The peroxisome proliferator-activated receptors under epigenetic control in placental metabolism and fetal development

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Lendvai Á, Deutsch MJ, Plösch T, Ensenauer R. The peroxisome proliferator-activated receptors under epigenetic control in placental metabolism and fetal development. Am J Physiol Endocrinol Metab 310: E797–E810, 2016. First published February 9, 2016; doi:10.1152/ajpendo.00372.2015.—The placental metabolism can adapt to the environment throughout pregnancy to both the demands of the fetus and the signals from the mother. Such adaption processes include epigenetic mechanisms, which alter gene expression and may influence the offspring’s health. These mechanisms are linked to the diversity of prenatal environmental exposures, including maternal under- or overnutrition or gestational diabetes. The peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that contribute to the developmental plasticity of the placenta by regulating lipid and glucose metabolism pathways, including lipogenesis, steroidogenesis, glucose transporters, and placental signaling pathways, thus representing a link between energy metabolism and reproduction. Among the PPAR isoforms, PPARγ appears to be the main modulator of mammalian placentation. Certain fatty acids and lipid-derived moieties are the natural activating PPAR ligands. By controlling the amounts of maternal nutrients that go across to the fetus, the PPARs play an important regulatory role in placenta metabolism, thereby adapting to the maternal nutritional status. As demonstrated in animal studies, maternal nutrition during gestation can exert long-term influences on the PPAR methylation pattern in offspring organs. This review underlines the current state of knowledge on the relationship between environmental factors and the epigenetic regulation of the PPARs in placenta metabolism and offspring development.

peroxisome proliferator-activated receptors; reproduction; placenta; metabolism; epigenetic programming

Although by definition a placenta is present in all placental mammals, its structure may vary tremendously between species. Not surprisingly, most information is available on mouse and human placentas (47), which, therefore, are central to this review. The placenta is formed starting from the trophectoderm of the early embryo, but also comprising large numbers of maternal-derived cells. In short, the main mass of both the human and mouse placenta is formed by trophoblast cells of trophectodermal origin. In the term placenta, they form the spongiosotrophoblasts and the syncytiotrophoblasts. Fetal cells (blood vessels, mesenchyme, and fetal blood cells) and maternal cells (decidual cells, maternal blood vessels, maternal blood cells, and maternal immune cells) account for the remaining mass. Because placentation is an invasive process, there is no obvious border between the tissue types; therefore, samples taken for epigenetic analysis often consist of several cell types.

One important group of nuclear receptors, the peroxisome proliferator-activated receptors (PPARs), contribute to the plasticity of the placenta by regulating important pathways of...
lipid and glucose metabolism. During the last few years, PPARs have been shown to be under epigenetic control and are now considered to be a part of the “DOHaD mechanisms” (94, 99, 113). The epigenetic regulation of the PPARs in placenta metabolism and offspring development is the main focus of this review.

PPARs are ligand-activated transcription factors, members of the nuclear hormone receptor superfamily, which are involved in embryonic development and differentiation of several tissues, including the placenta. Identified in the early 1990s in *Xenopus laevis* and the mouse (31, 58), they have been found to be key regulators of lipid homeostasis, energy metabolism, and inflammation through the positive and/or negative control of the expression of a variety of target genes (59, 139, 145). However, the range of genes regulated and the molecular mechanisms involved may be different for the three PPAR isoforms, PPARα (also known as NR1C1), PPARγ (also known as NR1C2), and PPARδ (also known as NR1C3). Similarly to other nuclear receptors, the transcriptional activity of PPARs is affected not only by direct ligand stimulation, such as by polyunsaturated fatty acids, but also by cross-talk with other transcription factors (96).

Consistent with their important role in energy metabolism, the members of the PPAR family are able to regulate gene expression of several key modulators of energy homeostasis, including major glycemic regulators such as glucose transporters (3). They also influence metabolic and endocrine pathways involved in, e.g., lipogenesis, steroidogenesis, ovulation, oocyte maturation, maintenance of the corpus luteum, the nitric oxide system, and activities of several proteases and the plasminogen activator (61). By regulating important placental signaling pathways such as phosphatidylinositol 3-kinase/Akt1 signaling (100), PPARs add to the plasticity of the developing placenta.

Taken together, alterations in PPAR metabolic pathways, induced by environmental factors, could be one candidate mechanism contributing to the pathogenesis of gestational diseases that can influence offspring growth and health.

**The PPAR Family and its Role in Reproduction**

Reproduction is a very energy-consuming process in all species. The PPARs represent a link between energy metabolism and reproduction implicated in early pregnancy development, including implantation, placentation, and trophoblast differentiation (Fig. 1) (26, 81, 118, 124). A conserved structural homology is present in all distinct PPAR isoforms, particularly in the DNA-binding domain and ligand-binding domain. However, each isoform is encoded by a separate gene with a tissue-specific expression pattern relating to their distinct functions (31, 38, 41, 58, 128, 144). We summarized the major studies of PPAR expression in reproduction in Tables 1, 2, and 3.

