International Journal of Medicaland Health Sciences

Journal Home Page:http://www.ijmhs.netISSN:2277-4505

Original article

Rehabilitation Mitigates Changes in Body Fat %, Visceral Adiposity and Lipid Metabolism in the Wistar Rat Offspring Induced by Maternal Vitamin B12 Restriction

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ABSTRACT

Background: Vitamin B12 supplementation to pregnant Indian mothers has attracted little attention despite high prevalence of its deficiency among them. Werecently reported that vitamin B12 rehabilitation during pregnancy and lactationmitigated the changes in growth, muscle development, glucose tolerance and metabolism in the offspring born to B12 restricted Wistarrat dams. We now report the prevention/reversibility by rehabilitation of the effects caused by chronic maternal dietary vitamin B12restriction on body fat %, visceral adiposity and lipid metabolism in Wistar rat offspring. **Methods:** Vitamin B12 restricted, pregnant rat dams were rehabilitated with control diet from conception or parturition and their offspring from weaning. Whereas offspring born to some vitamin B12 restricted rat dams were weaned on to control diet. Body composition was determined in dams before mating and in male offspring at 3, 6, 9 and 12 months of their age. Biochemical parameters like lipid profile, plasma and tissue adipocytokine levels, activity of fatty-acid-synthase & acetyl-CoA-carboxylase and plasma cortisol levels were analyzed. **Results:**Maternal vitamin B12 restriction increased body fat % (especially abdominal adiposity), altered lipid metabolism (lipid profile, plasma and tissue adipocytokine levels, activities of fatty-acid-synthase and acetyl-CoA-carboxylase) and increased the glucocorticoidstress in B12 restricted offspring. While rehabilitation from conception restored the changes to controls, rehabilitation from parturition and weaning corrected the changes only partially. **Conclusion:**The results appear to suggest that rehabilitation may alleviate changes in body fat%, visceral adiposity and lipid metabolism induced by maternal vitamin B12 restriction in Wistar rat offspring.

KEYWORDS: Vitamin B12, restriction, rehabilitation, lipid metabolism, adipocytokines, fetal programming

INTRODUCTION

Maternal under nutrition induced placental insufficiencyreduces nutrient transfer from mother to fetus resulting in fetal under-nutrition and IUGR [1].Vitamin B12, an important regulator of one carbon metabolism modulates DNA methylation and myelination of nervous-system. Its deficiency increases plasma homocysteine, an independent risk factor for recurrent, spontaneous, early pregnancy losses [2]. Around one in twenty Indian women are B12 deficient in early pregnancy [3].

We reported earlier that rat offspring born to B12but not folate or dual vitamin restricted dams had low birth weight [4]. Interestingly, offspring of folate and/or B12 restricted rat dams had higher body weight and body fat % (visceral adiposity) from weaning till 12 months of age at which time their lipid metabolism was altered and glucocorticoidstress

increased [4]. We reported recently that maternal vitamin B12 deficiency induced changes in percentage of lean body mass, fat free mass and glucose metabolism in the offspring were almost reversed by B12rehabilitation from conception and parturition but not weaning [5]. This finding highlights that vitamin B12status during pregnancy and lactation may be an important determinant of growth, muscle development, glucosetolerance and metabolism in offspring.

