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Leptin and Gestational Diabetes Mellitus

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Abstract

Emerging research has highlighted the importance of leptin in fetal growth and development, independent of its essential role in the regulation of feeding and energy metabolism. Leptin is now considered an important signaling molecule of the reproductive system, since it regulates the production of gonadotropins, the blastocyst formation and implantation, the normal placentation, as well as the feto-placental communication. Placental leptin is an important cytokine which regulates placental functions in an autocrine or paracrine manner. Leptin seems to play a crucial role during the first stages of pregnancy as it modulates critical processes like proliferation, protein synthesis, invasion, and apoptosis in placental cells. Furthermore, deregulation of leptin levels has been correlated with the pathogenesis of various disorders associated with reproduction and gestation, including gestational diabetes mellitus (GDM). Due to the relevant incidence of the GDM and the importance of leptin, we decided to review the latest information available about leptin action in normal and GDM pregnancies to support the idea of leptin as an important factor and/or predictor of diverse disorders associated with reproduction and pregnancy.

Keywords: leptin, reproduction, placenta, GDM, microRNAs

1. Introduction

Adipose tissue acts as an endocrine organ, secreting different molecules or adipokines. A link between body weight, adipokines, and success of pregnancy has been proposed, although it is not fully understood [1]. Leptin was the first adipokine claimed to be the “missing link” between fat and reproduction [2]. Leptin is considered as a pleiotropic hormone that regulates not only body weight but also many other functions, including the normal physiology of the reproductive system [3]. Importantly, this hormone is also produced by other tissues, especially placenta [4].

Placental formation during human gestation is crucial for embryonic progress and successful pregnancy outcome, allowing metabolic exchange and producing steroids, hormones, growth factors, and cytokines that are critical for the maintenance of pregnancy [5, 6]. Trophoblast cells play an essential role in the development of placenta. These cells differentiate in two distinct types: extravillous and villous trophoblast. In the extravillous pathway, cytotrophoblasts proliferate,

differentiate into an invasive phenotype, and penetrate in the maternal decidua and myometrium. Meanwhile, in the villous pathway, mononuclear cytotrophoblasts fuse to form a specialized multinuclear syncytium called syncytiotrophoblast [7]. In normal pregnancy, trophoblast invasion is a critical step in remodeling the maternal spiral arteries to adequately perfuse the developing placenta and fetus [8]. In this sense, deregulation of leptin levels has been implicated in the pathogenesis of gestational diabetes mellitus (GDM) [9].

2. Leptin and reproduction

Reproductive function depends on the energy reserves stored in the adipose tissue. The large energy needs for a hypothetical pregnancy was the original rationale to explain the disruption of reproductive function by low fat reserves. This led to the hypothesis of an endocrine signal that conveys information to the brain about the size of fat stores [10]. Thus, leptin was the first adipokine claimed to be the “missing link” between fat and reproduction [2]. Leptin modulates satiety and energy homeostasis [11, 12] but is also produced by the placenta. Thus, it was suggested that the effects of placental leptin on the mother may contribute to endocrine-mediated alterations in energy balance, such as the mobilization of maternal fat, which occurs during the second half of pregnancy [13, 14]. In addition, leptin has been found to influence several reproductive functions, including embryo development and implantation [15]. Moreover, animal models have demonstrated that leptin-deficient mice are subfertile and fertility can be restored by exogenous leptin [16]. This adipokine may therefore play a critical role in regulating both energy homeostasis and the reproductive system [17].

Leptin increments the secretion of gonadotropin hormones, by acting centrally at the hypothalamus [18]. In addition, because leptin has been shown to be influenced by steroid hormones and can stimulate LH release, leptin may act as a permissive factor in the development of puberty [19].

Leptin can also regulate ovary functions [20–23]. Thus, leptin resistance and hyperleptinemia in obesity lead to altered follicle function, whereas in conditions in which nutritional status is suboptimal, leptin deficiency results in hypothalamic-pituitary gonadal axis dysfunction [24, 25].

In addition, a significant role of leptin in embryo implantation was proposed. Leptin receptor (LEPR) is specifically expressed at the blastocyst stage [26], and it was also reported that leptin is present in conditioned media from human blastocysts, promoting embryo development, suggesting a function in the blastocyst-endometrial dialog [27].

3. Leptin and placenta

The implantation involves complex and synchronized molecular and cellular events between the implanting embryo and uterus, and these events are regulated by autocrine and paracrine factors [5]. Fetal growth depends on the ability of the placenta to supply nutrients adequate to meet fetal demand, which increases as gestation progresses. Villous cytotrophoblast is a progenitor cell population that produces daughter cells to support the expansion of the syncytium as placental surface area increases as well as the expansion of cytotrophoblast columns, which contain the cells destined to invade maternal decidua [28]. The placenta grows exponentially in the first and early second trimester, but growth has slowed down by term [29]. Therefore, placental growth, especially in early gestation, is a

prerequisite of a high-capacity transport interface. In 1997, leptin was described as a new placental hormone in humans [14]. In fact, during pregnancy, circulating leptin levels are also increased due to leptin production by trophoblastic cells [30]. After delivery, leptin levels return to normal levels [31].

