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High vitamin intake by Wistar rats during pregnancy alters tissue fatty acid concentration in the offspring fed an obesogenic diet

Sandra A. Reza-López[a], G. Harvey Anderson[a], Ignatius M.Y. Szeto[a], Ameer Y. Taha[a], David W.L. Ma[a,b,*]

[a]Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 3E2
[b]Department of Human Health and Nutritional Sciences, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

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Abstract

Diet during pregnancy affects the long-term health of the offspring. Vitamins are known to modulate lipid metabolism, which may be reflected in tissue fatty acid (FA) concentrations. The objective of this study was to investigate the effect of high vitamin intake during pregnancy on tissue FA concentration of the offspring. Wistar rats were fed an AIN-93G diet with either the recommended vitamin or 10-fold higher amounts (HV) during pregnancy. Afterward, offspring were weaned onto an obesogenic diet. Liver, quadriceps, adipose, and brain were collected over 48 weeks. Fatty acid concentration of tissue total lipids was analyzed by gas chromatography. At birth, the liver from HV offspring was higher in monounsaturated, stearic, and arachidonic acids. At weaning, the liver from HV offspring was higher in stearic and oleic acids; and in adipose tissue, n-6 and n-3 FAs were lower only in the male HV offspring ($P < .05$). At 12 weeks, HV offspring had higher concentrations of total fat, saturates, monounsaturates, and n-6 FA in muscle ($P < .05$), but not in other tissues. At 48 weeks, gestational diet did not affect tissue total lipid FA concentrations; but differences remained in specific tissue phospholipids species. Liver phospholipids from HV offspring were lower in monounsaturates and n-6 FA. Brain phosphatidylethanolamine was higher in oleic, n-6 FA, and docosahexaenoic acid in the HV offspring. Phosphatidylinositol was lower in saturates, monounsaturates, arachidonic, and docosahexaenoic acids only in HV female offspring. These observations demonstrate that high vitamin intake during pregnancy has short- and long-term effects on tissue FA concentration in the offspring.

1. Introduction

In humans and animals, the fat content of gestational diets is reflected in fetal tissues [1-3] and has been identified as a determinant of the risk for the offspring of developing obesity and chronic diseases [4-6]. Tissue fatty acid (FA) composition in the fetus is readily affected by the dietary fat in the maternal diet [7,8] and mobilization of maternal FA stores during pregnancy [9-11]. In addition to FA composition, the adequacy of energy and protein in the gestational diet affects fetal brain and liver FA content of n-3, n-6, and monounsaturated FA [12,13], possibly with long-term effects [14]. However, the effect of micronutrients in the maternal diet during pregnancy on tissue lipid metabolism and FA composition in the offspring has received little investigation.

A role for vitamins in FA metabolism and development is supported by 3 lines of evidence. First, vitamins influence gene expression and cell function, activating transcriptional factors or acting as methyl donors, coenzymes, or cofactors. For example, in the viable yellow Agouti mouse, feeding high intakes of folate, B12, and choline during pregnancy leads to changes in the expression of sensitive genes regulating coat color, adiposity, and glucose metabolism in the offspring [15]. Second, many vitamins directly affect FA metabolism. Vitamin A interacts with nuclear receptors, thus activating transcriptional factors [16-18]. Two of the genes regulated by peroxisome proliferators–activated receptors (PPARs) are those that encode the Δ-5 and Δ-6 desaturases, involved in the synthesis of long-chain polyunsaturated fatty acids (PUFA) from their precursors; and vitamins, such as folic acid, are

* Corresponding author. Department of Human Health and Nutritional Sciences, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Tel.: +1 519 824 4120x52272; fax: +1 519 763 5902.
E-mail address: davidma@uoguelph.ca (D.W.L. Ma).

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involved in the regulation of the gene expression by PPARs through an epigenetic mechanism [19,20]. The activity of Δ-5 and Δ-6 desaturases is also regulated by tocopherols [21] and probably B6 [22]. In addition, acyl–coenzyme A oxidase activity, a key enzyme involved in β-oxidation, is also involved in the conversion of eicosapentaenoic acid (EPA) to docosahexaenoic acid (DHA), which has been shown to be reduced by B6 deficiency [22]. Third, studies from our group have shown that high vitamin intake by Wistar rats during pregnancy leads to higher body weight and fat mass, and components of the metabolic syndrome in the adult offspring [23]. Because tissue FA content is used as a surrogate marker for diet-induced changes in lipid metabolism, the objective of this study was to investigate the effect of high multivitamin intake during gestation on tissue FA concentration of the offspring at birth, weaning, and 12 and 48 weeks postweaning (PW).

2. Materials and methods

2.1. Experimental design

Pregnant (2nd to 3rd day of pregnancy) Wistar rats (Charles River, Montreal, Quebec, Canada) were randomly allocated to receive either the AIN-93G diet [24] containing the regular amount of vitamins (RV) or a modified AIN-93G diet containing 10-fold higher vitamins (HV) during pregnancy. Except for the vitamin content, the rest of the components were the same in both diets (Table 1). Because the vitamin mix uses sucrose as a carrier (9.75 g for 10 g) [24], we adjusted this component to provide similar amount in both diets; and it is presented separately.

