Captopril Enhanced Insulin-Stimulated Glycogen Synthesis in Skeletal Muscle But Not Fatty Acid Synthesis in Adipose Tissue of Hereditary Hypertriglyceridemic Rats

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In addition to their hypotensive action, angiotensin-converting enzyme (ACE) inhibitors exert a beneficial effect on glucoregulation. In the present study, the effect of ACE inhibition by captopril on glucose utilization in peripheral tissues was investigated in non-obese rats with hereditary hypertriglyceridemia (HHTg) associated with hyperinsulinemia and insulin resistance. Normotriglyceridemic Wistar rats served as controls (C). Rats of both groups received a high-sucrose diet, and a half of each group also captopril in drinking water (10 mg/kg body weight [bw]) for 2 weeks. Captopril administration reduced fasting glycemia and postprandial triglyceridemia in HHTg rats, while the fasting levels of nonesterified fatty acids (NEFA), glycerol, and lactate were decreased in both groups. The sensitivity of skeletal muscle to insulin action evaluated as in vitro ^{14}C-glucose incorporation into glycogen was significantly increased by captopril treatment both in HHTg (3.51 ± 0.48 *v* 2.0 ± 0.12 μmol glucose/g wet weight [ww]) and C (3.32 ± 0.21 *v* 2.48 ± 0.09 μmol glucose/g ww). In isolated adipose tissue, the insulin-stimulated ^{14}C-glucose incorporation into neutral lipids was increased, after captopril administration, by 137% in C and by 35% only in HHTg. After captopril treatment, insulin-stimulated de novo fatty acid synthesis rose significantly in C while remaining low in HHTg. The increase in esterification was comparable in both groups. Separate experiments were designed to assess the possible involvement of bradykinin in mediating captopril action. Both C and HHTg rats fed a high-sucrose diet for 2 weeks were treated with captopril (50 mg/kg orally) for 1 hour; half of each group received the specific inhibitor of bradykinin receptor HOE-140 (100 μg/kg intraperitoneally [IP]) 1 hour before captopril administration. In C, captopril administration enhanced the insulin-stimulated in vitro glucose incorporation into lipids in adipose tissue by 255%, and into glycogen in the musculus soleus by 45%; this effect was eliminated by HOE-140. In HHTg, neither a single dose of captopril nor HOE-140 had any effect. We conclude that long-term captopril administration increased the insulin sensitivity of peripheral tissue in both C and HHTg rats, but with different efficacy. While the insulin-sensitizing action of captopril on skeletal muscle was comparable in HHTg and C rats, there were differences in the effect of captopril on adipose tissue. The difference became particularly manifest in de novo fatty acid synthesis.

DYSLIPIDEMIA, hyperinsulinemia, peripheral tissue resistance to insulin action, glucose intolerance, and hypertension often cluster into a condition referred to as insulin resistance syndrome.[1] A frequently used intervention for the treatment of high blood pressure have been angiotensin-converting enzyme (ACE) inhibitors. Several studies have demonstrated that, besides their main effect, ACE inhibitors improve glucose homeostasis and insulin action on peripheral glucose disposal. Enhanced insulin-stimulated whole-body glucose disposal after chronic treatment with ACE inhibitors has been reported in several clinical studies.[2-6] The beneficial effect of chronic treatment with ACE inhibitors (not depending on the presence or absence of a sulfhydryl group) on skeletal muscle glucose transport[7-10] and whole-body glucose disposal[10] has been described in obese Zucker rats, spontaneously hypertensive (SHR) rats,[11] and in fructose-fed Sprague-Dawley rats.[12,13] The effect of captopril on the plasma lipid profile is controversial—while, in humans, some studies[2,6,14] found no improvement in the plasma lipid profile after ACE inhibition, other studies reported a reduction in plasma nonesterified fatty acid (NEFA) content in humans[5] and in obese Zucker rats.[10] The improvement of insulin sensitivity of isolated adipocytes has also been reported in SHR rats after long-term enalapril treatment.[15] ACE inhibitors, in addition to inhibiting conversion of angiotensin I to angiotensin II, inhibit kininase II, which leads to decreased bradykinin degradation. Several lines of evidence suggest bradykinin involvement in the mechanism of captopril action on insulin-stimulated glucose disposal. Acutely administered bradykinin improved glucose disposal in obese Zucker rats during a glucose tolerance test.[8] In insulin-resistant dogs, infusion of a bradykinin antagonist abolished the positive effect of captopril during a hyperinsulinemic euglycemic clamp,[4] and bradykinin increased insulin-stimulated glucose uptake into isolated dog adipocytes.[16] Damas et al[17] showed that captopril had no effect, during the clamp, on kininogen-deficient rats unable to synthesize bradykinin.

