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REVIEW ARTICLE

Insulin receptor isoforms: an integrated view focused on gestational diabetes mellitus

F. Westermeier[1,2,3*], T. Sáez[1,4], P. Arroyo[1], F. Toledo[5], J. Gutiérrez[1,6], C. Sanhueza[1], F. Pardo[1], A. Leiva[1], L. Sobrevia[1,7,8*]

[1]*Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile*
[2]*Advanced Centre for Chronic Diseases (ACCDiS), Faculty of Chemical & Pharmaceutical Sciences and Faculty of Medicine, Universidad de Chile, Santiago, Chile*
[3]*Faculty of Science, Universidad San Sebastián, Santiago, Chile*
[4]*University Medical Centre Groningen (UMCG), Faculty of Medicine, University of Groningen, Groningen, The Netherlands*
[5]*Department of Basic Sciences, Faculty of Sciences, Universidad del Bío-Bío, Chillán, Chile*
[6]*Cellular Signalling and Differentiation Laboratory (CSDL), School of Medical Technology, Health Sciences Faculty, Universidad San Sebastian, Santiago, Chile*
[7]*Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, Seville, Spain*
[8]*University of Queensland Centre for Clinical Research (UQCCR), Faculty of Medicine and Biomedical Sciences, University of Queensland, Queensland, Australia*

*Correspondence to: Luis Sobrevia, Francisco Westermeier, Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, PO Box 114-D, Santiago 8330024, Chile.
E-mail: sobrevia@med.puc.cl; fdwester@ciq.uchile.cl

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Summary

The human insulin receptor (IR) exists in two isoforms that differ by the absence (IR-A) or the presence (IR-B) of a 12-amino acid segment encoded by exon 11. Both isoforms are functionally distinct regarding their binding affinities and intracellular signalling. However, the underlying mechanisms related to their cellular functions in several tissues are only partially understood. In this review, we summarize the current knowledge in this field regarding the alternative splicing of IR isoform, tissue-specific distribution and signalling both in physiology and disease, with an emphasis on the human placenta in gestational diabetes mellitus (GDM). Furthermore, we discuss the clinical relevance of IR isoforms highlighted by findings that show altered insulin signalling due to differential IR-A and IR-B expression in human placental endothelium in GDM pregnancies. Future research and clinical studies focused on the role of IR isoform signalling might provide novel therapeutic targets for treating GDM to improve the adverse maternal and neonatal outcomes.

Keywords insulin receptor; gestational diabetes; placenta

Introduction

Appreciation of the role of insulin in D-glucose homeostasis began with its discovery in 1922 [1]. The ability of insulin to induce D-glucose uptake [2] results from activating specific cell surface receptors on target tissues [3–5]. Insulin triggers the translocation of isoform 4 of the D-glucose transporter (GLUT4) to the plasma membrane in insulin-sensitive tissues [6,7]. However, both the physiological and pathophysiological impact of insulin signalling, considering the diversity of tissues, such as adipose tissue and skeletal muscle, upon which this hormone acts, is not clearly understood [8]. The insulin receptor (IR) exists in two splice variants, that is, IR A (IR-A) or B (IR-B), depending on the absence or presence of a 12-amino acid segment (encoded by exon 11) at the C-terminal of the extracellular α-subunit [5,9–11]. IR-A and IR-B are functionally distinct regarding their binding affinities for insulin, receptors internalization, receptors recycling time and intracellular signalling [12]. Additionally, IR-A and IR-B are expressed in a highly tissue-specific manner in humans, with IR-B as the dominant isoform in classical insulin-sensitive tissues, such as skeletal muscle, adipose tissue and liver, whereas IR-A is predominantly

expressed in cancer tissue, brain, haematopoietic cells, foetal tissue and the placenta [13].

Although a comprehensive understanding of the mechanisms underlying insulin action in the placenta is emerging, insulin impact in the human foetoplacental unit is addressed, showing modulation of a wide range of cellular processes, such as D-glucose [14,15], nucleoside [16–19], and amino acid [20–23] transport, placental growth [24], angiogenesis [25] and modulation of the expression of placental genes, both in trophoblast and endothelial cells [26]. Extensive experimental and clinical evidence indicates that gestational diabetes mellitus (GDM), characterized by D-glucose intolerance with onset or first recognition during pregnancy [27], is associated with placental defective insulin signalling [28,29] and elevated plasma adenosine in human umbilical veins [16,18]. Interestingly, adenosine and insulin cause concentration-dependent relaxation of umbilical vein rings, an effect that is less effective in GDM compared with normal pregnancies [16]. These findings suggest that these molecules might play crucial roles in regulating the vascular haemodynamics of human foetoplacental circulation under physiological conditions or insulin resistance states, such as GDM [29].

The biological effects of insulin are mediated by the activation of IR-A and IR-B in human umbilical vein endothelial cells (HUVECs) [17] and human placental microvascular endothelial cells (hPMECs) [18]. In these cell types, as in other mammalian cells, insulin-mediated activation of IR-A is associated with a mitogenic, p44 and p42 kDa mitogen-activated protein kinases (p44/42^{mapk})-mediated phenotypes, whereas IR-B activation is associated with a metabolic, phosphatidylinositol 3-kinase (PI3K)/Akt-mediated phenotype [17,29], raising new questions regarding the complex interplay between insulin signalling and human foetoplacental vasculature. In this review, we summarize the current knowledge regarding IR isoforms, focusing on their alternative splicing, expression, ligand binding and signalling in several tissues, with special emphasis on human foetoplacental vascular function. New molecular mechanisms associated with defective insulin signalling in GDM and therapeutic considerations are also discussed.

Insulin receptor gene and alternative splicing

The 22-exon gene encoding the human IR (*INSR*) is located in chromosome 19 and was first cloned and vsequenced in 1985 [5,9]. Subsequently, the presence of two isoforms, which differ by the absence (IR-A) or inclusion (IR-B) of 12 amino acids generated by alternative splicing of exon 11, a 36 base-pair exon, was described [10,11]. The regions of intron 10 and exon 11 involved in the alternative splicing of the *INSR* have been identified [30,31]. The splicing factors serine/arginine-rich protein 20 (SRp20) and serine/arginine-rich splicing factor 1 (SRSF1) increase exon 11 inclusion, whereas CUG-repeat binding protein 1 (CUG-BP1) causes exon skipping. Thus, the relative ratios of SRp20 and SRSF1 to CUG-BP1 in different cell types determine the degree of exon inclusion associated with stabilization of an RNA secondary structure that regulates IR alternative splicing [31]. Binding of muscleblind-like 1 protein to the conserved element in human intron 11 promotes IR exon 11 inclusion [32,33]. In this context, the characterization of the mechanism by which muscleblind-like 1 protein activates inclusion of IR exon 11 suggests the involvement of U2 small nuclear ribonucleoprotein auxiliary factor 65 kDa subunit (U2AF65) [34]. However, heterogeneous nuclear ribonucleoproteins (hnRNPs) have also been associated with the alternative splicing of IR. Inclusion of exon 11 is promoted by the subtype hnRNPF, whereas hnRNPA1 is associated with its exclusion [35]. Altogether, these mechanisms of inclusion and exclusion of exon 11 in the IR transcript result in structural and ligand binding affinity differences between both isoforms [12].

