Glucose uptake and glucose transporter proteins in skeletal muscle from undernourished rats

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Agote, María, Luis Goya, Sonia Ramos, Carmen Alvarez, M. Lucía Gavete, Ana M. Pascual-Leone, and Fernando Escrivá. Glucose uptake and glucose transporter proteins in skeletal muscle from undernourished rats. Am J Physiol Endocrinol Metab 281: E1101–E1109, 2001.—Undernutrition in rats impairs secretion of insulin but maintains glucose normotolerance, because muscle tissue presents an increased insulin-induced glucose uptake. We studied glucose transporters in gastrocnemius muscles from food-restricted and control anesthetized rats under basal and euglycemic hyperinsulinemic conditions. Muscle membranes were prepared by subcellular fractionation in sucrose gradients. Insulin-induced glucose uptake, estimated by a 2-deoxyglucose technique, was increased 4- and 12-fold in control and food-restricted rats, respectively. Muscle insulin receptor was increased, but phosphotyrosine-associated phosphatidyl-inositol 3-kinase activity stimulated by insulin was lower in undernourished rats, whereas insulin receptor substrate-1 content remained unaltered. The main glucose transporter in the muscle, GLUT-4, was severely reduced albeit more efficiently translocated in response to insulin in food-deprived rats. GLUT-1, GLUT-3, and GLUT-5, minor isoforms in skeletal muscle, were found increased in food-deprived rats. The rise in these minor glucose carriers, as well as the improvement in GLUT-4 recruitment, is probably insufficient to account for the insulin-induced increase in the uptake of glucose in undernourished rats, thereby suggesting possible changes in other steps required for glucose metabolism.

Diet protocols of food deprivation are commonly applied to animals for limited periods of their life span. We have previously established a rat model of undernutrition on the basis of a food restriction that begins in the fetal stage and continues until adulthood (12). This chronic deficiency better represents the condition of undernourished humans in developing countries. Food-restricted rats, according to this model, show normal glucose tolerance, despite the fact that the release of insulin is seriously impaired. Because skeletal muscle accounts for most of the whole body utilization of glucose, and white adipose tissue is severely reduced in these undernourished rats (12), an increased capacity to promote the uptake of glucose by muscle probably plays a major role in the enhanced insulin responses, as previously reported in other models of dietary restriction (7–9, 15).

The uptake of glucose depends on a facilitative glucose transporter family. GLUT-4, which is recruited to plasma membrane in response to insulin, is the main glucose carrier in skeletal muscle. Recent investigations have shown no effects of shorter dietary restriction on muscle GLUT-4 content in rats but a better recruitment after insulin (8, 9). Other GLUT isoforms, particularly GLUT-1, are also expressed in muscle fibers in a much lower proportion (reviewed in Ref. 41), and the effects of food restriction on them are not known at present. The goal of this work was to investigate the effect of chronic undernutrition on basal and insulin-induced glucose uptake in vivo in a represen-

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tative skeletal muscle such as the gastrocnemius by means of a hyperinsulinemic euglycemic clamp and to correlate this uptake with the content of the different glucose transporters present in muscle. Key proteins in the insulin-signaling cascade can be affected by nutrition (32, 39). Consequently, another purpose of the present study was to assess the effects of chronic undernutrition on different transducers in insulin-mediated glucose transport, especially considering the activation of phosphatidylinositol (PI) 3-kinase by insulin in vivo, which is an essential step in the hormone stimulation on glucose transport (3).

MATERIALS AND METHODS

Animals and diets. Wistar rats bred in our laboratory with controlled temperature and an artificial dark-light cycle (light from 0700 to 1900) were used throughout the study. Females were caged with males, and mating was confirmed by the presence of spermatozoa in vaginal smears. Each dam was housed individually from the 14th day of pregnancy. Food restriction was established from the 16th day of pregnancy. Control animals were fed a commercial standard laboratory diet ad libitum, containing by weight 19% protein, 56% carbohydrate (starch and sucrose), 3.5% lipid, 4.5% cellulose, 5% vitamin and mineral mix, and 12% water. Food-restricted animals were subjected to the following dietary pattern: pregnant rats received 10 g of the standard food daily until delivery. The number of pups in each litter was evened to eight. Lactating mothers received 15, 20, and 25 g of the standard diet daily during the 1st, 2nd, and 3rd wk of suckling, respectively. After weaning, only females were selected for this study. They received daily 35% of the diet consumed by controls until day 70 of their life. Water was given ad libitum. Food intake of control and undernourished rats has been previously reported (12).

