Early undernutrition induces glucagon resistance and insulin hypersensitivity in the liver of suckling rats

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Lizarraga-Mollinedo E, Fernández-Millán E, de Toro Martín J, Martínez-Honduvilla C, Escrivá F, Álvarez C. Early undernutrition induces glucagon resistance and insulin hypersensitivity in the liver of suckling rats. Am J Physiol Endocrinol Metab 302: E1070–E1077, 2012. First published February 14, 2011; doi:10.1152/ajpendo.00495.2011.—Developing brains are vulnerable to nutritional insults. Early undernutrition alters their structure and neurochemistry, inducing long-term pathological effects whose causal pathways are not well defined. During suckling, the brain uses glucose and ketone bodies as substrates. Milk is a high-fat low-carbohydrate diet, and the liver must maintain high rates of gluconeogenesis and ketogenesis to address the needs of these substrates. Insulin and glucagon play major roles in this adaptation: throughout suckling, their blood concentrations are low and high, respectively, and the liver maintains low insulin sensitivity and increased glucagon responsiveness. We propose that disturbances in the endocrine profile and available plasma substrates along with undernutrition-related changes in brain cortex capacity for ketone utilization may cause further alterations in some brain functions. We explored this hypothesis in 10-day-old suckling rats whose mothers were severely food restricted from the 14th day of gestation. We measured the plasma/serum concentrations of glucose, ketone body, insulin and glucagon, and hepatic insulin and glucagon responses. Undernutrition led to hypoglycemia and hyperketonemia to 84% (P < 0.001) and 144% (P < 0.001) of control values, respectively. Liver responsiveness to insulin and glucagon became increased and reduced, respectively; intraperitoneal glucagon reduced liver glycogen by 90% (P < 0.01) in control and by 35% (P < 0.05) in restricted. Cortical enzymes of ketone utilization remained unchanged, but their carrier proteins were altered: monocarboxylate transporter (MCT) 1 increased: 73 ± 14, controls; 169 ± 20, undernourished (P < 0.01; densitometric units); MCT2 decreased: 103 ± 3, controls; 37 ± 4, undernourished (P < 0.001; densitometric units). All of these changes, coinciding with the brain growth spurt, may cause some harmful effects associated with early undernutrition.

monocarboxylate transporter; glucose transporter

Some insults during critical phases of development can have long-term consequences. This fact has led to the concept of the developmental origin of adult disease (5). The nutritional status of the mother constitutes one of the main factors affecting the development of children. Long-term consequences of exposure to perinatal severe undernutrition, such as those that occurred in the Dutch Hunger Winter and Chinese famine, are currently being investigated. These studies show that this condition is associated with increased prevalence of pathologies like coronary diseases, diabetes, and mental disorders in adulthood (30, 37, 49, 52). Studies in animal models have indeed shown that early undernutrition can have an impact on the development programing of immature mammals, causing permanent dysfunction of specific tissues. The brain has much relevance in this regard. Since the mid-1960s, undernutrition endured early in life has been known to affect central nervous system development, causing impairment in brain functions, which may be irreversible and lead to clinical disease (15). However, in most cases, the physiological/biochemical mechanisms involved are still not fully defined (56). Most studies in this area have examined the consequences of early undernutrition on offspring into adulthood. However, the period in which tissues grow is of particular importance.

Milk is a high-fat low-carbohydrate food, and the glucose it provides is inadequate for developing brains of newborn rats and humans (10, 24). Moreover, energy metabolism in the brain during suckling relies to a large extent on ketone bodies, which are also used as precursors for various compounds (44). A number of essential adaptations in hepatic metabolism during suckling are required to cover substrate needs. The liver carries out 1) intense gluconeogenesis, to meet the glucose needs of organs, and 2) active fatty acid oxidation, to stimulate gluconeogenesis and enable active ketogenesis. These adaptations are mainly directed by insulin and glucagon (47). During the suckling period of species such as rats and humans, plasma insulin and glucagon concentrations are low and high, respectively; therefore, the insulin-to-glucagon ratio remains low (23). Changes to this balance can result in alterations in available substrates, and these hormone-substrate alterations may have an impact on the development of immature mammals (25). Significant disturbances in the biological effects of these hormones may also result from changes in liver responsiveness.

