Up-regulation of phosphatidylinositol 3-kinase and glucose transporter 4 in muscle of rats subjected to maternal undernutrition

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Abstract

Early postnatal nutrition has been associated with the long-term effects on glucose homeostasis in adulthood. To elucidate the molecular mechanisms by which undernutrition during early life leads to changes in insulin sensitivity, we investigated the insulin signaling in skeletal muscle of rats during development. Offspring of dams fed with either protein-free or normal diets during the first 10 days of lactation were studied from lactation period until adulthood. Early maternal undernutrition impaired secretion of insulin but maintained normal blood glucose levels until adulthood. Insulin receptor (IR) activation after insulin stimulation was decreased during the period of protein restriction. In addition, glucose uptake, insulin receptor substrate 1 (IRS-1) phosphorylation and glucose transporter 4 (GLUT-4) translocation in muscle were reduced in response to insulin during suckling. In contrast, non- or insulin-stimulated glucose uptake and GLUT-4 translocation were found significantly increased in muscle of adult offspring. Finally, basal association of phosphatidylinositol 3-kinase (PI3-kinase) with IRS-1 was increased and was highly stimulated by insulin in muscle from adult rats. Our findings suggest that early postnatal undernutrition increases insulin sensitivity in adulthood, which appears to be directly related to changes in critical steps required for glucose metabolism.

Keywords: PI3-kinase; GLUT-4; Skeletal muscle; Undernutrition; Insulin sensitivity

1. Introduction

Epidemiological and experimental studies have demonstrated that maternal nutrition environment during pre- and/or postnatal period may predispose to a higher susceptibility to chronic diseases in adulthood [1,2]. These studies provide evidence that specific biological mechanisms might operate at morphological, cellular or molecular level memorizing the metabolic effects of early nutritional environments [3,4]. The metabolic reorganization that might occur during developmental period could induce permanent effects on key organs activity, actively influencing the development of degenerative diseases, most of them associated to the impairment of hormonal homeostasis, such as cardiovascular disease, hypertension, obesity and type 2 diabetes [5–16].

Different studies have shown that deprivation of protein calories during early stages of development has effects on glucose metabolism in adulthood [2,9,17–20]. Thus, some investigations have shown an increased insulin sensitivity in adult offspring from dams fed a low-protein diet during pregnancy and/or lactation [14,21–23]. In addition, it has been observed that adult offspring from dams fed with a protein-free diet during early lactation displayed reduction in insulin secretion associated with increase in insulin sensitivity [24–27].

The effect of insulin acutely stimulating glucose uptake and metabolism in peripheral tissues is essential for normal glucose homeostasis [28]. Insulin action involves multiple
signaling pathways initiated by insulin binding to insulin receptor (IR), eliciting receptor autophosphorylation with receptor tyrosine kinase activation, resulting in tyrosine phosphorylation of several substrates [29–32]. One of these phosphorylated proteins, insulin receptor substrate 1 (IRS-1), is critical for the mitogenic and metabolic effects of the hormone [33]. The tyrosine phosphorylation of IRS-1 generates docking sites for several proteins containing Src homology 2 (SH2) domains [34]. Among these, the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3-kinase), a necessary step on glucose transport stimulation [29,35], which is brought about primarily by translocation of the major insulin-responsive glucose transporter, GLUT-4, from intracellular vesicles to the plasma membrane [36]. Alterations in insulin sensitivity have been frequently associated to insulin receptor and postreceptor signaling defects. Naturally occurring mutations in the primary sequence of the IR, a low expression of IR molecules on the plasma membrane of the target cells, or alterations in insulin postreceptor signaling can result in decreased insulin sensitivity [37–39]. Recent evidence showed that a reduction in the association of PI3-kinase p85 subunit with IRS-1 could be a key abnormality related to insulin resistance in skeletal muscle [40]. Studies with GLUT-4 knockout mice have shown a direct involvement of GLUT-4 in insulin resistance and glucose tolerance [41]. In addition to the association between the glucose transporter and the reported pathologies, Katsumata et al. [42] demonstrated that GLUT gene expression in muscle seems to be regulated by nutritional status. Along this line, Ozanne et al. [18] showed that maternal malnutrition during fetal life induces an increase in insulin sensitivity apparently dependent on GLUT-4 up-regulation. It has been also demonstrated that undernutrition improves glucose uptake as well as GLUT-4 content [43,44], indicating that alterations in early steps of insulin signaling pathway may play an important role regulating insulin sensitivity.

