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Does 11β-Hsd1 Associate with the Development of Visceral Adiposity in Maternal Mg Restricted Wistar/Nin Rat Offspring?

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Abstract

Maternal magnesium restriction irreversibly increased body fat %, specially the visceral adiposity in WNIN rat offspring. We have now investigated whether the increased visceral adiposity was associated with increased adipogenesis and glucocorticoid stress. Female, weanling WNIN rats were fed for 12 weeks, a control (AIN 93G) diet (MgC) or the same with 70% restriction of Mg (MgR) and mated with control males. Some of the pregnant MgR dams were rehabilitated from parturition and their pups weaned on to control diet (MgRP). At weaning half of the pups from MgR dams were shifted to control diet (MgRW) while the other half continued on Mg restriction (MgR). mRNA expression for fatty acid synthase (FAS) and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) was significantly higher in MgR adipose tissue offspring. However leptin mRNA expression was comparable among different groups. Both the rehabilitation regimes corrected the expression of 11β-HSD1 but not that of FAS. Maternal Mg restriction predisposed the offspring to increased glucocorticoid stress (11β-HSD1) that could underlie the increased body fat %/central adiposity.

Keywords: Magnesium; 11β-HSD1; Maternal under-nutrition; Fatty acid synthase; Visceral adiposity

Background

Adverse intrauterine environment predisposes the offspring to metabolic syndrome in adult life [1,2]. We showed earlier that maternal micronutrient (mineral and vitamin) restriction in WNIN rats increased the body fat%, altered plasma lipid and insulin levels and was associated with altered oxidative stress and adipocytokine levels in offspring suggesting that micronutrients were important in developmental programming of adiposity in the offspring [3,4]. Available literature suggests that modulation of epigenetics and stress (glucocorticoid and/or oxidative) could be some common pathways involved in developmental programming for adult diseases in the offspring due to maternal malnutrition during pregnancy and/or lactation [5,6]. High glucocorticoid stress as evident from 11β-HSD1 over expression is known to regulate adipose tissue differentiation, function, distribution and cause visceral adiposity, insulin resistance [7,8] and further 11β-HSD1 acts at the interface of inflammation and obesity [9]. Magnesium is important in the structure and function of the cell and is involved in more than 300 essential metabolic reactions. Epidemiological studies have shown that Mg deficiency resulted in the development of inflammation and was strongly associated with cardiovascular disease risk [10,11]. We recently showed that Mg restriction *in utero* predisposed the rat offspring to long term adiposity, specially visceral adiposity [12,13] and the offspring had increased expression (protein) of fatty acid synthase (FAS) and decreased leptin expression in adipose tissue [13]. This study aimed to determine whether or not altered mRNA expression (transcriptional regulation) underlies the changes in 11β-HSD1, FAS and leptin expression Vis-a-vis visceral adiposity in the offspring of Mg restricted WNIN rat dams.

Materials and Methods

Study design

Female, weanling WNIN rats ($n = 28$) were obtained from National Center for Laboratory Animal Sciences, National Institute of Nutrition (Hyderabad, India). The animal experimental procedure was approved by the "Institute's ethical committee on animal experiments" at National Institute of Nutrition, Hyderabad, India. They were housed individually in polypropylene cages with wire mesh bottom and maintained under standard lighting conditions (12-hour light/dark cycle). The animal feeding and experimental protocol for this study has been described by us earlier [12]. Briefly, a group of 21 rats was fed a basal diet (AIN-93G) containing 165 mg of Mg/kg diet (Mg restricted-MgR) for 12 weeks. The other group of seven rats received the control diet containing 650 mg of Mg/kg diet (Mg control-MgC). Rats were mated with control males and maintained on their respective diets throughout pregnancy. A subgroup ($n = 7$) of MgR dams was shifted to control diet on the day of parturition and their offspring from weaning (Mg rehabilitated from parturition-MgRP). At the time of weaning half the number of MgR pups were weaned on to control diet (Mg rehabilitated from weaning - MgRW) and the remaining half of the MgR pups continued on Mg restricted diet. The offspring (male) were continued on their respective diets till the time of sacrifice (18 months of age).