Insulin receptor isoforms structure and tissue distribution

The IR protein is a heterotetramer composed of two extracellular α-subunits and two transmembrane β-subunits. The α-chains and β-chains are both synthesized from unique mRNA, which is constituted by 22 exons. *INSR* mRNA encodes a protein of 1370 amino acids (~154 kDa), which is cleaved by furin into an α-subunit (723 amino acids, ~130 kDa) and a β-subunit (620 amino acids, ~95 kDa). The extracellular portion of IR includes the complete α-chain and a portion of 194 residues of the β-chain, whereas the cytoplasmic domain is composed of the other 403 residues of the β-chain, which contains the tyrosine kinase activity. Ligand binding to the IR α-subunit stimulates tyrosine kinase activity intrinsic to the β-subunit of the receptor (Figure 1) [42–45]. These topics have been recently covered in an excellent and comprehensive review [46].

The alternative splicing of exon 11 in the expression of IR-A and IR-B results in structural and therefore functional differences between the isoforms. The 12-amino acid segment confers different insulin ligand binding properties to the IR isoforms, showing that insulin association and dissociation from IR-A is faster than from IR-B [47–49]. Subsequently, several experimental approaches have been used for calculating the half maximal inhibitory

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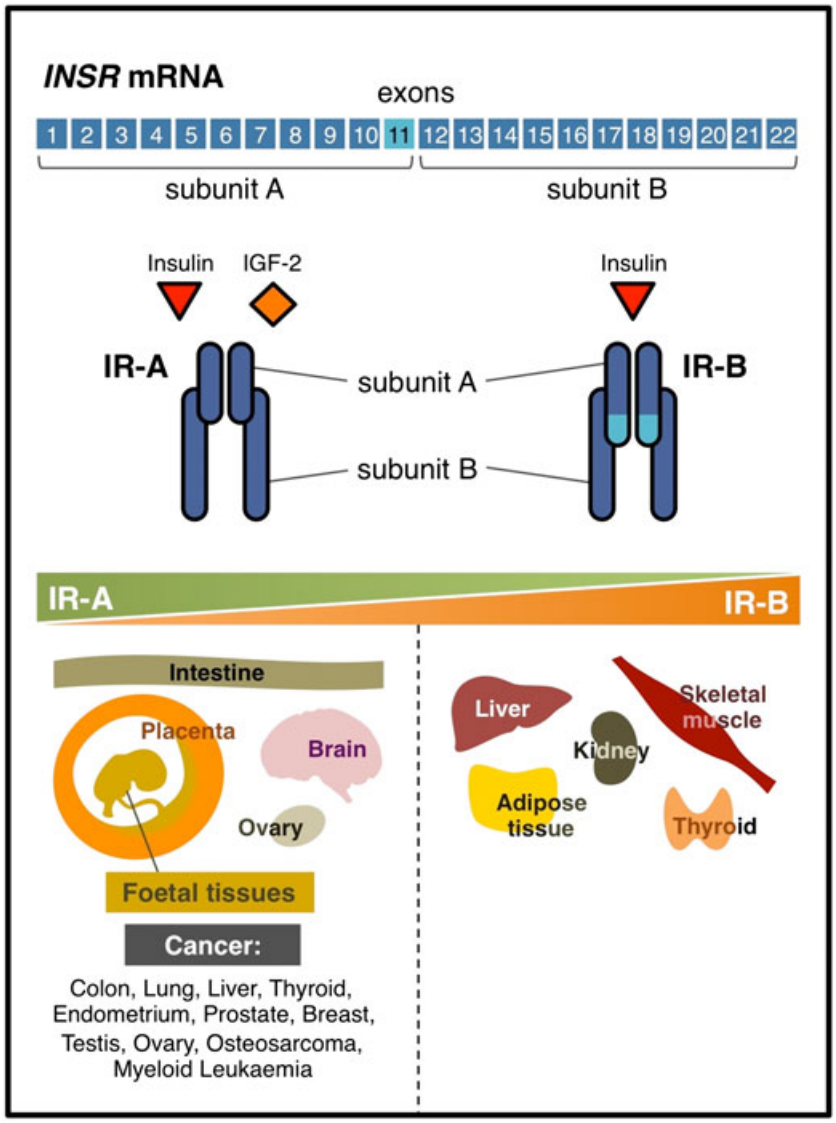

Figure 1. Insulin receptor gene, alternative splicing and tissue-specific distribution. ***Upper panel*****. The insulin receptor gene (*INSR*) is located in chromosome 19 and contains 22 exons. Exclusion or inclusion of exon 11 at the *INSR* mRNA, which belongs to the insulin receptor α-subunit (subunit A), generates two insulin receptor isoforms, IR-A or IR-B, respectively, that differ by a 12-amino acid fragment at this subunit with a full insulin receptor β-subunit (subunit B) derived from exons 12–22. IR-A binds insulin and insulin-like growth factor 2 (IGF-2), whereas IR-B binds only insulin.** ***Lower panel*****. IR-A is predominantly expressed in differentiated human intestinal epithelium, placental vascular cells, brain, ovary, and several types of cancer. IR-B is mainly expressed in skeletal muscle, liver, kidney, adipose tissue, and thyroid gland. From [16,36–41]**

concentration (IC_{50}) values for ligand binding to IR isoforms. Insulin-like growth factor 1 (IGF-1) receptor-null (R^-) mouse embryonic fibroblasts cells expressing IR-A (R^-/IR-A) showed that IR-A, but not IR-B, is a high-affinity receptor for IGF-2 ($IC_{50} \sim 3$ nmol/L) that binds with similar IC_{50} to the classical IGF receptor 1 (IGFR-1) [50]. IR-A/IR-B hybrids are randomly formed and exhibit different ligand binding to insulin, IGF-1 and IGF-2 [51]. Moreover, given the high degree of homology, the insulin and IGF-1 half-receptors (composed of one α-subunit and one β-subunit) can form heterodimers, leading to the formation of insulin/IGF-1 hybrid receptors (Hybrid-Rs) [12]. IR-A and IR-B also form hybrids with IGFR-1, of which hybrid IGFR-1/IR-B receptors (Hybrid-RsB) have high affinity only for IGF-1, whereas hybrid IGFR-1/IR-A receptors (Hybrid-RsA) not only have higher affinity for IGF-1 but also bind IGF-2 and insulin [52]. However, studies in Chinese hamster ovary cells suggest that IR-A–IGFR-1 and IR-B–IGFR-1 hybrids have similar, relatively low affinity binding for insulin and relatively high affinity for IGF-1 and IGF-2 [53]. Additionally, similar findings were reported for IR-A–IGFR-1 and IR-B–IGFR-1 hybrids in baby hamster

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kidney cells [54]. Thus, the possibility that regulation of the IR isoforms expression has implications in both insulin and IGFs signalling under physiological and pathological conditions is uncertain. Table 1 summarizes the currently available data regarding IR isoforms, hybrids receptors and ligand specificity related to insulin, IGF-1 and IGF-2 in mammalian cells.