Euglycemic insulin clamp. These studies were performed in control rats ~15 h after removal of food. In the undernourished group, they were performed 15 h after the restricted amount of food had been consumed. Rats were anesthetized with pentobarbital sodium (4 mg/100 g body wt), and after tracheotomy (to prevent respiratory problems), one carotid artery was catheterized for blood sampling. Once glycemia returned to the level observed before anesthesia (~40 min), insulin (Actrapid; Novo, Copenhagen, Denmark) was infused through a saphenous vein at a constant rate to reach an insulin dose of 5.0 IU·h⁻¹·kg⁻¹. A solution of glucose, 30 and 40% for control and food-restricted rats, respectively, was also infused through the other saphenous vein 5 min after the infusion of hormone was started. The difference in the concentration of glucose was necessary to infuse similar final volumes in both groups. The infusion rate was adjusted to clamp blood glucose at the level present in the conscious animals. To achieve this rate, blood samples were taken every 5 min from the carotid artery, blood glucose was determined within 2 min using a Reflolux II glucose analyzer (Boehringer Mannheim, Mannheim, Germany), and the pump dial was adjusted according to the changes in the level of blood glucose. Within 40 min of the start of the clamp, plasma insulin and glucose levels remained constant without further adjustment of the pump dial. At this steady state, insulin infusion was equal to insulin clearance, and the overall glucose utilization reached a constant value. This condition was maintained for 60 min, and then the rats were cervically dislocated. The gastrocnemius muscle of both hindlimbs was quickly excised, trimmed free of fat and connective tissue, freeze-clamped in liquid N₂, and stored at ~80°C until assayed. The clamp was also applied to a group of 35-day-old control rats that weighed approximately the same as the 70-day-old undernourished rats to quantify the glucose infusion rate to maintain euglycemia.

Estimation of glucose uptake. The uptake of glucose by gastrocnemius was estimated by measuring the accumulation of the phosphorylated form of the glucose analog 2-deoxy-D-glucose. A bolus of 80 μCi 2-deoxy-D-[¹³C]glucose (Amersham, Alesbury, UK) was injected intravenously 40 min after the clamp experiment was started, that is, under steady-state condition, as required by the theoretical model. The same bolus was administered 40 min after anesthesia to rats not infused with insulin to estimate basal uptake of glucose. Arterial blood was sampled for determination of the concentration of blood glucose and 2-deoxy-D-[¹³C]glucose radioactivity. At the end of the experiment, rats were killed, and gastrocnemius muscles were removed and stored as indicated. This tissue was digested at 60°C for 45 min in 1 M NaOH, and the 2-deoxy-D-[¹³C]glucose 6-phosphate content was determined as described previously (13). This method is based on the fact that both 2-deoxyglucose and 2-deoxyglucose 6-phosphate remain soluble in 6% HClO₄ extracts, whereas 2-deoxyglucose 6-phosphate precipitates in the Somogyi reagent [BaSO₄·Zn(OH)₂]. The rate of glucose utilization was calculated by dividing the disintegrations per minute of 2-deoxy-D-[¹³C]glucose 6-phosphate in the tissue by the calculated integral of the ratio of arterial blood 2-deoxy-D-[¹³C]glucose to glucose concentration.

