High multivitamin intake by Wistar rats during pregnancy results in increased food intake and components of the metabolic syndrome in male offspring

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Szeto IM, Aziz A, Das PJ, Taha AY, Okubo N, Reza-Lopez S, Giacca A, Anderson GH. High multivitamin intake by Wistar rats during pregnancy results in increased food intake and components of the metabolic syndrome in male offspring. Am J Physiol Regul Integr Comp Physiol 295: R575–R582, 2008. First published June 4, 2008; doi:10.1152/ajpregu.90354.2008.—The effect of high multivitamin intake during pregnancy on the metabolic phenotype of rat offspring was investigated. Pregnant Wistar rats (n = 10 per group) were fed the AIN-93G diet with the recommended vitamin (RV) content or a 10-fold increase [high vitamin (HV) content]. In experiment 1, male and female offspring were followed for 12 wk after weaning; in experiment 2, only males were followed for 28 wk. Body weight (BW) was measured weekly. Every 4 wk, after an overnight fast, food intake over 1 h was measured 30 min after a gavage of glucose or water. An oral glucose tolerance test was performed every 3–5 wk. Postweaning fasting glucose, insulin, ghrelin, glucagon-like peptide-1, and systolic blood pressure were measured. No difference in the phenotype of the Avy mice offspring and in histone modification (10). These effects are observed in a transgenic obese model, but nothing is known about the impact of micronutrient supplementation on phenotypic expression in a rodent model with no known genetic defects.

The relevance of these observations in rodents to humans is unclear, but bioactive compounds are consumed intentionally and unintentionally. The importance of vitamin-adequate diets in the prevention of birth defects before and during pregnancy is well known; therefore, multivitamins are recommended, leading to increased intake during pregnancy by the majority of women (29). However, unintentional consumption of bioactive compounds also occurs. An example is glycyrrhetinic acid, an active constituent of licorice that has been shown to lower the activity of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in the human placenta (3, 9). As a result, exposure of the fetus to gestational glucocorticoid is increased, and in utero development of the hypothalamic-pituitary-adrenal (HPA) axis is altered (42, 47, 48). Associated with chronic licorice consumption are a lower birth weight and a shorter gestation period, along with hypertension and hyperglycemia in adult life, in rats and humans.

The possibility of unintentional excess consumption of vitamins during pregnancy by women can be suggested for three reasons. 1) Multivitamins are the most popular supplement consumed in developing and developed countries (29). A recent survey of intakes by pregnant women in Boston, MA, found intakes by those in the upper third quartile to be two to seven times the Recommended Dietary Allowance for 10 vitamins (21). As a result, the intakes of some vitamins exceed the daily upper intake levels set by the National Academy of Sciences (12), and a chronic excess of intake may be occurring, with long-term consequences for the developing fetus (37). 2) One of the consequences may be a predisposition of the children to obesity and the metabolic syndrome. Thus it is of interest that increased use of multivitamin and other supplements has occurred (29) concurrently with the increased prevalence of obesity in the last three decades (13). 3) High intakes of vitamins involved in methyl group metabolism by pregnant viable yellow agouti (A+Y) mice alter expression of the hypothalamic orexigenic agouti-related protein in the offspring (56), suggesting that the development of food intake regulation was affected. Therefore, we hypothesized that high vitamin intake by the Wistar rat during pregnancy would affect the regulation of food intake, body weight, and metabolic phenotype of the offspring.

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MATERIALS AND METHODS

Animals and diets. First-time pregnant Wistar rats were purchased from Charles River (Montreal, QC, Canada). On arrival, at day 3 of pregnancy, they were housed individually in ventilated plastic transparent cages with bedding in a 12:12-h light-dark cycle (lights on at 0600) at 22 ± 1°C. An automated water system provided free access to water. The University of Toronto Animal Care Committee approved the protocols and maintenance of the animals in conformance with the guidelines of the Canadian Council on Animal Care.

