We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Adenosine–Insulin Signaling Axis in the Fetoplacental Endothelial Dysfunction in Gestational Diabetes

Enrique Guzmán-Gutiérrez, Pablo Arroyo,
Fabián Pardo, Andrea Leiva and Luis Sobrevia

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55627>

1. Introduction

Gestational diabetes (GD) is a syndrome associated with maternal hyperglycaemia and defective insulin signaling in the placenta (Metzger et al., 2007; Colomiere et al., 2009; ADA 2012). GD have been associated with abnormal fetal development and perinatal complications such as macrosomia, neonatal hypoglycaemia, and neurological disorders (Nold & Georgieff, 2004; Pardo et al., 2012). The main risk factor to predict the GD development are increased maternal age, overweight before pregnancy, a history of GD in the first pregnancy and history of intolerance abnormal D-glucose (Morisset et al., 2010). Clinical manifestations of GD have been attributed to conditions of hyperglycaemia, hyperlipidemia, hyperinsulinemia, and fetal endothelial dysfunction (Nold & Goergieff, 2004; Greene & Solomon, 2005; Sobrevia et al., 2011). Moreover, GD produces alterations in vascular reactivity (i.e., endothelium dependent vasodilation), which is considered a marker of endothelial dysfunction (De Vriese et al., 2000; Sobrevia et al., 2011; Westermeier et al., 2011; Salomón et al., 2012).

2. Gestational diabetes effect on endothelial function

GD generates structural and functional alterations, including placental microvascular and macrovascular endothelial dysfunction (Tchirikov et al., 2002; Biri et al., 2006; Sobrevia et al., 2011), observations showing an altered regulation of vascular tone in the fetal-placental circulation (San Martín & Sobrevia, 2006; Casanello et al., 2007; Sobrevia et al., 2011). The distal

segment of umbilical cord and the placenta correspond to vascular beds without innervation (Marzioni et al., 2004), therefore local regulation of vascular tone results from a balanced combination of the synthesis, release and bioactivity endothelium-derived vasodilators (i.e., nitric oxide (NO), prostanglandins, adenosine) and vasoconstrictors (i.e., endothelin-1, angiotensin II) (Olsson & Pearson, 1990; Becker et al., 2000). It was reported that arteries and veins in the human placenta from pregnancies with GD have an increase in NO synthesis (Figueroa et al., 2000). Furthermore, the same result was obtained from primary cultures of human umbilical vein endothelial cells (HUVEC) from pregnant women diagnosed with GD (Sobrevia et al., 1995). Therefore, vascular dysfunction resulting from GD may result from a functional dissociation between NO synthesis and its bioavailability in the human placental circulation (Sobrevia et al., 2011). Even when endothelial dysfunction is associated with GD, this is referred to as an alteration of NO synthesis and the uptake of cationic aminoacid L-arginine (i.e., L-arginine/NO pathway) (Figure 1) and a lack of mechanism behind these effects of GD is still a reality (Pardo et al., 2012). However, it is accepted that GD is a result of multiple mechanisms of metabolic alteration, including human fetal endothelial sensitivity to vasoactive molecules such as adenosine (Vásquez et al., 2004; San Martín & Sobrevia, 2006; Sobrevia et al., 2011; Pardo et al., 2012).

Figure 1. Fetal endothelial dysfunction in gestational diabetes. Human umbilical vein endothelial cells (HUVEC) from gestational diabetes (*Gestational diabetes*) exhibit increased human cationic amino acids transporter 1 (hCAT-1)–mediated L-arginine transport and endothelial nitric oxide synthase (eNOS)–dependent nitric oxide (NO) synthesis compared with HUVEC from normal pregnancies (*Normal*). From Vásquez et al (2004), San Martín & Sobrevia (2006), Westermeier et al (2011).

3. L-arginine transport in endothelial cells

L-arginine transport in human cells corresponding to different system of amino acids transports, someone of them, it is y^+ system (high affinity, sodium independent) and sodium dependent ($b^{0,+}$, $B^{0,+}$, $e y^+L$) (San Martín & Sobrevía, 2006; Wu, 2009). System y^+ has for five cationic amino acids transporters (CAT): CAT1, CAT2A, CAT2B, CAT3 and CAT4 (Closs et al., 2006; Grillo et al., 2008), considered the main L-arginine transport mechanism in different cell types (Tong & Barbul, 2004). In addition, CAT-1 isoform is the main L-arginine transporter in the placenta (Table 1) (Grillo et al., 2008).

4. Human cationic amino acids transporter 1

Human CAT-1 (hCAT-1) expression is modulated by cytokines (i.e., $TNF\alpha$, $TGF\beta$) (Irie et al., 1997; Visigalli et al., 2007; Vásquez et al., 2007) and hormones (i.e., insulin) (Simmons et al., 1996; González et al., 2004, 2011a). The gene coding for this protein is called *SLC7A1* and it was originally located on chromosome 13q12-q14 (Albritton et al., 1992; Hammermann et al., 2001) and now referred as 13q12.3 (Gene ID: 6541). This gene is formed by 13 exons and 11 introns, where exons -1 and -2 are untranslatable (Hammermann et al., 2001; Sobrevía & González 2009) and located at the start transcription in +1 exon (Sobrevía & González 2009, González et al., 2011a). hCAT1 is pH and sodium independent (Devés & Boyd, 1998; Cloos et al., 2006) with values for apparent K_m are between 100 and 150 μM , and subjected to *trans*-stimulation (uptake increased by its substrates at the *trans* side of the plasma membrane) (Cloos et al., 2006).

5. hCAT-1 mediated L-arginine transport regulation

L-Arginine transport via hCAT-1 is regulated by different conditions (Sobrevía & González, 2009; González et al., 2011a). In HUVEC, hCAT-1 expression increases by tumoral necrosis factor alpha ($TNF-\alpha$) (Irie et al., 1997; Visigalli et al., 2007) and transforming growth factor beta ($TGF-\beta$) (Vásquez et al., 2007), in the presence of free radicals such as superoxide anion (O_2^-) (González et al., 2011b), insulin (González et al., 2011a; Guzmán-Gutiérrez et al., 2012a), activation of A_{2A} adenosine receptors ($A_{2A}AR$) (Vásquez et al., 2004; Guzmán-Gutiérrez et al., 2012a), or high extracellular D-glucose concentration (25 mM) (Vásquez et al., 2007). Interestingly, insulin, $A_{2A}AR$ and extracellular D-glucose have been directly associated with GD (San Martín & Sobrevía, 2006). Notably, HUVEC from GD pregnancy have increased hCAT-1 expression (Vásquez et al., 2004). Moreover, oxidized low-density lipoprotein (oxLDL) and protein kinase C (PKC) activity increase this transporter abundance in the membrane in HEK293 (Zhang et al., 2008; Vina-Vilasaca et al., 2011). Based in a series of recent publications (reviewed in Leiva et al., 2011; Sobrevía et al., 2011; Pardo et al., 2012) it is proposed that hCAT-1 mediated L-arginine transport in HUVEC from GD could depend on the regulation of *SLC7A1* gene expression.