Table 1
Composition of experimental diets

Component (g/kg diet dry weight)	RV	HV	Ob-pup
Cornstarch[a]	529.5	529.5	278.4
Casein (>85% protein)[a]	200.0	200.0	147.3
Sucrose (added)[b]	100.0	10.0	397.2
Sucrose from vitamin mix	9.75	97.5	5.1
Sucrose from mineral mix	21.4	21.4	11.2
Lactose	0.0	0.0	36.5
Fat[c,e]	70.0	70.0	78.9
Fiber (cellulose)[a]	50.0	50.0	26.3
Mineral mix[d,f]	13.6	13.6	7.2
Vitamin mix[d,f]	0.25	2.5	0.2
L-Cystine[d]	3.0	3.0	1.6
Choline bitartrate[d]	2.5	2.5	1.3
tert-Butylhydroquinone[d] (mg/kg)	14.0	14.0	7.4

RV indicates regular vitamin diet; HV, high vitamin diet; Ob-pup, obesogenic liquid diet.

[a] From Harlan Teklad (Madison, WI).

[b] From Allied Food Service (Toronto, Ontario, Canada).

[c] From Loblaws (Toronto, Ontario, Canada).

[d] From Dyets (Bethlehem, PA).

[e] Fat source in RV and HV was soybean oil; in obesogenic diet, fat was derived from condensed milk and soybean oil.

[f] Absolute amount of mineral/vitamin, without sucrose used as a carrier. The content of vitamin mix is 10 and 100 g/kg in RV and HV, respectively. The sucrose content of the diet was adjusted to provide similar amount of sucrose in the RV and HV diets and is presented separately.

After delivery, all dams received the RV diet; and the litters were culled to 10 pups per dam. At 3 weeks of age, the offspring from both groups were sexed and weaned to an obesogenic liquid diet, described in Table 1 [25]. Animals were housed individually in a temperature-controlled environment (22°C ± 1°C) with a 12-hour dark-light cycle. The protocol was approved by the Animal Ethics Committee at University of Toronto. All diets were prepared in the laboratory and were provided ad libitum.

2.2. Tissue collection

Animals were terminated by decapitation after overnight fasting. Tissues were collected at birth (liver and muscle), weaning (liver, muscle, abdominal adipose tissue), and 12 and 48 weeks PW (liver, brain, adipose, muscle). All tissues were immediately frozen in liquid nitrogen and stored at −80°C until their analysis. At birth, the tissues from 2 pups per litter (when available) were analyzed; and their results were averaged. For each of the other time points, the tissues of 1 pup per dam were used for analyses, whereas part of the offspring was used for a separate experiment.

2.3. Lipid analysis

2.3.1. Chemicals

All solvents and reagents, unless other specified, were obtained from Sigma Chemical (St Louis, MO). Phospholipid standards were obtained from Matreya Biochemicals (Pleasant Gap, PA).

2.3.2. Analysis

Liver, adipose tissue, and muscle (approximately 0.5 g of tissue) were homogenized (CH-6010 homogenizer; Kinematica, Kriens, Lucerne, Switzerland) in 2.5 mL of 0.88% KCl. Lipids were extracted using the method of Folch et al [26]. In the case of the brain, the whole organ was homogenized for lipid analysis; and the solvent volume was adjusted according to the organ weight. Heptadecanoic acid (17:0) either as a free FA or as cocktail of triglycerides, phospholipids, and cholesterol ester was added as an internal standard. Liver lipid classes (at 12 and 48 weeks PW) were separated by thin-layer chromatography (TLC) to obtain triglycerides, phospholipids, and cholesterol esters. Thin-layer chromatography plates were used to separate lipid classes using a solvent mixture of petroleum ether, ethyl ether, and acetic acid (80:20:1 vol/vol/vol). Bands corresponding to phospholipids, triglycerides, and cholesterol esters were visualized under UV light after lightly spraying with 8-anilino-1-naphtalene-sulfonic acid (0.1% wt/vol).

Brain and muscle phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidylinositol [PI], phosphatidylethanolamine [PE]) and sphingomyelin from the offspring at 12 and 48 weeks PW were also separated by a similar procedure. The TLC plates were developed with a mixture of chloroform, methanol, 2-propanol, KCl (0.25% wt/wt),

Table 2
At birth, gestational diet influences liver FA concentrations (milligrams per gram tissue) of the unsexed offspring