Muscle fractionation. The procedure used to isolate plasma and intracellular membranes was similar to that described by Gümä et al. (18), with some modifications. Approximately 6 g of muscle (for the undernourished rats, the two gastrocnemius muscles were pooled) were minced and homogenized at 4°C in a Polytron at low speed (setting 4.8) for 20 s in 2,000 M KCl solution, left on ice for 15 min, and centrifuged for 10 min at 2,000 g. The two supernatants were pooled and subjected to ultracentrifugation at 190,000 g for 1 h. The resulting pellet, which contained crude membranes, was resuspended using a tissue grinder in 3 ml of buffer A [20 mM HEPES, 0.15 M KCl, containing 1 μM leupeptin and 100 μM phenylmethylsulfonyl fluoride (PMSF) as protease inhibitors, pH 7.4]. A solution of KCl was then added to the homogenate to a final concentration of 0.65 M, and it was left on ice for 15 min. It was then centrifuged at 2,000 g for 10 min. The supernatant was collected and kept on ice. The pellet was resuspended in 7 ml of buffer A, rehomogenized as indicated above for 10 s, treated with the KCl solution, left on ice for 15 min, and centrifuged for 10 min at 2,000 g. The two supernatants were pooled and subjected to ultracentrifugation at 190,000 g for 1 h. The resulting pellet, which contained crude membranes, was resuspended using a tissue grinder in 3 ml of buffer (0.25 M sucrose, 10 mM NaHCO₃, 5 mM Na₃cit, and 100 μM PMSF, pH 7.4). A 0.05-ml sample was removed for measurements of GLUT content, marker enzymes, and proteins. The rest was loaded onto the top of a discontinuous sucrose gradient, 25, 30, and 35% (wt/wt, in 20 mM HEPES, pH 7.4), and centrifuged for 16 h at 150,000 g. Fractions were collected from the top of the 25% gradient (25% fraction) and from interphases 25–30% (30% fraction) and 30–35% (35% fraction). The pellet was also collected (35P fraction). All of the fractions were diluted 10-fold with buffer A and centrifuged at 190,000 g for 90 min. The resulting pellets were resuspended in 20 mM HEPES, pH 7.4. Proteins were assayed, and fractions were kept frozen at ~80°C.

Western blot analyses. The fractions of muscle membrane were subjected to SDS-PAGE on 7–10% polyacrylamide gels according to Laemmli (28). Proteins were then electrophoretically transferred to polyvinylidene difluoride filters (PVDF) protein sequencing membrane, Bio-Rad Laboratories,
Alcobendas, Spain) for 2 h. After transfer, the filters were blocked with 5% (wt/vol) nonfat dry milk in phosphate-buffered saline with 3% bovine serum albumin and 0.02% sodium azide. Antibodies against the GLUT-1 and GLUT-4 glucose transporters were purchased from Biogenesis (Sandown, NH) and were used at dilutions 1:5,000 and 1:1,000, respectively. Antibodies against GLUT-3 and GLUT-5 (1:2,500 dilutions) were obtained from Chemicon (Temecula, CA). Anti-insulin receptor, β-subunit, and anti-rat α subunit of NADPH oxidase (Upstate Biotechnology, Lake Placid, NY) were diluted at 1:250. The PVDF filters were next washed four times for 10 min at 37°C with phosphate-buffered saline with 1% Tween 20, followed by a 1-h incubation with goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase (Sigma BioSciences, St. Louis, MO). The PVDF membranes were then washed as already indicated. Detection of antibody-antigen complexes was accomplished by the enhanced chemiluminescence method (BM Chemiluminescence, Boehringer Mannheim, Mannheim, Germany). Optical density of bands was determined by laser scanning densitometry (Molecular Dynamics, Sunnyvale, CA).

Immunoblots were performed under linear conditions with regard to the amount of protein loaded on the gel. The PVDF filters were finally stained with Coomassie blue to confirm, in the same Western assay, that equal amounts of protein were analyzed as well as the heterogeneity of the protein composition of the different sucrose fractions. 

RNA isolation and Northern blot analysis. RNA was extracted from gastrocnemius (500 mg) obtained from rats in the basal condition, by use of the guanidinum isothiocyanate-phenol-chloroform method (6). After quantification, total RNA (30 μg) was subjected to Northern blot analysis, following the method previously described (38). A 2.4-kb rat GLUT-4 cDNA insert subcloned from pBluescript KS†-EcoRI lane into the EcoRI site of pBluescript KS+ (Stratagene, Merck-Farma y Química, Barcelona, Spain) and a 2.6-kb rat GLUT-1 cDNA insert subcloned from pGT3 into pBluescript KS+ at the EcoRI site (Promega Innogenetics Diagnostica y Tecnología, Spain) were kindly provided by Dr. A. Zorzano (Dept. of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain) and were used as probes. Membranes were autoradiographed, and relative densitometric analysis of signals was determined by densitometric scanning of the autoradiograms in a laser densitometer. 

