Impact of diet and stress on the development of preeclampsia-like symptoms in p57\(^{kip2}\) mice

Stéphanie Falcao,1,2 Crina Solomon,1,2 Caroline Monat,1 Julie Bérubé,1 Jolanta Gutkowska,3,4 and Julie L. Lavoie1,4

1Research Centre, Centre Hospitalier de l’Université de Montréal-Technopôle Angus, Montréal, 2Department of Biomedical Sciences, Université de Montréal, Montréal, 3Research Centre, Centre hospitalier de l’Université de Montréal-Hôtel-Dieu, Montréal, and 4Department of Medicine, Université de Montréal, Montréal, Québec, Canada

Submitted 17 September 2008; accepted in final form 28 October 2008

Falcao S, Solomon C, Monat C, Bérubé J, Gutkowska J, Lavoie JL. Impact of diet and stress on the development of preeclampsia-like symptoms in p57\(^{kip2}\) mice. Am J Physiol Heart Circ Physiol 296: H119–H126, 2009. First published October 31, 2008; doi:10.1152/ajpheart.01011.2008.—The cyclin-dependent kinase inhibitor p57\(^{kip2}\) regulates the cell cycle of trophoblastic cells. It has been established by a Japanese group that the heterozygous p57\(^{kip2}\) knockout (p57\(^{+/−}\)) mice are a good model of preeclampsia as they develop hypertension, proteinuria, and placental pathology. However, apart from the placental pathology, we could not observe these symptoms in our laboratory. Hence, we investigated the impact of diet and stress on this model. To do so, we compared the effects of the Japanese diet to that of the North American diet used by our animal facility. Furthermore, the impact of stress was determined by placing the mice in a restraining device before and at the end of gestation. Although the Japanese diet did not have any impact on blood pressure or proteinuria, the mice did develop endothelial dysfunction, left ventricular hypertrophy, as well as increased placental pathology. Also, all mice had smaller litters when fed the Japanese diet. However, stress response of these mice was not increased during gestation; in fact, a decrease was observed in the p57\(^{−/−}\) mice, suggesting that this was probably not a player in the development of the pathology. Taken together, these results suggest that other environmental factors may have been implicated in the development of preeclampsia-like symptoms in this model. Hence, the mechanisms underlying the pathology have not yet been elucidated, and to date there are no therapies available. Indeed, the only “treatment” for PE is the induction of delivery, which can of course be problematic if the symptoms become severe early in the third trimester. Consequently, further studies are required to increase our knowledge of this disease.

Since we are describing a maternal-fetal phenomenon, many factors may contribute to the pathology, which makes it hard to establish its exact origin. Furthermore, accumulating data indicate that PE is not a homogeneous disease similar to essential hypertension and, thus, may not always be produced by the same factors. Hence, the mechanisms underlying the pathology have not yet been elucidated, and to date there are no therapies available. Indeed, the only “treatment” for PE is the induction of delivery, which can of course be problematic if the symptoms become severe early in the third trimester. Consequently, further studies are required to increase our knowledge of this disease.

Because the emergence of PE cannot be reliably predicted, and since it needs to be controlled immediately to prevent serious complications, the investigation of this pathology is difficult in women. On the other hand, in mice, environment and genetic background can be easily controlled. Furthermore, their gestation has many common characteristics with human pregnancy (32), which makes mice an interesting model of the disease. For instance, Kanayama’s group reported that breeding mice that are heterozygote for a p57\(^{kip2}\) gene deletion (p57\(^{−/+}\)) provoked PE-like symptoms (15) such as hypertension, proteinuria, thrombocytopenia, and excess trophoblast proliferation. P57\(^{kip2}\) normally inhibits many cyclin/cyclin-dependent kinase complexes, which are implicated in the trophoblastic cell cycle regulation. Impaired control of the trophoblastic cell proliferation is a key component of the abnormal placenta observed in PE (7). Furthermore, the p57\(^{kip2}\) gene is paternally imprinted and expressed in both humans and mice (12), and the homozygous deletion of the gene is lethal (38).

