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Long-term Consequences of Methyl Donor Deficiency during in Utero and Early Life Development on Markers of the Metabolic Syndrome

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Abstract

The aim of the present study was to assess the consequences of maternal vitamin B deficiency in mice during early development on the offspring's biochemical and biometric parameters. Female mice received vitamin deficient or standard control diets for one month prior to pregnancy, during pregnancy and lactation. Their offspring were then divided into three groups: Control "CT" (offspring of control, breastfed by control mothers), deficient pregnancy "DP" (offspring of deficient mothers, breastfed by control mothers) and deficient pregnancy and lactation "DPL" (offspring of deficient mothers, breastfed by deficient mothers). The body, perigonadal white adipose tissue (PWAT), pericardial white adipose tissue (PCWAT), liver, kidney, spleen, heart and brain were weighed, and blood glucose, total cholesterol and triglycerides evaluated in female and male offspring at postnatal day (PND) 0, 28, 90 and 210. The results indicated that the dams exposure to vitamin B2, B9, B12 and choline deficiencies contributed to increased events related to cannibalization and/or dead pups observed at PND 1 ($p<0.049$) and decreased the body weight at birth of female ($p<0.001$) and male ($p<0.02$) offspring. The body weight of both DPL female ($p<0.006$) and male ($p<0.01$) offspring remained lower at PND 28. At PND 210 no statistically significant differences were observed in female ($p>0.8$) and male ($p>0.09$) offspring body weight, however a decrease in PWAT of DPL males was observed. The early exposure to methyl donor deficiency was not associated with increased body weight or changes in glucose, triglycerides and cholesterol metabolism, typical markers of the metabolic syndrome.

Keywords: Maternal programming; Vitamin B deficiency; Organs weight; Body weight; Mice

Introduction

A series of pregnancy complications such as recurrent early pregnancy loss, placental abruption, preeclampsia, premature birth, intrauterine growth retardation, neural tube defects and fetal death have been associated with perturbations in one-carbon metabolism [1-5]. Low birth weight has been associated with increased rates of coronary heart disease and related disorders like stroke, hypertension and non-insulin-dependent diabetes later in life, according to epidemiological studies [6,7]. Differences in maternal diet may induce different long-term effects, and methionine content and sulfur amino acid balance in the maternal diet have been identified as one of the most important programming factors [8-10]. The methionine cycle successively involves the synthesis of S-adenosylmethionine (SAM) and transmethylation reactions of a large number of substrates that use SAM as a methyl donor. After transmethylation reactions, SAM is converted into S-adenosylhomocysteine and then hydrolysed to adenosine and homocysteine (Hcy). Hcy may then be remethylated to methionine by the ubiquitously distributed methionine synthase, a cobalamin dependent enzyme; or in the liver and kidney of some species by betaine-homocysteine methyltransferase (BHMT) that uses betaine, produced during choline oxidation as well as being provided by the diet [11]. Additionally, Hcy can be catabolized by its transsulfuration, through a series of reactions that end with the production of cysteine, which may be further used in glutathione (GSH) production [12].

Sulfur amino acids are involved in both methylation processes and lipid metabolism regulation (Oda), with SAM as the key intermediate. Studies have revealed a relation between folate-Hcy pathways and lipoprotein profile, liver steatosis, adiposity and fat distribution [13-15] and a study published by Stewart et al. [16] showed that folate supplementation during pregnancy reduced metabolic syndrome development in children at 6-8 years of age in a generally undernourished population. Furthermore, the increased amount of folate in protein-restricted mice during pregnancy tended to decrease the concentrations of non-esterified fatty acids, β-hydroxybutyrate and glucose [17].

In a previous study by our group, Swiss mice subjected to a vitamin B deficient diet during pregnancy and lactation presented offspring with elevated plasma Hcy concentrations and a deficit in brain antioxidant capacity at the end of the lactation period (PND 28). In addition, we observed decreased concentrations of plasma folate, plasma GSH and brain cortex concentrations of SAM in male offspring at PND 210 [18]. The hypothalamus is the main center of energy balance regulation, and in rodents, the differentiation of neural systems responsible for this control begins the last week of gestation and lasts until weaning. Based on these findings, the aim of the present study was to assess the consequences of methyl donor deficiency during early development on body composition and metabolic markers in adult mice offspring.

Materials and Methods

Animal treatment protocols

Swiss mice were used for the experiments, in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academy Press, Washington D. C., 2011), approved by the Institutional Animal Care and Use Committee of the Universidade Federal de São Paulo (#1169/08). On a 12 h light/dark cycle, adult female mice at 3 months of age (n=90) had access to food and water *ad libitum*, under standard laboratory conditions. Two female mice groups received different diets one month prior to mating, a control group (n=46) was fed with a standard diet (AIN-93M, LabDiet®, St. Louis, MO), while the experimental group (n=44) had a deficient diet (LabDiet®, St. Louis, MO). Standard diet contained the following levels of vitamins: B2 (6.2 mg/kg), B9 (2.2 mg/kg), B12 (25 µg/kg), and choline (1.250 mg/kg); while the deficient diet contained: B2 (0.938 mg/kg), B9 (0.290 mg/kg), B12 (2.37 µg/kg), and choline (0.1736 mg/kg). The latter diet increases the plasma Hcy concentration in dams and leads to a significant methyl-group deficit in the brain fetus, caused by vitamin B and choline deficiency [8]. For mating, a male mouse was introduced in female home cages with two or three females and the detection of vaginal plug was established as day zero of gestation [19]. All dams were assessed during the experiment for fertility and litter size. Coprophagy was not prevented during the experiment.

Female mice received vitamin deficient or standard control diets for one month prior to pregnancy, during pregnancy and lactation. Their offspring were then divided into three groups: Control "CT" (offspring of control, breastfed by control mothers), deficient pregnancy "DP" (offspring of deficient mothers, breastfed by control mothers) and deficient pregnancy and lactation "DPL" (offspring of deficient mothers, breastfed by deficient mothers). Dams of CT and DPL offspring were respectively supplied with standard and deficient diet during pregnancy and lactation. Dams of DP offspring were provided with deficient diet during pregnancy, and after birth the offspring were cross-fostered by control dams during lactation, fed with standard diet. In order to minimize the fostering effect, pups of CT and DPL groups were also cross-fostered by CT and DPL dams, respectively. The cross fostering of litters occurred on PND 0 and the litter size was adjusted to n=8 animals per dam (n=4 females; n=4 males) in all groups. All offspring groups were breastfed until PND 28, and received a standard diet after weaning. They were analyzed between thirteen and twenty-one litters per group; a schematic of overall study design is presented in Figure 1.

