Insulin receptors and renal sodium handling in hypertensive fructose-fed rats

CRISTIANA CATENA, ALESSANDRO CAVARAPE, MARILEDA NOVELLO, GILBERTA GIACCHETTI, and LEONARDO A. SECHI

Clinica Medica, Hypertension Unit, Department of Experimental and Clinical Pathology and Medicine, University of Udine, Udine, Italy; and Clinica di Endocrinologia, University of Ancona, Ancona, Italy

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Background. Insulin resistance and hypertension are present in Sprague-Dawley rats fed a fructose-enriched diet. In these rats, insulin might elevate blood pressure via an antinatriuretic action.

Methods. To investigate the sodium-insulin interaction in fructose-fed rats, we compared insulin sensitivity, insulin receptor binding, and insulin receptor mRNA levels in the kidney and skeletal muscle of rats that were fed standard rat chow or a fructose-enriched diet (66%) with either low (0.07%), normal (0.3%), or high (7.5%) NaCl concentrations for 3 weeks.

Results. Systolic blood pressure increased in the fructose-fed rats receiving the normal and high-salt diet, but not the low-salt diet. When the rats were fed the low-salt diet, the rate of glucose infusion required to maintain euglycemia during a hyperinsulinemic clamp and insulin receptor number and mRNA levels in skeletal muscle were lower in fructose-fed than control rats. High-salt diet decreased significantly the rate of glucose disposal during the clamp and muscular insulin receptor number and mRNA levels in control, but not fructose-fed rats. During the low-salt diet, renal insulin receptor number and mRNA levels were comparable in fructose-fed and control rats and hyperinsulinemia had comparable acute antinatriuretic effects in the two groups; when the rats were maintained on the high-salt diet, the expected decrease in renal insulin receptor number and mRNA levels occurred in control but not fructose-fed rats. An inverse relationship between dietary NaCl content and renal insulin receptor mRNA levels was observed in control but not fructose-fed rats.

Conclusion. Fructose-fed rats appear to have lost the feedback mechanism that limits insulin-induced sodium retention through a down-regulation of the renal insulin receptor when the dietary NaCl content is increased. This abnormality might possibly contribute to the elevation of blood pressure in these rats.

Key words: hyperinsulinemic clamp, insulin binding, insulin sensitivity, messenger RNA, salt.

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metabolic responses to an euglycemic-hyperinsulinemic clamp, insulin receptor binding parameters, and insulin receptor mRNA levels in fructose-fed and control rats maintained on diets with different salt content.

METHODS

All experiments were performed in male Sprague-Dawley rats (Banting Kingman, Fremont, CA, USA) weighing 150 to 180 g that were housed in climate-controlled conditions with a 12-hour light/12-hour dark cycle and provided tap water ad libitum. We performed three sets of rat experiments using two different protocols, as performed in previous studies [21].

Protocol A

Rats were fed standard rat chow for 3 days and were then divided into four groups: rats fed standard chow containing a low-salt concentration (NaCl 0.07%) (Harlan Teklad, Madison, WI, USA); rats fed standard chow containing a high-salt concentration (NaCl 7.5%); rats fed a fructose-enriched diet (fructose 66%, fat 5%, casein 20%, cellulose 7%, vitamins 1%) (Harlan Teklad) containing a low-salt concentration; and rats fed a fructose-enriched diet containing a high-salt concentration. Body weight and systolic blood pressure were measured every other day [20]. Systolic blood pressure was measured in conscious, prewarmed (light lamp), restrained rats by the tail-cuff method with plethysmography and a physiograph recorder (Pulse Amplifier) (ITTC Life Sciences, Woodland Hills, CA, USA). For these measurements, rats were trained adequately before the study. Two distinct sets of experiments were conducted in which, after 3 weeks on the experimental diets, 24 rats were prepared for the euglycemic-hyperinsulinemic clamp and 24 rats were killed by decapitation; in the latter group trunk blood was collected in ethylenediaminetetraacetic acid (EDTA) and skeletal muscle (always the same muscle groups from lower limbs) and kidneys were removed and snap-frozen in liquid nitrogen for insulin receptor binding study.