A unique model of insulin resistance, the non-obese hereditary hypertriglyceridemic rat (HHTg), was selected in our laboratory for the current study. This strain exhibits elevated triglyceridemia, especially when fed a simple carbohydrate-rich diet. A number of other previously recognized facets of the metabolic syndrome, such as hyperinsulinemia, resistance to insulin action, impaired glucose tolerance, and elevated blood pressure, have been observed in this HHTg strain.[18] The HHTg rat is a model different from the usual rat models of insulin resistance such as Zucker obese rats, SHR rats, or short-term sucrose-fed rats. They are non-obese, do not exhibit hyperphagia, and show milder hypertension compared to SHR rats. The specific aim of the present study was to compare the effect of

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long-term captopril administration on Wistar and HHTg rats fed a high-sucrose diet. A novel feature of our work, besides the use of the unique HHTg rats model, is the in vitro use of intact adipose tissue and a detailed study of the effect of captopril on 2 different pathways of glucose utilization in intact adipose tissue (de novo fatty acid synthesis and fatty acids esterification). Our study was designed: (1) to asses the effect of chronic captopril treatment on some parameters of carbohydrate and lipid metabolism in vivo; (2) to determine the effect of chronic captopril treatment on glucose utilization in adipose tissue in vitro, especially its division between esterification and de novo fatty acid synthesis; (3) to determine the effect of chronic captopril treatment on in vitro nonoxidative glucose utilization (glycogen synthesis) in the musculus soleus; and (4) to investigate the role of bradykinin in mediating captopril action in skeletal muscle and adipose tissue in HHTg and controls.

MATERIALS AND METHODS

Animals and Experimental Protocol

All procedures described here were approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine. Male HHTg and control Wistar rats (C) were kept in a temperature-controlled room. Animals had free access to drinking water and diet. Wistar rats were obtained from the Institute of Physiology, Czech Academy of Sciences, Czech Republic. The strain of HHTg rats was originally selected from Wistar strain rats in our laboratory.[18] Starting at 4 months of age (C: 335 ± 14 g; HHTg: 325 ± 15 g), all animals were fed a high-sucrose diet (70% of calories as sucrose) for 2 weeks. Half of the animals of each group received captopril in drinking water (100 mg/L) during this period. The captopril was provided in a water bottle. Before the experiment, the amount of water consumed daily by rats of similar weight on high-sucrose diet was determined to be 33 ± 5 mL. This amount and the weight of the animals at the beginning of the experiment were taken as the basis for calculating the actual amount of captopril consumed. When using starting weight, the actually consumed amount of captopril per day was 9.85 ± 0.5 mg/kg in C and 10.2 ± 0.3 mg/kg in HHTg. One day before the experiment, postprandial triglyceridemia was determined in tail-vein blood. All experiments were performed after overnight fasting (16 hours).

In separate experiments designed to study the role of bradykinin, rats were fed a high-sucrose diet for 2 weeks. On the day of experiment, half of the animals after overnight fasting were pretreated, for 1 hour before the commencement of captopril administration, with HOE-140 (100 μg/kg intraperitoneally [IP]). Captopril (50 mg/kg) was administered by gavage to all animals 1 hour before decapitation.

Muscle and Adipose Tissue Incubation Procedure

Tissue sensitivity to insulin was measured in vitro by ^{14}C-glucose incorporation into lipids (adipose tissue) or glycogen (musculus soleus). The animals were killed after overnight fasting by decapitation and tissues were collected for incubation. Distal parts of epididymal adipose tissue (150 ± 25 mg) and the m. soleus (160 ± 20 mg) were rapidly dissected. The m. soleus was attached to a stainless steel frame in situ at in vivo length and separated from other muscles. The tissues were incubated for 2 hours in Krebs-Ringer bicarbonate buffer with 5 mmol/L glucose, 0.1 μCi (U-^{14}C)-glucose /mL (UVVR, Prague, Czech Republic) and 2% (adipose tissue) or 0.5% (m. soleus) bovine serum albumin, gaseous phase 95% O_2 and 5% CO_2 in the presence (250 μU/mL) or absence of insulin in incubation medium. All incubations were performed at 37°C in sealed vials in a shaking water bath.

Glycogen Synthesis in Isolated Muscle

Glycogen synthesis was determined by ^{14}C-glucose accumulation in glycogen as previously described.[19] After 2 hours of incubation, muscles were removed from incubation medium, briefly rinsed in ice-cold physiological solution, and immediately put into chlorophorm:methanol (2:1). Muscles were digested by boiling in 1 mL 30% KOH containing 10 mg/mL oyster glycogen. After digestion, 2.5 mL of 96% ethanol was added and glycogen was allowed to precipitate at 4°C overnight. The precipitate was centrifuged, washed with 1 mL of 80% ethanol, and dissolved in 1 mL H_2O. Aliquots (0.2 mL) were used for scintillation counting.

Measurement of Lipogenesis and Esterification in Adipose Tissue In Vitro

Estimation of ^{14}C-glucose incorporation into neutral lipids was performed as described previously.[20] Briefly, adipose tissue was removed from incubation medium, rinsed in saline, and immediately put into chlorophorm. The pieces of tissue were dissolved using a Teflon peastle homogenizer, methanol was added (chlorophorm:methanol 2:1), and lipids were extracted at 4°C overnight. On the next day, the rest of tissue was removed and clear extract was taken for further analysis. An aliquot was evaporated, reconstituted in scintillation liquid, and the radioactivity measured by scintillation counting.