The goal of this work was to investigate the effect of early maternal undernutrition on basal and insulin-induced glucose uptake in muscle during development. These results were then related to key proteins in the insulin-signaling cascade.

2. Materials and methods

2.1. Animals and diets

The procedures used throughout this study were approved by our Institutional Ethics Committee and are in accordance to National Institutes of Health animal care guidelines. Wistar rats were housed in controlled temperature rooms at 23–25 °C. Virgin female rats were mated and the pregnant dams, housed in individual cages, were fed with a normal diet containing 23% protein during gestation. After delivery, each lactating dam was kept with six male pups and one group of dams received a protein-free diet during the first 10 days of lactation while the other group received a normal diet. Both groups were fed ad libitum and diets were isocaloric, including vitamins and mineral mixture. The composition of the diets is shown in Table 1. At the end of lactation (day 21), the pups were separated from dams and received the normal diet until 60 days of age. The animals were divided in two groups: control group (C-group: offspring from dams which received normal diet) and undernourished group (UN-group: offspring from dams which received protein-free diet) and studied at ages 4, 8, 15, 21, 30 and 60 days. In all experiments, animals with the same age of the UN-group matched the C-group. Body weights were measured daily. Blood was collected and glucose levels were monitored using Advantage II Glucose Blood monitor (Roche Diagnosis Co, Indianapolis, IN, USA). Plasma insulin concentrations were determined by radioimmunoassay kit (ICN Pharmaceuticals Inc., Orangeburg, NY, USA).

2.2. Glucose transport studies

The glucose transport into muscle tissue was assayed using the isolated muscle strip technique as described earlier [46]. Briefly, tibialis anterior muscles were removed and 30-mg tissue strips were preincubated in Krebs–Ringer buffer in the presence or in the absence of insulin (1 μM) for 20 min, at 37 °C under continuous gassing with 95% O2/5% CO2. Samples were incubated for 10 min in the presence of 1 mM 2-deoxy-D-[14C] glucose (15.4 Ci/mmol; New England Nuclear, Boston, MA, USA). After that, the muscle strips were homogenized in 1% trichloroacetic acid (TCA) and centrifuged at 10,000 × g. Radioactivity in the supernatant was determined by liquid scintil-
lation counting (Beta Counter, Beckman Instruments, Fullerton, CA, USA).

2.3. Subcellular fractionation of skeletal muscle

Skeletal muscle membranes were isolated as described by Ozanne et al. [18]. Briefly, tibialis anterior muscle strips (30 mg) were incubated for 30 min at 37 °C in phosphate buffered saline (PBS) with insulin (1 μM). After incubation, tissue was homogenized in ice-cold homogenization buffer containing 50 mM Mes, pH 6.4, 1 mM MgCl₂, 10 mM EDTA, the following protease inhibitors: 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM benzamidine, 1 μM aprotinin, 1 μM leupeptin and 1 μM soybean trypsin inhibitor (Sigma Chemicals Co, St. Louis, MO, USA). For fractionation experiments, tissue was homogenized in 50 mM Mes, pH 6.4, 1 mM MgCl₂, 10 mM EDTA, 1 μg/ml DNase, 0.5 μg/ml RNase and the following protease inhibitors: 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM benzamidine, 1 μM aprotinin, 1 μM leupeptin and 1 μM soybean trypsin inhibitor (Sigma Chemicals Co, St. Louis, MO, USA). For fractionation experiments, tissue was homogenized in 50 mM Mes, pH 6.4, 1 mM MgCl₂, 10 mM EDTA, 1 μg/ml DNase, 0.5 μg/ml RNase and protease inhibitors, centrifuged at 13,000 × g for 15 min and supernatant was centrifuged at 190,000 × g for 60 min. The pellet resuspended in homogenization buffer was overlayed onto a 25, 30 and 35% (wt/wt) step sucrose gradient and centrifuged at 190,000 × g for 75 min. Plasma membranes found on the sample layer—25% sucrose interface were washed in homogenization buffer and centrifuged at 150,000 × g for 60 min. Proteins were assayed and fractions were kept frozen at −70 °C.

