Expression and Regulation of Adiponectin and Receptor in Human and Rat Placenta

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Context: Adiponectin is an adipocyte hormone involved in glucose and lipid metabolism. Recently, two receptors of this protein, called adiponectin receptor 1 (Adipo-R1) and Adipo-R2, have been cloned.

Objective: The aim of this study was to examine whether adiponectin and its receptors are expressed in human and rat placentas and to evaluate the regulation of these factors by gestational age and nutritional status.

Results: Our results demonstrate that adiponectin and Adipo-R2 are localized in both human and rat placentas. Human adiponectin and Adipo-R2 are presented in cytotrophoblast and syncytiotrophoblast cells. However, rat adiponectin and Adipo-R2 change their specific cell type immunostaining during gestation. Furthermore, placental adiponectin mRNA expression is increased during pregnancy in the rat, whereas Adipo-R2 has the contrary pattern. We also assessed the effect of food restriction (30%) during gestation, and we observed that adiponectin mRNA levels decrease after 16 d of undernutrition. In contrast, placental Adipo-R2 mRNA is unchanged by undernutrition. Finally, treatment with adiponectin during gestation decreases Adipo-R2, glucose transporter 3, lipoprotein lipase, and TGF-β mRNA expression.

Conclusion: Taken together, our results suggest that, at least in rodents, adiponectin may be involved in the regulation of several placental functions. (J Clin Endocrinol Metab 90: 4276–4286, 2005)

WHITE ADIPOSE TISSUE (WAT) is an important endocrine organ that regulates energy and glucose metabolism and secretes a large number of physiologically active peptides, such as leptin (1), adiponectin (2, 3), TNF-α (4), and resistin (5), which often share structural properties with cytokines and are therefore collectively referred to as adipocytokines. Adiponectin is a novel adipose tissue-specific protein abundantly expressed in human and rodent fat and secreted from differentiated adipocytes (3, 6). Adiponectin is present at high concentrations in the circulation as a low molecular weight complex consisting of a dimer of trimers as well as a high molecular weight complex of up to six trimers (7). Circulating levels of adiponectin are approximately two to three times higher in females than in males (3, 8), are inversely related to the degree of adiposity, and are positively associated with insulin sensitivity in both healthy subjects and diabetic patients. Plasma adiponectin levels decreased in parallel to the progression of insulin resistance, suggesting that a reduction in circulating adiponectin may be related to the development of insulin resistance (9). The high-fat diet significantly increases adipocyte size and at the same time increases the levels of several molecules causing insulin resistance, such as TNF-α and free fatty acids, and lowers the levels of other molecules that improve insulin sensitivity, such as adiponectin (10). Adiponectin mRNA levels in adipose tissue and plasma adiponectin concentrations were elevated in mice on a calorie-restricted diet (11), and very recently it has been demonstrated that adiponectin acts in the brain to decrease body weight (12). Thus, dysregulation of the synthesis and/or secretion of adiponectin from adipose tissue may play a role in the pathogenesis of insulin resistance in both obesity and type 2 diabetes. Furthermore, mutations of the adiponectin gene that cause low plasma adiponectin levels are associated with the metabolic syndrome, including insulin-resistant diabetes and atherosclerotic disease (13). Very recently, cloning of two human and murine adiponectin receptors, Adipo-R1 and Adipo-R2, was reported. Adipo-R1 is abundantly expressed in muscle, whereas Adipo-R2 is predominantly expressed in liver. Both isoforms serve as receptors for globular and full-length adiponectin and mediate adiponectin stimulation of AMP kinase, peroxisomal proliferator-activated receptor-α ligand activities, fatty acid oxidation, and glucose uptake (14).

In contrast, pregnancy is characterized by a concerted and widespread series of changes in maternal physiology that increase food intake and produce a positive energy balance associated with the challenge of reproduction. Plasma adiponectin levels decrease in late gestation, but increase in calorie-restricted rats (8). Placental function is regulated at least in part by a wide spectrum of cytokines that are produced both locally and distally and are essential for adapting
the maternal metabolism to pregnancy to assure normal placental development and fetal growth.