The site (glycerol *v* acyl moiety) of glucose incorporated into neutral lipids was determined as described previously[21,22] with slight modifications. Briefly, an aliquot of clear extract was evaporated and saponified in ethanolic 15% potassium hydroxide at 70°C. Saponification was terminated by adding 5.4 mol/L H_2SO_4. After cooling, the released fatty acids were extracted repeatedly into petroleum ether. The pooled petroleum ether fractions of each sample were evaporated, reconstituted in scintillation liquid, and the radioactivity measured by scintillation counting. The amount of radioactivity incorporated into glycerol residue was calculated as the difference of total activity incorporated into neutral lipids and petroleum ether fraction of the same aliquot.

Biochemical Analysis

Serum NEFA, triglyceride, glycerol, and blood glucose contents were determined using commercially available kits (NEFA: FFA half micro test, Roche Diagnostics GmbH, Mannheim, Germany; triglycerides and glycerol: Triglyceride [GPO-Trinder] Sigma Diagnostics, St Louis, MO; glucose: Bio-La-Test Glucose GOD, Lachema, Brno, Czech Republic). Lactate was assayed as described.[23]

Statistical Analysis

Data are presented as the mean ± SE of multiple determinations. Two-way analysis of variance (ANOVA) was used to test for the effect of strain-drug-treatment, and strain-treatment effect. Differences within strain and treatment groups were evaluated by *t* test using InStat (GraphPad Sofware, San Diego, CA). Statistical significance was defined as $P < .05$.

RESULTS

Characteristics of Experimental Groups

Control and HHTg rats fed standard diet differ significantly in all tested parameters (fasting NEFA, glycerol, lactate, and postprandial triglyceridemia) except fasting glycemia. The body weight of animals in both groups did not differ significantly either at the beginning (C: 335 ± 14 g; HHTg: 325 ± 15 g) or at the end (C: 365 ± 15.2; C + captopril: 361 ± 10 g; HHTg: 350 ± 10 g; HHTg + captopril: 358 ± 14.4 g) of the high-sucrose feeding period. Sucrose feeding worsened all

Table 1. Characteristics of C and HHTg Groups

Diet	Control			HHTg		
	Standard	Sucrose	Sucrose + Captopril	Standard	Sucrose	Sucrose + Captopril
Fasting NEFA (mmol/L)	0.78 ± 0.03[a,e]	1.1 ± 0.07[a,c]	0.81 ± 0.05[c]	1.01 ± 0.08[b,e]	1.21 ± 0.05[b,d]	0.92 ± 0.04[d]
Fasting glycerol (mmol/L)	0.29 ± 0.02[a,e]	0.47 ± 0.04[a,c]	0.24 ± 0.03[c]	0.52 ± 0.03[b,e]	0.61 ± 0.02[b,d]	0.34 ± 0.04[d]
Fasting lactate (mmol/L)	3.8 ± 0.11[a,e]	6.01 ± 0.33[a,c]	4.85 ± 0.09[c]	7.8 ± 0.35[b,e]	8.92 ± 0.38[b,d]	4.04 ± 0.22[d]
Fasting glycemia (mmol/L)	3.85 ± 0.04	3.8 ± 0.09	3.9 ± 0.2	4 ± 0.1[b]	4.45 ± 0.13[b,d]	4.11 ± 0.09[d]
Postprandial triglycerides (mmol/L)	1.1 ± 0.1[e]	1.44 ± 0.26	1.27 ± 0.45	2.7 ± 0.23[b,e]	6.35 ± 0.65[b,d]	4.06 ± 0.94[d]

NOTE. Data are given as means ± SE, n = 5 to 7.

Values with the same index are statistically different. [a]Controls on standard *v* sucrose diet; [b]HHTg on standard *v* sucrose diet; [c]captopril-treated *v* untreated C on sucrose diet; [d]Captopril-treated *v* untreated HHTg on sucrose diet; [e]C *v* HHTg on standard diet.

tested parametres in both groups except of fasting glycemia and postprandial triglycerides in controls. Captopril treatment of sucrose-fed rats lowered the NEFA, glycerol, and lactate contents in serum in both groups to values reached on a standard diet; in HHTg, lactate and glycerol contents were decreased even more. Elevated fasting blood glucose and postprandial serum triglyceride contents in HHTg decreased after captopril treatment (Table 1).

Effect of Chronic ACE Inhibition on ^{14}C-Glucose Incorporation Into Glycogen in Skeletal Muscle

Chronic administration of captopril had no effect on in vitro glucose incorporation into glycogen in the medium without insulin in C (2.09 ± 0.26 *v* 1.84 ± 0.21 μmol glucose/g wet weight [ww]) and in HHTg (2.26 ± 0.42 *v* 2.3 ± 0.12 μmol glucose/g ww) either. Captopril treatment in both groups significantly increased insulin-stimulated glycogen synthesis (C: 3.32 ± 0.21 *v* 2.48 ± 0.09 μmol glucose/g ww, $P <$,01; HHTg: 3.51 ± 0.48 *v* 2.0 ± 0.12 μmol glucose/g ww, $P < .01$) (Fig 1).

