistance. Moxonidine, a highly selective $\alpha_1$ imidazole receptor agonist with weak central $\alpha_2$-receptor effects, has been shown to prevent the insulin resistance, hyperinsulinemia, and hypertension in rats fed a fructose-enriched diet\textsuperscript{17} and to reduce blood pressure, reduce triglycerides, and improve glucose tolerance in obese spontaneously hypertensive rats.\textsuperscript{18} However, in their fructose-fed rat model, Hwang et al\textsuperscript{19} failed to show a beneficial effect of clonidine in preventing insulin resistance despite demonstrating that clonidine did prevent the increase in blood pressure. The acute administration of clonidine has been shown to induce hyperglycemia and impaired glucose tolerance in rats and humans.\textsuperscript{20} However, chronic clonidine treatment does not appear to exert such effects.\textsuperscript{21}

The present study was not designed to determine the mechanism whereby clonidine prevents the development of insulin resistance that occurs in dogs fed a high fat diet. However, because of previous studies and our present study design, some inferences can be made into the mechanism of insulin resistance. Insulin-mediated glucose uptake is determined both by the ability of insulin to stimulate glucose extraction at the level of tissues and cells and by the rate of glucose and insulin delivery (blood flow). Thus, the relative contributions of tissue and blood flow actions of insulin will determine the overall rate of glucose uptake (ie, degree of insulin resistance). Baron et al\textsuperscript{22} observed that the reduced rate of insulin-mediated glucose uptake that occurs in non–insulin-dependent diabetes mellitus, obesity, and hypertension may be due in large part to an impairment in the action of insulin to increase skeletal muscle blood flow. In the present and other studies,\textsuperscript{1} we have demonstrated that obese dogs have a reduced ability of euglycemic hyperinsulinemia to increase cardiac output (Figure 3). Because clonidine treatment enabled euglycemic hyperinsulinemia to cause a dose-dependent increase in cardiac output even after the dogs gained weight, we believe that the increased blood flow responses to insulin may have contributed to the improvement in insulin resistance with clonidine. Vollweider et al\textsuperscript{23} demonstrated that insulin resistance in obese subjects is associated, in skeletal muscle, with a specific impairment of sympathetic neural and vasodilatory responsiveness to hyperinsulinemia. The impairment of insulin to increase skeletal muscle blood flow in obesity and in non–insulin-dependent diabetes is speculated to be related to an abnormality in the nitric oxide system.\textsuperscript{24} Insulin is known to interact with the sympathetic nervous system at the vascular level, predominantly through the $\alpha_2$-adrenergic pathway.\textsuperscript{25} Lacolley et al\textsuperscript{26} demonstrated that the sympathetic nervous system plays an important role in modulating the synthesis, release, or both of vascular nitric oxide. Thus, it is possible that clonidine treatment of dogs fed a high fat diet may have prevented insulin resistance by blocking the sympathetically mediated reduced vasodilator responsiveness to insulin known to occur in obesity.

Besides the affect of the sympathetic nervous system on glucose uptake by reducing blood flow, there is evidence that the sympathetic nervous system can directly influence the cellular uptake of glucose. Takahashi et al\textsuperscript{27} demonstrated in the rat that ventromedial hypothalamic stimulation can alter peripheral glucose uptake at the cellular level. Catecholamine treatment of rat adipocytes has also been demonstrated to reduce the tyrosine kinase activity of the insulin receptor.\textsuperscript{28} Finally, because clonidine is known to suppress free fatty acid levels,\textsuperscript{29} it is possible that glucose uptake in our obese dogs was improved by increasing oxidative glucose metabolism.

In summary, the results of the present study document that clonidine treatment of dogs fed a high fat diet blocks the development of both hypertension and insulin resistance even though the dogs still gained weight. Further studies will be necessary to better clarify how clonidine is able to dissociate weight gain from both hypertension and insulin resistance.

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References

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