Maternal and Perinatal Magnesium Restriction Predisposes Rat Pups to Insulin Resistance and Glucose Intolerance

Lagishetty Venu, Yedla Durga Kishore, and Manchala Raghunath

Division of Endocrinology and Metabolism, National Institute of Nutrition, Hyderabad 500 007 India

ABSTRACT According to the fetal programming hypothesis, impaired intrauterine development results in insulin resistance and associated metabolic disturbances. Recently, we reported increased body fat, a forerunner of insulin resistance, in the pups of mineral-restricted rat dams. To identify the causative mineral(s), the effect of magnesium restriction was assessed. Female weanling WNIN rats (n = 21) consumed ad libitum for 9 wk a 70% magnesium-restricted diet or were pair-fed a control (C) diet (n = 7). After 9 wk, they were mated with control males. Control dams and pups were fed the control diet throughout, whereas 7 Mg-restricted dams were switched to the control diet at parturition and their pups weaned onto the control diet (RP). Pups of the remaining 14 restricted dams were weaned onto the control diet (RW) or the Mg-restricted diet (R). All groups had 8 male pups from weaning. Pups were studied on postnatal d 90 and 180. R pups weighed less than C pups at weaning, but both RP and RW pups caught up with controls by d 90. At this time, R pups were neither insulin resistant nor glucose intolerant, but had a higher percentage of body fat and plasma triglycerides and lower lean body and fat-free mass than C pups. These variables were partially corrected in both RP and RW pups. On postnatal d 180, R, RP, and RW pups were insulin resistant and had a lower insulin response to a glucose challenge than C pups; however, glucose tolerance was impaired only in RW pups. Thus, maternal magnesium restriction irreversibly increases body fat and induces insulin resistance in pups by 6 mo of age, whereas additional perinatal Mg deficiency impairs glucose tolerance. J. Nutr. 135: 1353–1358, 2005.

KEY WORDS: • body fat • glucose tolerance • insulin resistance • magnesium • maternal undernutrition

Intrauterine growth retardation has been linked to the development of diseases such as hypertension, type 2 diabetes, and obesity in later life (1,2). Insulin resistance (IR) is the common underlying feature, but its causes are not clear. Epidemiologic data and studies in experimental animals suggest that fetal and early postnatal nutritional adaptations persist and are expressed in adulthood, even in the absence of the stimulus or stress that initiated them (3). Termed “metabolic programming,” this has important implications for adult-onset diseases and abundant evidence demonstrates that the quantity and quality of nutrition during critical periods of early development influence the organism’s susceptibility to adult-onset pathological conditions (4–6). However, most studies have considered only maternal macronutrient deficiency (7,8). Recently, maternal iron restriction was reported to cause hypertension and alter lipid metabolism in rat pups (9). We observed recently that maternal mineral restriction altered the body fat content, plasma lipids, and oxidative stress in rat pups and may thus predispose them to IR in later life (10). However, the causative mineral(s) or their role in this process is not yet known.

Magnesium, the 4th most common cation in the body, affects numerous biological processes by modulating cell cycle progression, differentiation, and proliferation (11). As a cofactor of several enzymes, it modulates energy metabolism, carbohydrate oxidation, and glucose transport across the cell membrane (12,13). Mg also regulates insulin at levels such as secretion, receptor-binding, and activity (14,15).

Intestinal absorption and cellular distribution are among the factors important in maintaining intra- and extracellular Mg concentrations (16). Intracellular Mg concentration and transport are correlated with insulin-mediated glucose uptake (17,18). Indeed, intracellular accumulation of Mg depends upon insulin action, and subclinical Mg deficiency is common in people with diabetes and cardiovascular disorders (12,19). Alternatively, intracellular Mg deficiency could be due to IR, and it is also suggested that Mg deficiency may worsen IR (16,20). Interestingly, intracellular Mg concentrations are lower in hypertensive people, and Mg depletion from cells of normotensive people was reported to render them insulin resistant (21).

Data on the prevalence of Mg deficiency are scarce. The incidence is reported to be 25–39% in patients with diabetes.
mellitus (22) and ~45% in pregnant women of developing countries such as India (23). Despite this and the known effects of Mg on insulin secretion and action, the role of maternal Mg deficiency in predisposing the pups to IR has not been assessed. In light of our earlier studies (10), we hypothesized that maternal Mg restriction predisposes the pups to IR in later life. The present study was carried out in Wistar/NIN (WNIN) rats to validate or negate this hypothesis.