6. Regulation of *SLC7A1* gene expression

The amino acid cationic transporters family are coding by *SLC7A* (1-4) gene (Verrey et al., 2004), where *SLC7A1* is coding for hCAT-1 (Hammermann et al., 2001). Among the genes coding for CAT-1 in rat, mouse and human there are several common characteristics, i.e., the promoter region lack TATA box, have multiple binding sites for specific protein 1 (Sp1) and have an extensive 3'-untranslated region (3'UTR) which could play roles in the regulation of RNA stability or translation (Aulak et al., 1996, 1999; Fernández et al., 2003; Hatzoglou et al., 2004). *SLC7A1* gene has multiple sites for different types of transcription factors such as nuclear factor κ B (NF- κ B) and Sp1, which is regulated by insulin or inflammatory processes (Sobrevia & González 2009). In HUVEC from normal pregnancies it has been described that insulin increased the *SLC7A1* transcriptional activity (González et al., 2011a; Guzmán-Gutiérrez et al., 2012a), a mechanism that is Sp1 dependent (between -177 and -105 pb from ATG), However, at present there are not studies in HUVEC from GD (Figure 2).

Figure 2. *SLC7A1* gene proximal promoter. The locus 13q12.3 codes for *SCL7A1*. In the proximal promoter of *SLC7A1* there are several consensus sequences for transcription factors, including the nuclear factor κ B (NF- κ B) and specific protein 1 (Sp1) between -115 and -736 pb from the transcriptional start point (ATG). In HUVEC from gestational diabetes (*Gestational diabetes*) NF- κ B and Sp1 could bind to *SLC7A1* proximal promoter inducing its transcriptional activity. However, in HUVEC from normal pregnancies (*Normal*) basal transcriptional activity is commanded mainly by Sp1. The *SLC7A1* contains an ATG within the untranslatable region (3'-UTR) and 2 exons (exon -2 and exon -1). This region could be involved in post-transcriptional regulation of hCAT-1 protein. (1) regards exon 1 of the translatable region. From Hammerman et al. (2001), Hatzoglou et al. (2004), Sobrevia & González (2009).

Specific protein 1 (Sp1). The transcriptional factor Sp1 belongs to the super family Sp/Krupel-like factor, which is divided into Sp subfamilies, with 8 members (Sp1-Sp8) and KLF subfamily, with 15 members (Solomon et al., 2008). Then, Sp subfamily is divided into 2 groups Sp1-Sp4 (604-785 amino acids) and Sp5-Sp8 (394-785 amino acids) (Solomon et al., 2008; Wierstra, 2008). Sp1 has several consensus sites for various kinases, including calmodulin kinase

(CaMK), casein kinases (CK), protein kinase A (PKA), PKC, and p44/42^{mapk} (Samsons et al., 2002; Sobrevia & González 2009). Interestingly, insulin increases Sp1 activity in HepG2 cells, where raised genes transcription such as plasminogen activator inhibitor 1 (Banfi et al., 2001) and Apo A1 lipoprotein (Murao et al., 1998, Lam et al., 2003). In addition, in the skeletal muscle L6 cell line it has been demonstrated that insulin increases PKC expression via a Sp1-independent mechanism (Horovitz-Fried et al., 2007).

Nuclear factor κ B. Nuclear factor κ B (NF- κ B) participates in inflammation being a main element in many diseases whose activation is induced and is protein synthesis independent, requiring post-translational changes to migrate to the nucleus (Grimm et al., 1993). NF- κ B was described as a transcriptional factor activated by several immunological stimuli, for example, TNF α and LPS (Crisóstomo et al., 2008; Nakao et al., 2002), or interleukine 1 (IL-1) (Jung et al., 2002). NF- κ B activity is related with inhibitor κ B (I κ B), which is an inhibitor when is attached to NF- κ B (Baldwin, 1996). Hyperglycaemia increases NF- κ B protein abundance in the nucleus in HUVEC, a PI3K/Akt mechanism dependent (Sheu et al., 2005). Insulin acting in a short time (30 minutes) inhibits NF- κ B activations (Zhang et al., 2010). High D-glucose is associated with NF- κ B activation in human aortic endothelial cells (HAEC), and bovine aortic endothelial cells (BAEC) (Mohan et al., 2003; Sobrevia & González 2009; González et al., 2011b). In BAEC, insulin blocks high D-glucose effects on NF- κ B activity (Aljada et al., 2001). This insulin effect has been seen in mononuclear cells from obese subjects, who have an increase in NF- κ B activity (Dandona et al., 2001). NF- κ B is also regulated by A_{2A}AR activation leading to inhibition in HUVEC (Sands et al., 2004). In other hands, in astrocytes A_{2A}AR activation leads to increased NF- κ B activity (Ke et al., 2009). Probably, NF- κ B has different functions depending on the cell type. Furthermore, A₃AR activates NF- κ B in thyroid carcinoma (Morello et al., 2009; Bar-Yehuda et al., 2008) and in mononuclear cells from rheumatoid arthritis patients (Fishman et al., 2006; Madi et al., 2007). NF- κ B activity has been associated with cationic amino acid transporter 2B (CAT-2B) in human saphenous vascular endothelial cells (HSVEC) in response to TNF α (Visigalli et al., 2007). In animal models it has been demonstrated that mCAT-2B requires activation of NF- κ B in macrophages (Huang et al., 2004), and that LPS increases mCAT2 levels via NF- κ B in these cells (Tsai et al., 2006). In animal models of GD it has been demonstrated that NF- κ B inhibition leads to an increase in insulin sensitivity in cheeps skeletal muscle (Yan et al., 2010), and increases GLUT-4 expression in GD rat uterus. However, there is no information regarding the role of NF- κ B in human tissues or cells from GD.

7. Gestational diabetes effect on L-arginine transport in HUVEC

It has been reported that NO levels in amniotic fluid (von Mandach et al., 2003) and NO synthesis in placental vein and artery (Figuroa et al., 2000) are increased in GD. Early studies in HUVEC from GD pregnancies show increased NO synthesis and L-arginine transport (Sobrevia et al., 1995, 1997). These results were associated with an increase in eNOS number of copies for mRNA, protein level and activity (Vásquez et al., 2004; Farías et al., 2006, 2010; Westermeier et al., 2011). Moreover, HUVEC from GD pregnancies exhibit a higher number of copies of mRNA for hCAT-1 (Vásquez et al., 2004). Interestingly, HUVEC incubated with

high D-glucose show increased NO synthesis and intracellular cGMP levels (Sobrevia et al., 1997; González et al., 2004, 2011a). In this phenomenon a role has been proposed for cell signaling pathways including PKC and p44/p42^{mapk} (Montecinos et al., 2000; Flores et al., 2003). Thus, in GD there is an increase in NO level associated with an increase in hCAT-1 mediated L-arginine transport.