Fatty Acid	From RV	From HV	*P* value[a]
	Mean ± SEM	Mean ± SEM	
14:0	1.0 ± 0.2	1.6 ± 0.3	.11
16:0	12.2 ± 1.3	18.2 ± 2.3	.05
18:0	4.3 ± 0.3	5.4 ± 0.3	.03
Saturates[b]	18.6 ± 1.8	27.0 ± 3.2	.04
16:1 c9	0.7 ± 0.1	1.3 ± 0.2	.04
18:1 c9	7.2 ± 0.9	12.7 ± 2.0	.03
18:1 c11	1.0 ± 0.1	1.7 ± 0.2	.03
Monounsaturates[b]	9.2 ± 1.1	16.0 ± 2.5	.03
18:2 n-6	10.8 ± 1.1	16.4 ± 2.5	.07
18:3 n-6	0.5 ± 0.1	0.9 ± 0.2	.05
20:3 n-6	0.8 ± 0.1	1.2 ± 0.2	.06
20:4 n-6	10.2 ± 0.9	14.2 ± 1.6	.05
22:5 n-6	0.8 ± 0.1	1.0 ± 0.1	.10
Sum n-6[b]	24.9 ± 2.3	36.3 ± 4.8	.06
18:3 n-3	0.7 ± 0.1	1.0 ± 0.2	.11
20:5 n-3	1.3 ± 0.1	1.8 ± 0.2	.09
22:5 n-3	2.0 ± 0.2	2.6 ± 0.3	.10
22:6 n-3	5.6 ± 0.5	6.8 ± 0.4	.08
Sum n-3[b]	9.6 ± 0.9	12.3 ± 1.1	.07
n-6/n-3 ratio	2.6 ± 0.0	2.9 ± 0.2	.10
Total mg/g tissue[b]	62.4 ± 6.0	91.8 ± 11.5	.04

n = 6 to 7 per group.
[a] By unpaired *t* test.
[b] Total includes other minor FAs not reported.

and triethylamine (30:9:25:6:18 vol/vol). Bands corresponding to the phospholipid fractions were visualized as previously described. Fatty acids from total lipid extracts, lipid classes, and phospholipid fractions were converted to methyl esters (FA methyl esters [FAMEs]) using 14% methanolic boron trifluoride. Fatty acid methyl esters were separated on an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector and separated on a fused-silica capillary column (SP2560, Sulpeco, Bellefonte, PA; 100 m, 0.25 μm film thickness, 0.25 mm internal diameter). Samples were injected in splitless mode. The injector and detector ports were set at 250°C. The FAMEs were eluted using a temperature program set initially at 60°C, increased at 10°C/min, and held at 170°C for 5 minutes; increased at 5°C/min, then at 2°C/min and at 1°C/min until reaching 175°/min, 185°C, and 190°C, respectively; and finally increased at 10°C/min until reaching 240°C and held for 18 minutes to complete the run. The carrier gas was helium, set to a 1.3-mL/min constant flow rate. Peaks were identified by comparison with authentic FA standards (GLC463; Nu-Chek Prep, Elysian, MN) and quantified using heptadecanoic acid as the internal standard. Peaks were quantified using ChemStation software (Version B.01.01, Agilent). Results from total lipid analyses are presented as milligrams per gram of wet tissue for the sum of saturated, monounsaturated, n-6 and n-3 FA, and the main individual FAs corresponding to these series.

2.4. Statistical analysis

Values are presented as the mean and standard error of the mean. Means were compared by 2-way analysis of variance (ANOVA), including gestational diet and sex as main factors and their interaction term. Unpaired *t* test was

Table 3
At weaning, gestational diet and sex influence adipose tissue FA concentration (milligrams per gram tissue) of the offspring

Fatty Acid	Males		Females	
	From RV	From HV	From RV	From HV
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
14:0	44.6 ± 2.0	35.6 ± 2.5	40.1 ± 3.5	39.0 ± 1.5*
16:0	149.1 ± 4.9	133.0 ± 8.5	154.4 ± 12	151.1 ± 8.0
18:0	18.7 ± 0.6	17.1 ± 1.2	19.9 ± 1.3	19.7 ± 1.0
Saturates[a]	239.9 ± 7.2	207.3 ± 13.6	233.4 ± 18	227.7 ± 10.2
16:1 c9	19.4 ± 1.2	19.6 ± 1.9	22.2 ± 2.2	21.5 ± 2.0
18:1 c9	132.7 ± 5.4	122.4 ± 8.1	138.6 ± 9.8	139.5 ± 7.4
18:1 c11	10.2 ± 0.5	9.5 ± 0.7	10.8 ± 0.8	11.1 ± 0.5
Monounsaturates[a]	165 ± 6.9	154.0 ± 10.3	174.1 ± 13	174.8 ± 10.0
18:2 n-6	157.2 ± 6.1	138.4 ± 11.3	155.1 ± 12	157.7 ± 5.4
18:3 n-6	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.3 ± 0.1* ‡
20:3 n-6	1.7 ± 0.1	1.3 ± 0.1	1.5 ± 0.2	1.7 ± 0.1‡
20:4 n-6	4.9 ± 0.2	4.1 ± 0.3	4.8 ± 0.4	5.4 ± 0.3‡
Sum n-6[a]	168.4 ± 6.5	147.7 ± 12	166.4 ± 13	169.7 ± 6.0
18:3 n-3	19.9 ± 0.9	17.9 ± 1.5	20.2 ± 1.8	20.3 ± 0.7
20:5 n-3	0.7 ± 0.1	0.5 ± 0.04	0.6 ± 0.1	0.7 ± 0.1
22:6 n-3	1.5 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.5 ± 0.1‡
Sum n-3[a]	23.9 ± 1.2	20.8 ± 1.7	23.9 ± 2.1	24.4 ± 0.9
n-6/n-3	7.1 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.0 ± 0.1
Total mg/g[a]	597.7 ± 19.3	530.3 ± 36.3	598.3 ± 45	597.2 ± 25.6

P less than .05 for *gestational diet effect, ¶sex of the pup effect, and ‡gestational diet × sex interaction, by 2-way ANOVA; n = 7 to 9 per group.
[a] Includes other minor FAs not reported.

used for comparison of means of the FA concentration at birth and to test means of FAs within each sex. Statistical significance was declared at P less than .05. The software SAS version 9.1 (SAS Institute, Cary, NC) was used for statistical analyses.