2.4. Immunoprecipitation

After treatment with insulin, muscle strips (30 mg) were lysed in 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1.5 mM MgCl₂, 1.5 mM EDTA, 0.1% Triton X-100, 10% glycerol, 10% aprotinin, 10 μg/ml leupeptin, 2 μg/ml pepstatin and 1 mM PMSF. Lysates (2 μg/μl) were incubated overnight at 4 °C with polyclonal goat anti-IRS-1 antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Then, protein A/G agarose (20 μg/ml protein; Santa Cruz Biotechnology) was added and samples were incubated at 4 °C under rotation for 2 h. The content of IRS-1 and PI3-kinase associated to IRS-1 was analyzed by Western blotting as subsequently described.

2.5. Western blotting

Cellular proteins (30 μg total) were subjected to 10% SDS-PAGE, transferred to polyvinylidene difluoride filters (PVDF Hybond-P, Amersham Pharmacia Biotech) and blocked with Tween-TBS (20 mM Tris–HCl, pH 7.5, 500 mM NaCl, 0.01% Tween-20) containing 1% bovine serum albumin. Primary antibodies used in Western analysis were anti-insulin receptor β-subunit (1:1000; Santa Cruz Biotechnology); anti-IRS-1 (1:1000; Santa Cruz Biotechnology); anti-GLUT-4 (1:1000; Santa Cruz Biotechnology) or anti-PI3-kinase p85 subunit (1:1000; Santa Cruz Biotechnology). The PVDF filters were next incubated with appropriate secondary antibody conjugated to biotin (Santa Cruz Biotechnology) followed by 1-h incubation with horseradish peroxidase-conjugated streptavidin (1:1000; Caltag Laboratories, Burlingame, CA, USA). Immunoreactive proteins were visualized by 3,3'-diaminobenzidine (Sigma) staining. The bands were quantified by densitometry, using Scion Image Software (Scion Co, Frederick, MD, USA).

2.6. Statistical analysis

Data were analyzed using two-way ANOVA with Fisher’s post-hoc test. A P value <0.05 was considered significant. Data are shown as means ± S.E.

3. Results

3.1. Body weight and growth rate during development

Maternal protein restriction during the first 10 days of lactation affected the offspring body weight at all ages analyzed (Table 2). Rats from UN-group presented a reduc-

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<th>Age (days)</th>
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<td>8</td>
<td>21 ± 2.4&lt;sup&gt;a&lt;/sup&gt;, 10.7 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.4 ± 2.6&lt;sup&gt;c&lt;/sup&gt;, 18.4 ± 3.2&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>15</td>
<td>18.4 ± 0.11&lt;sup&gt;e&lt;/sup&gt;, 1.8 ± 0.14&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.8 ± 0.57&lt;sup&gt;g&lt;/sup&gt;, 1.0 ± 0.17&lt;sup&gt;h&lt;/sup&gt;</td>
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<td>21</td>
<td>56.9 ± 8.6&lt;sup&gt;i&lt;/sup&gt;, 1.5 ± 0.12&lt;sup&gt;j&lt;/sup&gt;</td>
<td>27.2 ± 2.2&lt;sup&gt;k&lt;/sup&gt;, 1.5 ± 0.12&lt;sup&gt;l&lt;/sup&gt;</td>
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<td>30</td>
<td>93.2 ± 9.1&lt;sup&gt;m&lt;/sup&gt;, 4.0 ± 0.13&lt;sup&gt;n&lt;/sup&gt;</td>
<td>74.0 ± 5.5&lt;sup)o&lt;/sup&gt;, 5.2 ± 0.39&lt;sup&gt;p&lt;/sup&gt;</td>
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<tr>
<td>60</td>
<td>258.0 ± 31.8&lt;sup&gt;q&lt;/sup&gt;, 5.5 ± 0.68&lt;sup&gt;r&lt;/sup&gt;</td>
<td>203.0 ± 18.0&lt;sup&gt;s&lt;/sup&gt;, 4.3 ± 0.38&lt;sup&gt;t&lt;/sup&gt;</td>
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<th>Weight (g)</th>
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<td>8</td>
<td>5.5 ± 0.7</td>
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<td>6.1 ± 0.9</td>
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Values are means ± S.E., n = 16. Data in a row without a common letter differ, P<0.05.

