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Maternal dietary folate and/or vitamin B_{12} restrictions alter body composition (adiposity) and lipid metabolism in Wistar rat offspring☆

Kalle Anand Kumar[a], Anumula Lalitha[a], Dhandapani Pavithra[b], Inagadapa J.N. Padmavathi[a], Manisha Ganeshan[a], Kalashikam Rajender Rao[a], Lagishetty Venu[a], Nagala Balakrishna[c], Nemani Hari Shanker[d], Singi Umakar Reddy[b], Giriraj Ratan Chandak[b], Shantanu Sengupta[e], Manchala Raghunath[a,*]

[a]Division of Endocrinology and Metabolism, National Institute of Nutrition, Indian Council of Medical Research (ICMR), Hyderabad-500 604, India
[b]Centre for Cellular and Molecular Biology (CCMB), Council of Scientific and Industrial Research (CSIR), Hyderabad-500 007, India
[c]Division of Statistics, National Institute of Nutrition, Indian Council of Medical Research (ICMR), Hyderabad-500 604, India
[d]National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Indian council of Medical Research (ICMR), Hyderabad-500 604, India
[e]Institute of Genomics and Integrative Biology, Council of Scientific and Industrial Research (CSIR), New Delhi-110 007, India

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Abstract

Maternal vitamin deficiencies are associated with low birth weight and increased perinatal morbidity and mortality. We hypothesize that maternal folate and/or vitamin B_{12} restrictions alter body composition and fat metabolism in the offspring. Female weaning Wistar rats received *ad libitum* for 12 weeks a control diet (American Institute of Nutrition-76A) or the same with restriction of folate, vitamin B_{12} or both (dual deficient) and, after confirming vitamin deficiency, were mated with control males. The pregnant/lactating mothers and their offspring received their respective diets throughout. Biochemical and body composition parameters were determined in mothers before mating and in offspring at 3, 6, 9 and 12 months of age. Vitamin restriction increased body weight and fat and altered lipid profile in female Wistar rats, albeit differences were significant with only B_{12} restriction. Offspring born to vitamin-B_{12}-restricted dams had lower birth weight, while offspring of all vitamin-restricted dams weighed higher at/from weaning. They had higher body fat (specially visceral fat) from 3 months and were dyslipidemic at 12 months, when they had high circulating and adipose tissue levels of tumor necrosis factor α, leptin and interleukin 6 and low levels of adiponectin and interleukin 1β. Vitamin-restricted offspring had higher activities of hepatic fatty acid synthase and acetyl-CoA-carboxylase and higher plasma cortisol levels. In conclusion, maternal and peri-/postnatal folate and/or vitamin B_{12} restriction increased visceral adiposity (due to increased corticosteroid stress), altered lipid metabolism in rat offspring perhaps by modulating adipocyte function and may thus predispose them to high morbidity later.
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Keywords: Vitamin restriction; Body composition; Insulin resistance; Lipid profile; Adipocytokines

1. Introduction

Nutritional and hormonal environment experienced by the fetus determines the degree of adult risk and the risk of developing chronic diseases. For instance, reports indicate that metabolic syndrome in adulthood can be determined by conditions experienced *in utero* [1]. Inadequate supply of nutrients forces the fetus to adapt, down-regulate growth and prioritize the development of essential tissues, which enhance its immediate survival but may carry a long-term price. An association between low birth weight (LBW) and insulin resistance (IR) in later life, a strong risk factor for cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM), is a consistent finding in a number of populations [2].

The role of micronutrients in determining pregnancy outcome is well established. Their deficiencies have profound and often persistent effects on fetal tissues and organs, even in the absence of clinical signs of their deficiency in the mother [3]. Further, consequences of vitamin imbalance during fetal development may not be apparent at the time of nutritional insult, but manifest later during development. Maternal vitamin deficiencies widely prevalent in the developing world could be a leading cause of LBW and consequent risk for adiposity and IR in the later life of the offspring [4,5].

Emerson et al. (1949) showed that female rats deficient in vitamin B_{12} had decreased number and size of the progeny compared to those

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* Corresponding author. Tel.: +91 40 27197235; fax: +91 40 27019074.
E-mail address: mraghunath55@yahoo.com (M. Raghunath).

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fed a complete ration [6]. However, the effects of peri-/postnatal deficiency of folate and/or vitamin B_{12} on birth weight, body adiposity and lipid metabolism in the offspring have not been well established yet. Hence, the present study was conducted to assess the effects of maternal folate and/or vitamin B_{12} restriction on body fat content, its distribution, adipose tissue function and lipid metabolism in Wistar rat offspring.

2. Materials and methods

2.1. Animal experimentation

The experiment was carried out in Wistar rats in accordance with the 'Principles of Laboratory Animal Care' [7] and with the approval of the 'Institute's Ethical Committee on Animal Experiments' at the National Institute of Nutrition, Hyderabad, India.