From day 3 of pregnancy to full term, dams were fed the AIN-93G diet (38), which contains the recommended vitamin (RV) level, or the AIN-93G diet + AIN-93 vitamin mix to achieve 10 times the RV level [high vitamin (HV) content]. The composition (in g/kg) of the AIN-93G diet was 200 casein, 529.4 cornstarch, 100.1 sucrose, 70 soybean oil, 50 cellulose, 10 vitamin mixture, 35 mineral mixture, 2.5 choline bitartrate, and 0.01% tert-butyl hydroquinone. Cornstarch, high-protein casein (87%), and cellulose were purchased from Harlan Teklad (Madison, WI). Vitamin mixture, mineral mixture, choline bitartrate, and tert-butyl hydroquinone were purchased from Dyets (Bethlehem, PA), sucrose from Allied Food Service (Toronto, ON, Canada), and soybean oil from Loblaw’s (Toronto, ON, Canada). Each 10 g of vitamin mix contains 9.75 g of sucrose as a carrier. Therefore, in the HV diet, we reduced the added sucrose to 9.5 g to adjust for the 97.5 g from the vitamin mix. The vitamin additions used in the HV diet were well below those expected to have a teratogenic effect and half the lowest intake of vitamins known to have an adverse effect in rats (1, 49).

During lactation, dams were fed the RV diet. At weaning, pups were housed individually in ventilated plastic transparent cages with bedding. Pups in experiments 1 and 2 were weaned to the RV diet. The powdered diet was provided in stainless steel cups; each cup was equipped with a mesh disk insert to reduce spillage. All gestational and pup diets were provided ad libitum in experiments 1 and 2.

Design. The study consisted of two experiments. In both experiments, two groups of pregnant female rats (n = 10 per group) were fed the RV or HV diet from the day of pregnancy until labor. At birth, each litter was culled to 10 pups to minimize the difference in milk availability in each litter. Litters of <10 pups were excluded. Two litters were excluded in each experiment: one from the control dams and one from the HV dams (n = 5 and 8 pups per litter, respectively). During lactation, the dams were fed only the RV diet. At weaning, male and female (experiment 1) and male (experiment 2) offspring from the RV and HV diets were fed the RV diet. Groups of 10 (1 pup per dam) were formed for each of the dependent measures.

Experiment 1: effect of multivitamin supplementation during pregnancy on body weight, food intake, and glucose tolerance of the offspring to 12 wk after weaning. Body weight was measured weekly from weaning to 12 wk after weaning. Twenty-four-hour food intake was measured once every 3 wk. Fat pad mass [FPM: abdominal + perirenal + epididymal (males only) fat pads] was measured at death at 12 wk after weaning.

An oral glucose tolerance test (OGTT) was performed at 0, 3, 6, and 9 wk after weaning. Rats were fasted overnight for 10 h before the OGTT. At the beginning of the OGTT, a blood sample was withdrawn from the capillary bed of the tail tip, and baseline glucose was immediately measured using a commercial glucometer (MediSense Precision Xtra). The rats were then gavaged (0.375 g glucose/ml, 5 g glucose/kg body wt), and blood glucose concentrations were determined 15, 30, and 60 min later.

Experiment 2: effect of multivitamin supplementation during pregnancy on regulation of food intake and appearance of components of the metabolic syndrome in male offspring to 28 wk after weaning. The objective of experiment 2 was threefold. 1) We aimed to reproduce observations from experiment 1 showing higher food intake, body weight, and adipose tissue and development of glucose intolerance in the male offspring from HV dams. 2) We measured hormones [ghrelin, glucagon-like peptide-1 (GLP-1), and insulin] that are known to regulate food intake (30) at weaning and at 14 wk after weaning to derive physiological evidence of altered regulation of food intake in offspring from HV dams. 3) We measured body weight, insulin resistance, and blood pressure to 28 wk after weaning to provide further evidence that the rats had developed components of the metabolic syndrome (2, 46).

Body weight was measured at birth (day 1, after litters were culled to 10 pups) and on days 7, 14, and 21. At weaning, 20 rats (10 per mother group) were killed, and 20 offspring from each gestational group were assigned to the RV diet. At 14 wk after weaning, 10 rats from each gestational group were killed. The remaining 10 rats per group were maintained to 28 wk after weaning. Postweaning body weight was measured weekly, and 24-h food intake was measured every 3–28 wk after weaning. An OGTT was performed every 4–5 wk to 28 wk after weaning.

