Changes in dietary sodium consumption modulate GLUT4 gene expression and early steps of insulin signaling

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1Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, São Paulo; 2Department of Nephrology, School of Medicine, University of São Paulo, São Paulo; and 3Experimental Medicine Department, Institute of Cardiology of the State of Rio Grande do Sul/University Foundation of Cardiology, Rio Grande do Sul, Brazil

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Okamoto, Maristela Mitiko, Dóris Hissako Sumida, Carla Roberta Oliveira Carvalho, Alessandra Martins Vargas, Joel Cláudio Heimann, Beatriz D’Agord Schaan, and Ubiratan Fabres Machado. Changes in dietary sodium consumption modulate GLUT4 gene expression and early steps of insulin signaling. Am J Physiol Regul Integr Comp Physiol 286: R779–R785, 2004. First published December 11, 2003; 10.1152/ajpregu.00396.2003.—Previous studies have shown that chronic salt overload increases insulin sensitivity, while chronic salt restriction decreases it. In the present study we investigated the influence of dietary sodium on 1) GLUT4 gene expression, by Northern and Western blotting analysis; 2) in vivo GLUT4 protein translocation, by measuring the GLUT4 protein in plasma membrane and microsome, before and after insulin injection; and 3) insulin signaling, by analyzing basal and insulin-stimulated tyrosine phosphorylation of insulin receptor (IR)-β, insulin receptor substrate (IRS)-1, and IRS-2. Wistar rats were fed normal-sodium (NS-0.5%), low-sodium (LS-0.06%), or high-sodium diets (HS-3.12%) for 9 wk and were killed under pentobarbital anesthesia. Compared with NS rats, HS rats increased (P < 0.05) the GLUT4 protein in adipose tissue and skeletal muscle, whereas GLUT4 mRNA was increased only in adipose tissue. GLUT4 expression was unchanged in LS rats compared with NS rats. The GLUT4 translocation in HS rats was higher (P < 0.05) both in basal and insulin-stimulated conditions. On the other hand, LS rats did not increase the GLUT4 translocation after insulin stimulus. Compared with NS rats, LS rats showed reduced (P < 0.01) basal and insulin-stimulated tyrosine phosphorylation of IRS-1 in skeletal muscle and IRS-2 in liver, whereas HS rats showed enhanced basal tyrosine phosphorylation of IRS-1 in skeletal muscle and IRS-2 in liver. In summary, increased insulin sensitivity in HS rats is related to increased GLUT4 gene expression, enhanced insulin signaling, and GLUT4 translocation, whereas decreased insulin sensitivity of LS rats does not involve changes in GLUT4 gene expression but is related to impaired insulin signaling.

Salt restriction, together with weight loss and physical exercise, is the mainstay of the nonpharmacological approach to the treatment of hypertension (38, 39, 43), and its effects on insulin sensitivity have already been studied (36). Most of these studies showed that salt restriction reduces insulin sensitivity (11, 12); however, the opposite was also reported (10). Because most data concerning the effects of salt consumption on insulin sensitivity were obtained in short-term studies (10–12), long-term studies are necessary. Recently, we showed that chronic salt restriction in rats, compared with salt overload, is associated with lower blood pressure and decreased insulin-dependent glucose uptake in isolated epididymal adipocytes (7). Moreover, by using the euglycemic hyperinsulinemic clamp, we demonstrated that chronic salt restriction decreases, while chronic salt overload increases, glucose uptake and blood pressure (33).

The insulin-sensitive glucose transporter GLUT4 plays a key role in the insulin-stimulated glucose uptake in white adipose tissue and skeletal muscle (17, 37), which greatly contribute to glucose homeostasis. Adipose tissue and skeletal muscle specifically express GLUT4 protein, which is responsible for both basal and insulin-stimulated glucose uptake. Conspicuously, changes in GLUT4 gene expression in adipose tissue and muscles have been reported as involved in several conditions of both increased and reduced insulin sensitivity (21, 30). In resting cells, GLUT4 is highly sequestered into the intracellular compartment, comprising up to 95% of its total cellular content (34). Insulin stimulation causes the translocation of GLUT4 from its intracellular compartment to the plasma membrane, acutely increasing glucose uptake, playing an important role in the regulation of blood and tissue glucose levels (14). The key role of GLUT4 in glucose disposal has been recognized for a long time, and it is independent of changes in glycogen synthesis, glycolytic pathway, glucose oxidation, and hexokinase activity (25, 42). Since it is not generally feasible to determine the rate-limiting step of the glucose disposal pathway, other approaches, such as the use of transgenic animal models, were used. In GLUT4-transgenic mice, striking results clearly demonstrate the rate-limiting nature of this transporter, suggesting that overexpression of GLUT4 is an obvious tool to employ toward insulin resistance correction (23).