To alter intracellular signaling and function, leptin must bind to the receptor (LEPR) [32]. There are six different isoforms of LEPR (a–f) that are produced by alternative RNA splicing [33]. The only isoform that has a transmembrane domain that is capable of activating signal transduction pathways is LEPRb, whereas the other five short LEPR isoforms have either a truncated or no transmembrane domain and are unable to activate signaling pathways [33]. Activation of LEPRb results in an upregulation of a number of signal transduction pathways, including the Janus kinase/signal transducers and activators of the transcription pathway (JAK/STAT), as well as the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways [34]. Research findings do indicate that there may be fetal-to-maternal leptin exchange across the placenta [35]. However, to date, it is not known which receptor is mediating this transportation.

Leptin has physiological effects on the placenta, including angiogenesis, growth, and immunomodulation [13]. Leptin is now considered an important regulator during the first stages of pregnancy, modulating proliferation, invasion, apoptosis, and protein synthesis, in placenta [36–41].

The control of cell proliferation is critical for a correct placental development, and it is finely regulated [42]. Altered rates of cytotrophoblast proliferation are associated with different pathologies; levels are enhanced with increased fetal growth (macrosomia) and diminished in fetal growth restriction [42]. Others factors in maternal circulation might coordinately stimulate proliferation, differentiation, and survival [43, 44] through the activation of multiple kinases [43–45] and phosphatases [45].

During placentation, cytotrophoblasts and syncytiotrophoblast keep a subset of cells in direct contact to the villous basement membranes. In the extravillous compartment, cell proliferation favors the invasion of the uterine stroma. Similarly, in the villous compartment, cells undergo syncytial fusion directed by specific transcription factors [46].

The role of MAPK in regulating trophoblast turnover is well documented in both human and animal systems [43, 44, 47]. Moreover, it was shown that leptin induces proliferative activity in many human cell types [48–50], mainly via MAPK activation [51]. We have demonstrated that leptin promotes proliferation of trophoblast cells by this MAPK pathway [41, 52]. We have also found that leptin dose-dependently stimulates protein synthesis by the activation of translation machinery [36, 53].

In this context, it is interesting to mention the role of Sam68, an RNA-binding protein originally identified as the substrate of Src during mitosis and a member of the signal transduction and activation of RNA metabolism (STAR) family [54, 55]. Leptin stimulates Tyr-phosphorylation of Sam68 in the trophoblast, mediating the dissociation from RNA, suggesting that leptin signaling could modulate RNA metabolism [48, 56]. Recent data indicate that microRNAs have a fundamental role in a variety of physiological and pathological processes. In this context, studies of microRNA expression have revealed that some microRNAs are abundantly expressed in the placenta [57]. However, the signature of miRNAs in the placenta has yet to be elucidated.

In placental villi, cell turnover is tightly regulated, via apoptotic cascade [49]. In normal pregnancy, apoptosis is an essential feature of placental development, and it is well established that trophoblast apoptosis increases with placental growth and advancing gestation [50]. Leptin prevents early and late events of apoptosis via MAPK pathway [41, 52]. The role of leptin was also studied under different stress

conditions like serum deprivation, hyperthermia, and acidic stress [39, 40]. Under serum deprivation, leptin increased the anti-apoptotic BCL-2 protein expression, while it downregulated the pro-apoptotic BAX and BID proteins expression as well as caspase-3 active form and cleaved PARP-1 fragment in Swan-71 cells and placental explants. In addition, it was demonstrated that p53 and its phosphorylation in Ser-46 are downregulated by leptin suggesting that leptin plays a pivotal role for apoptotic signaling by p53 [37]. Recent studies have demonstrated that MAPK and PI3K pathways are necessary for this anti-apoptotic leptin action, and it was also demonstrated that MDM-2 expression is regulated by leptin [38]. In placental explants cultured at high temperatures (40 and 42°C) and a pH acid (<7.3), the expression of Ser-46 p53, p53AIP1, p21, and caspase-3 is increased, and, these effects are significantly attenuated by leptin, indicating that leptin is a pro-survival placental cytokine [39, 40].