The IR promoter contains multiple transcription sites and positive/negative regulatory elements [11,61–64], suggesting that its expression is highly regulated in cells in a developmental-specific and tissue-specific manner [12]. Although typical insulin-responsive tissues include the liver, adipose tissue and skeletal muscle, IR is ubiquitously expressed in mammalian tissues; however, the inclusion/exclusion of exon 11 is differentially regulated in various tissues [12]. In human adult mammalian tissues associated with the metabolic effects of insulin, such as the liver, skeletal muscle, adipose tissue and kidney, the *IR-B/IR-A* mRNA ratio is predominant, whereas in foetal and cancer tissues where insulin acts as mitogenic agent, the *IR-A/IR-B* mRNA ratio predominates (Figure 1 and Table 2). Nevertheless, *IR-A* mRNA expression predominates in rat and mouse extensor *digitorum longus* and *soleus* skeletal muscles [36], suggesting that tissue distribution is not completely conserved among species. It was also reported that the *IR-A/IR-B* mRNA ratios are conserved in the liver and brain in mice, rats and pigs [36]; however, this is not the case in the skeletal muscle and adipose tissue, agreeing with findings in humans [10], rhesus monkeys [49] and sheep [73]. Because each tissue is composed of several morphologically and functionally heterogeneous cells, that is, skeletal muscle (slow-twitch and fast-twitch fibres), endothelium (microvascular and macrovascular endothelial cells) and heart (cardiomyocytes and fibroblasts), findings regarding the expression of IR isoforms cannot be extrapolated without considering the framework of the specific cell types and tissues. The concept that IR isoform tissue distribution is well-conserved among species and therefore is likely to have distinctive functional roles may have to be reconsidered and explored for clearer and better therapy protocols, as recently discussed for patients with GDM requiring insulin therapy [29]. The latter is based on the fact that from a physiological and pathophysiological point of view, the relative abundance of the IR isoforms could be essential in regulating these receptor-specific mitogenic/metabolic actions of insulin in target tissues.

Table 1. IC_{50} values for ligand binding to insulin receptor isoforms and hybrids

Receptor	Insulin	IGF-1	IGF-2	Reference
IR-A	0.8	—	3.0	[50]
	0.9	41	—	[48]
	2.8	120	18	[55]
	0.2	>30	0.9	[52]
	0.3	9.0	2.2	[53]
	0.5	65	6.2	[56]
	0.4	68	—	[57]
	0.8	—	—	[58]
	0.9	80	3.3	[59]
	1.2	26	5.9	[60]
IR-B	1.1	—	24	[50]
	1.6	390	—	[48]
	1.4	366	68	[55]
	0.3	>30	11	[52]
	0.5	90	10	[53]
	0.6	171	47	[56]
	0.5	>100	—	[57]
	0.7	—	—	[58]
	1.0	>100	36	[59]
	0.6	41	12	[60]
IGFR-1/IR-A	3.7	0.3	0.6	[52]
IGFR-1/IR-B	>100	2.5	15	
IGFR-1/IR-A	70	0.5	0.7	[53]
IGFR-1/IR-B	76	0.3	0.3	
IGFR-1/IR-A	2.6	0.02	—	[54]
IGFR-1/IR-B	2.8	0.01	—	
IR-A/IR-B	1.0	>50	10	[51]

Half maximal inhibitory concentration (IC_{50}) values (given in nM) were obtained using different experimental approaches (ligand competition assay or bioluminescence resonance energy transfer (BRET), intact cells or solubilized receptors, incubation at 4 °C or room temperature and ^{125}I-insulin, Eu-labelled insulin or ^{125}I-insulin-like growth factor (IGF-1) as tracer). IGF-1, insulin-like growth factor 1; IGF-2, insulin-like growth factor 2; IR-A, insulin receptor A; IR-B, insulin receptor B; IGFR-1, IGF receptor 1; –, not determined.

Insulin receptor isoforms signalling in physiology and disease

The biochemical processes related to insulin binding/activation of IR and its complex signal transduction networks that regulate diverse cellular function have been well-documented [44,132]. Insulin binds to the extracellular IR α-subunit, promoting its conformational change, which subsequently generates autophosphorylation of the IR β-subunit. The activated IR tyrosine kinase phosphorylates several intracellular substrates, including IR substrate (IRS) and the Src homology 2 domain containing (Shc) family members serving as docking proteins for downstream signalling [133]. Tyrosine phosphorylation of the IRS family members at multiple sites provides docking sites for effectors containing Shc domains that recognize different phosphotyrosine residues, including PI3K and the growth factor receptor-bound protein 2 (Grb-2). Thus, two major signalling pathways mediate metabolic or mitogenic effects in response to PI3K and Grb-2 activation, respectively [132,133]. The PI3K/Akt branch of insulin signalling regulates D-glucose metabolism (D-glucose uptake, gluconeogenesis and glycogen synthesis) in skeletal muscle, adipose tissue and liver [8]. Moreover, increased activity of this signalling branch caused by insulin leads to increased nitric oxide production and vasodilatation in the vascular endothelium

Table 2. Insulin receptor isoforms expression, signalling and related pathologies