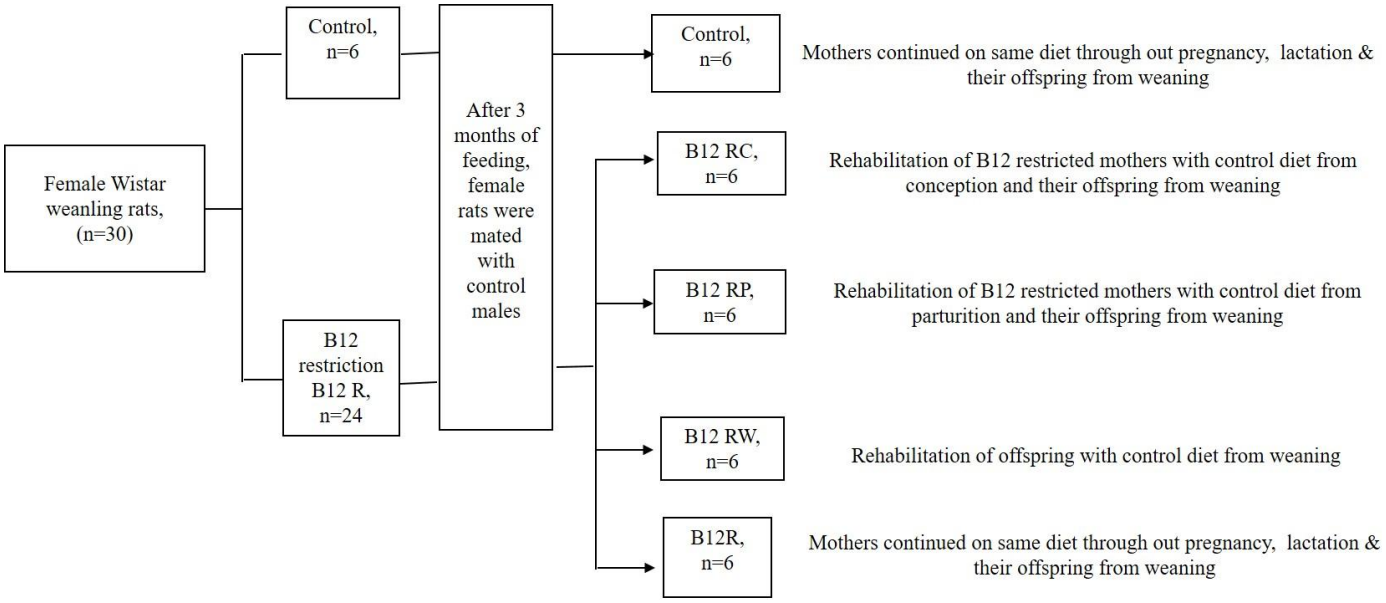
Considering our earlier observation that maternal B12 restriction increased body adiposity and impaired adipose function in rat offspring, the objective of this study was to assess whether or not the maternal vitamin B12restriction induced changes in adipose development, function and lipid metabolism in offspring are reversible by B12 rehabilitation and if yes from which time point onwards.

MATERIALS AND METHODS

The schematic design of animal experiment is given in figure 1, and as described by us earlier [5]. Briefly, female weaning Wistar rats (n=30) were fed *ad libitum* for 12 weeks, a control diet (AIN 76A casein based) (n=6) or the same with 40% restriction of vitamin B12 (B12R) (n=24). After confirming deficiency(by the plasma levels of vitamin B12 and homocysteine), they were mated with control males. Six each of pregnant B12R dams were rehabilitated from conception (B12RC)or parturition (B12RP) and their offspring were weaned to control diet.

Offspring of six B12R dams were weaned to control diet (B12RW), whereas those of remaining six B12R dams continued on restricted diet.Considering that estrogens influence insulin sensitivity, action and glucose homeostasis [6-8]we monitored the effects only in the male offspring (n= 6 per group). Plasma vitamin B12, homocysteine, body fat%, adiposity index, lipid profile, plasma &tissue adipocytokines, fatty-acid biosynthetic enzyme (fatty-acid-synthase and acetyl-CoA-carboxylase) activities and plasma cortisol levels were determined as described by us earlier [4,5]. Data were analyzed statistically by one-way ANOVA followed by post-hoc least significant difference test using SPSS software package, version19.0.

Figure 1: Schematic representation of the feeding protocol



RESULTS

BMI was higher in B12R offspring than controls at 12 months of their age and this was restored to that of controls by B12RC but not by B12RP or B12RW.Indeed, at all the time points studied, B12R offspring had significantly higher body fat %than controls and B12RC and B12RP, but not B12RW appeared to correct the changes*albeit* partially.Interestingly however, all three rehabilitation regimes appeared to restore the increased visceral adiposity to that of controls (as evident from the changes in adiposity index) (Table 1, figure 2).

The significantly higher (than controls) plasma triglycerides in B12R was restored to control levels by B12RC and B12RW. A significant increase in plasma total cholesterol was observed at 12 months of age in B12R and this was restored to controls by all three rehabilitation regimes. (Table 1 and figure 3).

