The Human Placenta in Gestational Diabetes Mellitus

The insulin and cytokine network

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The placenta is a complex fetal organ that fulfills pleiotropic roles during fetal growth. It separates the maternal and fetal circulation, with which it is in contact through different surfaces, i.e., the syncytiotrophoblast exposes the placenta to the maternal circulation and the endothelium is in contact with fetal blood. Because of this unique position, the placenta is exposed to the regulatory influence of hormones, cytokines, growth factors, and substrates present in both circulations and, hence, may be affected by changes in any of these. In turn, it can produce molecules that will affect mother and fetus independently.

The human placenta expresses virtually all known cytokines including tumor necrosis factor (TNF)-α, resistin, and leptin, which are also produced by the adipose cells. The discovery that some of these adipokines are key players in the regulation of insulin action suggests possible novel interactions between the placenta and adipose tissue in understanding pregnancy-induced insulin resistance. The interplay between the two systems becomes more evident in gestational diabetes mellitus (GDM).

In diabetes, the placenta undergoes a variety of structural and functional changes (rev. in 1–3). Their nature and extent depend on a range of variables including the quality of glycemic control achieved during the critical periods in placental development, the modality of treatment, and the time period of severe departures from excellent metabolic control of a nondiabetic environment.

Placental development is characterized by three distinct periods. At the beginning of gestation, a series of critical proliferation and differentiation processes predominantly of the trophoblast eventually lead to the formation of villous and extravillous structures. The latter anchor the placenta in the uterus and remodel the uterine spiral arteries into low resistance vessels. Then the newly formed villi differentiate through various steps of maturation. The end of gestation is associated with placental mass expansion, i.e., villous growth (Fig. 1). During the first half of gestation, the trophoblast is the key tissue that undergoes the most profound alterations, whereas extensive angiogenesis and vascularization occur in the second half of gestation, i.e., the endothelium is the site of the more prominent processes, although there is overlap. This period is also accompanied by extensive vascular remodeling and stabilization of the vascular bed (4,5).

Diabetic insults at the beginning of gestation as in many pregestational diabetic pregnancies may have long-term effects on placental development. These adaptive responses of the placenta to the diabetic environment, such as buffering excess maternal glucose or increased vascular resistance, may help limit fetal growth within a normal range. If the duration or extent of the diabetic insult, including maternal hyperglycemia, hyperinsulinemia, or dyslipidemia, exceeds the placental capacity to mount adequate responses, then excessive fetal growth may ensue.

Diabetic insult at later stages in gestation, such as may occur in gestational diabetes, will foremost lead to short-term changes in a variety of molecules for key functions including gene expression (6).

The diabetic environment can be regarded as a network of substances (hormones, nutrients, cytokines) with altered concentrations. The current view is that the abnormal maternal metabolic environment may generate stimuli within the adipose tissue and the placental cells resulting in the increased production of inflammatory cytokines whose expression is minimal under normal pregnancy. One leading hypothesis is that changes in circulating TNF-α, adiponectin, leptin, and resistin link inflammation to metabolic changes by enhancing insulin resistance in the mother. Likewise, the fetal environment is also changed in diabetes, and elevated levels of insulin, leptin, and other cytokines have been well documented. This review will concentrate on insulin and cytokines as contributors to this network and potential regulators of placental function in GDM.

THE INSULIN RECEPTOR NETWORK

— Despite improvements in care over the past decades to achieve adequate maternal glucose control, fetal hyperinsulinemia is quite common in GDM pregnancies. Intensive research has tried to establish alterations in maternal-fetal transport of the most important insulin secretagogues, i.e., glucose and amino acids. Although the placental glucose transporter GLUT1 is subject to changes by the ambient level of glycemia, i.e., it can be downregulated and translocated into the cell interior by hyperglycemia in vitro (7–9), maternal-fetal glucose transport is lower than normal in diet-treated GDM subjects and near normal in insulin-treated GDM subjects. This will only have an effect if maternal glucose concentrations are high above postprandial glucose levels (10,11), because of the