A 1-h food intake assessment was conducted every 4 wk for 20 wk after weaning. After a 10-h overnight fast, rats were randomly assigned to be gavaged with a glucose preload (0.375 g glucose/ml, 5 g glucose/kg body wt) or a water preload. At 30 min after the gavage, food intake was measured for 1 h. After a washout day, rats were again fasted for 10 h overnight and gavaged with the opposite preload; 30 min after the gavage, food intake was measured for 1 h.

Systolic and diastolic blood pressure (SBP and DBP, mmHg) were measured 24 and 28 wk after weaning by a noninvasive, light-based indirect blood pressure monitor (BP-2000 Series II, Visitech Systems, Apex, NC) (21). Rats were acclimatized daily to the device, beginning 5 days before the measurement. On the day of measurement, between 1000 and 1300, five mock measurements preceded a series of 10 measurements, of which only the latter were averaged to produce the blood pressure values.

At weaning and 14 and 28 wk after weaning, plasma samples were collected. Plasma glucose was measured from trunk blood obtained immediately via the neck opening upon decapitation using a plasma-compatible glucose oxidase kit (Ascensia Elite XL, Bayer). Plasma insulin, ghrelin (total), and corticosterone were determined using radioimmunoassay kits (catalog nos. RI-13K and GHRT-89HK, Linco Research, St. Charles, MO; catalog no. 07-120103, MP Biomedicals, Orangeburg, NY). Active GLP-1 concentration in plasma was determined using an enzyme-linked immunosorbent assay (catalog no. EGPL-35K, Linco Research).

Statistical analysis. For analysis of the individual times of measurement, cumulative food intake was calculated as the sum of the five 24-h food intake measurements. For the 1-h food intake assessments, food intake after the water and glucose preload, as well as the difference (Δ) in food intake after preloads, was analyzed. For the OGTT, the blood glucose response was calculated as the net incremental area under the curve (iAUC) of the blood glucose concentration from 0 (fasting) to 60 min after the glucose gavage. The insulin resistance index was calculated as fasting glucose multiplied by fasting insulin (8). In experiment 2, from weaning to 14 wk after weaning, 2 male pups from the same dam were included in each group for a total of 20 pups per group. However, body weights of pups born to the same dam were averaged to obtain one value for the litter, as recommended previously (55).

Treatment effects on body weight, 24-h and short-term food intake, glucose response, SBP, and DBP in the offspring were analyzed using the PROC MIXED procedure in SAS (version 9.1, SAS Institute, Cary, NC) with gestational diets and age (postweaning period) as main factors in both experiments. In addition, the unpaired r-test was used to compare the means for the dependent measures at each of the postweaning time points. Cumulative 24-h food intake, fasting glucose, insulin, ghrelin, corticosterone, GLP-1, and FPM between the groups were analyzed by unpaired r-test. Significance of difference was considered if P < 0.05. Values are means ± SE.
RESULTS

Experiment 1: effect of multivitamin supplementation during pregnancy on body weight, food intake, and glucose tolerance of the offspring to 12 wk after weaning. Gestational diet did not affect litter size at birth (13.2 ± 0.5 and 13.0 ± 0.5 pups per litter in RV and HV groups, respectively), but at weaning, body weight of males from the HV dams was 4% lower than body weight of males from the RV dams: 69.5 ± 1.0 vs. 72.1 ± 0.9 g (P < 0.05). Similarly, at weaning, body weight of females from the HV dams was 4% lower than body weight of females from the RV dams: 63.9 ± 1.0 vs. 66.8 ± 1.3 g (P < 0.05). Gestational diet (P < 0.05) and age (P < 0.0001) affected postweaning body weight in male offspring, and there was a significant gestational diet × age interaction (P < 0.01; Fig. 1A). Beginning at 10 wk after weaning, body weight of males from HV dams was higher (P < 0.05), and at 12 wk after weaning, males from the HV dams weighed 6% more than males from the RV dams: 559.7 ± 13.9 g vs. 530.6 ± 7.4 g (P < 0.05). Age (P < 0.0001), but not gestational diet, affected postweaning body weight in female offspring (Fig. 1B).