TNFα, IL6 were increased and adiponectin was decreased (compared to controls) in the adipose tissue of B12R offspring and this was not restored by any of the three rehabilitation regimes. While B12RC alone (but not B12RP or B12RW) restored the increased plasma IL6 and partially the decreased IL1β (plasma and adipose), the increased levels of the adipose tissue MCP in B12R offspringwasmitigated *albeit* partially by all three regimes. In general, the changes in plasma leptin, TNFα and adiponectin levels in B12R offspring were prevented / restored to control levels by all the rehabilitation regimes (Table 2).

While the significantly increased activity of fatty-acid-synthase (FAS) in B12R offspring was restored by all the three rehabilitation regimes, the increased activity of acetyl-CoA-carboxylase was restored by rehabilitation from

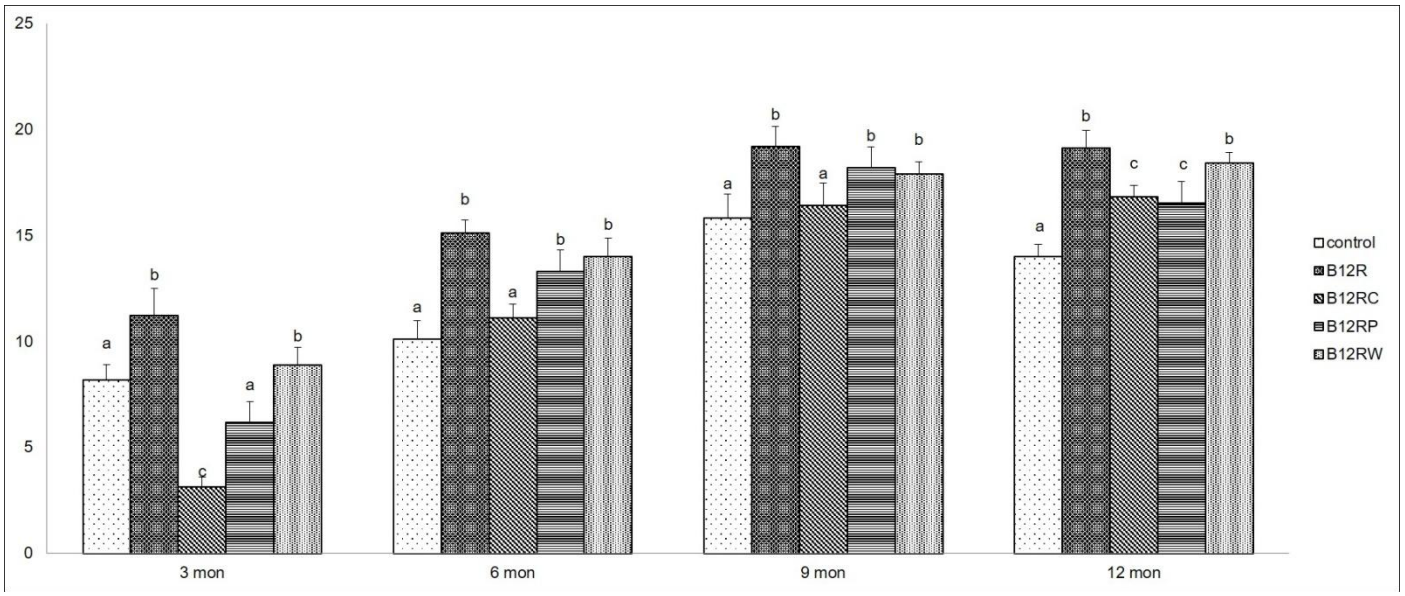
conception alone but not from parturition or weaning.Similarly, the increased plasma cortisol levels observed in B12R rat offspring was restored to that of controls by only B12RC but not by B12RP & B12RW (Table 2).