We developed a rat model of severe food restriction during the last week of gestation, which was prolonged into adulthood (18). This model is an approach to situations in which human beings have experienced severe famines. Undernourished rats underwent β-cell failure (39, 40), leading to chronic hypoinsulinemia (18, 39). Surprisingly, these rats were not glucose intolerant since they experienced increased insulin sensitivity in adipose tissue, skeletal muscle, and heart (3, 18, 22). Recently, we reported that suckling-restricted pups showed enhanced amounts of cortical glycogen, which were confined to astrocytes, in combination with increased GLUT-1 (38). Both results prove the occurrence of alterations in this brain area of undernourished pups.

Only a few reports have explored the effects of early undernutrition on the liver’s ability to respond to hormones that control the availability of metabolic substrates. The main goal

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of this study was to investigate the effects of this restriction on the endocrine and substrate profiles during suckling. Our key findings support the view that early undernutrition alters plasma insulin and glucagon levels, as well as the availability of circulating glucose and ketone bodies. This condition induces opposite changes in the hepatic response to both hormones, causing the liver of undernourished suckling rats to experience a situation that is in sharp contrast to what would normally be appropriate for this period, namely low insulin sensitivity and high glucagon responsiveness. In addition, specific monocarboxylate transporters are altered in the cortex by undernutrition. We suggest that all of these changes may be causative in regard to long-term consequences.

MATERIALS AND METHODS

Animals, diets, and hormone treatments. Wistar rats bred in our laboratory were used. Animals were fed a standard laboratory diet. Two groups of rats were studied. One consisted of pups from severely undernourished dams (n = 15) with 65% food restriction during the last week of gestation and lactation. Controls were pups whose mothers (n = 15) were given access to food ad libitum. Other characteristics of this experimental model have been previously described in detail (18). We observed no differences in the number of rats born or the survival when comparing both groups of animals. The number of pups in each litter was evened to eight. Only females were selected, to rule out potential effects due to gender differences. They were studied at age 10 days. To avoid biased results due to the peculiarity of a given litter, we have randomly selected only one pup from each litter (control or undernourished) to form the analyzed groups. The pups were killed by decapitation without anesthesia, blood was harvested from the trunk, and plasma was stored at −80°C. The liver was quickly removed and freeze-clamped in liquid N2. The brains were also rapidly dissected, and cerebral cortices were harvested and immersed in N2. Liver and cortices were kept at −80°C until assayed. In a set of experiments, conscious pups were given an intraperitoneal injection of insulin (5 IU/kg, Actrapid; Novo, Copenhagen, Denmark) or saline and killed 15 min after injection. Other groups of rats were anaesthetized with pentobarbital (2 mg/100 g body wt), the abdominal cavity was exposed, and glucagon was injected into the cavity vein with the proximal end toward the liver (2 µg/g body wt, in 40–90 µl solution, over 15- to 25-s period, GlucaGen Hypokit; Novo Nordisk, Bagsvaerd, Denmark). A gauze pad was placed over the exposed abdomen and kept moist with saline. At different times (as indicated in Figs. 1–5), blood was collected from the tail. At the end of experiment, the pups were killed, and the liver was harvested, as indicated above. All animals were handled in accordance with the principles and procedures of and were approved by The Committee for Animal Experimentation of the Universidad Complutense, Madrid.

Analytic procedures. Plasma insulin was determined with a rat insulin RIA (Linco Research, St. Louis, MO), with rat insulin for the standard curve. Sensitivity of 0.1 ng/ml was achieved with overnight equilibrium using a 100-µl plasma sample. Serum glucagon was determined with a glucagon RIA (Linco Research). Sensitivity of 20 pg/ml was achieved using a 100-µl serum sample in a 2-day disequilibrium assay. The coefficients of variation within and between assays were 10%. Blood or serum glucagon was determined using an Accurated Glucagon Analyzer (Roche) or by a glucose oxidase method, respectively. Blood 3-hydroxybutyrate and acetocetate were determined in ClO4H neutralized deproteinized extracts (58). Total ketone bodies are expressed as the sum of the concentrations of 3-hydroxybutyrate and acetocetate. For determination of metabolites, liver frozen samples were extracted with 6% cold ClO4H and centrifuged. The supernatant was neutralized with 0.6 M KOH and used to quantify 3-hydroxybutyrate, acetocetate, lactate, and pyruvate (58). Glucose was analyzed by the glucose oxidase method. Glycogen content was quantified according to Belke et al. (6); glucose resulting from enzymatic hydrolysis was quantified by the glucose oxidase method.