Expanding knowledge about the behavior of GLUT4 gene expression, protein translocation to the plasma membrane, and the metabolic syndrome was first introduced as a concept by Reaven in 1988 to cluster cardiovascular risk factors such as hypertension, type 2 diabetes, obesity, and dyslipidemia, which culminate in high risk for atherosclerotic cardiovascular disease. Insulin resistance has been proposed as the metabolic link between all these cardiovascular risk factors (16, 24, 35).

THE METABOLIC SYNDROME was first introduced as a concept by Reaven in 1988 to cluster cardiovascular risk factors such as hypertension, type 2 diabetes, obesity, and dyslipidemia, which culminate in high risk for atherosclerotic cardiovascular disease. Insulin resistance has been proposed as the metabolic link between all these cardiovascular risk factors (16, 24, 35).
insulin signaling under different sodium dietary manipulation may provide an attractive target for pharmacological intervention strategies so as to control sodium-induced glucose homeostatic changes. In this context, the purpose of the present study was to investigate the influence of dietary sodium content on 1) GLUT4 gene expression, by analyzing GLUT4 mRNA and protein content in white adipose tissue, skeletal muscle, and heart; 2) GLUT4 protein translocation to the plasma membrane in white adipose tissue; and 3) insulin signaling in liver and skeletal muscle.

MATERIALS AND METHODS

All experiments reported here were in accordance with guidelines of the Brazilian College of Animal Experimentation and were approved by the Ethics Committee for Animal Research of the Institute of Biomedical Sciences, University of São Paulo (certificate 05/2000).

Animals. Male Wistar rats were fed from weaning either normal-sodium (NS; 0.5% Na⁺, TD-92140; n = 18), low-sodium (LS; 0.06% Na⁺, TD-92141; n = 20), or high-sodium (HS; 3.12% Na⁺, TD-92142; n = 18) diets (Harlan Teklad Chow). Rats were housed in a controlled-temperature environment (23 ± 2°C), with controlled light off cycling, and with free access to chow and tap water.

Experimental protocol. Experiments were performed in 3-mo-old rats. On the day of death, the animals were anesthetized with pentobarbital sodium (50 mg/kg body wt ip). The abdominal venous cava was exposed, and blood was collected for insulin and glucose determinations in the fed state. Then the epididymal white adipose tissue, skeletal muscle gastrocnemius, and heart were removed and immediately weighed, frozen, and stored at −70°C for further GLUT4 protein and mRNA analysis. Additional groups of animals in the fasting state were used for in vivo insulin stimulation of GLUT4 translocation and tyrosine phosphorylation of insulin receptor (IR) and insulin receptor substrates (IRS), as described below.

GLUT4 protein and mRNA analysis. Three subcellular membrane fractions of adipose tissue were prepared as previously described (21). Tissues were homogenized and processed to obtain the following fractions: fat-free extract, plasma membrane, and microsome. Skeletal muscle and heart were processed to obtain a total cellular membrane fraction (21). GLUT4 protein was assessed by Western blotting method, as previously described (29). Immunoblotting was performed with an antisem developed against the COOH terminus of the rat GLUT4 protein followed by 125I-protein A (Amersham, Buckinghamshire, UK). Autoradiograms were analyzed by densitometry, using the Image Master1D software (Pharmacia Biotech, Upsalla, Sweden). Results were expressed as arbitrary units (AU) per microgram of total protein subjected to electrophoresis (AU/µg of protein), and the GLUT4 content per gram of body weight (AU/g body wt) was calculated considering the total tissue protein yield and the body weight.