Table 1: Body composition and lipid profile in B12 rehabilitated offspring at 12 months of their age

Parameter	Control	B12R	B12RC	B12RP	B12RW
Body composition and distribution of abdominal body fat					
BMI	6.17 ± 0.230 [a]	6.96± 0.079 [b]	6.29 ± 0.289 [a]	6.47 ± 0.133 [b]	6.44 ± 0.247 [b]
Total body fat %	14.0 ± 0.560 [a]	19.1 ± 0.851 [b]	16.8 ± 0.562 [c]	16.5 ± 1.02 [c]	18.4 ± 0.524[b]
Retroperitoneal fat (g/100g body wt)	2.25 ± 0.333 [a]	4.75 ± 0.275[b]	1.08 ± 0.160 [c]	2.06 ± 0.123 [a]	1.90 ± 0.060 [a]
Mesenteric fat (g/100g body wt)	0.686 ± 0.062 [a]	1.15 ± 0.075 [b]	0.503 ± 0.080 [c]	0.707 ± 0.018 [a]	0.532 ± 0.320 [a]
Epidydimal fat (g/100g body wt)	1.22 ± 0.227 [a]	2.28 ± 0.073 [b]	0.554 ± 0.043 [c]	0.894 ± 0.079 [a]	0.982 ± 0.091 [a]
Adiposity index	4.16 ± 0.603 [a]	8.19 ± 0.288 [b]	2.14 ± 0.12 [c]	3.67 ± 0.199 [a]	3.41 ± 0.158 [a]
Lipid profile					
Triglycerides (mmol/L)	0.440 ± 0.086 [a]	1.37 ± 0.297 [b]	0.394 ± 0.040 [a]	0.870 ± 0.391 [b]	0.745 ± 0.090 [a]
Total cholesterol (mmol/L)	1.55 ± 0.073 [a]	2.98 ± 0.325 [b]	1.43 ± 0.129 [a]	1.87 ± 0.281 [a]	2.02 ± 0.156 [a]
HDL Cholesterol (mmol/L)	0.888 ± 0.064 [a]	1.11 ± 0.080 [a]	0.912 ± 0.090 [a]	0.991 ± 0.113 [a]	1.19 ± 0.079 [a]
Non esterified fatty acids (mmol/L)	0.636 ± 0.026 [a]	0.590 ± 0.027 [a]	0.562 ± 0.023 [a]	0.550 ± 0.042 [a]	0.528 ± 0.018 [a]

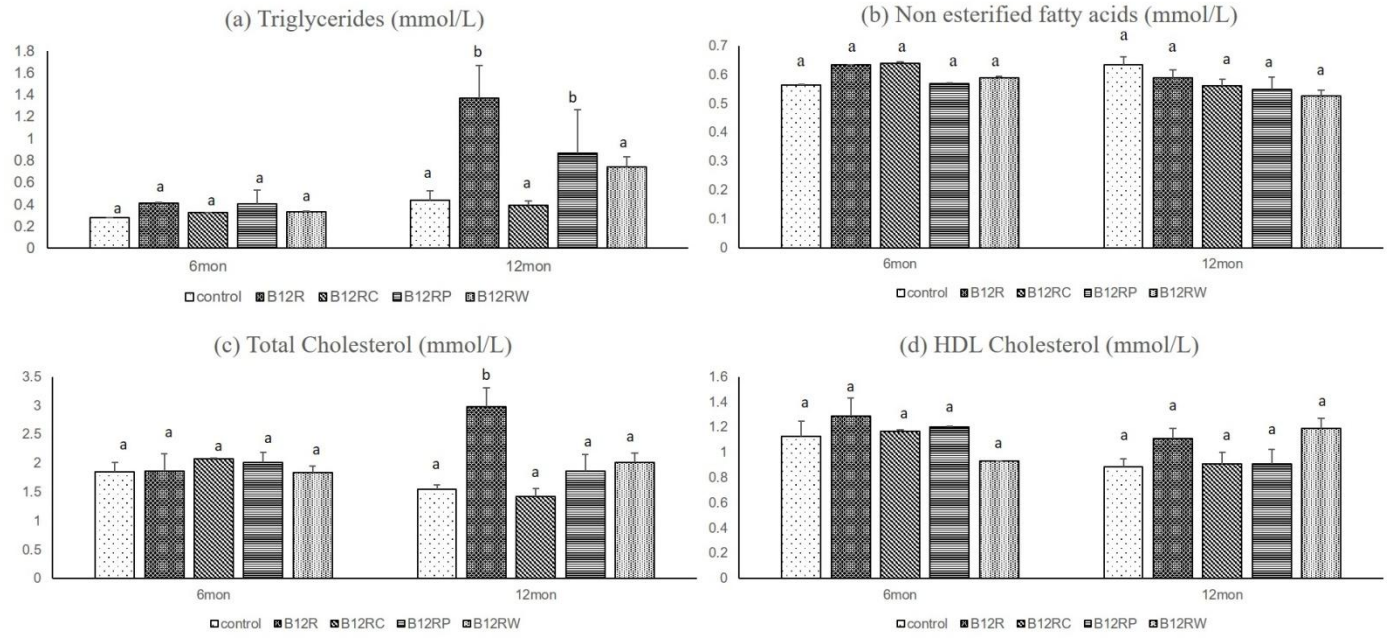
Body mass index, body fat%, retroperitoneal, mesenteric & epidydimal fat pads and lipid profile in male offspring at 12 months of age; Control, B12 restriction (B12R), B12 rehabilitation from conception (B12RC), B12 rehabilitation from parturition (B12RP), B12 rehabilitation from weaning (B12RW). Values are mean ± SE (n=6). Values in a row with different superscripts (a/b/c) are significantly different from one another at $p < 0.05$ by one way ANOVA / LSD tests.

