Interactions of Captopril and Verapamil on Glucose Tolerance and Insulin Action in an Animal Model of Insulin Resistance

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We have shown previously that the combination of a long-acting, non–sulfhydryl-containing angiotensin-converting enzyme (ACE) inhibitor (trandolapril) and the Ca^{2+} channel blocker verapamil improve insulin-stimulated glucose transport in skeletal muscle of the obese Zucker rat, a model of insulin resistance, hyperinsulinemia, and dyslipidemia. In the present study, we investigated the interactions of chronic treatment (28 days) with verapamil (20 mg/kg) and a short-acting, sulfhydryl-containing ACE inhibitor (captopril, 50 mg/kg) in combination on insulinemia, lipidemia, glucose tolerance, and insulin action on skeletal muscle glucose transport (2-deoxyglucose uptake in epitrochlearis) in lean and obese Zucker rats. In lean animals, verapamil alone and in combination with captopril actually increased ($P < .05$) plasma insulin, whereas in obese animals, verapamil alone worsened the hyperinsulinemia already present, and this effect was abolished by cotreatment with captopril. Captopril alone or in combination with verapamil reduced plasma free fatty acid (FFA) levels in obese rats, but not in lean rats. Captopril alone reduced the glucose-insulin index in obese animals given an oral glucose load, and this was associated with a significant increase in insulin-mediated muscle glucose transport. The greatest improvement in these responses was elicited in obese animals receiving combined captopril and verapamil treatment, and was associated with increases in muscle GLUT-4 glucose transporter protein and hexokinase and citrate synthase activities. In conclusion, these findings indicate that the short-acting, sulfhydryl-containing ACE inhibitor captopril can elicit beneficial metabolic effects on the hyperinsulinemia, dyslipidemia, glucose intolerance, and insulin resistance of muscle glucose transport of the obese Zucker rat. Moreover, there is a positive interactive effect on these pathophysiological parameters between captopril and verapamil in this animal model of insulin resistance.

INDIVIDUALS WITH essential hypertension frequently display a clustering of atherogenic risk factors, including insulin resistance of whole-body glucose disposal, hyperinsulinemia, dyslipidemia, and central adiposity, a condition referred to as the insulin resistance syndrome or syndrome X.[1,2] The insulin resistance and its accompanying hyperinsulinemia are thought to be important factors in the development of this condition,[1-4] and can themselves contribute, directly or indirectly, to a greater risk of cardiovascular disease.[5-7] Therefore, any intervention that may ameliorate insulin resistance may also help to reduce this increased risk of cardiovascular disease.

Several animal model[8-11] and clinical[4,8,12-17] investigations have demonstrated that chronic treatment with angiotensin-converting enzyme (ACE) inhibitors leads to improvements in insulin-stimulated whole-body or muscle glucose disposal. Whereas Ca^{2+} channel blockers, which may be administered together with ACE inhibitors in the treatment of hypertension, are themselves essentially metabolically neutral,[4,10,18,19] recent evidence indicates that Ca^{2+} channel blockers, such as verapamil, can enhance the beneficial effects of long-acting, non–sulfhydryl-containing ACE inhibitors (trandolapril) on ameliorating insulin resistance.[10] This interactive effect of trandolapril and verapamil on muscle glucose transport was associated with enhanced levels of glucose transporter (GLUT-4) protein level and total hexokinase activity, indicating a greater capacity to transport and phosphorylate glucose following combined treatment. However, it is currently unknown whether similar cellular adaptations underlie the effect of the short-acting, sulfhydryl-containing ACE inhibitor, captopril, or whether the interactive effect on insulin action between captopril and verapamil also occurs. It would be important to know whether this observation represents a general or specific effect of ACE inhibitors, or whether the interactive effect is restricted to long-acting, non–sulfhydryl-containing ACE inhibitors.

In this context, the present study was designed (1) to characterize the effect of chronic (28 days) treatment with the short-acting, sulfhydryl-containing ACE inhibitor, captopril, on insulinemia, lipidemia, oral glucose tolerance, and insulin-stimulated skeletal muscle glucose transport activity in lean and obese Zucker rats, the latter being a well-established model of insulin resistance, hyperinsulinemia, and dyslipidemia[20]; (2) to determine if these effects of chronic captopril treatment are modulated by simultaneous treatment with the Ca^{2+} channel blocker, verapamil; and (3) to determine the accompanying adaptations in muscle GLUT-4 protein level and marker enzymes of intracellular glucose phosphorylation capacity (hexokinase) and oxidative capacity (citrate synthase).