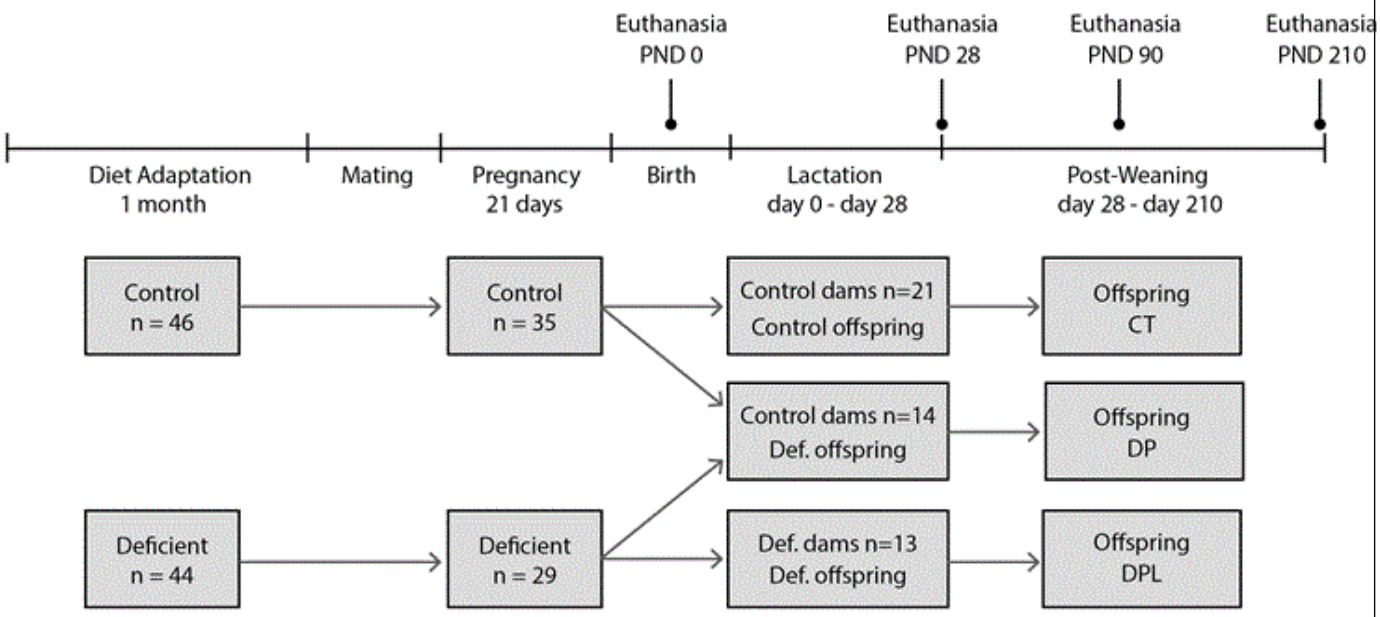

Figure 1: Overall study design [PND: Postnatal day; Def: Deficient; CT: Control; DP: Deficient diet during pregnancy; DPL: Deficient during pregnancy and lactation. Mating=4 days. Litter adjustment to n=8 animals per dam during lactation period].

Sample collection

Euthanasia of male and female mice at different developmental stages (PND 0, 28, 90 and 210) was performed by decapitation. These time points were chosen to allow the consequences of vitamin B2, B9, B12 and choline deficiency during pregnancy and pregnancy/lactation to be observed in the short-term (PND 0 and 28), as well as the appearance or maintenance of alterations following weaning, when standard diet was introduced, that could possibly be noticed in the medium- and long-term (PND 90 and 210, respectively). Males and females were analyzed separately because gender differences in several parameters have been widely described in the literature, including after manipulation during pregnancy and postnatal period.

Following euthanasia procedures, total blood from all offspring groups was collected in heparin containing tubes (Fisher Scientific, USA) to evaluate glucose, total cholesterol and triglycerides levels through photometric measurement of reflection by Accutrend Plus (Roche).

Biometric evaluation

All mice were weighed prior to euthanasia and, afterwards, perigonadal white adipose tissue (PWAT), pericardial white adipose tissue (PCWAT), liver, kidney, spleen, heart and brain weight were determined using an analytical balance (Bioprecisa, Model-FA2104N, Curitiba, Paraná, Brazil), accurate to 0.001 g. The classic dissection of PWAT described by Johnson and Hirsch in 1972 was followed in this experiment. Pericardial fat has been defined as the combination of paracardial and epicardial fat [20], and the PCWAT dissection was performed accordingly. Relative tissue weights were calculated by dividing organ weight by the whole animal weight.

Statistical analyses

All variables were checked for normality (Shapiro-Wilk's test) and/or homogeneity (Levene's test) and, when necessary, normalized using the Z-score. Analysis of offspring PND 0 was performed using t-test for independent groups and the events related to cannibalized or dead animals at PND 1 using Fisher's exact test. Additionally, data from offspring at PND 28, 90 and 210 were compared using the one-way analysis of variance (ANOVA), followed by Fisher post hoc, when necessary. Data is presented as mean ± standard error, and the significance level was set at $p \leq 0.05$. Analyses were performed using STATISTICA 8.0 software.

Results

Pregnancy and early life

The level of vitamin B deficiency in the deficient diet was not associated with changes in female fertility and litter size. Female fertility evaluation was based on the number of successful pregnancies resulting from four nights of mating, being 78.3% for the control group and 65.9% for the deficient group ($p>0.125$). The average litter size was 8.9 pups per dams for the control group and 8.3 for the deficient group ($p>0.240$). Birth weight was significantly reduced in both female ($p<0.001$) and male ($p<0.02$) offspring born to methyl donor deficient mothers (Figures 2A and 2B respectively). We also considered events related to cannibalization and/or death of one pup or whole offspring, as shown in Figure 3. There was an increase of these events in the DPL (38.5%) and DP (14.3%) groups when compared to the CT (4.8%) groups ($p<0.036$). However, dead animals were observed only in the DPL group and represented 80% of the events; CT and DP groups presented only cannibalized animals.