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Abbreviations: GDM, gestational diabetes mellitus; IL, interleukin; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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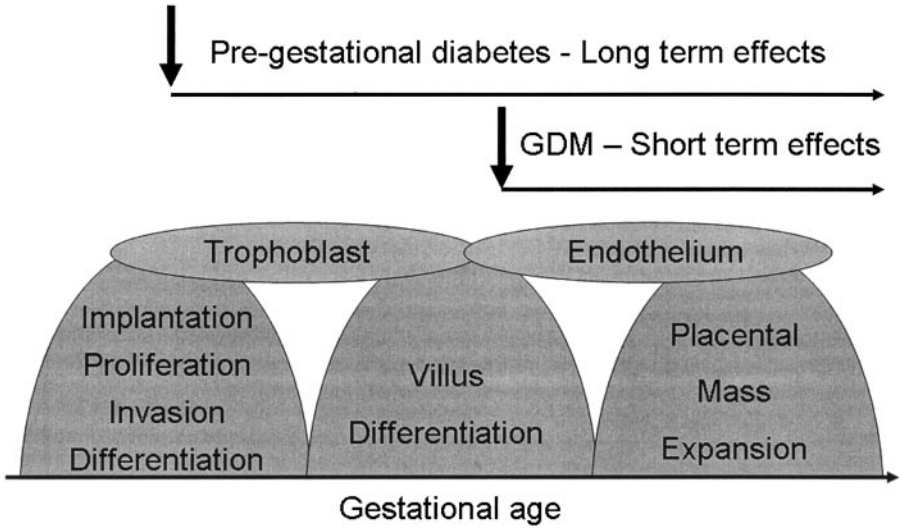

Figure 1—*Placental growth and development are separated in three distinct, yet overlapping phases, which are predominantly associated with the trophoblast in the first half of gestation and with the endothelium in the second half of gestation. Any insult of the diabetic environment early in pregnancy will alter the placenta in a period critical for later development and, hence, have long-term effects unless counteracted by adaptive responses. Diabetic insults at later stages in gestation such as in GDM will only have short-term effects predominantly on placental function rather than its structure.*

high capacity of the transplacental glucose transport system (12). Changes in placental amino acid transporters, if at all, are not associated with maternal diabetes, but rather with elevated fetal weight (13). However, because of the complex nature of amino acid transporter systems in the human placenta, any generalization has to be avoided, and perfusion studies across the intact organ are still pending. Yet, according to current knowledge, fetal hyperinsulinemia in diabetes is the result of the steeper transplacental glucose gradient associated with maternal hyperglycemia and is not accounted for by placental transporter changes.

The placenta expresses high amounts of insulin receptors relative to other tissues in the body. Their location undergoes developmental changes. At the beginning of gestation, they are located at the microvillous membrane of the syncytiotrophoblast, whereas at term, they are predominantly found at the endothelium (14,15). This strongly suggests a shift in control of insulin-dependent processes from the mother at the beginning of pregnancy to the fetus at the end. The spatiotemporal change in insulin receptor location is paralleled by a change in function, since insulin-induced gene expression is highest in first trimester trophoblast (16). At term, insulin has a stronger effect on the endothelium than on the trophoblast. This is important for diabetic pregnancies in general and for GDM in particular, because it can be assumed that the fetal hyperinsulinemia will affect the placental endothelium.

As a current concept (Fig. 2), at the beginning of pregnancy, maternal insulin will regulate the placenta by interacting with the syncytiotrophoblast. This may lead to altered synthesis and secretion of hormones and cytokines that in turn will act back on the mother, thus forming a feedback loop. As gestation advances, the fetus, i.e., fetal insulin, will gradually take over control from the mother and directly or indirectly affect the endothelium or tissue-resident macrophages (Hofbauer cells). Whether one of the results will be the placental release of molecules or nutrients to the fetus as another feedback loop is currently under investigation. Changes in the number, affinity, and signaling properties of placental insulin receptors may confound this concept, but available information is scant. In diet-treated GDM, the amount of trophoblast insulin receptors is lower than in nondiabetic pregnancies, whereas in insulin-treated GDM, the placenta contains more insulin receptors (17). Whether endothelial insulin receptors are also altered is unknown.