The aim of this study was originally to elucidate the role of placental pathology in the development of PE-like symptoms in p57\(^{−/−}\) mice. However, the blood pressure and proteinuria changes reported by Kanayama’s group (15) could not be attempted to find nutrients involved in eliciting or protecting against PE but without major success (30). Although the data on the effect of maternal stress on the risk of preeclampsia is controversial, many studies have shown that it may contribute to the development of the disease (11, 16, 19, 21). Indeed, stress has been shown to stimulate the sympathetic nervous system, which in turn can modulate peripheral vascular resistance (33) as well as the immune system (3, 24) during pregnancy and thus could be implicated in the development of PE.

PREECLAMPSIA (PE) is the most important cause of maternal and perinatal morbidity and mortality (34). It is human pregnancy-associated syndrome diagnosed with a new appearance of hypertension and proteinuria (23), which resolve completely after delivery (31), suggesting that feto-placental factors are the main origin of the pathology. Many studies have demonstrated the significant impact of abnormal placenta in the development of PE, although maternal factors have also been found to promote the disease (4). Indeed, even though no PE gene has yet been characterized, a genetic basis for the pathology has been postulated, and familial predisposition has been implicated (34). Still, as in many cardiovascular diseases, environmental and behavioral factors may influence development of the pathology. Many studies and clinical trials have

Address for reprint requests and other correspondence: J. L. Lavoie, Centre de Recherche, CHUM-Technopôle Angus, 2901 Rachel St. East, Suite 310, Montréal, Québec, Canada H1W 4A4 (e-mail: julie.lavoie.3@umontreal.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Laboratory Diets, Madison, WI or standard Japanese laboratory chow [CA-1 Japanese (JPN); CLEA Japan, Tokyo, Japan]. Mice in these experiments were 12–15 wk of age, and their care met the standards set forth by the Canadian Council on Animal Care for the use of experimental animals. All procedures were approved by the University Animal Care Committee of the CHUM Research Centre.

To investigate the role of placental pathology in PE-like symptoms, female p57<sup>−/+</sup> or wild-type (p57<sup>+/+</sup>) mice were time-mated with identical genotype males. Indeed, Kanayama et al. (15) showed that, when p57<sup>−/+</sup> females were bred with p57<sup>−/+</sup> males, they developed PE-like symptoms.

**Blood pressure measurement.** Female mice were anesthetized with isoflurane and then implanted with TA11PA-C10 radiotelemeters (Data Sciences International, St. Paul, MN) in the left carotid artery for the direct measurement of arterial pressure (AP) and heart rate (HR), as described previously (20). The mice were given 7–10 days to recover, after which HR and AP were recorded for 3 consecutive days. Male mice where then placed in the cages for timed mating. Gestation was confirmed by the presence of a vaginal plug and was considered day 1. Starting on this day, AP and HR were measured every 2 days until pups were born. To assess the impact of immobilization stress, AP and HR were measured continuously for 1 h before and during 30-min immobilization in a restraining device (IITC Life Science, Woodland Hills, CA). This procedure was undertaken before and at day 18 of gestation.

**Proteinuria.** Urine samples were collected by briefly restraining the mice manually and directly retrieving urine in 1.5-ml tubes (6). This method avoids the unnecessary stress of placing the animals in metabolic cages for 24 h. It has been shown that mice of both sex present a significant rise in mean AP (MAP) and HR when placed in metabolic cages (13). Samples were taken in all mice before and at day 18 of gestation and they were kept frozen at −80°C until assayed. For mice being assessed by telemetry, urine was collected on days where AP was not recorded. Albumin and creatinine urinary concentrations were measured with the Albuwell and Creatinine companion mouse Elisa kits (Exocell, Philadelphia, PA), according to the manufacturer’s protocol. Each sample was thawed and diluted 1:10 before measurement in duplicate. Proteinuria was evaluated as the albumin/creatinine ratio.