MATERIALS AND METHODS

Animals and Chronic Treatments

All procedures described here were approved by the University of Arizona Animal Use and Care Committee. Female obese Zucker rats (Hsd/Ola:ZUCKER-fa; Harlan, Indianapolis, IN) and lean littermates (Fa/−) were received at 6 to 7 weeks of age and were housed two per cage in a temperature-controlled room (20 to 22°C) at the Central Animal Facility of the University of Arizona. A 12-:12-hour light-dark cycle was maintained and animals had free access to water and chow (Purina, St Louis, MO). Starting at 8 weeks of age, lean and obese animals received one of the following treatments by gavage for 28 consecutive days: vehicle (water) at 3.0 mL/kg body weight, captopril (50 mg/kg), verapamil (20 mg/kg), or a combination of captopril and verapamil.

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Oral Glucose Tolerance Tests

Animals were food-restricted (4 g of chow given at 5 PM) the evening before the experiment. Between 8 AM and 10 AM, approximately 20 hours after the most recent treatment (to avoid the known acute effects of captopril on insulin action[9]), the animals underwent an oral glucose tolerance test (OGTT) using a 1-g/kg body weight glucose feeding by gavage.[21] Blood was drawn from a cut at the tip of the tail at 0, 15, 30, and 60 minutes following the glucose feeding. This whole blood was thoroughly mixed with EDTA (18 mmol/L final concentration) and centrifuged at 13,000 × *g* to separate the plasma. Plasma samples were analyzed for glucose (Sigma), insulin (Linco Research, St Charles, MO), and free fatty acid (FFA; Wako, Richmond, VA). Immediately after the completion of the OGTT, all animals received 2 mL of sterile 0.9% saline subcutaneously to compensate for plasma loss and treatments resumed for 2 further days.

Glucose Transport Activity

At 8 AM, approximately 20 hours after the final drug treatment and having been restricted to 4 g of chow in the previous 15 hours, animals were deeply anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally). Both epitrochlearis muscles were surgically removed and prepared for in vitro incubation. Epitrochlearis muscles were initially incubated (without tension throughout) for 60 minutes in 3 mL of oxygenated Krebs-Henseleit buffer (KHB) containing 8 mmol/L glucose, 32 mmol/L mannitol, and 0.1% bovine serum albumin (BSA; radioimmunoassay grade). One muscle from each animal was incubated in the absence of insulin, while the contralateral muscle was incubated in medium containing a maximally effective concentration of pork insulin (2 mU/mL; Eli Lilly, Indianapolis, IN). The flasks were shaken in a Dubnoff incubator at 37°C and had a gas phase of 95% O_2:5% CO_2. Following the initial treatments, all muscles were rinsed for 10 minutes at 37°C in 3 mL of oxygenated KHB containing 40 mmol/L mannitol, 0.1% BSA, and, if present previously, insulin. The muscles were then transferred to flasks containing 2 mL of oxygenated KHB, 0.1% BSA, 1 mmol/L 2-deoxy [1,2-^{3}H]glucose (2-DG) (300 mCi/mol; Sigma), 39 mmol/L [U-^{14}C]mannitol (0.8 mCi/mol) (ICN Radiochemicals, Irvine, CA), and insulin, if present previously. After this final 20-minute incubation period at 37°C, muscles were trimmed of fat, extraneous muscle, and connective tissue, frozen between aluminum blocks cooled to the temperature of liquid N_2, and weighed.

This frozen muscle was divided into two pieces and each piece was weighed. One piece was dissolved in 0.5 mL of 0.5 mol/L NaOH and used to determine glucose transport activity as described by Henriksen and Ritter.[22] Incubated epitrochlearis muscles of this size remain metabolically viable[23] and this method for assessing glucose transport activity in the epitrochlearis muscles has been validated.[24]

Muscle Biochemistry

The remaining muscle piece was homogenized in 40 vol of ice-cold buffer containing 20 mmol/L HEPES (pH 7.4), 1 mmol/L EDTA, and 250 mmol/L sucrose. The homogenates were then frozen at −70°C until analysis of total protein concentration (bicinchoninic acid method; Sigma), GLUT-4 protein, [25] total hexokinase activity,[26] and citrate synthase activity.[27]

Statistical Analysis

All data are presented as means ± SE. The significance of differences between multiple groups was assessed by ANOVA with Duncan's multiple-range post-hoc tests. Statistical significance was set at the .05 probability level.

RESULTS

Plasma Glucose, Insulin, and FFA

As shown in Table 1, there were no significant differences within the lean or obese animals between the various treatment groups for body weight, epitrochlearis muscle weight, or plasma glucose concentration. In the lean animals, chronic administration of verapamil caused a 64% ($P < .05$) increase in plasma insulin, and this elevation in insulin was still observed when captopril was coadministered with verapamil. Neither captopril nor verapamil significantly altered plasma FFA levels in lean animals.