PI 3-kinase assay. To determine insulin-stimulated PI 3-kinase activity, rats were anesthetized with pentobarbital sodium as indicated, the abdominal cavity was opened, the portal vein was exposed, and 5 IU of insulin were injected. After 90 s, gastrocnemius muscles were quickly removed and freeze-clamped with liquid N2 and stored at −80°C until assayed. Muscles (100 mg) from basal and insulin-injected rats were homogenized with a Polytron operated at maximum speed in 1 ml of lysis buffer, composed of 50 mM HEPES (pH 7.4), 1% Triton X-100, 50 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF, 2 mM benzamidine, and 20 μM leupeptin. The homogenates were left on ice for 30 min and then subjected to centrifugation at 180,000 g for 60 min at 4°C. The supernatants were used as samples for protein and PI 3-kinase determinations. Aliquots containing 2 mg of protein were immunoprecipitated with monoclonal anti-phosphotyrosine antibody (Santa Cruz Quimigranel, Madrid, Spain), and immunocomplexes were collected with anti-mouse IgG agarose (Sigma BioSciences).

PI 3-kinase activity was assayed by phosphorylation of PI with [32P]ATP (Amersham, Bucks, UK). The phosphorylated PI was analyzed by thin-layer chromatography by use of previously described procedures (38). The products of the radioactive reaction were visualized by autoradiography and quantified by densitometry. 

Determination of insulin receptor substrate-1. Gastrocnemius muscles from rats in the basal state were extracted as aforementioned, and samples containing 0.5 mg of protein were immunoprecipitated with polyclonal anti-rat insulin receptor substrate-1 (IRS-1; Upstate Biotechnology). The complexes were bound to anti-mouse IgG agarose as described. The agarose beads were treated with Laemmli sample buffer with 100 mM dithiothreitol at 95°C for 5 min and subjected to SDS-PAGE (6%). The rest of the Western blot procedure was performed as described for GLUT4 determinations, with polyclonal anti-rat IRS-1 as primary antibody, diluted at 1 μg/ml. 

Other analytical procedures. The concentration of protein was determined by the Bradford method (2) with the Bio-Rad protein assay and with γ-globulin as standard. The specific activity of phosphodiesterase-I was assayed as a plasma membrane marker (27). Plasma insulin was determined by RIA with rat insulin as standard (Incstar, Stillwater, MN). This method allows the determination of 2.0 ng/ml, with a coefficient of variation within and between assays of 10%. 

Expression of the results. All of the data are reported as means ± SE. A difference between two mean values was assessed with the Student’s t-test. For multiple comparisons, significance was evaluated by ANOVA, followed by the protected least significant difference test. 

RESULTS

Body weight of food-restricted rats was reduced to 50% compared with the control value at 70 days of life

| Table 1. Weight, blood glucose, plasma insulin, glucose infusion rate, and glucose utilization in control and undernourished 70-day-old rats |
|--------------------------------------------------|-----------------|-----------------|
|                                                  | Basal           | Insulin Treated |
|                                                  | Control         | Undernourished  |
|                                                  | Control         | Undernourished  |
| Weight, g                                        | 188.0 ± 11.2    | 93.5 ± 7.4‡     |
| Blood glucose, mg/ml                             | 0.93 ± 0.04     | 0.89 ± 0.02     |
| Plasma insulin, μU/ml                            | 24.9 ± 3.4      | 16.8 ± 2.4*     |
| Steady-state glucose infusion rate, mg·min⁻¹·kg⁻¹ | 26.3 ± 4.9      | 42.5 ± 3.0†     |
| Muscle glucose utilization rate, ng·min⁻¹·mg protein⁻¹ | 6.2 ± 0.5     | 3.3 ± 0.3†      |
|                                                  | 21.2 ± 0.2      | 1.3 ± 0.4†      |
|                                                  | 9.0 ± 1.4       | 17.0 ± 1.3†     |

Results are means ± SE for 8–12 independent experiments. Significant differences between control and undernourished rats: *P < 0.05, †P < 0.01, ‡P < 0.001.
Insulin did not affect the content of this protein marker, being predominant in the plasma membranes. This marker phosphodiesterase-I was 26.6-fold higher in undernourished rats submitted to fractionation in sucrose gradients (Table 1). The uptake of 2-deoxyglucose in the food-deprived rats in the basal state was 50% that of controls. After insulin, glucose utilization was activated in the two groups of rats. However, the food-restricted group underwent a remarkably higher increase (12- vs. 4-fold), and their values exceeded those of controls (Table 1).