Protocol B

Rats were fed standard rat chow for 3 days and were then divided into six groups: rats fed standard chow containing low-salt (NaCl 0.07%), normal-salt (NaCl 0.3%), or high-salt concentration (NaCl 7.5%); rats fed a fructose-enriched diet (66%) containing low-salt, normal-salt, or high-salt concentration. After 3 weeks, rats were decapitated, trunk blood was collected for measurement of plasma glucose, insulin, and triglycerides, and skeletal muscle and kidneys were removed and snap-frozen for total RNA isolation.

Euglycemic-hyperinsulinemic clamp study

The euglycemic-hyperinsulinemic clamp was used to determine insulin sensitivity and to evaluate the renal effects of insulin in 24 rats that were housed and fed as in protocol A, following a procedure that has been described previously [20]. On the day of the experiment, rats were anesthetized with an intraperitoneal injection of 100 mg/kg of thiobutabarbital (Inactin) (Andrew Lockwood, Sturtevant, WI, USA) and placed on a heated table to maintain rectal temperature at 37°C. Animals underwent tracheostomy and breathed spontaneously. Thereafter, they were prepared for acute experimentation as described previously [22]. Briefly, catheters were placed in the right jugular vein for insulin infusion, the left jugular vein for the infusion of glucose and saline, and the right femoral artery for blood sampling and blood pressure monitoring via a Gemini 7070 pressure transducer (Ugo Basile, Comerio, Italy). A flanged catheter was placed in the bladder through a suprapubic incision for urine collection. To replace fluid losses during the surgical preparation, rats received a constant intravenous infusion of plasma substitute (Hespan, 6% hetastarch in 0.9% NaCl) (Du Pont Pharmaceuticals, Wilmington, DE, USA) at a rate of 40 μL/min until a total volume of 0.5% body weight was administered. The rate of the infusion was subsequently adjusted to match the urinary output and was not significantly different between control and fructose-fed rats fed either low- or high-salt diet. Urine was collected from the bladder in preweighed polypropylene tubes over a 45-minute period for determination of baseline urine flow rate and sodium concentration. Blood was obtained every 15 minutes for glucose measurement (Accu-check II) (Boheringer Mannheim, Indianapolis, IN, USA) and at the beginning and end of this interval for sodium determination. After the 45-minute baseline period, rats received an insulin load of 85 mU/kg followed by a continuous insulin infusion of 8 mU/kg per minute. As expected, this infusion produced comparable plasma insulin levels in fructose-fed and control rats fed with both low-salt and high-salt chow. After injection of the insulin bolus, a 25% glucose solution was infused for a 40-minute stabilization interval, during which blood glucose was determined at 5-minute intervals and the rate of glucose infusion was adjusted to maintain blood glucose at the average level measured at baseline. Thereafter, a second 45-minute experimental period was begun, during which urine flow rate, urinary and plasma sodium concentration, and blood glucose concentration were determined, and the rate of glucose infusion was adjusted as indicated above. The glucose disposal rate was calculated during the 45 minutes of the experimental period from the amount of glucose required to maintain euglycemia.