Plasma insulin was 15-fold higher and plasma FFA levels were nearly twofold higher in obese control animals compared with lean control animals. Captopril treatment resulted in a nonsignificant decrease (14%) in plasma insulin in obese animals. As in lean animals, verapamil treatment in obese animals caused a significant increase (33%, $P < .05$) in plasma insulin. However, this verapamil-induced response was completely prevented by coadministration of captopril, with insulin values being 20% lower than obese control values. Whereas plasma FFA levels were not significantly affected by captopril or verapamil treatments in lean animals, in obese animals, captopril alone lowered FFA levels by 30% ($P < .05$) and verapamil actually increased this variable by 20% ($P < .05$). This verapamil-induced elevation in plasma FFA levels was completely prevented by captopril coadministration.

OGTT Responses

In lean animals, treatment with captopril alone or verapamil alone had no significant effect on glucose disposal during the

Table 1. Final Body Weights, Epitrochlearis Wet Weights, and Plasma Glucose, Insulin, and FFA Levels Following Chronic Administration of Captopril and Verapamil to Lean and Obese Zucker Rats

Group	Final Body Weight (g)	Epitrochlearis Wet Weight (mg)	Plasma Glucose (mg/dL)	Plasma Insulin (μU/mL)	Plasma FFA (mEq/L)
Lean vehicle-treated	179 ± 5	40 ± 2	127 ± 5	11 ± 1	0.69 ± 0.04
Lean captopril-treated	178 ± 5	41 ± 2	127 ± 5	12 ± 1	0.74 ± 0.07
Lean verapamil-treated	195 ± 4	40 ± 1	132 ± 7	18 ± 3*	0.83 ± 0.08
Lean captopril + verapamil-treated	183 ± 6	40 ± 1	134 ± 4	18 ± 2*	0.84 ± 0.05
Obese vehicle-treated	372 ± 5	46 ± 2	133 ± 4	167 ± 27	1.22 ± 0.09
Obese captopril-treated	361 ± 8	46 ± 2	129 ± 3	144 ± 8	0.86 ± 0.05*
Obese verapamil-treated	360 ± 11	42 ± 2	138 ± 10	222 ± 13*	1.46 ± 0.05*
Obese captopril + verapamil-treated	381 ± 4	42 ± 2	128 ± 6	134 ± 25	1.09 ± 0.04

NOTE. Values are means ± SE for 5 to 10 animals per group.
*$P < .05$ *v* corresponding vehicle-treated group.

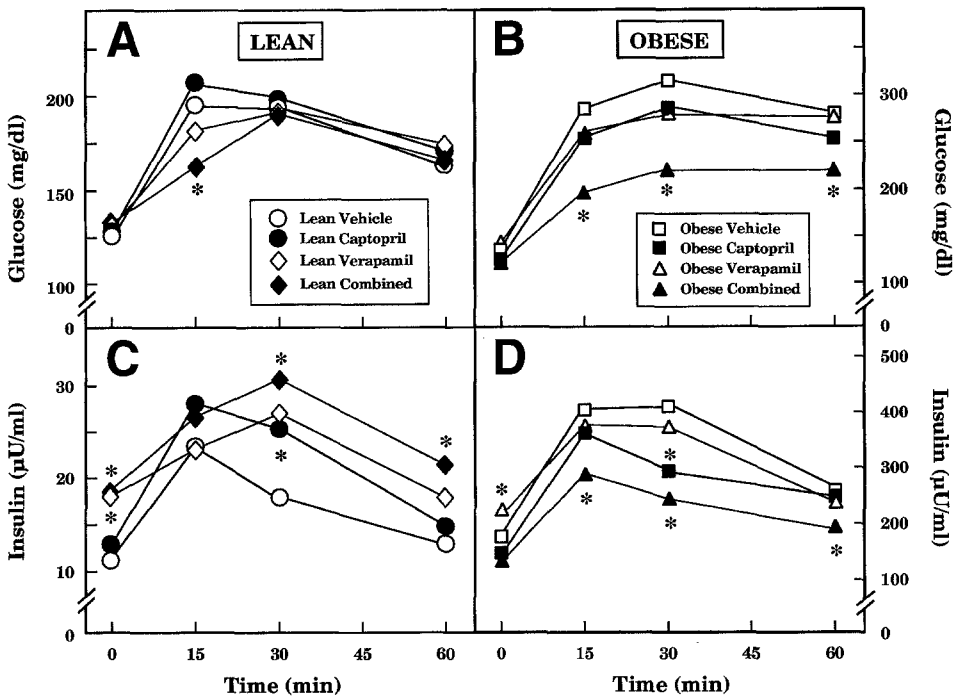

Fig 1. Effects of chronic treatment on glucose tolerance in lean and obese Zucker rats. Animals were treated with either vehicle, captopril (50 mg/kg), verapamil (20 mg/kg), or captopril and verapamil in combination and the glucose and insulin responses to a 1-g/kg oral glucose load assessed. Values are means for 5 to 10 animals per group. SE values (not shown) ranged from 2.6% to 8.8% of the mean for glucose determinations and from 4.1% to 18.8% of the mean for insulin determinations. *$P < .05$ *v* vehicle-treated group at same time point.