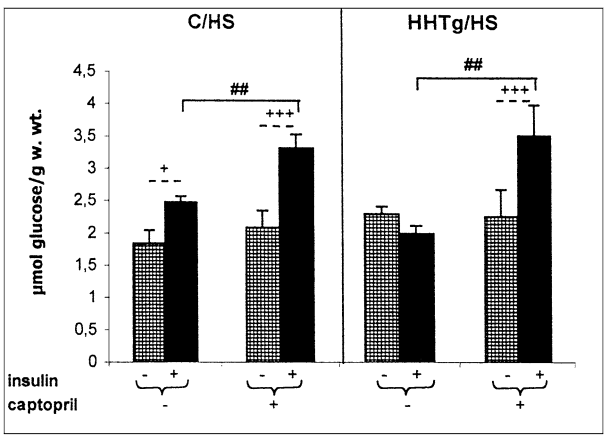

Fig 1. Effect of chronic ACE inhibition on ^{14}C-glucose incorporation into glycogen in skeletal muscle (m. soleus) of C and HHTg rats during incubation in vitro. Captopril (10 mg/kg) was administered for 14 days in drinking water together with a high-sucrose (HS) diet. Insulin (250 μU/mL) was added into incubation medium in vitro. Data are given as means ± SE for 5 to 7 animals per group. +, Statistically significant difference (ssd) between samples incubated in the absence *v* in the presence of insulin (+*P* < .05, +++*P* < .001); #, ssd between captopril-treated *v* untreated animals (##*P* < .01).

Effect of Chronic ACE Inhibition on ^{14}C-Glucose Incorporation Into Neutral Lipids, Acyl Groups, and Glycerol Residues of the Lipid Molecule

Data on ^{14}C-glucose incorporation into total lipids in C and HHTg are summarized in Table 2. The C and HHTg groups differed in the distribution of incorporated glucose between the glycerol residue (esterification) and acyl (de novo fatty acid synthesis) groups of the lipid molecule. Captopril increased both basal and insulin-stimulated esterification in C; in HHTg, it raised significantly only insulin-stimulated esterification. Basal de novo fatty acid synthesis was not influenced by captopril in C or in HHTg. Captopril highly significantly increased insulin-stimulated de novo fatty acid synthesis in C, which remained low in HHTg (Fig 2).

Effect of Acute ACE Inhibition With or Without Bradykinin Receptor Inhibition on ^{14}C-Glucose Incorporation Into Glycogen in Skeletal Muscle

Single-dose administration of captopril affected only insulin-stimulated glucose incorporation into glycogen in C (−insulin: 1.78 ± 0.12; +insulin: 3.59 ± 0.44 μmol glucose/g ww, $P <$ .001). HOE-140 abolished this increase (−insulin: 2.18 ± 0.15; +insulin: 2.38 ± 0.19 μmol glucose/g ww, $P < .05$). In HHTg, single-dose captopril administration or concomitant administration of captopril and HOE-140 did not induce any significant changes (Fig 3).

Effect of Acute ACE Inhibition With or Without Bradykinin Receptor Inhibition on ^{14}C-Glucose Incorporation Into Acyl Groups and Glycerol Residues of the Lipid Molecule In Vitro

Basal ^{14}C-glucose incorporation into adipose tissue lipids in vitro was increased in C ($P < .05$), but was not affected by single-dose captopril administration in HHTg rats. After adding insulin into the incubation medium in vitro, glucose incorporation into lipids after a single dose of captopril rose highly significantly in C ($P < .001$). Administration of HOE-140 significantly attenuated the effect of captopril in C ($P < .05$), while not exerting an effect on insulin-stimulated glucose incorporation into lipids in HHTg (Table 2). In C, single-dose captopril increased both insulin-stimulated glucose incorporation into acyl groups of the lipid molecule and insulin-stimulated esterification, and HOE-140 significantly reduced this effect. In HHTg, de novo fatty acid synthesis was very low and was not affected by single-dose captopril. Esterification was not

Table 2. Incorporation of ^{14}C-Glucose Into Neutral Lipids in Adipose Tissue In Vitro After Chronic or Acute ACE Inhibition

Treatment	C/HS		HHTg/HS	
	− Insulin	+ Insulin	− Insulin	+ Insulin
− Captopril	1.23 ± 0.12[a,b]	2.33 ± 0.26[a,c]	1.35 ± 0.19	1.68 ± 0.23[e]
+ Long-term captopril	2.18 ± 0.13[a,b]	5.53 ± 0.56[a,c]	1.25 ± 0.04[a]	2.27 ± 0.25[a,e]
+ Single-dose captopril	2.95 ± 0.21[a]	8.29 ± 0.04[a,d]	1.57 ± 0.16[a]	2.28 ± 0.21[a]
+ Single-dose captopril + HOE-140	2.58 ± 0.2[a]	4.86 ± 0.32[a,d]	1.73 ± 0.16[a]	2.74 ± 0.19[a]

NOTE. Data are given as means ± SE (in μmol of ^{14}C-glucose/g ww), n = 5 to 7.