**Tissue collection and histology.** On day 18 of gestation, the mice were anesthetized with ketamine/xylazine. Kidneys, heart, placentas, and pups were all weighed individually, and tails from the pups were snipped for genotyping. Kidneys and placentas were then placed overnight in 4% paraformaldehyde for fixation. The next day, they were rinsed with phosphate buffer and embedded in paraffin. Kidneys and placentas were cut cross-sectionally in a microtome. Sections were stained with HPS to assess overall renal and placental morphol-ogy. Embedding, sectioning, and staining were performed by the histology platform of the Research Institute in Immunology and Cancerology at the Université de Montréal.

Placental alterations were characterized by five criteria: necrosis, hyalinization, microcalcification, giant cell island loss, and labyrinthine trophoblast structure loss. For each criterion, changes were assigned a score from 0 to 3, where 0 was the absence of, 1 was mild, 2 was moderate, and 3 was severe alteration. Furthermore, all scores

Table 1. Characteristics of pups and placentas in p57 mice on the JPN vs. the N-A rodent diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Mother’s Genotype</th>
<th>Placental Weight, mg</th>
<th>Pup Weight, mg</th>
<th>Pups/Litter (average)</th>
<th>Nonviable Fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-A</td>
<td>p57&lt;sup&gt;−/+&lt;/sup&gt;</td>
<td>112.0±8.5</td>
<td>741.3±39.9</td>
<td>6.6±0.7†</td>
<td>1.6±0.4†</td>
</tr>
<tr>
<td></td>
<td>p57&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>99.2±1.9</td>
<td>802.9±21.6</td>
<td>9.1±0.6</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>JPN</td>
<td>p57&lt;sup&gt;−/+&lt;/sup&gt;</td>
<td>112.2±4.5</td>
<td>887.4±49.7†</td>
<td>4.0±1.2+*</td>
<td>1.7±0.9†</td>
</tr>
<tr>
<td></td>
<td>p57&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>111.7±3.2</td>
<td>662.0±37.9†</td>
<td>8.1±0.9*</td>
<td>0.0±0.0</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. N-A, North American diet; JPN, Japan diet. N values for placental and fetal weight are as follows: p57<sup>−/+</sup>-JPN = 25; p57<sup>−/+</sup>-N-A = 31; and p57<sup>−/+</sup>-N-A = 94. Statistically different from mice on the N-A diet: *P ≤ 0.05; †P ≤ 0.001. ‡Statistically different from p57<sup>−/+</sup> mice (P ≤ 0.001).
were summed up for total evaluation of the placental pathology present. The investigator scoring the placentas was blinded to the genotype of the pups and mothers to avoid any bias.

Placental expression of p57kip2. Since p57kip2 is a paternally imprinted gene (12), p57\(^{-/-}\) mice may express the gene depending on from which parent it was inherited. Hence, we determined RNA expression of p57kip2 by PCR. For placentas evaluated histologically, p57 expression was determined in the respective pups. To do so, RNA from the placentas was extracted with Trizol. Single-stranded cDNA was synthesized by reverse-transcriptase reaction with M-MLV (Invitrogen, Burlington, Ontario, Canada). Polymerase chain reaction was performed with specific primers for p57 [forward (F) 5'-TG-CACCTGAGAGCGAGTAGAGATT-3'; reverse (R) 5'-TAGAG-GCTAACGCCGAGGACA-3'] and 18s (F 5'-AAACGGCTACCATCCAAAG-3'; R 5'-CTCTCAATTGGATCTTGTA-3') using Taq polymerase (Invitrogen, Burlington).

Vessel reactivity. During tissue collection, the mesentery was removed and placed in cold Krebs solution (118.6 mM NaCl, 4.7 mM KCl, 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 25.1 mM NaHCO\(_3\), 26 μM EDTA, 0.18% glucose, 2.5 mM CaCl\(_2\)). A segment of the mesenteric artery was isolated then transferred to an arteriograph chamber filled with oxygenated Krebs and mounted on glass capillaries, as described by Takase et al. (36). The artery was then perfused intraluminally and equilibrated at constant 30-mmHg pressure for 45 min before the experiments. Vascular reactivity was studied under four protocols, and dose-response curves were charted: 1) norepinephrine (10\(^{-9}\) to 10\(^{-5}\) M), 2) acetylcholine (10\(^{-9}\) to 10\(^{-4}\) M), 3) sodium nitroprusside (SNP) (10\(^{-9}\) to 10\(^{-4}\) M), and 4) endothelin-1 (10\(^{-11}\) to 10\(^{-8}\) M). All drugs were administered extraluminally, and each protocol was separated by a 30-min washout period to allow vessel diameter to return to baseline values. Before vasodilatation measurements, the artery was precontracted by arterenol, 8M, with an appropriate amount of norepinephrine.