Weight gain

Results concerning body weight gain in females and males during development, from PND 28 to PND 210, are shown in Figures 4A and 4B, respectively. At the end of the breastfeeding period (PND 28), we found a statistically significant increase of body weight in DPL group in both female ($p<0.006$) and male ($p<0.01$), but this increase was smaller than in the CT and DP groups. A difference between the experimental groups DP and DPL was observed in PND 90 female ($p<0.01$) and no differences were found in the body weight of PND 90 male ($p>0.07$) and in both genders at PND 210 (female, $p>0.8$; male, $p>0.09$).

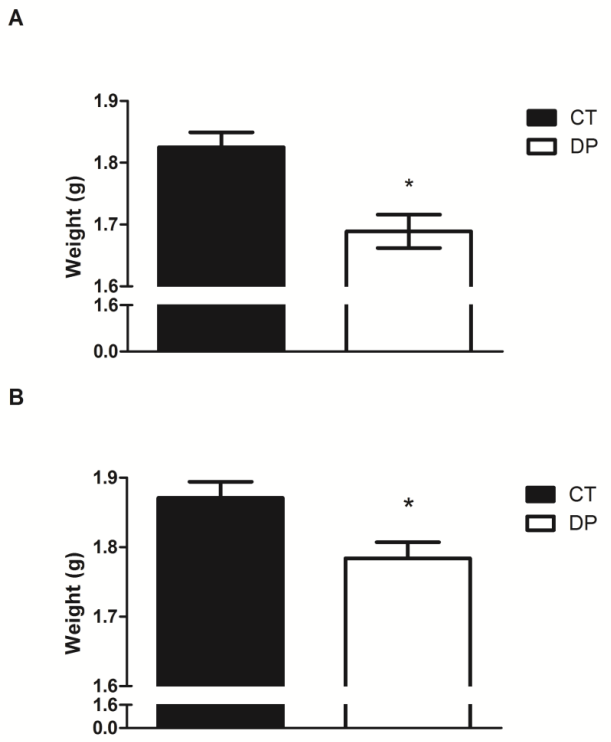

Figure 2: Body weight after birth in female and male offspring [(A) Body weight after birth in female, CT, n=146, DP, n=119. (B) Body weight after birth in male, CT, n=168, DP, n=122. Values are presented as the mean ± S.E.M. *Difference between CT and DP groups, $P \leq 0.05$; t test].

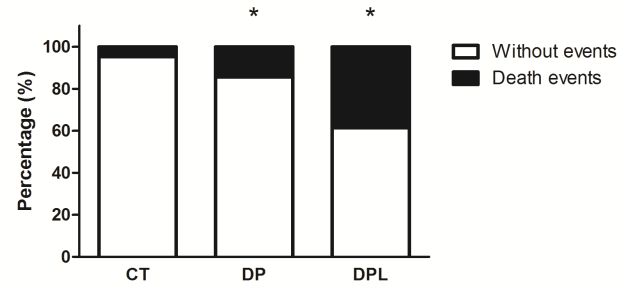

Figure 3: Percentage of events related to cannibalized and/or dead animals on dams litter at PND 1 [CT=Control, n=21; DP=Deficient diet during pregnancy, n=14; DPL=Deficient during pregnancy and lactation, n=13. *Different from the CT group. Fisher's exact test, $P \leq 0.05$].

Organs

Data related to organ weight from female and male mice at PND 28 are presented in Table 1. In females, the DPL group showed significantly less perigonadal white adipose tissue ($p<0.003$) than the

CT and DP groups. A similar result was observed when the organ weight was expressed as a percentage of total body weight ($p<0.001$). A significant decrease in organ weight was also observed in the spleen ($p<0.006$) and brain ($p<0.004$) of DPL female mice when compared to CT and DP groups, although their expression as a percentage of total body weight revealed no differences ($p>0.09$ and $p>0.2$, respectively). By contrast, the liver ($p>0.6$) and heart ($p>0.8$) showed no statistically significant differences in total weight, but were increased in the DPL group when expressed as a percentage of the total body weight ($p<0.001$ and $p<0.001$, respectively). No statistical differences were found in the total or relative weight of pericardial white adipose tissue and kidney between female groups. As for males, there was no difference in perigonadal white adipose tissue weight. Likewise, no differences were found in pericardial white adipose tissue, liver, kidney and heart weights. However, increased relative weight was observed in the liver ($p<0.007$) and heart ($p<0.002$) of DPL males when compared to the CT and DP groups. Additionally, increased relative weight of pericardial white adipose tissue was observed in DPL only in comparison to the CT group ($p<0.02$) and in the kidney of DPL when compared to DP group weights ($p<0.01$). Conversely, a decrease in spleen ($p<0.006$) and brain ($p<0.03$) weights were observed in the DPL group when compared to the CT and DP groups, although such differences disappeared after being analyzed as a percentage of total body weight (spleen, $p>0.1$ and brain, $p>0.07$).

In female mice, some of the changes observed in DPL PND 28 were found at PND 90, such as decreased brain weight ($p<0.047$) and increased liver ($p<0.03$) and heart ($p<0.02$) weight percentage in relation to total body weight. No other statistical differences in organ weight ($p>0.2$) and relative organs weight ($p>0.1$) were observed in perigonadal white adipose tissue, pericardial white adipose tissue, kidney and spleen (Table 2). Data related to male PND 90 are also shown in Table 2. An increase in relative liver weight ($p<0.02$) is observed in DPL only when compared to the DP group. No differences were observed in spleen ($p>0.1$) and heart ($p>0.2$) weight; however, an increase in relative organ weight was observed in the spleen ($p<0.01$) of DPL when compared to the CT group and in the heart ($p<0.04$) of DPL when compared to the CT and DP groups. Furthermore, DPL kidney weight ($p<0.02$) was increased when compared solely with the DP group, whereas increased relative kidney weight ($p<0.05$) was found in DPL when compared to the CT and DP groups. No statistical differences were observed in perigonadal white adipose tissue ($p>0.06$), pericardial white adipose tissue ($p>0.1$) and the brain ($p>0.5$).