3. Results

3.1. FA concentration of the offspring at birth and weaning

Total FA, including many saturated FA, monounsaturated FA, and arachidonic acid (20:4n-6) concentrations (Table 2) were higher in the liver of the unsexed offspring from HV dams relative to RV dams. Muscle FA concentration (not reported) was not different between groups. Abdominal adipose tissue was not detected at this age.

At weaning, the concentrations (milligrams per gram tissue) of stearic (males: RV 6.1 ± 0.2, HV 6.6 ± 0.2; females: RV 6.2 ± 0.2, HV 6.6 ± 2.2) and oleic acids (males: RV 1.3 ± 0.1, HV 1.5 ± 0.1; females: RV 1.4 ± 0.1, HV 1.5 ± 0.1) were higher in the liver of HV offspring ($P < .05$). No other significant changes were observed in other FAs. In muscle, there was no effect of gestational diet on FA concentrations. In adipose tissue, an interaction was observed between gestational diet and sex for total n-6 FA and DHA (22:6n-3). Males, but not females from HV dams, were observed to have lower values of these FAs (Table 3).

3.2. FA concentration of the offspring at 12 weeks PW

At 12 weeks PW, liver total FA concentration was different between sexes; but gestational diet had no effect. Therefore, data from RV and HV were pooled for further analysis to explore sex differences. Males had higher concentrations than females in the following FAs (milligrams per gram tissue): 16:0 (11.02 ± 0.7 vs 7.6 ± 1.0), 18:1 c11 (1.8 ± 0.1 vs 0.8 ± 0.1), 18:2 n-6 (7.3 ± 0.7 vs 4.8 ± 0.4), 20:3n-6 (0.3 ± 0.01 vs 0.2 ± 0.01), 22:4n-6 (0.1 ± 0.01 vs 0.04 ± 0.01), 18:3n-3 (0.5 ± 0.1 vs 0.3 ± 0.04), monounsaturates (12.9 ± 0.9 vs 8.5 ± 1.6), and n-6/n-3 ratio (5.4 ± 0.2 vs 3.8 ± 0.1). Females were higher in 18:0 (7.5 ± 0.2 vs 5.2 ± 0.1) and the sum of n-3 (3.0 ± 0.1 vs 2.6 ± 0.2) milligrams per gram tissue ($P < .05$). In addition to total lipid analyses, liver lipid classes were separated; and as observed for total lipids, phospholipids and cholesterol esters, FAs differed by sex, but were not influenced by the gestational diet (data not shown).

The gestational diet markedly affected muscle FA concentration (Table 4). The HV male offspring had higher concentration of total fat, saturates, monounsaturates, and n-6 FA (milligrams per gram tissue). Adipose tissue FA concentration showed a difference by sex, but no effect of gestational diet was detected. Males had higher concentration (milligrams per gram tissue) of 16:1 (41.7 ± 1.9 vs 28.8 ± 1.4) and 18:1c11 (24.9 ± 1.1 vs 17.0 ± 0.5), and lower 22:4n-6 (0.1 ± 0.05 vs 0.4 ± 0.05) and DHA (0.2 ± 0.2 vs 0.4 ± 0.2) than females ($P < .05$). Similarly, in the brain, differences by sex were observed in the concentration of 22:6 n-3 (females 4.7 ± 0.2, males 4.2 ± 0.11); but no differences by gestational diet were found in total lipids.

3.3. FA concentration of the offspring at 48 weeks PW

In the offspring at 48 weeks PW, there was no effect of gestational diet on liver total lipids. However, there were differences in FA concentration (milligrams per gram tissue) due to sex. Males were lower in 18:0 (6.0 ± 0.2 vs 8.6 ± 0.9) and 22:5n-6 (0.04 ± 0.02 vs 0.13 ± 0.04), and higher in 18:3n-3 (0.8 ± 0.1 vs 0.4 ± 0.1) than females. Further

Table 4
At 12 weeks PW, gestational diet and sex influence muscle FA concentration (milligrams per gram tissue) of the offspring