In HUVEC from GD pregnancies insulin reduces L-arginine transport-increased observed in this cells compared with HUVEC from normal pregnancies (Sobrevia et al., 1998). Moreover, it was observed that insulin reduce NO synthesis-increased (Sobrevia et al., 1998). Another vasoactive molecule, including adenosine, increases L-arginine transport and eNOS activity (Vásquez et al., 2004; San Martín & Sobrevia 2006; Farías et al., 2006, 2010; Westermeier et al., 2011). It was observed by assays *in vitro* that the adenosine level in the culture medium of HUVEC from GD pregnancies (2.5 μ M) is higher than HUVEC from normal pregnancies (50 nM) (Vásquez et al., 2004; Westermeier et al., 2011). Moreover, in HUVEC from normal pregnancies incubated with nitrobenzylthioinosine (NBTI, equilibrative adenosine transporters inhibitor) exhibit increased L-arginine transport, a phenomenon blocked by antagonists of A_{2A}AR, indicating that elevated extracellular adenosine level and A_{2A}AR activation are factors involved in the stimulation of L-arginine transport by NBTI (San Martín & Sobrevia, 2006; Westermeier et al., 2009; Sobrevia et al., 2011).

8. Adenosine receptors

Adenosine is a purine nucleoside associated with several biological functions, such as nucleotides synthesis or cellular energetic metabolism (Eltzschig, 2009). Moreover, this nucleoside is a vasodilator in coronary, cerebral, and muscular circulation, in several conditions including hypoxia and exercise (Berne et al., 1983). Extracellular adenosine is a signaling molecule that activates adenosine receptors (ARs). ARs belonging purinergic receptor P1 family, are coupled to G-protein and only four subtypes ARs, A₁, A_{2A}, A_{2B} y A₃ have been described (Fredholm et al., 2001, 2007, 2011; Burnstock et al., 2006, 2010). ARs stimulation generates several biological effects which are related with the expression pattern and membrane disponibility in a certain cellular type or tissue (Liu et al., 2002; Wyatt et al., 2002; Feoktistov et al., 2002). The protein assembly exhibits a short N-terminal (7-13 amino acids) compared with the C-terminal (32-120 amino acids) (Burnstock, 2006). Humans ARs transmembrane domains have between 39–61% of identical sequence and 11-18% with P2 family (nucleotide receptors) (Burnstock, 2006). The A₁AR, A_{2A}AR y A₃AR are activated by adenosine at nanomolar concentration, while A_{2B}AR requires micromolar concentration for its activation (Fredholm et al., 2001; 2011; Schulte & Fredholm, 2003; Eltzschig, 2009; Mundell & Kelly, 2010). A₁AR and A₃AR are clasically associated with inhibitory signaling receptors coupled to G_i/G_o protein; however, A_{2A}AR and A_{2B}AR are associated with stimulatory signaling receptors coupled to G_s protein (Klinger et al., 2002).

ARs activation depends on the adenosine extracellular level, a characteristic that is mainly regulated by adenosine membrane transporters (Baldwin et al., 2004; Burnstock, 2006;

Westermeier et al., 2009; Burnstock et al., 2010; Sobrevia et al., 2011). In HUVEC and in human placental microvascular endothelial cells (hPMEC) the extracellular adenosine is taken up mainly via the equilibrative nucleoside transporters (ENTs) (Westermeier et al., 2009; 2011; Sobrevia et al., 2011; Salomón et al., 2012). Interestingly, the sodium dependent, concentrative nucleoside transporters (CNT) have not been described in HUVEC or hPMEC (Sobrevia et al., 2011; Pardo et al., 2012). Several studies have described endothelial effects of adenosine, including a rise in the oxygen demand/delivery relation in human heart due to A_{2A}AR activation-associated vasodilation (Shryock et al., 1998; Sundell et al., 2003), or reduction on norepinephrine release and peripheral vascular resistance by A₁AR activation in rat sympathetic nerve (Burgdorf et al., 2001, 2005). A summary of the potential biological effects resulting from activation of ARs is given in Table 2.

9. Role of adenosine receptors in gestational diabetes

The vasodilatory effect of adenosine, which is endothelial-derived NO-dependent, is mediated by activation of ARs (Sobrevia & Mann, 1997; Edmunds & Marshall, 2003; Vásquez et al., 2004; San Martín & Sobrevia, 2006; Ray & Marshall, 2006; Casanello et al., 2007; Escudero et al., 2008, 2009; Westermeier et al., 2009; Sobrevia et al., 2011). This is also seen in primary cultures of HUVEC from GD (Vásquez et al., 2004; San Martín & Sobrevia, 2006; Casanello et al., 2007; Westermeier et al., 2009; Farías et al., 2006, 2010) or in HUVEC from normal pregnancies exposed to high D-glucose (Muñoz et al., 2006; Puebla et al., 2008). The functional link between adenosine and L-arginine/NO pathway in HUVEC has been referred as the ALANO signalling pathway (i.e., *Adenosine/L-Arginine/Nitric Oxide*) (San Martín & Sobrevia, 2006). This mechanism has been proposed as a key new element for a better understanding of the endothelial dysfunction in conditions of hyperglycaemia, such as that seen in GD (Figure 3) (Pandolfi & Di Pietro, 2010).

The increased activity of ALANO pathway in GD involves extracellular adenosine accumulation resulting from reduced of adenosine uptake into endothelial cells (Vásquez et al., 2004; Farías et al., 2006, 2010). This means that changes in plasma adenosine concentration in the fetoplacental circulation could result in an altered blood flux control in the human placenta (Westermeier et al., 2009; Sobrevia et al., 2011). It was demonstrated that resistance of umbilical vessels from GD do not change with respect to vessels from normal pregnancies (Brown et al., 1990; Biri et al., 2006; Pietryga et al., 2006). It has been reported that plasma adenosine level in umbilical vein whole blood is higher in GD with respect to normal pregnancies (Westermeier et al., 2011). In addition, umbilical vein blood contained more adenosine compared with umbilical cord arteries in GD, thus suggesting that an altered placental metabolism of this nucleoside is likely in this syndrome (Salomón et al., 2012). These results complement other studies showing increased adenosine concentration in umbilical vein blood from GD compare to normal pregnancies (Maguire et al., 1998) or in the extracellular medium in primary cultures for HUVEC and hPMEC from GD (Vásquez et al., 2004; Farías et al., 2006, 2010; Westermeier et al., 2011; Salomón et al., 2012). Even when all these observation have been made, there is not a full consense between the findings showing increased plasma level of adenosine and

Figure 3. Endothelial ALANO pathway. The ALANO (Adenosine/L-Arginine/Nitric oxide) pathway is initiated by a low adenosine uptake via equilibrative nucleoside transporters (ENT) (dotted arrow) leading to increased extracellular adenosine concentration. Accumulation of adenosine activates A_{2A} adenosine receptors (A_{2A}) resulting in increased human cationic amino acid (hCAT-1)-mediated L-arginine transport and endothelial nitric oxide synthase (eNOS)-dependent nitric oxide (NO) synthesis. eNOS is activated by preferential Serine¹¹⁷⁷ phosphorylation (p-eNOS). ALANO pathway has been associated as a new mechanism for the understanding of gestational diabetes effects on human fetal endothelial cells. From Vásquez et al. (2004), San Martín & Sobrevia (2006), Pandolfi & Di Pietro (2010).

endothelial dysfunction in GD pregnancies (Baldwin et al., 2004; San Martín & Sobrevia, 2006; Casanello et al., 2007; Westermeier et al., 2009; Sobrevia et al., 2011; Pardo et al., 2012).