Drugs. The following drugs were purchased for mouse anesthesia: ketamine (Bimeda-MTC, Cambridge, Ontario, Canada), xylazine (Bayer, Toronto, Ontario, Canada) and isofluorane (Abbott, St.-Laurant, Quebec, Canada). For vessel reactivity, the following drugs were dissolved in distilled water and diluted with Krebs' solution: arterenol bitartrate salt (norepinephrine) (Sigma A-0937, Oakville, Ontario, Canada); acetylcholine chloride (Sigma A-6625, Oakville, Ontario, Canada); endothelin-1, human, porcine (Sigma E-7764), SNP (Calbiochem 567538, Mississauga, Ontario, Canada); and endothelin-1, human, porcine (Sigma E-7764).

Statistical analysis. All data are expressed as means ± SE. Two-way ANOVA was used to determine the impact of diet and genotype on most parameters. However, repeated-measures ANOVA were performed to assess the impact on vessel reactivity and the effect of immobilization stress, followed by Tukey's post hoc test when an interaction was detected. Placental pathology scores were analyzed by nonparametric Mann-Whitney U-tests.

RESULTS

AP and proteinuria were first quantified during gestation to confirm the phenotype observed by Kanayama et al. (15). To our surprise, we detected no increases in AP in p57\(^{-/-}\) mice during pregnancy. Indeed, MAP was 99 ± 2 and 92 ± 5 mmHg at baseline, and 107 ± 4 and 108 ± 7 mmHg in p57\(^{+/+}\) (n = 3) and p57\(^{-/-}\) (n = 5) mice, respectively.

Furthermore, we found no changes in proteinuria during gestation as the albumin-to-creatinine ratio ranged from 1.48 ± 0.29 and 1.57 ± 0.27 at baseline to 2.93 ± 1.4 and 3.65 ± 1.09 at day 18 of gestation in p57\(^{-/-}\) (n = 5) and p57\(^{+/-}\) (n = 6) mice, respectively. This is in contrast to the data published by Kanayama’s group who reported a 30-mmHg increase in systolic blood pressure and ∼10-fold elevation in urinary protein excretion. However, we did not note some phenotypes similar to
those reported previously. Indeed, pronounced placental pathology could be seen in placentas that did not express p57kip2 (Fig. 1). Furthermore, p57\(^{-/-}\) mothers had significantly smaller litters than p57\(^{+/-}\) mice, since they carried an average of 6.6 ± 0.7 fetuses compared with 9.1 ± 0.6 fetuses in p57\(^{+/-}\) mice (Table 1). In addition, nonviable fetuses were apparent in p57\(^{+/-}\) pregnancies, whereas this phenomenon was not present in p57\(^{+/-}\) controls (Table 1). However, we did not observe any preterm delivery in the p57\(^{-/-}\), as previously reported (15). Indeed, in our study, all mice gave birth, on average, at 20 days of gestation.

Since the mice in our study came from the same source as in Kanayama’s paper and were maintained on the same background (C57Bl/6), their genetic backgrounds were the same. This implicated an environmental factor in the differences observed in our two groups. Therefore, we investigated the role of diet in these mice. Indeed, the rodent chow fed in Japan (JPN) is very different in content from that of North America (N-A) (Table 2). Interestingly, although fat and fiber content were the same, protein content was much greater in the JPN diet: 26.8% compared with 18.9% in the N-A diet. Also, noticeably, sodium content in the JPN diet was twice that of the N-A diet (0.41 vs. 0.23 g/100 g). Hence, the potassium-to-sodium ratio was decreased in the JPN diet (2.28 vs. 2.96). In addition, folic acid content was lower in the JPN diet (0.24 vs. 0.33). We thus repeated the afore-mentioned experiments in mice born from parents fed the JPN diet. Indeed, previous papers have reported that diet may affect phenotype in specific strains of rodents (37).