Reported evidence	References
Relative expression and tissue distribution	
IR-B > IR-A in post-mitotic and differentiated cells from human intestinal epithelium at the villus	[37]
IR-A > IR-B in proliferating and undifferentiated cells from post-mitotic and differentiated cell from human intestinal epithelium at the crypt	
IR-A > IR-B in mouse aortic VSMCs	[65]
IR-B > IR-A in mature human osteoblasts	[66]
IR-A > IR-B in human osteoblast precursors	
IR-A = IR-B in hPMECs from normal pregnancies	[18]
IR-A > IR-B in human astrocytes	[39]
IR-A > IR-B in rat and mouse brain, spleen, skeletal muscle (extensor *digitorum longus* and *soleus*)	[36]
IR-B > IR-A in rat and mouse epididymal adipose tissue, liver and kidney	
IR-A = IR-B in rat and mouse mesenteric and retroperitoneal adipose tissue, and heart	
IR-A > IR-B in HUVECs from normal pregnancies	[16]
IR-B > IR-A in adult and prenatal mouse liver	[67]
IR-B > IR-A in human thyroid	[40]
IR-B > IR-A in differentiated mouse mammary gland	[68]
IR-A > IR-B in rat skeletal muscle (*soleus* and white quadriceps) pancreas and rat pre-adipocytes	[69]
IR-B > IR-A in rat liver, epididymal white adipose tissue, brown adipose tissue, and kidney	
IR-A > IR-B in human ovarian follicles	[70]
IR-A > IR-B in human mural granulosa and *cumulus* cells	[70]
IR-A > IR-B in bovine *corpora lutea*	[71]
IR-B > IR-A in bovine late luteal stage and during early pregnancy	
IR-B > IR-A in human skeletal muscle	[38]
IR-B > IR-A in rat liver and kidney	[72]
IR-A > IR-B in rat pancreas, sciatic nerve, anterior tibial muscle, dorsal root ganglion, spinal cord and brain	
IR-A > IR-B in human fetal fibroblasts	[50]
IR-B > IR-A in human adult fibroblasts	
IR-B = IR-A in ovine liver and skeletal muscle (*rectus capitis* and *longissimus dorsi*)	[73]
IR-B > IR-A in rat liver, adipose tissue, kidney and adrenal gland	[74]
IR-A > IR-B in rat cerebral cortex, hypothalamus and skeletal muscle	
IR-A > IR-B in rat skeletal muscle	[75]
IR-B > IR-A in rat liver	[75,76]
IR-A > IR-B in human skeletal muscle	[77,78]
IR-B > IR-A in human quadriceps *femoris* muscle	[79]
IR-B > IR-A in human liver	[80,81]
IR-A > IR-B in human leukocytes	
IR-A = IR-B in human placenta, skeletal muscle and adipose tissue	
IR-A > IR-B in human muscle, adipocytes and fibroblasts	[81]
Insulin signalling	
IR-A and IR-B maturation depends on furin	[82]
IR-B is matured by proprotein convertase PACE4 when furin activity is reduced	
IGF-2/IR-A pathway is downregulated by decorin	[83]
F19A/IR-A pathway promotes neural stem cell expansion	[84]
IR-A, but not IR-B, associates with proliferative phenotype in VSMCs in response to proatherogenic stimuli (TNF-α, Ang II, ET-1, U46619)	[65]
IR-A trafficking and stability is differentially regulated by IGF-2 and insulin	[85]
Proinsulin/IR-A pathway promotes cell proliferation and migration via ERK/p70S6 kinase	[59]
Identification of IR-A substrates recruited after insulin exposure	[86–88]
Insulin/IR-A pathway promotes pancreatic β-cell signal transduction via PI3K-C2α/Akt	[89]
IGF-2/IR-A pathway promote p70S6 kinase, and ERK and Akt activation	[90]
IR-A and IR-B modulate the susceptibility to apoptosis in mouse immortalized neonatal hepatocytes	[91]
Insulin/IR-B pathway induces transcription of GK via PI3K-C2α–like/PDK1/Akt	[92]
Insulin/IR-B pathway induces transcription of c-*fos* genes via PI3K class Ia/Shc/MEK1/ERK	
IGF-2 C domain is critical for signalling, cell survival, and migration induced by IGF-2/IR-A pathway	[93]
Insulin/IR-A pathway promotes activation of the insulin promoter	[94]
Insulin/IR-B pathway promotes activation of the β-GK promoter	
IR-A and IR-B exhibit different plasma membrane domains	
IR-A is downregulated by prostaglandin F2α in bovine *corpora lutea*	[71]
IGF-2/IR-A pathway induces nuclear IRS-1 translocation and relates to mitogenic and antiapoptotic signals	[95]
Insulin/IR-B pathway promotes differentiation signals	
IGF-2/IR-A pathway promotes cell migration via Shc/ERK	[96]
Insulin/IR-B pathway blocks apoptosis via PI3K/Akt	
IGF-2/IR-A pathway is associated with Akt/Gsk3β activation	[97]
Insulin/IR-B pathway induces transcription of GK via PI3K class II–like activity/Akt	[94]
Insulin/IR-A pathway induces insulin transcription via PI3K class Ia/p70S6 kinase	
IGF-2 binds and activates IR-A, but not IR-B	[50]
Insulin/IR-A pathway promotes metabolic effects	
IGF-2/IR-A pathway induces mitogenic effects	

(continues)

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Table 2. (continued)

Reported evidence	References
Faster IR-A internalization and recycling induced by pp120 compared with IR-B	[98,99]
IR-B is upregulated by dexamethasone in HepG2 cells	[100]
IR-B exhibits higher tyrosine kinase activity than IR-A in response to insulin	[101]
Insulin stimulates PLC activity by signalling via IR-A and IR-B	[102]
Insulin stimulates PI3K activity by signalling via IR-A and IR-B	[103]
Insulin affinity is higher for IR-A compared with IR-B	[49]
Cancer	
IR-A > IR-B in human colorectal adenoma in patients with high plasma insulin	[41]
IR-A > IR-B in human non-small cell lung cancers	[104]
IR-A overexpression in human hepatoma carcinoma cells associates with upregulation of CUGBP1, hnRNPH, hnRNPA1, hnRNPA2B1, and SRSF1	[105]
IR-B overexpression reduces proliferation in human intestinal and colorectal cancer cell lines	[37]
IR-A > IR-B in human endometrial carcinoma	[106]
IR-A > IR-B in human prostate cancer	[107]
IR-A > IR-B in human breast cancer	[108,109]
IR-A > IR-B in human thyroid cancer cells	[40]
IR-A knockdown promotes formation of IGFR-1 homodimers and enhances the viability in human colorectal cancer cell lines	[110]
Insulin activates ERK and Akt pathways via IR-A and increase cell proliferation in a human acute myeloid leukaemia cell line	[111]
IR-A > IR-B in human osteosarcoma	[112]
Inhibition of IR-A signalling restores sensitivity to gefitinib in resistant colon cancer line cells	[113]
IR-B > IR-A in seminoma testis tissue	[114]
IR-B > IR-A in ovarian carcinoma cell lines associates with proliferation after IGF-2 stimulation	[115]
IR-B > IR-A in thyroid cancer cells associates with mitogenic effects and tumour de-differentiation after IGF-2 stimulation	[116]
IR-B > IR-A in human breast cancer associates with mitogenic effects after IGF-2 stimulation	[117]
IR-B > IR-A in human breast, lung and colon cancer	[50]
Myotonic dystrophy	
IR-B expression is lower in type 1 and type 2 skeletal muscle from MD1 and MD2 patients	[118]
IR-B expression is lower in *vastus lateralis* muscle from MD1 and MD2 patients	[119,120]
IR-B expression is lower in skeletal muscle from MD1 patients associated with impaired metabolic responsiveness to insulin	[38]
Diabetes	
Insulin restores GDM–reduced adenosine transport via IR-A in HUVECs	[17]
Reduced hENT2-adenosine transport in hPMECs from GDM is reverse by insulin involving IR-A and IR-B activation	[18]
IR-A expression is higher in HUVECs from GDM compared with normal pregnancies, whereas the IR-B expression is unaltered	[16]
IR-A and IR-B unaltered expression in skeletal muscle and liver of diabetic rats	[75]
IR-A > IR-B in liver from T2DM monkeys	[121]
IR-B expression is higher in fat and muscle from obese and T2DM patients	[122]
IR-A = IR-B in muscle from STZ-induced diabetic rats	[123]
IR-A expression is higher in *vastus lateralis* muscle from T2DM monkeys	[124]
IR-A expression is higher in skeletal muscle from T2DM patients	[125]
IR-B expression is higher in skeletal muscle from T2DM patients	[77–79,126]
IR-A = IR-B in skeletal muscle from lean, obese and T2DM patients	[127]
IR-A = IR-B in skeletal muscle from T2DM patients	[80]
IR-B expression is higher in isolated adipocytes from T2DM patients	[128]
Insulin analogues	
GLA-M1 correlates with serum-induced IR-A, but not IR-B expression	[129]
GLA does not alters IGF via IR signalling in long-term insulin therapy in T2DM	
INS-A/IR-A pathway promotes muscle glycogen synthesis	[36]
INS-B/IR-B pathway promotes glycogen accumulation and lipogenesis in hepatocytes and adipocytes	
Glargine and detemir increase cell proliferation via IR-A/ERK pathway	[57]
X-10/IR-A induces higher mitogenic pathway compared with insulin	[130]
S597/IR-A induces glycogen synthesis that is comparable with activation by insulin via Akt in rat muscle L6 cells	[131]

