

High multivitamin intakes during pregnancy and postweaning obesogenic diets interact to affect the relationship between expression of PPAR genes and glucose regulation in the offspring[☆]

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Abstract

High multivitamin intake (HV) during pregnancy increases body fat and weight and alters glucose and fatty acid metabolism in Wistar rat offspring. This study investigated the expression of peroxisome-proliferator activated receptors (PPARs) genes involved in regulation of glucose and fatty acid metabolism in their tissues. Dams received the AIN-93G diet with either the regular (RV) or 10-fold multivitamins (HV) during pregnancy. Male offspring were weaned to either the RV diet (RV-RV and HV-RV) or an obesogenic diet (RV-Ob and HV-Ob). Gene expression of PPARs in tissues was analyzed by real-time reverse transcriptase polymerase chain reaction. Gestational diet (GD) did not affect PPARs gene expression in offspring at either birth or weaning. In liver, at 14 weeks postweaning, PPAR- γ was 30% lower in the HV-RV and 30% higher in HV-Ob than in the RV-RV group [GD $P=.76$, postweaning diet (PD) $P=.19$, interaction $P=.02$, by two-way analysis of variance]. In muscle, PPAR- α expression was affected by GD and PD (GD $P=.05$, PD $P<.01$, interaction $P=.07$). In adipose tissue, PPAR- α expression was higher in all groups compared to RV-RV (GD $P=.25$, PD $P=.85$, interaction $P=.03$). PPAR- γ mRNA levels correlated with abdominal fat ($r=0.45$, $P<.05$) and insulin resistance index ($r=0.39$, $P<.05$). In liver, PPAR- γ expression correlated with insulin resistance index in offspring from RV ($r=-0.62$, $P<.05$), but not in those from HV dams ($r=0.13$, $P>.05$). In conclusion, the HV diet during pregnancy interacts with postweaning diets in determining the expression of PPARs genes in a tissue- and age-dependent manner and uncouples the relationship between these genes and glucose regulation and fat mass in the rat offspring.

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1. Introduction

Maternal nutrition during pregnancy plays a fundamental role in fetal development, and growing evidence suggests a linkage to later outcomes in life associated with the risk of metabolic disturbances in the offspring [1]. Whereas altered metabolic responses due to gestational nutrient-deficient diets are commonly recognized [2], nutrient excess during pregnancy also has adverse consequences in offspring and increases their risk for chronic diseases [3]. As shown by animal studies, maternal overnutrition due to high-fat diet consumption affected offspring responses to postnatal diets, resulting in higher

body weight and alterations in glucose response in the offspring, effects that were amplified by feeding offspring a high-fat diet after weaning [3–5]. Other nutrients, such as vitamins, when consumed above recommendations during pregnancy also result in higher body weight and components of the metabolic syndrome in offspring of Wistar rats [6]. These effects were further amplified by feeding the offspring a liquid obesogenic diet after weaning and resulted in higher body weight, abdominal fat mass, as well as altered glucose homeostasis and tissue fatty acid composition [7,8].

Glucose and fatty acid metabolism is partially regulated by nuclear transcription factors known as peroxisome-proliferator activated receptors (PPARs) [9]. Each PPAR isotype, namely, PPAR- α , PPAR- β/δ and PPAR- γ , has specific actions according to the tissue in which it is expressed, but in general, PPAR- α and PPAR- β/δ regulate the expression of enzymes and transporters that promote fatty acid uptake and oxidation, whereas PPAR- γ is related to increased adipogenesis and insulin sensitivity [10]. Thus, metabolic disturbances may be related to changes in the gene expression of these transcriptional factors.

Gene expression of PPARs has been shown to be affected by obesity and dietary factors in different tissues of animal models. For

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example, gene expression of PPAR- γ is altered in cardiac tissue of obese lambs [11], and a high-fat postweaning diet increases PPAR- γ expression in mouse liver and adipose tissue [12]. PPARs gene expression is also sensitive to the nutrient content of maternal diets, as exemplified by studies in sheep showing that a global nutrient restriction results in higher expression of PPAR- α in adipose tissue of the lamb fetuses [13]. In rats, a protein-restricted diet during pregnancy also increases the expression of PPAR- α in the offspring liver, but the expression of this gene was comparable to that from offspring of control mothers when the protein-restricted diet given to the dams was supplemented with folic acid [14]. However, whether the vitamin content of the maternal diet, without protein or energy restriction, alters the expression of PPARs in the offspring tissues is unknown.