Figure 2: Body fat % of different groups of male offspring at different time points of their age



Body fat % at different time points in Wistar rat male offspring fed different diets: Control(C), B12 restricted (B12R), B12 rehabilitation from conception (B12RC), B12 rehabilitation from parturition (B12RP), B12 rehabilitation from weaning (B12RW). Each bar represents a mean ± SE (n = 6). Bars without a common superscript (a/b/c) are significantly different at $p \leq 0.05$ by one way ANOVA followed by post hoc LSD (least significant difference) test.

Figure 3: Lipid profile of different groups of male offspring at different time points of their age



Lipid profile of different groups of male offspring at different time points of their age fed different diets: Control(C), B12 restricted (B12R), B12 rehabilitation from conception (B12RC), B12 rehabilitation from parturition (B12RP), B12 rehabilitation from weaning (B12RW). Each bar represents a mean ± SE (n = 6). Bars without a common superscript (a/b/c) in a panel are significantly different at $p \leq 0.05$ by one way ANOVA followed by post hoc LSD

Table 2: Adipocytokine levels, activities of fattyacid biosynthesis enzymes & plasma cortisol levels in B12 rehabilitated offspring at 12 months of their age

Parameter		Control	B12R	B12RC	B12RP	B12RW
Plasma and adipose tissue levels of various adipocytokines						
MCP1	Plasma (ng/ml)	226± 4.10	236± 1.70	242± 8.51	230± 2.45	233± 1.91
	Adipose (ng/mg protein)	7.46± 1.33 [a]	15.5± 0.531 [b]	11.2± 0.920 [c]	11.4±0.610 [c]	11.4± 0.757 [c]
Leptin	Plasma (ng/ml)	196± 9.20 [a]	112± 13.7 [b]	230± 9.69 [a]	208± 16.5 [a]	202± 18.3 [a]
	Adipose (µg/mg protein)	0.748± 0.133	1.20± 0.195	0.427± 0.129	0.875± 0.199	1.01± 0.261
IL1 β	Plasma (ng/ml)	62.1± 3.04 [a]	23.9± 2.45 [b]	38.3± 1.63 [c]	27.3± 3.72 [b]	21.0± 4.02 [b]
	Adipose (ng/mg protein)	5.78± 0.124 [a]	1.46± 0.105 [b]	3.69± 0.493 [c]	2.70± 0.234 [b]	2.30± 0.403 [b]
IL6	Plasma (ng/ml)	16.8± 0.654 [a]	22.8± 0.404 [b]	20.9± 0.940 [a]	23.3± 0.335 [b]	25.2± 0.347 [b]
	Adipose (ng/mg protein)	2.77± 0.129 [a]	6.44± 0.414 [b]	6.07± 0.528 [b]	6.26± 0.471 [b]	6.87± 0.300 [b]
TNF α	Plasma (ng/ml)	0.174±0.012 [a]	0.265±0.009 [b]	0.203±0.018 [a]	0.186±0.006 [a]	0.197±0.012 [a]
	Adipose (pg/mg protein)	30.3± 2.54 [a]	57.7± 4.37 [b]	54.7± 4.00 [b]	58.1± 4.47 [b]	68.0± 3.21 [b]
PAI	Plasma (ng/ml)	308± 19.0 [a]	328± 18.3 [a]	363± 26.2 [b]	328± 14.0 [a]	338± 8.50 [a]
	Adipose (ng/mg protein)	49.7± 12.2	66.1± 11.6	45.9± 7.43	51.3± 10.7	67.6± 7.49
Adiponectin	Plasma (µg/ml)	46.5± 1.02 [a]	11.4± 1.60 [b]	45.6± 6.72 [a]	43.2± 7.42 [a]	41.3± 9.58 [a]
	Adipose (µg/mg protein)	12.8± 1.67 [a]	6.72± 0.193 [b]	9.07± 0.597 [b]	9.52± 0.917 [b]	8.58± 1.20 [b]
Activities of fatty acid biosynthesis enzymes and plasma cortisol levels						
Acetyl-CoA-Carboxylase (units/ml/mg protein)		0.439 ± 0.063 [a]	0.749 ± 0.076b	0.571 ± 0.061 [a]	0.633 ± 0.052 [b]	0.086± 0.064 [b]
Fattyacid-synthase (units/ml/mg protein)		0.771 ± 0.098 [a]	1.71 ± 0.111 [b]	0.911 ± 0.150 [a]	1.12 ± 0.125 [a]	1.23 ± 0.201 [a]
Plasma Cortisol (ng/ml)		29.2 ± 2.05 [a]	43.7 ± 1.45 [b]	29.7 ± 1.94 [a]	39.1 ± 0.792 [b]	43.3 ± 0.843 [b]