Unfortunately, mice placed on the JPN diet did not exhibit any increase in MAP with pregnancy as baseline values were 95 ± 4 and 94 ± 2 mmHg, and 120 ± 7 and 111 ± 6 mmHg at the end of gestation in p57\(^{+/-}\) (n = 5) and p57\(^{-/-}\) (n = 7) mice, respectively. Furthermore, we did not detect any increase in proteinuria, as the albumin-to-creatinine ratios were 1.55 ± 0.37 and 2.44 ± 1.68 at baseline and 1.04 ± 0.21 and 5.58 ± 3.21 at day 18 of gestation for p57\(^{-/-}\) and p57\(^{+/-}\) mice, respectively. The change of diet also had no significant effect on hematocrit values (Table 3).

However, mice on the JPN diet were smaller before and at the end of pregnancy than those on the N-A diet, independently of genotype (Table 3). Furthermore, as expected, heart mass was lower (Table 4) in mice receiving the JPN diet compared with those on the N-A diet, whereas the heart-to-body weight ratio was unaffected (Table 3). Interestingly, left ventricle (LV) weight was not different in mice fed the JPN diet compared with those receiving the N-A diet, although all other compartments were significantly smaller (Table 4). Furthermore, when looking at the LV-to-body-weight ratio, we observed a significant increase with the JPN diet (Table 4) again, independently of genotype.

In addition, even though mice on the JPN diet were smaller, their kidneys were significantly larger in both p57\(^{+/-}\) and p57\(^{-/-}\); thus the kidney-to-body weight ratio was significantly increased (Table 3). However, histological analysis of the kidneys did not reveal any signs of noticeable associated pathology.

In addition, we confirmed that placental pathology was present mainly in placentas that did not express p57kip2 mRNA independently of the diet given to their mothers (Table 5). This seemed to be due to the significant presence of a labyrinthine trophoblast structure loss and microcalcification. This loss of labyrinthine structure is most probably a result of an increased trophoblastic proliferation, as has been reported previously (15). Interestingly, although total placental pathology was not increased with diet in the p57\(^{-/-}\) mice, we did observe a higher incidence of microcalcification in placentas from these mothers (Table 5). Furthermore, we did find a higher incidence of pathology in placentas expressing p57kip2 from p57\(^{+/-}\) females fed the JPN diet (Table 5).

Additionally, to better characterize the impact of diet on blood pressure regulation, we investigated the vessel reactivity of mesenteric arteries collected at the end of gestation. We noted that the JPN diet had a negative impact on endothelial function. Indeed, vessels from p57\(^{-/-}\) mice fed the JPN diet were more responsive to norepinephrine (Fig. 2) than p57\(^{+/-}\) mice as well as mice receiving the N-A diet. Furthermore, arteries from p57\(^{+/-}\) mice on the JPN diet were less reactive to

### Table 3. Characteristics of female p57 mice on the JPN diet vs. N-A rodent diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Mother’s Genotype</th>
<th>N</th>
<th>BW (Baseline)</th>
<th>BW (End of Gestation)</th>
<th>Heart-to-BW Ratio</th>
<th>Kidney-to-BW Ratio</th>
<th>Htc, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-A</td>
<td>p57(^{-/-})</td>
<td>14</td>
<td>23.2±0.9</td>
<td>35.8±1.08</td>
<td>6.08±0.48</td>
<td>7.23±0.25</td>
<td>45.5±2.0</td>
</tr>
<tr>
<td></td>
<td>p57(^{+/-})</td>
<td>15</td>
<td>23.7±0.7</td>
<td>39.4±1.3</td>
<td>6.19±0.81</td>
<td>7.08±0.18</td>
<td>43.1±5.0</td>
</tr>
<tr>
<td>JPN</td>
<td>p57(^{-/-})</td>
<td>7</td>
<td>19.4±0.55</td>
<td>30.5±2.2</td>
<td>7.04±0.25</td>
<td>9.67±0.41</td>
<td>49.5±4.8</td>
</tr>
<tr>
<td></td>
<td>p57(^{+/-})</td>
<td>7</td>
<td>21.4±0.7†</td>
<td>34.4±1.5*</td>
<td>6.41±0.21</td>
<td>9.17±0.23†</td>
<td>39.5±10.8</td>
</tr>
</tbody>
</table>