Values with the same index are statistically different. [a]Samples incubated without *v* with insulin; [b]C without insulin (− captopril *v* + captopril); [c]C with insulin (− captopril *v* + captopril); [d]C with insulin (+ captopril + HOE-140 *v* + captopril alone); [e]HHTg + insulin (−captopril *v* + captopril).

significantly affected with HOE or HOE + captopril administration in HHTg (Fig 4).

DISCUSSION

In the present study, the effect of chronic or acute ACE inhibition by captopril on insulin action in skeletal muscle and adipose tissue in HHTg rats was compared with that in short-term sucrose-fed controls (C). What was actually new about our study was the fact we used a unique model of genetically fixed insulin resistance, non-obese strain of hereditary hypertriglyceridemic rats. Although most human non–insulin dependent diabetes mellitus patients are obese, there is a significant subgroup of non-obese patients[24] and a model that offers the possibility to study the underlying mechanisms of this type of metabolic disorder may provide relevant data for further clinical study.

We found decreased serum levels of NEFA and glycerol after chronic captopril treatment in both groups, a finding suggesting decreased lipolysis in adipose tissue. In vitro experiments showed that, in adipose tissue, chronic captopril treatment stimulated glucose incorporation into glycerol–triglyceride, ie, esterification, which may represent another mechanism contributing to the lowering of circulating NEFA levels. The lower serum NEFA content can then lead to improvement of the insulin-resistant state as the decreased supply of NEFA to the tissues made the oxidative capacity of the cell available for glucose oxidation.[25-27] This interpretation is supported by the reduction of serum lactate content seen in both groups. Captopril treatment reduced postprandial triglyceridemia in HHTg, which may be just another consequence of decreased serum NEFA levels.

Several studies reported improved insulin sensitivity after ACE inhibition during clamp studies[5,6,17] in both humans and rats. These in vivo effects of ACE inhibitors may be a combination of increased muscle blood flow and a direct effect of ACE inhibitors on tissues. We studied the insulin action in vitro on isolated tissues of animals treated in vivo with ACE inhibitors during incubation, which allows a more precise control of conditions under which glucose utilization is measured and eliminates other factors contributing to the final effect in vivo.

Chronic captopril treatment increased the insulin sensitivity of skeletal muscle (measured as in vitro ^{14}C-glucose incorporation into glycogen) comparably both in HHTg and C. Our findings are in accordance with those of Henriksen et al[8,28] and Dal Ponte et al,[10] who reported increased glucose transport into the cell after captopril treatment in skeletal muscle of obese Zucker rats.

A growing body of data indicates an important role of

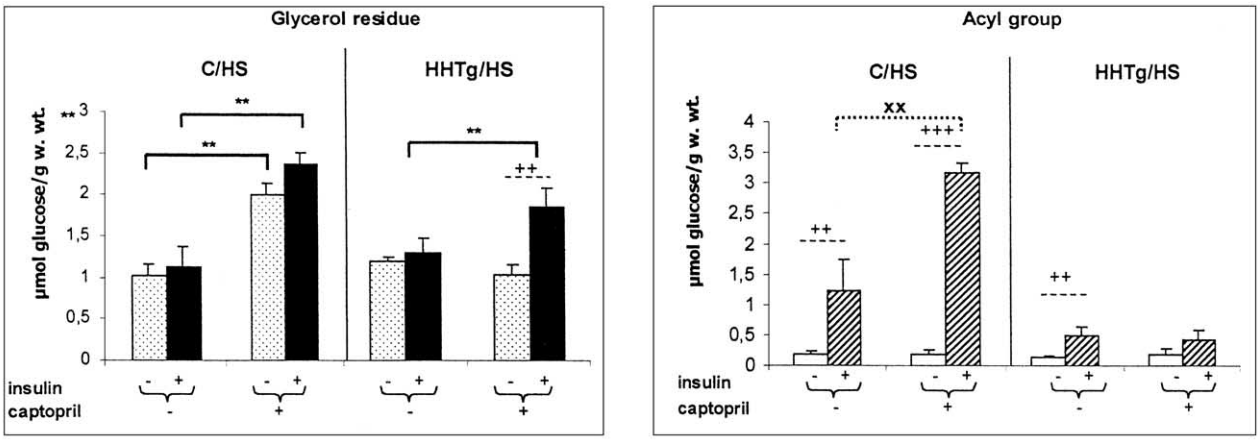

Fig 2. Effect of chronic ACE inhibition on ^{14}C-glucose incorporation into acyl group and glycerol residue in adipose tissue of C and HHTg rats during incubation in vitro. Captopril (10 mg/kg) was administered for 14 days in drinking water together with HS diet. Insulin (250 μU/mL) was added into incubation medium in vitro. Data are given as means ± SE for 5 to 7 animals per group. +, ssd between samples incubated in the absence *v* in the presence of insulin (++*P* < .01, +++*P* < .001); *, ssd in the ^{14}C-glucose incorporation into glycerol residue in captopril treated *v* untreated animals (*P* < .01); x, ssd in the ^{14}C-glucose incorporation into acyl group in captopril-treated *v* untreated animals (xx*P* < .01).**