PPARγ is highly expressed in adipose tissue, in which it tightly controls adipogenic differentiation (121, 138). It has the most important role in mammalian placentation through the regulation of trophoblast differentiation, the control of the thickness of the spongiosotrophoblast layer, and in tropho-
Table 1. Summary of studies on Ppara in the reproductive system

<table>
<thead>
<tr>
<th>Organism (Tissue)</th>
<th>Expression of PPARs</th>
<th>Effect on DNA Methylation</th>
<th>Condition/Intervention</th>
<th>Detection Method</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (amnion)</td>
<td>No difference</td>
<td>NA</td>
<td>Term vs. preterm</td>
<td>RT-qPCR, Western blot</td>
<td>54</td>
</tr>
<tr>
<td>Human (placenta)</td>
<td>No difference</td>
<td>No difference</td>
<td>Obese vs. normal-weight women, term</td>
<td>RT-qPCR, Western blot</td>
<td>32</td>
</tr>
<tr>
<td>Human (chorionic villi samples and cord blood)</td>
<td>NA</td>
<td>Decreased methylation associated with GDM</td>
<td>Dietetically treated GDM; insulin-dependent GDM; control (non-GDM group)</td>
<td>Bisulfite pyrosequencing</td>
<td>36</td>
</tr>
<tr>
<td>Human (placenta at term)</td>
<td>Increased in preeclampsia</td>
<td>NA</td>
<td>Preeclampsia</td>
<td>RT-qPCR, Western blot</td>
<td>50</td>
</tr>
<tr>
<td>Human (placenta at term)</td>
<td>Located in the nuclei of syncytiotrophoblasts and reduced in GDM placentas</td>
<td>NA</td>
<td>GDM vs. control</td>
<td>Immunohistochemistry, Western blot</td>
<td>154</td>
</tr>
<tr>
<td>Human (placenta at term)</td>
<td>Decreased in preeclampsia</td>
<td>NA</td>
<td>Preeclampsia</td>
<td>RT-qPCR</td>
<td>27</td>
</tr>
<tr>
<td>Human (esophagus, stomach, intestine, and colon from 7- to 22-wk fetuses)</td>
<td>More localized in the cytoplasm than in the nucleus at the early stages (7–15 wk) of esophageal and stomach development; at later stages, predominantly nuclear</td>
<td>NA</td>
<td>Normal pregnancy</td>
<td>Immunohistochemistry</td>
<td>56</td>
</tr>
<tr>
<td>Rat (labyrinth + basal zone trophoblast)</td>
<td>Higher in labyrinth trophoblast than basal trophoblast, increased from day 16 to near term</td>
<td>NA</td>
<td>Normal gestation</td>
<td>RT-qPCR, immunohistochemistry</td>
<td>53</td>
</tr>
<tr>
<td>Sheep (superficial endometrium)</td>
<td>Peak on day 9, then linear decline</td>
<td>NA</td>
<td>Normal gestation/peri-implantation</td>
<td>RT-qPCR, immunohistochemistry</td>
<td>18</td>
</tr>
<tr>
<td>Sheep (trophoblast)</td>
<td>Expressed almost exclusively on day 7</td>
<td>NA</td>
<td>Normal gestation/peri-implantation</td>
<td>RT-qPCR, immunohistochemistry</td>
<td>18</td>
</tr>
<tr>
<td>Rat (placenta on E13.5)</td>
<td>Increased in diabetic placentas</td>
<td>NA</td>
<td>Diabetic dams (STZ-induced) vs. controls</td>
<td>Western blot</td>
<td>90</td>
</tr>
<tr>
<td>Mouse (placenta on E14)</td>
<td>Decreased in male placentas from obese fathers compared with normal, but not in female placentas</td>
<td>NA</td>
<td>Obese vs. normal-weight fathers</td>
<td>RT-qPCR array, global DNA methylation ELISA, immunohistochemistry</td>
<td>14</td>
</tr>
<tr>
<td>Rat (placenta on E21)</td>
<td>Reduced in diabetic placentas</td>
<td>NA</td>
<td>Diabetic vs. control treated with PPARα agonists</td>
<td>RT-PCR, Western blot</td>
<td>91</td>
</tr>
<tr>
<td>Rat (fetal lung)</td>
<td>Decreased in male offspring from diabetic rat</td>
<td>NA</td>
<td>STZ-induced diabetic condition</td>
<td>Immunohistochemistry</td>
<td>78</td>
</tr>
<tr>
<td>Rat (fetal heart)</td>
<td>Decreased in male offspring from diabetic rat</td>
<td>NA</td>
<td>STZ-induced diabetic condition</td>
<td>RT-qPCR</td>
<td>79</td>
</tr>
<tr>
<td>Mouse (oocytes)</td>
<td>NA</td>
<td>Decreased methylation in high-fat diet-fed dams; increased methylation in oocytes of obese vs. controls</td>
<td>Combined bisulfite restriction analysis and bisulfite sequencing</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Rat (offspring liver)</td>
<td>Increased in offspring liver upon maternal protein restriction</td>
<td>Decreased methylation upon protein restriction</td>
<td>Protein-restricted vs. restricted + folic acid vs. control</td>
<td>Methylation-sensitive PCR, RT-qPCR</td>
<td>87</td>
</tr>
<tr>
<td>Rat (offspring liver)</td>
<td>No significant difference in expression</td>
<td>Ppara promoter, lower methylation level</td>
<td>Protein-restricted maternal diet vs. control</td>
<td>RT-qPCR, methylation-sensitive real-time PCR</td>
<td>17</td>
</tr>
<tr>
<td>Mouse (offspring liver)</td>
<td>Decreased</td>
<td>Highest level in liver from protein-restricted dams and reduced by folic acid supplementation</td>
<td>Repeated abortion model Protein-restricted maternal diet with or without folic acid supplementation vs. control</td>
<td>RT-qPCR</td>
<td>88</td>
</tr>
<tr>
<td>Rat (offspring liver)</td>
<td>Decreased</td>
<td>Highest level in liver from protein-restricted dams and reduced by folic acid supplementation</td>
<td>Repeated abortion model Protein-restricted maternal diet with or without folic acid supplementation vs. control</td>
<td>RT-qPCR</td>
<td>21</td>
</tr>
</tbody>
</table>