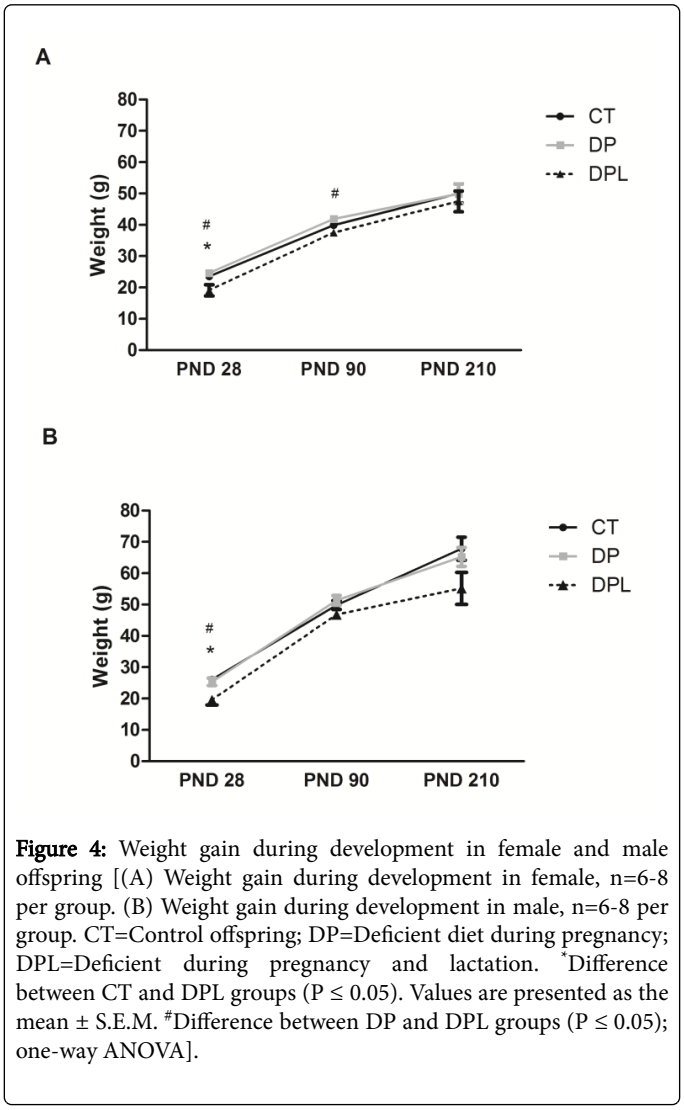

Figure 4: Weight gain during development in female and male offspring [(A) Weight gain during development in female, n=6-8 per group. (B) Weight gain during development in male, n=6-8 per group. CT=Control offspring; DP=Deficient diet during pregnancy; DPL=Deficient during pregnancy and lactation. *Difference between CT and DPL groups ($P \leq 0.05$). Values are presented as the mean ± S.E.M. #Difference between DP and DPL groups ($P \leq 0.05$); one-way ANOVA].

PND 28	Female				Male			
	CT (n=8)	DP (n=7)	DPL (n=7)	p	CT (n=6)	DP (n=8)	DPL (n=8)	p
PWAT (g)	0.33 ± 0.03	0.36 ± 0.04#	0.14 ± 0.03*	<0.003	0.51 ± 0.03	0.42 ± 0.05	0.32 ± 0.06	0.070
PWAT (%)	1.40 ± 0.14	1.14 ± 0.14#	0.58 ± 0.14*	<0.001	1.96 ± 0.07	1.62 ± 0.14	1.65 ± 0.31	0.466
PCWAT(g)	0.12 ± 0.00	0.14 ± 0.01	0.10 ± 0.01	0.059	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.456
PCWAT (%)	0.53 ± 0.02	0.56 ± 0.02	0.55 ± 0.05	0.762	0.45 ± 0.02	0.50 ± 0.02	0.56 ± 0.03*	<0.02
Liver (g)	1.15 ± 0.04	1.14 ± 0.05	1.22 ± 0.09	0.612	1.24 ± 0.08	1.25 ± 0.09	1.20 ± 0.11	0.914
Liver (%)	4.88 ± 0.11	4.65 ± 0.17	6.63 ± 0.48*	<0.001	4.76 ± 0.16	4.90 ± 0.17#	6.00 ± 0.40*	<0.007
Kidney (g)	0.14 ± 0.01	0.14 ± 0.00	0.13 ± 0.01	0.443	0.16 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.127
Kidney (%)	0.59 ± 0.03	0.58 ± 0.02	0.67 ± 0.02	0.055	0.61 ± 0.02	0.58 ± 0.02#	0.65 ± 0.01	<0.01
Spleen (g)	0.17 ± 0.01	0.15 ± 0.01#	0.13± 0.01*	0.006	0.20 ± 0.02	0.19 ± 0.01#	0.13 ± 0.02*	<0.006
Spleen (%)	0.75 ± 0.05	0.61 ± 0.03	0.64 ± 0.05	0.093	0.80 ± 0.11	0.76 ± 0.02	0.60 ± 0.07	0.112

Heart (g) Heart (%)	0.11 ± 0.00 0.48 ± 0.01	0.12 ± 0.00 0.48 ± 0.02#	0.12 ± 0.01 0.62 ± 0.03*	0.882 <0.001	0.12 ± 0.01 0.47 ± 0.01	0.12 ± 0.01 0.48 ± 0.01#	0.13 ± 0.02 0.62 ± 0.04*	0.976 <0.002
Brain (g) Brain (%)	0.45 ± 0.01 1.95 ± 0.04	0.44 ± 0.01# 1.82 ± 0.03	0.40 ± 0.01* 2.24 ± 0.30	<0.004 0.234	0.47 ± 0.01 1.80 ± 0.08	0.48 ± 0.01# 1.81 ± 0.06	0.42 ± 0.01* 2.32 ± 0.30	<0.03 0.071

PND: Postnatal day; CT: Control offspring; DP: Deficient diet during pregnancy; DPL: Deficient during pregnancy and lactation; PWAT: Perigonadal white adipose tissue; PCWAT: Pericardial white adipose tissue Values are presented as the mean ± S.E.M

*Different from the CT group (P ≤ 0.05)

#Difference between DP and DPL groups (P ≤ 0.05); One-way ANOVA

Table 1: Organ weights (g) and relative organs weights (%) in female and male offspring at PND 28 from dams on vitamin B deficient diet during pregnancy and pregnancy/lactation.