Because we previously observed that a maternal high-vitamin (HV) diet followed by a postweaning obesogenic diet results in alterations in glucose and fatty acid metabolism, the objective of this study was to investigate the effects of the HV diet during pregnancy on gene expression of PPAR- γ , PPAR- β/δ and PPAR- α in adipose tissue, liver and muscle and their relationship with fat mass and insulin resistance in the male offspring at birth, at weaning and when fed either a regular-vitamin (RV) or obesogenic diet for 14 weeks postweaning (PW).

2. Methods and materials

2.1. Animals and diets

All procedures were approved by the Animal Ethics Committee at University of Toronto. Twenty Wistar rats in the second to third day of their first pregnancy were purchased from Charles River (Montreal, QC, Canada). Animals were housed individually in transparent cages with free access to water in facilities with controlled temperature ($22^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and illumination (12-h dark–light cycle, lights on at 7:00 a.m.). The regular AIN-93G diet [15] with either the regular amount (RV) or 10 times the amount of vitamin mix (HV) and a liquid obesogenic diet (Ob) were used. Feeding this Ob diet postweaning has shown to be highly palatable and to induce overeating and to accelerate the development of obesity and metabolic diseases [7,16,17]. The composition of these diets has been previously reported [8].

2.2. Experimental design

Dams were randomly allocated to receive either the RV or the HV diet during pregnancy (gestational diet). During lactation, all dams received the RV diet. The litters were culled to 10 animals per dam on postnatal day 1 and were weaned at postnatal day 21. From weaning to 14 weeks postweaning, the offspring (one pup/dam) were fed either the RV diet or the Ob diet (postweaning). Thus, after weaning, four groups of animals were formed: (a) from RV dams weaned to RV diet (RV-RV), (b) from RV dams weaned to Ob diet (RV-Ob), (c) from HV dams weaned to RV diet (HV-RV) and (d) from HV dams weaned to Ob diet (HV-Ob).

2.3. Tissue collection and analysis

Tissues from the unsexed offspring at birth (liver) and from males at weaning and 14 weeks PW (liver, quadriceps muscle and abdominal fat) were collected, immediately frozen in liquid nitrogen and kept at -80°C until analysis. Samples of tissues were homogenized in Trizol (Invitrogen, Carlsbad, CA, USA), and mRNA was isolated. After quantification using a UV–Agilent 8453, mRNA from adipose (1 μg), liver (3–4 μg , from weaning and adult samples, respectively) and muscle tissue (~4 μg) was used for cDNA synthesis using a High Capacity cDNA archive kit (Applied Biosystems Inc., Foster City, CA, USA). The mix was incubated for 10 min at 25°C , followed by 120 at 37°C in an ABI Gene Amp PCR System 2700. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed using Taqman assays for the following genes: PPAR- α (cat. # Rn00566193_m1), PPAR- β/δ (cat. # Rn00565707_m1), PPAR- γ (cat. # Rn00440945_m1), glucokinase (GK, cat. # Rn00688285_m1), carnitine-palmitoyl transferase 1 (CPT1, cat. # Rn00580702_m1) and glucose transporter 4 (Glut4, cat. # Rn00562597_m1) from Applied Biosystems, in the 7900HT Fast Real-Time RT-PCR System also from Applied Biosystems (Foster City, CA, USA). The cycle conditions were as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles for 95°C for 15 s and 60°C for 1 min. The relative quantification method was performed using glyceraldehyde-3-phosphate dehydrogenase (cat. # Rn99999916-s1) as an endogenous control for liver samples and β -2-microglobulin for muscle and adipose tissue samples (cat. # 4331182-Rn00560865-m1). Results are expressed as fold-change, obtained by the $2^{-\Delta\Delta\text{Ct}}$ method [18]. The ΔCt of each sample was calculated as the difference between the Ct of the target gene and the Ct of the endogenous control. The mean of the RV group (birth and weaning) or the RV-RV group (postweaning) was used as the

reference to obtain the value of the $\Delta\Delta\text{Ct}$ for each sample ($\Delta\Delta\text{Ct} = \Delta\text{Ct}$ of each sample – mean of the ΔCt in the reference group). The fold change was calculated as $2^{-\Delta\Delta\text{Ct}}$, and their mean and standard error were calculated.