IR-A, insulin receptor A; IR-B, insulin receptor B; VSMCs, vascular smooth muscle cells; hPMECs, human placental microvascular endothelial cells; HUVECs, human umbilical vein endothelial cells; PACE4, paired basic amino acid-cleaving enzyme 4; IGF-2, insulin-like growth factor 2; F19A, IGF-2 analogue; TNF-α, tumour necrosis factor α; Ang 2, angiotensin 2; ET-1, endothelin 1; U46619, prostaglandin H_2/thromboxane A_2 receptor agonist; ERK, extracellular-signal-regulated kinases; p70S6 kinase, 70 kDa ribosomal protein S6 kinase; PI3K, phosphatidylinositol 3-kinase; PI3K-C2α, PI3K class II α-isoform; Akt, protein kinase B; GK, glucokinase; PDK1, pyruvate dehydrogenase lipoamide kinase isozyme 1; Shc, Src homology 2 domain-containing transforming protein 1; MEK1, mitogen-activated protein kinase 1; β-GK, beta-cell-specific glucokinase; IRS-1, insulin receptor substrate 1; Gsk3β, glycogen synthase kinase 3 beta; pp120, 120-kDa glycoprotein substrate; PLC, phospholipase C; HepG2, human hepatocellular liver carcinoma cell line; CUG-BP1, CUG-repeat binding protein 1; hnRNPH, heterogeneous nuclear ribonucleoprotein H; hnRNPHA1, hnRNPH A1; hnRNPHA2B1, hnRNPH A2B1; SRSF1, serine/arginine-rich splicing factor 1; IGFR-1, IGF receptor 1; MD1, myotonic dystrophy type 1; MD2, myotonic dystrophy type 2; GDM, gestational diabetes mellitus; T2DM, type 2 diabetes mellitus; STZ, streptozotocin; GLA-M1, glargine metabolite M1; INS-A, insulin receptor A analogue; INS-B, insulin receptor B analogue; Detemir, X-10 and S597 are insulin analogues.

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[16,134,135]. In addition, the Grb-2/p44/42^{mapk} branch ubiquitously regulates gene transcription, protein synthesis, cell growth and differentiation, and also controls the secretion of vasoconstrictor endothelin-1 in endothelial cells [12,136]. Thus, the differential action of insulin on IR subtypes causes a differential regulation of these phenomena [17,29].

Activation of IR-A by the 120-kDa glycoprotein substrate (pp120) undergoes faster internalization and recycling compared with IR-B in NIH3T3 fibroblasts [98,99] and is differentially regulated by IGF-2 and insulin [85]. In addition, IGF-2 binding to IR-A is associated with stimulation of cell growth and invasion and nuclear IRS type 1 (IRS-1) translocation [50,95], whereas IR-B, which does not bind IGF-2, is associated with differentiation and metabolic signals following insulin stimulation [95]. In human uterine leiomyosarcoma-derived cells, IGF-2 via IR-A was a more potent activator of the Shc/p44/42^{mapk} signalling pathway and stimulator of cell migration than insulin, whereas this hormone was a more potent stimulator of the PI3K/Akt pathway and a better protector from apoptosis [96]. In R^{-}/IR-A, IGF-2 induces mitogenic effects associated with Akt/glycogen synthase kinase-3β (Akt/Gsk3β) activation [97]; however, it also stimulates p70S6 kinase (p70S6K), p44/42^{mapk} and Akt [90]. These findings suggest a complex role of IR-A, in addition to eliciting a unique signalling pattern after IGF-2 binding. In pancreatic β-cell lines, glucokinase (GK) gene transcription is promoted by insulin through IR-B/PI3K class II-like activity/Akt, whereas insulin expression is regulated through PI3K class Ia/p70s6k [94], which is attributed to the different localization of IR isoforms at the plasma membrane subdomains [137]. It was recently shown that both IR isoforms are cleaved by the protein convertase furin; however, when its activity is reduced, the paired basic amino acid-cleaving enzyme 4 promotes IR-B maturation, highlighting the importance of pharmacological inhibition of furin to modulate the IR-A-induced mitogenic effects [82]. In addition, the proteoglycan decorin significantly inhibited IGF-2–mediated activation of the Akt signalling pathways, without affecting insulin and proinsulin-dependent signalling in R^{-}/IR-A cells. Thus, decorin activity might play a pivotal role in tumour initiation/progression in cancer cells, which exhibit increased IGF-2/IR-A signalling pathway activity [83].

Several types of cancer have been associated with IR-A overexpression (Figure 1 and Table 2). IGF-2 binds with high affinity to IR-A, promoting its activation and mitogenic, rather than metabolic, effects in breast, colon and lung human carcinoma [50]. Human acute myeloid leukaemia cell lines incubated with insulin exhibit increased cell proliferation through the p44/42^{mapk} and Akt signalling pathways via a mechanism that involves activation of IR-A [111]. Conversely, inhibition of IR-A signalling restored sensitivity to gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor [113], and elevated IR-B expression reduced the proliferation of human colon cancer cell lines [37]. Recently, it was reported that IR-A overexpression is associated with upregulation of the splicing factors CUG-BP1, hnRNPH, hnRNPA1, hnRNPA2B1 and SRSF1 in human haepatoma carcinoma cells [105], which have also been related to myotonic dystrophy type 1-associated aberrant IR splicing [38,138]. Myotonic dystrophy type 1 is characterized by lower IR-B expression in skeletal muscles (Figure 2 and Table 2), muscle hyperexcitability (myotonia), progressive muscle wasting, cardiac conduction defects, cataracts, alterations in smooth muscle function, neuropsychiatric disturbances and insulin resistance [38].