PND 90	Female				Male			
	CT (n=8)	DP (n=8)	DPL (n=8)	p	CT (n=8)	DP (n=8)	DPL (n=7)	p
PWAT (g)	1.65 ± 0.18	1.78 ± 0.19	1.31 ± 0.24	0.284	1.84 ± 0.32	1.80 ± 0.22	1.04 ± 0.19	0.067
PWAT (%)	4.07 ± 0.38	4.20 ± 0.40	3.40 ± 0.58	0.431	3.60 ± 0.55	3.45 ± 0.35	2.25 ± 0.42	0.09
PCWAT(g)	0.14 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.763	0.13 ± 0.01	0.16 ± 0.02	0.11 ± 0.01	0.105
PCWAT (%)	0.36 ± 0.02	0.32 ± 0.03	0.35 ± 0.02	0.537	0.26 ± 0.03	0.31 ± 0.03	0.24 ± 0.03	0.288
Liver (g)	1.62 ± 0.06	1.54 ± 0.06	1.70 ± 0.09	0.267	2.09 ± 0.03	1.92 ± 0.07	2.06 ± 0.09	0.164
Liver (%)	4.05 ± 0.08	3.66 ± 0.11*#	4.53 ± 0.15*	<0.03	4.22 ± 0.13	3.76 ± 0.17#	4.42 ± 0.21	<0.02
Kidney (g)	0.18 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.208	0.27 ± 0.01	0.24 ± 0.02#	0.31 ± 0.02	<0.02
Kidney (%)	0.45 ± 0.02	0.49 ± 0.02	0.54 ± 0.04	0.116	0.55 ± 0.03	0.46 ± 0.05#	0.67 ± 0.03*	<0.05
Spleen (g)	0.16 ± 0.02	0.15± 0.01	0.18 ± 0.02	0.617	0.14 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.143
Spleen (%)	0.40 ± 0.03	0.37 ± 0.03	0.48 ± 0.05	0.169	0.28 ± 0.03	0.33 ± 0.02	0.37 ± 0.03*	<0.01
Heart (g)	0.16 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.299	0.20 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.202
Heart (%)	0.40 ± 0.01	0.40 ± 0.02#	0.47 ± 0.17*	<0.02	0.40 ± 0.01	0.37 ± 0.02#	0.45 ± 0.02*	<0.04
Brain (g)	0.50 ± 0.05	0.47 ± 0.07*#	0.45 ± 0.09*	<0.047	0.47 ± 0.10	0.47 ± 0.08	0.45 ± 0.01	0.526
Brain (%)	1.27 ± 0.03	1.13 ± 0.03	1.21 ± 0.05	0.107	0.95 ± 0.03	0.92 ± 0.03	0.97 ± 0.02	0.532

PND: Postnatal day; CT: Control offspring; DP: Deficient diet during pregnancy; DPL: Deficient during pregnancy and lactation; PWAT: Perigonadal white adipose tissue; PCWAT: Pericardial white adipose tissue

Values are presented as the mean ± S.E.M

*Different from the CT group (P ≤ 0.05)

#Difference between DP and DPL groups (P ≤ 0.05); One-way ANOVA

Table 2: Organ weights (g) and relative organs weights (%) in female and male offspring at PND 90 from dams on vitamin B deficient diet during pregnancy and pregnancy/lactation.

At PND 210, no statistical differences in organ weight ($p>0.07$) or relative organ weight ($p>0.1$) were observed in females (Table 3). In male mice, a decrease in PWAT ($p<0.003$) was observed in the DPL group when compared to the CT and DP groups, similarly to the total body weight percentage result ($p<0.03$). Additionally, an increase in DPL relative weight of kidney ($p<0.001$), spleen ($p<0.007$) and heart ($p<0.02$) was found when compared to the CT and DP groups. No statistical differences were observed in PCWAT ($p>0.05$) and liver ($p>0.2$) (Table 3).

Biochemical parameters

Results concerning total blood measurements in female and male mice at PND 28, 90 and 210 are summarized in Table 4. At PND 28, we found increased triglyceride concentrations ($p<0.002$) in DPL females when compared to the CT and DP groups and no statistical differences in glucose ($p>0.6$) and cholesterol ($p>0.4$). As for males, we observed higher glucose concentrations ($p<0.04$) in DP mice compared to the CT and DPL groups, without statistical differences in triglycerides ($p>0.1$) and cholesterol ($p>0.1$). We did not find differences in glucose

(female, p>0.3; male, p>0.4), triglycerides (female, p>0.7; male, p>0.5) and cholesterol (female, p>0.08; male, p>0.2) at PND 90. At PND 210, female mice likewise did not show any differences in glucose (p>0.7), triglycerides (p>0.3) and cholesterol (p>0.9), although DPL males showed lower cholesterol concentrations (p<0.004) than the CT and DP groups. No statistical differences were observed in glucose (p>0.06) and triglycerides (p>0.3) in male PND 210.