Weanling female Wistar rats (n=48) were housed individually in polypropylene cages with wire mesh bottom and maintained at 22°C±2°C under standard lighting conditions (12-h light/dark cycle). They were divided into four groups (n=12 each) and fed *ad libitum* for 12 weeks a casein-based (20% protein) (AIN 76 A) control diet (M/S Research Diets Inc, USA) or the same diet restricted in folate (FR) or vitamin B_{12} (B_{12}R) or both (dual deficient: DD) (Fig. 1). Vitamin B_{12} content of the B_{12}R diet was approximately 60% of that of the control diet (0.006 vs. 0.010 mg/kg diet); the folate-deficient diet had less than 10% of the folate content of the control diet (0.08 vs. 2.00 mg/kg diet), and the dual-deficient diet was a combination of folate- and B_{12}-deficient diet (Supplemental Table 1). DD and B_{12}R diets contained 50 g pectin/kg diet (in addition to cellulose) because it has been shown earlier that pectin binds vitamin B_{12} in the intestine and makes it less bioavailable [8]. Animals had free access to deionized water and respective diets until their offspring were weaned, and the offspring continued on their respective mothers' diets until 12 months of age. Daily food intake and weekly body weights were determined until 12 weeks of feeding, whereas plasma folate, vitamin B_{12} and homocysteine levels were determined at the end of 12 weeks of feeding.

After ensuring vitamin deficiency, they were mated with control males (@ two females per male), and the dams continued on their respective diets throughout pregnancy and lactation. A uniform litter size of eight pups (equal numbers of males and females wherever possible) was maintained with each mother from day 3 of lactation. Male offspring (n=24 per group) were weaned on postnatal day 21 and continued on their respective mothers' diet until they were sacrificed (in batches of six animals each) at 3, 6, 9 and 12 months of age. Body weights of the offspring were determined at birth, weaning and 3, 6, 9 and 12 months of age, whereas plasma folate, vitamin B_{12} and homocysteine levels were determined in the offspring at 3, 6, 9 and 12 months of age.

2.2. Body composition

Body composition was determined in the female Wistar rats after 3 months of feeding different diets (just before mating) and in the offspring at quarterly intervals between 3 and 12 months of age using total body electrical conductivity, a small-animal body composition analysis system (model SA 3000 multidetector; EMSCAN, Springfield, IL, USA), as described by us earlier [4,5]. Body fat percentage was computed mathematically according to Morbach and Brans [9].

Adiposity index (AI), an index of visceral adiposity, was computed according to Taylor and Philips [10]. For this purpose, retroperitoneal, mesenteric and epididymal fat pads were quickly excised from the offspring at the time of their sacrifice (time points mentioned above), their fresh weights were determined, and AI was computed as follows:

$$AI = (\text{sum of the fresh weights of the three fat deposits}/\text{body weight}) \times 100.$$

2.3. Biochemical parameters

Plasma vitamin B_{12} and folate levels were determined by a radioimmunoassay kit (SIEMENS Medical Solutions Diagnostics) based on a dual-count, solid-phase no-boil assay, and homocysteine concentrations were analyzed in plasma by high-performance liquid chromatography equipped with a fluorescence detector as described by us earlier [11].

Total cholesterol, triglycerides (TGs) and high-density lipoprotein (HDL) cholesterol were determined in plasma using enzymatic assay kits from Biosystems