The gene expression of adiponectin or its receptors in placenta has not been previously reported. Thus, the aim of this study was to assess whether adiponectin and both receptors are expressed in human and rat placentas as occurs with other hormones expressed mainly in WAT, such as leptin (15), TNF-α (16), and resistin (17). Assessment of adiponectin expression in human and rat placentas was carried out using different experimental approaches. First, placental adiponectin and adiponectin receptor gene expressions were evaluated by different techniques. Secondly, expression and cellular location of adiponectin and Adipo-R2 within placental and adipose tissue from human and female rat specimens were studied by immunohistochemistry. Thirdly, we examined by real-time semiquantitative RT-PCR analysis the adiponectin and Adipo-R2 mRNA expressions in rat placenta at different gestational ages. Placenta adiponectin and Adipo-R2 mRNA expression were assessed at different stages of pregnancy in rats subjected to chronic undernutrition. Finally, the functional significance of the placental adiponectin system was studied by assessing the influence of chronic adiponectin administration on the mRNA levels of several genes known to be involved in placental physiology.

Materials and Methods

Tissues explant

Virgin female Sprague Dawley rats (225–250 g) bred in the vivarium of the University of Santiago de Compostela were used. Rats were mated overnight, and the day on which spermatozoa were present in vaginal smear was designated gestational d 1, after which dams were randomly assigned to one of two dietary groups (18). The animals were housed under controlled light (12-h light, 12-h dark cycle) and temperature (21 ± 3 C). All experimental procedures were approved by the animal care committee on research of University of Santiago de Compostela and were conducted in accordance with the European Union normative for care and use of experimental animals. Restricted and control pregnant dams were assigned to one of two dietary groups (18). The animals were housed overnight, and the day on which spermatozoa were present in vaginal smear was designated gestational d 1, after which dams were randomly assigned to one of two dietary groups (18). The animals were housed under controlled light (12-h light, 12-h dark cycle) and temperature (21 ± 3 C). All experimental procedures were approved by the animal care committee on research of University of Santiago de Compostela and were conducted in accordance with the European Union normative for care and use of experimental animals. Restricted and control pregnant rats at different stages of gestation were anesthetized (80 mg/kg ketamine hydrochloride and 10 mg/kg xylazine) and killed by decapitation. Additional placental samples were collected from the following age previously described (19, 20), then processed for routine paraffin histology.

Chronic adiponectin treatment

The effects of the adipocyte-derived hormone adiponectin on the mRNA expression of several placental hormones involved in insulin resistance were evaluated. In this setting, on d 13 of pregnancy, rats were infused with globular mouse adiponectin (2.5 μg/d) for 7 d by osmotic minipump (model 2ML1, Alza Corp., Palo Alto, CA). An sc pocket on the dorsal surface was created using blunt dissection, and the osmotic minipump was inserted. The incision was closed with sutures, and rats were kept warm until they had fully recovered.

Northern hybridization

Adiponectin mRNA expression was assessed by Northern blot in rat placenta. Total RNA was isolated from rat placental samples from different experimental settings using the single-step acid guanidinium thiocyanate-phenol-chloroform extraction method as previously described (20). Filters containing 25 μg total rat placental RNA were prepared; as a positive control, 4 μg total WAT RNA were used. Northern blot analyses were performed as described previously (20). The density of 18S rRNA was used to monitor the amount of total RNA in each lane. The cDNA probe for Northern blot and Southern blot analysis of adiponectin was a 658-bp fragment prepared by RT-PCR using first-strand cDNA from rat fat mRNA. The PCR primers used to generate an adiponectin (ACRP30)-specific probe were as follows: forward, 5'-AAT CCT GCC CAG TCA TGA AG-3'; and reverse, 5'-GTC CCC TTC CCC ATAC ATC T-3' (GenBank accession no. NM144740). The putative adiponectin cDNA fragment was subcloned using the In-Fusion cloning kit (BD Clontech), and the specificity was checked by nucleotide sequencing. Digitized images were subsequently processed using microcomputer imaging device software, and the signal was normalized using an image analyzer (Gel 200, Bio-Rad Laboratories, Richmond, CA).