4. Leptin and immune system in placenta

One of the most important placental functions is to prevent embryo rejection by the maternal immune system to enable its correct development [51]. To ensure normal pregnancy, trophoblast differentiation requires potent immunomodulatory mechanisms to prevent rejection of syncytiotrophoblast and invasive trophoblast by alloreactive lymphocytes and natural killer (NK) cells present in maternal blood and decidua [58]. Inflammatory mediators such as IL-6, IL-1 β , TNF α , and prostaglandins are produced and secreted by the human placenta, and these cytokines play an important role in a number of normal and abnormal inflammatory processes, including the initiation and progression of human labor [59–61]. There are several homologies between the expression and regulation of cytokines and inflammation-related genes in the placenta and in the white adipose tissue. In this regard, leptin effects include the promotion of inflammation and the modulation of adaptive and innate immunity [56, 62, 63]. Thus, placental leptin acts as an immune modulator, regulating the generation of matrix metalloproteinases, arachidonic acid products, nitric oxide production, and T cell cytokines [61]. Interestingly, leptin expression is also regulated by IL-6, IL-1 α , IL-1 β , and IFN- γ [31, 64, 65].

It was reported that leptin stimulates IL-6 secretion in human trophoblast cells [66, 67]. In addition, TNF α release from human placenta is also stimulated by leptin, and it was demonstrated that NF- κ B and PPAR- γ are important mediators of this effect [68]. Recently, we have found that leptin induces HLA-G expression in placenta. HLA-G has potent immunosuppressive effects promoting apoptosis of activated CD8 $^{+}$ T lymphocytes, the generation of tolerogenic antigen-presenting cells, and the prevention of NK cell-mediated cytotoxicity. These data place leptin as a placental cytokine which confers to trophoblast cells a tolerogenic phenotype to prevent immunological damage during the first steps of pregnancy [69].

Pro-inflammatory leptin actions may also have significant implications in the pathogenesis of various disorders during pregnancy, such as GDM, which is characterized by increased leptin expression. In this sense, placental leptin may contribute to the incremented circulating levels of pro-inflammatory mediators that are evident in these pregnancy diseases, whereas successful pregnancy is associated with downregulation of intrauterine pro-inflammatory cytokines [9, 70, 71].

5. Leptin and gestational diabetes mellitus

Gestational diabetes mellitus, characterized by glucose intolerance diagnosed during pregnancy, is one of the most common complications in pregnancy and

affects 3–8% of all pregnancies [72, 73]. The prevalence of GDM has increased in recent decades due to increased average age of pregnant females and increased risk of obesity [74]. However, GDM is associated with numerous complications including macrosomia, neonatal metabolic disorders, respiratory distress syndrome, and neonatal death as well as a predisposition for the development of metabolic syndromes and type 2 diabetes [75, 76].

The placenta is thought to have a critical role in the pathogenesis of gestational diabetes mellitus, as GDM-associated complications resolve following delivery. Therefore, aberrant development and functions of the placenta, including placental overgrowth, have been implicated as important factors that contribute to GDM-associated complications [77, 78]. GDM is associated with insulin resistance, hyperinsulinemia, and hyperleptinemia, and these GDM-associated conditions disturb placental nutrient transport and fetal nutrient supply [79, 80].

It has been found that leptin and LEPR expressions are increased in placenta from GDM [9, 70], and, in fact leptin was proposed as a first-trimester biochemical predictor of GDM [81, 82]. In addition it was suggested that hyperinsulinemia may regulate placental leptin production acting as a circulating signal to control fetal homeostasis [73, 83]. Furthermore, it is thought that maternal glucose regulates cord blood leptin levels, and this could explain why newborns exposed to GDM have an increased risk of obesity [84]. Comparison of the placental gene expression profile between normal and diabetic pregnancies indicates that increased leptin synthesis in GDM is correlated with higher production of pro-inflammatory cytokines such as IL-6 and TNF α , causing a chronic inflammatory environment that enhances leptin production [85].

Our group has reported that insulin induces leptin expression in trophoblastic cells by increasing leptin promoter activity [86]. It is known that leptin and insulin share several signaling pathways, such as JAK2/STAT-3, MAPK, and PI3K. Moreover, we could demonstrate that in GDM, the basal phosphorylation of STAT-3, MAPK 1/3, and Akt is increased in the placenta, with resistance to a further stimulation with leptin or insulin in vitro, suggesting synergistic interaction between insulin and leptin signaling and action in human placenta [9].

On the other hand, GDM is associated with increased incidence of polyhydramnios, due to an increase in amniotic fluid volume, suggesting that aquaporins (AQP), such as AQP9 expression, could be altered in GDM [87, 88]. Besides, when maternal circulating glucose levels are controlled, they have normal amniotic fluid volume. AQP9 is also a transporter for glycerol and may also provide this substrate to the fetus. In this context, we have found that AQP9 mRNA and protein expressions are overexpressed in placentas from women with GDM. These data could suggest that during GDM the overexpression of AQP9, which correlates with higher leptin plasma levels, increments glycerol transport to the fetus which may help to cover the increase in energy needs that may occur during this gestational metabolic disorder [89].