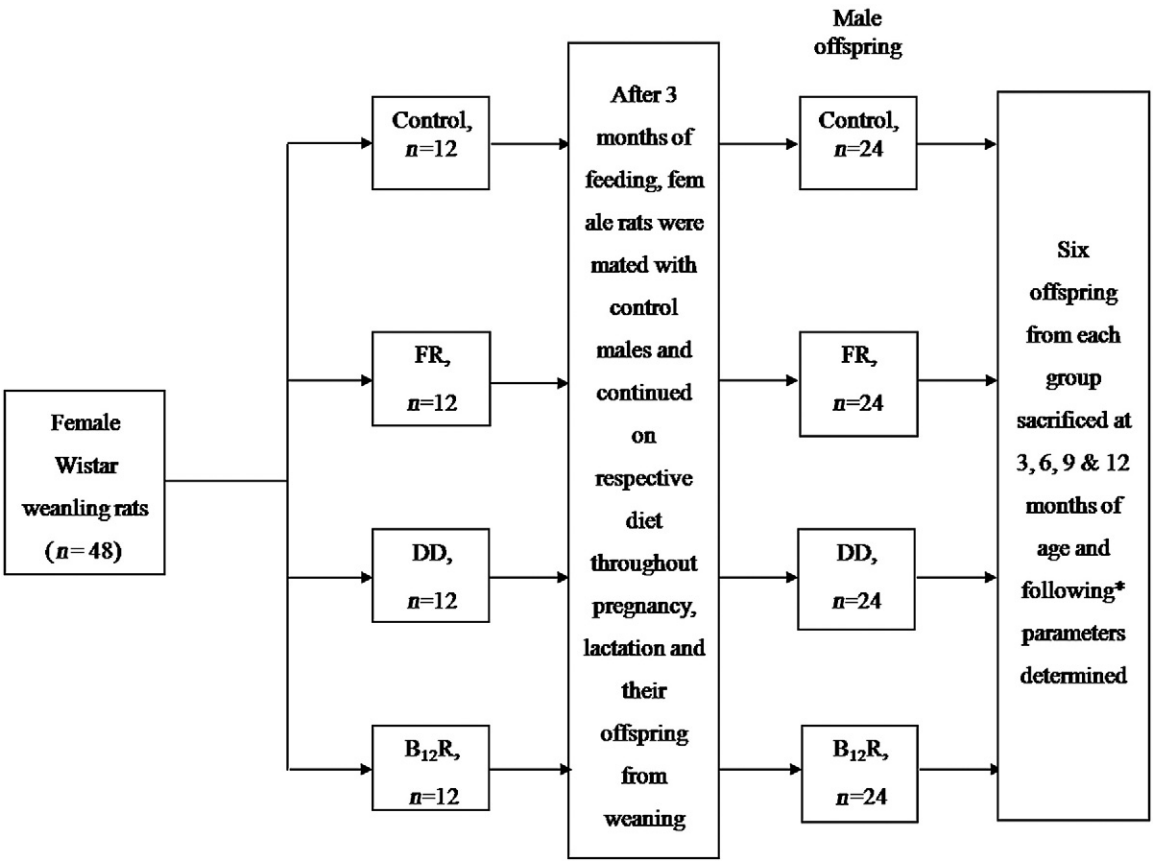

Fig. 1. Schematic representation of the feeding protocol for different groups of Wistar female rats. Control(C), folate (FR), dual-deficient (DD) and B_{12}-restricted (B12R) *Body weights at birth, weaning and 3, 6, 9 and 12 months of age. Body fat %, AI, plasma lipid profile and cortisol levels at 3, 6, 9 and 12 months of age. Adipocytokine profile (plasma and adipose tissue) and hepatic fatty acid synthase/acetyl-CoA-carboxylase at 12 months of age.

Table 1
Food intake, body weight, plasma vitamin B_{12}, folate and homocysteine levels of female, weaning Wistar rats fed different diets for 3 months

Parameter	Control	FR	DD	$B_{12}R$
Food intake (g)	12.9±0.466	13.3±0.424	13.1±0.610	13.5±0.340
Body wt (g)	184±4.46	218±9.10[a]	204±8.14	213±7.82[a]
Plasma folate (ng/ml)	35.6±1.61	17.7±2.02[c]	10.2±1.14[c]	37.4±1.79
Plasma vitamin B_{12} (pg/ml)	1164±8.1	1026±0.3	219±4.31[c]	277±4.54[c]
Plasma homocysteine (μM)	4.89±0.358	5.57±0.244	8.26±0.285[c]	8.03±0.208[c]
Body fat %	9.41±0.919	8.70±1.33	11.8±1.46	12.5±0.685[a]
AI	2.40±0.189	6.25±0.380[c]	2.71±0.227	2.56±0.172

Food intake, body weights, plasma folate, vitamin B_{12}, homocysteine, body fat % and AI in Wistar female rats fed control (C), folate (FR), dual-deficient (DD) and B_{12}-restricted (B_{12} R) diets for 3 months from weaning. Values given are mean±S.E. (n=6). Values in a row (comparison among groups) not sharing a common superscript are significantly different from control by one-way ANOVA/LSD tests: a=P<.05 and c=P<.001.

(Barcelona, Spain). Plasma nonesterified fatty acids (NEFA) were determined using the enzymatic kit from Randox (Antrim, UK).

Concentrations of adiponectin, leptin, plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein 1 (MCP-1), interleukin 6 (IL-6), interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) were determined in fasting plasma and adipose tissue using Lincoplex research kits (Linco Research, St. Louis, MO, USA) on a BIOPLEX platform (BioRad). For this purpose, adipose tissue homogenate was prepared as described earlier [12], and the protein content was determined in the homogenate by bicinchoninic acid assay [13].

Fatty acid synthase activity was estimated in liver cytosol (100,000 g supernatant) according to Linn et al. [14], whereas acetyl-CoA-carboxylase activity was determined according to Tanabe et al. [15].

Cortisol levels were determined in the early morning plasma samples using a chemiluminescence enzyme assay kit (SIEMENS Medical Solutions Diagnostics) according to manufacturer's instructions.

2.4. Statistical analysis

We compared all the parameters in VR rats (before mating) and their pups with the respective controls in addition to analyzing the time course effect (differences in various parameters between 3 and 12 months of age) in the offspring of different groups. The data were analyzed using SPSS statistics package (version 19.0). All values are presented as mean±S.E. One-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) method was used to analyze the differences among female Wistar rats fed different diets (before mating) and among the offspring of different groups at a given time point, whereas the time course effect in a given group of offspring was determined using the Student's t test. Differences were considered significant if P was at least ≤.05.