Recent evidence demonstrated that insulin receptors at the different locations preferentially activate different intracellular signaling pathways. Whereas in the trophoblast compartment the mitogen-activated protein kinase pathway is predominantly activated, insulin stimulates

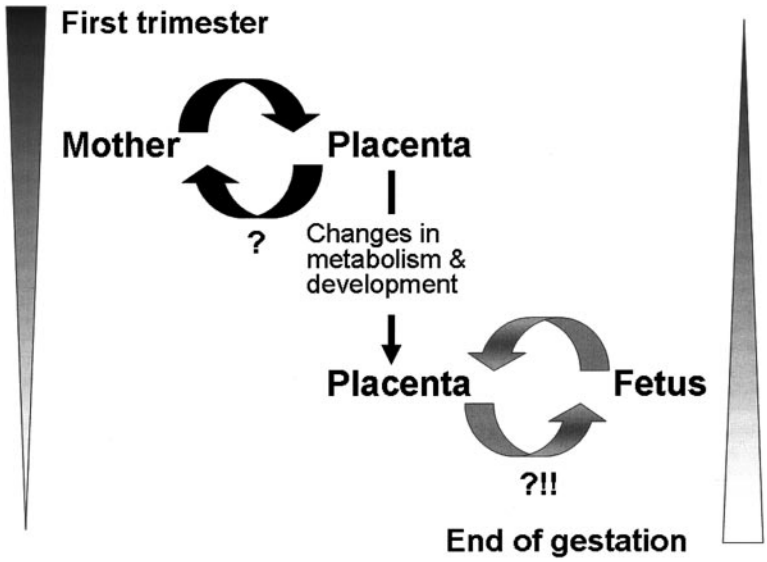

Figure 2—*Generalized hypothetical model of the gestational shift in placental control from mother to fetus. The maternal changes in the diabetic environment will predominantly alter placental development at the beginning of gestation. Changes in placenta function may include altered synthesis and/or secretion of growth factors, hormones, and cytokines that will act back on the mother. As gestation advances, the placenta is beginning to be affected by the diabetic environment in the fetus. Whether this results in altered release of placental signals to the fetus is currently unknown. Taken from Hiden et al. (16) with kind permission of Springer Science and Business Media.*

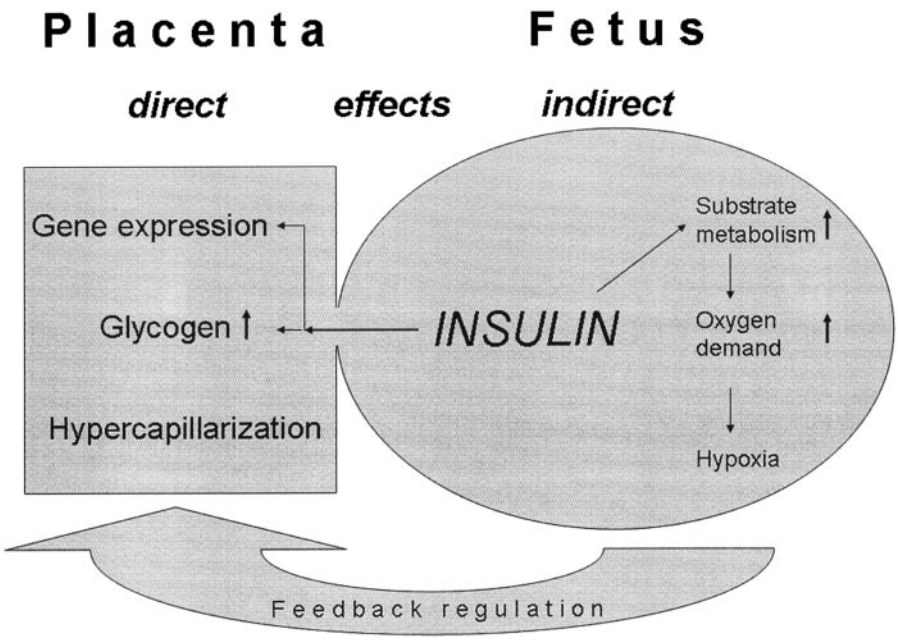

Figure 3—*Fetal insulin will have direct effects on the placenta, such as inducing alterations in gene expression and stimulating endothelial glycogen synthesis. In addition, indirect effects mediated by fetal hypoxia on placental structure can be regarded as adaptive feedback responses to ensure adequate oxygen supply.*