Undernutrition did not affect the recoveries of protein in the fractions of membrane obtained from gastrocnemius (Table 2). Specific activities of the enzyme marker phosphodiesterase-I were 7- to 15-fold higher in the fraction enriched in the plasma membranes (25% sucrose) compared with those in the crude membranes. No changes in this enzyme activity were produced by insulin, whereas undernutrition significantly increased phosphodiesterase-I activity (Table 2).

The α1-subunit of Na+-K+-ATPase was barely detected in the fraction enriched in intracellular membranes, being predominant in the plasma membranes. Insulin did not affect the content of this protein marker (Fig. 1A).

The distribution of GLUT-4 and GLUT-1 is shown in Fig. 1B. Every fraction of membrane contained significant amounts of GLUT-4. However, when the proteins recovered were considered (Table 2), this carrier isoform was found predominantly in intracellular membranes. In contrast, GLUT-1 was mainly collected in the plasma membrane fraction (Fig. 1B). As shown in Fig. 2A, the content of GLUT-1 in plasma membranes was increased 2- to 3.5-fold in food-restricted rats above control values, without changes after insulin treatment. The whole content of GLUT-4 underwent a remarkable decrease (~70%) after undernutrition, as shown in Fig. 2B. When gastrocnemius muscle was fractionated, this decrease was evident in plasma as well as in intracellular membranes (Fig. 3). In response to insulin, an increase in GLUT-4 present in plasma membranes was concomitant to a decrease in the intracellular content in both groups of rats. However, the relative quantity of GLUT-4 translocated was different: whereas a 1.6-fold increase in plasma membrane and a 1.3-fold decrease in intracellular membrane with respect to the contents found in the basal state were observed in control rats, a 3.0-fold increase and a 3.3-fold decrease, respectively, were observed in those food deprived (Fig. 3).

Figure 4 depicts representative experiments showing the Northern blot analysis of muscle GLUT-4 and GLUT-1 mRNA. The GLUT-4 mRNA content was decreased in gastrocnemius muscle of undernourished rats compared with controls. In contrast, GLUT-1 mRNA remained unchanged. The mean content of

Table 2. Protein recovery and phosphodiesterase-I activities in gastrocnemius from control and undernourished rats submitted to fractionation in sucrose gradients

<table>
<thead>
<tr>
<th>Fraction</th>
<th>CM</th>
<th>25%</th>
<th>30%</th>
<th>35%</th>
<th>35 P</th>
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<tbody>
<tr>
<td>Protein recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16.9±0.6</td>
<td>50±3</td>
<td>165±8</td>
<td>602±64</td>
<td>6.7±0.3</td>
</tr>
<tr>
<td>Undernourished</td>
<td>15.7±0.3</td>
<td>49±3</td>
<td>172±9</td>
<td>510±64</td>
<td>5.8±0.2</td>
</tr>
<tr>
<td>Phosphodiesterase-I activities</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Insulin</td>
<td></td>
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<tr>
<td>Control</td>
<td>23±3</td>
<td>24±3</td>
<td>338±42</td>
<td>334±35</td>
<td>267±40</td>
</tr>
<tr>
<td>Undernourished</td>
<td>37±7†</td>
<td>36±7†</td>
<td>453±92†</td>
<td>429±90*</td>
<td>390±72†</td>
</tr>
</tbody>
</table>

Protein recovery: values are means ± SE for 12 experiments. CM, crude membranes. Data are expressed as milligrams per gram of muscle (CM and 35P) and micrograms per gram of muscle (25, 30, 35%). Phosphodiesterase activities: data are means ± SE for 6 different experiments expressed as nanomoles of p-nitrophenol released per milligram of protein per minute. Significant differences between control and undernourished in the same group: †P < 0.05, **P < 0.01.
mRNA corresponding to β-actin, used for normalization, was similar in both groups. The presence of GLUT-3 and GLUT-5 was barely evident in muscle crude membranes of control rats (Fig. 5). However, both protein transporters underwent large increases after food restriction: three- and sixfold above the control values for GLUT-3 and GLUT-5, respectively. Insulin receptor (β-subunit) was significantly increased in gastrocnemius muscles of undernourished rats, as shown in Fig. 6A. However, food restriction did not alter the muscle content of IRS-1 (Fig. 6B).