10. Insulin

Insulin is a polypeptide hormone of 51 amino acid residues, synthesized and secreted by β cells in the Langerhans islets of pancreas as an inactive single polypeptide, i.e., preproinsulin, with an N-terminal signal sequence that determines its incorporation to secretory vesicles (Mounier et al., 2006). The proteolytic elimination of the signal sequence and the formation of three di-sulfur bridges yield the proinsulin. This molecule goes to the Golgi apparatus where it is modified and stored in secretory vesicles (Shepherd, 2004). The raise of D-glucose in the blood triggers insulin production through conversion of proinsulin to active insulin by proteases that will cut two peptide bonds to form the mature form of insulin in equimolar quantities of C peptide (Shepherd, 2004). Insulin is the archetypal growth hormone during fetal development, promotes the deposit of carbohydrates, lipids and protein in the tissues and D-glucose uptake. This hexose is the main source of energy in the fetus and its metabolism responds to fetal insulin since the 12th week of gestation (first trimester) (Desoye et al., 2007). Intracellular hormones and signals regulate insulin secretion, also the autonomous nervous system and the interaction of substrates like amino acids and mainly D-glucose (Shepherd,

2004). Once secreted from the pancreas, insulin exerts several effects on its target cells and regulates a myriad of processes in the organism (Muniyappa et al., 2007).

11. Role of insulin in gestational diabetes

The studies ‘Summary and Recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus’ (Metzger & Coustan, 1998) and ‘Gestational Diabetes Mellitus, Position Statement of the American Diabetes Association’ (2004) list different priority areas regarding gestational diabetes research, proposing the characterization of regulatory mechanisms of fetal blood flow as a necessary attention sector, based in the lack of information about the effect of gestational diabetes over the fetoplacental circulation. Furthermore, some reports (i.e., ‘Summary and Recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus’) (Metzger et al., 2007) include recommendations for research in several aspects of placental function in the context of gestational diabetes. These recommendations include characterization of insulin resistant mechanisms and identification of cellular mechanism that reduces insulin signal in GD (Metzger et al., 2007). Although the role of insulin is accepted in GD, cellular signaling and the fetoplacental tissue response to insulin in this syndrome is not well understood (Hiden et al., 2009; Westermeier et al., 2009; Sobrevia & González, 2009; Sobrevia et al., 2009). Even when insulin receptors are expressed in human placental vasculature (Hiden et al., 2009; Westermeier et al., 2011; Salomón et al., 2012), there is limited information available about the biological action of insulin receptors activation and the vascular effects of insulin in the placental circulation in GD (Desoye & Hauguel-de Mouson, 2007; Barret et al., 2009; Genua et al., 2009; Sobrevia et al., 2011).

GD leads to abnormalities in the transplacental transport, an event that happens, among others factors, due to a lost in the hormonal balance induced by changes in the synthesis and signaling of insulin (Kuzuya & Matsuda, 1997; Metzger & Custan, 1998; Greene & Solomon, 2005; Biri et al., 2006; Sobrevia & González, 2009; Barret et al., 2009). Insulin causes vasodilation in normal subjects via a mechanism that is dependent on endothelium-derived NO (Steinberg & Baron, 2002; Sundell & Knuuti, 2003; Barret et al., 2009; Sonne et al., 2010; Timmerman et al., 2010). Furthermore, *in vitro* studies show that insulin activates L-arginine/NO signaling pathway in HUVEC (González et al., 2004, 2011a; Muñoz et al. 2006), hPMEC (Salomón et al., 2012) and in other endothelia (Sundell & Knuuti, 2003; Barrett et al., 2009). Initial observations suggested a differential vasodilatory effect of insulin between the macro and microvasculature of the human placenta from fetuses that were appropriate (AGA) or large (LGA) for gestational age in GD (Jo et al., 2009). This study shows that insulin-associated vasodilation depends on endothelium-derived NO in umbilical arteries and veins from normal pregnancies or GD, and that insulin did not alter chorionic vessels of normal pregnancies, but generated chorionic vessel relaxation in pregnancies with this syndrome. These observations were accompanied by increased level of insulin in the plasma from umbilical cord blood, confirming earlier observations (Westgate et al., 2006; Lindsay et al., 2007; Colomiere et al., 2009). Interestingly, there is not information regarding the potential mechanism(s) associated with this specific

response to insulin by the fetoplacental unit in GD (Youngren et al., 2007, Barrett et al., 2009; Sobrevia & González, 2009; Sobrevia et al., 2011).

12. hCAT-1-mediated L-arginine transport regulated by insulin

In primary cultures of HUVEC in euglycemia conditions (i.e., containing 5 mM D-glucose) and in the presence of physiological concentrations of insulin (0.1-1 nM) it has been observed an increase of the maximum velocity of L-arginine transport ($V_{\max}$), with no significant changes in the apparent K_m (González et al., 2004). This phenomenon was seen in a higher maximum transport capacity (i.e., $V_{\max}/K_m$) (Casanello et al., 2007; Vásquez et al., 2004; Escudero et al., 2008). The reported magnitude of stimulatory effect of L-arginine transport (~5 fold) was comparable to *trans*-stimulation with lysine of L-arginine uptake (González et al., 2004). These results are complemented by an increased number of copies of mRNA for hCAT1 (González et al., 2004) and protein abundance (González et al., 2011a; Guzmán-Gutiérrez et al., 2012a). These studies suggest that L-arginine transport is stimulated by insulin by increasing the expression of hCAT1 in HUVEC (Sobrevia & González, 2009). Furthermore, it has been proposed that the effect of insulin on transport of L-arginine is a cellular signaling mechanism including phosphatidylinositol 3 kinase (PI3K), protein kinase C (PKC) and mitogen-activated protein kinases p44 and p42 (p42/p44^{mapk}) (González et al., 2004). This phenomenon will increase the binding of Sp1 to SLC7A1 promoter in the consensus area between -177 to -105 bp from the ATG (González et al., 2011) (Figure 4). Furthermore, in HUVEC from pregnancies with GD in the presence of 5 mM D-glucose, insulin (1 nM) decreases overall L-arginine transport, whereas in 25 mM D-glucose, insulin insensitivity is seen (0.1-10 nM) (Sobrevia et al., 1996, 1998). These findings open the option of studying a mechanism of insulin resistance mediated by insulin receptor or post-insulin receptor defects. Recently it has been reported that HUVEC (Westermeyer et al., 2011) and hPMEC (Salomón et al., 2012) express at least two insulin receptor subtypes, IR type A (IR-A) and IR type B (IR-B). In HUVEC from GD the IR-A/IR-B ratio is 1.6 fold compared with cells from normal pregnancies, an effect due to increased IR-A mRNA expression. However, in hPMEC from GD pregnancies the IR-A mRNA expression was reduced, while IR-B mRNA expression was increased compared with cells from normal pregnancies. Thus, a differential and cell specific involvement of these IR subtypes in GD and perhaps other pathologies of pregnancy, such as pre-eclampsia (Mate et al., 2012) could occur.