RT-PCR/Southern blot

Real-time RT-PCR analyses were performed in a fluorescent temperature cycler (LightCycler, Roche, Mannheim, Germany) according to the manufacturer’s instructions (21). Two micrograms of total RNA placenta were used for each RT reaction. The 20-μl amplification mixture contained 1.2 μl RT reaction products plus 3 mM MgCl2, 0.5 μM of each primer, and 1 × LightCycler DNA Master SYBR Green I mix (Roche). The PCR cycling conditions included an initial denaturation at 96 C for 20 sec, followed by 40 cycles at 96 C for 2 sec, 60 C for 15 sec, and 72 C for 15 sec.

The oligonucleotide-specific primers for rat adiponectin, Adipo-R2 and HPRT, are described in Table 1. After PCR, a cycle threshold value was obtained using the software provided by the manufacturer. Relative quantification of PCR products were then based on value differences using the comparative cycle threshold method. Adiponectin mRNA levels were normalized with respect to the HPRT level in each sample. This experiment was performed on six animals per group.
Immunoblotting

Western blot analysis of human and rat adiponectin and Adipo-R2 was performed as previously described (22). Portions of placental and adipose tissue were homogenized at 4 °C in ice-cold lysis buffer [50 mM HEPES (pH 7.5), 1% Triton X-100, 10 mM Na3VO4, 10 mM sodium fluoride, 2 μg/ml aprotinin, 10 μg/ml antipain, 5 μg/ml leupeptin, 0.5 μg/ml pepstatin A, 17 μg/ml phenylmethylsulfonyl fluoride, and the commercial protease-inhibitor cocktail Complete (Roche)] and centrifuged at 11,000 χ g for 30 min. The supernatant protein concentration was determined (protein assay kit, Bio-Rad Laboratories). Samples were loaded onto SDS-PAGE (10% separating) and transferred to nitrocellulose membrane (Hybond C-Super, Amersham Biosciences, Arlington Heights, IL). Consistency of protein loading and transfer among samples was confirmed by Ponceau S dye staining (ICN Biomedicals, Inc., Aurora, OH). The membrane was incubated with rabbit adiponectin primary antibody for human (adiponectin, recombinant antibody, H-ADI-01; 1:100; Phoenix Pharmaceuticals, Inc., Belmont, CA), rat (ACRP 301-A; 1:100; a Diagnostic International, San Antonio, TX), Adipo-R2 primary antibody (Phoenix Pharmaceuticals, Inc.; 1:100), and monoclonal α-tubulin (Sigma-Aldrich Corp., St. Louis, MO; 1:1000). The filters were then washed before incubation with secondary antibody for immunoblotting, and detection was performed using a chemiluminescent system (Tropix, Bedford, MA) (22).

Immunohistochemical analysis

Rat placentas [6 and 21 days post coitum (dpc)] and human placentas from first trimester and term placentas (n = 4 each) were immersion-fixed in 4% buffered (0.1 mol/liter NaCl) formaldehyde for 18–24 h and included routinely in paraffin. Sections (4 μm thick) were mounted on Histobond adhesion microslides (Marienfeld, Lauda-Königshofen, Germany), dewaxed, and rehydrated. Immunohistochemistry was performed as follows.

Adiponectin (ACRP-30)

In step 1, epitope retrieval was carried out in 0.1 M sodium citrate buffer, pH 6, using a water bath for 40 min at 95–99 °C, and the slides were allowed to cool for 20 min at room temperature. For step 2, incubation with the goat antiadiponectin (ACRP 30 N-20; Santa Cruz Biotechnolog­gy, Inc., Santa Cruz, CA) antibody at a dilution of 1:200 in ChemMate antibody diluent (DakoCytomation, Glostrup, Denmark) was performed overnight at 4 °C. In step 3, hydrogen peroxide (3%; Merck & Co., Darmstadt, Germany) was added for 10 min to block endogenous peroxidase. In step 4, multilink biotinylated antimouse, rabbit, and goat IgG (15 nmol/liter; LSAB System HRP, DakoCytomation, Carpinteria, CA) were added for 30 min. In step 5, streptavidin conjugated to horseradish peroxidase (LSAB System HRP) was added for 30 min, and in step 6, 3,3′-diaminobenzidine-tetrahydrochloride (Liquid DAB + Substrate-Chromogen System, DakoCytomation) was added for 10 min (prepared according to the manufacturer’s instructions). Between steps, sections were washed twice for 5 min with 0.05 mol/liter Tris buffer (pH 7.6) containing 0.3 mol/liter NaCl, and after step 5, sections were washed with distilled water. The sections were faintly counterstained with Harris’ hematoxylin for 1 min.