Anti-phosphotyrosine immunoprecipitable PI 3-kinase activities were the same in both groups of rats in the basal state. The enzyme was remarkably stimulated 90 s after a single insulin dose (see MATERIALS AND METHODS), although the peak was significantly smaller in undernourished rats than in controls. In the hyperinsulinemic condition established for 60 min (clamp technique), PI 3-kinase was decreased compared with controls.
the levels found immediately after insulin injection, and again enzyme activity was lower in the food-restricted rats (Fig. 7).

**DISCUSSION**

In a previous study (12), we showed that rats subjected to undernutrition from the fetal stage until adulthood are glucose tolerant, despite the fact that food restriction alters β-cell function (22, 30) and induces hypoinsulinemia. This result is linked to the fact that both muscle and adipose tissues from chronically undernourished rats present a compensatory increase in insulin-induced glucose uptake (12). Because the livers of food-restricted rats seem to undergo insulin resistance and the amount of adipose tissue is largely reduced, the increased glucose disposal in vivo after insulin treatment observed in the present as well as in the previous study (12) must be contributed mainly by skeletal muscle. In fact, gastrocnemius muscle from these rats exhibits a much greater increment in uptake of 2-deoxyglucose under the euglycemic insulin clamp than in their controls. A similar insulin-sensitizing effect of calorie restriction on the transport of muscle glucose has been found in rats and mice subjected to reduced food consumption for shorter periods (7, 9, 15, 25). However, in such cases, the uptake of basal glucose by incubated muscles remains unaltered, whereas it is significantly diminished in vivo in the present model of chronic undernutrition. Because a primary regulated step in glucose utilization is the membrane transport, the possibility arises that this discrepancy could result from a different effect of the type of food deprivation on the amount and/or functional activity of muscle glucose carriers. The abundance of GLUT-4 is not altered in skeletal muscle from rats or rhesus monkeys subjected to...
GLUCOSE TRANSPORTERS IN UNDERNOURISHED RATS

Fig. 7. Phosphatidylinositol 3-kinase (PIP) in gastrocnemius muscle from C and U rats in the basal condition, treated with a single insulin dose and subjected to hyperinsulinemic clamp. Blots show 1 representative experiment. Bars correspond to means ± SE from 5 independent determinations. Differences between C and U rats: *P < 0.05, **P < 0.001.

to caloric restrictions without malnutrition (8, 9, 16). Consequently, we studied this and other glucose carrier isoforms in gastrocnemius muscles from chronically undernourished rats, analyzing specifically the subcellular distribution of GLUT-4 and GLUT-1.

Undernutrition leads to a large decrease in GLUT-4 protein and mRNA in skeletal muscle. On the other hand, GLUT-1 is significantly increased without changes in the corresponding mRNA. The decline in GLUT-4 probably leads to the impaired basal uptake of glucose shown in undernourished rats, which would not be sufficiently compensated for by the rise in GLUT-1. Although it is currently accepted that GLUT-1 is the main carrier for unstimulated glucose transport, our results support the idea that GLUT-4 might also be important in mediating this transport, as recently proposed by other authors (16). In favor of this idea, it could be argued that GLUT-4 intrinsic activity is higher than that of GLUT-1 (21), which accounts for only a minor part of total glucose transporters (41) and seems to be primarily present in the perineural sheaths rather than in muscle fiber (20). Moreover, although GLUT-4 is located mainly in intracellular membranes in the basal state, our data as well as those of the others (31) show a significant presence of this carrier in the plasma membrane in such a condition, probably due to the exposure of muscle fibers to basal insulinemia and tonal contraction, both inducers of GLUT-4 translocation (29).