3. Results

3.1. Effects in female Wistar rats

Food intake was comparable among the four groups of rats (Table 1). After 3 months of feeding, FR rats had ~50% lower concentration of folate and $B_{12}R$ rats had ~75% lower levels of vitamin B_{12}, respectively, in plasma than controls. On the other hand, plasma levels of these vitamins were ~75% lower in DD rats (Table 1). Plasma homocysteine levels were higher in all the VR groups (than controls), but was significant in only DD and $B_{12}R$ rats.

After 3 months on their respective diets, VR rats weighed higher than controls, albeit the difference was significant in only FR and $B_{12}R$ rats (Table 1). Whereas $B_{12}R$ rats had higher body fat % (but not visceral adiposity) than controls, FR rats had higher visceral adiposity but not body fat % (Table 1 and Supplemental Table 2). DD rats showed no changes in any of these parameters, and plasma lipid profile was comparable among all the groups. Except for some minor differences, the adipocytokine levels in both plasma and adipose tissue were comparable among the groups (Supplemental Table 3 and 4). Reproductive performance of the VR and control rats was also comparable (Supplemental Table 5).

3.2. Effects in offspring

Birth weight of the offspring was comparable among control, FR and DD groups, whereas $B_{12}R$ offspring weighed lower than controls (Table 2). As expected, in all the groups, the body weights were significantly higher at 12 months than 3 months of age. All VR offspring weighed higher than controls at weaning (Table 2) and continued so until 12 months of age, despite comparable (to controls) food intake (Supplemental Figure 1; Supplemental Table 6). Plasma folate and/or vitamin B_{12} levels continued to be less in the respective VR offspring compared to those of controls until the time of their sacrifice (Supplemental Tables 7, 8). In offspring of all the groups, plasma homocysteine levels were higher at 12 months than 3 months of age. Although variable at earlier age, notwithstanding the differences, plasma homocysteine levels were comparable among different groups at 12 months of age (Supplemental Table 9).

In all groups, body fat % was significantly higher at 12 months than 3 months of age. In line with their higher body weight, all VR offspring had higher body fat % at 12 months of age, whereas at 3 months of age, only FR and $B_{12}R$ offspring had higher body fat% (Table 2) than controls (Supplemental Figure 2). Interestingly, at 9 and 12 months of age, FR offspring had three- to fourfold higher body fat % than the other groups. Adiposity index, an indicator of visceral adiposity, was significantly higher in all VR offspring at 12 months than 3 months but not in controls. While VR offspring had higher visceral adiposity at 12 months (Table 3) (Supplemental Figure 3) than controls, the visceral adiposity of FR, DD and $B_{12}R$ offspring was higher from/at 3, 9 and 12 months, respectively.

Plasma total cholesterol and TG levels but not those of HDL cholesterol and NEFA were significantly higher at 12 than 3 months of age, albeit only in VR but not in control offspring (Table 4). At 12 months of age, plasma total cholesterol and TG levels were higher in all VR offspring (Table 4) than controls. Only FR (but not DD or $B_{12}R$) offspring had higher plasma HDL levels than controls both at 3 and 12 months of age, whereas no differences were observed in plasma NEFA levels among the different groups (Table 4) at these two time points. Plasma TNF-α was higher, whereas adiponectin was lower, in FR and $B_{12}R$ offspring than controls.

Table 2
Body weight and body fat % of offspring of different groups at different ages

Group	Time point	Control	FR	DD	$B_{12}R$
Body weight (g)	Birth	6.41±0.407	6.43±0.105	6.01±0.116	5.61±0.212[c]
	Weaning	36.0±2.72	52.5±1.33[c]	45.0±2.38[b]	51.1±4.64[c]
	3 months	227±13.7	434±8.61[c]	358±3.37[c]	356±5.42[c]
	12 months	390±18.8*$	552±17.8[c]*$	518±16.6[c]*$	577±9.93[c]*$
Body fat %	3 months	8.16±0.734	18.1±0.830[c]	10.2±0.817	11.2±1.31[a]
	12 months	14.0±0.560*$	56.0±0.992[c]*$	19.6±0.384[c]*$	19.1±0.851[c]*$

Body weight and body fat % in offspring of different groups at different time points. Values given are mean±SE (n=6). Values in a row (comparison among groups at the given time point) not sharing a common superscript are significantly different from control by one-way ANOVA /LSD tests: a=P<.05, b=P<.01 and c=P<.001. Comparison of the value of the parameter in the given group between 3 and 12 months of age (age-dependent change) significantly different by Student's t test: *$=$P$<.001.