OGTT (Fig 1A). The combination of captopril and verapamil treatment in lean animals resulted in a significantly lower (17%, $P < .05$) 15-minute glucose value compared with control. However, total areas under the curve (AUC) for glucose responses in lean animals were not significantly affected by any of the treatments (Fig 2A). Insulin values during the OGTT in the lean treatment groups were generally greater than control (Fig 1C), and the insulin AUC was significantly greater than control in lean animals treated with both captopril and verapamil (Fig 2B). The glucose-insulin index, calculated as the product of the glucose and insulin AUCs and an indirect index of peripheral insulin action on glucose disposal,[21] was signifi-

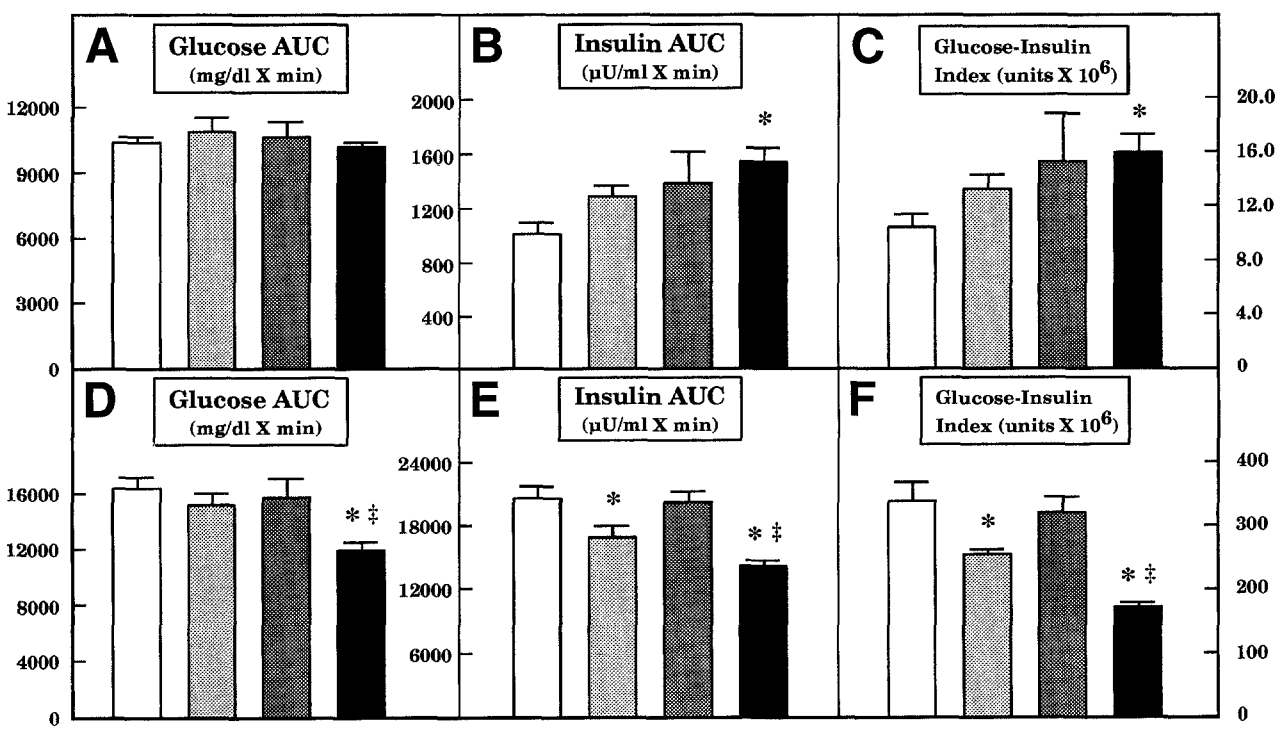

Fig 2. Effects of chronic treatment with vehicle (□), captopril (▨), verapamil (▨), or captopril and verapamil in combination (■) on AUCs for glucose and insulin responses during OGTT in lean (A-C) and obese (D-F) Zucker rats. Total areas under glucose and insulin curves were calculated using data in Fig 1. Glucose-insulin index was calculated as the product of glucose AUC and insulin AUC for each animal. Values are means ± SE for 5 to 10 animals per group. *$P < 0.05$ *v* respective vehicle-treated group; ‡$P < .05$, combined treatment *v* captopril-treated group.

cantly increased (indicating worsened insulin resistance) in this latter group compared with the lean control group (Fig 2C).