Fatty Acid	Males		Females	
	From RV	From HV	From RV	From HV
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
14:0	0.2 ± 0.02	0.5 ± 0.10	0.2 ± 0.05	0.2 ± 0.04*
16:0	2.5 ± 0.20	4.8 ± 0.90	3.0 ± 0.35	3.4 ± 0.30*
18:0	1.0 ± 0.00	1.3 ± 0.10	1.1 ± 0.05	1.1 ± 0.03
Saturates[a]	3.7 ± 0.30	6.6 ± 1.20	4.3 ± 0.41	4.7 ± 0.30*
16:1	0.6 ± 0.10	1.2 ± 0.30	0.6 ± 0.17	0.6 ± 0.10
18:1 c9	1.7 ± 0.20	4.5 ± 1.20	2.5 ± 0.56	2.8 ± 0.40*
18:1 c11	0.4 ± 0.03	0.7 ± 0.10	0.4 ± 0.03	0.4 ± 0.03*†
Monounsaturates[a]	2.7 ± 0.30	6.6 ± 1.70	3.4 ± 0.77	3.9 ± 0.50*
18:2 n-6	1.7 ± 0.20	3.3 ± 0.70	2.1 ± 0.25	2.2 ± 0.20*
20:4 n-6	0.9 ± 0.10	0.9 ± 0.03	0.8 ± 0.07	0.9 ± 0.03
Sum n-6[a]	2.7 ± 0.20	4.3 ± 0.70	3.0 ± 0.25	3.3 ± 0.20*
18:3 n-3	0.04 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	0.06 ± 0.03
22:6 n-3	0.7 ± 0.10	0.7 ± 0.03	0.8 ± 0.10	0.9 ± 0.10
Sum n-3[a]	0.9 ± 0.10	1.0 ± 0.04	1.0 ± 0.10	1.2 ± 0.10
n-6/n-3 ratio	3.0 ± 0.10	4.5 ± 0.80	3.7 ± 0.90	2.9 ± 0.30
Total mg/g	10.1 ± 0.90	18.6 ± 3.60	11.8 ± 1.30	13.1 ± 1.00*

P less than .05 for *gestational diet effect, †sex of the pup effect, and ‡gestational diet × sex interaction, by 2-way ANOVA; n = 7 to 9 per group.

[a] Includes other minor FAs not reported.

analysis of liver lipid classes showed that the gestational diet affected phospholipid FA concentrations in which monounsaturates, n-6 FAs, and n-6/n-3 ratio were lower in HV offspring (Table 5). No effect of gestational diet was observed in liver triglycerides or cholesterol esters.

Brain total lipids at 48 weeks did not show differences by gestational diet. Small differences by sex were observed in several FAs. Males had higher concentration (milligrams per gram tissue) of 18:0 (7.1 ± 0.1 vs 6.7 ± 0.06), 18:1c11 (1.6 ± 0.03 vs 1.6 ± 0.05), 20:4n-6 (3.2 ± 0.05 vs 3.0 ± 0.4), 22:4n-6 (0.8 ± 0.01 vs 0.9 ± 0.02), and 18:3n-3 (0.3 ± 0.01 vs 0.2 ± 0.03) than females ($P < .05$). Additional analysis showed that gestational diet affected the FA concentration (micrograms per gram tissue) of phospholipid classes, specifically on brain PI and PE FA concentrations (Table 6).

Muscle total lipid FA concentrations were influenced by sex, but not by gestational diet. Males had lower concentration (milligrams per gram tissue) than females in the following FAs: 14:0 (0.4 ± 0.04 vs 0.7 ± 0.07), 16:0 (5.9 ± 0.4 vs 8.2 ± 0.6), total saturates (8.1 ± 0.04 vs 10.7 ± 0.7), 16:1 (1.4 ± 0.1 vs 2.1 ± 0.2), 18:1c9 (5.7 ± 0.4 vs 8.7 ± 0.9), monounsaturates (8.2 ± 0.6 vs 12.0 ± 1.2), and total FAs (23.5 ± 1.2 vs 30.6 ± 2.1) in the total lipids ($P < .05$). Phospholipid FA concentration in this tissue also was influenced by sex, but not gestational diet (data not shown). Similarly, adipose tissue was not affected by gestational diet; and only sex was observed to affect the concentration of 18:1c11 (males 32.6 ± 1.2, females 23.0 ± 0.7 mg/g tissue) and 18:2n-6 (males 126.8 ± 5.6, females 99.6 ± 7.5 mg/g tissue) ($P < .05$).

4. Discussion

The results of this study support the hypotheses that high multivitamin intake during pregnancy affects tissue FA concentration and composition in the offspring from Wistar rats and that the effect is tissue, sex, and age dependent.

Effects of the gestational diet were evident in early life, as shown by the higher concentration of saturates, monounsaturates, and n-6 PUFA in the liver at birth of the offspring from dams fed the HV diet compared with those from dams fed the RV AIN-93G diet. In contrast, in the adipose tissue, the maternal high vitamin intake resulted in lower concentration of n-6 PUFA and DHA in male offspring at weaning. Long-lasting effects of the gestational diet were observed in specific tissues and classes of lipids in muscle (Table 4), liver (Table 5), and brain (Table 6) at 12 and 48 weeks PW.