Insulin receptor binding studies

The distribution and binding characteristics of insulin receptors in skeletal muscle and kidney were assessed in 24 rats that were housed and fed as in protocol A, by an in situ autoradiographic technique associated with
computerized microdensitometry, following a procedure that has been described previously and to which readers are referred for further details [19]. Briefly, adjacent tissue sections (20 μm thick) were cut on a cryostat, liophilized, preincubated twice for 10 minutes in KCl (30 mmol/L), and incubated for 120 minutes in a buffer containing 200 pmol/L 125I-Tyr-insulin (2200 Ci/mmol) (DuPont-NEN, Boston, MA, USA) in the presence of increasing concentrations (from 10 pmol/L to 1 mmol/L) of unlabeled insulin (Humulin R) (Eli-Lilly, Indianapolis, IN, USA), then rinsed in ice-cold buffer, and dried for 2 hours in a stream of cool air. In the kidney, regional analysis of insulin binding was performed in renal cortex, and outer and inner renal medulla by use of film autoradiographs obtained by exposing the tissue sections on LKB-Ultrofilm (Leica, Inc., Deerfield, IL, USA). Optical density in the different regions was measured by computerized microdensitometry. Scatchard analysis of equilibrium binding data was done with the Ligand program of Munson and Rodbard and resulted in curvilinear profiles indicating either two classes of insulin receptors (R1: high-affinity, low-capacity; R2: low-affinity, high-capacity) or a negative cooperative hormone-receptor interaction [19]. In all the experiments, data were analyzed for a two-site model.

**Insulin receptor mRNA studies**

The levels of insulin receptor mRNA in skeletal muscle and kidney were assessed in 36 rats that were housed and fed as in protocol B. Total RNA was isolated from frozen tissue, as described previously [19]. The RNA pellet was dissolved in sterile water and quantitated by ultraviolet absorbance at 260/280 nm. A 0.92 kb 32P-labeled antisense insulin receptor cRNA probe encoding the 5′ end of the rat insulin receptor cDNA was synthesized using a PPMul linearized rat insulin receptor clone, T3 RNA polymerase, and 32P-CTP (800 Ci/mmol). Probe purification was performed by spin column centrifugation using a G-50 Sephadex RNA Purification Quick Spin Column (Boehringer Mannheim).

Insulin receptor mRNA levels were measured by slot-blot hybridization analysis. Nitrocellulose filters were prehybridized in a solution containing 50% formamide, 2× standard sodium citrate (SSC), 10× Denhardt’s, 10 mmol/L Tris (pH 7.6), 200 μg/mL sheared denatured salmon sperm DNA, and 0.2% sodium dodecyl sulfate (SDS) for 2 to 4 hours at 65°C. Hybridization was performed using fresh prehybridization solution, to which 32P-labeled rat insulin receptor probe was added at a final concentration of 2.5 to 3.0 × 106 cpm/mL of hybridization solution, and incubated for 18 to 20 hours at 65°C. Following hybridization, the membranes were washed for 30 minutes at 65°C in 2× SSC-0.1% SDS, 0.5× SSC-0.1% SDS, 0.1× SSC-0.5% SDS, and finally in 0.1× SSC-0.5% SDS. Autoradiographs were obtained by exposing the filters to Cronex x-ray film (Du Pont) with an intensifying screen at −80°C for 3 to 5 days and were analyzed by scanning densitometry (GS670 Imaging Densitometer) (Bio-Rad, Hercules, CA, USA).

To ensure equivalent loading conditions, duplicate blots were prepared and hybridized with a 32P-labeled oligonucleotide probe complementary to bases 4011 to 4036 of 28S ribosomal RNA [19]. Probe purification was performed by spin column centrifugation using a G-25 Sephadex DNA Purification Quick Spin Column. After prehybridization, hybridization was performed in a solution containing 4× SSC, 5× Denhardt’s, 2% SDS, and 1% Ppi, to which 32P-labeled probe was added at a final concentration of 1.0 × 106 cpm/mL of hybridization solution, and incubating for 18 to 20 hours at 40°C.

**Plasma glucose, insulin, triglycerides, and serum and urinary sodium**

Sodium concentrations in serum and urine were measured by flame photometry (Klina Flame) (Beckman, Fullerton, CA, USA). Blood glucose concentration in trunk blood was determined by the glucose oxidase method (Beckman Glucose Analyzer) (Beckman). Plasma triglycerides were assayed enzymatically by an automated method. Plasma insulin was measured by radioimmunoassay (Behring, Marburg, Germany) [20].