Crude mitochondria preparations. Frozen liver and brain cortex samples were homogenized with a Teflon pestle in sucrose-Tris buffer (10 mM Tris HCl, pH 7.4, 0.25 M sucrose, and 0.5 mM EDTA) at 4°C and centrifuged at 600 g for 10 min. The supernatants were then centrifuged at 15,000 g for 10 min. The resulting supernatants were discarded, and the mitochondrial pellets were resuspended in the buffer and sonicated. These preparations were used for the spectrophotometric analysis of 3-hydroxybutyrate dehydrogenase, acetoacetyl-CoA thiolase, or 3-oxo acid CoA transferase (57). The units of the enzyme activity are nanomoles of substrate transformed per minute, and specific activity is expressed as units per milligram protein. Protein concentration was estimated by the Bradford (12) method.

Preparation of lysates. Sample tissues were homogenized with a Polytron in ice-cold lysis buffer, composed of 50 mM HEPES (pH 7.4), 150 mM NaCl, 1 mM MgCl2, 10 mM sodium pyrophosphate, 10 mM sodium fluoride, 2 mM EDTA, 10% glycerol, 1% Nonidet P-40, 2 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine, 15 µM leupeptin, 10 µg/ml aprotinin, and 2 mM sodium orthovanadate. The homogenate was incubated for 30 min at 4°C with gentle stirring and then centrifuged at 100,000 g for 60 min. The supernatants were collected, assayed for protein concentration, separated into aliquots, and stored at −80°C until used.

Western blot analyses. The samples were subjected to SDS-PAGE on 6.5–10% polyacrylamide gels according to Laemmli (32). Proteins were then electrophoretically transferred to polyvinylidene difluoride (PVDF) filters (PVDF Protein Sequencing Membrane; Bio-Rad Laboratories, Alcobendas, Spain) for 2 h. After being transferred, the PVDF filters were blocked with 5% nonfat dry milk (for general antibodies) or with 3% BSA (for anti-phosphotyrosine antibodies) in Tris-buffered saline (TBS) followed by incubation with primary antibodies overnight. The PVDF filters were washed four times for 10 min each time with TBS and 0.1% Tween 20, followed by 1 h incubation with appropriate secondary antibody conjugated to horseradish peroxidase (Sigma). The PVDF filters were then washed as indicated above and subsequently exposed to an enhanced chemiluminescence reagent (Amersham Life Science, Little Chalfont, Buckinghamshire, UK). The bands were quantified by laser scanning densitometry (Molecular Dynamics, Sunnyvale, CA). The presence of linearity between the time of X-ray film exposure and the optical density of the bands was initially ensured. Protein loading was assessed by reprobing the blots with mouse anti-rat β-actin.

Immunoprecipitation. Lysates containing 500–1,000 µg proteins were immunoprecipitated overnight at 4°C with gentle rotation in the presence of 2–5 µg of the corresponding primary antibody, followed by the addition of protein A-agarose (Roche Diagnostics, Indianapolis, IN) or antismouse IgG-agarose (Sigma BioSciences, St. Louis, MO) for the rabbit polyclonal and mouse monoclonal antibodies, respectively. After being mixed for 2 h, the pellets were collected by centrifugation, and the supernatants were discarded. Next, the pellets were washed and saved for Western blot analysis.

Phosphatidylinositol 3-kinase assay. To determine the basal and insulin-stimulated phosphatidylinositol 3-kinase (PI 3-kinase), aliquots of liver lysates from basal and insulin-treated rats containing 1 mg protein were immunoprecipitated with anti-insulin receptor substrate (IRS)-2 polyclonal antibody (Millipore, Temecula, CA). Immunocomplexes were collected with protein A-agarose (Roche Diagnostics). PI 3-kinase activity was assayed by phosphorylation of phosphatidylinositol with [32P]ATP (Amersham International). The radioactive phosphorylated phosphatidylinositol was analyzed and quantified using previously described procedures (55).