Continued
blast maturation to establish and maintain maternal-fetal transport (see Refs. 8 and 68 for review). These functions have been clearly established in rodent models (7, 77, 101, 123). Expression of \( Pparg \) mRNA was detected in rat placenta on day 11 of pregnancy, followed by a peak on day 13 and a decrease toward later gestation (4) (Table 3). Higher \( Pparg \) mRNA expression was detected in the labyrinth placental zone, the layer of actual maternal-fetal transfer, compared with the basal zone during the last third of rat gestation (53). Although evidence from human placental cell studies is not as clear and results from expression analysis have been less consistent (Table 3), PPAR\( \gamma \) appears to also play a role for human trophoblast differentiation (134), invasion (135), and placental lipid metabolism (148).

\( Ppard \) mRNA is expressed ubiquitously, including in the digestive tract and the placenta, with a role in fatty acid catabolism and energy uncoupling in adipose tissue and muscle (6, 128). This isoform is also important for placentation through the regulation of lipid homeostasis in trophoblast cells, as shown in mice (6, 100). Whereas data on temporal placental expression were inconsistent among various animal models (Table 2), an increase in mRNA and protein expression of

### Table 1.—Continued

<table>
<thead>
<tr>
<th>Organism (Tissue)</th>
<th>Expression of PPARs</th>
<th>Effect on DNA methylation</th>
<th>Condition/Intervention</th>
<th>Detection Method</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (neonatal and adult offspring heart)</td>
<td>Increased in adult offspring of protein-restricted dams</td>
<td>( Ppara ) promoter methylation significantly lower in both neonatal and adult hearts from offspring of protein-restricted dams</td>
<td>Protein-restricted maternal diet vs. control</td>
<td>Methylation-sensitive PCR, RT-qPCR</td>
<td>129</td>
</tr>
</tbody>
</table>

RT-qPCR, reverse transcription-quantitative real-time PCR; \( Ppara \), peroxisome proliferator-activated receptor-\( \alpha \); NA, not available; GDM, gestational diabetes; E, embryonic day; STZ, streptozotocin.

### Table 2. Summary of studies on \( Ppard \) in the reproductive system

<table>
<thead>
<tr>
<th>Organism (Tissue)</th>
<th>Expression of PPARs</th>
<th>Effect on DNA methylation</th>
<th>Condition/Intervention</th>
<th>Detection Method</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (amnion)</td>
<td>No difference</td>
<td>NA</td>
<td>Term vs. preterm</td>
<td>RT-qPCR, Western blot</td>
<td>54</td>
</tr>
<tr>
<td>Human (choriodeciduum)</td>
<td>Significantly higher</td>
<td>NA</td>
<td>Preterm vs. term</td>
<td>RT-qPCR, Western blot</td>
<td>54</td>
</tr>
<tr>
<td>Human (trophectoderm cells from blastocysts)</td>
<td>Induced in trophectoderm and maintained in placenta</td>
<td>NA</td>
<td>Normal</td>
<td>Microarray analysis</td>
<td>5</td>
</tr>
<tr>
<td>Human (placenta)</td>
<td>Significantly higher</td>
<td>NA</td>
<td>Term vs. preterm</td>
<td>RT-qPCR, Western blot</td>
<td>54</td>
</tr>
<tr>
<td>Human (placenta at term)</td>
<td>No difference</td>
<td>NA</td>
<td>Obese vs. normal-weight women</td>
<td>RT-qPCR, Western blot</td>
<td>32</td>
</tr>
<tr>
<td>Human (placenta from 1st, 2nd, and 3rd trimester)</td>
<td>Increased from 1st to 3rd trimester</td>
<td>NA</td>
<td>Normal pregnancy</td>
<td>Immunohistochemistry, Northern blot, Western blot</td>
<td>120</td>
</tr>
<tr>
<td>Human (placenta from 3rd trimester)</td>
<td>Unchanged</td>
<td>NA</td>
<td>Preeclampsia and intrauterine growth restriction vs. control</td>
<td>Immunohistochemistry, Northern blot, Western blot</td>
<td>120</td>
</tr>
<tr>
<td>Rat (labyrinth + basal zone trophoblast)</td>
<td>Decreased in labyrinth and basal zone from days 16 to 22</td>
<td>NA</td>
<td>Normal gestation</td>
<td>RT-qPCR, immunohistochemistry</td>
<td>53</td>
</tr>
<tr>
<td>Sheep (superficial endometrium)</td>
<td>Low on day 7, then consistently expressed</td>
<td>NA</td>
<td>Normal gestation/peri-implantation</td>
<td>RT-qPCR, immunohistochemistry</td>
<td>18</td>
</tr>
<tr>
<td>Sheep (trophoblast)</td>
<td>Gradual increase from days 7 to 14</td>
<td>NA</td>
<td>Normal gestation/peri-implantation</td>
<td>RT-qPCR, immunohistochemistry</td>
<td>18</td>
</tr>
<tr>
<td>Mouse (placenta on E4, E5, and E6)</td>
<td>PPAR( \delta ) deficiency disrupts normal placentation</td>
<td>NA</td>
<td>( Ppard ) null mice</td>
<td>Immunofluorescence, in situ and Northern hybridization</td>
<td>146</td>
</tr>
<tr>
<td>Mouse (placenta on E10.5 and E13.5)</td>
<td>Abundant; associated with prenatal mortality</td>
<td>NA</td>
<td>( Ppard ) null mice</td>
<td>Northern blot</td>
<td>6</td>
</tr>
<tr>
<td>Rat (placenta on E21)</td>
<td>Increased in placenta from obesity-prone dams</td>
<td>NA</td>
<td>Obesity-prone vs. obesity-resistant dams</td>
<td>RT-qPCR</td>
<td>131</td>
</tr>
<tr>
<td>Mouse (offspring liver)</td>
<td>No statistical differences</td>
<td>NA</td>
<td>Repeated abortion model</td>
<td>RT-qPCR</td>
<td>88</td>
</tr>
</tbody>
</table>