Gene expression analysis

Adipose tissue (retroperitoneal) was collected from different groups of offspring at the time of their sacrifice, frozen immediately and stored at - 80°C for molecular studies. Total RNA was isolated from ~ 100 mg of the frozen tissue by TRIzol reagent according to manufacturer's instructions (Invitrogen Life technologies, Carlsbad, CA). 2 µg of total RNA was used to synthesize cDNA using Invitrogen kit (Invitrogen Life technologies, Carlsbad, CA). The primer sequences for 11β-HSD1,

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leptin, fatty acid synthase (Fasn) and 18S rRNA were shown in table 1. The expressions of different genes were analyzed by semi-quantitative PCR. The PCR products were resolved electrophoretically on 1.2% agarose gel pre-stained with ethidium bromide. The quantitative expression of the genes was analyzed by Quantity One software (Bio-Rad Laboratories, Herculus, CA).

Statistical analysis

The values were presented as means ± S.E. Data was analyzed using one-way ANOVA followed by the multiple range test or least significant difference method. Wherever heterogeneity of variance was observed, differences between groups were tested using non-parametric Mann-Whitney U test. The differences were considered significant only if p was < 0.05.

Results

Fatty acid synthase mRNA expression

The mRNA expression of fatty acid synthase was significantly increased in MgR compared to MgC offspring and this result was not corrected by either of the rehabilitation regimes (Figure 1).

11β-HSD1 mRNA expression

The mRNA expression of 11β-HSD1 was significantly upregulated in MgR offspring compared to controls. Interestingly, this change was restored to control levels by both the rehabilitation regimes (Figure 1).

Leptin mRNA expression

The expression of leptin mRNA was comparable among the different groups (Figure 1).

Discussion

Magnesium intake is important in maintaining intracellular Mg homeostasis and is hypothesized to be one of the common antecedents for the pathogenesis of insulin resistance, type 2 diabetes, hypertension, and cardiovascular disease (CVD) [14,15]. Evidence from observational studies showed that dietary Mg intake was inversely correlated to the development of the adult diseases and its deficiency may promote inflammatory response [16]. We showed earlier that maternal Mg restriction increased the body fat %, specially the visceral adiposity in WNIN rat offspring and was associated with altered protein expression of fatty acid synthase (increased) and leptin (decreased) in the adipose tissue of the MgR offspring [12,13]. Altered expression of leptin and FAS proteins in adipose tissue has indeed been linked earlier to the development of visceral adiposity, obesity, insulin resistance and type 2 diabetes [17]. Considering these facts we deciphered whether the altered expression of the above proteins in the MgR offspring was at the transcriptional level and if yes determine the effects of rehabilitation. The significant over expression of fatty acid synthase mRNA in MgR offspring in the present study correlates with its increased protein expression reported by us earlier [13]. Taken together these results suggest maternal Mg restriction modulates FAS in the offspring at transcription and/or translation level and this may be responsible

Gene Name	Accession No	Forward Primer (5'-3')	Reverse primer (5'-3')
11β-HSD1	NM 017080.2	CTCTTGGGTGGACTGGACAT	TGCTCAGGACCACATAGCTG
leptin	NM 013076	GAGACCTCCTCCATCTGCTG	CATTCAGGGCTAAGGTCCAA
Fasn	NM 017332	TCGAGACACATCGTTTGAGC	TCAAAAAGTGCATCCAGCAG
18S rRNA	M 11188.1	CCAGAGCGAAAGCATTTGCCAAGA	AATCAACGCAAGCTTATGACCCGC

Table 1: Accession numbers and primer sequences of different genes.