MATERIALS AND METHODS

All animal experimental procedures were carried out with the approval of the Ethical Committee on Animal Experiments at the National Institute of Nutrition, Hyderabad, India.

Animals: feeding, maintenance and breeding. Female weanling WNIN rats (n = 28) were obtained from National Centre for Laboratory Animal Sciences, National Institute of Nutrition. They were divided into 2 groups of 7 and 21, housed individually in polypropylene cages with wire mesh bottoms and maintained at 22 ± 2°C, under standard lighting conditions (12-h light:dark cycle). The group of 21 rats consumed ad libitum for 9 wk the AIN-93G diet (24) containing 165 mg Mg/kg diet (Mg restricted: R), and the group of 7 rats was pair-fed (offered the mean intake of the R group) the control AIN-93 G diet (C) containing 650 mg Mg/kg diet. All rats had free access to deionized water. After 9 wk of feeding, the hemoglobin level and the concentrations of Mg, glucose, insulin, cholesterol, and triglycerides were determined in blood plasma.

The rats were mated with control males (2 females to 1 male), and the pregnant rats continued to consume their respective diets throughout gestation. The control dams and their pups consumed the control diet throughout (C). At parturition, 7 of the 21 R dams were switched to the control diet (RP), and the others consumed a restricted diet during lactation. In all groups, a uniform litter size of 8 pups/dam (equal number of male and females) was maintained from postnatal d 3, until weaning on postnatal d 21. C and RP pups were weaned onto the control diet, whereas some R pups were weaned onto control diet (RW) and some continued to be Mg restricted (R).

From weaning, 8 male pups from 4–5 dams of the corresponding group were maintained in each group and they consumed their respective diets and deionized water ad libitum until postnatal d 180. To avoid the possible effects of estrous cycle on glucose and fat metabolism and IR, only male pups were included in this study. Figure 1 gives the schematic representation of the feeding protocol used. Food intake (daily) and body weights (once a week) were recorded in dams and pups.

Biochemical measurements in pups’ plasma. After overnight food deprivation, blood was collected from the supraorbital sinus of pups on d 90 and 180 of age; plasma was separated and stored at −20°C until analysis. Mg was quantified in plasma by atomic absorption spectroscopy (25). Glucose (glucose oxidase/peroxidase kit), triglycerides (glycerol phosphate oxidase kit), total cholesterol, and HDL cholesterol (cholesterol oxidase-peroxidase kit) were measured in plasma using enzymatic assay kits from Biosystems. Plasma free-fatty acids (FFA) were measured using an acyl CoA synthetase-oxidase-peroxidase kit from Randox Laboratories, and plasma insulin by RIA using a kit from BRIT.

Glucose tolerance test. An i.p. glucose tolerance test (IPGTT) was performed on 8 pups of each of the groups on postnatal d 90 and 180. Briefly, after overnight food deprivation (16 h), glucose (250 g/L) was administered i.p. as a bolus, at a dose of 1 g/kg body weight, and blood samples were collected at 0, 15, 30, 60, and 120 min for determining plasma glucose and insulin concentrations. Glucose and insulin responses during the glucose-tolerance test were computed from the area under the curve (AUC) for glucose and insulin, respectively, using the trapezoidal method (26).

Indices of insulin resistance. Indices based on fasting glucose and insulin, homeostasis model assessment of insulin resistance (HOMA-IR), and one that takes into account the pups’ response to a challenge with glucose (ratio of glucose AUC: insulin AUC) were computed as described previously (10).

Body composition. Body composition of the pups was determined on d 90 and 180 using the total body electrical conductivity (TOBEC) small animal body composition analysis system (EMSCAN, Model SA-3000 Multi detector) (27). Lean body mass (LBM), the percentage of body fat, and fat-free mass (FFM) were computed mathematically according to Morbach et al. (28) as described previously (10). The wet weights of the epididymal, mesenteric, and retroperitoneal fat pads were determined on d 180 and the adiposity index computed as described previously (29).