\( Ppard \), peroxisome proliferator-activated receptor-\( \delta \); NA, not available; E, embryonic day; RT-qPCR, reverse transcription-quantitative real-time PCR.
Table 3. Summary of studies on Pparg in the reproductive system

<table>
<thead>
<tr>
<th>Organism (Tissue)</th>
<th>Expression of PPARs</th>
<th>Effect on DNA methylation</th>
<th>Condition/Intervention</th>
<th>Detection Method</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (amnion)</td>
<td>No difference</td>
<td>NA</td>
<td>Term vs. preterm</td>
<td>RT-qPCR, Western blot</td>
<td>54</td>
</tr>
<tr>
<td>Human (trophectoderm cells from blastocysts)</td>
<td>Induced in trophectoderm and maintained in placenta</td>
<td>NA</td>
<td>Normal</td>
<td>Microarray analysis</td>
<td>5</td>
</tr>
<tr>
<td>Human (placental venous endothelial cells isolated from term placenta)</td>
<td>Higher in human placental venous endothelial cells</td>
<td>PPARG showed coordinated (less) methylation in human placental venous endothelial cells</td>
<td>Full-term control pregnancy</td>
<td>Gene expression microarray and genome-wide DNA methylation (Infinium BeadChip)</td>
<td>67</td>
</tr>
<tr>
<td>Human (placenta at term)</td>
<td>Reduced in placenta of small-for-gestational age infants</td>
<td>NA</td>
<td>Pregnancy with normal vs. small-for-gestational age infants</td>
<td>RT-qPCR</td>
<td>30</td>
</tr>
<tr>
<td>Human (placenta at term)</td>
<td>Lower than in controls</td>
<td>NA</td>
<td>Diabetic pregnancies vs. control</td>
<td>Western blot</td>
<td>62</td>
</tr>
<tr>
<td>Human (placenta at term)</td>
<td>No difference</td>
<td>NA</td>
<td>Obese vs. normal-weight women</td>
<td>RT-qPCR, Western blot</td>
<td>32</td>
</tr>
<tr>
<td>Human (placenta at term)</td>
<td>Decreased in preeclampsia</td>
<td>NA</td>
<td>Preeclampsia</td>
<td>RT-qPCR, Western blot</td>
<td>50</td>
</tr>
<tr>
<td>Human (placenta, term/preterm)</td>
<td>Increased</td>
<td>NA</td>
<td>Intrahepatic cholestasis of pregnancy</td>
<td>RT-qPCR, Western blot, immunohistochemistry</td>
<td>153</td>
</tr>
<tr>
<td>Human (term placenta and cord blood)</td>
<td>NA</td>
<td>PPARG methylation level is lower in placenta compared to cord blood</td>
<td>Normal pregnancy</td>
<td>Bisulfite pyrosequencing</td>
<td>60</td>
</tr>
<tr>
<td>Human (placenta at term)</td>
<td>Not significantly decreased</td>
<td>NA</td>
<td>Preeclampsia</td>
<td>RT-qPCR</td>
<td>27</td>
</tr>
<tr>
<td>Human (placenta)</td>
<td>PPARγ expression decreased in the syncytiotrophoblast and the extravillous trophoblast of GDM placentas compared with normal controls</td>
<td>NA</td>
<td>GDM vs. control</td>
<td>Immunohistochemistry, RT-qPCR</td>
<td>75</td>
</tr>
<tr>
<td>Human (placenta from 1st, 2nd, and 3rd trimester)</td>
<td>Unchanged between the 1st and 3rd trimester</td>
<td>NA</td>
<td>Normal pregnancy</td>
<td>Immunohistochemistry, Northern blot, Western blot</td>
<td>120</td>
</tr>
<tr>
<td>Human (placenta from 3rd trimester)</td>
<td>Unchanged</td>
<td>NA</td>
<td>Preeclampsia and intrauterine growth restriction vs. control</td>
<td>Immunohistochemistry, Northern blot, Western blot</td>
<td>120</td>
</tr>
<tr>
<td>Sheep (superficial endometrium)</td>
<td>Inconsistent expression</td>
<td>NA</td>
<td>Normal gestation/peri-implantation</td>
<td>RT-qPCR, immunohistochemistry</td>
<td>18</td>
</tr>
<tr>
<td>Sheep (trophoblast)</td>
<td>Increase from days 7 to 17</td>
<td>NA</td>
<td>Normal gestation/peri-implantation</td>
<td>RT-qPCR, immunohistochemistry</td>
<td>18</td>
</tr>
<tr>
<td>Rat (labyrinth + basal zone trophoblast)</td>
<td>Constitutively expressed in basal zone trophoblast, increased in labyrinth zone from days 16 to 22</td>
<td>NA</td>
<td>Normal gestation</td>
<td>RT-qPCR, immunohistochemistry</td>
<td>53</td>
</tr>
<tr>
<td>Rat (placenta)</td>
<td>Increase from days 11 to 13 and slight decrease to day 15</td>
<td>NA</td>
<td>Normal gestation</td>
<td>RT-PCR, immunohistochemistry</td>
<td>4</td>
</tr>
<tr>
<td>Mouse (placenta on E10.5)</td>
<td>Deficiency led to abnormal establishment and maintenance of fetal-maternal vascular system</td>
<td>NA</td>
<td>Pparg null mice</td>
<td>Northern blot, RNase protection assay</td>
<td>7</td>
</tr>
<tr>
<td>Bovine [peri-implantation on E15, E18, and E25 (trophoblast)]</td>
<td>Highest expression on day 25</td>
<td>NA</td>
<td>control</td>
<td>RT-qPCR, immunofluorescence</td>
<td>26</td>
</tr>
<tr>
<td>Rat (fetal adipose tissue)</td>
<td>Increased in visceral adipose tissue of male newborns with intrauterine growth restriction</td>
<td>NA</td>
<td>Intrauterine growth restriction vs. control</td>
<td>RT-qPCR, Western blot</td>
<td>28</td>
</tr>
<tr>
<td>Rat (fetal lung)</td>
<td>Decreased in male offspring from diabetic rat</td>
<td>NA</td>
<td>STZ-induced diabetic condition</td>
<td>Immunohistochemistry</td>
<td>78</td>
</tr>
<tr>
<td>Rat (offspring liver)</td>
<td>No differences</td>
<td>No differences in Pparg methylation</td>
<td>Protein-restricted vs. restricted + folic acid vs. control</td>
<td>Methylation-sensitive PCR, RT-qPCR</td>
<td>87</td>
</tr>
</tbody>
</table>
PPARs operate mainly by directing the expression of certain sets of target genes through specific promoter elements, the PPAR response elements (PPREs). Upon binding of specific ligands to their ligand-binding domain, PPARs bind to their PPREs as a heterodimer complex with the retinoid X receptor (RXR), followed by recruitment of coactivator proteins and dissociation of corepressors (29, 96). Numerous coactivators involved in PPAR-dependent target gene transcription have been identified (119), including PPARγ coactivator-1α (PGC-1α), cAMP-responsive element binding proteins, and the nuclear receptor coactivator 6 (also known as PPAR-interacting protein). Cofactor recruitment is initiated throughout an increase in activity of histone acetyltransferase, histone methyltransferase, and subsequent nucleosome remodeling. However, there are no receptor-specific coactivators that direct the overall transcriptional activity of the members of the PPAR subfamily (151).