Total RNA was extracted from 0.1 g of frozen tissue using Trizol reagent (GIBCO BRL, Life Technologies, Gaithersburg, MD) according to manufacturer’s instructions. Total RNA (20 µg) was subjected to electrophoresis in 1.0% formaldehyde-agarose gel, blotted, cross-linked onto nylon filter, and then hybridized with rat GLUT4 cDNA probe. The filter was washed and exposed to autoradiography. After that, the filter was stripped and hybridized with β-actin cDNA probe. The blots were analyzed by scanner densitometry as described for Western blotting analysis, and the results obtained for the GLUT4 mRNA blots were normalized by the respective β-actin value, and expressed as AU.

In vivo insulin-stimulated GLUT4 translocation in white adipose tissue. The experiment was carried out in rats previously deprived of food for 12 h. The animals were anesthetized, blood samples were collected from the tail, and the epididymal fat pads were collected before (right pad) and 10 min after (left pad) regular insulin injection (portal vein, 4 U/rat). From these adipose tissues, plasma membrane and microsomal fractions were obtained, as described in Western blotting. The GLUT4 translocation index was calculated using the following formula: GLUT4 translocation index (%) = plasma membrane GLUT4 × 100/(plasma membrane GLUT4 + microsomal GLUT4).

In vivo insulin-stimulated tyrosine phosphorylation of IR-β, IRS-1, and IRS-2 in liver and skeletal muscle. The experiment was carried out in rats previously deprived of food for 12 h. The animals were anesthetized, and the samples of liver were removed before (basal) and 30 s after regular insulin injection (portal vein, 4 U/rat). Liver samples were removed and homogenized (Polytron; Brickmann Instruments, Westbury, NY) in 4 ml of extraction buffer [1% Triton X-100, 100 mM Tris (pH 7.4), 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF, and 0.1 mg/ml aprotinin] at 4°C. Samples of skeletal muscle (SM) were removed before and 90 s after insulin injection and homogenized as described for liver. Following the method described by Carvalho et al. (6), equal amounts of protein from liver or SM of NS, LS, and HS rats were immunoprecipitated overnight at 4°C with specific antibodies (anti-IR-β, anti-IRS-1, anti-IRS-2). Immunoprecipitated samples were mixed with protein A-Sepharose for 4 h at 4°C and then submitted to electrophoresis and immunoblotted by anti-phosphotyrosine (anti-PY) antibody to observe the effect of insulin on the phosphorylation of proteins (IR-β, IRS-1, IRS-2). The contents of these proteins in SM and liver were analyzed by Western blotting.

Analytical methods. Plasma samples were assayed for glucose by the glucose oxidase method (Glicose Enzimatica, ANALISA Diagnostica, Belo Horizonte, MG, Brazil) and for insulin by RIA (Coat-A-Count, DPC Diagnostics Products, Los Angeles, CA). Insulin resistance was assessed from fasting insulin and glucose levels and the previously validated homeostasis model assessment (HOMA-IR) (4) as follows: HOMA-IR = fasting glucose (mmol/l) × fasting insulin (µU/ml)/22.5. The total protein concentration of membrane samples was assayed by Lowry’s method.

Statistical analysis. All values are reported as means ± SE. Data were analyzed with the GraphPad InStat Software, using ANOVA (1-way), when three groups were compared and Student-Newman-Keuls as a post hoc test. Basal and insulin-stimulated conditions in the same individuals were compared by paired Student’s t-test.

RESULTS

Baseline data. As shown in Table 1, body weight was lower in HS rats than in LS (P < 0.01) and NS rats (P < 0.05). The

Table 1. Characteristics of rats fed on NS (0.5% Na⁺), LS (0.06% Na⁺), and HS (3.12% Na⁺) diets from weaning to adulthood