Gestational diet (P < 0.05) and age (P < 0.0005) affected 24-h food intake in male offspring, but there was no gestational diet × age interaction (Fig. 2A). In males from dams fed the HV diet, 24-h food intake was higher at 1 wk (16.7 ± 0.9 g, P < 0.05) and 12 wk (31.4 ± 1.1 vs. 27.7 ± 1.3 g, P < 0.05) after weaning. In males from HV dams, cumulative 24-h food intake was 10% higher from weaning to 12 wk after weaning: 138.1 ± 3.1 vs. 125.5 ± 3.7 g (P < 0.05). Age (P < 0.0001), but not gestational diet, was a significant factor in 24-h food intake in female offspring (Fig. 2B). Cumulative 24-h food intake from weaning to 12 wk after weaning was not statistically different in females from RV or HV dams: 117.2 ± 4.8 vs. 121.3 ± 3.7 g.

A significant interaction between gestational diet and age was observed for postweaning blood glucose response (P < 0.01; Table 1) in the male, but not the female, offspring. At 9 wk after weaning, the blood glucose response (IAUC) was 69% higher during the OGTT in males from the HV dams than in males from the RV dams (P < 0.05), and at 12 wk after weaning, FPM was 18% greater in males from the HV dams than in males from the RV dams (P < 0.05; Table 2).

Experiment 2: effect of multivitamin supplementation during pregnancy on regulation of food intake and appearance of components of the metabolic syndrome in male offspring to 28 wk after weaning. Gestational diet did not affect litter size (13.0 ± 0.6 and 13.5 ± 0.5 pups per litter in RV and HV groups, respectively) or body weight at birth of the offspring from RV and HV dams (8.3 ± 0.3 vs. 8.3 ± 0.2 g, n = 10 means per dam group). Gestational diet (P < 0.01) and age (P < 0.0001) affected postweaning body weight in male offspring, and there was a significant gestational diet × age interaction (P < 0.05; Fig. 1C). Body weight was 5% lower in male pups from the HV dams than in male pups from the RV dams at weaning (68.2 ± 1.0 g vs. 72.2 ± 1.6 g, P < 0.05) and 2 wk after weaning (168.7 ± 2.0 g vs. 181.0 ± 2.1 g, P < 0.01). Beginning at 11 wk after weaning, body weight was higher in offspring from the HV dams than in offspring from the RV dams (P < 0.05), and at 28 wk after weaning, offspring from the HV dams weighed 8% more (782.4 ± 14.0 g vs. 725.0 ± 19.7 g, P < 0.05; Fig. 1C).

Fig. 1. A: postweaning body weight of male offspring (experiment 1). RV, recommended-vitamin diet; HV, high-vitamin diet. Values are means ± SE (n = 14 per group). Gestational diet (P < 0.05) and age (P < 0.0001) affected postweaning body weight, and there was a significant gestational diet × age interaction (P < 0.01). *P < 0.05 (by unpaired t-test). B: postweaning body weight of female offspring (experiment 1). Values are means ± SE (n = 14 per group). Age (P < 0.0001), but not gestational diet with no interaction, led to higher postweaning body weight. C: postweaning body weight of male offspring (experiment 2). Values are means ± SE (n = 10 means per group from week 1 to week 14, where each mean is average weight of 2 pups from the same litter). At 14 wk after weaning, 1 rat from each litter was killed, leaving 10 pups per group from week 15 to week 28. Gestational diet (P < 0.01) and age (P < 0.0001) affected postweaning body weight, and there was a significant gestational diet × age interaction (P < 0.05). *P < 0.05 (by unpaired t-test).
Gestational diet (P < 0.05) and age (P < 0.0005) affected 24-h food intake in male offspring, but there was no gestational diet × age interaction (Fig. 2C). Twenty-four-hour food intake was higher at 1 wk (15.4 ± 0.4 vs. 13.4 ± 0.4 g, P < 0.01), 12 wk (32.3 ± 1.1 vs. 30.0 ± 0.8 g, P = 0.07), 18 wk (35.2 ± 1.1 vs. 31.7 ± 0.9 g, P < 0.05), and 28 wk (30.8 ± 1.0 vs. 28.2 ± 1.1 g, P < 0.05) after weaning in male offspring from the HV dams than in male offspring from the RV dams. Cumulative 24-h food intake was 7% higher in males from the HV dams than in male offspring from the RV dams.