13. Insulin receptors

Insulin generates its biological effects via activation of insulin receptors in the plasma membrane of endothelial cells of human umbilical vein (Zheng & Quon, 1996; Nitert et al., 2005) and placental microvasculature (Desoye & Hauguel-de Mouzon, 2007; Hiden et al., 2009). The gene coding the human insulin receptor is located on the short arm of chromosome 19 and consists of 22 exons and 21 introns (Seino et al., 1989). The mature insulin receptor is a glycoprotein composed of two β subunits (transmembrane domain) joined by disulfide

Figure 4. Insulin modulation of hCAT-1 mediated L-arginine transport in HUVEC. Insulin activates insulin receptors (IR) triggering signaling cascades involving phosphoinositide 3 kinase (PI3K), Akt and protein kinases C (PKC), and in parallel p44 and p42 mitogen-activated protein kinases (p44/42^{mapk}). These molecules induce an increase in Sp1 cytoplasm levels, which then promotes *SLC7A1* transcriptional activity due to higher Sp1 activity (between the region -331 to -130 bp from the transcriptional start point (3'UTR)) leading to an increase in the hCAT-1 mRNA expression and protein abundance at the plasma membrane allowing higher L-arginine uptake. From González et al. (2004, 2011).

bridges. The *N*-terminal extracellular α -subunit (exons 1-2) and the cysteine-rich domain (exons 3-5) are responsible for the high affinity for the insulin in combination with C-terminal domain (amino acid residues 704-719) (Kristensen et al., 1998; Thørsoe et al., 2010). Insulin signaling involves the participation of PI3K as regulatory protein of D-glucose metabolism in tissues such as skeletal muscle and adipocytes promoting translocation of isoform 4 of D-glucose transporter (GLUT4) to the plasma membrane and stimulating NO production and endothelium-dependent vasodilation (Bergandi et al., 2003). The mitogenic effect is primarily mediated by MAPK, which regulates the growth, differentiation and control, for example, the synthesis of vasoconstrictor molecules such as ET-1 (Kim et al., 2006; Muniyappa et al., 2007).

With the cloning of the two isoforms of the insulin receptor, i.e., IR-A and IR-B, the possibility of a differential response to insulin by selective activation (or semi selective) of these isoforms has been proposed (Ullrich et al., 1985; Ebina et al., 1985; Frasca et al., 1999; Sesti et al., 2001; Belfiore et al., 2009; Genua et al., 2009; Sciacca et al., 2003, 2010; Thørsoe et al., 2010; Sen et al., 2010; Westermeier et al., 2011; Sobrevia et al., 2011; Leiva et al., 2011; Pardo et al., 2012; Salomón et al., 2012). The IR-A cDNA (exon 11-) lacks exon 11, and IR-B (exon 11+) contains exon 11 (Genua et al., 2009; Thørsoe et al., 2010; Sen et al., 2010). Both isoforms are expressed in insulin-sensitive tissues (liver, muscle and adipose tissue) (Moller et al., 1989; Mosthaf et al., 1990), but IR-A is predominantly expressed in the fetus and placenta, where it plays a role in embryonic development (Frasca et al., 1999). These isoforms are also expressed in adult tissue, especially in the brain (Belfiore et al., 2009). Moreover, IR-B is expressed mainly in differentiated adult tissues, such as the liver, and associates with increased metabolic effects of insulin (Sciacca et al., 2003, 2010; Genua et al., 2009; Sen et al., 2010). Dysregulation of the insulin receptor splicing

in key tissues responsive to insulin may occur in patients with insulin resistance, but this role is unclear in diabetes mellitus (Belfiore et al., 2009) and not reported in GD (Sobrevia et al., 2011; Leiva et al., 2011; Pardo et al., 2012). A recent study shows that IR-A activation by insulin activates a predominant metabolic signaling pathway ($p42/p44^{\text{mapk}}/\text{Akt}$ activity ratio >1) instead of a predominant mitogenic signaling pathway ($p42/p44^{\text{mapk}}/\text{Akt}$ activity ratio <1), as described in response to IR-B activation in the R- cell line of mouse embryonic fibroblasts (Sciacca et al., 2010). These results suggest differential cell signaling pathways activated by these insulin receptor subtypes (Genua et al., 2009; Sciacca et al., 2010). In fact, recently was shown that hPMEC from GD exhibit a predominant metabolic phenotype compared with cells from normal pregnancies, and that this phenotype could be reversed to a mitogenic, normal phenotype (Salomón et al., 2012). Thus, a modulation of the expression level will, perhaps, has a consequence in the metabolism of the endothelial cells of the fetoplacental unit in GD. Other evidence suggests that a decrease in insulin response, as in Type 2 Diabetes Mellitus where the predominant isoform is IR-A (Norgren et al., 1994), and in states of insulin resistance where IR-A/IR-B increases in the skeletal muscle of patients with myotonic dystrophy type 1 (Savkur et al., 2001) and 2 (Savkur et al., 2001; Phillips et al., 1998).

Insulin, insulin-like growth factor 1 (IGF1) and 2 (IGF2) generate various metabolic and mitogenic effects through activation of receptors associated with tyrosine kinase activity on the surface of the target cells. These hormones have high structural homology. The two receptors may act as ligands for these molecules. At physiological concentrations insulin and IGF1 are attached only to the insulin and IGF receptors, respectively. While, IGF2 receptor binds to IGF1 (IGFR1) and IR-A (Frasca et al., 1999). The affinity of IGF2 by IR-A is less than that of insulin for this receptor (EC_{50} 0.9 versus 2.5 nM, respectively), while the binding of IGF1 to IR-A is very high ($EC_{50} > 30$ nM). The affinity of IGF2 to IGFR1 is comparable to that of IGF1 (EC_{50} 0.6 versus 0.2 nM, respectively), where insulin binds weakly to this receptor ($EC_{50} > 30$ nM) (Pandini et al., 2002). Therefore, there may be a differential response to insulin in the fetoplacental vasculature given by preferential activation of one insulin receptor subtype in pregnancies with GD.