PPARs, Epigenetic Regulation, and the Placenta

Epigenetic regulation refers to changes in gene expression that occur via modification of the DNA without a change in the DNA sequence itself. Epigenetic modifications include DNA methylation, covalent modifications of histone proteins, chromatin conformation, and microRNA (miRNA)- or noncoding RNA-mediated control of gene expression. The different epigenetic mechanisms are interdependent and determine chromatin packaging of DNA and nuclear organization. This review focuses mainly on DNA methylation (63, 66) as a critical factor in the environmental control of placental metabolic pathways and fetal development, with a specific focus on PPAR regulation. This includes the regulation of both PPAR gene expression and PPAR activity on target gene transcription.

The 5' regulatory regions of PPARs belong to the CpG island promoters (Fig. 2) and could be potential “fertile soil” for epigenetic regulation of the expression of these genes. The potential transcription factor-binding sites in PPAR promoters might particularly support the idea that these genes are under epigenetic control. For PPARG, transactivation of its promoter is achieved by members of the E2F family of transcription factors relevant to cell cycle regulation, which influence the expression of PPARG1 (39). E2F1 induces PPARG transcription during clonal adipocyte expansion, whereas E2F4 represses PPARG expression during terminal adipocyte differentiation (95). Similarly, recent data have shown that PPARD is regulated by E2Fs (155), which also regulate maintenance DNA methyltransferase DNMT1 (93). Availability of data on DNA methylation of the PPAR genes in the placenta is still limited (Tables 1, 2, and 3). In humans, lower methylation levels of PPARG were identified in placenta-derived venous

### Table 3.—Continued

<table>
<thead>
<tr>
<th>Organism (Tissue)</th>
<th>Expression of PPARs</th>
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<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (offspring liver)</td>
<td>Reduced by folic acid supplementation</td>
<td>NA</td>
<td>Protein-restricted maternal diet with or without folic acid supplementation vs. control</td>
<td>RT-qPCR</td>
<td>21</td>
</tr>
<tr>
<td>Mouse (offspring liver)</td>
<td>Decreased</td>
<td>NA</td>
<td>Repeated abortion model</td>
<td>RT-qPCR</td>
<td>88</td>
</tr>
<tr>
<td>Mouse (offspring liver)</td>
<td>Upregulated in obese group</td>
<td>NA</td>
<td>Diet-induced maternal obesity vs. control</td>
<td>Western blot</td>
<td>2</td>
</tr>
<tr>
<td>Mouse (offspring liver)</td>
<td>Increased in high-fat diet group</td>
<td>NA</td>
<td>Maternal high-fat diet vs. control</td>
<td>RT-qPCR</td>
<td>24</td>
</tr>
</tbody>
</table>

STZ, streptozotocin; *Pparg*, peroxisome proliferator-activated receptor-γ; NA, not available; GDM, gestational diabetes; E, embryonic day; RT-qPCR, reverse transcription-quantitative real-time PCR.
expression is related to epigenetic regulation of the target gene in controlling PPAR transcriptional function (73, 116). Acetylation, or sumoylation contributes to the complex interplay of PPARs by mechanisms involving, e.g., phosphorylation, ubiquitination. In addition, posttranslational modification of the PPARs by mechanisms involving, e.g., phosphorylation, ubiquitination, or sumoylation contributes to the complex interplay in controlling PPAR transcriptional function (73, 116).