Table 2

Weight, growth rate, blood glucose and plasma insulin in rats from control (C) and undernourished (UN) groups during development.
tion (about 50%) in body weight during the suckling period (ages 8 until 21 days). After weaning, age 21 days, the differences in body weight between rats from both UN- and C-groups decreased to only about 20% (ages 30 and 60 days). The growth rate in rats from UN and control groups during the postnatal development was also measured (Table 2). The undernutritional conditions of dams during lactation resulted in a minor weight gain during lactation period when compared to controls. However, UN-group showed an accelerated gain of weight after the weaning, with a significant increase in growth rate at age 30 reaching values significantly higher than control rats.

3.2. Blood glucose and plasma insulin levels during development

Blood glucose and plasma insulin levels were evaluated throughout the postnatal development of rats from UN- and C-groups (Table 2). The glucose levels, analyzed from ages 8 until 60 days, were not different between both groups. Insulin levels, despite being quite similar in rats from C- and UN-groups until age 30 days, were significantly lower (30%) in rats from UN-group at age 60 days.

3.3. Effect of insulin on glucose uptake in skeletal muscle during development

Basal glucose uptake (i.e., the rate in the absence of acute insulin stimulation) was diminished in skeletal muscle from UN-group during the period of maternal undernutrition (age 8 d), as presented in Fig. 1. However, a significant increase in basal glucose uptake was observed in muscle from UN-group at ages 15 and 60 days. As expected, insulin increased muscle glucose uptake in C-group at all ages studied, especially after weaning (age 21 days: 1.78-fold; age 30 days: 1.93-fold; and age 60 days: 1.37-fold over basal values). In contrast, insulin was unable to stimulate glucose uptake by skeletal muscle from UN-group from age 8 until 21 days, although the insulin stimulation was restored at age 30 days. Although basal glucose uptake in muscle from UN-group at age 60 days was significantly higher when compared to C-group with the same age, insulin stimulation of muscle tissue from those rats was able to further increase glucose uptake.

3.4. Expression and tyrosine phosphorylation of IR in skeletal muscle

IR immunocontent was significantly reduced in muscle from UN-group at ages 4, 8 and 15 days, when compared to controls, as shown in Fig. 2. However, IR expression was completely restored in muscle tissue from UN-group, at ages 21 until 60 days.

The stimulatory effect of insulin on tyrosine kinase activity of IR (β-subunit) in muscle of rats during postnatal development is shown in Fig. 3. IR basal activity (non-stimulated) did not change throughout development in C-group. After insulin stimulation, tyrosine phosphorylation of IR increased at all ages analyzed ranging from two- to fourfold. On the other hand, UN-group at ages 4 and 8 days displayed a significant reduction in IR tyrosine phosphorylation when compared to C-group, either in non- or insulin-stimulated tissues. However, tyrosine kinase activity of IR was restored in skeletal muscle from UN-group at ages 15 until 60 days (Fig. 3A and B).
3.5. IRS-1 expression and activity in skeletal muscle

IRS-1 protein content associated to plasma membrane and IRS-1 tyrosine phosphorylation were analyzed by Western blotting in muscle from C- and UN-groups (Fig. 4A and C). The expression of IRS-1 in muscle from C-group was not affected during postnatal development, at ages 4 until 60 days (Fig. 4A). Maternal undernutrition during lactation did not induce significant changes in the IRS-1 levels during postnatal development, except at age 8 days when an impressive decrease in protein content was observed in muscle from UN-group (Fig. 4A and B). IRS-1 tyrosine phosphorylation induced by insulin stimulation was observed in muscle from C-group at ages 4 until 60 days reflecting a physiological response of the tissue (Fig. 4C and D). However, insulin failed to induce IRS-1 phosphorylation in tissue from UN-group at ages 4 and 8 days. After this period, the phosphotyrosine content of IRS-1 progressively increased, although the response was still under control values being restored at ages 30 and 60 days, and reaching a similar level to C-group (Fig. 4C and D).