Table 3
Wet weights of the visceral fat depots (g/100 g body wt) and AI of the offspring of different groups at 3 and 12 months of age

Group	Time point	Control	FR	DD	$B_{12}R$
Retroperitoneal fat	3 months	1.66±0.253	2.67±0.242[a]	2.22±0.200	2.11±0.209
	12 months	2.25±0.333	5.05±0.563[c#]	3.78±0.416[c#]	4.75±0.275[c*$]
Mesenteric fat	3 months	0.597±0.075	0.806±0.028	0.738±0.076	0.715±0.067
	12 months	0.686±0.062	0.875±0.055[a]	1.08±0.110[c*]	1.15±0.075[c#]
Epididymal fat	3 months	0.988±0.164	1.31±0.177	1.22±0.118	1.19±0.080
	12 months	1.22±0.227	1.65±0.126 [a]	1.64±0.150[a*]	2.28±0.073[c*$]
AI	3 months	3.25±0.434	4.79±0.396[a]	4.19±0.250	4.02±0.293
	12 months	4.16±0.603	7.58±0.693[c#]	6.51±0.557[c#]	8.19±0.288[c*$]

Wet weights of visceral fat pads and AI in offspring of different groups at 3 and 12 months of age. Values given are mean±S.E. (n=6). Values in a row (comparison among groups at the given time point) not sharing a common superscript are significantly different from control by one-way ANOVA/LSD tests: a=P<.05 and c=P<.001. Comparison of the value of the parameter in the given group between 3 and 12 months of age (age-dependent change) significantly different by Student's t test: *=P<.05; #=P<.01 and *$=$P$<.001.

Plasma IL-6 levels were higher in only $B_{12}R$ and DD offspring, whereas plasma leptin levels were unaffected in FR and DD but lower in $B_{12}R$ rat offspring. It was surprising that plasma IL-1β levels were lower in all VR offspring, whereas MCP-1 and PAI were comparable among groups (Table 5). On the other hand, in the adipose tissue, MCP-1, IL-6 and TNF-α were higher in all the VR offspring, whereas leptin was higher in only FR. While PAI was comparable among groups, IL-1β and adiponectin were lower, albeit in $B_{12}R$ and DD offspring only (Table 6).

Specific activities of hepatic acetyl-CoA-carboxylase and fatty acid synthase were higher in all VR offspring than in controls (Fig. 2). It was interesting to note higher plasma cortisol levels in all VR offspring compared to controls (Fig. 3).

4. Discussion

In this study, we assessed the effect of specific micronutrient deficiencies in mothers on body composition (specially the adiposity), adipocyte function and lipid metabolism in the offspring. Pectin used in DD and $B_{12}R$ diets not only decreases the bioavailability of vitamin B_{12} but also promotes the depletion of endogenous vitamin B_{12} due to its enterohepatic circulation [16].

The fact that plasma levels of folate and/or vitamin B12 observed in the female Wistar VR rats were similar to those reported in the folate- and/or vitamin-B12-deficient subjects [17,18] suggests that the magnitude of deficiency produced was comparable to that reported in humans, despite no differences being observed in their food intake. The increase in body weight (FR and $B_{12}R$), body fat percent ($B_{12}R$) and visceral adiposity (FR) observed in the VR female Wistar rats is in contrast to our failure to observe such effects in female, weaning WNIN rats fed diets restricted in vitamins, minerals, chromium and magnesium [4,5,19,20]. However, they agree with reports that deficiency of micronutrients such as calcium accelerated weight gain and fat accretion, whereas high-calcium diets inhibited lipogenesis and accelerated lipolysis [21,22]. Indeed, zinc deficiency has also been reported to alter adipose metabolism, IR and obesity [23,24]. The finding that $B_{12}R$ *per se* increased body fat % (but not visceral adiposity), FR increased visceral adiposity but not body fat %, whereas DD did not affect either of these parameters establishes the importance of these vitamins, especially the ratio of folate to vitamin B_{12}, in modulating the body fat content and distribution, albeit the underlying mechanisms need to be deciphered. That chronic dietary VR *per se* did not affect plasma lipid profile and adipocytokines appears to suggest that they may not modulate adipocyte function or lipid metabolism in these rats. Considering that maternal undernutrition is reported to program the body composition of the fetus in a way that the fetus is predisposed to adult-onset degenerative diseases, it was felt important to assess the effects, if any, of maternal folate and/or vitamin B_{12} deficiency on body composition (adiposity) and lipid metabolism in the offspring.

That $B_{12}R$ offspring had lower birth weight than controls is in line with an earlier observation of a similar nature in vitamin-B_{12}-deficient rats [25]. Although VR female rats were not insulin resistant before conception, considering that birth weight of the offspring is reported to be inversely related to maternal IR, the present findings suggest that $B_{12}R$ dams were probably insulin resistant during pregnancy. Despite comparable food intakes, the observation that maternal and postnatal folate and/or vitamin B_{12} restriction significantly increased the offspring's body weight at weaning and thereafter is in line with our findings of a similar nature in the offspring of chromium-restricted rat dams [20] but in contrast to our observations in the pups born to magnesium-restricted rat dams [19]. However, the reasons for the increased body weight in the VR offspring are not clear yet.