*Values are expressed as means ± SE. BW, body weight; Htc, hematocrit. Statistically different from mice on the N-A diet: *P ≤ 0.05; †P ≤ 0.005; ‡P ≤ 0.001. §Statistically different from p57\(^{-/-}\) mice (P ≤ 0.05).

### Table 4. Weight of the whole heart and compartments in female p57 mice on the JPN diet vs. N-A rodent diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Mother’s Genotype</th>
<th>N</th>
<th>Heart, mg</th>
<th>LV, mg</th>
<th>LV/BW, Ratio</th>
<th>RV, mg</th>
<th>LA, mg</th>
<th>RA, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-A</td>
<td>p57(^{-/-})</td>
<td>7</td>
<td>149±5</td>
<td>90±3</td>
<td>4.1±0.3</td>
<td>27.2±1.2</td>
<td>3.5±0.4</td>
<td>3.5±0.4</td>
</tr>
<tr>
<td></td>
<td>p57(^{+/-})</td>
<td>5</td>
<td>153±6</td>
<td>92±4</td>
<td>4.1±0.2</td>
<td>26.0±1.4</td>
<td>3.9±0.4</td>
<td>3.9±0.4</td>
</tr>
<tr>
<td>JPN</td>
<td>p57(^{-/-})</td>
<td>7</td>
<td>136±8*</td>
<td>93±4</td>
<td>4.8±0.7*</td>
<td>18.1±1.8†</td>
<td>2.9±0.3*</td>
<td>2.9±0.3*</td>
</tr>
<tr>
<td></td>
<td>p57(^{+/-})</td>
<td>6</td>
<td>137±6*</td>
<td>96±6</td>
<td>4.5±0.2*</td>
<td>22.6±1.6†</td>
<td>2.8±0.3*</td>
<td>2.8±0.3*</td>
</tr>
</tbody>
</table>

*Values are expressed as means ± SE. LA, left atria; LV, left ventricle; RA, right atria; RV, right ventricle. Statistically different from mice on the N-A diet: *P ≤ 0.05; †P ≤ 0.001.
both acetylcholine (Fig. 3A) and SNP (Fig. 3B). In addition, they were less responsive to acetylcholine than their p57<sup>−/−</sup> littermates. No effect of diet or genotype was observed in response to endothelin (data not shown).

Kanayama’s group used the tail-cuff method to assess AP, whereas we used telemetry. To investigate the impact of immobilization stress on the AP of our mice fed the JPN diet, we placed them in a restraining device before and at the end of immobilization stress. Moreover, no changes in response to immobilization stress on the AP of our mice fed the JPN diet, whereas we used telemetry. To investigate the impact of immobilization stress on the AP of our mice fed the JPN diet, we placed them in a restraining device before and at the end of immobilization stress. In fact, although Kanayama’s group used the tail-cuff method to assess AP, we placed them in a restraining device before and at the end of immobilization stress. Moreover, no changes in response to immobilization stress were observed with gestation. Indeed, no significant change in MAP could be found in the p57<sup>−/−</sup> mice throughout the stress, whereas a significant MAP decrease was observed in the p57<sup>−/−</sup> mice (Fig. 4). Moreover, no changes in response to immobilization stress were observed with gestation.

**DISCUSSION**

The main finding of this study was that genetic predisposition and placental pathology were not sufficient to induce PE-like symptoms. Indeed, we demonstrated that mice with the same genetic background did not develop the disease under different environmental conditions. In fact, although Kanayama’s group showed that p57<sup>kip2</sup> deficiency provoked PE-like symptoms (15), their results could not be replicated in our laboratory, indicating that environmental factors might have been implicated in triggering the pathology.