Adiponectin receptor type 2

In step 1, incubation with the rabbit anti adiponectin receptor 2 (4–39, Phoenix Pharmaceuticals, Inc.; 1:50) was performed overnight at room temperature. In step 2, 3% hydrogen peroxide was added for 10 min. In step 3, Envision/HRP rabbit/mouse (DakoCytomation) was added for 30 min, and in step 4, 3,3′-diaminobenzidine (DakoCytomation) was added for 10 min. Between steps 1 and 4, the sections were washed twice for 5 min each time in 0.05 mol/liter Tris buffer (pH 7.6) containing 0.3 mol/liter NaCl, and after step 4 sections were washed in distilled water. Counterstaining was performed using hematoxylin.

Positive controls included adipose tissue and liver. Negative controls were performed by 1) preadsorbing the antiadiponectin antibody with the homologous peptide (blocking peptide sc-17044, Santa Cruz Biotechnology, Inc.; 10 nmol/ml), 2) replacing the primary antibody with normal goat serum and with normal rabbit serum at the same concentration of the primary antibody, and 3) omitting any essential step of the reaction.

RIA for rat adiponectin

Plasma levels of adiponectin were assayed using reagent kit and methods provided by Linco Research, Inc. (St. Charles, MO). Truncal vein plasma was obtained by decapitation from the various groups of rats, collected in tubes containing EDTA (1 mg/ml blood) and aprotinin (500 U/ml blood; Sigma-Aldrich Corp.), and centrifuged immediately. Plasma adiponectin was determined using a double-antibody RIA provided by Linco Research, Inc. All samples were assayed in duplicate within one assay, and results were expressed in terms of the adiponectin standard. The limit of assay sensitivity was 1 ng/ml, the intra- and interassay levels were 4.11 and 6.56%, respectively.

TABLE 1. Primers

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<th>Oligo</th>
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Statistical analysis

Instat (GraphPad, Inc., San Diego, CA) was used to perform statistical calculations. All data are expressed as the mean ± SEM, and a minimum of six animals were used for each experimental variable. Differences between groups were evaluated using one-way ANOVA, followed by post hoc, multiple-comparison tests. The level of significance for all tests was set at P < 0.05.

Results

Adiponectin and Adipo-R2 are expressed in placenta

The expression of adiponectin in placenta has not been previously reported. By using specific primers for rat adiponectin, products of the expected size (224 bp) were amplified from placental tissues by RT-PCR (Fig. 1A). Sequence analysis confirmed that these amplicons were derived from adiponectin mRNA, and omission of the reverse transcriptase enzyme from the RT reaction demonstrated that the product was not the result of contaminating genomic DNA. For comparative purposes, RT-PCR amplification of adiponectin mRNA was also conducted in RNA samples from rat WAT, the major source of systemic adiponectin. These reactions yielded strong amplification signals (Fig. 1A). Having confirmed that adiponectin transcript and protein were expressed in rat placenta, we assessed the expression of its receptors Adipo-R1 and Adipo-R2. Both receptors were expressed in rat placenta (Fig. 1B); however, we wanted to rule out the possibility that the positive result obtained by RT-PCR was a consequence of the expression of Adipo-R1 and Adipo-R2 in blood cells. To verify this fact, we performed RT-PCR, and our results showed clear expression of Adipo-R1 in rat blood cells, whereas Adipo-R2 was negative in the same cells (Fig. 1C). The difficulty of differentiating whether the expression of Adipo-R1 mRNA was due to contamination or was actually localized in placental cells led us to study only the expression and regulation of Adipo-R2.