Effects of the gestational diet at birth and through to 48 weeks of the life suggest that they were imprinted in utero. At birth, the FA concentration of the tissues would primarily reflect the effect of vitamin intake on the maternal, placental, and fetal lipid metabolism. The influence of the gestational period remained even though the offspring started to eat the AIN-93G diet with the regular content of vitamins by their third week of lactation. Furthermore, both males and females received the same diet (same dam's milk and diet); but only the male offspring of HV dams showed lower concentration of FAs in adipose tissue, particularly n-6, thus suggesting that gestational diet altered the offspring FA metabolism in a sex-dependant manner.

Table 5
At 48 weeks PW, gestational diet and sex influence FA concentrations (milligrams per gram tissue) of liver phospholipids in the offspring

Fatty Acid	Males		Females	
	From RV	From HV	From RV	From HV
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
14:0	0.8 ± 0.21	0.4 ± 0.12	2.9 ± 2.10	0.8 ± 0.21
16:0	3.8 ± 0.12	3.5 ± 0.15	2.6 ± 0.12	2.8 ± 0.10†
18:0	4.5 ± 0.52	4.5 ± 0.62	6.9 ± 0.37	6.4 ± 0.22†
Saturates[a]	9.2 ± 0.42	8.6 ± 0.80	12.6 ± 2.08	10.2 ± 0.34†
141 c9	ND	ND	ND	ND
161 c9	0.3 ± 0.03	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.03
181 c9	0.8 ± 0.04	0.7 ± 0.03	0.7 ± 0.07	0.6 ± 0.03†
181 c11	0.7 ± 0.06	0.5 ± 0.04	0.4 ± 0.04	0.4 ± 0.03†
Monounsaturates[a]	1.8 ± 0.11	1.4 ± 0.06	1.3 ± 0.11	1.3 ± 0.09*†
18:2 n-6	1.9 ± 0.12	1.4 ± 0.09	1.4 ± 0.27	1.1 ± 0.10*†
20:3 n-6	0.3 ± 0.04	0.2 ± 0.02	0.2 ± 0.03	0.2 ± 0.03*
20:4 n-6	5.7 ± 0.18	5.4 ± 0.29	5.9 ± 0.28	5.7 ± 0.19
22:5 n-6	0.04 ± 0.01	0.1 ± 0.02	0.1 ± 0.05	0.2 ± 0.03†
Sum n-6[a]	8.0 ± 0.15	7.2 ± 0.28	7.7 ± 0.34	7.2 ± 0.24*
18:3 n-3	0.01 ± 0.01	ND	ND	ND
20:5 n-3	0.05 ± 0.02	0.01 ± 0.01	0.1 ± 0.04	0.04 ± 0.02
22:6 n-3	1.8 ± 0.10	2.03 ± 0.07	2.0 ± 0.27	2.2 ± 0.16
Sum n-3[a]	2.0 ± 0.09	2.2 ± 0.08	2.2 ± 0.22	2.4 ± 0.14
n-6/n-3	4.1 ± 0.18	3.3 ± 0.17	3.5 ± 0.39	3.1 ± 0.19*
Total (mg/g)[a]	21.1 ± 0.56	19.4 ± 1.07	23.9 ± 2.26	21.1 ± 0.66*†

Males: RV n = 9, HV n = 7; females: RV n = 4, HV n = 7. ND indicates not detected.
P less than .05 for *gestational diet effect and †sex of the pup effect, by 2-way ANOVA.
[a] Includes other minor FAs not reported.

Table 6
At 48 weeks PW, gestational diet and sex influence FA concentration (micrograms per gram tissue) of brain phospholipids in the offspring