Nevertheless, even though any nutritional or lifestyle intervention aimed to reduce weight produce a decrease in leptin levels, both in gestational diabetes and in general population, no therapeutic intervention, using leptin as a pharmacological target, has so far been used in the management of gestational diabetes.

6. Leptin and microRNAs

Gene expression can be regulated by short (18–22-nucleotide) noncoding RNAs, microRNAs, derived from long primary transcripts (pre-microRNAs) through sequential processing by two enzymes, Drosha and Dicer, and then incorporated

into the RNA silencing complex, where they target homologous mRNAs. In mice, loss or inactivation of Dicer leads to multiple developmental defects [90, 91], and it has been demonstrated that in human placenta, cytotrophoblast proliferation is increased following Dicer [92]; however, the individual microRNAs responsible for these effects are unknown. In silico network analysis identified microRNAs (miR-145 and let-7a) that influence the expression of components of nodal signaling pathways. The large network is bridged by nodal molecules, such as mitogen-activated protein kinase (MAPK1/2), and AKT, which are recognized components of pro-mitogenic signaling pathways [20]. In fact, the role of MAPK1/2 in regulating trophoblast turnover is well documented in both human and animal systems [43, 44, 47]. In this context, we have reported an increased activation of MAPK 1/2 in response to leptin in trophoblastic cells from the human placenta. Thus, it is tempting to speculate that altered microRNAs expression influences the leptin expression and contributes to the pathogenesis of the GDM. However, the signature of microRNAs in the leptin expression in the placenta both in normal pregnancy and GDM remains to be elucidated. Therefore, it will be interesting to determine, in future studies, the combined role of these microRNAs in the leptin expression in normal placenta and in placenta from pregnancy pathology associated with altered placental growth (e.g., GDM) in order to clarify the regulation of placental growth by leptin.

7. Leptin in fetal development

Obesity is associated with significantly elevated plasma leptin concentrations due to an increase in white adipose tissue compared with healthy individuals [93]. As obesity rates are increasing rapidly in the Western world, so is increasing the number of obese women who become pregnant. Importantly, obese pregnant women have significantly elevated plasma leptin concentrations compared with nonobese pregnant women throughout pregnancy [94]. Even though no differences in placental leptin production has been shown, there is a downregulation of LEPRb expression in the placenta of obese mothers, which would cause placental leptin resistance (in addition to the central leptin resistance that occurs during normal pregnancy) that may be attempting to modulate fetal growth under high-energy conditions [95, 96]. Despite the complications associated with pregnancies in obese women, the offspring may be growth restricted, normal weight, or macrosomic. However, after birth, babies born from obese mothers are exposed to elevated leptin concentrations in the maternal milk [97], which suggests that the postnatal environment may increase infant growth and development, increasing the risk of developing a number of diseases in adulthood. Therefore, alterations in maternal-placental-fetal leptin exchange may modify the development of the fetus and contribute to the increased risk of developing disease in adulthood.

8. Conclusions

In conclusion, it could be affirmed that leptin controls reproduction depending on the energy state of the body and sufficient leptin levels are a prerequisite for the maintenance of reproductive capacity. The present review was focused in placental leptin effects during gestation, when leptin levels are increased due to leptin production by trophoblastic cells. Thus, leptin has a wide range of biological functions on trophoblast cells and a role in successful establishment of pregnancy. In this sense, leptin promotes proliferation, protein synthesis, and survival of placental

cells. These actions are very important since cell proliferation and apoptotic cascades are critical for the correct placental development and function. Moreover, an important role of leptin in the regulation of immune mechanisms at the maternal interface has been suggested.

Observational studies have demonstrated that states of leptin overabundance or resistance can be associated with GDM. Moreover, it is also established that obesity may lead to deregulation in leptin function that results in maternal disease and clinical studies demonstrate an impact of obesity with an increased risk of a number of diseases in adulthood, including metabolic disease. In this context, leptin deregulation has been implicated in the pathogenesis of GDM. It is well accepted that leptin and LEPR expressions are increased in placentas from GDM, which may be relevant to control fetal homeostasis. Moreover, a role for microRNAs in the regulation of placental growth has been suggested, and expression profiling in the studies has shown expression and gestational changes in microRNA levels that demand functional evaluation. Further investigation is needed to fully elucidate the association of leptin with GDM and to establish leptin as a biomarker for this pathology or the development of microRNA-based approaches to therapeutic targeting for correcting the abnormal placental growth and cell turnover seen in GDM.

Disclosure of interests

The authors declare no conflict of interest.

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