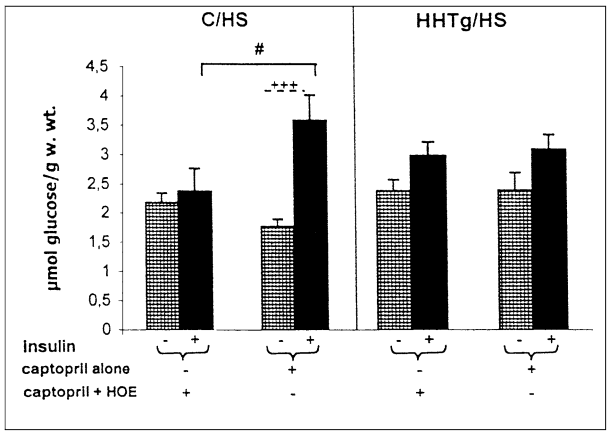

Fig 3. Effect of acute ACE inhibition with or without bradykinin receptor inhibition via HOE-140 on ^{14}C-glucose incorporation into glycogen in skeletal muscle (m. soleus) of C and HHTg rats during incubation in vitro. Rats were fed HS diet for 2 weeks. Captopril (50 mg/kg) was administered on the day of experiment by gavage with or without pretreatment with HOE-140 (100 μg/kg IP). Insulin (250 μU/mL) was added into incubation medium in vitro. Data are given as means ± SE for 5 to 7 animals per group. +, ssd between samples incubated in the absence *v* in the presence of insulin (+++*P* < .001); #, ssd between captopril-treated *v* untreated animals (#*P* < .05).

adipose tissue in the development of insulin resistance.[29] Adipose tissue of untreated HHTg rats was almost completely resistant to insulin action. Chronically administered captopril increased insulin-stimulated glucose incorporation into lipids but, in HHTg, this effect was 4 times lower than in controls. The site of glucose incorporation (glycerol *v* acyl moiety) was entirely different in C and HHTg. To our knowledge, no data are available about de novo fatty acid synthesis after captopril treatment. Under basal conditions (medium without insulin), captopril did not influence fatty acid synthesis in vitro in C or in HHTg. Insulin-stimulated de novo fatty acid synthesis was significantly enhanced by captopril (+150%) in C, while in HHTg, captopril had no effect and fatty acid synthesis was nearly 8 times lower than that in captopril-treated controls. As expected, captopril did not influence basal esterification. Insulin-stimulated esterification was enhanced by captopril both in C and in HHTg but with a lower efficacy in the latter (by 115% and 40%, respectively). Lipogenesis depends on the cell's nutritional state. Tozzo et al[30] showed, in murine adipocytes incubated at low glucose concentrations, that, in a situation when glucose transport into the cell is limited, most of the transported glucose is metabolized to triglyceride-glycerol and a very small amount to de novo fatty acid synthesis. On the other hand, using a model of murine adipocytes overexpressing GLUT4, Tozzo et al demonstrated that increased glucose transport into cells stimulated fatty acid synthesis. We have previously shown that the ability of HHTg to translocate GLUT4 to the plasma membrane after a glucose load is impaired as against C.[31] Recent results suggest that bradykinin delays dephosphorylation of the β-subunit of insulin receptor while intensifying insulin signal.[16] The sucrose-fed controls have probably preserved a functioning insulin cascade, which can be modulated by bradykinin, whereas insulin signal transmission is disturbed in HHTg.[31] In this setting, insulin receptor stimulation by bradykinin after chronic captopril administration was less effective in HHTg compared with C. The low rate of fatty acid synthesis may reflect the limited rate of glucose transport into the adipocytes in HHTg. Fatty acid esterification was increased by captopril treatment in both groups, which may have contributed to the reduction of serum NEFA content, but de novo fatty acid synthesis rose only in C after captopril administration. Given these facts, our results can be interpreted as showing that captopril caused only a mild increase in glucose transport into adipocytes in HHTg, meeting the demands for esterification. In C, the increase in glucose transport into