14. Insulin effects are modulated by adenosine

Insulin sensitivity is increased in rats supplemented with adenosine in the diet (Ardiansyah et al., 2010). These data are complemented by similar observations described in diabetic rat adipocytes (Joost & Steinfelder, 1982), nondiabetic rat skeletal muscle (Vergauwen et al., 1995), and patients with T1DM who received an infusion of adenosine (Srinivasan et al., 2005) (Table 3). In other studies, adenosine, agonists and antagonists concentration of adenosine receptors, and insulin used was greater than 100 nM, suggesting for adenosine receptors, that the activation and inhibition of this receptors was complete, and for insulin, involving IR-A, IR-B, and IGF receptors in the system. However, in some studies the concentration of insulin that was used is relatively selective for the receptors of insulin, suggesting the possibility that activation of adenosine receptors increases insulin effect (Webster et al., 1996; Ciaraldi et al., 1997; Sundell et al., 2002; Srinivasan et al., 2005). Similarly, other groups show that inhibition of adenosine receptors blocks the effect of insulin mediated only by the insulin receptor (Pawelczyk et al.,

2005; Dhalla et al., 2008). Moreover, the expression and activation of adenosine receptors reduces plasma levels of D-glucose, due to increased release and the biological effect of insulin in diabetic rats (Johansson et al., 2006; Németh et al., 2007; Töpfer et al., 2008). Activation of A_1 AR (Vergauwen et al., 1994) or decreased expression A_{2B} AR (Ardiansyah et al., 2010; Figler et al., 2011), results in an increased sensitivity to insulin, but there is no information about the specific mechanisms explaining the biological actions of adenosine (Burnstock et al., 2006; San Martín & Sobrevia, 2006; Mundell & Kelly, 2010). The activation of the A_{2B} AR, but to a lesser degree than the A_1 AR, prevents the development of diabetes in mouse (Németh et al., 2007). However, a study in C57BL/6J mice suggests that insulin sensitivity decreases by activation of A_{2B} AR, except in the knockout mouse for this receptor, suggesting that A_{2B} AR is involved in the phenomenon of insulin resistance (Figler et al., 2011). This finding opens the possibility that the increase and/or inhibition of the expression or activity of ARs may be associated as a protective mechanism against this syndrome. Recently, we have published that A_{2A} AR activation in HUVEC from normal pregnancies modulate insulin effect on hCAT-1-mediated L-arginine transport and expression (Guzmán-Gutiérrez et al., 2012a). Interestingly, we saw that in HUVEC from GD insulin reversed GD-increased hCAT-1-mediated L-arginine transport, a mechanism that is dependent on A_1 AR activation (Guzmán-Gutiérrez et al., 2012b). Based on these findings, a possible cross talk between the adenosine receptors and insulin receptors is feasible. This phenomenon could create a potential regulatory mechanism of the biological actions of insulin in the fetoplacental vasculature in GD (Figure 5).

Figure 5. Adenosine/insulin signaling axis involvement in the L-arginine transport in HUVEC. Insulin acting via insulin receptors (IR) increases (+) human cationic amino acid (hCAT-1)-mediated L-arginine transport in HUVEC from normal pregnancies (*Normal*). This biological action of insulin requires (+) activation of A_{2A} (A_{2A}), but not on A_1 adenosine receptors (A_1) by adenosine. In HUVEC from gestational diabetes insulin decreases (-) the hCAT-1 mediated L-arginine transport (dotted arrow), which was elevated in cells from this syndrome to values in cells from normal pregnancies. This biological action of insulin requires (+) activation of A_1 , but not on A_{2A} adenosine receptors by adenosine. From Guzmán-Gutiérrez et al. (2011, 2012a,b)

15. Concluding remarks

GD associates with endothelial dysfunction in the fetoplacental macro and microcirculation associated with an increase in NO synthesis and hCAT-1 mediated L-arginine transport. Hyperinsulinemia and high plasma adenosine in umbilical blood in GD, suggest the involvement of these molecules in this syndrome. A_{2A} AR and insulin receptors increase hCAT-1 and eNOS activity and expression in HUVEC from normal, while HUVEC from GD, activation of A_{2A} AR would be part of mechanism that explain the increase of NO synthesis (i.e., ALANO pathway). In other hands, it has been proposed that insulin acts as a factor that reverses GD-increased NO synthesis and L-arginine transport to values in cells from normal pregnancies. This insulin dual effect can be explained for a differential expression of IR-A and IR-B in normal and GD pregnancies. Insulin effects are dependet on activation of ARs in several cell types, suggesting that adenosine should be act as an isoform insulin receptor activity regulator. Thus, regarding the GD association with increased hCAT-1 expression and activity, there are several not still answered, for example, how is insulin decreasing hCAT-1 activity and expression?, and is adenosine a modulator of the expression and associated signaling of the isoforms of insulin receptors in GD?. Answering these (and other) questions will help us understand insulin mechanisms, opening the possibility to study potential treatment for insulin resistance pathologies including GD.

Acknowledgements

We are thankful to the personnel at the Hospital Clínico Pontificia Universidad Católica de Chile labour ward for their support in the supply of placentas. This research was supported by Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT 1110977, 11110059, 3130583), Programa de Investigación Interdisciplinario (PIA) from Comisión Nacional de Investigación en Ciencia y Tecnología (CONICYT, Anillos ACT-73) (Chile) and CONICYT Ayuda de Tesis (CONICYT AT-24120944). EG-G and PA hold CONICYT-PhD (Chile) fellowships. FP was the recipient of a postdoctoral position (CONICYT PIA Anillos ACT-73 postdoctoral research associate at CMPL, Pontificia Universidad Católica de Chile (PUC)). PA is the recipient of a Faculty of Medicine (PUC) PhD fellowship.

Author details

Enrique Guzmán-Gutiérrez*, Pablo Arroyo, Fabián Pardo, Andrea Leiva and Luis Sobrevia

*Address all correspondence to: elguzman@uc.cl, sobrevia@med.puc.cl

Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

References

- [1] Albritton LM, Bowcock AM, Eddy RL, Morton CC, Tseng L, et al. The human cationic amino acid transporter (ATRC1): physical and genetic mapping to 13q12-q14. *Genomics* 12: 430-434.
- [2] Aljada A, Ghanim H, Saadeh R, Dandona P. (2001) Insulin inhibits NFkappaB and MCP-1 expression in human aortic endothelial cells. *J Clin Endocrinol Metab* 86: 450-453.
- [3] American diabetes association (ADA).(2004) Gestational diabetes mellitus. *Diabetes Care* 27: S88-S90.
- [4] American Diabetes Association.(2012) Diagnosis and Classification of Diabetes Mellitus. *Diabetes care* 35: S64-S71.
- [5] Arancibia-Garavilla Y, Toledo F, Casanello P, Sobrevia L. (2003) Nitric oxide synthesis requires activity of the cationic and neutral amino acid transport system y+L in human umbilical vein endothelium. *Exp Physiol* 88: 699-710.
- [6] Ardiansyah HS, Yumi S, Takuya K, Michio K. (2010) Anti-metabolic syndrome effects of adenosine ingestion in stroke-prone spontaneously hypertensive rats fed a high-fat diet. *Br J Nutr* 104: 48-55.
- [7] Aulak KS, Liu J, Wu J, Hyatt SL, Puppi M, et al. (1996) Molecular sites of regulation of expression of the rat cationic amino acid transporter gene. *J Biol Chem* 271: 29799-29806.
- [8] Aulak KS, Mishra R, Zhou L, Hyatt SL, de Jonge W, et al. (1999) Post-transcriptional regulation of the arginine transporter Cat-1 by amino acid availability. *J Biol Chem* 274: 30424-30432.
- [9] Baldwin AS Jr. (1996) The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol* 14: 649-683.
- [10] Baldwin SA, Beal PR, Yao SY, King AE, Cass CE, et al. (2004) The equilibrative nucleoside transporter family, SLC29. *Pflugers Arch* 447: 735-743.
- [11] Banfi C, Eriksson P, Giandomenico G, Mussoni L, Sironi L, et al. (2001) Transcriptional regulation of plasminogen activator inhibitor type 1 gene by insulin: insights into the signaling pathway. *Diabetes* 50: 1522-1530.
- [12] Barrett HL, Morris J, McElduff A. (2009) Watchful waiting: a management protocol for maternal glycaemia in the peripartum period. *Aust N Z J Obstet Gynaecol* 49: 162-167.
- [13] Bar-Yehuda S, Stemmer SM, Madi L, Castel D, Ochaion A, et al. (2008) The A3 adenosine receptor agonist CF102 induces apoptosis of hepatocellular carcinoma via de-