Glucose and insulin AUCs in the obese control animals were 1.6-fold and 20-fold greater ($P < .05$), respectively, than those values in the lean control group. In obese animals, treatment with verapamil alone or captopril alone had no significant effect on glucose response during the OGTT (Fig 1B, and Fig 2D). Combined treatment of obese animals with captopril and verapamil resulted in a 27% decrease ($P < .05$) in the glucose AUC (Fig 2D), so that this parameter was only 15% greater than the lean control value. The insulin response was not altered in obese animals by verapamil treatment. Treatment of obese animals with captopril alone caused a significant (16%, $P < .05$) decrease in the insulin AUC, and combined treatment with both captopril and verapamil led to a 31% ($P < .05$) lower insulin AUC relative to obese control values (Fig 2E). The insulin AUC in the combined obese group was 18% less ($P < .05$) than that in the captopril-treated obese group, but still remained 14-fold greater than the lean control value.

The glucose-insulin index was more than 31-fold greater ($P < .05$) in the obese control animals compared to the lean control animals. Whereas the glucose-insulin index in the obese verapamil-treated group was not different from the obese control group, captopril treatment of obese animals resulted in a 23% lower ($P < .05$) value for this parameter (Fig 2F). The greatest decrease in the glucose-insulin index was observed in the combined obese group, with this parameter being 50% lower ($P < .05$) than in the obese control group, and also significantly less than in the obese group receiving captopril alone. The difference in the glucose-insulin index between the lean and obese groups was reduced by more than half following treatment of the obese group with both captopril and verapamil. These data imply that peripheral insulin action on glucose disposal was enhanced in the obese animals receiving the ACE inhibitor alone, with the most marked effect being observed in obese animals receiving both ACE inhibitor and the Ca^{2+} channel blocker.

Muscle Adaptive Responses

In all of the groups studied, the rate of 2-deoxyglucose uptake in the absence of insulin was not altered by any of the interventions (Fig 3). In lean animals, chronic treatment with either captopril alone, verapamil alone, or the two interventions in combination had no significant effect on insulin-mediated glucose transport activity in the isolated epitrochlearis muscle (Fig 3). Insulin-mediated glucose transport activity in muscle from the obese control group was less than half of that observed in the lean control group. In obese animals, chronic treatment with verapamil did not alter insulin action on muscle glucose transport activity (Fig 3). In contrast, chronic treatment with captopril significantly increased (67%, $P < .05$) insulin-mediated glucose transport activity in the epitrochlearis muscle compared with the obese control group. Treatment of obese animals with captopril and verapamil in combination induced the greatest enhancement (84%, $P < .05$) of insulin-mediated glucose transport activity, and improved this parameter to 84% of the value in the lean control group.

No alterations in total hexokinase activity, citrate synthase activity, or GLUT-4 protein levels were induced by the interventions in epitrochlearis muscle of lean animals (Table 2).

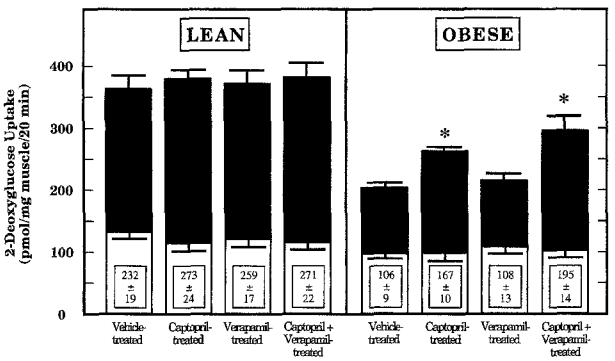

Fig 3. Effect of chronic treatment on skeletal muscle glucose transport activity in lean and obese Zucker rats. Animals were treated with either vehicle, captopril (50 mg/kg), verapamil (20 mg/kg), or captopril and verapamil in combination, and 2-deoxyglucose uptake in the isolated epitrochlearis muscle was determined in the absence (open portion of bar) or presence of (total height of bar) of 2 mU/mL insulin. Black portion of the bar represents the net effect of insulin above basal and is shown numerically in the box at the bottom of each bar. Values are means ± SE for 5 to 10 animals per group. *$P <$ .05 *v* obese vehicle-treated group.

However, in muscle of obese animals, hexokinase was significantly ($P < .05$) elevated by captopril treatment alone (33%) and in combination with verapamil (43%). Citrate synthase activity (19%) and GLUT-4 protein levels (36%) were significantly ($P < .05$) increased only in obese animals receiving captopril and verapamil in combination.