Furthermore, PPAR transcriptional activity on target gene expression is related to epigenetic regulation of the target gene promoter (132). In human placental cells, promoter regulation and expression of the growth hormone 2 (GH2; also known as hGH-V) gene coding for placental growth hormone were shown to be sensitive to the presence of 5-azacytidine, a demethylating agent and inhibitor of DNA methyltransferases at low doses, resulting in increased GH2 gene expression (25).

Concerning the transcriptional function of PPARγ at target gene-specific sites, a possible molecular route is the interaction of PPARγ with cofactors, including the cell cycle regulator cyclin D1, an inhibitor of PPARγ-dependent activity (44). The underlying mechanism was suggested to involve the activity of epigenetic regulators, the histone deacetylases (HDACs), based on data pointing toward, e.g., transcriptional downregulation of PPARγ through recruitment of HDAC3 (95). PPARγ transcriptional activity was shown to be enhanced in cyclin D1−/− cells, whereas reintroduction of cyclin D1 into cyclin D1−/− murine embryonic fibroblasts inhibited basal and ligand-induced PPARγ activity, increased HDAC1 activity, and enhanced HDAC repression of PPARγ activity on the promoter of its target gene lipoprotein lipase (44). These data suggested an important role for cyclin D1 in the regulation of PPARγ-mediated adipocyte differentiation via recruitment of HDACs to the PPRE local chromatin structure and PPARγ activity (44). Besides this example, there is advancing knowledge on the regulation of transcriptional activity of all PPAR isoforms by various interacting proteins (see Refs. 98 and 143 for an overview). This has been researched particularly for PPARγ, which is of specific interest for the development of antidiabetic drugs such as the thiazolidinediones, a group of PPAR γ ligands (73). In addition, postranslational modification of the PPARs by mechanisms involving, e.g., phosphorylation, ubiquitination, or sumoylation contributes to the complex interplay in controlling PPAR transcriptional function (73, 116).

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This study also suggested that a certain DNA methylation background appears to be required to maintain the ability of placental transcription factors such as PPARγ to bind and regulate proximal promoter regions of GH2 in placental cells. Knowledge on the placental target genes of PPARγ and the other PPAR isoforms is still limited, and the interrelationships of regulatory factors upstream and downstream of the PPARs are currently far from being understood.

Furthermore, the global epigenetic landscape of the placenta may be an active venue of PPAR regulation. Interestingly, placental tissue has been shown to be relatively hypomethylated, being the organ with the lowest 5-methylcytosine content of all human tissues (35). This content was found to increase across gestational age (19, 45, 107), corresponding with temporal gene expression changes (97). However, a recent study analyzing the human placenta methylome focused on genomic hypomethylation within partially methylated domains, which were shown to be stable through gestation and among individuals (125). In this study, the PPAR genes were found to be located in highly methylated regions of term placenta.

Cell-specific gene expression might be explained by cell-specific epigenetic changes. The placenta is a complex system of embryonic and maternal cell types. In placental development, cytotrophoblasts (embryonic origin) and fibroblasts (maternal origin) are the two major cell types. Grigoriu et al. (48) analyzed the promoter methylation patterns of these cell types, which showed global similarity. However, at the same time, certain genes, including tumor suppressor genes, showed differential promoter methylation, highlighting the epigenetic

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### Fig. 2. CpG island arrangement in the promoter region of PPAR genes.

Areas of significant similarity between human and murine promoter regions of PPARA, PPARD, and PPARG are depicted. Other than the PPARD promoter region, the PPARG and PPARA promoter regions of humans and mice contain CpG islands.

**PPARα promoter region**

- **Homo sapiens**
  - Variant 1
  - Variant 2

- **Mus musculus**
  - No CpG island

**PPARγ promoter region**

- **Homo sapiens**
  - No CpG island

- **Mus musculus**
  - No CpG island

**PPARδ promoter region**

- **Homo sapiens**
  - No CpG island

- **Mus musculus**
  - No CpG island

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The diagram illustrates the CpG island arrangement in the promoter region of PPAR genes, highlighting areas of significant similarity and the presence or absence of CpG islands.
marks of the placenta. Furthermore, there are differences in methylation patterns between specific human placental tissue types, such as higher global methylation in extraembryonic mesoderm cells than cytotrophoblasts, with a differential methylation of genes involved in DNA binding, transcription factor activity, and protein transport (137). As described above, the term placenta consists of many more cell types; however, epigenetic studies on single cell types are largely lacking. Specifically, more research is required to understand the role of DNA methylation in trophoblast lineages of different types and stages of development. Several papers report PPAR expression patterns in trophoblasts of various origins (Refs. 5, 18, and 53 and Tables 1, 2, and 3), but none of them in the context of epigenetic regulation of the PPAR genes. Overall, although a growing number of potential mechanisms link the regulation of PPAR gene expression and PPAR function to placental metabolism and disease development, they require further study.

The DNA methylation signature established in the early embryo is thought to be stably carried throughout life, but it has its own dynamics (69). Some epigenetic marks, such as that in classical genomic imprinting, are very stable, as they are tightly regulated by the cell. However, the “variable” epigenetic marks demonstrate developmental plasticity, with some changes being introduced by environmental exposures and others by random chance. Dietary restriction or overnutrition, hypoxia, or other early-life exposures may induce persistent adaptive changes via epigenetic mechanisms that possibly influence an individual’s susceptibility toward environmental factors later in life and the development of adult-onset disease (20, 51).