Northern blot analysis of rat adiponectin gene expression in placenta at term and fat tissue was performed with a specific cDNA fragment to adiponectin (Fig. 2). When these samples were probed for adiponectin under stringent conditions, a single hybridization mRNA band was present in rat placenta. Three distinct bands (2.5, 1.8, and 1.2 kb) were detected in WAT as previously described (23) (Fig. 2). Surprisingly, rat placenta showed a transcript with an electrophoretic mobility less than that of the rat fat 1.2-kb transcript. Adiponectin mRNA species detected by Northern blot anal-

![Fig. 1. A, Expression of adiponectin gene in rat placenta on d 20 of pregnancy. Upper panel, Representative RT-PCR assay of the expression levels of adiponectin mRNA in three independent term placental samples. In addition, positive (rat WAT), negative, and genomic DNA controls are shown. A 100-bp molecular weight marker (MW) was used. The integrity and loading of RNA were confirmed by amplification of rat HPRT. Lower panel, Specificity of the amplicons is demonstrated by Southern hybridization using a specific cDNA probe to adiponectin. B, Representative RT-PCR assay of the expression levels of adiponectin mRNA in three independent human term placental samples (last trimester). In addition, positive (human WAT), negative, and genomic DNA controls are shown. A 100-bp molecular weight marker (MW) was used. The integrity and loading of RNA were confirmed by amplification of human HPRT. C, Expression of rat Adipo-R1 and Adipo-R2 in blood cells. D, Representative RT-PCR assay of the expression levels of Adipo-R2 mRNA in three independent term placental samples. In addition, positive (rat liver), negative, and genomic DNA controls are shown. A 100-bp molecular weight marker (MW) was used. The integrity and loading of RNA were confirmed by amplification of rat HPRT.](https://www.endojournals.org/content/jcem/90/7/4276.full)

![Fig. 2. Representative results of Northern blot analysis of total rat RNA isolated from WAT (4 μg/lane) and placenta on d 20 of gestation (25 μg/lane), using rat adiponectin cDNA and oligonucleotide 18S probes.](https://www.endojournals.org/content/jcem/90/7/4276.full)
ysis and amplicons of RT-PCR analysis indicate that the difference in size of rat placental and fat mRNAs is in the 3'-untranslated region, formed by alternative polyadenylation. The blot was stripped and reprobed with an 18S probe as the loading control.

Having found by Northern blot and RT-PCR/Southern blot analysis, that the placenta is a source of adiponectin mRNA, we employed Western blot analysis and immunohistochemistry to determine whether the message is translated into protein. Using a polyclonal rabbit adiponectin primary antibody for human (ACRP302-A) and rat (ACRP301-A), we detected a major band at approximately 30,000 molecular weight in protein extracts of human and rat placental tissue at term (Fig. 3). Placental adiponectin and Adipo-R2 protein determined by Western blotting in human and rat tissues showed a pattern similar to that in WAT.

Cellular localization of adiponectin and Adipo-R2 in placenta

Immunohistochemistry showed that adiponectin (ACRP-30) was expressed in rat (6 and 21 dpc) and human (first trimester and term) placentas. Adiponectin was mainly expressed in decidual and ectoplacental cone (Fig. 4, A–C) in 6 dpc rat placenta. Furthermore, adiponectin immunoreactivity was localized in the basolateral layer of rat placenta on d 21 of gestation (Fig. 4). In this case, immunostaining was primarily localized in vacuolated glycogen cells and was less intense in the small basophilic cells (Fig. 4D). Human placenta also showed a positive reaction for adiponectin. In first trimester placenta, adiponectin immunostaining was localized in cytotrophoblast and syncytiotrophoblast cells (Fig. 4E). In contrast, adiponectin immunoreactivity was localized in syncytiotrophoblast cells of human villi at term (Fig. 4F). WAT was used as a positive control. Strong adiponectin immunostaining was detected in human and rat WAT cells (Fig. 4G). In addition, controls for the specificity of the primary antibody and the immunohistochemical reaction were carried out in WAT (Fig. 4H) and rat (Fig. 4I) and human (Fig. 4J) placentas, resulting in negative staining.

Adipo-R2 expression was also demonstrated in rat and human placentas of early gestation and term. Rat placentas, 6 dpc, showed higher Adipo-R2 immunoreactivity than those at 21 dpc. In humans, first-trimester placenta immunostaining was predominantly localized in primary giant trophoblast cells (Fig. 5, A–C) and was less intense in the ectoplacental cone. Adipo-R2 immunoreactivity was localized in peripheral giant spongiotrophoblast cells of rat term placentas (Fig. 5D). Vitellic membranes were also intensely stained (Fig. 5E). Like adiponectin, Adipo-R2 was expressed in cytotrophoblast and syncytiotrophoblast cells of first-trimester human placentas (Fig. 5F) and in syncytiotrophoblast cells of term human placentas (Fig. 5G). Positive controls (liver and adipose tissue) showed intense im-

![Fig. 3.](image-url)
munostaining (not shown), whereas negative controls showed no immunostaining (Fig. 5I).