DISCUSSION

In the present study, we have extended our previous investigations[9,10] of the chronic effects of ACE inhibitor treatment on insulin action in the obese Zucker rat, an animal model of insulin resistance of glucose metabolism, glucose intolerance, hyperinsulinemia, and dyslipidemia.[20] We investigated the effect of chronic administration of the short-acting, sulfhydryl-containing ACE inhibitor, captopril, with and without coadministration of the Ca^{2+} channel blocker verapamil, on whole-body glucose tolerance, insulinemia, and lipidemia, on insulin action on skeletal muscle glucose transport activity, and on associated adaptive responses of glucose transporter protein level and

Table 2. Hexokinase and Citrate Synthase Activities and GLUT-4 Protein Level in Epitrochlearis Muscle Following Chronic Administration of Captopril and Verapamil to Lean and Obese Zucker Rats

Group	Hexokinase (μmol/mg/min)	Citrate Synthase (μmol/mg/min)	GLUT-4 Protein (relative units)
Lean vehicle-treated	1.29 ± 0.04	12.9 ± 0.7	100 ± 6
Lean captopril-treated	1.15 ± 0.03	12.9 ± 0.3	108 ± 5
Lean verapamil-treated	1.21 ± 0.05	14.1 ± 0.6	113 ± 7
Lean captopril + verapamil-treated	1.11 ± 0.03	14.4 ± 0.6	100 ± 16
Obese vehicle-treated	0.82 ± 0.06	13.4 ± 0.4	100 ± 4
Obese captopril-treated	1.09 ± 0.07*	12.5 ± 0.4	112 ± 5
Obese verapamil-treated	1.00 ± 0.12	13.2 ± 1.1	96 ± 8
Obese captopril + verapamil-treated	1.17 ± 0.08*	15.9 ± 0.6*	136 ± 16*

NOTE. Values are means ± SE for 5 to 10 animals per group.
*$P <$ .05 *v* corresponding vehicle-treated group.

enzymes associated with intracellular glucose phosphorylation and oxidation. The primary new information in this study is that chronic captopril administration in the insulin-resistant obese Zucker rat enhanced whole-body glucose disposal and decreased the insulin response following an oral glucose load (Fig 2), and this improvement was associated with a greater effect of insulin to stimulate glucose transport activity in isolated skeletal muscle (Fig 3) and a significant lowering of plasma FFA levels (Table 1). Moreover, the beneficial effects of chronic captopril administration on the insulin response during an OGTT (Fig 1), on the glucose-insulin index (Fig 2), and on insulin action on muscle glucose transport (Fig 3) were further enhanced when the Ca^{2+} channel blocker verapamil was simultaneously provided. These findings indicate that the combination of a sulfhydryl-containing ACE inhibitor and a Ca^{2+} channel blocker may provide a greater metabolic benefit in conditions of insulin resistance than either intervention individually.

We have also identified specific muscle cellular adaptive responses to chronic administration of captopril with and without verapamil. In obese animals, captopril alone induced an increase in total hexokinase level in the epitrochlearis muscle (Table 2), indicating that the muscle's ability to phosphorylate the greater amount of glucose transported in response to insulin is likely not compromised. This enhanced activity of hexokinase was also observed when captopril was administered in combination with verapamil. The level of GLUT-4 protein and activity of citrate synthase (an index of muscle oxidative capacity) were also increased in muscle from obese animals chronically treated with both captopril and verapamil (Table 2). Thus, in this combined treatment group, there appeared to be a coordinated upregulation of the muscle's ability to transport, phosphorylate, and oxidize glucose.

These local adaptive responses in skeletal muscle of the obese Zucker rat to chronic combined captopril and verapamil treatment are similar to recent findings on combined trandolapril, a long-acting, non–sulfhydryl-containing ACE inhibitor, and verapamil treatment in this same animal model[10] and also in the spontaneously hypertensive rat.[11] This implies that the presence or absence of sulfhydryl groups in the ACE inhibitor and the ACE inhibitor's pharmacokinetics are likely not a critical factors in the ability of the ACE inhibitor to interact with simultaneous Ca^{2+} channel blocker treatment and elicit the increases in GLUT-4 protein, hexokinase, and citrate synthase. Moreover, the present findings extend our previous investigations on ACE inhibitor treatment alone[9,10] and in combination with a Ca^{2+} channel blocker[10] by demonstrating that the local muscle adaptations in glucose transport capacity are of physiological relevance in that they are associated with a marked improvement in the animal's ability to dispose of an oral glucose load.

The exact mechanism of action underlying captopril's ability to enhance insulin-stimulated muscle glucose transport and glucose tolerance in the insulin-resistant obese Zucker rat is not apparent from the present findings. Several possibilities exist. First, while captopril treatment did not significantly enhance the muscle level of GLUT-4 protein (Table 2), it is possible that insulin's ability to cause translocation of the existing pool of GLUT-4, a process that is defective in muscle of the obese Zucker rat,[28,29] was increased following captopril treatment. Second, captopril possesses antioxidant properties and can act as a free radical scavenger,[30,31] and we have shown that chronic administration of antioxidants to obese Zucker rats improves insulin-stimulated glucose transport in skeletal muscle.[32,33] Third, captopril treatment resulted in a substantial decrease in circulating FFA levels (Table 1), and it is known that FFA levels are inversely related to rates of insulin-stimulated skeletal muscle glucose disposal (see review by Boden[34]). It is possible that the enhanced insulin action elicited by chronic captopril was secondary to the decrease in plasma FFA levels.