3.6. GLUT-4 translocation in skeletal muscle

The translocation of GLUT-4 glucose transporter to plasma membrane after insulin stimulation was investigated in skeletal muscle from UN- and C-groups. Western blotting protein analysis with anti-GLUT-4 antibody in C-group indicated that non-stimulated tissues presented low levels of membrane-associated GLUT-4 that remained unaltered during postnatal development (Fig. 5A and B). The basal content of immunoreactive protein was low in muscle from UN-group at ages 4 and 8 days, although no differences were observed after ages 15 until 30 days, when compared to C-group. However, a significant increase of the basal...
GLUT-4 was observed in muscle plasma membrane from UN-group at age 60 days. Upon insulin stimulation, an increase of GLUT-4 translocation (threefold over basal levels) was measured in muscle from C-group and the levels of membrane-bound protein were quite similar at ages from 8 to 60 days. In contrast, acute insulin stimulation did not alter GLUT-4 content in muscle from UN rats at ages from 4 to 15 days. After that, GLUT-4 translocation to plasma membrane progressively increased upon insulin stimulation, being completely restored at age 30 days. An expressive change was the insulin-induced GLUT-4 translocation, which was found significantly increased in tissue of 60-day-old rats from UN-group when compared to control.

3.7. Association of PI3-kinase with IRS-1 in skeletal muscle

It is well established that activation of PI3-kinase mediates GLUT-4 redistribution to the plasma membrane. The fact that non- or insulin-induced GLUT-4 translocation and glucose uptake were significantly higher in muscle from UN-group at age 60 days prompted us to investigate whether this effect could be related to PI3-kinase interaction with IRS-1. After insulin stimulation, muscle tissue from rats at age 60 days (C- and UN-groups) was lysed and IRS-1 was immunoprecipitated for PI3-kinase associated with IRS-1 analysis. Insulin induced an increase in IRS-1-associated PI3-kinase in muscle tissue from C-group when compared to non-stimulated tissue, as shown in Fig. 6. However, in muscle from UN-group, it can be observed that basal association of PI3-kinase with IRS-1 reached similar levels to those found in tissue from C-group after insulin stimulation. Upon insulin stimulation, IRS-1-associated PI3-kinase was highly increased in muscle from UN-group as compared to control (Fig. 6A and B).

4. Discussion

Different studies have shown that nutritional deprivation during early stages of development is critical to glucose metabolism in adulthood [2,9,17–20,43,44]. Maternal nutritional status during early life has been often described as a determinant factor for programming alterations in insulin sensitivity and insulin-mediated effects in adulthood [8,18,20]. The molecular events by which food deprivation leads to changes in insulin sensitivity are not well understood, and the development of animal models of nutritional restriction becomes important to assemble...
weight gain was also described in previous studies on body weight in early life with a subsequent acceleration in developmental period. This capacity to compensate the lost of increased rate of growth of UN-group at the end of developmental period until the adult age with an significant reduction of body weight of UN offspring during development.

During early lactation, we investigated the insulin-mediated insulin programming induced by maternal undernutrition to elucidate the molecular mechanisms involved in the those of the human situation. Therefore, in order to elucidate the molecular mechanisms involved in the insulin programming induced by maternal undernutrition during early lactation, we investigated the insulin-mediated signaling responses in skeletal muscle of rats during development.

Undernutrition status during early lactation resulted in a significant reduction of body weight of UN offspring during the developmental period until the adult age with an increased rate of growth of UN-group at the end of developmental period. This capacity to compensate the lost of body weight in early life with a subsequent acceleration in weight gain was also described in previous studies on maternal undernutrition [9,15,16,25].