Because the muscle uptake of glucose is increased in hyperinsulinemic, undernourished rats over the control values, we studied insulin’s effect on the translocation of GLUT-4 from the intracellular to the plasma membrane. When the decrease in the abundance of GLUT-4 in the chronically food-restricted rats is taken into account, the ability of insulin to recruit this carrier seems to be improved by undernutrition. An increase in GLUT-4 translocation in response to insulin has also been reported in skeletal muscle from calorie-restricted rats, and in this case, it leads to a higher amount of this carrier in the plasma membrane (9). However, in the present undernutrition model, the final amount of GLUT-4 located at the cell surface in hyperinsulinemic state is still lower than in control rats. Consequently, we thought that merely a better GLUT-4 translocation cannot be sufficient to explain the insulin-sensitizing effect of chronic undernutrition on the muscle glucose uptake. Thus we studied other GLUT isoforms to explore whether they could explain the enhanced muscle uptake of glucose. Undernutrition led to an increase in both GLUT-3, which is barely detectable in control rats, and GLUT-5, which is mainly a fructose carrier but has a low capacity to transport glucose (17). To the best of our knowledge, we have established the first evidence that GLUT-5 is present in rat gastrocnemius muscle and that it is enhanced by undernutrition. Because fructose contributes to carbohydrate metabolism in muscle (40), the increase in GLUT-5 could be an adaptation to the chronic reduction in food consumption, perhaps to favor a better utilization of fructose supplied in the diet. In any case, we think that the improvement in muscle insulin-induced glucose uptake, as seen in this work, cannot be accounted for by increases in these minor carrier isoforms. The possibility arises from the fact that chronic undernutrition will affect the GLUTs’ intrinsic activity, although this parameter is not altered in rats food restricted for limited periods of life (9).

Improvements in the uptake of glucose might be secondary to several systemic changes. One of them could be the basal rate of blood flow, which is also increased by insulin (33). This hormonal effect is improved in muscle after physical training (37). A similar change elicited by undernutrition, which in turn would affect the uptake of glucose, may also be considered to explain our results. Plasma fatty acids can also influence insulin sensitivity (5, 36), but in a previous study (12), we did not find differences between control and undernourished rats in plasma fatty acids and ketone body. Changes in counterregulatory hormones might improve insulin actions in food-restricted rats. However, increased insulin-induced glucose uptake has been reported in isolated muscle preparations in vitro from rodents submitted to dietary restriction (7–9, 15), a result which seems to be consistent with our in vivo results.

Nonetheless, few studies have determined the effects of undernutrition on the insulin-signaling pathways, an important question to identify the cellular mechanism that could explain the improved muscle insulin sensitivity. Therefore, in the present work, we have shown that this condition upregulates the insulin receptor, as occurs in prolonged fasting (34). An increase in tyrosine-phosphorylated insulin receptor has been reported in gastrocnemius from calorie-restricted rats injected with insulin (10). In the present model, undernutrition did not alter muscle IRS-1 content, whereas
it decreased in rodents subjected to calorie restriction for 20 days (9, 15). Despite the increase in GLUT-4 recruitment, we found that insulin activation on PI 3-kinase, which plays an important role in this recruitment (3), was partially impaired in undernourished rats, in contrast with the lack of changes after shorter periods of food restriction (9, 10). These results could indicate, as previously suggested by others (10), that the improved uptake of glucose secondary to undernutrition does not necessarily involve a concomitant change in the PI 3-kinase sensitivity to insulin. An alternative explanation could be based on the fact that PI 3-kinase is compartmentalized in adipocytes (35), which opens up the possibility of a similar situation in the muscle and suggests different effects of undernutrition on distinct PI 3-kinase pools. The increase in insulin action secondary to undernutrition could also be derived from alterations in other pathways related to the effects of insulin on the uptake of glucose. Food restriction may affect protein kinase C isoforms involved in insulin signaling, leading to GLUT-4 translocation in muscle primary cultures (4) or influence c-Cbl-associated proteins, recently proposed as another carrier (1). Finally, a new glucose carrier, designated GLUT-X1 (24) may be enhanced by food restriction. This carrier seems not to be involved in basal uptake of glucose, which is decreased in our undernourished rats.

An understanding of the adaptive mechanisms elicited to compensate for the partial loss of GLUT-4 when glucose normotolerance is maintained could be beneficial in the treatment of insulin resistance states in the long run. To this effect, undernutrition represents a useful experimental model to be added to others currently studied.

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