**Statistical analysis**

Data are expressed as mean ± SE. Comparisons among groups were done by Student t test for unpaired data or analysis of variance (ANOVA) for multiple comparisons or repeated measures (GB-Stat) (Dynamic Microsystems, Inc., Silver Spring, MD, USA) when appropriate. Correlations are expressed by the correlation coefficient r. Differences were considered as statistically significant when P was less than 0.05.

**RESULTS**

**Body weight, systolic blood pressure, and plasma glucose, insulin, and triglycerides**

As partially reported in a previous study [21], plasma triglyceride levels were significantly greater in the fructose-fed rats independent of dietary salt content, whereas body weight, fasting plasma glucose, and fasting plasma insulin did not differ significantly among groups (Table 1). Systolic blood pressure levels were significantly greater in the fructose-fed rats that received either the normal or high-salt diet but no significant difference was observed in the fructose-fed rats that received the low-salt diet (Table 1).
During the clamp study, plasma insulin levels increased seven- to eightfold, eucremia was maintained adequately in all groups, and blood pressure did not change significantly in any group. During the low-salt diet, the amount of glucose infused to maintain eucremia during the hyperinsulinemic clamp was significantly less in fructose-fed rats than controls, indicating reduced sensitivity to insulin; high-salt diet decreased significantly insulin-induced glucose utilization in control but not fructose-fed rats (Table 2). Baseline urine flow rate and urinary sodium excretion were comparable in fructose-fed and control rats fed both low- and high-salt chow. During the low-salt diet, hyperinsulinemia caused in both fructose-fed and control rats a significant reduction of urine flow rate and urine sodium excretion. When rats were fed the high-salt diet, hyperinsulinemia maintained its antidiuretic and antinatriuretic effect in fructose-fed, but not control rats (Table 2).

### Insulin receptor binding studies

Radiolabeled insulin binding was homogeneously distributed in the skeletal muscle of fructose-fed and control rats fed both low- and high-salt diet. Analysis of binding data showed a significantly decreased $B_{\text{max}}$ of the R1 (high-affinity, low-capacity receptor) in the muscle of fructose-fed rats maintained on a low-salt diet as compared to controls, but no difference in the $K_d$ of R1 and in both the $K_d$ and $B_{\text{max}}$ of R2 (low-affinity, high-capacity receptor). When rats were fed high-salt, a significant decrease in the $B_{\text{max}}$ of R1 was observed in control but not fructose-fed rats.
whereas other binding parameters remained unchanged (Table 3).

In the kidney, insulin binding was more abundant in the renal cortex than medulla and the distribution was comparable in all four groups of rats. Analysis of binding data showed no differences in the $K_d$ and $B_{max}$ of both insulin receptor sites between fructose-fed and control rats maintained on a low-salt diet. When control rats were fed high-salt, a significant decrease in the $B_{max}$ of R1 was observed in comparison to control rats fed low-salt, whereas other binding parameters did not change. In fructose-fed rats, the high-salt diet did not affect the $B_{max}$ and $K_d$ of both insulin receptor sites (Table 3). Analysis of regional binding to renal cortex, outer medulla, and inner medulla showed that insulin receptor density was comparable in all regions of the kidneys obtained from fructose-fed and control rats fed a low-salt diet. High-salt diet decreased insulin receptor density in all regions of the kidney in control but not fructose-fed rats (Fig. 1).

### Table 3. Insulin binding parameters in skeletal muscle and kidney of control and fructose-fed rats fed low-salt and high-salt diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control rats</th>
<th>Fructose-fed rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-salt ($N = 6$)</td>
<td>High-salt ($N = 6$)</td>
</tr>
<tr>
<td><strong>Skeletal muscle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_d, \times 10^{-10}$ M</td>
<td>4.3 ± 0.9</td>
<td>4.8 ± 1.1</td>
</tr>
<tr>
<td>$B_{max}, \times 10^{13}$ receptors/mm$^3$</td>
<td>12.1 ± 1.4</td>
<td>7.4 ± 1.5$^b$</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_d, \times 10^{-10}$ M</td>
<td>3.1 ± 1.5</td>
<td>2.7 ± 1.1</td>
</tr>
<tr>
<td>$B_{max}, \times 10^{13}$ receptors/mm$^3$</td>
<td>6.6 ± 2.0</td>
<td>6.9 ± 2.1</td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_d, \times 10^{-7}$ M</td>
<td>3.6 ± 1.1</td>
<td>3.2 ± 1.2</td>
</tr>
<tr>
<td>$B_{max}, \times 10^{10}$ receptors/mm$^3$</td>
<td>6.5 ± 1.2</td>
<td>3.8 ± 1.7$^b$</td>
</tr>
</tbody>
</table>