Statistical analysis. Data were analyzed statistically using SPSS (version 10.0). All values are presented as means ± SEM. Differences between control and Mg-restricted dams and their pups (up to weaning) were analyzed by Student’s t test. Data from pups were analyzed on d 90 and 180, using one-way ANOVA followed by post-hoc least significant difference (LSD) tests. If heterogeneity was observed in the variance, differences between groups were tested by the nonparametric Mann-Whitney U test.

RESULTS

Dams. Body weight gain in WNIN female rats was not affected by Mg restriction for 9 wk from weaning, although plasma Mg concentrations were lower (P < 0.001) in the R group (0.57 ± 0.02 mmol/L) compared with pair-fed controls (0.72 ± 0.02 mmol/L). Despite being Mg deficient, R rats did not differ from controls in fasting plasma glucose, insulin, or HOMA-IR index. Plasma total cholesterol and triglycerides also did not differ between these 2 groups (data not shown). Chronic Mg restriction had no adverse effect on the dams’ reproductive performance, pups’ birth weight, or their mortality during parturition and lactation (data not shown).

Pups: growth characteristics. Despite comparable birth weights and maintaining a uniform litter size of 8 pups/dam from postnatal day 3, R pups had lower (P < 0.05) weaning weights than control pups (Table 1). R pups weighed less even on d 180, whereas RP and RW pups caught up with controls by d 90 (Table 1). However, BMI did not differ among pups of the 4 groups at either time point (data not shown). As expected, R pups had lower (P < 0.05) plasma Mg concentrations than controls until d 180, whereas RP and RW pups caught up with controls by d 90 and remained at those levels (Table 1).

HOMA-IR. At 90 d of age, C and R pups did not differ in fasting plasma glucose, insulin, and HOMA-IR (data not shown). Although RW but not RP pups had higher fasting
glucose concentrations than controls, HOMA-IR values did not differ among the 4 groups (data not shown).

Even on d 180, C and R pups had comparable fasting glucose concentrations, and RW, but not RP pups, had higher (P < 0.05) fasting glucose concentrations than controls (Fig. 2A). However, at this time, R pups had higher (P < 0.05) fasting insulin concentrations than controls and neither rehabilitation regimen mitigated this change (Fig. 2B). On the contrary, RP and RW pups had higher (P < 0.01) fasting insulin concentrations than R pups. As a result, HOMA-IR values computed from fasting glucose and insulin were higher (P < 0.05) in R pups than in controls (Fig. 2C) and rehabilitation from parturition or weaning increased the values further (P < 0.001).

**Glucose tolerance and insulin response.** Glucose tolerance was assessed by an IPGTT in pups on d 90 and 180. On d 90, RW pups had a higher glucose AUC than the other groups, which did not differ, indicating impaired glucose tolerance in RW pups (data not shown). At this time, the insulin AUC did not differ among the groups. Nevertheless, the ratio of glucose AUC:insulin AUC did not differ among the 4 groups (data not shown).

On d 180, the glucose AUC did not differ between C and R pups (Fig. 2D). Only RW pups had a higher (P < 0.01) glucose AUC than the other groups. Also, RW pups, but not others, had impaired glucose tolerance. Interestingly, the insulin AUC was lower (P < 0.01) in R pups than in controls, and it was not affected by either rehabilitation regimen (Fig. 2E). As a consequence, the ratio of glucose AUC:insulin AUC was higher (P < 0.01) in R pups than in controls and neither rehabilitation regimen mitigated this change (Fig. 2F).

**Plasma lipid profile.** In keeping with high percentage of body fat than controls (Fig. 4A) on d 90 and 180, whereas their LBM (Fig. 4B) and FFM (Fig. 4C) were lower (P < 0.05). These changes tended to be normalized in the RP (P = 0.07) and RW (P = 0.08) by d 180 (Fig. 4).

Changes in the body adiposity of pups determined by TOBEC measurements were confirmed by the higher (P < 0.01) than control wet weights of the epididymal, retroperitoneal, and mesenteric fat deposits in R, RP, and RW pups on d 180 (Table 2). As a result, the adiposity index was also higher (P < 0.001) in these pups than in controls.