the protein kinase B/Akt pathway in the endothelium (18). This may indicate a mitogenic effect of insulin on the trophoblast, predominantly at the beginning of pregnancy, whereas fetal insulin will stimulate metabolic processes within the endothelium. In fact, in vitro studies confirmed the mitogenic potency of insulin in trophoblast models (19). This may explain the biphasic growth of the placenta and fetus at around mid-gestation in type 1 and experimental diabetes (20,21).

Fetal insulin in normal pregnancies and even more so in diabetic pregnancies with hyperinsulinemia may have direct and indirect effects on the placenta (Fig. 3). In addition to altering the expression of genes (16), it will stimulate endothelial glycogen synthesis (22). Although diet-treated GDM is associated with even lower than normal glycogen levels, elevation of placental glycogen levels in all other forms of diabetes has been well established (rev. in 3). In this respect, the placenta is a paradoxical tissue, since in the classic insulin target tissues, glycogen levels are reduced in diabetes because of the insulin resistance. Insulin does not change glycogen levels in the trophoblast. Glycogen increments in diabetes are found around the villous vessels and capillaries, suggesting that the glycogen stores are built up by glucose derived from the fetal circulation. In fact, not only the ubiquitous glucose transporter GLUT1, but also the high affinity transporter GLUT3, is expressed in the placental endothelium, where it colocalizes with glycogenin, the protein precursor for glycogen synthesis (23). Increased glycogenin gene expression in placenta with GDM supports our hypothesis (6). In addition, the insulin-sensitive GLUT4 is located on the endothelium (24). Fetal glucose can be transported back into the placenta (25), and this back transport is increased in diabetic rats (26). The placenta is the only fetal tissue that can store excess fetal glucose. The buffer function of the placental endothelium will be stimulated by insulin, not only in vitro, as in human, but also in vivo in the rodent (27). This has led to a hypothesis proposing that some types of fetal macrosomia are the result of placental failure to store excess fetal glucose (28).

In addition to the direct effects of fetal insulin on the placenta that have been established so far, i.e., gene expression and glycogen synthesis, indirect effects can also be seen.

Insulin stimulates fetal aerobic glucose metabolism and will hence increase oxygen demand of the fetus. If adequate supply is not available because of reduced oxygen delivery to the intervillous space as a result of the higher oxygen affinity of glycated hemoglobin (29), thickening of the placental basement membrane (30,31), and reduced utero-placental or fetoplacental blood flow (32,33), fetal hypoxemia will ensue (34). Hypoxia is a potent stimulator of hypoxia-sensitive transcription factors such as the hypoxia inducible factor (HIF) and will therefore lead to the stimulated expression and synthesis of a variety of molecules, some of which are key players, especially in angiogenesis (35,36). Diabetic pregnancies are associated with elevated fetal levels of fibroblast growth factor-2 (37,38), which will stimulate placental angiogenesis and lead to the hypercapillarization seen in placentas of type 1 diabetic pregnancies. Reports in GDM are conflicting (39–41). Some, but not all, studies found increased longitudinal vascular growth and enhanced branching angiogenesis, which may reflect different time points of GDM onset in gestation either within or after the critical developmental stages of vasculogenesis and angiogenesis (42).

One of the characteristic features of a placenta in GDM is its increased weight, which is accompanied by enlarged surface areas of exchange on the maternal (syncytiotrophoblast) and fetal (endothelium) side (3). Teleologically, it may appear paradoxical that in a situation of maternal nutritional oversupply, the placenta increases its surface, thus potentially contributing to enhanced maternal fetal transport, but this reflects the prime importance of adequate oxygen supply to the fetus and the effect of excess growth factors such as insulin, which collectively dictate some of the placental changes even at the cost of adverse side effects.