R1 is high-affinity, low-capacity insulin binding site; R2 is low-affinity, high-capacity insulin binding site. Binding parameters ($K_d$, dissociation constant; $B_{max}$, maximum binding capacity) were derived from Scatchard analysis of equilibrium binding data from competition experiments performed on adjacent tissue sections that, after incubation with radioligand, were placed in a gamma counter. Values are means ± SE. Comparisons were done by two-way analysis of variance (ANOVA).

$^aP < 0.05$ vs. the respective control; $^bP < 0.05$ vs. low-salt diet.

#### DISCUSSION

The findings of this study demonstrate that the density and mRNA levels of insulin receptors in the kidney of fructose-fed rats do not differ from controls when rats are fed low and normal-salt diet. High-salt diet decreases the insulin receptor number and mRNA levels in the kidney of control rats, as expected from the results of previous studies [19], but this does not occur in rats fed a fructose-enriched diet. Consistent with receptor findings, the acute antinatriuretic effect of insulin is significantly decreased by the high-salt diet in controls but not fructose-fed rats, suggesting that the feedback mechanism that normally limits insulin-induced sodium reabsorption when dietary salt is increased is lacking in this experimental model of hypertension associated with insulin resistance.

Different from the kidney, evaluation of the effects of insulin in a tissue, such as the skeletal muscle, that has a primary role in the metabolic actions of the hormone, demonstrates that insulin sensitivity is significantly reduced in fructose-fed rats fed low and normal-salt diets as compared to controls. Decreased insulin-stimulated glucose utilization in fructose-fed rats is associated with decreased insulin receptor number and mRNA levels, providing a possible molecular explanation for insulin resistance and indicating tissue-specific regulation of the insulin receptor in this model. Similar to the kidney, the high-salt diet down-regulates muscular insulin receptor number and mRNA levels in control but not fructose-fed rats.

#### Insulin receptor mRNA studies

Insulin receptor mRNA levels in the muscle were significantly lower in fructose-fed than control rats fed low and normal-salt diet. High-salt diet induced a significant decrease in insulin receptor mRNA levels in the muscle of control but not fructose-fed rats (Fig. 2). In the kidney of fructose-fed and control rats maintained on a low-salt diet, insulin receptor mRNA levels were comparable. High-salt diet decreased significantly renal insulin receptor mRNA in control but not fructose-fed rats (Fig. 2). In both muscle and kidney of control rats, insulin receptor mRNA levels were inversely correlated with dietary salt content ($r = −0.594, P < 0.01$, and $r = −0.639, P < 0.01$, respectively) but no relationship was observed in fructose-fed rats.
Possible contribution of insulin to the pathogenesis of arterial hypertension has been suggested following the demonstration of hyperinsulinemia and insulin resistance in essential hypertensive patients [1, 2] as well as hypertensive rat models [4–6]. Chronic fructose feeding induces hypertension associated with insulin resistance and compensatory hyperinsulinemia in normal rats [7] and this model has been largely employed to investigate the mechanisms of insulin-induced hypertension. Some controversy about fructose-induced hypertension is mainly related to the possibility to induce hyperinsulinemia [21, 23] and hypertension [23] and many potential explanations have been offered, such as differences in the technique used to measure blood pressure [23, 24], rat strains [25], and dietary components (e.g., lipids or sodium) [26, 27]. More specifically, in our and in other authors’ [23] hands, fasting hyperinsulinemia cannot be obtained by chronically feeding rats a fructose-enriched diet and, as indicated above, this fact resides among the many disputes regarding this experimental model. In both protocol A and protocol B and independent of salt intake, the fructose-fed rats had greater average fasting insulin levels than controls, although these differences did not reach statistical significance. An explanation might be that the reduced sample size could have limited the power of the study to detect such a difference (β-error). Most important, however, we have previously demonstrated that plasma insulin is significantly increased in fructose-fed rats as compared to controls after a standard oral glucose load [21], suggesting that hyperinsulinemia might be more relevant in the postprandial state. In our opinion, the fructose-model is definitely an hyperinsulinemic rat model of hypertension and, as such, findings on these rats might be extrapolated to human conditions in which hypertension is associated with hyperinsulinemia.