Table 1. Blood glucose response during the OGTT in the postweaning period

<table>
<thead>
<tr>
<th>iAUC, min·mM</th>
<th>RV Diet</th>
<th>HV Diet</th>
<th>P</th>
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<tr>
<td>Week 1</td>
<td>151 ± 12</td>
<td>169 ± 15</td>
<td>NS†</td>
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<td>Week 3</td>
<td>130 ± 10</td>
<td>117 ± 16</td>
<td>NS‡</td>
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<tr>
<td>Week 6</td>
<td>98 ± 16</td>
<td>97 ± 14</td>
<td>&lt;0.01§</td>
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<td>Week 9</td>
<td>95 ± 8</td>
<td>161 ± 16†</td>
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Table 2. Fat pad mass at 12 wk (experiment 1) and 14 wk (experiment 2) after weaning

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<tr>
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<tr>
<td>Body weight</td>
<td>530.6 ± 7.4</td>
<td>559.7 ± 13.3*</td>
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<tr>
<td>Fat pad mass</td>
<td>29.4 ± 2.0</td>
<td>34.7 ± 1.6*</td>
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<tr>
<td>Body weight</td>
<td>330.4 ± 8.4</td>
<td>315.2 ± 6.7</td>
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<tr>
<td>Fat pad mass</td>
<td>18.9 ± 2.0</td>
<td>15.6 ± 1.0</td>
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<tr>
<td>Body weight</td>
<td>564.2 ± 11.3</td>
<td>607.0 ± 7.7*</td>
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<tr>
<td>Fat pad mass</td>
<td>37.7 ± 3.9</td>
<td>48.0 ± 2.9*</td>
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Values are means ± SE in g; n = 9–10 per group. Fat pad mass = abdominal + epididymal (only in males) + perirenal fat pads. *P < 0.05 (by unpaired t-test).
more after the glucose preloads at 4 wk (P < 0.05) and 12 wk (P < 0.05) after weaning. Although the response to the glucose preload (Δ) was not affected by gestational diet or age and there was no interaction, post hoc analysis at 20 wk after weaning by unpaired t-test indicated a greater response to the glucose load, perhaps because of the higher intake of males from the HV dams after the water preload. The 1-h food intake was lower after the glucose preload than after the water preload in both groups (P < 0.005).

Gestational diet did not affect fasting glucose and insulin concentration at weaning (Table 4). However, fasting ghrelin and GLP-1 of offspring from the HV dams were 27% (P < 0.05) and 32% (P < 0.05) higher, respectively. At 14 wk after weaning, FPM (Table 2), fasting glucose, insulin, insulin resistance index, and ghrelin were 27% (P < 0.05), 11% (P < 0.05), 62% (P = 0.07), 80% (P < 0.05), and 41% (P < 0.005) higher (Table 4). At 28 wk after weaning, fasting ghrelin of offspring from HV dams was 32% (P < 0.005) lower. No difference was observed for fasting corticosterone at 0, 14, and 28 wk after weaning, and there was no difference for fasting insulin and insulin resistance index at 28 wk after weaning.

Gestational diet (P < 0.01) and postweaning age (P < 0.001) interacted (P < 0.01) in their effect on the blood glucose response during the OGT (Table 1). Response decreased with age in males from the RV, but not the HV, dams. At 23 and 28 wk after weaning, the blood glucose response (iAUC) of male offspring from the HV dams was 46% (P < 0.05) and 47% (P < 0.05) higher (Table 1). Maternal diet (P < 0.05), but not age, affected SBP (Table 5). At 24 and 28 wk after weaning, SBP of male offspring from the HV dams was 5% (P < 0.05) and 8% higher (P < 0.05), respectively.

**DISCUSSION**

The results of the present study show that high multivitamin intakes during pregnancy in Wistar rats resulted in increased food intake, body weight, FPM, and insulin resistance and elevated blood pressure in male offspring fed a diet that is not known to be obeseogenic. Fetal programming of obesity and metabolic disease during gestational nutrient deprivation is well recognized (4, 5, 53, 54), but these results are the first to show that vitamins alone, albeit at high levels during pregnancy, potentially program the offspring for increased food intake, obesity, and metabolic disease. Furthermore, it can be suggested that the phenotype was driven by in utero programming of the regulation of intake control, predisposing the offspring to eat excess food.