This study also revealed that placental pathology is not sufficient to generate PE-like symptoms, as it was observed in...
p57\(^{-/-}\) mice without any rise in AP and proteinuria. Indeed, abnormal placentalation is thought by many groups to be central in the development of PE (4). In humans, placental pathology in PE is usually characterized by impaired remodeling and trophoblastic invasion of the uterine spiral arteries (22), which results in decreased placental perfusion. This is well illustrated by the fact that surgical reduction of uterine perfusion during pregnancy in rats results in PE-like symptoms (1). In addition, necrosis of syncytiotrophoblasts (14) and marked proliferation of cytotrophoblasts (14, 28) are also usually observed. Histological analysis of placental tissue in p57\(^{-/-}\) mice uncovered necrosis, hyalinization, microcalcification, loss of giant cell islands, and labyrinthine trophoblast structure. We can, therefore, establish that the placental pathology we encountered is typical of PE, although it did not produce the disease.

The fact that we used mice from the same genetic background and could not reproduce the model described in Kanayama’s paper suggested that environmental factors may be implicated in the development of PE. The diets used by our two groups were evidently different since the main components were clearly dissimilar, for example, in ion concentrations. Indeed, the JPN diet contains more sodium and iron, and the potassium-to-sodium ratio is lower compared with the N-A diet. Sodium could have been involved in the increase of AP, since its content is higher in the JPN diet, and sodium intake has been related to the development of hypertension (8). Indeed, sodium retention is a characteristic of PE, but sodium intake does not affect the incidence of PE (5). However, it has been reported that a high-sodium diet is associated with an increase in vascular reactivity in pregnant rats (2). Normal pregnancy is known to be an inflammatory state, which is also known to be more pronounced in PE (29). Some nutrients such as vitamin E decrease the inflammatory response, and iron status can influence it by catalyzing free radical formation and therefore cytokine production (9). Interestingly, the N-A diet had more vitamin E than the JPN diet, which might have protected the p57\(^{-/-}\) mice on the N-A in our study.

Although the JPN diet did not enhance AP or proteinuria, it did have other impacts on the mice and may have been implicated in development of the disease. First, mice receiving the JPN diet were smaller at the beginning and end of gestation than those on the N-A diet. Furthermore, although the heart-to-body-weight ratio was not different, LV-to-body-weight ratio was increased, which suggests that mice fed the JPN diet are developing LV hypertrophy, which might reflect underlying cardiovascular pathologies.

Furthermore, an increase in the kidney-to-body-weight ratio was observed in animals receiving the JPN diet, suggesting that the diet may affect kidney function. However, there was no indication of renal pathology by histology in the p57\(^{-/-}\) mice, on any diet, contrary to what had been previously published (15). Indeed, PE is often associated with kidney glomerulosclerosis, which leads to proteinuria (4). Since no proteinuria was apparent in this model in our laboratory, we think that the diet, in addition to another environmental factor, could trigger kidney physiopathology, leading to proteinuria, as was observed by Kanayama et al. (15). It is possible that the use of metabolic cages for the urine collection may have contributed to this. Indeed, it has been shown that females present a significant rise in MAP and HR when placed in metabolic cages (13). Hence, as the mice were placed many times in these cages for urine collection for the Japanese study, it is possible that it may have produced an increase in BP, which may have triggered renal problems.

In addition, although all p57\(^{-/-}\) mice had smaller litters, this was accentuated by the JPN diet reflecting intrauterine growth restriction in these litters, which is often found in PE (25). However, the placental pathology observed in p57\(^{-/-}\) mice was not worsened by the JPN diet, although p57\(^{-/-}\) animals showed elevated symptoms compared with those on the N-A diet. These results indicate a role of diet in the development of placental pathology.