Environmentally Induced Epigenetic Alterations of the Placenta

The placenta is able to respond to maternal and fetal environmental exposures (“placental exposome”) in growth and development (84, 86), as supported, for example, by alterations in both methylation and expression of human placental glucose transport across pregnancy (105). The independent phenotypic traits of the placenta itself have predictive value for the offspring outcome. Size and shape of an individual’s placenta are associated with increased risks for high blood pressure and heart failure later in life (10). In particular, the maternal phenotype such as obesity or GDM is a determining feature in the placenta-disease risk (24, 65). However, the mechanisms by which placentation growth and metabolism strongly affect offspring outcomes are not well understood. Growing evidence supports the role of epigenetic modifications as a disease-causing mechanism via modulation of gene expression.

Three mechanisms have been proposed by which environmental factors can alter the epigenetic pattern of placental genes (see Ref. 22 for an overview). Alterations could be caused first by directly interfering with the process of DNA or histone methylation by affecting the supply of methyl donors in the diet. Second, the expression or activity of relevant enzymes responsible for DNA methylation, DNA demethylation, or histone modifications could be altered. Third, another possible mechanism is by varying the accessibility of DNA to epigenetic enzymes by modulating chromatin structure and function.

Although environmental factors such as drugs can interfere with the enzymes that modify DNA and histone proteins (133), nutritional exposures affecting the availability of methyl donors (e.g., folate) can also have an impact on these processes (112). The availability of methyl groups for epigenetic modification is mediated by an intermediate of one-carbon metabolism, S-adenosyl methionine (SAM). SAM is produced indirectly from dietary folate and reflects a metabolic link between compromised folate status and methylation levels. An impaired maternal folate metabolism by exposure to folic acid antagonists appears to increase the risk of placenta-mediated unfavorable outcomes of human pregnancy, i.e., intrauterine growth restriction (IUGR) or preeclampsia (147).

Interestingly, human term placentas associated with IUGR fetuses were shown to have an increased transport of folic acid and other bioactive molecules compared with healthy placentas, possibly as a compensatory mechanism counteracting insufficiencies of the IUGR placenta (70). The placental transport systems of folate and the essential amino acid methionine are established early in human gestation and are dependent upon a continuous maternal supply (130). Therefore, restriction of the maternal B vitamin supply during the periconceptional period might have the potential to alter the methylation state of the entire offspring genome, as shown in sheep (127). Furthermore, epigenetic modulation of vitamin D bioavailability at the fetomaternal interface (106) supports the notion that epigenetic regulation of normal placental nutritional functions may play a relevant role in human development.

Maternal Nutrition as a Modifier of Epigenetic Regulation of PPARs in the Offspring

Studies involving specific animal models, in which the diet of the dams was modified during early developmental phases, including pregnancy and lactation, have allowed for investigating the offspring outcome and support an intergenerational transmission of cardiovascular and metabolic disease (37, 64, 115). Changes in gene methylation have been identified to be important in the context of upregulation of genes that control hepatic fatty acid oxidation and gluconeogenesis in energy-demanding tissues (i.e., liver, heart muscle; Tables 1 and 3). Whereas recent studies in rodent models of maternal overnutrition identified a sex-specific upregulation of hepatic expression of Pparg (Table 3) (2, 24), nutritional effects in animal models of poor nutrition were shown to be mediated through Ppara and the glucocorticoid receptor (Nr3c1, also known as Gr; Table 1 and Fig. 1) (17, 87, 89, 129). Following exposure to maternal protein restriction in rodents, promoter methylation of the Ppara and Gr genes was found to be reduced in different offspring organs and among generations (17, 87, 129). Hepatic Ppara gene expression was increased, and hypomethylation of the Ppara promoter persisted in offspring until after weaning (21, 87). At that time, any influence of the maternal diet on the offspring had ceased, suggesting stable epigenetic modification in a gene-specific manner.

Hepatic PPARα expression induces an increase in PPARα signaling, which might be inferred from upregulated expression of acyl-coenzyme A oxidase, a PPARα target gene, in livers of rat offspring “programmed” by maternal protein restriction (87). Nutritional alterations may also affect sirtuin 1 (SIRT1), a positive regulator of hepatic PPARα (118) shown to influence chromatin remodeling in oxidative stress (108). SIRT1 possesses eNAD+ -dependent histone deacetylase activ-
ity that contributes to cellular regulation. Indeed, it appears to have an important role in adaptation, stress response, inflammatory response, and metabolism during pregnancy, and it activates anti-inflammatory transcription factors such as the PPARs (117). In the human placenta, the localization of SIRT1 extends to the syncytiotrophoblast layer and the cytotrophoblasts, amnion epithelium, trophoblast layer of the chorion, and decidual cells (82), which also express PPARγ, resulting in anti-inflammatory effects (1, 83). Future studies are required to investigate the effect of the relationship between SIRT1, PPARγ, and other transcription factors in human gestational tissues and relate them to processes of stress adaptation in adverse nutritional conditions.

Offspring exposed to maternal low-protein diet during pregnancy and lactation are small and more susceptible to the development of obesity, glucose intolerance, and diabetes with aging or when fed a high-energy diet. Investigation of underlying cellular responses revealed that prenatal protein restriction in rats can be associated with a higher rate of preadipocyte proliferation (15, 152), which was consistent with a higher activity of PPARγ. Furthermore, increased PPARγ signaling enhances glucose uptake in adipocytes (114), a feature also observed in rat offspring that had been exposed to a maternal low-protein diet during pregnancy and lactation (110).