regulation of the Wnt and NF-kappaB signal transduction pathways. *Int J Oncol* 33: 287-295.

- [14] Becker BF, Kupatt C, Massoudy P, Zahler S. (2000) Reactive oxygen species and nitric oxide in myocardial ischemia and reperfusion. *Z Kardiol* 89: 88-91.
- [15] Belfiore A, Frasca F, Pandini G, Sciacca L, Vigneri R. (2009) Insulin Receptor Isoforms and Insulin Receptor/Insulin-Like Growth Factor Receptor Hybrids in Physiology and Disease. *Endocr Rev* 30: 586-623.
- [16] Bergandi L, Silvagno F, Russo I, Riganti C, Anfossi G, et al. (2003) Insulin stimulates glucose transport via nitric oxide/cyclic GMP pathway in human vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 23: 2215-2221
- [17] Berne RM, Knabb RM, Ely SW, Rubio R. (1983) Adenosine in the local regulation of blood flow: a brief overview. *Fed Proc* 42: 3136-3142.
- [18] Biri A, Onan A, Devrim E, Babacan F, Kavutcu M, et al. (2006) Oxidant status in maternal and cord plasma and placental tissue in gestational diabetes. *Placenta* 27: 327-32.
- [19] Brown MA, North L, Hargood J. (1990) Uteroplacental Doppler ultrasound in routine antenatal care. *Aust N Z J Obstet Gynaecol* 30: 303-307.
- [20] Burgdorf C, Richardt D, Kurz T, Seyfarth M, Jain D, et al. (2001) Adenosine inhibits norepinephrine release in the postischemic rat heart: the mechanism of neuronal stunning. *Cardiovasc Res* 49: 713-720.
- [21] Burgdorf C, Schütte F, Kurz T, Dendorfer A, Richardt G. (2005) Adenylyl cyclase-dependent inhibition of myocardial norepinephrine release by presynaptic adenosine A1-receptors. *J Cardiovasc Pharmacol* 45 :1-3.
- [22] Burnstock G, Fredholm BB, North RA, Verkhratsky A. (2010) The birth and postnatal development of purinergic signalling. *Acta Physiol* 199: 93-147.
- [23] Burnstock G. (2006) Vessel tone and remodeling. *Nat Med* 12:16-17.
- [24] Bussolati O, Sala R, Astorri A, Rotoli BM, Dall'Asta V, et al. (1993). Characterization of amino acid transport in human endothelial cells. *Am J Physiol* 265: C1006-C1014.
- [25] Carr CS, Hill RJ, Masamune H, Kennedy SP, Knight DR, et al. (1997) Evidence for a role for both the adenosine A1 and A3 receptors in protection of isolated human atrial muscle against simulated ischaemia. *Cardiovasc Res* 36: 52-59.
- [26] Casanello P, Escudero C, Sobrevia L. (2007) Equilibrative nucleoside (ENTs) and cationic amino acid (CATs) transporters: implications in foetal endothelial dysfunction in human pregnancy diseases. *Curr Vasc Pharmacol* 5: 69-84.
- [27] Casanello P, Sobrevia L. (2002) Intrauterine growth retardation is associated with reduced activity and expression of the cationic amino acid transport systems y+/-

hCAT-1 and y+/hCAT-2B and lower activity of nitric oxide synthase in human umbilical vein endothelial cells. *Circ Res* 91: 127-134.

- [28] Ciaraldi TP, Morales AJ, Hickman MG, Odom-Ford R, Olefsky JM, et al. (1997) Cellular insulin resistance in adipocytes from obese polycystic ovary syndrome subjects involves adenosine modulation of insulin sensitivity. *J Clin Endocrinol Metab* 82: 1421-1425.
- [29] Closs EI, Boissel JP, Habermeier A, Rotmann A. (2006) Structure and function of cationic amino acid transporters (CATs). *J Membr Biol* 213: 67-77.
- [30] Colomiere M, Permezel M, Riley C, Desoye G, Lappas M. (2009) Defective insulin signaling in placenta from pregnancies complicated by gestational diabetes mellitus. *Eur J Endocrinol* 160: 567-578.
- [31] Crisostomo PR, Wang Y, Markel TA, Wang M, Lahm T, et al. (2008) Human mesenchymal stem cells stimulated by TNF-alpha, LPS, or hypoxia produce growth factors by an NF kappa B- but not JNK-dependent mechanism. *Am J Physiol Cell Physiol* 294: C675-C682.
- [32] Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, et al. (2001) Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab* 86: 3257-3265.
- [33] Danialou G, Vicaute E, Sambe A, Aubier M, Boczkowski J. (1997) Predominant role of A1 adenosine receptors in mediating adenosine induced vasodilatation of rat diaphragmatic arterioles: involvement of nitric oxide and the ATP-dependent K⁺ channels. *Br J Pharmacol* 121: 1355-1363.
- [34] De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. (2000). Endothelial dysfunction in diabetes. *Br J Pharmacol* 130: 963-974.
- [35] Derave W, Hespel P. (1999) Role of adenosine in regulating glucose uptake during contractions and hypoxia in rat skeletal muscle. *J Physiol* 515: 255-263.
- [36] Desoye G, and Hauguel-de Mouzon S. (2007) The human placenta in gestational diabetes mellitus. The insulin and cytokine network. *Diabetes Care* 30: S120-S126.
- [37] Devés R, Boyd CA. (1998) Transporters for cationic amino acids in animal cells: discovery, structure, and function. *Physiol Rev* 78: 487-545.
- [38] Dhalla AK, Santikul M, Chisholm JW, Belardinelli L, Reaven GM. (2009) Comparison of the antilipolytic effects of an A1 adenosine receptor partial agonist in normal and diabetic rats. *Diabetes Obes Metab* 11: 95-101.
- [39] Dye JF, Vause S, Johnston T, Clark P, Firth JA, et al. (2004) Characterization of cationic amino acid transporters and expression of endothelial nitric oxide synthase in human placental microvascular endothelial cells. *FASEB J* 18: 125-127.