Furthermore, all mice receiving the JPN diet manifested endothelial dysfunction compared with those on the N-A diet. Indeed, many authors have reported an impaired response to vasodilators and chronic vasoconstriction during preeclamptic pregnancies, which could thus be implicated in the development of hypertension in this disease (27). Since the JPN diet had almost twice the amount of sodium than that contained in the N-A diet, we can hypothesize that it might have impaired vascular reactivity since it has been demonstrated that endothelium-dependent vascular relaxation is diminished in pregnant rats receiving a high-sodium diet (2). This further suggests...
that the JPN diet may have indeed been an environmental factor involved in our model.

Another factor that may have contributed to the results obtained by Kanayama’s group was the method of AP assessment. Indeed, AP was measured by the tail-cuff in Kanayama’s laboratory. This method requires the mice to be restrained as well as human presence. In certain strains, the increased AP observed may be the product of greater sensitivity to stress (10, 26). Telemetry has been shown to be the best way to measure AP accurately in mice (18). To assess the impact of this stress, p57/+/ and p57/+ mice receiving the JPN diet and assessed by telemetry underwent immobilization stress before and at the end of gestation. Although restraint in p57/+ mice caused the usual AP rise, this reaction was lessened in p57/+- animals at the end of gestation, suggesting that AP measurement may not be the culprit in the development of hypertension in the p57/+ model in Japan. Supporting these results, Baker’s group (17) recently reported that no difference in systolic AP could be observed in pregnant p57/+- mice in their laboratory, even though they also assessed AP by tail-cuff. Taken together with the fact that no difference was observed before and at the end of gestation in p57/+- mice, we suggest that the AP method was not the main factor implicated in the development of PE-like symptoms in these animals.

Determining the exact environmental factor that triggered PE-like symptoms in the p57/+ model in Japan would be almost impossible. Indeed, many factors, put together or alone, may have been implicated in the development of the disease. For instance, it would be extremely difficult to determine the exact ion composition of the water in the animal facilities at the time they made the study. Furthermore, it would be unfeasible for us to verify every single parameter in their animal facility that may be implicated, such as routine mouse handling and luminosity of the room in which the mice were housed during experimentation. Rodents are very sensitive to environmental changes, and their blood pressure is easily altered by human presence, noise, and stress. Put together with the diet, any of these factors may have triggered the release of factors implicated in the rise in BP in these animals.

PE has been thought to be caused by abnormal placentation in many studies. It is thus important to acknowledge that placental pathology may be responsible for the development of PE but is not sufficient by itself to trigger the disease. Furthermore, even though diet did not induce PE-like symptoms, it surely contributed to the disease, since it induced endothelial dysfunction and placental pathology, landmarks of PE. Moreover, diet might have influenced renal pathology, since, although no kidney histological pathology could be detected, kidney hypertrophy was present, which could have become pathological if another risk factor, such as hypertension, was added. Hence, we can think of PE as a pathology that needs a certain level of factors to be triggered, and thus our study shows that diet brought us closer to the threshold. Further experiments are required to assess the implication of environmental factors in PE. Indeed, there is presently no treatment for this disease, which is the most common cause of maternal and fetal death. Hence, given that environment can be controlled, better knowledge could strongly impact its treatment. By revealing environmental aspects implicated in the trigger of PE, clinicians would be able to better prevent it in women at high risk.

ACKNOWLEDGMENTS

We thank Dr. Louis Gaboury for help in the evaluation of pathological symptoms present in placentas and kidneys. We also thank Catherine Michel for excellent technical assistance in all the mice studies. We are indebted to Dr. Johanne Tremblay for guidance and for letting us use her telemetry equipment.

GRANTS

This research was supported by grants from the Canadian Institutes of Health and Research and Fonds de la Recherche en Santé du Québec.

REFERENCES

23. Moutquin JM, Garner PR, Burrows RF, Rey E, Helewa ME, Lange IR, Rabin SW. Report of the Canadian Hypertension Society Consensus

AJP-Heart Circ Physiol • VOL 296 • JANUARY 2009 • www.ajpheart.org

Downloaded from http://ajpheart.physiology.org/ by 10.220.33.2 on April 7, 2017