In recent years, it has become clear that the nuclear receptors affect the circadian clock system (see Refs. 85 and 136 for an overview). There is evidence that the PPARs are rhythmically expressed in mammalian tissues and interact with the core clock genes (57, 149), thus representing a link between circadian rhythm and cellular metabolism. However, the underlying mechanisms by which nutritional challenges influence this important molecular system are just about to be uncovered.

High-fat diet feeding of mice has recently been shown to alter important molecular systems just about to be uncovered. A recent study investigating PPARγ expression in placenta from AGA and LGA (34). Desynchronization of the biological clock as a result of nutritional exposures during early placenta and fetal development might increase the susceptibility toward the development of 21st century disorders, including obesity, diabetes, and cancer.

**Human Perspective**

Evidence from animal models implies that the intrauterine period is critical in prompting disease risk for a range of disorders that develop later in life (109). The reproductive system and specifically the placentas of different mammalian species vary considerably. Besides structural variation between mouse and human placentas, they also show many differences in placental metabolism and expression profiles of genes and proteins, although some markers and signaling pathways are shared, such as PPARγ (111, 134). Obviously, most data on placental expression and methylation patterns are derived from animal experiments, since systems to study human trophoblast differentiation and placental development still need to be optimized. Therefore, it is a great challenge to determine the underlying mechanisms triggered by the maternal nutrition and metabolic environment during early life in humans.

Diabetes during pregnancy is one such exposure that leads to dramatic changes in the maternal metabolic milieu (52, 80) and the proinflammatory environment of the intrauterine tissues. This has been shown in human studies contributing to knowledge on developmental alterations that increase the risk of overweight and type 2 diabetes later in the offspring’s life (36, 102, 126). Considering that the PPAR system regulates many metabolic and anti-inflammatory pathways, it is plausible to hypothesize that it also plays a role in the development of maternal and fetal alterations of diabetic pregnancies. Reduced expression of placental PPARγ and PPARα proteins has been identified at term in diabetic pregnancies of both animals and humans (61, 75).

Jawerbaum et al. (62) examined the involvement of vasoactive agents such as nitric oxide (NO) and PPARγ ligands in healthy placental remodeling processes and human diabetic pregnancies, with the latter showing higher NO levels in term placentas. This could imply underlying epigenetic influences on endothelial nitric oxide synthase (NOS3; also known as eNOS), which was shown recently to be regulated by DNA methylation in human endothelial cells from the placental vasculature (67). PPARγ activation resulted in suppression of placental NO levels (62). Furthermore, in rat offspring exposed to maternal diabetes, PPAR ligands beneficially modulated lipid composition and metabolic enzyme expression in lungs and led to a reduction in NO production and lipoperoxidation in hearts (78, 79).

There is overwhelming evidence that low birth weight with relative thinness at birth, and particularly if followed by rapid postnatal growth (“mismatch”), is associated with an increased prevalence of obesity, insulin resistance, cardiovascular disease, and certain cancers in later life (see Refs. 12, 55, 103, and 141 for excellent reviews). A recent study specifically investigated **PPARG** gene expression levels in human placentas associated with small- (SGA), appropriate- (AGA), and large-for-gestational age (LGA) births (30). Low **PPARG** expression in placentas of SGA fetuses was associated with low fetal and placental weights within this subpopulation. This finding and a higher **PPARG** expression in placenta from AGA and LGA newborns may suggest an underlying epigenetic regulation. These authors speculated that increased placental gene methylation in the SGA population, due to an adverse intrauterine environment, may result in a decreased expression of **PPARG** and possibly other coregulators (30).

Further studies are warranted to understand the underlying mechanisms of intergenerational transmission of disease risk and a potential role of PPAR signaling herein. Placental epigenetic regulation appears to provide a reasonable link between environmental exposures and fetal development, which may be further elucidated by technological advances, including next-generation sequencing. Recent studies investigating the placenta methylome identified changes in placental DNA methylation patterns and associations with fetal growth following exposure to maternal risk conditions such as GDM, obesity, and preeclampsia (104, 122). Differentially methylated genes in GDM placentas were suggested to be related to metabolic disease pathways potentially involved in fetal metabolic programming (36, 40, 122). Prospectively, such aberrant methylation profiles may be of use as epigenetic biomarkers for a risk of developmental abnormalities and to be applied for the design of prevention strategies.
Conclusions

The PPARs play a crucial role in metabolism during the gestational period. They are key metabolic regulators of placental adaptive responses to environmental stressors, which include both controlling the amounts of maternal nutrients that go across to the fetus and adjusting to maternal physiology. Their expression pattern is at least partially regulated by DNA methylation in the placenta, whereas other PPAR regulatory processes are just about to be uncovered, such as the involvement of placenta-specific miRNAs (154). Besides understanding placental PPAR gene regulation, more research is required to elucidate the mechanistic pathways and interacting partners involved in modulating the complexity of signaling of the PPARs and their ligands throughout pregnancy. Animal studies have already shown that early-life environmental exposures can influence the PPAR methylation pattern and PPAR expression in both offspring and mothers. However, intensive investigations of placental epigenetic regulation are required to understand the interplay of the maternal environment and offspring health-disease relationships in both animals and humans and the potential of such alterations as prognostic indicators.

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AUTHOR CONTRIBUTIONS
A.L., T.P., and R.E. conception and design of research; A.L., M.J.D., T.P., and R.E. approved final version of manuscript.

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Review

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