In this study, we showed that no significant variations in blood glucose and plasma insulin were observed in rats submitted to maternal protein restriction in early lactation at ages 8 until 30 days, when compared to control group. However, it was detected a significant decrease in insulin-induced glucose uptake into muscle tissue from UN-group, which was completely restored 30 days after birth. In addition, muscle tissue from early undernourished rats presented a compensatory increase in basal glucose uptake at age 60 days, which was further increased after insulin stimulation. Interestingly, in spite of presenting blood glucose levels similar to C-group, UN-group at age 60 days showed an expressive reduction in plasma insulin levels. These data suggest that UN-group could not overcome the metabolic damage caused by earlier nutritional environment, although they might use some other adaptive mechanisms in order to maintain glucose homeostasis. Taken together, these data suggest an increased sensitivity to the insulin actions programmed by the early adverse nutritional environment that lasts throughout the whole life. These results are in accordance to previous studies showing that such alterations in insulin secretion as a consequence of the metabolic programming induced by perinatal nutritional status [8,18–20,25].

The impairment of insulin-stimulated glucose uptake in early life and an apparent increase of insulin sensitivity in adulthood suggest alterations of insulin signaling in receptor and/or postreceptor levels. The present work shows that in spite of IR expression being down-regulated in muscle from UN-group at age 15 days, tyrosine kinase activity of IR was completely restored at age 10 days, when the malnutrition conditions were reversed. The recovery of IR activity detected before the restoration of IR expression suggests an adaptive mechanism to maintain glucose homeostasis during early development.

Interestingly, the time course of the low profile of IRS-1 activity is compatible with the decrease in glucose uptake by muscle tissue seen in UN-group during the whole period of lactation. Activation of IRS-1 is a key step by which insulin exerts its metabolic effects that culminate in glucose transport [33]. Insulin increases glucose incorporation mainly due to its ability to induce translocation of GLUT-4 from intracellular pool to plasma membrane increasing glucose transport into the cell [47–49]. An increase in GLUT-4 translocation in response to insulin has also been reported in skeletal muscle from calorie-restricted rats [43,44,50]. In our experimental model, the basal content of GLUT-4 was significantly decreased in muscle during the maternal undernutrition period in contrast to the significant increase in muscle from UN-group at age 60 days. Insulin-induced GLUT-4 translocation to plasma membrane was profoundly diminished during the whole period of lactation followed by a significant increase in the cell surface of tissue in adulthood. These results demonstrate that energy imbalance during early development affects GLUT-4 trafficking from the intracellular pool to the plasma membrane in muscle and suggest that postnatal GLUT-4 regulation is sensitive to prominent changes in early nutritional status. Our findings are in accordance to previous studies showing that such adaptations associated with undernutrition may account to the improvement of insulin-induced glucose uptake [43,44]. Many studies have concluded that the increase in the cell surface of GLUT-4 does not correlate quantitatively with the degree of stimulation of glucose uptake [51,52].

The insulin-stimulated PI3-kinase pathway seems to be the main route involved in glucose uptake in most insulin-responsive cells, and IRS-1 interaction with and subsequent activation of PI3-kinase mediates this process [29,34]. To test this hypothesis, we investigated the association of PI3-kinase with IRS-1 in muscle from UN-group at age 60 days. Our results showed that, when compared to control, UN-group presented a significant increase in IRS-1-associated PI3-kinase in muscle under basal (non-stimulated) conditions reaching similar levels to those of insulin-stimulated control tissues. After insulin stimulation, PI3-kinase interaction with IRS-1 in tissue of UN-group increased 63% over control levels, a clear indication of PI3-kinase activation [34]. These alterations may contribute to the increase in insulin sensitivity observed in adult rats from UN-group. In addition to metabolic regulation, insulin also controls the transcription of important genes in its target cells [53]. This action is crucial for insulin metabolic effects and also for organism adaptation to environmental changes. Moreover, altered transmission of the insulin signal through the PI3-kinase pathway could be involved in the impaired regulation of gene expression [54].

It has been proposed that a stimulus or insult operating at a critical or sensitive period of development could result in a long-standing effect on the structure of the organism and that biological mechanisms may exist to memorize the metabolic effects of early nutritional environments [1–3]. 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. Our findings provide evidence that maternal protein restriction in a short period of early life may affect insulin-dependent signaling in muscle...
that could be involved with metabolic alterations in glucose homeostasis in adult life. Early undernutrition impairs secretion of insulin but maintains glucose normotolerance in adulthood. The muscle tissue presents an increase of non- or insulin-induced GLUT-4 translocation and basal glucose uptake. These alterations are probably related to an up-regulation of PI3-kinase interaction with IRS-1, however, the precise mechanisms involved require further elucidation. The basis for increase in insulin sensitivity in adulthood appears to be directly related to the altered protein content in the maternal diet imposed during early lactation, which could affect expression and activity of key proteins in the insulin signaling cascade.