In line with our earlier observation of increased body fat % in the micronutrient restricted WNIN rat offspring [4], we observed that, compared to controls, VR offspring had higher (than control) body fat percentage at 12 months of age. However, it was of interest that despite the significant increase in body fat % in all groups at 12 months of age (compared to that at 3 months), there was significant time-/age-dependent increase in visceral adiposity in only the VR but not control offspring. These observations appear to indicate the importance of maternal vitamin B_{12} and/or folate status not only in aggravating the age-dependent increase in body fat % but also in

Table 4
Plasma lipid profile of the offspring of different groups at 3 and 12 months of age

Group	Time point	Control	FR	DD	B_{12} R
Cholesterol mmol/L	3 months	1.43±0.008	1.77±0.116 [a]	1.50±0.148	1.75±0.009[a]
	12 months	1.55±0.073	3.12±0.268 [c#]	2.56±0.180[c#]	2.98±0.325[c#]
Triglycerides mmol/L	3 months	0.392±0.009	0.719±0.089 [a]	0.271±0.123	0.313±0.002
	12 months	0.440±0.086	1.21±0.090[c#]	0.928±0.137[a#]	1.37±0.297[c#]
HDL cholesterol mmol/L	3 months	1.16±0.009	1.62±0.115 [b]	1.11±0.108	1.20±0.006
	12 months	0.888±0.064*$	1.93±0.198 [c]	1.04±0.067	1.11±0.080
NEFA mmol/L	3 months	0.594±0.005	0.547±0.033	0.425±0.008	0.553±0.003
	12 months	0.636±0.026	0.663±0.056	0.626±0.045	0.590±0.027

Plasma lipid profile in offspring of different groups at 3 and 12 months of age. Values given are mean±S.E. (n=6). Values in a row (comparison among groups at the given time point) not sharing a common superscript are significantly different from control by one-way ANOVA/LSD tests: a=P<.05; b=P<.01 and c=P<.001. Comparison of the value of the parameter in the given group between 3 and 12 months of age (age-dependent change) significantly different by Student's t test: #=P<.01 and *$=$P$<.001.

Table 5
Plasma adipocytokine levels in the offspring of different groups at 12 months of age

Adipocytokine	Control	FR	DD	$B_{12}R$
TNF-α (ng/ml)	0.174±0.012	0.215±0.004[b]	0.183±0.006	0.265±0.009[c]
Adiponectin (μg/ml)	46.5±1.02	15.6±3.36[c]	34.3±2.92	11.4±1.60[c]
IL-6 (ng/ml)	16.8±0.654	20.6±0.556	24.8±0.686[c]	22.8±0.404[b]
Leptin (ng/ml)	196±9.20	175±6.69	183±14.8	112±13.7[c]
IL-1β (ng/ml)	62.1±3.04	28.4±8.21[c]	19.6±1.61[c]	23.9±2.45[c]
MCP-1 (ng/ml)	226±4.10	240±3.79	228±2.00	236±1.70
PAI (ng/ml)	308±19.0	332±19.2	292±13.6	328±18.3

Plasma adipocytokines concentrations in offspring of different groups at 12 months of age. Values given are mean±S.E. (n=6). Values in a row (comparison among groups) not sharing a common superscript are significantly different from control by one-way ANOVA/LSD tests: b=P<.01 and c=P<.001.

increasing the visceral adiposity specifically. Although we have no suitable explanation for these observations and have not determined the plasma methyl malonyl CoA levels, the report that high methylmalonyl-CoA seen in vitamin B_{12} deficiency increases lipogenesis by inhibiting beta-oxidation appears to support/explain the present findings at least partially [26]. Altered body adiposity and/or lipid metabolism usually precede tissue IR [27,28], and it is hypothesized that IR originates in increased adipogenesis and/or lipid metabolism [26,28,29]. Taken together with these reports, we feel that our observations, for the first time to the best of our knowledge, demonstrate that maternal vitamin B_{12} and/or folate restriction modulate/program the body fat % as well as its distribution (central adiposity) in the offspring. However, the underlying/associated mechanisms remain to be elucidated.