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NS</th>
<th>LS</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>395±15</td>
<td>422±16</td>
<td>343±13*</td>
</tr>
<tr>
<td>Food intake, mg/g body wt −1 • 24 h</td>
<td>0.052±0.004</td>
<td>0.050±0.002</td>
<td>0.065±0.002†</td>
</tr>
<tr>
<td>Water intake, ml/24 h</td>
<td>28.9±1.94</td>
<td>27.15±1.56</td>
<td>92.97±7.46‡</td>
</tr>
<tr>
<td>White adipose tissue, g</td>
<td>5.29±0.48</td>
<td>6.78±0.56*</td>
<td>4.55±0.45‡</td>
</tr>
<tr>
<td>Skeletal muscle, g</td>
<td>2.29±0.08</td>
<td>2.43±0.08</td>
<td>1.97±0.06†</td>
</tr>
<tr>
<td>Heart, g</td>
<td>1.26±0.07</td>
<td>1.24±0.06</td>
<td>1.22±0.04</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>69.0±6.67</td>
<td>52.2±8.30</td>
<td>62.3±7.56</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>8.84±0.38</td>
<td>9.89±0.34</td>
<td>9.08±0.53</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>1.89±0.11</td>
<td>2.60±0.36*</td>
<td>1.09±0.15‡</td>
</tr>
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Values are means ± SE from 10–16 rats. Plasma insulin and glucose are referred to fed state; HOMA-IR index was calculated using plasma insulin and glucose in fasting state. HS, high sodium; †P < 0.05 vs. normal sodium (NS); ‡P < 0.01 vs. low sodium (LS); §P < 0.01 vs. NS; §P < 0.001 vs. NS, P < 0.001 vs. LS, 1-way ANOVA, Student-Newman-Keuls post hoc test.
24-h food and water intake was higher in HS rats compared with NS and LS rats \((P < 0.01\) and \(P < 0.001\), respectively). Adipose tissue weight was higher in LS than in NS \((P < 0.05)\) and HS \((P < 0.01)\); skeletal muscle weight \((g)\) was lower in HS than in NS \((P < 0.01)\) and LS \((P < 0.01)\). No differences were observed in heart weight and plasma glucose and insulin of animals in fed state. On the other hand, compared with NS rats, the HOMA-IR index, calculated from fasting glycemia and insulinemia, was increased and decreased in LS and HS rats, respectively \((P < 0.05)\).

**GLUT4 expression in white adipose tissue.** Compared with NS and LS rats, HS rats showed an increase of \(\sim 40\% \ (P < 0.05)\) in GLUT4 mRNA (Fig. 1A) and \(\sim 130\% \ (P < 0.05)\) in GLUT4 protein content of fat-free extract (Fig. 1B). GLUT4 protein in plasma membrane, expressed per microgram of protein subjected to electrophoresis, was \(\sim 48\% \ (P < 0.05)\) higher in HS rats than in NS and LS rats (Fig. 2A), and no significant differences were observed in microsomal GLUT4 content among the groups (Fig. 2B). When the total tissue content of plasma membrane GLUT4 was related to the body weight, a twofold increase \((P < 0.01)\) was found in HS rats compared with NS and LS rats (data not shown).

**GLUT4 expression in skeletal muscle and heart.** Figure 3 shows the GLUT4 analysis in skeletal muscle. No differences were observed in the GLUT4 mRNA content among NS, LS, and HS rats (Fig. 3A). However, Western blotting analysis showed that HS rats increased their GLUT4 protein content by \(\sim 20\% \ (P < 0.05)\) when expressed by microgram of protein subjected to electrophoresis (Fig. 3B) and by \(\sim 57\% \ (P < 0.01)\) when expressed by gram body weight (data not shown) compared with NS and LS rats. Finally, in the heart, there were no differences among NS, LS, and HS rats in GLUT4 protein \((84.3 \pm 11.9, 84.4 \pm 12.6, \text{and } 89.8 \pm 5.39 \text{ AU/\(\mu\)g of protein in NS, LS, and HS, respectively, } P = 0.893, n = 7)\) or GLUT4 mRNA \((184 \pm 39, 176 \pm 31, \text{and } 154 \pm 17 \text{ AU in NS, LS, and HS, respectively, } P = 0.759, n = 6)\).

**In vivo GLUT4 protein translocation in white adipose tissue.** Figure 4 shows the plasma membrane GLUT4 protein before and after acute insulin stimulation. HS rats had higher \((P < 0.05)\) GLUT4 content either in basal \((\sim 65\%)\) or under insulin-stimulated \((\sim 160\%)\) conditions (Fig. 4A). Moreover, compared with basal condition, we can observe that insulin stimulus significantly increased the plasma membrane GLUT4 protein in NS and HS rats and had no effect on LS rats (Fig. 4A). When the plasma membrane GLUT4 was related to the total cellular GLUT4 (Fig. 4B), HS rats showed a higher \((P < 0.05)\) basal \((\sim 31\%)\) and insulin-stimulated \((47\%)\) translocation index compared with NS and LS rats. Once more we observed that only HS and NS rats showed significant increase in the GLUT4 protein translocation index after the insulin stimulus (Fig. 4B).