The increase in body weight and the expression of components of the metabolic syndrome may be due to the effect of the
HV diet on the early development of intake regulatory mechanisms in the central nervous system. HV intake during pregnancy produced male rats that were hyperphagic in early life, as shown by the higher 24-h food intake at 1 wk after weaning in both experiments. Furthermore, higher fasting ghrelin and GLP-1 levels were found at weaning. Ghrelin is an orexigenic hormone (30), and its higher concentration in blood at weaning suggests that offspring from HV dams may be programmed to increase food intake from an early age. Fasting concentrations of GLP-1, an anorexigenic hormone (30), were also higher in the offspring of HV dams at weaning.

The effect of age on the fasting plasma concentration of ghrelin and GLP-1 suggests that compensatory mechanisms were occurring that would prevent further hyperphagia. The concept of compensatory adaptation favoring reduced energy intake in hyperinsulinemia has been observed. Similar to the offspring from the HV mothers, men with elevated insulin and body mass index reduced food intake more after a glucose preload than normal insulinemic controls (41). Thus the development of insulin resistance in the HV offspring may have been a factor in their stronger response to the glucose preload (Table 3), the normalization of GLP-1, and the reduction of ghrelin at 28 wk after weaning (Table 4).

The effect of the HV diet on the phenotype of the offspring is unlikely to be attributable to a stress response of the dams. First, there was no difference in litter size and birth weight of the offspring. We chose not to employ close-monitoring techniques, including blood sampling for measurement of plasma corticosterone, because such procedures would add stress to the dams and unborn fetus. Restraining the dams only once during late pregnancy and tail prickling for a blood sample have been shown to elevate corticosterone concentration in the offspring (6). However, we recorded the body weight of the dams 1 day before delivery and 1 day after delivery in subsequent experiments and found no evidence that they were avoiding or were stressed by the HV diet. The body weights of the RV and HV dams were not different 1 day before (369.3 ± 8.7 vs. 367.7 ± 11.1 g) or 1 day after (287.5 ± 7.9 vs. 289.7 ± 8.8 g) delivery.

Additions of 8 of the 12 required vitamins in the HV diet were on average many times lower than the levels reported to cause adverse effects on the fetus, although it must be recognized that the amounts have not been fully quantified (38, 49). Folate at 10 times the recommended level in the diet was estimated to be closest to an adverse-effect level (49). Folate added at 20 times the level in the AIN-93G diet (2 mg/kg) and fed during pregnancy to Wistar rats led to lower birth weight but not litter size (1). Moreover, the HV diet was much lower in vitamins involved in methyl group metabolism than the diet used in previous studies showing the epigenetic effects of these vitamins on the viable yellow agouti mouse. Folate, vitamin B12, and choline fed at 9, 60, and 9 times levels in the control diet during pregnancy and lactation were not reported to affect litter size or weaning weights (57, 59).

The lower body weight of the pups at weaning cannot be explained, but the similar fasting plasma corticosterone levels of the two groups at weaning suggest that stress, either arising from the gestational diet or nursing behavior of the mother, was not a factor. Dams that are stressed provide less nursing or access to the milk to the pups. Pups that receive less grooming, licking, and arched-back nursing from the dams during lactation have excess HPA responses to stress as early as 7 days after birth (58). However, it is possible that milk volume or composition was in some way affected or that sucking and intake control of the offspring were factors.

The increased postweaning food intake may support the predictive adaptive response hypothesis, which assumes that in utero adaptive responses prepare the offspring for survival in a nutrient environment similar to that of the mothers (15, 16). On the basis of the predictive adaptive response hypothesis (14, 15), it would be predicted that the HV gestational diet would program the offspring to require a high-vitamin postnatal environment. There is evidence that rats have a nutrient-selective appetite that leads to increased food intake of the diets with marginal nutrient (25). If this is an explanation, then it would be predicted that, by allowing the offspring access to a high-vitamin diet similar in vitamin content to their mother’s diet, the overeating and development of the components of the metabolic syndrome would be ameliorated.

The absence of an effect of the gestational diet on the body weight and food intake of female offspring is consistent with other studies showing that the effect of the gestational diet is dependent on the sex of the offspring (18). Male offspring are more susceptible to changes in gestational nutrition (22, 50), possibly because of their faster growth rate and, thus, their more critical nutrient needs (27). Because of the absence of phenotypic changes in experiment 1, the focus was on male offspring in experiment 2. Because females have been shown to develop insulin resistance later in life than males (11) and a previously formulated obesogenic diet has been proved to accelerate the development of insulin resistance (34, 35), the effect of weaning the female offspring to the obesogenic diet needs to be explored in future studies.