It is documented that administration of captopril enhances circulating bradykinin levels in rodents and humans,[8,35] and several studies have demonstrated that direct bradykinin administration can improve insulin action on glucose uptake by muscle.[11,36,37] Importantly, it has also been shown recently that bradykinin B_2 receptors are present in the sarcolemmal membrane of rat skeletal muscle.[38] We have found that chronic treatment of obese Zucker rats with bradykinin causes a significant improvement in whole-body disposal of an oral glucose load, as well as an enhancement of insulin-mediated glucose transport activity by skeletal muscle and a reduction in circulating FFA levels.[39] These findings are consistent with the concept that bradykinin could also be a potential mediator of the beneficial effects of captopril on whole-body and muscle insulin action on glucose disposal. It is clear that further investigations regarding the metabolic effects of bradykinin in models of insulin-resistance are warranted.

It is noteworthy that the beneficial effects of chronic treatment with captopril alone and the combination of captopril and verapamil were observed only in obese animals and not in lean animals. Indeed, the chronic treatment of lean Zucker rats with captopril and verapamil actually caused an increase in basal and glucose-stimulated plasma insulin levels (Table 1 and Fig 1). These findings indicate that treatment with ACE inhibitors alone and in combination with a Ca^{2+} channel blocker overcomes a defect specific to the insulin-resistant animal. While this specific defect is remains unclear, it may involve systemic and/or local kallikrein-kinin systems.

In conclusion, we have demonstrated that chronic administration of the short-acting, sulfhydryl-containing ACE inhibitor, captopril, can improve whole-body glucose tolerance in the obese Zucker rat, an animal model of insulin resistance, hyperinsulinemia, glucose intolerance, and dyslipidemia. This effect is likely mediated by an increase in insulin action on skeletal muscle glucose transport and by a reduction in circulating FFA levels. Moreover, the greatest improvements in glucose tolerance, insulin action on muscle glucose transport, and lipidemia were brought about when captopril was chronically administered in combination with the Ca^{2+} channel blocker verapamil. The results from the present and previous[10] findings support the contention that substantial improvements in several metabolic defects associated with insulin resistance of muscle-glucose disposal may be realized by combining an ACE inhibitor with a Ca^{2+} channel blocker.