Plasma & adipose tissue levels of various adipocytokine levels, activities of fatty acid biosynthesis enzymes (Fattyacid synthase, acetyl-CoA-carboxylase) and plasma cortisol levels in male offspring at 12 months of their age; Control, B12 restriction (B12R), B12 rehabilitation from conception (B12RC), B12 rehabilitation from parturition (B12RP), B12 rehabilitation from weaning (B12RW). Values are mean ± SE (n=6). Values in a row with different superscripts (a/b/c) are significantly different from one another at $p < 0.05$ by one way ANOVA / LSD tests.

DISCUSSION

Several studies have demonstrated that maternal mineral and vitamin deficiencies influence pregnancy outcome. We recently reported the body composition and biochemical parameters in Wistar female rats fed control or vitamin B12 restricted diets for three months [4, 5]. As expected, plasma vitamin B12 levels in restricted rats were ~75% lower than those of controls. We also reported that maternal vitamin B12 restriction altered birth and weaning weights of rat offspring. The differences among groups of offspring (control, B12R and rehabilitated) in their food intake, body weights, plasma vitamin B12 and homocysteine levels at different time points of their age was also recently reported by us[5]. Further, we also reported that the body weights and body fat% (especially abdominal fat) were increased with altered lipid metabolism [4]. Only B12 but not folate or dual (vitamin B12+folate) deficient rat offspring had low birth weight and most changes in glucose metabolism in B12R offspring were corrected by B12RC; while B12RP and B12RW corrected the changes *albeit* partially [4,5]. Therefore,we considered it pertinent to assess the reversibility/preventability if any of changes in reported by us earlier in the body fat content, distribution (specially the visceral adiposity) and the lipid metabolism of B12R offspring by vitaminB12 rehabilitation from different points of initiation/duration.

All three rehabilitation regimes prevented / corrected the increased body weight of B12R offspring, but only B12RC corrected the altered BMI corroborating the significance of gestational B12 status in programming growth and development of the fetus and offspring [5].To assess whether or not maternal vitamin B12 restriction altered the function of adipose tissue in addition to the increased body fat % and visceral adiposity reported earlier [4], we analyzed the plasma lipid profile and adipokine levels in adipose tissue as well as in circulation. Consistent with the literature that altered adiposity and lipid metabolism are the earliest changes [9, 10], altered adipose function (adipokine levels) was associated with higher central adiposity. That altered lipidprofilein B12R offspring was mostly reversed by all rehabilitation regimes appears to suggest a causal relationship.