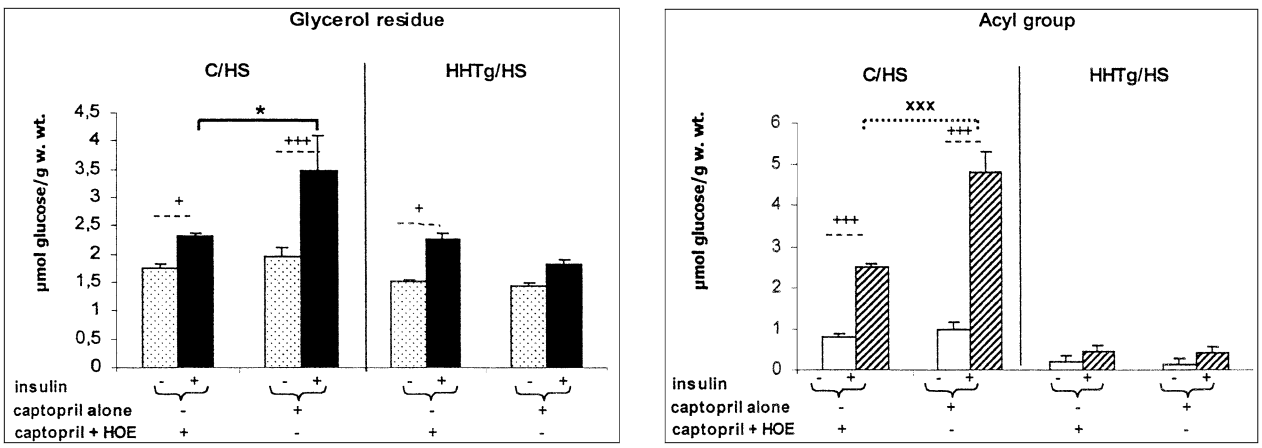

Fig 4. Effect of acute ACE inhibition with or without bradykinin receptor inhibition via HOE-140 on ^{14}C-glucose incorporation into glycerol residue and acyl group in adipose tissue of C and HHTg rats during incubation in vitro. Rats were fed HS diet for 2 weeks. Captopril (50 mg/kg) was administered on the day of experiment by gavage with or without pretreatment with HOE-140 (100 μg/kg IP). Insulin (250 μU/mL) was added into incubation medium in vitro. Data are given as means ± SE for 5 to 7 animals per group. +, ssd between samples incubated in the absence *v* in the presence of insulin (+*P* < .05, +++*P* < .001); *, ssd in the ^{14}C-glucose incorporation into glycerol residue in captopril-treated *v* untreated animals (P* < .05); x, ssd in the ^{14}C-glucose incorporation into acyl group in captopril-treated *v* untreated animals (xxx*P* < .001).**

cells was apparently much higher, manifesting itself both by an increase in esterification rates and in the rates of de novo fatty acid synthesis.

ACE inhibitors enhance bradykinin levels by inhibiting the kininase II–mediated degradation of this nonapeptide. The increased tissue bradykinin levels after captopril administration may play a significant role in improving insulin sensitivity.[4,8,16,28,32] Bradykinin has been shown to affect cellular metabolism through B2 receptors, which are expressed in many cell types,[33] including tissues dependent on insulin for glucose uptake such as skeletal muscle and adipocytes.[34] In our experimental design (single-dose administration of captopril with or without pretreatment with a bradykinin-receptor inhibitor), we were able to demonstrate bradykinin involvement in captopril action in sucrose-fed C, but not in HHTg. Nevertheless, in HHTg, single-dose captopril did not lead to significant improvement so we logically could not find any inhibitory effect of HOE-140. Our results do not allow us to confirm or exclude the role of bradykinin in mediating captopril action in HHTg.

We conclude that, in HHTg rats, chronic captopril treatment decreased postprandial triglyceridemia, fasting blood glucose, and serum NEFA, glycerol, and lactate contents. These changes are associated with the effect of captopril on in vitro glucose utilization in tissue. The skeletal muscle responds to chronic ACE inhibition similarly in C and HHTg. In adipose tissue, we found major differences between C and HHTg in the response to captopril. In HHTg, captopril enhanced insulin-stimulated glucose incorporation into lipids, but only into the glycerol moiety, ie, esterification. In C, captopril enhanced both insulin-stimulated esterification and fatty acid synthesis. These differences can be explained by impaired glucose transport into HHTg adipose cells.