**In vivo insulin-stimulated tyrosine phosphorylation of IR-\(\beta\), IRS-1, and IRS-2 in liver and skeletal muscle.** Figure 5 shows basal and insulin-stimulated tyrosine phosphorylation of IR-\(\beta\), IRS-1, and IRS-2 in liver and skeletal muscle.
In healthy humans, decreased insulin sensitivity was observed after 3 days but not after 7 days of LS diets (12); however, increased insulin sensitivity after 5 days on LS diet was also reported (10). Moreover, salt-sensitive but not salt-resistant normotensive individuals present resistance to insulin-mediated glucose disposal or a hyperinsulinemic response to oral glucose when submitted to a high-sodium diet (40). In rats, chronic salt overload (2 wk) decreased insulin sensitivity despite increased insulin signaling (27). Taking these studies together, the effect of sodium intake on insulin sensitivity seems to be time dependent, and the long-term effects of dietary sodium on insulin sensitivity are not clear.

In chronically treated rats (from weaning until the age of 3 mo), we reported lower insulin-dependent and -independent glucose uptake in isolated adipocytes from LS rats compared with HS rats (7). By using the euglycemic hyperinsulinemic clamp, we have recently confirmed that chronic salt restriction decreases while chronic salt overload increases insulin sensitivity (33). In the present study, by using a surrogate index for insulin resistance, the HOMA-IR, we have confirmed the decreased and increased insulin sensitivity in LS and HS rats, respectively. In fact, the HOMA-IR index was described as an excellent parameter of insulin sensitivity when analyzed in overnight-fasted subjects, closely mimicking the evaluation of insulin sensitivity by the glucose-clamp technique (4).

Although the LS rats did not significantly increase their body weight, an increased epididymal adipose tissue mass was observed, which we have already reported (33), suggesting that an obese condition may be present. In the same study, we observed that LS-treated rats are characterized by high plasma IRS-1, and IRS-2 in liver. LS rats showed reduced ($P < 0.01$) basal and insulin-stimulated tyrosine phosphorylation of IRS-2 ($−50\%$ and $31\%$, respectively) compared with NS and HS rats. HS rats showed enhanced ($P < 0.05$) insulin-stimulated IRS-1 ($−13\%$) and basal IRS-2 ($−56\%$) tyrosine phosphorylation. Insulin stimulus significantly increased tyrosine phosphorylation in each group compared with basal condition. Figure 6 shows the insulin-stimulated basal and insulin-stimulated tyrosine phosphorylation of IR-$\beta$, IRS-1, and IRS-2 in skeletal muscle. LS rats showed reduced ($P < 0.01$) basal ($−37\%$) and insulin-stimulated ($−56\%$) tyrosine phosphorylation of IRS-1 compared with NS and HS rats. HS rats showed enhanced ($P < 0.05$) basal ($−24\%$) tyrosine phosphorylation of IRS-1 compared with NS rats. Acute insulin stimulus significantly increased tyrosine phosphorylation in each group compared with basal condition.

**DISCUSSION**

In healthy humans, decreased insulin sensitivity was observed after 3 days but not after 7 days of LS diets (12); however, increased insulin sensitivity after 5 days on LS diet was also reported (10). Moreover, salt-sensitive but not salt-resistant normotensive individuals present resistance to insulin-mediated glucose disposal or a hyperinsulinemic response to oral glucose when submitted to a high-sodium diet (40). In rats, chronic salt overload (2 wk) decreased insulin sensitivity despite increased insulin signaling (27). Taking these studies together, the effect of sodium intake on insulin sensitivity seems to be time dependent, and the long-term effects of dietary sodium on insulin sensitivity are not clear.