Antibodies used. The following antibodies were used: anti-MCT2, anti-phosphotyrosine, anti-glucagon receptor (Santa Cruz Biotechnol-
Results

The body and liver of undernourished rats weighed 55% less than those of control rats; however, brain weight was reduced by only 18%, and so the brain-to-body mass ratio was significantly greater for the restricted rats. Serum glucose, plasma insulin, and serum glucagon concentrations were reduced in the undernourished rats (15, 65, and 60%, respectively), and blood ketone bodies were 1.5-fold higher (Table 1). Liver glycogen and lactate contents were decreased by undernutrition, whereas acetoacetyl-CoA thiolase increased by 7, specific activities for undernourished and control groups.

Table 1. Body, liver, and brain weights and circulating glucose, ketone body, insulin, and glucagon in undernourished and control 10-day-old rats

<table>
<thead>
<tr>
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<th>Control</th>
<th>Undernourished</th>
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<tr>
<td>Body wt, g</td>
<td>21.8 ± 0.7</td>
<td>12.3 ± 0.2***</td>
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<tr>
<td>Liver wt, g</td>
<td>0.621 ± 0.015</td>
<td>0.347 ± 0.005***</td>
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<tr>
<td>Liver/body, %</td>
<td>2.84 ± 0.07</td>
<td>2.75 ± 0.04</td>
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<tr>
<td>Brain wt, g</td>
<td>0.745 ± 0.006</td>
<td>0.613 ± 0.005***</td>
</tr>
<tr>
<td>Brain/body, %</td>
<td>3.33 ± 0.07</td>
<td>4.97 ± 0.11***</td>
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<tr>
<td>Plasma glucose, mg/dl</td>
<td>153 ± 4</td>
<td>128 ± 3***</td>
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<tr>
<td>Blood ketone body, μmol/ml</td>
<td>0.83 ± 0.07</td>
<td>1.20 ± 0.11***</td>
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<tr>
<td>Plasma insulin, ng/ml</td>
<td>0.87 ± 0.14</td>
<td>0.29 ± 0.06***</td>
</tr>
<tr>
<td>Serum glucagon, pg/ml</td>
<td>128 ± 12</td>
<td>48 ± 2***</td>
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Values are means ± SE of 6-8 independent determinations. **P < 0.01 and ***P < 0.001.

We analyzed the effects of undernutrition on liver contents of glucose transporters GLUT-1/2 and found that this condition led to an increase in both transporters (Fig. 1A). Our results showed that MCT1, the predominant form in rat livers, was markedly upregulated by undernutrition, which, however, did not affect MCT2 (Fig. 1B). We also analyzed the protein expression of GS and GP, which are key rate-limiting enzymes of glycogen metabolism. GS protein underwent a large increase in restricted rats, approximately a fivefold increase above control values, whereas GP was decreased (Fig. 1C).

Table 2. Liver content of glucose, glycogen, lactate, pyruvate, and ketone body in undernourished and control 10-day-old rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Undernourished</th>
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<tbody>
<tr>
<td>Glucose, mg/g</td>
<td>1.13 ± 0.04</td>
<td>0.57 ± 0.03***</td>
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<tr>
<td>Glycogen, mg/g</td>
<td>20.5 ± 1.3</td>
<td>33.4 ± 1.3***</td>
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<tr>
<td>Lactate, μmol/g</td>
<td>1.23 ± 0.04</td>
<td>0.72 ± 0.07***</td>
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<tr>
<td>Pyruvate, μmol/g</td>
<td>0.09 ± 0.01</td>
<td>0.07 ± 0.01</td>
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<tr>
<td>Ketone body, μmol/g</td>
<td>0.88 ± 0.05</td>
<td>1.58 ± 0.09***</td>
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Values are means ± SE of 6-8 independent determinations. ***P < 0.001.
We next analyzed several steps in the liver insulin-signaling pathway. We found no alterations in the content of the following proteins: insulin receptor, IRS-1 and α- and β-isoforms of p85, the PI 3-kinase regulatory subunit (Fig. 2A). However, we found that IRS-2 abundance, IRS-2 basal phosphorylation, and the basal level of IRS-2 associated with insulin receptor were significantly increased in undernourished rats (Fig. 2B). The content of Akt, a substrate of PI 3-kinase, and basal phospho-Akt were upregulated by undernutrition (Fig. 2C). Basal PI 3-kinase activity associated with IRS-2 was very low in the two populations of rats; however, following insulin stimulation, this activity increased more in the restricted animals than in the control animals (Fig. 2D). GSK3, which is implicated in the regulation of glycogen synthesis, is a downstream target of Akt. As shown in Fig. 3A, phospho-GSK3β, the most abundant isoform in the liver, was higher in undernourished rats than in control rats after insulin treatment. We found that insulin induced an increase in P70 S6 kinase phosphorylation in both populations of rats, as expected; however, basal levels of phospho-P70 S6 kinase were already higher in the undernourished rats (Fig. 3B).