REFERENCES

1. Reaven GM: Role of insulin resistance in human disease: An expanded definition. Annu Rev Med 44:121-131, 1993
2. Pollare T: Insulin sensitivity and blood lipids during antihypertensive treatment with special reference to ACE inhibition. J Diabetes Complications 4:75-78, 1990
3. Paolisso G, Gambardella A, Verza M, et al: ACE inhibition improves insulin sensitivity in aged insulin-resistant hypertensive patients. J Hum Hypertens 6:175-179, 1992
4. Uehara M, Kishikawa H, Isami S, et al: Effect on insulin sensitivity of angiotensin converting enzyme inhibitors with or without sulphydryl group: Bradykinin may improve insulin resistance in dogs and humans. Diabetologia 37:300-307, 1994
5. Torlone E, Britta M, Rambotti A, et al: Improved insulin action and glycemic control after long-term angiotensin-converting enzyme inhibition in subjects with arterial hypertension and type II diabetes. Diabetes Care 1610:1347-1355, 1993
6. Giordano M, Matsuda M, Sanders L, et al: Effects of angiotensin converting enzyme inhibitors, Ca^{2+} channel antagonists, and α-adrenergic blockers on glucose and lipid metabolism in NIDDM patients with hypertension. Diabetes 44:665-671, 1995
7. Henriksen EJ, Jacob S: Effects of captopril on glucose transport activity in skeletal muscle of obese Zucker rats. Metabolism 44:262-272, 1995
8. Henriksen EJ, Jacob S, Kinnick TR, et al: ACE inhibition and glucose transport in insulin resistant muscle: Roles of bradykinin and nitric oxide. Am J Physiol 277:R332-R336, 1999
9. Jacob S, Henriksen JE, Fogt DL, et al: Effects of trandolapril and verapamil on glucose transport in insulin resistant rat skeletal muscle. Metabolism 45:535-541, 1996
10. Dal Ponte D, Fogt DL, Jacob S, et al: Interactions of captopril and verapamil on glucose tolerance and insulin action in an animal model of insulin resistance. Metabolism 47:982-987, 1998
11. Leighton B, Sanderson AL, Young ME, et al: Effect of treatment of spontaneously hypertensive rats with the angiotensin converting enzyme inhibitor trandorapril 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. Shimamoto K, Ura N, Nakagawa M, et al: The mechanisms of the improvement of insulin sensitivity by angiotensin converting enzyme inhibitor. Clin Exp Hypertens 18:257-266, 1996
13. Iimura O, Shimamoto K, Matsuda K, et al: Effects of angiotensin receptor antagonist and angiotensin converting enzyme inhibitor on insulin senzitivity in fructose fed rats and essential hypertensives. Am J Hypertens 8:353-357, 1995
14. Jauch KW, Hartl W, Guenther 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. Caldiz CI, de Cingolani GE: Insulin resistance in adipocytes from spontaneously hypertensive rats: Effect of long-term treatment with enalapril and losartan. Metabolism 48:1041-1046, 1999
16. Isami S, Kishikawa H, Araki E, et al: Bradykinin enhances GLUT4 translocation through the increase of insulin receptor tyrosine kinase in primary adipocytes: Evidence that bradykinin stimulates the insulin signalling pathway. Diabetologia 39:412-420, 1996
17. Damas J, Bourdon V, Lefebvre P: Insulin sensitivity, clearance and release in kininogen-deficient rats. Exp Physiol 84:549-557, 1999
18. Vrána A, Kazdová L: Increased adipose tissue lipolysis in hypertriglyceridemic rat line. Lipids and syndrome of insulin resistance. Ann NY Acad Sci 827:510-513, 1997
19. Burant CF, Lemmon SK, Treutellar MK: Insulin resistance of denervated rat muscle: A model for impaired receptor-function coupling. Am J Physiol 247:E657-E666, 1984
20. Folch J, Lees M, Stanley GHS: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509, 1957
21. Sreenan S, Keck S, Fuller T, et al: Effects of troglitazone on substrate storage and utilization in insulin-resistant rats. Am J Physiol 276:E1119-E1129, 1999
22. Vrána A, Fábry P, Kazdová L: Effect of dietary fructose on fatty acid synthesis in adipose tissue and on triglyceride concentration in blood in the rat. Nutr Metab 15:305-313, 1973
23. Gutmann J, Wahlefeld AD: L-(+) lactate. Determination with lactate dehydrogenase and NAD, in Bergmeyer HU, Gawehn K (eds): Methods of Enzymatic Analysis, vol 4. New York, NY, Academic Press, New 1974, pp 1464-1469
24. Thornburn AW, Gumbiner B, Bulacan F, et al: Intracellular glucose oxidation and glycogen synthase activity are reduced in non-insulin-dependent (type II) diabetes independent of impaired glucose uptake. J Clin Invest 85:522-529, 1990
25. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46:3-10, 1997
26. Petersen KF, Shulman G: Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. Am J Cardiol 90:11G-18G, 2002 (suppl)
27. Schulmann G: Cellular mechanisms of insulin resistance. J Clin Invest 106:171-176, 2000
28. Henriksen EJ, Jacob S, Augustin HJ, et al: Glucose transport activity in insulin resistant rat muscle: Effects of ACE inhibitors and bradykinin antagonism. Diabetes 45:S125-S128, 1996 (suppl 1)
29. Smith U: Impaired (diabetic) insulin signalling and action occur

in fat cells long before glucose intolerance—Is insulin resistance initiated in adipose tissue? Int J Obesity 26:897-904, 2002

30. Tozzo E, Shepherd PR, Gnudi L, et al: Transgenic GLUT-4 overexpression in fat enhances glucose metabolism: Preferential effect on fatty acid synthesis. Am J Physiol 268:E956-E964, 1995

31. Cahova M, Vavrinkova H, Markova I, et al: The GLUT4 translocation and glycogen synthase activity are impaired in hereditary hypertriglyceridemic rats. Diabetologia 45:A230, 2002 (suppl 2, abstr)

32. Carvalho CRO, Thirone ACP, Gontijo JAR, et al: Effect of captopril, losartan, and bradykinin on early steps of insulin action. Diabetes 46:1950-1957, 1997

33. Figueroa C, Dietze GJ, Muller-Esterl W: Immunolocalization of bradykinin B2 receptors on skeletal muscle cells. Diabetes 45:S24-S28, 1996 (suppl 1)

34. Duka I, Shenouda S, Johns C, et al: Role of B2 receptor of bradykinin in insulin sensitivity. Hypertension 38:1355-1360, 2001