Studies evaluating whether IR isoform regulation could be associated with insulin resistance and type 2 diabetes mellitus (T2DM) have yielded inconsistent results (Figure 2 and Table 2), possibly because T2DM is a heterogeneous and complex disease with variable levels of insulin resistance. The patient age and body mass index, as well as different therapies (i.e. insulin, insulin analogous, insulin sensitizers, controlled calories ingest and physical activity), influence IR isoform expression. Unfortunately, given this level of complexity, the available data are not sufficient to draw a firm conclusion on the role, if any, of IR isoforms in T2DM and insulin resistance. However, IR isoforms might play a crucial role in maintaining the insulin signalling cascades in the microvascular and macrovascular endothelium in the human foetoplacental unit in GDM [16–18,29].

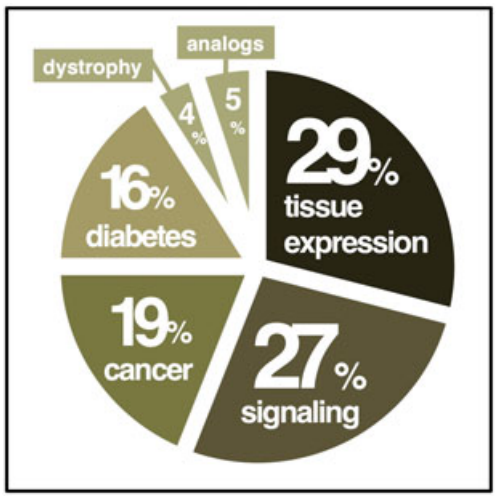

Figure 2. The pie chart expresses the evidence collected from state-of-the-art research for the past 30 years (from PubMed database; http://www.ncbi.nlm.nih.gov/pubmed/). The chart shows the percentage of the predominant topics (signalling, tissue expression, cancer, diabetes, dystrophy and analogues) reported in 96 original research articles

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Insulin signalling in the human placenta

The placenta is an organ of foetal origin that acts as a selective natural barrier between maternal and foetal circulation and is essential for foetal growth and development [139]. During its transient existence, the placenta performs a wide range of functions, such as transport of maternal O_2 and other nutrients to the foetus, synthesis of several hormones and release of growth factors that may affect the mother, the foetus or both [140,141]. Placental growth and development is associated with trophoblasts-dependent proliferation and differentiation processes and with endothelium-dependent angiogenesis and vascularization in the first and the second half of the gestational period [142]. In addition, a wide range of hormones, cytokines and growth factors narrowly regulates placenta formation and development and substrate uptake of the maternal and foetal circulation in close contact with trophoblasts and endothelial cells. Transgenic mice expressing human placental growth hormone (hPGH) at levels that are comparable with those in the third trimester of human pregnancy display severe insulin resistance [143]. Expression of hPGH was associated with higher expression of the p85α monomer of PI3K, which competes in a dominant negative manner with the p85-p110 heterodimer, resulting in marked reduction in IRS-1/PI3K kinase activity [144]. Moreover, a prospective observational cross-sectional study including 180 normal pregnant women showed that maternal hormonal and metabolic factors related to the placenta, adipose tissue and the growth hormone axis are associated with the variation in insulin sensitivity seen during normal human pregnancy [145]. Altogether, these effects might explain the development of maternal pregnancy-induced insulin resistance, a pivotal physiological process designed to limit maternal D-glucose uptake, ensuring a proper supply of nutrients to the growing foetus. However, none of these studies characterized the potential differential involvement of the IR isoforms in these phenomena.

The placenta expresses high amounts of IR compared with other tissues, and its location undergoes developmental changes. At the beginning of gestation, IR is mainly located in trophoblasts, whereas at term, it is predominantly found at the endothelium [146–148]. Unfortunately, previous research did not study whether both IR-A and IR-B account for this distribution in the placenta or whether one form predominates. However, the spatiotemporal shift in the IR expression has been associated with different insulin-induced intracellular signalling depending on the placental cell type [141]. Signalling associated with the IR-A or IR-B forms is different in placenta endothelial cells [16–18]. In this context, insulin induced the expression of 236 genes in human primary trophoblasts in the first trimester (HPTs-ft), whereas only the expression of six genes was induced in human primary trophoblasts in term (HPTs-tp) placenta. Conversely, 146 transcripts were regulated by insulin in human placental endothelial cells, suggesting that the shift regarding the control of insulin-dependent processes throughout pregnancy might regulate placental insulin effects from mother to foetus [26].

The insulin effect in human placental trophoblasts is not fully elucidated [29,149]. In this regard, insulin increases D-glucose uptake [150] and lipid deposition [151], but does not alter L-alanine transport in HPTs-ft [150]. However, insulin increased both L-alanine and L-leucine in HPTs-tp via a mechanism that is mediated by the mammalian target of rapamycin (mTOR) [152]. Insulin increased D-glucose uptake but decreased the human chorionic gonadotrophin (hCG) secretion in the first trimester, but not in term placenta explants [153], showing that its effect depends on the placenta developmental stage. Accordingly, insulin increases the expression of the membrane-type matrix-metalloproteinase 1 in HPTs-ft [154], a process that might be crucial for placental tissue remodelling. Conversely, in HPTs-tp, interleukin-1β (IL-1β) caused inhibition of insulin-stimulated L-arginine, but not L-leucine uptake, which was associated with higher phosphorylation of IRS-1 at Ser^{307} (inhibitory residue), lower total IRS-1 protein abundance and unaltered IR β-subunit expression [22]. Other studies show that adipose tissue-derived adiponectin is associated with insulin-sensitizing action in the liver and muscle and inhibits the insulin-stimulated L-alanine transport in HPTs-tp [155], following activation of placental peroxisome proliferator-activated receptor α (PPARα) and ceramide synthesis [156]. This information reinforces the concept that adiponectin and IL-1β play pivotal roles in foetal growth/development by reducing the insulin-regulated placental amino acid transport. The latter might be a link between GDM and/or maternal obesity or excessive pregnancy weight gain and defective placental insulin signalling in the foetoplacental vasculature [157]. Lower DNA methylation in the promoter of the adiponectin gene (*ADIPOQ*) on the foetal and maternal sides of the placenta was correlated with higher maternal D-glucose concentration and higher insulin resistance index throughout pregnancy, suggesting that epigenetic changes in *ADIPOQ* might be one of the mechanisms involved in the foetal programming of metabolic disorders in adult life [158]. Insulin induces 2785 and 87 genes in HPTs-ft from lean and obese women, respectively [159]. These results highlight the role of insulin in early gestation and show the impact of maternal obesity on insulin-regulated placental gene expression.