**Placental expression of adiponectin and Adipo-R2 mRNA throughout pregnancy and influence of chronic adiponectin infusion on placental gene expression**

Adiponectin and Adipo-R2 mRNA expression were examined in rat placental tissues during the second half of gestation (d 12, 16, 19, and 21) by real-time RT-PCR (Fig. 6). We found that placental adiponectin mRNA was expressed throughout pregnancy, and adiponectin levels increased during the progression of gestation, reaching the highest expression at the end of this period ($P < 0.01$; Fig. 6A). In contrast, Adipo-R2 mRNA expression decreased during pregnancy ($P < 0.05$; Fig. 6B). In addition, to determine whether adiponectin and Adipo-R2 patterns of expression...
throughout gestation could be modified by exogenous factors known to be associated with placental development, we assessed the effect of undernutrition. Using real-time RT-PCR, we studied the relative expression of placental adiponectin and Adipo-R2 mRNA throughout gestation in fed *ad libitum* vs. restricted rats (fed 30% of the daily *ad libitum* diet). Adiponectin was slightly lower in restricted fed dams compared with control animals fed *ad libitum*, although this difference was only significant on d 16 of gestation (Fig. 7A); however, Adipo-R2 was not modified by food restriction throughout pregnancy (Fig. 7B).

Finally, in pregnant rats, treatment with adiponectin did
not affect food intake (Fig. 8A) or body weight (Fig. 8B). Moreover, we observed a significant decrease in GLUT3 placental mRNA levels and lesser decreases in Adipo-R2, lipoprotein lipase (LPL), and TGF-β. However, insulin receptor sustrate-1 (IRS-1) and vascular endothelial growth factor (VEGF) mRNA expression remained unchanged in the placenta (Fig. 8C). Plasma adiponectin levels were assayed in virgin rats, pregnant rats, and pregnant rats treated with adiponectin, and we detected higher adiponectin levels in the treated group compared with vehicle animals (Fig. 8D), similar to levels achieved at midpregnancy in nontreated rats (Fig. 8E).

**Discussion**

In the present work, adiponectin and Adipo-R2 have been unambiguously identified in human and rat placentas. The identification of both factors has been accomplished by several different approaches to assess mRNA and protein expression. Furthermore, with immunohistochemistry we were able to demonstrate the cell types responsible for their expression in both human and rat placentas. Adiponectin and Adipo-R2 seem to be specifically related to some functions, because their expression was localized to only some cells of the placenta, with important differences between humans and rats. In fact, cytotrophoblast and syncytiotrophoblast cells showed immunostaining for both the ligand and the receptor in humans. In contrast, trophoblast and glycogen cells in the rat showed adiponectin expression, whereas giant cells and viteline membranes were immunostained for Adipo-R2.

In our study we detected the expression of adiponectin and Adipo-R2 in trophoblast cells on day 6 of gestation. These cells are situated between maternal and embryonic compartments and permit the embryo to develop within the female reproductive tract. The growth and differentiation of these cells are essential for the establishment and maintenance of pregnancy. Moreover, in mice and rats, the trophoblast giant cells are one of the first differentiated cells to arise in the developing embryo. The physiological role, if any, of adiponectin and Adipo-R2 in trophoblast cells remains to be determined.
Adiponectin expression in these cell types is presently under investigation in our laboratory.

In our study human adiponectin and Adipo-R2 were localized in the cytotrophoblast and syncytiotrophoblast in first-trimester placentas and in the syncytiotrophoblast in term placentas. This pattern of expression is the same for other hormones, such as leptin (24) and IGF-I (25). However, the roles of both factors in these cells are unknown, and additional experiments are needed to elucidate this question.