	Female				Male			
PND 210	**CT (n=8)**	**DP (n=7)**	**DPL (n=8)**	**p**	**CT (n=8)**	**DP (n=8)**	**DPL (n=6)**	**p**
PWAT (g)	3.58± 0.59	2.65 ± 0.74	3.94 ± 0.79	0.568	3.28 ± 0.25	2.57 ± 0.29	1.56 ± 0.49*	<0.003
PWAT (%)	6.85 ± 0.77	3.78 ± 0.40	6.90 ± 1.38	0.356	4.80 ± 0.15	3.90 ± 0.33#	2.62 ± 0.63*	<0.03
PCWAT(g)	0.14 ± 0.02	0.17 ± 0.02	0.14 ± 0.02	0.351	0.23 ± 0.03	0.20 ± 0.02	0.14 ± 0.06	0.269
PCWAT (%)	0.27 ± 0.02	0.35 ± 0.04	0.29 ± 0.03	0.251	0.33 ± 0.03	0.30 ± 0.03	0.23 ± 0.07	0.264
Liver (g)	1.62 ± 0.01	1.83 ± 0.13	1.54 ± 0.09	0.167	2.54 ± 0.22	2.63 ± 0.17	2.47 ± 0.17	0.843
Liver (%)	3.25 ± 0.09	3.78 ± 0.40	3.29 ± 0.21	0.293	3.72 ± 0.15	4.04 ± 0.16	4.60 ± 0.40	0.054
Kidney (g)	0.20 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.708	0.32 ± 0.02	0.31 ± 0.01	0.37 ± 0.03	0.138
Kidney (%)	0.42 ± 0.02	0.44 ± 0.03	0.46 ± 0.06	0.686	0.48 ± 0.02	0.47± 0.01#	0.68 ± 0.05*	<0.001
Spleen (g)	0.13 ± 0.01	0.26 ± 0.08	0.12 ± 0.01	0.075	0.15 ± 0.01	0.15 ± 0.01	0.20 ± 0.03	0.131
Spleen (%)	0.27 ± 0.03	0.55 ± 0.18	0.25 ± 0.02	0.124	0.22 ± 0.01	0.23 ± 0.01#	0.38 ± 0.07*	<0.007
Heart (g)	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.477	0.22 ± 0.01	0.21 ± 0.01	0.23 ± 0.02	0.611
Heart (%)	0.32 ± 0.01	0.35 ± 0.03	0.35 ± 0.04	0.637	0.33 ± 0.01	0.32 ± 0.02#	0.43 ± 0.05*	<0.02
Brain (g)	0.51 ± 0.03	0.48 ± 0.01	0.48 ± 0.09	0.092	0.47 ± 0.10	0.48 ± 0.07	0.46 ± 0.09	0.161
Brain (%)	1.04 ± 0.07	1.01 ± 0.08	1.04 ± 0.10	0.951	0.71 ± 0.04	0.78 ± 0.03	0.86 ± 0.07	0.136

PND: Postnatal day; CT: Control offspring; DP: Deficient diet during pregnancy; DPL: Deficient during pregnancy and lactation; PWAT: Perigonadal white adipose tissue; PCWAT: Pericardial white adipose tissue

Values are presented as the mean ± S.E.M

*Different from the CT group (P ≤ 0.05)

#Difference between DP and DPL groups (P ≤ 0.05); One-way ANOVA

Table 3: Organ weights (g) and relative organs weights (%) in female and male offspring at PND 210 from dams on vitamin B deficient diet during pregnancy and pregnancy/lactation

Discussion

Metabolic disease is linked to genetic and environmental factors and the perinatal/postnatal period has been considered a critical window for the effect of stressors, such as under nutrition or over nutrition [21,22]. Evidence that impaired fetal growth, increased lipogenesis, obesity and insulin resistance in children is related to deficiencies of maternal B vitamins raises questions about the risk of metabolic adult syndrome being related to early deprivation of B9 and B12 vitamins [23-27]. To test this hypothesis, the offspring from methyl donor deficient dams were followed during development and parameters such as body and organ weights and biochemical measurements were analyzed.

Studies in rodents and humans have reported that low maternal folate status during pregnancy leads to lower birth weight [28,29]. In the current study, both female and male offspring from deficient dams presented a statistically significant decrease in birth body weight. An increase in the incidence of cannibalism and/or death events at PND 1 was observed in DP and DPL groups; It is probable that maternal deficiency has been the predominant factor in this cannibalistic behavior because of their inability to produce milk, also, the damage caused by early offspring deficiency may be involved, since some animals were found dead, but not cannibalized. Data published by McKay et al. [29] showed 64% excess of miscarriage or postpartum litter death for low folate fed dams, suggesting that inadequate folate supply decreased capacity to carry pregnancy to term or rear pups effectively. Additionally, we did not observe significant difference between the groups in terms of fertility or litter size, corroborating the findings in the study by McKay et al. [29]. However, other diets with even lower levels of vitamin B2, B9, B12 and choline were previously tested by our group but not used because they led to absence of pregnancy or low fertility (data not shown). At PND 28, the DPL female (breastfed by deficient diet dams) presented low body weight associated with low perigonadal white adipose tissue and triglycerides. A study published by Gupta and Powers [30] showed an association between vitamin B12 deficiency and severe weight loss in the elderly. Regarding the role of vitamin B in the metabolism of fats, carbohydrates and proteins [31,32], the low body weight observed in DPL females (PND 28) could be directly related to the deficient diet. Similarly to females at PND 28, DPL male mice presented low body

weight, although perigonadal white adipose tissue weight was not altered. Considering the difficulty to compare organs weight when total body weight is different, we used the organ/body weight ratio as an auxiliary parameter. We observed that low body weight was not related to changes in pericardial white adipose tissue, liver, kidney and heart weights in the DPL group from PND 28 females and males. Conversely, perigonadal white adipose tissue in females, and spleen and brain weight in both genders of the DPL group were negatively affected. On the other hand, the relative weights of female' and male' hearts, and male' livers, hearts and white adipose tissue were increased.

The long-term consequences of the deficient diet during pregnancy and pregnancy/lactation were analyzed at PND 90 and 210. In females at PND 90, body weight differences were observed in DP only when compared to DPL; No significant difference was observed in female body weight at PND 210. The decreased brain weight also observed in these animals suggests that early exposure to a low vitamin B2, B9, B12 and choline diet could impair brain development; however, at PND 210 this difference is no longer observed. In males, the body weight evaluation at PND 90 and PND 210 showed no statistically significant changes. However, the decrease in body weight observed in DPL males during development may have a biological significance, since perigonadal white adipose tissue weight is reduced and statistically significantly different at PND 210. Additionally, these animals showed low cholesterol concentrations in total blood, although in this case the result seems to have no biological significance, since the decrease is less than 3% compared to controls. Regarding organ weight, increased kidney, spleen and heart relative weights were found in DPL males in both PND 90 and 210.