**FIGURE 2.** Fasting plasma glucose (A), insulin (B), HOMA-IR (C), and AUC during an IPGTT of plasma glucose (D), insulin (E), and the AUC glucose:AUC insulin ratio (F) on postnatal d 180 in C, R, RP, and RW pups. Each bar represents the mean ± SEM, n = 8. Means without a common letter differ, P < 0.05.
HDL cholesterol did not differ among the groups at either time studied (data not shown).

**DISCUSSION**

We reported recently the increased percentage of body fat in pups of rat dams subject to mineral restriction and their possible predisposition to IR (10). Mg deficiency modulates insulin sensitivity and may be associated with impaired insulin secretion (14,18,20). In light of our earlier findings (10) and the widespread incidence of Mg deficiency among pregnant and lactating women in developing countries (23), we hypothesized that maternal Mg restriction leads to IR and impaired glucose tolerance in the offspring. Our present results in WNIN rats demonstrate that Mg restriction during maternal growth, pregnancy, and lactation causes IR, glucose intolerance, and irreversible changes in body composition in pups.

Mg restriction for 9 wk lowered plasma Mg concentrations in female WNIN rats but did not affect their food intake, body weight gain, IR status, plasma glucose, and lipids, probably indicating that they were moderately Mg deficient. This could be a reason for the lack of IR in these rats, unlike earlier studies reporting IR in Mg deficiency (14,17). That Mg restriction had no effect on the dams' reproductive performance or the pups' birth weight corroborates the above inference and agrees with similar reports (30). However, maternal Mg restriction that continued through lactation and weaning decreased the pups' body weight at weaning and thereafter, indicating the importance of neonatal and postnatal Mg nutrition in developing rat pups (31,32). That both the rehabilitation regimens could partly correct these changes suggests that maternal and perinatal Mg restriction may modulate or program the pups' body composition and lipid metabolism.

It was interesting that on d 180, R pups had higher fasting plasma insulin and HOMA-IR than controls, emphasizing the importance of maternal and perinatal Mg deficiency in IR development in pups. That neither rehabilitation regimen affected these indices on d 180 not only suggests the importance of maternal Mg deficiency in predisposing pups to fasting hyperinsulinemia and IR but also the irreversibility of the defect. Further, impaired glucose tolerance seen only in RW pups is similar to our finding in vitamin-deficient and rehabilitated pups (34) and stresses that perinatal Mg nutrition is important in modulating glucose tolerance in pups. This view seems to be corroborated by our observation that RP pups had normal glucose tolerance.

These findings are in agreement with our earlier observations in pups of mineral-restricted rat dams (10) and in both studies, pups appeared predisposed to IR. However, a closer

![FIGURE 3](https://academic.oup.com/jn/article-abstract/135/6/1353/4663819) Time course of plasma glucose (A) and insulin (B) during an IPGTT on postnatal d 180 in C, R, RP, and RW pups. Each point on the curve represents the mean ± SEM, n = 8. Asterisks indicate that RW (panel A) or C (panel B) differed from the other groups: *P < 0.05; ***P < 0.001.

![FIGURE 4](https://academic.oup.com/jn/article-abstract/135/6/1353/4663819) The percentage of body fat (A), LBM (B), and FFM (C) by TOBEC on postnatal d 90 and d 180 in C, R, RP, and RW pups. Each bar represents the mean ± SEM, n = 8. Means without a common letter differ, P < 0.05.
MAGNESIUM AND FETAL ORIGIN OF INSULIN RESISTANCE

TABLE 2

<table>
<thead>
<tr>
<th>Fat pad</th>
<th>C</th>
<th>R</th>
<th>RP</th>
<th>RW</th>
</tr>
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<tbody>
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<td></td>
<td>g/100 g body weight</td>
<td></td>
<td></td>
<td></td>
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<td>Epidermal</td>
<td>1.40 ± 0.052b</td>
<td>1.65 ± 0.068a</td>
<td>1.73 ± 0.056a</td>
<td>1.69 ± 0.068a</td>
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<td>Retroperitoneal</td>
<td>2.59 ± 0.178b</td>
<td>3.19 ± 0.077a</td>
<td>3.42 ± 0.201a</td>
<td>3.13 ± 0.121a</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>0.91 ± 0.090b</td>
<td>1.33 ± 0.072a</td>
<td>1.20 ± 0.079a</td>
<td>1.23 ± 0.086a</td>
</tr>
<tr>
<td>Adiposity index</td>
<td>4.77 ± 0.288b</td>
<td>6.26 ± 0.166a</td>
<td>6.42 ± 0.246a</td>
<td>6.05 ± 0.191a</td>
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† Values are means ± SEM, n = 8. Means in a row without a common letter differ, P < 0.01.