The mechanism by which the HV gestational diet modified the phenotype has not been defined. However, placental transfer of vitamins would be expected to result in elevated concentrations in the developing fetus and epigenetic modification of gene expression. Increased cytosine methylation of the Aβ gene has been found after pregnant mice were fed high dietary levels of methyl donors (betaine and choline) or methyl metabolism cofactors (folate and vitamin B12) (56), which led to a change in the coat color of the offspring (57, 59). In addition, higher intake of fat-soluble vitamins not known to be involved in the gene methylation process may also contribute to the observations. Intake of vitamins A and D during pregnancy affects gene expression in the offspring (26, 51). Vitamin A, in the form of 9-cis retinoic acid, binds to retinoic X receptors, which allows for interaction with retinoic acid receptors and response elements present on particular genes (28). For example, retinoic acid has been shown to stimulate the expression of 11β-HSD2 in a trophoblast-like cell line that displays a number of functional similarities to the placental syncytiotrophoblast (52). 11β-HSD2 is a crucial enzyme that metabolizes glucocorticoids transported to the placenta from the maternal side, and lower 11β-HSD2 activity in the placenta due to stress can lead to higher exposure of the glucocorticoid to the fetus and, consequently, alter the development of the fetal HPA axis (23, 24, 43). Gestational vitamin D status also modulates gene expression. Vitamin D acts like a steroid hormone and interacts with cell membrane receptors and nuclear vitamin D receptor proteins to affect gene transcription, which affects cell differ-
entiation and proliferation and mineral homeostasis in the offspring (26).

It is also possible that the effect on the pups of the feeding high amounts of fat-soluble vitamins to the dams during pregnancy is not limited to the pregnancy period. Fat-soluble, in contrast to water-soluble, vitamins are stored in the liver and adipose tissue (7, 32, 33, 39). Mobilization and transfer through the milk to the pups in the early stage of lactation may have occurred. Also, the pups may have accumulated stores of the fat-soluble vitamins in the liver and in their very limited fat stores. This may be significant, because the newborn rat continues to undergo significant developmental changes (36, 40).

In conclusion, high multivitamin intake during pregnancy programmed the male offspring for development of the components of the metabolic syndrome in adulthood, possibly by its effects on central mechanisms of food intake control.

Perspectives and Significance

The past few decades have provided pregnant women an unparalleled opportunity to obtain an intake of vitamins in amounts that may not have been predicted from evolution. The effect of excess vitamin intakes by pregnant women on genetic/epigenetic changes that adversely affect the adaptability of the offspring to their environment remains to be determined, but there are several reasons to be concerned. 1) There is a potential for pregnant women who select a diet of foods that are rich natural sources and those that contain added vitamins and also to consume vitamin supplements to have a chronic consumption of vitamins at the levels above the daily upper intake levels set by the National Academy of Sciences (12). The high vitamin intakes at several times the requirement during pregnancy, as found in a recent survey (21), can be easily explained. Multivitamin supplements targeted to women during pregnancy contain vitamins that exceed the daily recommended intakes (29, 37). 2) In addition to an abundance of vitamin supplements, many foods and beverages consumed during pregnancy contain added nutrients, and the amounts are likely underestimated, because, by law, manufacturers must provide at least the labeled amounts of vitamins. Thus higher amounts are probable, inasmuch as manufacturers provide a surplus to ensure that they meet label specifications (31). 3) Of further concern are recent recommendations to increase intakes of specific vitamins during pregnancy. Motherrisk (Toronto) has proposed increasing the supplement dose of folic acid from 400 μg to 5 mg daily (12 times the RDA) for women of child-bearing age and/or pregnant women. Adequate intake of vitamin D for 19- to 50-y-old women is 200 IU, but the Canadian Cancer Society is recommending 1,000 IU. Much higher intakes (4,000 IU) are being suggested as needed during pregnancy on the basis of 25-hydroxyvitamin D status in plasma (17). 4) Relevant to the present study, consumption of a prenatal vitamin pill has been shown to produce a higher blood glucose response upon an oral sucrose load than consumption of a placebo pill in women with gestational diabetes (45). Gestational glycemic control affects gestational outcomes in normal and diabetic pregnant women (19, 44).

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