Glucagon receptor content underwent a sharp reduction in undernourished pups (Fig. 4A). We measured the effects of intravenous glucagon on glycemia and hepatic glycogen. Blood glucose increased gradually in control rats during the 30 min following this treatment; however, glycemia only showed a modest initial tendency to increase in the restricted rats and after it remained unchanged, under the control values (Fig. 4B). In terms of liver glycogen content, glucagon caused a large reduction (90%) in the well-fed animals. In the restricted rats, whose basal proportion was increased, the reduction promoted by this hormone was significantly weaker (65%) (Fig. 4C).

We then analyzed the ketone body metabolizing enzymes in cortical mitochondria from the two groups of rats: 3-hydroxybutyrate dehydrogenase, 3-oxoacid CoA-transferase, and acetocetyl-CoA thiolase. When the specific activities were compared, no differences were found: 14 ± 1 vs. 12 ± 0.5 for dehydrogenase; 5 ± 0.4 vs. 4 ± 0.8 for transferase, and 14 ± 2 vs. 16 ± 2 for thiolase, in the control and undernourished animals, respectively. Finally, we evaluated the effects of undernutrition on the cortical amount of three monocarboxylate transporters and found that this condition upregulated...
MCT1 and reduced the content of MCT2, without changing MCT4 (Fig. 5).

**DISCUSSION**

**Characteristics of undernourished rats.** There is evidence that some long-term consequences of early undernutrition are sex-specific, such as obesity (49) or schizophrenia (52). In the present work, we have studied only females, so possible gender differences in the found effects cannot be inferred. At 10 days of age, the rat brain undergoes a transient growth spurt, which involves an increase in metabolism and is a period of enhanced vulnerability. The offspring of rat dams that were undernourished during the last week of pregnancy and lactation were restricted in their growth, although the brain was less affected than the rest of the body (“brain sparing effect”) (35). To maintain its high growth rate, the developing brain is supplied with glucose and ketone bodies (14). We found that the decrease in plasma glucose associated with undernutrition was accompanied by a rise in ketone bodies, as reported in models of specific protein restriction (21), showing that this adaptation is independent of the type of restriction. In adults, brain glucose consumption slows down with prolonged fasting, a condition that enhances cerebral ketone body extraction from blood. Nevertheless, during immaturity, increased plasma levels of ketone bodies coinciding with hypoglycemia may not necessarily be beneficial. Thus, an increase of 3-hydroxybutyrate in plasma in rat pups submitted to hypoglycemic coma worsened their recovery (51). In addition, ketosis can alter the transport of amino acids across the blood-brain barrier (BBB) (60). Glucose and ketone bodies play complementary roles in the brain, and both are used for energy production. Glucose also provides precursors for neurotransmitter synthesis and the pentose phosphate cycle. In contrast, ketone bodies mainly serve to generate acetyl-CoA, which is used to synthesize lipids and amino acids (43). Changes in their availability during the brain growth spurt may have deleterious impacts on these pathways.