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renin activity, and consequently, higher levels of ANG II are expected (7). Because ANG II acts through prostacyclin in promoting adipocyte proliferation and/or differentiation (9), we hypothesized that the increased adipose mass observed in LS rats could be caused by their characteristic renin-angiotensin hyperactivity (7). In fact, the fat pad weight related to the body weight (data not shown) showed that the fat mass is increased in LS rats and preserved in HS rats, reinforcing the idea that low sodium intake induces obesity, whereas salt overload must not be seen as a low fat mass state but as a reduced whole body developmental condition.

Epididymal white adipose tissue is a visceral fat, and there is substantial evidence that visceral adiposity represents a powerful risk factor for insulin resistance and diabetes (1). In humans, adipocytes in the central depot are particularly resistant to the antilipolytic action of insulin and can release free fatty acids into the portal vein, exposing the liver to high free fatty acid concentration. Thus the possibility might be considered that free fatty acids themselves participate in various functional organ defects such as reduced insulin-stimulated glucose uptake in skeletal muscle, reduced insulin secretion, hepatic overproduction of glucose, and reduced insulin clear-

Fig. 5. Insulin receptor (IR)-β (A) and IR substrate (IRS)-1 (B) and IRS-2 phosphorylation (C) in liver from NS (0.5% Na⁺), LS (0.06% Na⁺), or HS (3.12% Na⁺) diet-treated rats. Liver samples were collected before (−) and 30 s after (+) insulin injection (4 U/rat) and homogenized. Aliquots containing the same amount of protein were immunoprecipitated with anti-IR-β, anti-IRS-1, or anti-IRS-2 antibodies and immunoblotted with anti-phosphotyrosine antibody (PY). Data are means ± SE of 4–5 animals. **P < 0.01 vs. respective basal condition; ***P < 0.001 vs. respective basal condition; §P < 0.05 vs. NS and HS in the same condition; §§P < 0.01 vs. NS and HS in the same condition; †P < 0.05 vs. NS and LS in the same condition; ††P < 0.01 vs. NS and LS in the same condition. One-way ANOVA, Student-Newman-Keuls post hoc test was used when the same condition was compared in different groups; paired Student’s t-test was used when basal and insulin-stimulated conditions into the same group were compared.

Fig. 6. IR-β (A), IRS-1 (B), and IRS-2 phosphorylation (C) in skeletal muscle from NS (0.5% Na⁺), LS (0.06% Na⁺), or HS (3.12% Na⁺) diet-treated rats. Skeletal muscle samples were collected before (−) and 90 s after (+) insulin injection (4 U/rat) and homogenized. Aliquots containing the same amount of protein were immunoprecipitated with anti-IR-β, anti-IRS-1, or anti-IRS-2 antibodies and immunoblotted with anti-phosphotyrosine antibody (PY). Data are means ± SE of 4–5 animals. *P < 0.05 vs. respective basal condition; **P < 0.01 vs. respective basal condition; ***P < 0.001 vs. respective basal condition; §P < 0.05 vs. NS and HS in the same condition; §§P < 0.01 vs. NS and HS in the same condition; †P < 0.05 vs. NS and LS in the same condition. One-way ANOVA, Student-Newman-Keuls post hoc test was used when the same condition in different groups was compared; paired Student’s t-test was used when basal and insulin-stimulated conditions into the same group were compared.
Dietary sodium and GLUT4

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We observed that GLUT4 gene expression is unchanged in LS rats, whereas in HS rats an increase in GLUT4 protein was observed in white adipose tissue and skeletal muscle. These results showed that changes in GLUT4 protein content may be involved in the increased insulin sensitivity of HS rats but not in the reduced sensitivity observed in LS rats. Additionally, the calculated total tissue GLUT4, expressed by gram of body weight, was markedly increased in adipose tissue and skeletal muscle of HS rats, reinforcing an in vivo role of these tissues in glucose clearance, and in the increased insulin sensitivity of HS rats.