In the consideration of possible mechanisms that could mediate the prohypertensive action of insulin, particular attention has been paid to the renal action of the hormone. Insulin, in fact, activates renal sodium reabsorption during acute infusions [15], but the exact site of the tubular action of insulin is unclear and previous reports have variously indicated an effect in the proximal tubule [16], distal tubule [17], and loop of Henle [18]. Indirect
evidence of the possible relevance of insulin in the regulation of sodium balance was provided by experiments in which an inverse relationship between dietary sodium intake and renal insulin receptor number and mRNA levels was demonstrated [19], indicating the existence of a feedback mechanism that limits insulin-induced sodium retention when extracellular fluid volume is expanded. Of particular interest is that, in our present experiments, a significant and comparable insulin-induced antinatriuretic effect occurred in fructose-fed and control rats during a low-salt diet, and that this effect normally disappeared in control but not fructose-fed rats during a high-salt diet. Evaluation of renal insulin receptor binding and mRNA data indicate that this difference was mediated at the level of insulin receptor, inasmuch as the high-salt diet down-regulated both receptor number and mRNA levels in control but not fructose-fed rats, thereby suggesting a molecular explanation for this observation.

It must be acknowledged, however, that other mechanisms beside abnormal regulation of renal insulin receptors by salt might have contributed to impaired natriuresis in fructose-fed rats maintained on a high-salt diet, including changes in the renin-angiotensin system, atrial natriuretic peptides, nitric oxide production, activity of renal nerves, and intrarenal hemodynamics. Particularly relevant in mediating insulin-induced sodium retention might be the role of angiotensin II. In fact, fructose hypertension has been demonstrated to be, at least in part, angiotensin II-dependent [28] and inappropriate suppression of angiotensin II levels in fructose-fed rats maintained on a high-salt diet might contribute to persistence of insulin-induced renal sodium reabsorption. However, given our present demonstration of an effect of dietary salt mediated at the level of insulin receptor gene expression, this should not be the case. In fact, we have previously demonstrated that renal insulin receptor binding and mRNA levels are relatively insensitive to chronic angiotensin II infusion in both normal and streptozotocin-diabetic rats [29], facts that clearly argue against a role for this peptide in the modulation of renal sensitivity to insulin.

It is interesting to notice that the abnormal response of insulin receptor mRNA to high-sodium was not uniquely found in the kidney but was present also in the muscle of fructose-fed rats. Although changes in mRNA do not necessarily indicate changes in the transcription of the gene, this observation might suggest the presence of a ubiquitous defect in the transcriptional response of the gene to manipulation of dietary salt in these rats. This defect might achieve different pathophysiologic relevance depending on the functional role of the specific tissue and might explain why despite being insulin resistant, the fructose-fed rats fed a low-salt diet do not have hypertension.