THE CYTOKINE NETWORK —Cytokines are mainly but not exclusively produced by cells of the immune system, NK cells, and macrophages in response to an external stimulus such as stress, injury, and infection. Adipose tissue represents an additional source of cytokines, making possible a functional cooperation between the immune system and metabolism (43,44). The placenta also synthesizes a variety of cytokines, adding an additional level of complexity to the immune-metabolic network existing in pregnant individuals. This raises the possibility that placenta cytokine production contributes to a low-grade inflammation developing during the third trimester of pregnancy (45). In pregnancy complicated with GDM or obesity, there is a further dysregulation of metabolic, vascular, and inflammatory pathways supported by

increased circulating concentration of inflammatory molecules (46,47). Studies of transcriptional profiling have shown that adipose tissue and the placenta express a common repertoire of cytokines and inflammation-related genes, which become overexpressed in a diabetic environment (48). The current view is that maternal adipose tissue as well as the placenta contribute to the inflammatory situation by releasing common molecules, the relative contribution of which has yet to be determined.

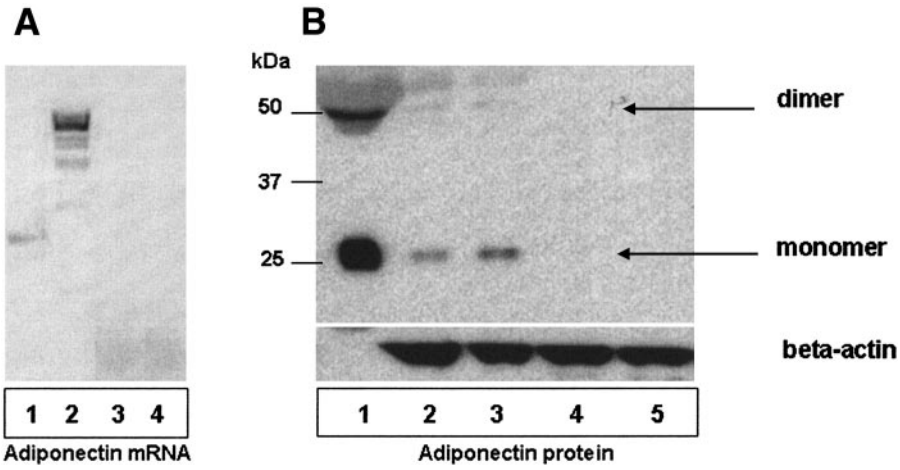

Figure 4—*Adiponectin expression.* A*: Adiponectin mRNA expression measured by real-time PCR (amplification run of 40 cycles) is detected in maternal white adipose tissue* (lane 1) *but not in human placenta at 39 weeks of gestation* (lanes 3 *and* 4). B*: Immunodectection of adiponectin protein. Signals for monomeric (25 kDa) and dimeric (50 kDa) adiponectin complexes were detected in maternal serum (1 μl) after electrophoresis under reducing conditions* (lane 1)*, in white adipose tissue (50 μg total protein,* lane 2*), and in crude placenta tissue (50 μg total protein,* lane 3*). Once the placenta is being washed out from intervillous blood, the adiponectin signals are no longer detectable* (lanes 4 *and* 5*). This indicates that adiponectin protein expression detected in term placenta is accounted for by the systemic blood trapped within the intervillous blood space.*

The placenta is a source of cytokines: placental influence

The concept of the endocrine function of the human placenta as being restricted to the production of gestational hormones is rapidly being challenged. The human placenta has been found to express virtually all known cytokines (49).