2.4. Statistical analyses

The effects of gestational diet and/or postweaning diet were analyzed by unpaired t test and general linear models. Two-way analysis of variance (ANOVA) was used to analyze the effects of the gestational and postweaning diets and their interaction on mRNA levels of the selected genes. Pearson correlation coefficients were calculated to analyze the relationship between gene expression and the insulin resistance index (IRI), previously calculated as glucose*insulin plasma concentrations [6] and abdominal fat mass. Correlation coefficients were calculated for the entire group of animals (pooled), as well as within each gestational and postweaning diet groups. SAS v. 9.2. (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis of the data.

3. Results

Results describing the phenotypic characteristics of the offspring (body weight, glucose metabolism and fatty acid concentration of tissues) have been previously reported [6–8]. In brief, the gestational HV diet did not affect litter size and birth weight, but resulted in greater weight gain after weaning and differences in fatty acid composition in different tissues than in offspring of dams fed the AIN-93G diet with the regular amount of vitamins [6–8]. At 14 weeks, male offspring of HV dams also showed higher abdominal fat mass and higher IRI [6,7]. In this study, we further analyzed the tissues for the expression for selected genes related to glucose and lipid metabolism.

Gene expression of PPARs in tissues from the offspring at birth and weaning did not differ by gestational diet (Table 1). However, mRNA levels of PPAR- β/δ in the liver of offspring at weaning were correlated with the IRI ($r = 0.73$, $P < .01$) in the whole group of animals (pooled data), and the direction of the association did not differ by gestational diet (RV $r = 0.6$, $P = .08$; HV $r = 0.78$, $P = .01$). The correlation between the expression of PPAR- α or PPAR- γ in this tissue and IRI was not significant ($r = 0.35$, $P = .15$ and $r = 0.04$, $P = .88$, respectively). Abdominal fat mass (g) was not correlated with the expression of any of the PPARs in the liver (PPAR- α $r = 0.15$, $P = .60$; PPAR- β/δ $r = 0.34$, $P = .26$; PPAR- γ $r = 0.39$, $P = .16$). Glucose concentrations, but not insulin or IRI, were negatively correlated with the expression of PPAR- α ($r = -0.52$, $P = .05$), PPAR- β ($r = -0.55$, $P = .03$) and PPAR- γ ($r = -0.57$, $P = .03$) in the muscle of the entire group of animals at

Table 1
Gene expression of PPARs on tissues from offspring at birth (unsexed) and males at weaning

Gene	From RV dams	From HV dams	P value ^a
Birth			
Liver			
PPAR- α	1.0 $\pm$ 0.1	0.8 $\pm$ 0.2	.36
PPAR- β/δ	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	.27
PPAR- γ	1.0 $\pm$ 0.1	1.2 $\pm$ 0.2	.34
Weaning			
Liver			
PPAR- α	1.1 $\pm$ 0.2	1.5 $\pm$ 0.2	.24
PPAR- β/δ	1.0 $\pm$ 0.1	1.2 $\pm$ 0.2	.51
PPAR- γ	1.2 $\pm$ 0.2	1.5 $\pm$ 0.2	.39
Muscle			
PPAR- α	1.3 $\pm$ 0.3	1.6 $\pm$ 0.5	.63
PPAR- β/δ	1.1 $\pm$ 0.2	1.3 $\pm$ 0.2	.66
PPAR- γ	1.1 $\pm$ 0.2	1.7 $\pm$ 0.4	.26
Adipose tissue			
PPAR- α	1.1 $\pm$ 0.3	0.9 $\pm$ 0.1	.48
PPAR- β/δ	1.1 $\pm$ 0.2	1.2 $\pm$ 0.1	.57
PPAR- γ	1.2 $\pm$ 0.3	1.7 $\pm$ 0.3	.26

Results are presented as fold change with RV group as reference.

RV=from dams fed AIN-93G regular diet; HV=from dams fed HV diet (AIN-93G+10-fold vitamin mix), $n = 7$ –10 per group, except adipose tissue $n = 5$ per group.

^a Unpaired t test.

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