Although the functional role of the abnormal response of renal insulin receptors to dietary salt in fructose-fed rats remains to be clarified, it may be related to the issue of salt-dependence of fructose-induced hypertension. This issue has been investigated in previous studies and although Donnelly, Ho, and Reaven [30] reported that the blood pressure response to a fructose-enriched diet was not modified by either reducing salt intake or unilateral nephrectomy and Iyer and Katovich [31] showed that hypertension in fructose-fed rats does not occur directly via an increase in fluid volume, Johnson, Zhang, and Kotchen [26] and, more recently, Nishimoto et al [27] demonstrated that blood pressure was not affected by high dietary carbohydrate when rats were maintained...
on a low-salt diet. The present study argues in favor of salt-dependence of fructose hypertension, inasmuch as hypertension did not occur in fructose-fed rats that were fed a low-salt diet.

Broadly defined, insulin resistance refers to a subnormal response to a physiologic concentration of insulin, but this term is commonly used to describe an impairment of insulin-stimulated glucose disposal, as evaluated by the euglycemic-hyperinsulinemic clamp. In our study, insulin-stimulated glucose disposal was significantly lower in fructose-fed than control rats, indicating insulin resistance in tissues involved in the metabolic actions of the hormone. Different cellular mechanisms can cause insulin resistance [32], including defects in insulin receptors, signal transduction pathways, and postreceptor defects at the level of substrates of phosphorylation or effector molecules such as glucose transporters and enzymes involved in glucose metabolism. Previous studies suggested that insulin resistance in fructose-fed rats is not due to a decrease in insulin-stimulated glucose uptake by skeletal muscle [33] and no changes in insulin receptor binding and tyrosine kinase activity were found in the liver of these rats [34]. Postreceptor defects, including decreased phosphorylation of insulin receptor substrate (IRS-1) and decreased association of IRS-1 with phosphatidylinositol 3-kinase and phosphotyrosine-phosphatase have been reported in more recent studies [35]. We have found that both insulin receptor number and mRNA levels are significantly decreased in the skeletal muscle of fructose-fed rats fed low and normal-salt diet as compared to controls. This finding indicates a possible mechanism for insulin resistance in fructose-fed rats, reasonably explaining why insulin-induced glucose utilization is decreased in these rats when fed low and normal-salt diet. Our observation of salt-dependence of insulin-induced glucose utilization and muscular insulin receptor number and mRNA levels in control rats is consistent with the findings of a recent study [36] in which rats fed an 8% NaCl diet showed decreased sensitivity to insulin in both skeletal muscle and liver. Similar association between salt and sensitivity to insulin has been reported in both normotensive and hypertensive humans independent of obesity [37].

In our study, insulin resistance in fructose-fed rats maintained on a low or normal-salt diet was limited to the skeletal muscle, where receptor number and mRNA levels were significantly decreased, whereas in the kidney no difference was found in comparison to controls. Consistent with our data, comparable density of insulin receptors was found in all nephron segments of kidneys obtained from fructose-hypertensive and normotensive rats [38]. Different findings in muscle and kidney should not be surprising because it is known that resistance to the action of insulin may differ among different tissues. For instance, it has been shown that spontaneously hypertensive rats have a decreased sensitivity to insulin while they maintain the same antinatriuretic response to this hormone as their normotensive controls Wistar-Kyoto [20, 39]. The present study confirms, in fructose-fed rats, the possibility of tissue-specific regulation of insulin receptors and suggests a possible mechanism to explain the different functional response to insulin in muscle and kidney.

CONCLUSION

This study identifies a possible mechanism to link insulin resistance to hypertension in rats fed a fructose-enriched diet and demonstrates tissue-specific regulation of insulin receptor in this model. The fructose-fed rats appear to have lost the capability to down-regulate insulin receptors in the kidney when dietary salt intake is chronically increased, leading to the persistence of an antinatriuretic effect of the hormone. This abnormality might possibly contribute to elevation of blood pressure in these rats.

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Reprint requests to Leonardo A. Sechi, M.D., Clinica Medica, Hypertension Unit, DPMSC, University of Udine, Ospedale Civile, Padiglione Medicina, 33100 Udine, Italy.
E-mail: Sechi@uniud.it

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