In term placenta, insulin has a stronger effect on the endothelium than in trophoblasts because the majority of placental IR is located in this cell type [26]. Insulin

regulates genes related to growth factors, the cell cycle and apoptosis, suggesting a metabolic/mitogenic effect in human placental endothelial cells (HPECs) [26]. Insulin and IGF-2 also induce membrane-type matrix-metalloproteinase 1 expression in HPECs; however, this effect is absent in cells treated with wortmannin (PI3K inhibitor) but not with U0126 (p44/42^{mapk} inhibitor), suggesting the involvement of IR-A in response to this hormone [159]. Moreover, insulin induces a metabolic effect by increasing L-arginine transport via human cationic amino acid transporter 1 in human umbilical vein endothelium from normal pregnancies [23], which requires functional A_{2A} adenosine receptor (A_{2A}AR) activation to lead to human umbilical vein ring dilation [20]. Accordingly, placental vascular function in response to insulin might be dependent on adenosine receptor activation. In the absence of autonomic innervation, vasomotor control of foetoplacental circulation is regulated by the release of local vasoactive factors. In this regard, human equilibrative nucleoside transporter 2 (hENT2)-mediated adenosine transport is reduced in IR-A knockdown (^{KD}IR-A) hPMECs from normal pregnancies stimulated with insulin [18]. However, the insulin-reduced hENT1-mediated adenosine transport in HUVECs from normal pregnancies was blocked both in ^{KD}IR-A and IR-B knockdown (^{KD}IR-B) cells [17]. These findings suggest that the insulin effect on microvascular and macrovascular endothelial cells involves a different expression pattern of IR isoforms, confirming the differences in the functionality of these types of endothelial cells in the human placenta [29,149,160]. Cell signalling mechanisms involved in the reduced activity of hENT2 in response to stimulation of hPMECs with insulin are associated with a p44/42^{mapk}/Akt ratio >1, supporting the need for the expression of IR-A in this cell type to mediate the insulin effect in this particular phenomenon. In HUVECs exposed to insulin, p44/42^{mapk}/Akt was ~ 1, suggesting equal or balanced cell signalling associated with IR-A and IR-B activation by this hormone in this cell type.

Placental defective insulin signalling in gestational diabetes mellitus

The state of insulin resistance described in normal pregnancy has been related to a decrease in insulin signalling pathways associated with several hormonal and metabolic factors [145], including maternal tumour necrosis factor-α (TNF-α) [161], a phenomenon reversed postpartum [28]. Conversely, in GDM, insulin resistance is exacerbated because of increased TNF-α, leptin and resistin plasma levels [142,161]. In addition, GDM women with impaired glucose tolerance postpartum have insulin resistance at the skeletal muscle, which is associated with high risk of developing T2DM [162]. GDM leads to adverse maternal and neonatal outcomes [163,164] and defective placental insulin signalling [16–18,140,165]. Despite the impaired action of insulin and differential IR form activation and subsequent cell signalling described earlier, studies in humans and primates have reported that hyperinsulinemia is correlated with higher birth weight [166,167] and contributes to a larger placental glycogen content [168], highlighting the role of insulin either as a mitogenic or metabolic placental factor.

Differential expression of IR, IRS-1 and PI3K p85α is reported between the apical (maternal side) and basal (foetal side) membrane of the trophoblasts in normal or GDM pregnancies [169] and in abdominal subcutaneous adipose tissue from GDM women [170]. These findings are related to the reported decrease in D-glucose uptake seen in the placenta in GDM pregnancies [171,172]. Differential expression of IRS-1, IRS-2, PI3K p85α, PI3K p110α, GLUT-1 and GLUT-4 in the placenta in normal or GDM pregnancies has been shown; however, these changes were treatment-dependent (diet-controlled or insulin-controlled GDM) and/or associated with GDM and maternal obesity [165]. The effectiveness of insulin therapy of pregnant women with GDM that are unresponsive to diet, in terms of glycaemia control, is not fully understood [29]. Indeed, there is no information regarding insulin therapy and foetal outcome in terms of foetal endothelial dysfunction [29,173–175]. Thus, the role of IR forms in this intervention in patients and the consequences for foetoplacental vascular function is unknown [29].

The fact that placental hypervascularization is induced by angiogenesis in GDM [176] highlights the role of the insulin/IR axis in placenta vascular growth [25,177]. In addition, placenta vascular alterations [160], in both the macrovascular and microvascular endothelium [149] in GDM, are well-documented. Insulin and adenosine cause relaxation of umbilical vein rings, an effect that is less common in GDM compared with normal pregnancies and is blocked by ZM-214385, an A_{2A}AR antagonist [178], and N^G-nitro-L-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor) [179]. Moreover, the adenosine concentration in the umbilical vein blood in GDM was higher than in normal pregnancies, suggesting that abnormal adenosine plasma levels and A_{2A}AR play critical roles in GDM [16]. Increased A_{2B}AR expression in leukocytes is associated with hyperglycaemia in GDM women [180]. Moreover, A_{2B}AR expression was shown to correlate with altered expression of 19 genes involved in insulin signalling, including insulin action, D-glucose and lipid metabolism, oxidative stress and inflammation [180]. HUVECs from GDM pregnancies show lower expression and activity of hENT1 [16,181,182] and accumulate extracellular adenosine *in vitro* [16], which is associated with the A_{2A}AR-dependent increase in eNOS and p42/44^{mapk} activity [183]. Because insulin reverses the GDM-reduced hENT1-mediated adenosine transport

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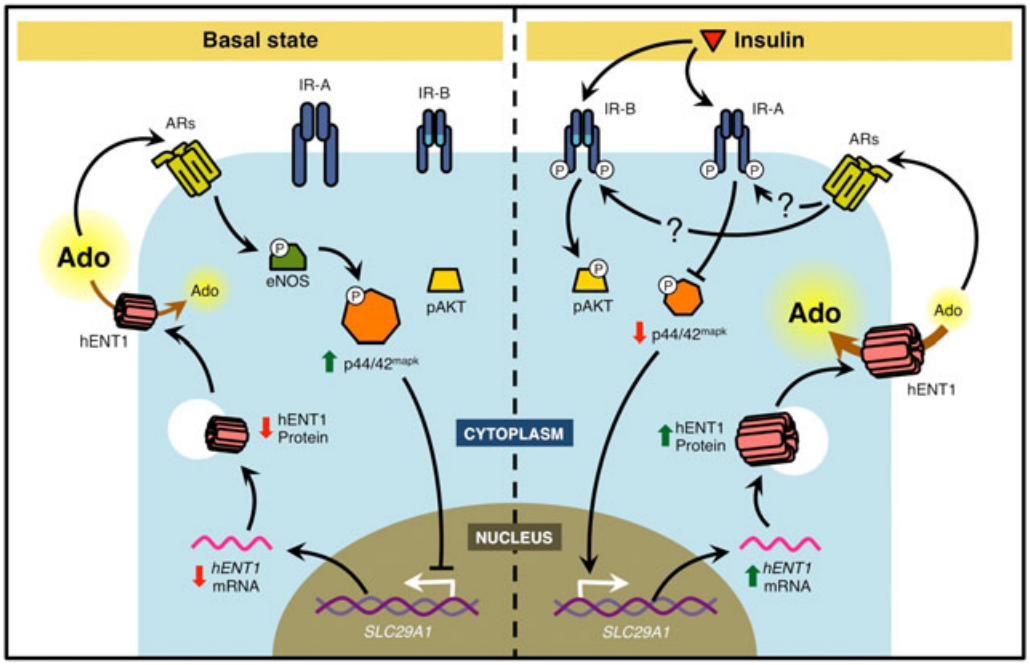