We investigated next whether or not maternal folate and/or vitamin B_{12} restriction modulated adipocyte function and lipid metabolism. Adipose tissue secretes various adipocytokines that regulate several metabolic pathways and the inflammatory state. Although inflammation is a secondary response to some disease-causing substances/events, it can protect/damage the cell depending on its severity and length of exposure. Proinflammatory adipocytokines are reported to alter endocrine function and impinge insulin signaling/action in adipocytes, liver and muscle, leading to development and/or aggravation of IR [30]. Leptin, a proinflammatory adipokine, is considered an important link between obesity, IR and atherosclerosis [31]. In the present study, although plasma leptin concentration was decreased in $B_{12}R$ offspring, it was high in the adipose tissue of the FR offspring, albeit the reasons for this apparently discordant result need to be deciphered. We observed increased levels of proinflammatory cytokines such as IL-6 and TNF-α in plasma and adipose tissue of all VR offspring, and this is in line with reports that circulating IL-6 stimulates the hypothalamic–pituitary–adrenal axis which is associated with central obesity, hypertension and IR [32], some of which have indeed been observed in the VR offspring. Although IL-6 increases lipolysis and NEFA release, plasma NEFA levels were however comparable among groups in this study. While IL-6 decreases adiponectin secretion and decreases insulin sensitivity [33], TNF-α promotes IR in skeletal muscle and liver and contributes to endothelial dysfunction. Indeed, chronic exposure to TNF-α may adversely affect pancreatic beta-cell function [34]. Obesity-associated increase in circulating TNF-α is primarily secreted by macrophages accumulated in adipose tissue [35,36] and is implicated in adipocyte IR [37,38].

In contrast to the increased expression of IL-1β and its receptor reported in visceral adipose tissue of obese subjects [39], we observed decreased IL-1β in both plasma and adipose tissue of $B_{12}R$ and DD rats. Although PAI is reported to be elevated in inflammatory/obese states and in metabolic syndrome, circulating as well as adipose tissue PAI concentrations were comparable among all groups in this study. Considering that MCP-1 initiates macrophage infiltration of the adipose tissue and induces systemic IR [40], the finding that MCP-1 levels were significantly increased in adipose tissue but not in circulation in all the VR offspring appears to suggest greater IR at least of the adipose tissue.

Adiponectin, an anti-inflammatory cytokine, is known to be antidiabetic and antiatherosclerotic. It is possible that adipogenic gene expression and adiponectin production which are activated during adipogenesis undergo feedback inhibition resulting in lower levels of adiponectin in the obese state [41]. Although circulating adiponectin levels were lower in only FR and $B_{12}R$ offspring, its concentration in adipose tissue was lower in DD and $B_{12}R$ offspring, suggesting a reduced anti-inflammatory status in the VR offspring. Overall, our findings on circulating and adipose tissue adipocytokine levels appear to indicate that the VR offspring were in a proinflammatory state that is known to be conducive to IR and associated diseases.

Considering that adipocytokines modulate lipid metabolism, we also investigated the effects of vitamin restriction on plasma lipid profile and activities of two key enzymes of lipid metabolism in liver. Since hypertriglyceridemia and hypercholesterolemia are associated with coronary artery disease and T2DM, significantly higher plasma TG and cholesterol levels in all VR offspring appear to suggest their predisposal to these diseases. Increased TG storage (visceral or deep subcutaneous depots) leads to large adipocytes that resist suppression of lipolysis by insulin (hence, low circulating NEFA levels) and aggravates IR in skeletal muscle and liver [42]. However, in the present study, plasma NEFA levels were comparable among all groups despite high visceral adiposity in VR offspring. Indeed, our finding that plasma TGs and total cholesterol were higher in all the VR offspring is in line with the reported association between IR and elevated total cholesterol [43].

Although plasma total cholesterol levels were higher in all VR offspring, HDL cholesterol levels were comparable, suggesting a probable increase in low-density lipoprotein and very low-density lipoprotein cholesterol, known risk factors for CVDs and T2DM. The fact that activities of the two rate-limiting enzymes of fatty acid synthesis — fatty acid synthase and acetyl-CoA-carboxylase — were

Table 6
Adipocytokine levels in adipose tissue of the offspring of different groups at 12 months of age

Adipocytokine	Control	FR	DD	$B_{12}R$
TNF-α (ng/ml)	30.3±2.54	65.2±6.25[c]	53.6±4.85[c]	57.7±4.37[c]
Adiponectin (μg/ml)	12.8±1.67	12.1±2.15	7.14±1.18[b]	6.72±0.193[c]
IL-6 (ng/ml)	2.77±0.129	5.39±0.664[c]	6.02±0.661[c]	6.44±0.414[c]
Leptin (ng/ml)	0.748±0.133	2.06±0.137[c]	0.674±0.147	1.20±0.195
IL-1β (ng/ml)	5.78±0.124	7.42±0.146	3.80±0.739[a]	1.46±0.105[c]
MCP-1 (ng/ml)	7.46±1.33	14.3±1.05[c]	10.6±0.858[c]	15.5±0.531[c]
PAI (ng/ml)	49.7±12.2	70.5±5.84	47.0±11.0	66.1±11.6

Adipocytokine concentrations in adipose tissue of male offspring at 12 months of age. Values given are mean±S.E. (n=6). Values in a row (comparison among groups) not sharing a common superscript are significantly different from control by one-way ANOVA/LSD tests: b=P<.01 and c=P<.001.