**Insulin and glucagon liver sensitivity.** Insulin resistance during suckling contributes to the high maintenance rate of hepatic gluconeogenesis. In addition, the low stimulation of glucose utilization by insulin in peripheral tissues increases its availability to the brain. Early undernutrition enhances insulin

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**Fig. 3.** Content of phospho-glycogen synthase kinase (GSK) 3 (A) and phospho-P70 S6 kinase (B) in liver of suckling rats, in the basal and insulin-treated conditions, as indicated in MATERIALS AND METHODS. Representative blots are shown. Data in bars represent means ± SE from quantitative analysis of 6–8 independent determinations. Black bars, controls; white bars, undernourished. Differences between control and undernourished and between basal and insulin-treated: *P < 0.05, **P < 0.01, and ***P < 0.001.

**Fig. 4.** A: glucagon receptor (GR) content in liver from control and undernourished 10-day-old rats. Representative blots are shown. Bars represent the means ± SE for 6–8 independent determinations. Black bars, control; white bars, undernourished. **B:** plasma glucose levels in the basal condition and after glucagon injection in control (●) and undernourished (□) suckling rats, as described in MATERIALS AND METHODS. Each point represents the mean ± SE for 8 independent determinations. C: glycogen content in the liver of suckling rats in the basal condition and 30 min after glucagon treatment, as indicated in MATERIALS AND METHODS. Bars represent the means ±SE from 8 independent analyses. Filled bars, control; open bars, undernourished. Differences between control and undernourished and between basal and glucagon-treated: *P < 0.05, **P < 0.01, and ***P < 0.001.
sensitivity in extrahepatic tissues, such as muscle and adipose tissue, in adulthood (16, 18, 27, 45), but the impact of this restriction on the adult or immature liver has been less frequently addressed. Suckling rats from dams on hypoproctic diets throughout pregnancy and lactation show increased hepatic glycogen (21, 26), as found herein in pups from globally restricted rats, indicating that this response is independent of the type of restriction. The increased amounts of GLUTs and GS and the low levels of GP suggest elevated rates of glucose uptake and glycogen production, which is supported by reduced hepatic contents of free glucose and lactate. These results provide for the first time an explanation of the increased glycogen associated with early undernutrition. Insulin increases GS and inhibits GP (20, 53). Therefore, considering the hypoinsulinemia of restricted rats, our findings are consistent with liver insulin hypersensitivity associated with glucose metabolism, as previously seen in extrahepatic tissues but never explored in the liver. Hepatic levels of the insulin receptor, IRS-1, and the regulatory subunits of PI 3-kinase were not affected. However, IRS-2 and Akt levels were enhanced. In addition, activation of IRS-2-associated PI 3-kinase form of p70 S6 kinase was increased in undernourished rats at baseline. Taken together, these findings support an insulin-sensitizing effect of maternal food restriction on the liver of suckling rats. Hepatic IRS-1 and IRS-2 are preferentially linked to glucose and lipid metabolism, respectively (54). However, while IRS-1 remained unaltered, the increased glycogen stores in undernourished rats coincided with upregulated IRS-2, which suggests its involvement in glucose homeostasis during suckling.

Plasma glucagon levels were very low in undernourished rats, as reported by others in fetal and suckling rats from food-restricted mothers (4). Because this hormone decreased in a similar proportion to insulin, the insulin-to-glucagon ratio was not altered. However, food restriction strongly reduced the content of liver glucagon receptors. Ozanne et al. (46) also reported a decrease in glucagon receptors in the adult male offspring of dams fed a protein-restricted diet during pregnancy and lactation (46). We found that some in vivo glucagon responses were significantly diminished in the restricted pups, a result not previously reported: stimulation of liver glycogenolysis was weak, and there was virtually no increase in glycemia. Thus it seems clear that early undernutrition leads to hepatic glucagon resistance with insulin hypersensitivity.

Liver ketogenesis. Although reduced utilization cannot be ruled out to explain the hyperketonemia of undernourished rats, it seems to be primarily attributable to increased hepatic ketogenesis, as deduced from the elevated liver ketone body content. The rise in acetoacetyl-CoA thiolase activity is consistent with this proposition. The activation of ketogenesis takes place despite the reduced supply of milk fat, which suggests that fatty acid oxidation is increased compared with esterification. Carnitine acyltransferase I is inhibited by malonyl-CoA, whose major source in the liver is glucose derived from glycogen (41). Therefore, the blunted glycogenolysis in the restricted rats should facilitate the diversion of fatty acid metabolism toward ketone body production.