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REFERENCES

1. Reaven GM: Role of insulin resistance in human disease (syndrome X): An expanded definition. Annu Rev Med 44:121-131, 1993
2. DeFronzo RA, Ferrannini E: Insulin resistance: A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991
3. Foster DW: Insulin resistance—A secret killer? N Engl J Med 320:733-734, 1989
4. Pollare T, Lithell H, Berne C: A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. N Engl J Med 321:868-873, 1989
5. Alberti KG: The clinical implications of impaired glucose tolerance. Diab Med 13:927-937, 1996
6. Haffner SM: Cardiovascular risk factors and the prediabetic syndrome. Ann Med 28:363-370, 1996
7. King GL: The role of hyperglycaemia and hyperinsulinaemia in causing vascular dysfunction in diabetes. Ann Med 28:427-32, 1996
8. Uehara M, Kishikawa H, Isami S, et al: Effect on insulin sensitivity of angiotensin converting enzyme inhibitors with or without a sulphydryl group: Bradykinin may improve insulin resistance in dogs and humans. Diabetologia 37:300-307, 1994
9. Henriksen EJ, Jacob S: Effects of captopril on glucose transport activity in skeletal muscle of obese Zucker rats. Metabolism 44:267-272, 1995
10. Jacob S, Henriksen EJ, Fogt DL, et al: Effects of trandolapril and verapamil on glucose transport in insulin-resistant rat skeletal muscle. Metabolism 45:535-541, 1996
11. Leighton B, Sanderson AL, Young ME, et al: Effects of treatment of spontaneously hypertensive rats with the angiotensin-converting enzyme inhibitor trandolapril and the calcium antagonist verapamil on the sensitivity of glucose metabolism to insulin in rat soleus muscle in vitro. Diabetes 45:S120-124, 1996 (suppl 1)
12. Ferriere M, Lachkar H, Richard JL, et al: Captopril and insulin sensitivity. Ann Intern Med 102:134-135, 1985
13. Gans ROB, Biol HJG, Nauta JJP, et al: The effect of angiotensin-I converting enzyme inhibition on insulin action in healthy volunteers. Eur J Clin Invest 21:527-533, 1991
14. Jauch KW, Hartl W, Günther B, et al: Captopril enhances insulin responsiveness of forearm muscle tissue in non–insulin-dependent diabetes mellitus. Eur J Clin Invest 17:448-454, 1987
15. Shieh S-M, Sheu WH-H, Shen DD-C, et al: Improvements in metabolic risk factors for coronary heart disease associated with cilazapril treatment. Am J Hypertens 5:506-510, 1992
16. Torlone E, Rambotti AM, Perriello G, et al: ACE-inhibition increases hepatic and extrahepatic sensitivity to insulin in patients with type 2 (non–insulin-dependent) diabetes mellitus and arterial hypertension. Diabetologia 34:119-125, 1991
17. Vuirinen-Markkola H, Yki-Järvinen H: Antihypertensive therapy with enalapril improves glucose storage and insulin sensitivity in hypertensive patients with non–insulin-dependent diabetes mellitus. Metabolism 44:85-89, 1995
18. Guigliano D, Saccomanno F, Paolisso G, et al: Nicardipine does not cause deterioration of glucose homeostasis in man: A placebo controlled study in elderly hypertensives with and without diabetes mellitus. Eur J Clin Pharmacol 43:39-45, 1992
19. Lithell HOL: Effect of antihypertensive drugs on insulin, glucose, and lipid metabolism. Diabetes Care 14:203-209, 1991
20. Mathe D: Dyslipidemia and diabetes: Animal models. Diab Metab 21:106-111, 1995
21. Cortez MY, Torgan CE, Brozinick JT, et al: Insulin resistance of obese Zucker rats exercise trained at two difference intensities. Am J Physiol 261:E613-E619, 1991
22. Henriksen EJ, Ritter LS: Effect of soleus unweighting on stimulation of insulin-independent glucose transport activity. J Appl Physiol 74:1653-1657, 1993
23. Gulve EA, Henriksen EJ, Rodnick KJ, et al: Glucose transporters and glucose transport in skeletal muscles of 1 to 25 month old rats. Am J Physiol 264:E319-E327, 1993
24. Hansen PA, Gulve EA, Holloszy JO: Suitability of 2-deoxyglucose for in vitro measurement of glucose transport activity in skeletal muscle. J Appl Physiol 76:979-985, 1994
25. Henriksen EJ, Halseth AE: Early alterations in soleus GLUT-4, glucose transport, and glycogen in voluntary running rats. J Appl Physiol 76:1862-1867, 1994
26. Uyeda K, Racker E: Regulatory mechanisms in carbohydrate metabolism. VII. Hexokinase and phosphofructokinase. J Biol Chem 240:4682-4688, 1965
27. Srere PA: Citrate synthase. Methods Enzymol 13:3-5, 1969
28. Etgen GJ, Wilson CM, Jensen J, et al: Glucose transport and cell surface GLUT-4 protein in skeletal muscle of the obese Zucker rat. Am J Physiol 271:E294-301, 1996
29. King PA, Horton ED, Hirshman MF, et al: Insulin resistance in obese Zucker rat (fa/fa) is associated with a failure of glucose transporter translocation. J Clin Invest 90:1568-1575, 1993
30. Chopra M, Beswick H, Clapperton M, et al: Antioxidant effects of angiotensin-converting enzyme (ACE) inhibitors: Free radical and oxidant scavenging are sulfhydryl-dependent, but lipid peroxidation is inhibited by both sulfhydryl- and nonsulfhydryl-containing ACE inhibitors. J Cardiovasc Pharmacol 19:330-340, 1992
31. DeCavanagh EM, Fraga CG, Ferder L, et al: Enalapril and captopril enhance antioxidant defenses in mouse tissues. Am J Physiol 272:R514-R518, 1997
32. Jacob S, Streeper RS, Fogt DL, et al: The antioxidant alpha-lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant skeletal muscle. Diabetes 45:1024-1029, 1996
33. Streeper RS, Henriksen EJ, Jacob S, et al: Differential effects of lipoic acid stereoisomers on glucose metabolism in insulin-resistant skeletal muscle. Am J Physiol 273:E185-E191, 1997
34. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46:3-10, 1997
35. Decarie A, Adam A, Couture R: Effects of captopril and Icatibant on bradykinin (BK) and des [Arg9] BK in carrageenan-induced edema. Peptides 17:1009-1015, 1996
36. Dietze GJ, Wicklmayr M, Rett K, et al: Potential role of bradykinin in forearm muscle metabolism in humans. Diabetes 45:S110-114, 1996 (suppl 1)
37. Mann WR, Villauer EB, Barilla D, et al: Effects of bradykinin on glucose metabolism in isolated rat soleus muscle and on blood glucose levels in Ob/Ob mice. Diabetes 44:133A, 1995 (suppl 1)
38. Figueroa CD, Dietze G, Müller-Esterl W: Immunolocalization of bradykinin B_2 receptors on skeletal muscle cells. Diabetes 45:S24-S28, 1996 (suppl 1)
39. Henriksen EJ, Jacob S, Fogt DL, et al: Effect of chronic bradykinin administration on insulin action in an animal model of insulin resistance. Am J Physiol (in press)