In addition to immune-related cytokines and growth factors, the placenta synthesizes resistin and leptin, two adipose tissue–specific proteins (adipokines) implicated in the regulation of insulin action (50). Whether the placenta at term is able to synthesize the insulin sensitizer, adiponectin, is still being debated (Fig. 4) (51). Cytokines are produced by three different placental cell types: the Hofbauer cells, the trophoblast cells, and cells of the vascular endothelium, albeit with cell type–specific cytokine patterns. For example, TNF-α is produced by the Hofbauer cells (52,53), whereas the syncytiotrophoblast is the major site of leptin synthesis, and interleukin (IL)-6 expression is found in both trophoblast and endothelial cells (54–56). Studies of the pattern of production and release of placental cytokines into the systemic circulation have provided valuable information relating to their mechanism and site of action. Leptin and IL-6 are released into the fetal and maternal systemic circulation. Thus, they can exert endocrine action by acting at sites remote from the production site (57–59). In contrast to leptin, TNF-α is poorly released from the placenta (Table 1) and hence is more likely to exert local paracrine effects. There is an overproduction of placenta leptin and TNF-α in type 1 diabetes and GDM (60; Fig. 5). In GDM, the overexpression of placenta TNF-α is associated with increased fetal adiposity (6). The stimuli and mechanisms responsible for increased leptin and TNF-α gene expression and production are not known at present; however, the consequences of their overproduction are just beginning to be unraveled (61). Both cytokines activate phospholipase A2, a family of lipolytic enzymes that generate eicosanoid precursors such as docosahexaenoic acid, an essential ω-3 polyunsaturated fatty acid. Recent evidence demonstrated an accumulation of ω-3 fatty acids in placenta of offspring of GDM mothers with increased adiposity at birth (62).

This may be one potential mechanism linking local placental inflammatory responses with increased lipid substrate availability for fetal fat deposition, in addition to increased maternal supply. TNF-α may also participate in the endocrine mechanism of pregnancy-induced insulin resistance by adding a placental component to the insulin resistance developing in the mother. The TNF-α induction of IRS-1 serine phosphorylation links inflammation to defective insulin action in pregnancy (58,63). In GDM, the activation of the proximal cytoplasmic proteins of TNF-α signaling such as TNFR1 associated death domain (TRADD) protein, TNFR2-associated death domain (TRAF2) protein, and Fas-associated death domain (FADD) protein is an indication of the recruitment of TNF-α R1 and R2 receptors (6). This raises the possibility that placenta TNF-α downregulates insulin action through serine phosphorylation of placental insulin receptors, as shown in skeletal muscle of women with GDM (64).

The placenta is a target of cytokines: maternal and fetal influences

The placenta is at the same time source and target for cytokines. The type and the location of the cytokine receptors present on the placental cells will determine whether signals are generated by placentally (internal), maternally (presumably adipose-derived), or fetally derived cytokines. This emphasizes the possibility of an external control of placental function that can become dysregulated when the cytokine levels are augmented, such as in GDM or obesity (45,65).

One leading hypothesis is that increased TNF-α, leptin, and resistin contribute to enhancing insulin resistance in the GDM mother (58). In addition, adiponectin may be implicated in the loss of insulin sensitivity with advancing gestation in normal pregnancy and in pregnancy with GDM through a decrease in maternal concentrations (66–68). It is worthwhile to emphasize here that, whereas it will be difficult to dissect out the relative contribution of the placental and maternal tissues for regulation through TNF-α, leptin, or resistin, the influence of adiponectin will be exclusively of maternal origin because of the absence of ligand but expression of adiponectin receptors in the placenta (Fig. 4; 69,70).

Table 1—*Leptin and TNF-α release by human term placenta perfused in vitro*

	Placental tissue accumulation (ng/min)	Release into the fetal perfusate (ng/min)	Release into the maternal perfusate (ng/min)	Placenta production ($ng \cdot min^{-1} \cdot g^{-1}$)
Leptin	1.52 ± 0.75	0.13 ± 0.04	1.99 ± 0.73	0.036 ± 0.15
TNF-α	−2.30 ± 1.67	0.015 ± 0.004	0.49 ± 0.25	0.12 ± 0.05

Placentas from uncomplicated term pregnancy (39–40 weeks) were analyzed with a dual cotyledon in vitro perfusion technique. Cytokine concentrations were measured by enzyme-linked immunosorbent assay from three distinct sources: placental tissue, maternal perfusate, and fetal perfusate. Tissue accumulation is calculated over the 60 min of perfusion time. The negative number for TNF-α indicates that TNF-α accumulates in the tissue during the time of perfusion besides being released in both maternal and fetal circulations. The positive value for leptin indicates that leptin is washed out from the tissue during the time of perfusion. Placental production is calculated as total release (M + F) minus tissue accumulation. Adapted from Lepercq et al. (57) and Kirwan et al. (58).