		Female				Male			
		n	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	n	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)
DPN 28	CT	(n=8)	188.7 ± 7.0	270.9 ± 13.1	155.8 ± 1.9	(n=6)	183.7 ± 11.5	264.3 ± 40.3	158.6 ± 1.8
	DP	(n=7)	179.6 ± 6.8	269.3 ± 23.6#	156.0 ± 1.6	(n=8)	219.4 ± 9.1#	293.1 ± 27.3	154.6 ± 0.9
	DPL	(n=7)	188.3 ± 10.4	174.1 ± 16.7*	159.0 ± 2.1	(n=8)	176.5 ± 11.5	209.5 ± 14.1	157.0 ± 1.4
	P		0.679	<0.002	0.437		>0.04	0.118	0.168
DPN 90	CT	(n=8)	151.5 ± 9.3	251.3 ± 30.0	165.0 ± 0.8	(n=8)	173.3 ± 11.7	378.1± 30.8	163.1 ± 1.0
	DP	(n=8)	153.6 ± 7.7	230.8 ± 22.2	164.1 ± 1.6	(n=8)	167.1 ± 7.5	323.3 ± 35.3	164.3 ± 1.0
	DPL	(n=8)	135.4 ± 12.8	226.9 ± 13.0	161.1 ± 1.1	(n=7)	155.9 ± 8.5	360.4 ± 41.6	165.7 ± 0.8
	P		0.397	0.722	0.083		0.451	0.537	0.2
DPN 210	CT	(n=8)	164.0 ± 7.2	273.5 ± 56.4	167.1 ± 1.4	(n=8)	207.9 ± 24.4	339.1 ± 28.2	168.6 ± 0.8
	DP	(n=7)	170.4 ± 9.4	202.1 ± 30.3	167.1 ± 1.4	(n=8)	181.9 ± 10.9	357.3 ± 21.1	168.4 ± 0.7#
	DPL	(n=8)	160.0 ± 9.7	287.6 ± 38.8	166.6 ± 1.1	(n=6)	139.5 ± 15.6	304.3 ± 24.4	164.3 ± 1.1*
	P		0.71	0.378	0.95		0.061	0.367	<0.004

PND: Postnatal day; CT: Control offspring; DP: Deficient diet during pregnancy; DPL: Deficient during pregnancy and lactation

Values are presented as the mean ± S.E.M

*Different from the CT group (P ≤ 0.05)

#Difference between DP and DPL groups (P ≤ 0.05); One-way ANOVA

Table 4: Blood glucose, triglycerides and cholesterol in offspring from dams on vitamin B deficient diet during pregnancy and pregnancy/lactation.

In a similar study, reduced maternal folate intake during pregnancy and lactation did not affect the body weight of adult offspring [29]. However, these animals presented increased triacylglycerol when exposed to a high-fat diet in a post-weaning period [29]. Additionally, since the third month, offspring from vitamin B12 and folate restricted rats presented higher body fat (especially visceral fat) and were dyslipidemic at 12 months [33]. Interestingly, reduced plasma triacylglycerol concentration was observed in rat offspring from dams submitted to a low-protein diet supplemented with folate during pregnancy [17,34]. In humans, the Indian population is a good model for long-term analyses of vitamin B12 and protein deficiencies. These deficiencies are consequences of personal beliefs, religion, cultural practices and poverty [35,36]. The observation that newborn Indian babies, who are on average 700 g lighter than European babies, and additionally have increased subcutaneous adiposity [37], intra-abdominal fat [24]and higher concentrations of insulin and leptin in cord blood [27], may be associated to an increased prevalence of metabolic syndrome in this population [38,39]. The review by Rush and collaborators [40] draws attention to the fact that understanding the factors which regulate maternal-fetal one-carbon metabolism and its role in fetal programing of non-communicable diseases, could help design effective interventions, starting with maternal nutrition before conception.

Based on the results presented, offspring from methyl donor deficient dams did not present fat accumulation or exacerbated weight gain during development. On the other hand, the initial imbalance in the brain one carbon metabolism presented by this experimental model [18] may be associated to the low body weight and the lower PWAT observed in DPL males. Although these parameters are not associated with metabolic syndrome, we cannot exclude the possibility of these biometric characteristics are associated with changes in the methylation pattern of hypothalamic genes involved in the control of energy metabolism.

Even though we did not observe the involvement of vitamin B2, B9, B12 and choline deficiency in increased body weight or changes in glucose, triglycerides and cholesterol metabolism, typical markers of the metabolic syndrome, it is important emphasize that all groups received standard diet after weaning in the current study. In this sense, the metabolic syndrome markers could be more evident when associated to high carbohydrate intake, a common practice in modern society.