Both human and rodent pregnancies are characterized by several metabolic changes that promote adipose tissue accretion in early gestation and later onset of insulin resistance. In rats, from d 10 of pregnancy, glucose-stimulated insulin secretion (26) and islet cell proliferation (27) increase. Food intake is also increased, whereas the sensitivity of maternal tissues to insulin decreases (28). Both factors cause an increased demand for insulin. In keeping with this, it has been shown that circulating adiponectin levels are suppressed from midgestation until weaning (8), probably reflecting the impaired insulin sensitivity during pregnancy. Thus, it was of great interest to assess whether placental adiponectin levels follow a similar pattern. Contrary to our expectations, we found an increase in placental adiponectin mRNA levels as pregnancy progressed, with the highest levels achieved at the end of gestation. These data suggest that adiponectin gene expression during pregnancy is regulated in a tissue-specific manner.

In addition, we assessed adiponectin receptor mRNA expression in placenta. Our results demonstrated that Adipo-R1 was expressed in blood cells, and because placenta showed a clear expression of the mononuclear cell marker CD45 (data not shown), we decided to study only the levels...
of Adipo-R2. We observed that Adipo-R2 was decreased during gestation, whereas the ligand was increased in the last part of gestation. Moreover, when pregnant rats were treated with adiponectin, placental Adipo-R2 mRNA expression was inhibited. These results suggest that, at least in placenta, the high expression of the ligand may down-regulate its receptor.

To further characterize the regulatory mechanisms involved in placental adiponectin gene expression, we assessed the influence of chronic undernutrition. It is well known that chronic undernutrition increases insulin sensitivity in adult rodents compared with ad libitum feeding (29, 30). Our results demonstrate that placental adiponectin mRNA levels were lower in food-restricted than in ad libitum-fed rats on d 16 of gestation, but not during the last part of pregnancy. These data follow a pattern opposite that found in plasma adiponectin levels reported in this study as well as in food-restricted mice (8). In contrast, it is known that mothers subjected to 50% food restriction over a long term have decreased plasma glucose levels, but this does not affect placental weight. However, Adipo-R2 mRNA expression is unchanged with undernutrition, indicating that the expression of this receptor is not affected under chronic food restriction conditions.

Finally, we assessed the effect of chronic adiponectin treatment during gestation on several genes involved in different placental functions, such as nutrient transfer or trophoblast proliferation. Glucose transporter-3 (GLUT3) is the transporter isoformal characterist of cells with high glucose requirements, such as neurons or tumor cells (31). It has also been reported that GLUT3, and not GLUT1, is the functional transporter for maternal glucose to trophectoderm cells, which are the precursors of trophoblast cells (32). Our results demonstrated that adiponectin decreased GLUT3 mRNA expression in the placenta, suggesting a role for adiponectin in the transport of maternal glucose to trophoblast cells. LPL is primarily responsible for the accumulation of triglycerides, and in our experiment, we observed an inhibition of placental LPL mRNA expression after adiponectin treatment, indicating that adiponectin could decrease the transport of triglycerides to the fetus. TGF-β is a cytokine family of three closely related peptides (TGF-β1, -β2, and -β3) that promote cellular actions through membrane receptors. We observed that adiponectin decreased TGF-β mRNA expression. Because it is known that TGF-β inhibited the proliferation of trophoblast cultures in a dose-dependent manner (33) and increased plasminogen activator inhibitor-1 expression (34), our results suggest that adiponectin might play a role in the proliferation of trophoblast cells and in the invasive mechanisms. In contrast, adiponectin had no effect on other target genes, such as IRS-1 and VEGF, indicating the specificity of the changes in the above-mentioned genes.

In summary, we have demonstrated for the first time that adiponectin and Adipo-R2 are expressed in human and rodent placental tissues. Furthermore, we showed that their expression is regulated by pregnancy and undernutrition differently than previously reported in relation to adiponectin circulating levels. Finally, our results suggest that adiponectin may be involved in the regulation of several placental functions.

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Erratum

In the article “Onset of Overweight during Childhood and Adolescence in Relation to Race and Sex” by C. Saha, G. J. Eckert, J. H. Pratt, R. R. Shankar (The Journal of Clinical Endocrinology & Metabolism 90:2648–2652, 2005), Dr. Chandan Saha should have been listed as first corresponding author and Dr. R. Ravi Shankar as second corresponding author. Dr. Saha’s mailing address is RG4101, 1050 Wishard Boulevard, Indianapolis, IN 46202-2872; Dr. Saha’s e-mail address is cksaha@iupui.edu. The printer regrets the error.

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