Similar to other peptide hormones such as insulin or glucagon, there is minimal trans-placental transfer of cytokines from mother to fetus (71,72). Hence, the origin of the cytokines found in the fetal circulation can be twofold, either released from the placenta or synthesized within the fetus. There is a critical lack of information regarding most fetal cytokines and adipokines. There is now clear evidence that placental leptin is poorly released into the fetal circulation (Table 1) and that leptin synthesized by fetal adipose tissue can be taken as a marker of fetal adiposity (61,73). Some of the stimuli that disturb placental metabolism may also be conveyed through the vascular endothelium as oxidative stress, endothelial injury, etc., thus bringing into the picture a control from the fetus, through alterations induced by circulating fetal TNF-α, leptin, and IL-1 and IL-6 (74).

Thus, the maternal-fetal control of the placenta is a cumulative result of cell cooperation that may propagate a vicious cycle for enhancement of cytokine production, which may eventually have an impact on insulin action in the feto-placental unit and possibly obesity in utero. The discovery that some adipokines are produced by the placenta opens novel perspectives for understanding the specificity of pregnancy-induced insulin resistance. It also emphasizes the importance of functional interplays between the placenta and maternal white adipose tissue in GDM. The signals that regulate the secretion of these molecules are far from clear. Further studies in this area may provide a clue for understanding the inflammatory processes in GDM and obesity and potentially in utero programming of obesity.

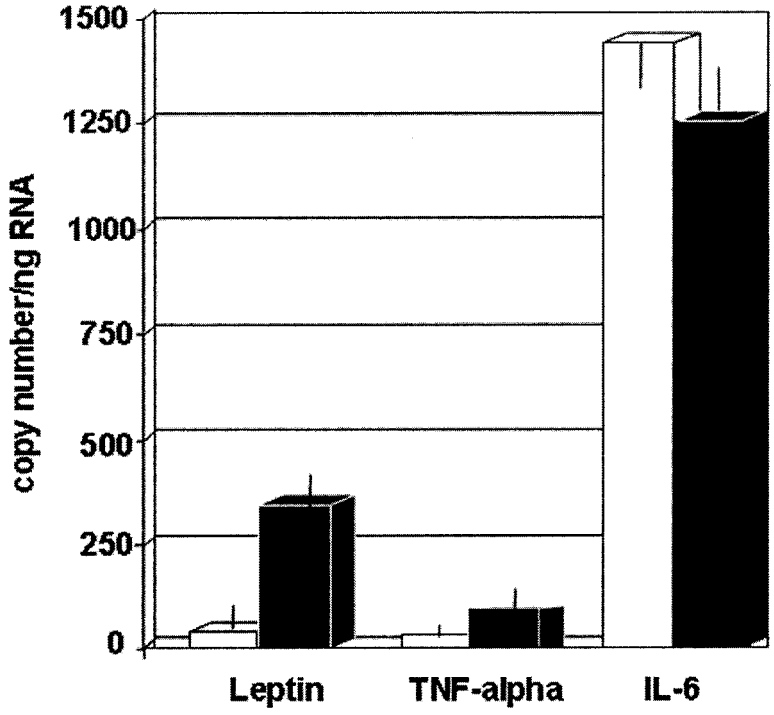

**Figure 5—*Expression of leptin, TNF-α, and IL-6 in term placenta (38–39 weeks). Absolute mRNA levels for leptin, TNF-α, and IL-6 were measured by real-time RT-PCR using actin as the normalization control.* □, *Control subjects,* n = *8;* ■, *GDM patients,* n = *7. Data are expressed as means ± SE. The statistical difference has been calculated using a nonpaired Student's* t *test.* *P *value <0.05 was considered significant.*

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