comparison of the 2 studies is perplexing. Maternal Mg restriction induced IR, glucose intolerance, and increased body adiposity in pups, whereas multiple mineral restriction increased pups’ percentage of body fat only by 6 mo of age. Although reasons for these discrepancies are not clear, it appears that the effects of maternal Mg deficiency on some of the variables were countered by the deficiencies of one or more of the other minerals in our earlier study (10). Alternatively, it could also be due to greater restriction of dietary Mg (to 30% of controls) in this study than in the previous one (50% of controls).

Although R pups showed an early insulin response on challenge with glucose load (peaking at 15 min compared with 30 min in controls), peak insulin concentrations were markedly lower than those of controls. Moreover, in R pups, plasma insulin fell drastically below the fasting concentrations by 30 min, when glucose concentrations actually peaked. Together with fasting hyperinsulinemia, lower insulin AUC values in R pups indicate that postnatal continuation of maternal Mg restriction not only induced fasting hyperinsulinemia and IR in R pups but also impaired their insulin response to glucose challenge. That only RW but not RP pups had IR as well as glucose intolerance strongly suggests a significant role for perinatal Mg nutrition in modulating pups’ glucose metabolism and agrees with similar previous reports (39). Overall, these observations agree with Barker’s hypothesis (1,2), are similar to those typical of type 2 diabetic patients (40), and appear to suggest an irreversible exhaustion of β cells in Mg-restricted pups.

The persistent increase in body adiposity of R pups on d 180 and the inability of either rehabilitation regimen to correct it suggest that the changes were probably programmed during intrauterine growth, persisted throughout life, and were not reversible by rehabilitation from as early as parturition. Further, the significantly higher wet weights of fat deposits, epididymal, retroperitoneal, and mesenteric, in R, RP, and RW pups relative to the controls not only corroborate the TOBEC measurements but also indicate the distribution of increased body fat throughout the abdomen and not specific to any tissue. These findings are consistent with observations in the literature that abdominal adiposity is an important factor in the development of insulin resistance syndrome (37). Also, the high percentage of body fat, low body weight, and decreased LBM and FFM observed in R pups are similar to those reported in “thin fat babies” in India, an abnormal condition attributed to maternal malnutrition (41).

One study (42) reported altered lipid metabolism in pups of rat dams subjected to protein malnutrition or iron deficiency during pregnancy. However, we observed hypertriglyceridemia in R pups on d 90 but not d 180, suggesting that the changes could be transient. Similarly, plasma total cholesterol, which did not differ among pups of the 4 groups on d 90, was significantly lower in R pups on d 180, and both rehabilitation regimens mitigated these changes. Whether the change in total cholesterol is transient and the nature of its role, if any, in the development of IR and/or body composition change in pups remains to be deciphered. However, maternal Mg restriction did not affect plasma HDL cholesterol and FFAs.

The body composition of R pups was altered by d 90, but they did not become insulin resistant until d 180; this agrees with evidence indicating that altered adiposity and/or lipid metabolism is seen much before IR manifests (35,36) and supports the hypothesis that IR originates in impaired adipogenesis and/or lipid metabolism (36,37). From our results, the recent increase in IR and associated diseases among Indians appear to be due in part to the widely prevalent Mg deficiency among pregnant and lactating women (23).

In conclusion, our results indicate that maternal Mg restriction alters body adiposity early in the life of pups. They stress the importance of maternal Mg status in regulating insulin synthesis and/or secretion in pups, their insulin response to a challenge of glucose, and their progressive development of IR. Also, they indicate that perinatal Mg restriction modulates glucose tolerance and/or metabolism in pups, leading to impaired glucose tolerance. Interestingly, the changes appear to be irreversible by rehabilitation either from parturition or weaning.

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LITERATURE CITED