Elevated MCP-1 induces adipocyte dedifferentiation and contribute to pathologies underlying hyperinsulinemia and obesity [11]. That theB12R offspring had higher MCP-1 levels than controls appears to suggest their predisposal to insulin resistance. The finding that B12 rehabilitation corrected this change only partially, probably suggests the involvement of other mechanisms. Hypoleptinemia seen in B12R offspring is in line with similar observation in magnesium restricted rat offspring [12], which in turn are in agreement with similar observations in diabetic conditions [13]may suggest the risk of these offspring to develop Diabetes. The correction of this condition by B12 rehabilitation probably suggests/ confirms their causal relationship. The decreased levels of adiponectin in plasma and adipose tissue in B12R offspring indicates their decreased sensitivity to insulin and that they were corrected partially by B12 rehabilitation is in line with partial correction observed in their body fat %and suggest that maternal vitamin B12 status not only programs adipose development but also its function.

The finding that decreased IL1β levels in plasma and adipose tissue of B12R offspring were partially corrected by B12RC alone but not others specially in the adipose tissue appears to reiterate the differential effects of rehabilitation on changes in the expression of different adipocytokines in plasma and adipose tissue; and are difficult to explain at this time point.That the expression/levels of pro-inflammatory adipocytokines were in general higher in the B12R offspring probably suggests that this (inflammatory condition) could be an underlying / associated mechanism of maternal vitamin B12 restriction induced adiposity and insulin resistance in the offspring

Our results indicate that increased body fat % and central adiposity in B12R offspring could be due to increased fatty-acid synthesis as evident from the increased activities of *Acetyl-CoA-carboxylase* and *Fatty-acid-synthase*[4]. That increased *Fatty-acid-synthase* activity alone was corrected by B12 rehabilitation in general, whereas only B12RC but not B12RP and B12RW corrected the changes in *Acetyl-CoA-carboxylase a*ctivity seems not only to suggest the importance of B12 in modulating their activities but also reiterates the differential effects of B12 status on the expression of different enzymes regulating the lipid metabolism. This is in line with earlier reports of similar nature in B12R rat offspring [14]

The higher plasma cortisol levels in B12R offspring at 12 months of age suggests that maternal vitamin B12 restriction may program HPA axis (maternal B12 restriction induced corticosteroid stress in the offspring)and is in line with our earlier report of increased 11β-HSD1 expression in the chromium restricted rat offspring which was associated / was underlying similar changes observed in chromium restricted rat offspring [15-17]. That the increased plasmacortisol was restored by only B12RC stresses that gestational vitamin B12 status is important in modulating glucocorticoid stress and attendant phenotypic changes in offspring.

CONCLUSION

In conclusion, maternal B12 restriction not only altered body composition of the offspring, but also adipose function as indicated by altered lipid profile, adipogenic enzymes and adipocytokine levels indicative of pro-inflammatory status and increased corticosteroid stress. For the first time to the best of our knowledge the present study has shown that changes in adiposity and lipid metabolism were mitigated by B12 rehabilitation from conception (B12RC)and partly by rehabilitation from parturition (B12RP)and weaning (B12RW), which appear to suggest a probable causal relationship between the maternal vitamin B12 status and the phenotypic and metabolic changes in offspring. The observation that B12R offspring had a pro-inflammatory status and higher glucocorticoid stress (than controls) appear to implicate them in the etiology of maternal vitamin B12 restriction induced changes in lipid metabolism in the rat offspring. That rehabilitation from conception if not from parturition or weaning could prevent / mitigate the change appears to confirm their causal relationship.

ACKNOWLEDGEMENTS

This work was supported by a research grant from the Department of Biotechnology, Government of India, New Delhi, India (Project # BT/PR-7506/PID/20/294/2006) to M.R., G.R.C. and S.S. The authors acknowledge the support and encouragement received from the Directors of National Institute of Nutrition and the Centre for Cellular and Molecular Biology, Hyderabad, in the conduct of these studies. KAK & MR also thank Dr. M.J. Mahesh Kumar for his help in the conduct of the animal experiments at CCMB. KAK is grateful to the Indian Council of Medical Research (ICMR), India, for awarding senior research fellowship.

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