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Botto LD, Lisi A, Robert-Gnansia E, Erickson JD, Vollset SE, et al. (2005) International retrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working? BMJ 330: 571.
2. Goddijn-Wessel TA, Wouters MG, Van de Molen EF, Spuijbroek MD, Steegers-Theunissen RP, et al. (1996) Hyperhomocysteinemia: a risk factor for placental abruption or infarction. Eur J Obstet Gynecol Reprod Bio 66: 23-29.
3. Mislanova C, Martsenyuk O, Huppertz B, Obolenskaya M (2011) Placental markers of folate-related metabolism in preeclampsia. Reproduction 142: 467-476.
4. Nelen WL, Blom HJ, Steegers EA, Den Heijer M, Eskes TK (2000) Hyperhomocysteinemia and recurrent early pregnancy loss: a meta-analysis. Fertil Steril 74: 1196-1199.
5. Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, et al. (2000) Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine study. Am J Clin Nutr 71: 962-968.
6. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME (1989) Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ 298: 564-567.
7. Godfrey KM, Barker DJ (2000) Fetal nutrition and adult disease. Am J Clin Nutr 71: 1344S-1352S.
8. Langley-Evans SC (2000) Critical differences between two low protein diet protocols in the programming of hypertension in the rat. Int J Food Sci Nutr 51: 11-17.
9. Rees WD, Hay SM, Cruickshank M (2006a) An imbalance in the methionine content of the maternal diet reduces postnatal growth in the rat. Metabolism: Clinical and Experimental 55: 763-770.
10. Rees WD, Wilson FA, Maloney CA (2006b) Sulfur amino acid metabolism in pregnancy: the impact of methionine in the maternal diet. J Nutr 136: 1701S-1705S.
11. Stead LM, Brosnan JT, Brosnan ME, Vance DE, Jacobs RL (2006) Is it time to reevaluate methyl balance in humans? Am J Clin Nutr 83: 5-10.
12. Vitvitsky V, Mosharov E, Tritt M, Ataullakhanov F, Banerjee R (2003) Redox regulation of homocysteine-dependent glutathione synthesis. Redox Rep 8: 57-63.
13. de Carvalho SC, Muniz MT, Siqueira MD, Siqueira ER, Gomes AV, et al. (2013) Plasmatic higher levels of homocysteine in non-alcoholic fatty liver disease (NAFLD). Nutr J 12: 37.
14. Mahabir S, Ettinger S, Johnson L, Baer DJ, Clevidence BA, et al. (2008) Measures of adiposity and body fat distribution in relation to serum folate levels in postmenopausal women in a feeding study. Eur J Clin Nutr 62: 644-650.
15. Semmler A, Moskau S, Grigull A, Farmand S, Klockgether T, et al. (2010) Plasma folate levels are associated with the lipoprotein profile: a retrospective database analysis. Nutr J l 9: 31.
16. Stewart CP, Christian P, Schulze KJ, Leclerq SC, West KP, et al. (2009) Antenatal micronutrient supplementation reduces metabolic syndrome in 6- to 8-year-old children in rural Nepal. J Nutr 139: 1575-1581.
17. Burdge GC, Lillycrop KA, Jackson AA, Gluckman PD, Hanson MA (2008) The nature of the growth pattern and of the metabolic response to fasting in the rat are dependent upon the dietary protein and folic acid intakes of their pregnant dams and post-weaning fat consumption. Br J Nutr 99: 540-549.
18. da Silva VC, Fernandes L, Haseyama EJ, Agamme AL, Guerra Shinohara EM, et al. (2014) Effect of vitamin B deprivation during pregnancy and lactation on homocysteine metabolism and related metabolites in brain and plasma of mice offspring. PloS One 9: e92683.
19. Calegare BF, Fernandes L, Tufik S, D'almeida V (2010) Biochemical, biometrical and behavioral changes in male offspring of sleep-deprived mice. Psychoneuroendocrinology 35: 775-784.
20. Liu J, Fox CS, Hickson D, Sarpong D, Ekunwe L, et al. (2010) Pericardial adipose tissue, atherosclerosis, and cardiovascular disease risk factors: the Jackson heart study. Diabetes Care 33: 1635-1639.
21. Painter RC, de Rooij SR, Bossuyt PM, Simmers TA, Osmond C, et al. (2006) Early onset of coronary artery disease after prenatal exposure to the Dutch famine. Am J Clin Nutr 84: 322-327.
22. Wang PX, Wang JJ, Lei YX, Xiao L, Luo ZC (2012) Impact of fetal and infant exposure to the Chinese Great Famine on the risk of hypertension in adulthood. PloS One 7: e49720.
23. Frery N, Huel G, Leroy M, Moreau T, Savard R, et al. (1992) Vitamin B12 among parturients and their newborns and its relationship with birthweight. Eur J Obstet Gynecol Reprod Biol 45: 155-163.
24. Modi N, Thomas EL, Uthaya SN, Umranikar S, Bell JD, et al. (2009) Whole body magnetic resonance imaging of healthy newborn infants demonstrates increased central adiposity in Asian Indians. Pediatr Res 65: 584-587.
25. Muthayya S, Kurpad AV, Duggan CP, Bosch RJ, Dwarkanath P, et al. (2006) Low maternal vitamin B12 status is associated with intrauterine growth retardation in urban South Indians. Eur J Clin Nutr 60: 791-801.
26. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, et al. (2008) Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. Diabetologia 51: 29-38.
27. Yajnik CS, Lubree HG, Rege SS, Naik SS, Deshpande JA, et al. (2002) Adiposity and hyperinsulinemia in Indians are present at birth. J Clin Endocrinol Metab 87: 5575-5580.
28. Furness DL, Yasin N, Dekker GA, Thompson SD, Roberts CT (2012) Maternal red blood cell folate concentration at 10-12 weeks gestation and pregnancy outcome. J Matern Fetal Neonatal Med 25: 1423-1427.
29. Mckay JA, Xie L, Manus C, Lanie SA, Maxwell RJ, et al. (2014) Metabolic effects of a high-fat diet post-weaning after low maternal dietary folate during pregnancy and lactation. Mol Nutr Food Res 58: 1087-1097.

30. Gupta NK, Powers JS (2008) Vitamin B12 deficiency and severe weight loss in an elderly patient. Tenn Med 101: 35-36.
31. Chow BF, Stone HH (1957) The relationship of vitamin B12 to carbohydrate metabolism and diabetes mellitus. Am J Clin Nutr 5: 431-439.
32. Mchenry EW, Gavin G (1941) The B vitamins and fat metabolism: IV. Synthesis of fat from proteins. J Biol Chem 138: 471-475.
33. Kumar KA, Lalitha A, Pavithra D, Padmavathi IJ, Ganeshan M, et al. (2013) Maternal dietary folate and/or vitamin B12 restrictions alter body composition (adiposity) and lipid metabolism in Wistar rat offspring. J Nutr Biochem 24: 25-31.
34. Chmurzynska A, Stachowiak M, Gawecki J, Pruszynska-Oszmalek E, Tubacka M (2012) Protein and folic acid content in the maternal diet determine lipid metabolism and response to high-fat feeding in rat progeny in an age-dependent manner. Genes Nutr 7: 223-234.
35. Antony AC (2003) Vegetarianism and vitamin B-12 (cobalamin) deficiency. Am J Clin Nutr 78: 3-6.
36. Misra A, Vikram NK, Pandey RM, Dwivedi M, Ahmad FU, et al. (2002) Hyperhomocysteinemia, and low intakes of folic acid and vitamin B12 in urban North India. Eur J Nutr 41: 68-77.
37. Yajnik CS, Fall CH, Coyaji KJ, Hirve SS, Rao S, et al. (2003) Neonatal anthropometry: the thin-fat Indian baby. The Pune Maternal Nutrition Study. Int J Obes Relat Metab Disord 27: 173-180.
38. Joshi SR, Saboo B, Vadivale M, Dani SI, Mithal A, et al. (2012) Prevalence of diagnosed and undiagnosed diabetes and hypertension in India-results from the Screening India's Twin Epidemic (SITE) study. Diabetes Technol Ther 14: 8-15.
39. Ramachandran A, Snehalatha C, Kapur A, Vijay V, Mohan V, et al. (2001) High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. Diabetologia 44: 1094-1101.
40. Rush EC, Katre P, Yajnik CS (2014) Vitamin B12: one carbon metabolism, fetal growth and programming for chronic disease. Eur J Clin Nutr 68: 2-7.