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References

insulin receptor substrate-1 is required for insulin-stimulated mitogen-

[34] M.F. White, The IRS-signalling system: a network of docking pro-

of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes: studies with a selective inhibitor

Molecular basis of insulin-stimulated GLUT4 vesicle trafficking: lo-

[37] E. Imano, H. Kadowaki, T. Kadowaki, N. Iwana, T. Watari, R.
Kawamori, T. Kamado, S.I. Taylor, Two patients with insulin resis-
tance due to decreased levels of IR mRNA, Diabetes 40 (1991) 548–557.

[38] M.J. Saad, F. Folli, J.A. Kahn, C.R. Kahn, Modulation of IR, IR
substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of

McKeon, Mutations in insulin-receptor gene in insulin-resistant pa-

Pratipanawat, R. DeFronzo, C.R. Kahn, L.J. Mandarino, Insulin resis-
tance differentially affects the PI 3-kinase- and MAP kinase-medi-

[41] A. Zisman, O.D. Peroni, E.D. Abel, M.D. Michael, F. Mauvais-Jarvis,
B.B. Lowell, et al., Targets disruption of the glucose transporter 4 se-
lectively in muscle causes insulin resistance and glucose intolerance,

[42] M. Katsumata, K.A. Burton, J. Li, M. Dauncey, Suboptimal energy
balance selectively up-regulates muscle GLUT gene expression but
reduces insulin-dependent glucose uptake during postnatal develop-

[43] M. Agote, L. Goya, S. Ramos, C. Alvarez, M.L. Gavete, A.M. Pas-
cual-Leone, F. Escriva, Glucose uptake and glucose transporter pro-
teins in skeletal muscle from undernourished rats, Am. J. Physiol:

of chronic undernutrition on glucose uptake and glucose transporter

[45] P.G. Reeves, F.H. Nielsen, G.C. Fahey, AIN-93 purified diets for
laboratory rodents: final report of the American Institute of Nutrition
Ad Hoc Writing Committee on the Reformulation of AIN-76A, J.

[46] S. Lund, G.D. Holman, O. Schmitz, O. Pedersen, Contraction stim-
ulates translocation of glucose transporter Glut4 in skeletal muscle
through a mechanism distinct from that of insulin, Proc. Natl. Acad.

Fukumoto, S. Seino, Molecular biology of mammalian glucose trans-

[48] S.W. Cushman, L.J. Wardzala, Potential mechanism of insulin action
on glucose transport in the isolated rat adipose cell. Apparent trans-
location of intracelular transport systems to the plasma membrane, J.

Vranic, J.O. Holloszy, A. Klip, Exercise induces recruitment of the
“insulin-responsive glucose transporter”. Evidence for distinct intra-
cellular insulin and exercise-recruitable transporter pools in skeletal

[50] D.J. Dean, J.T. Brozinick Jr., S.W. Cushman, G.D. Cartee, Calorie
restriction increases cell surface GLUT-4 in insulin-stimulated skel-

Huang, T. Ramlal, A. Klip, Differential effects of phosphatidylinosi-
tol 3-kinase inhibition on intracellular signals regulating Glut4
translocation and glucose transport, J. Biol. Chem. 276 (2001)
46079–46087.

[52] G. Sweeney, R. Somwar, T. Ramlal, A. Volchuk, A. Ueyama, A. Klip,
An inhibitor of p38 mitogen-activated protein kinase prevents insulin-
stimulated glucose transport but not glucose transporter translocation
in 3T3-L1 adipocytes and L6 myotubes, J. Biol. Chem. 274 (1999)
10071–10078.

[53] R.M. O’Brien, D.K. Granner, Regulation of gene expression by in-

Khalfallah, J.-P. Riou, H. Vidal, Defective regulation of phosphatidy-
linositol 3-kinase gene expression in skeletal muscle and adipose tis-
sue of non-insulin-dependent diabetes mellitus patients, Diabetologia