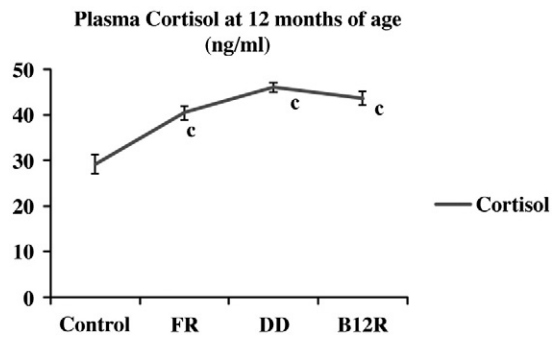

Fig. 3. Plasma cortisol concentrations in different groups of male offspring at 12 months of age. Values are mean±S.E. (n=6). Points with superscript c are significantly different from control by one-way ANOVA/LSD tests by P<.001.

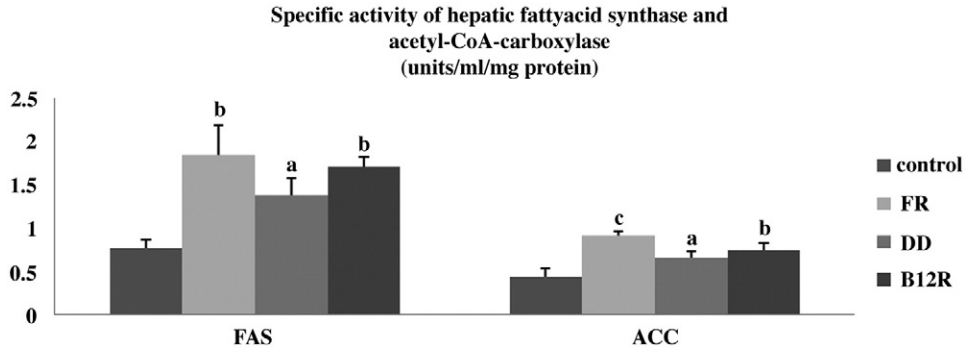

Fig. 2. Effect of maternal vitamin B_{12} and/ or folate restriction on the activity of fatty acid synthase and acetyl-CoA-carboxylase in the liver tissue in different groups of male offspring at 12 months of age. Values are mean±S.E. (n=6). Bars not sharing a common superscript are significantly different from control by one-way ANOVA/LSD tests: a=P<.05, b=P<.01, c=P<.001.

significantly higher in all the VR offspring probably suggests increased adipogenesis in the offspring of folate- and/or vitamin-B_{12}-restricted rat dams. These findings are in line with the reports that fatty acid synthase expression in adipose tissue is directly related to obesity (predominantly visceral fat accumulation) and impaired insulin sensitivity [44] and that pharmacological inhibition of fatty acid synthase blocks adipocyte differentiation and reduces adipocytes number [45]. Further, mice mutant for the lipogenic enzyme acetyl-CoA-carboxylase beta are protected against obesity and diabetes induced by high-energy diets [46]. The increased activity of the acetyl-CoA-carboxylase observed in the VR offspring that had higher body fat % and visceral adiposity indeed appears to be in line with these reports. Considering that IR is hypothesized to originate in increased adipogenesis and/or lipid metabolism [26,28,29], the increased adipogenesis observed here, taken together with high levels of TGs and total cholesterol also observed in the VR offspring, may suggest their predisposal to IR and associated diseases.

Increased corticosteroid stress is one of the common pathways suggested to underlie the maternal-undernutrition-induced programming of the offspring for adult diseases [47]. Higher plasma cortisol levels in all the VR offspring perhaps suggest that it could be one of the factors/mechanism(s) responsible for the increased visceral adiposity in the offspring. These findings agree with the reports that elevated plasma cortisol could be a link between low birth weight and IR syndrome [48]. Considering that high levels of cortisol decrease glucose metabolism which increases blood glucose levels and increases fat mobilization and metabolism which contribute to IR, the VR offspring appear to be predisposed to both hyperglycemia and IR.

To conclude, our study demonstrates, for the first time to the best of our knowledge, that maternal folate and/or vitamin B_{12} status plays an important role in regulating body composition (fat content and distribution), lipid metabolism and insulin sensitivity in the offspring and that their restriction *in utero* may predispose them to the increased risk for obesity, dyslipidemia and IR in later life. Our data also provide preliminary evidence that these effects may be mediated by altering the adipocyte function (balance between pro-and anti-inflammatory adipocytokines) and/or through increased corticosteroid stress. It will be interesting to understand the molecular mechanism(s) underlying/associated with the above-mentioned phenotypic changes in these offspring in addition to deciphering whether the offspring born to B_{12}- and/or folate-restricted dams have altered glucose metabolism and/or IR.

5. Duality of interest

None of the authors have any conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jnutbio.2012.01.004.

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