Fatty Acid	Males		Females	
	From RV	From HV	From RV	From HV
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
PI				
16:0	92.7 ± 5.9	120.7 ± 10.5	130.7 ± 30.9	87.5 ± 4.9‡
18:0	407.4 ± 38.4	384.2 ± 27.3	700.4 ± 86.4	420.7 ± 55.1*†‡
Saturates[a]	502.9 ± 41.1	509.5 ± 32.0	855.3 ± 130.0	508.2 ± 57.8*†‡
16:1	ND	ND	ND	ND
18:1 c9	133.3 ± 10.5	147.2 ± 8.8	228.2 ± 60.4	137.8 ± 15.7‡
18:1 c11	25.3 ± 2.4	33.8 ± 2.8	42.6 ± 12.0	18.3 ± 6.2‡
Monounsaturates[a]	180.2 ± 19.7	206.6 ± 10.7	316.7 ± 94.5	175.4 ± 32.4‡
18:2 n-6	2.6 ± 1.8	2.9 ± 1.9	7.2 ± 4.0	ND
20:3 n-6	ND	ND	ND	ND
20:4 n-6	323.7 ± 21.5	347.2 ± 33.2	489.4 ± 48.9	321.7 ± 39.4‡
22:4 n-6	20.7 ± 6.3	15.3 ± 2.9	54.6 ± 7.7	24.9 ± 8.3*†‡
22:5 n-6	ND	ND	ND	ND
Sum n-6[a]	347.0 ± 25.5	365.5 ± 33.3	551.3 ± 59.2	346.5 ± 45.7*†‡
18:3 n-3	ND	ND	4.5 ± 4.5	ND
20:5 n-3	ND	ND	ND	ND
22:6 n-3	98.8 ± 23.1	67.0 ± 6.4	247.0 ± 31.6	119.5 ± 30.7*†
Sum n-3[a]	98.8 ± 23.1	73.9 ± 11.0	251.6 ± 35.6	119.5 ± 30.7*†‡
n-6/n-3 ratio	5.12 ± 1.25	5.42 ± 0.69	2.23 ± 0.19	3.92 ± 1.39
PE				
16:0	254.4 ± 31.5	291.7 ± 44.5	197.4 ± 3.4	273.5 ± 30.2
18:0	642.9 ± 76.3	864.7 ± 183.1	508.5 ± 6.0	724.2 ± 79.8
Saturates[a]	1117.5 ± 133.5	1437.5 ± 294.1	888.7 ± 15.5	1234.2 ± 137.6
16:1	12.9 ± 2.5	17.2 ± 1.9	10.7 ± 0.7	16.5 ± 1.9*
18:1 c9	725.8 ± 86.0	1137.8 ± 285.7	536.0 ± 12.2	771.0 ± 85.4*
18:1 c11	137.5 ± 15.9	217.0 ± 54.8	99.8 ± 4.1	137.8 ± 16.1
Monounsaturates[a]	1192.0 ± 131.5	1846.9 ± 412.8	893.0 ± 20.4	1248.9 ± 129.1*
18:2 n-6	25.5 ± 3.4	28.0 ± 3.4	16.9 ± 1.7	23.1 ± 2.6
20:3 n-6	16.5 ± 1.9	25.7 ± 6.1	13.5 ± 1.4	19.4 ± 2.0*
20:4 n-6	451.1 ± 49.8	616.9 ± 131.5	354.3 ± 8.2	508.7 ± 56.1*
22:4 n-6	190.9 ± 21.2	307.5 ± 81.5	151.1 ± 2.7	216.7 ± 24.4*
22:5 n-6	19.3 ± 2.4	22.7 ± 3.8	16.2 ± 0.9	25.1 ± 3.6
Sum n-6[a]	716.4 ± 79.0	1016.3 ± 227.5	561.1 ± 13.2	803.0 ± 88.3*
18:3 n-3	37.2 ± 3.6	68.6 ± 21.7	25.6 ± 0.7	32.9 ± 4.3†
20:5 n-3	ND	ND	ND	ND
22:6 n-3	580.0 ± 65.9	851.0 ± 202.1	497.1 ± 11.8	697.9 ± 76.0*
Sum n-3[a]	640.4 ± 72.6	945.5 ± 223.1	542.7 ± 9.0	756.3 ± 81.0*
n-6/n-3 ratio	1.12 ± 0.01	1.09 ± 0.02	1.03 ± 0.02	1.06 ± 0.01†‡

Males: RV n = 9, HV n = 7; females: RV n = 4, HV n = 7.
P less than .05 for *gestational diet effect and †sex of the pup effect, by 2-way ANOVA.
[a] Includes other minor FAs not reported.

Moreover, after the pups were weaned to the obesogenic diet, which provided a higher content of saturates (35%) and lower n-6 (33.7%) and n-3 (6.7%) with traces of their elongated products than the AIN-93G diet, the gestational diet remained a factor in determining the FA concentration of their tissue. The FA of the diet is known to be reflected in tissues such as muscle [27], but this alone did not explain the differences in their elongated products of n-6 and n-3. Whereas, in RV males, the products from the precursor linoleic acid accounted for 37% of the total n-6, in the HV group, they accounted for only 23%. This suggests that the elongation and desaturation processes or the oxidation rate may have been altered by the gestational diet.

Functional changes in the peripheral tissues due to the HV diet may also be predicted from the phospholipid content of the tissues. We have reported that high vitamin intakes during pregnancy led to impairment of glucose metabolism [23] in male offspring consistent with the observed lipid composition of their peripheral tissues, as reported here. Phospholipids are mainly found in tissue cell membranes, and their FA composition modulates cell physiology [28-30]. For instance, studies in cell culture have shown that muscle cells treated with EPA (20:5 n-3) have higher uptake of glucose and FAs. Treatment with EPA has been shown to increase glucose transport more than 2-fold in the presence of insulin, demonstrating that long-chain FAs contribute to

glucose uptake [29]. Furthermore, a lower concentration of PUFA is associated with lower insulin sensitivity in skeletal muscle [31]; and specifically, lower content of n-3 PUFA in membrane phospholipids is associated with reduced insulin sensitivity [32-34]. The male offspring (at 12 weeks) of HV dams had higher concentration of saturates and monounsaturates and higher n-6 FA in muscle. Although the n-6/n-3 ratio was not significantly different, the HV offspring tended to have higher values ($P = .078$). The offspring of HV dams also had higher total fat (milligrams per gram tissue) in the muscle. Both a lower proportion of n-3 FA and higher intramuscular fat are related to insulin resistance [33] and support earlier observations that male HV offspring have impaired glucose metabolism [23].