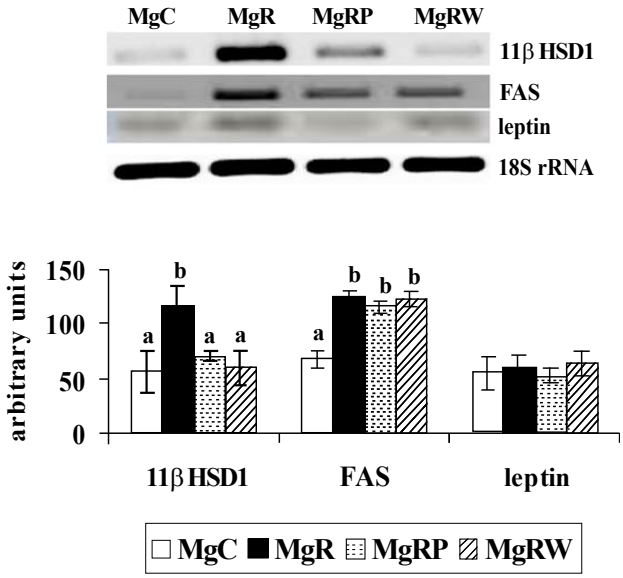

Figure 1: Effect of maternal Mg restriction and rehabilitation on expression of genes by semi-quantitative PCR in the adipose tissue of the offspring (at 18 months of age); white bars = MgC (control) group, black bars = MgR (Mg restriction) group, horizontal dotted bars = MgRP (Mg rehabilitation from parturition) group, diagonal stripe bars = MgRW (Mg rehabilitation from weaning). Gel picture for each gene is the representation of different groups. Values are mean ± SE (n=6). Bars without a common superscript 'a' and 'b' are significantly different at p< 0.05 by one way ANOVA & Post Hoc LSD.

for the increased adiposity/central adiposity in the offspring. That the expression of FAS mRNA was not corrected by either of the rehabilitation regimes probably indicates the importance of Mg in maintaining fat metabolism throughout gestation and lactation.

Despite the decreased leptin protein expression observed earlier in the MgR offspring [13], there was no change in leptin mRNA expression in the present study. This probably suggests that modulation of leptin expression in MgR offspring may be at post transcriptional and/or translational level rather than at transcription. This observation is not in agreement with our finding that maternal Cr restriction increased the expression of leptin in the offspring at both transcription and translation levels [18]. Developmental programming of adult onset diseases in the offspring due to maternal under-nutrition appears to occur through some common pathways/mechanisms involving stress and epigenetic changes. The effects of maternal under-nutrition on fetal programming are reported to be regulated either by modulating the glucocorticoid stress and cortisol hormone [19] or through release of oxidative free radicals and/or decreased antioxidant defense mechanism. Our earlier reports showed that maternal Mg restriction did not induce oxidative stress in rat offspring [13] probably suggesting the importance of the glucocorticoid stress in the adiposity and insulin resistance in MgR rat offspring. Therefore we assessed the expression of 11β-HSD1 in the MgR offspring to understand the role if any of the glucocorticoid stress.

Interestingly there was elevated expression of 11β-HSD1 mRNA in the adipose tissue of MgR offspring indicating that maternal Mg restriction probably programs/increases glucocorticoid mediated stress in the offspring. This is in line with our similar observations in the offspring of chromium restricted WNIN rat dams [18] and increased levels of plasma cortisol in the offspring of folate and/or vitamin B12 restricted Wistar rats (unpublished observations). This finding assumes significance considering that mRNA over expression of 11β-HSD1 in adipose tissue has earlier been linked to adipogenesis [20] and 11β-HSD1 has been suggested to be the potent enzyme mediating the function of glucocorticoid to manifest adiposity and inflammation [21]. Our finding also suggests that increased expression of 11β-HSD1 in adipose tissue could be a probable contributing factor for the developmental programming of adiposity and insulin resistance in MgR rat offspring. That rehabilitation could mitigate the change in 11β-HSD1 reiterates the importance of Mg in modulating the corticosteroid stress and hence adiposity in the offspring.

Conclusions

In conclusion maternal Mg restriction triggers corticosteroid stress as evident from the overexpression of 11β-HSD1 which may activate adipose tissue differentiation and result in visceral adiposity. This further could alter fat metabolism as suggested by the elevated levels of FAS protein and mRNA. Whether the altered expression of these genes in MgR offspring is due to epigenetic changes however need to be deciphered.

Competing Interests

The authors declare that they have no competing interests.

Author's Contributions

MR designed the project. LV conducted the animal experiment. IJNP and KRR analyzed and interpreted the data. KRR, IJNP and MR prepared the manuscript. All authors read and approved the final version of the manuscript to be submitted.

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