Figure 3. Proposed model for the requirement of IR-A by insulin to reverse gestational diabetes mellitus (GDM)–reduced hENT1-mediated adenosine transport. *Left panel*. In human umbilical vein endothelial cells (HUVECs) obtained from GDM and exposed to basal levels of insulin (Basal state), the expression of insulin receptor A (IR-A) is higher than IR-B and higher than in HUVECs from normal pregnancies. In GDM, the circulating concentration of adenosine (Ado) in the umbilical vein is higher than in normal pregnancies, a finding that is proposed to result from lower Øplasma membrane. Reduced adenosine transport via this membrane transporter isoform results from a reduced (⇩) hENT1 protein abundance due to reduced *hENT1* mRNA and lower transcription of *SLC29A1* (for hENT1) in this cell type. This phenomenon could result from activation of adenosine receptors (ARs), which, via increased phosphorylation (P)–dependent activity of the endothelial nitric oxide synthase (eNOS), leads to higher (⇧) $p44/42^{mapk}$ activity with non-significant modifications in the protein kinase B/Akt (pAKT) activity. Activated $p44/42^{mapk}$ inhibits (⊥) *SLC29A1* expression in HUVECs from GDM. *Right panel*. In the presence of insulin at a concentration over the basal condition (Insulin), IR-A expression is reversed to the values in HUVECs under basal conditions. Additionally, insulin causes phosphorylation of IR-A and IR-B, activating Akt by phosphorylation (pAKT) and reducing $p44/42^{mapk}$ phosphorylation, thus reducing the inhibitory effect on *SLC29A1* expression and allowing this gene to generate normal hENT1 mRNA and hENT1 protein abundance and location at the plasma membrane. Therefore, hENT1–mediated adenosine transport is restored, normalizing the extracellular level of adenosine and reducing the effect of this nucleoside on ARs. Additionally, a physiological extracellular level of adenosine is required (?) to facilitate the insulin biological effect via IR-A and/or IR-B in HUVECs from GDM. From [16–18,20,29,135,165,178,181–185]

[16] involving the expression and activation of IR-A and requiring normal $p42/44^{mapk}$/Akt signalling in HUVECs (Figure 3) [17] and because insulin restores the GDM-reduced, hENT2-mediated adenosine transport requiring both IR isoforms in hPMECs [18], it is likely that these IR isoforms play differential roles, depending on the vascular bed the endothelial cells are from and on which insulin is acting. More recently, it was shown that insulin reverses GDM-increased L-arginine and nitric oxide synthesis in HUVECs [E Guzmán-Gutiérrez, L Sobrevia, *unpublished*]. This phenomenon is due to activation of IR-A requiring A_1AR instead of A_2AR as in this cell type in normal pregnancies. Thus, adenosine receptors and IR act in concert depending on whether the cells are from GDM or normal pregnancies, which demonstrates the differential biological effect of insulin in the macrovascular and the microvascular endothelium of the human placenta [29].

Concluding remarks, perspectives and open questions

Despite the evidence available regarding IR isoforms, there are many open questions that require answers in order to understand the precise differential roles of these receptors both in health and disease. A better understanding of the apparent controversial studies about the relative IR-A/IR-B expression ratio among species, organs and cells in the several pathologies is essential. Additional studies are therefore required to determine the specific roles of IR isoforms in the regulation of tissue-specific insulin sensitivity, which should provide new detailed insight into the complex relationship described, for example, in cancer and diabetes mellitus [186]. Pregnant women that are diagnosed with GDM have higher risk of developing postpartum T2DM [187]. At the same time,

GDM confers future risk of T2DM and obesity to the developing foetus through 'developmental programming' [188]. To date, DNA methylation variable positions have been identified in 1485 samples of cord blood and 1708 placenta samples from GDM pregnancies [189], and 127 genes (i.e. growth factors and insulin signalling proteins) are differentially expressed in HUVECs from this disease [190]. In addition, microRNA-101 (miR-101) was related with GDM-impaired endothelial function in HUVECs [191]. Thus, one of the unanswered questions is whether miR-101 could be used as a predictive biomarker or target for potential therapies in GDM. In addition, no information is available regarding a potential mechanistic link between miR-101 and insulin signalling pathways in GDM. Thus, the concept that GDM alters the foetoplacental unit, playing a role in foetal programming, is proposed. In addition, whether altered IR alternative splicing and downstream signalling pathways have a pathophysiological role in human placental tissue in GDM pregnancies and how they affect the normal endothelial function during pregnancy remain to be elucidated. We propose that future research should be conducted considering the fact that placental endothelial cells are heterogeneous in their genotype and phenotype, even throughout the same vascular bed [140].

In a recent study, it was reported that the insulin-analogue glargine (GLA) metabolite 1 (GLA-M1) was found in circulation in patients with T2DM after long-term GLA therapy and is correlated with serum-induced IR-A, but not IR-B, activation. In addition, GLA did not increase IGFR-1 signalling during long-term insulin therapy in T2DM [129]. Thus, a new perspective in the treatment of GDM patients could be considered. Furthermore, evaluation of insulin analogues in the clinical treatment of GDM patients [192,193] and their effects on IR isoforms in different experimental models might be useful to reduce vascular function alterations in the mother and the foetus suffering from this disease. A recent study performed in blood samples from 390 children whose mothers were diagnosed with mild GDM during the pregnancy revealed that a randomized treatment trial for mild GDM was associated with lower fasting D-glucose levels in female but not in male offspring at ages 5–10 years [194]. Thus, the physiological differences of sex among the offspring might be a field for future research and clinical studies related to GDM. Another research line is adenosine regulation of insulin signalling in several tissues, including the placenta [16,20], pancreas, adipose tissue, muscle and liver [184], under physiological conditions. Nevertheless, adenosine could also contribute to endothelial dysfunction in HUVECs from GDM pregnancies [183]. However, because A_{2A}AR [20,21] in GDM and A_{2B}AR [21] in preeclampsia are required for insulin biological effects in the human foetoplacental endothelium, a potential beneficial role of this nucleoside is under consideration [29,149]. Thus, an open question is the potential impact of adenosine biological effects on placental insulin signalling in GDM. In addition, the involvement of the subtypes of the adenosine receptors and/or IR isoforms in the effect of adenosine in GDM is unknown. Answers to these questions will help to elucidate the complex interplay among adenosine receptors and the insulin receptor subtypes as key pieces of the adenosine/insulin axis [29,149,184,185], leading to endothelial dysfunction in the GDM-puzzle.

Conflict of interest

There is no conflict of interest.

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