The observed effects of the gestational diet are likely to result from its impact on in utero development of FA metabolism. However, the mechanism by which programming of lipid metabolism occurred in utero is unknown at present; but there are several possibilities. Both epigenetic regulation of transcriptional factors and its activation may provide a possible explanation [35]. Epigenetic regulation of gene expression is known to occur through changes in DNA methylation [16,36], as observed in the offspring of animals supplemented with high amounts of folic acid and group B vitamins during pregnancy [15,36-38]. Methylation of genes encoding transcriptional factors such as PPARs is also possible [20], thus altering the expression of other genes encoding enzymes involved in lipid and glucose metabolism [39]. The PPARs are also regulated by vitamin A derivatives, which activate nuclear receptors such as retinoic acid receptor and retinoid X receptor [18]. Thus, the high dietary intake of vitamin A may also have contributed to the activation of these transcriptional factors and an interaction of retinoids with the 1-carbon metabolism [40]. However, we did not measure gene expression at any time point; therefore, further research is needed to investigate this potential mechanism.

Another mechanism that has been proposed for in utero programming of metabolism is increased glucocorticoid concentrations [41-44]. Glucocorticoids have been shown to increase FA synthase expression [45], to mediate the programming effects of gestational diet on muscle and adipose tissue [46], and to mediate the effect of folic acid on the FA composition of brain in the context of a protein-deficient diet [47]. However, as previously reported [23], the corticosterone concentrations of the offspring from HV dams were not different from RV dams, suggesting that the observed changes in tissue FA concentration and composition in the offspring were not mediated by glucocorticoids.

Our results confirm previous studies reporting the influence of sex in tissue lipid profile [47,48]. The earliest that we observed an effect of the sex of the offspring in the FA concentration of tissues in the offspring was at weaning because the pups were not sexed at birth. Mechanisms mediating sex differences in FA metabolism may involve sexual hormones [49], which also modulate the expression of hepatic transcriptional and fat oxidation rate [50]. Furthermore, hepatic clearance of FAs is faster in females, probably reflecting a higher concentration of FA transporters [51-53].

Fatty acid concentration of tissues also showed age-related changes. Adipose tissue shows a decrease of saturates and increase of monounsaturates with aging [54], and a similar age-related increase in monounsaturated FAs has been also reported in liver [55]. These reports are consistent with our observations that monounsaturates concentration was found to be higher in aged rats and that both groups followed similar pattern, thus confirming that this is probably an age-related effect. Others have found that gestational diet affects lipid metabolism in an age-dependant manner [56]. In our study, liver FA concentration was different by gestational diet at birth and at 48 weeks, whereas muscle only showed differences at 12 weeks PW. Fatty acid concentration of brain also showed differences at 48 weeks but not at 12 weeks PW, the 2 time points in which it was evaluated. However, the results from the offspring at 48 weeks PW must be interpreted with caution because the sample size was reduced at this time point.

The high-vitamin gestational diet had long-lasting effects on FAs concentrations in muscle, liver, and brain of the offspring, which may affect insulin sensitivity in peripheral tissues and possibly brain development and function [7,31,57-64]. Thus, changes in FA concentrations in these tissues may have long-term consequences for the health of the offspring. This is important in the context of high vitamin intakes observed in developed countries. The use of multivitamin supplements during pregnancy is a common practice; and many foods are fortified with vitamins, thus increasing the likelihood of intakes higher than current recommendations. A study in pregnant women in Boston reported vitamin intakes that exceed 2 to 5 times the recommendations in the upper quartile for several vitamins, reaching levels that border or exceed the upper limit for several vitamins, including folic acid [65]. Recently, a study in humans showed that high-folate and low-B12 status in mothers was related to higher adiposity and risk for insulin resistance in their children [66]. Clearly, the effects of high intakes of vitamins during pregnancy by humans require further study.

We fed the offspring with an obesogenic diet. However, it may be that the effects on the offspring of the high vitamin intake during pregnancy would be ameliorated if they were fed a high-vitamin diet, as is proposed by the predictive adaptive hypothesis [67]. However, whether the effects of the gestational diet are modulated by exposure of the offspring to a high-vitamin diet deserves further investigation.

The vitamin content of the HV diet was high but less than toxic levels [68]. Although high amount of retinoic acid is teratogenic, the dose of vitamin A used in the HV diet was 8 to 10 times lower than the dose known to cause congenital malformations in mice [69,70]. Furthermore, the vitamin mix contains *all-trans*-retinyl palmitate, a form of vitamin A less toxic and less teratogenic than retinoic acid [68]. Vitamins

involved in the 1-carbon metabolism (folic acid, B12) have been used in amounts 9 and 60 times the amount of control diets without an effect on litter size or weight [15]. Folic acid, however, when provided 20-fold the recommended amount during pregnancy, was found to decrease birth weight and length in Wistar rats [71]. We did not observe differences in birth weight or litter size with our 10-fold multivitamin diet.

In summary, high intake of multivitamins during pregnancy resulted in altered tissue FA concentration and composition in the offspring, suggesting that the diet during pregnancy has long-lasting effects in the lipid metabolism of the offspring. Furthermore, the effect is dependent on the sex and age of the offspring, and can vary between and within the tissues. However, the mechanisms underlying these effects of the gestational diet are still unclear. On the basis of these findings, further investigations are warranted.

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