Several models of insulin resistance in rats have shown that in white adipose tissue, cellular content of GLUT4 protein and mRNA are similarly modulated. However, no such correlation has been found in skeletal muscle (19). These discrepancies may derive from the fact that in muscles, contractile activity also plays a role in the regulation of GLUT4 beyond the well-known metabolic-hormonal regulation (20). Higher GLUT4 mRNA and protein contents in the adipose tissue of HS rats suggest a transcriptional modulation of the GLUT4 gene. On the other hand, higher levels of GLUT4 protein, without changing GLUT4 mRNA, as observed in skeletal muscle of HS rats, suggest a posttranscriptional modulation of the GLUT4 gene. In streptozotocin-induced diabetes, reduced GLUT4 protein content in red and white portions of skeletal muscle was reported, concomitantly with either decreased (red muscle) or augmented (white muscle) GLUT4 mRNA levels. In contrast, in fasting, GLUT4 protein is decreased in red muscle in the presence of unaltered mRNA levels, whereas GLUT4 protein does not change in white muscle, and this is concomitant with a marked increase in GLUT4 mRNA levels (5). These data express a differential regulation of GLUT4 expression in red and white muscle and show the differences between protein and mRNA contents in muscles.

Several studies involving hypertension in humans (26) and in experimental models (44) have detected increased glucose uptake in cardiomyocytes. In the present study, although GLUT4 gene expression was increased in adipose and skeletal muscle of hypertensive HS rats, no changes were observed in the heart, suggesting a tissue-specific modulation of GLUT4 in this model.

In the present study, the GLUT4 translocation was analyzed only in white adipose tissue. However, considering the tyrosine phosphorylation of IRS-1 protein observed in skeletal muscle, we may assume that GLUT4 subcellular distribution in muscle was similar to that observed in white adipose tissue. The higher basal and insulin-stimulated translocation index of GLUT4 observed in adipose tissue of HS rats may be merely a consequence of increased GLUT4 expression, but it could also involve other mechanisms, such as the renin-angiotensin system and the bradykinin.

HS rats showed decreased plasma renin activity (33), which would reduce angiotensin activity. Angiotensin could impair the insulin signaling through both the ANG II type 1 receptor (AT1) (41) and the ANG II type 2 receptor (AT2) (8). Thus, in HS rats, reduced angiotensin activity may be involved in the increased insulin sensitivity. On the other hand, sodium overload increases bradykinin (31), which has been shown to increase the insulin-induced (15) and insulin-independent (18) glucose uptake. Additionally, it was reported that bradykinin can potentiate the insulin-induced GLUT4 translocation, by enhancing the phosphorylation of IR-β subunit and IRS-1 (15), as well as directly inducing GLUT4 translocation, independently of the phosphatidylinositol 3-kinase activation (18).

With regard to the low-sodium diet, our results showed a clear reduction in the insulin-stimulated GLUT4 translocation, possibly explaining the in vivo insulin resistance observed in LS rats (33). Increased plasma renin and angiotensin activity characteristic of LS rats (33) could impair insulin action (41). In fact, the treatment of LS rats with the angiotensin-convert-
ing enzyme inhibitor captopril improved their insulin resistance (33). However, the AT1 receptor blocker losartan was unable to improve insulin sensitivity (33), and AT2 receptors are not usually detected in the involved tissues (22, 28). Therefore, the low sodium intake effect seems not to be a consequence of the angiotensin and insulin signaling cross-talk. Nevertheless, because reduced insulin sensitivity in LS-treated rats could be caused by decreased bradykinin levels (13), and the blockade of angiotensin-converting enzyme increases bradykinin, the captopril effect in LS rats might be a consequence of increased bradykinin, as discussed above.

In humans, there are no strong randomized placebo-controlled trials showing that the angiotensin-converting enzyme inhibitors improve insulin sensitivity by reducing ANG II. Additionally, in human adipocytes, no effect of ANG II on the kinase activity of insulin receptor (2) and on the insulin-mediated glucose transport or suppression of lipolysis (32) was observed. Considering these observations, we speculate that bradykinin is the main modulator of insulin sensitivity changes induced by dietary sodium content, but this hypothesis requires further investigations.

In conclusion, in chronic experiments with HS rats, the present study demonstrated a direct relationship between insulin sensitivity, GLUT4 gene expression, and GLUT4 protein translocation to the plasma membrane. In LS rats, decreased insulin sensitivity was not related to the GLUT4 gene expression; however, reduced insulin-stimulated GLUT4 translocation may be involved. On the other hand, improvement in HS rats and impairment in LS rats of insulin signaling in skeletal muscle and liver may be additionally involved in the dietary sodium-induced modulations of insulin sensitivity.

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REFERENCES


Dietary Sodium and GLUT4