Insulin and glucagon play major opposing roles in the control of ketogenesis. This metabolic pathway increases concomitantly to low and high liver responses to insulin and glucagon, respectively, which is the opposite condition set by undernutrition in the suckling rats. This fact has not been previously described, to our knowledge. These results show that an increase in hepatic insulin sensitivity during suckling does not necessarily prevent high rates of ketone body production. Regarding glucagon resistance, it seems that stimulated ketogenesis associated with undernutrition is derived from nonglucagon mechanisms (33). It has been shown that catecholamines, which induce lipolysis, have no direct ketogenic effects on the liver (9). In contrast, glucocorticoids may be hormones that are directly involved in the activation of ketogenesis (13). Offspring from protein-restricted pregnant rats have increased liver expression of glucocorticoid receptors during suckling and adulthood (8). Moreover, these hormones, in synergy with insulin, increase both ketogenesis and glycogen synthesis in hepatocytes in vitro (2), two conditions seen in the liver of the undernourished pups. In any case, this condition of hyperproduction of ketones along with increased and decreased insulin and glucagon actions, respectively, has not been previously reported.

Because undernutrition induced a rise in MCT1, it seems likely that the livers of restricted rats develop higher capacities for exporting ketone bodies. The modulation of MCT1 synthesis is not well understood, but it has been shown that it is upregulated by peroxisome proliferator-activated receptor-α activation in fasting (29). It may be the same for restricted pups.

Ketone body transporters in the cortex. Enzyme activity for metabolizing ketone bodies remained unaltered in the cortex of restricted pups. However, the main factor controlling their rate of utilization is availability (28). Three main MCT forms have been identified in the central nervous system. The only one contained by BBB endothelial cells is MCT1, while neurons coexpress MCT1 and MCT2 and glial cells coexpress MCT1 and MCT4 (42). The cortical content of MCT1 was increased in undernourished hyperketonemic rats, which is in accordance with the upregulation reported in brain endothelial cells of rats on ketogenic diets (36). This adaptation probably contributes to increase the ketone body uptake by astrocytes surrounding the
cortical capillaries. MCT2, the exclusively neuronal form (7), underwent a marked decrease. This reduction may limit the entry of ketone bodies into neurons, which is perhaps an adaptation to prevent its buffering capacity from overloading and the fall of cytosolic pH. Some MCT2 is found in immature undernourished rats, which blockade has deleterious effects in vivo (1). In this regard, it should be noted that MCT2 has the highest affinity for lactate (42). Ketone bodies are involved in hypothalamic control of feeding by insulin and leptin (31). The inhibition of food intake is blunted in rats that develop hyperketonemia and high transport rates of ketone bodies across the BBB (19). The possibility arises that changes in plasma ketone bodies and cortical MCTs found in immature undernourished rats alter hypothalamic control of feeding and lead to increased obesity risk.

According to programming hypothesis, undernutrition during immaturity predisposes to disturbances that resemble the metabolic syndrome (5, 59). Offspring nursed by dams globally food restricted during part of pregnancy and throughout lactation, a model close to that studied in this work, present increased risk of obesity in adulthood (17) and deficiencies in endocrine pancreas adaptation to gestation (11), showing long-lasting effects of early undernutrition. On the other hand, a number of the metabolic/endocrine alterations associated with the global restriction applied in the present study are comparable to other reported models based on protein restriction, a similarity that strengthens the importance of specific protein deficiency on the consequences of early undernutrition.

As stated, one of these consequences is predisposition to obesity, probably because of changes in the appetite hypothalamic regulatory systems, since they are vulnerable to early endocrine and metabolic disturbances (48). In fact, early undernutrition can also lead to alterations in brain functions, as reviewed by Laus et al. (34). The present work shows that undernourished rats experience hormonal and glucoregulation alterations that could affect the appetite regulation. Within this context, we are analyzing the hypothalami of these animals in a work in progress. Preliminary unpublished data show modifications in hypothalamic insulin signaling when these rats reach adulthood, so we are also testing whether these changes could increase the risk of obesity.

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