- [40] Ebina Y, Ellis L, Jarnagin K, Edery M, Graf L, et al. (1985) The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. *Cell* 40: 747-758.
- [41] Edmunds NJ, Marshall JM. (2003) The roles of nitric oxide in dilating proximal and terminal arterioles of skeletal muscle during systemic hypoxia. *J Vasc Res* 40: 68-76.
- [42] Eltzschig HK. (2009) Adenosine: an old drug newly discovered. *Anesthesiology* 111: 904-915.
- [43] Escudero C, Bertoglia P, Hernadez M, Celis C, González M, et al. (2012) Impaired A(2A) adenosine receptor/nitric oxide/VEGF signaling pathway in fetal endothelium during late- and early-onset preeclampsia. *Purinergic signaling* 10.1007/s11302-012-9341-4 (In Press).
- [44] Escudero C, Puebla C, Westermeier F, Sobrevia L. (2009) Potential cell signalling mechanisms involved in differential placental angiogenesis in mild and severe preeclampsia. *Curr Vasc Pharmacol* 7: 475-485.
- [45] Escudero C, Sobrevia L. (2008) A hypothesis for preeclampsia: adenosine and inducible nitric oxide synthase in human placental microvascular endothelium. *Placenta* 29: 469-483.
- [46] Espinal J, Dohm GL, Newsholme EA. (1983) Sensitivity to insulin of glycolysis and glycogen synthesis of isolated soleus-muscle strips from sedentary, exercised and exercise-trained rats. *Biochem J* 212: 453-458.
- [47] Farías M, Puebla C, Westermeier F, Jo MJ, Pastor-Anglada M, et al. (2010) Nitric oxide reduces SLC29A1 promoter activity and adenosine transport involving transcription factor complex hCHOP-C/EBPalpha in human umbilical vein endothelial cells from gestational diabetes. *Cardiovasc Res* 86: 45-54.
- [48] Farías M, San Martin R, Puebla C, Pearson JD, Casado JF, et al. (2006) Nitric oxide reduces adenosine transporter ENT1 gene (SLC29A1) promoter activity in human fetal endothelium from gestational diabetes. *J Cell Physiol* 208: 451-460.
- [49] Feoktistov I, Goldstein AE, Ryzhov S, Zeng D, Belardinelli L, et al. (2002) Differential expression of adenosine receptors in human endothelial cells: role of A2B receptors in angiogenic factor regulation. *Circ Res* 90: 531-538.
- [50] Fernandez J, Lopez AB, Wang C, Mishra R, Zhou L, et al. (2003) Transcriptional control of the arginine/lysine transporter, cat-1, by physiological stress. *J Biol Chem* 278: 50000-50009.
- [51] Fernandez P, Jara C, Aguilera V, Caviedes L, Diaz F, et al. (2012) Adenosine A_{2A} and A₃ receptors are involved in the human endothelial progenitor cells migration. *J Cardiovasc Pharmacol* 59: 397-404.

- [52] Figler RA, Wang G, Srinivasan S, Jung DY, Zhang Z, et al. (2011) Links between insulin resistance, adenosine A2B receptors, and inflammatory markers in mice and humans. *Diabetes* 60: 669-679.
- [53] Figueroa R, Martinez E, Fayngersh RP, Tejani N, Mohazzab-H KM, et al. (2000) Alterations in relaxation to lactate and H(2)O(2) in human placental vessels from gestational diabetic pregnancies. *Am J Physiol Heart Circ Physiol* 278: H706-H713.
- [54] Fishman P, Bar-Yehuda S, Madi L, Rath-Wolfson L, Ochaion A, et al. (2006) The PI3K-NF-kappaB signal transduction pathway is involved in mediating the anti-inflammatory effect of IB-MECA in adjuvant-induced arthritis. *Arthritis Res Ther* 8:R33.
- [55] Flores C, Rojas S, Aguayo C, Parodi J, Mann G, et al. (2003) Rapid stimulation of L-arginine transport by D-glucose involves p42/44(mapk) and nitric oxide in human umbilical vein endothelium. *Circ Res* 92: 64-72.
- [56] Frasca F, Pandini G, Scalia P, Sciacca L, Mineo R, et al. (1999) Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. *Mol Cell Biol* 19: 3278-3288.
- [57] Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. (2001) Nomenclature and Classification of Adenosine Receptors. *Pharmacol Rev* 53; 527-552.
- [58] Fredholm BB, Ijzerman AP, Jacobson KA, Linden J, Müller CE.(2011) International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors--an update. *Pharmacol Rev* 63: 1-34.
- [59] Fredholm BB. (2007) Adenosine, an endogenous distress signal, modulates tissue damage and repair. *Cell Death Differ* 14: 1315-1323.
- [60] Genua M, Pandini G, Cassarino MF, Messina RL, Frasca F. (2009) c-Abl and insulin receptor signalling. *Vitam Horm* 80: 77-105.
- [61] González M, Flores C, Pearson JD, Casanello P, Sobrevia L. (2004) Cell signalling-mediated insulin increase of mRNA expression for cationic amino acid transporters-1 and -2 and membrane hyperpolarization in human umbilical vein endothelial cells. *Pflugers Arch* 448: 383-394.
- [62] González M, Gallardo V, Rodríguez N, Salomón C, Westermeier F, et al. (2011a) Insulin-stimulated L-arginine transport requires SLC7A1 gene expression and is associated with human umbilical vein relaxation. *J Cell Physiol* 226: 2916-2924.
- [63] González M, Muñoz E, Puebla C, Guzmán-Gutiérrez E, Cifuentes F, et al. (2011b) Maternal and fetal metabolic dysfunction in pregnancy diseases associated with vascular oxidative and nitrative stress. In: *The Molecular Basis for Origin of Fetal Congenital Abnormalities and Maternal Health: An overview of Association with Oxidative Stress*. Eds. BM Matata, M Elahi. Ed. Bentham, USA. Chapter 8, pp 98-115.

- [64] Green A. (1987) Adenosine receptor down-regulation and insulin resistance following prolonged incubation of adipocytes with an A₁ adenosine receptor agonist. *J Biol Chem* 262: 15702-15707.
- [65] Greene MF, Solomon CG. (2005) Gestational diabetes mellitus -- time to treat. *N Engl J Med* 352: 2544-2546.
- [66] Grillo MA, Lanza A, Colombatto S. (2008) Transport of amino acids through the placenta and their role. *Amino Acids* 34: 517-523.
- [67] Grimm S, Baeuerle PA. (1993) The inducible transcription factor NF-kappa B: structure-function relationship of its protein subunits. *Biochem J* 290: 297-308.
- [68] Guzmán-Gutiérrez E, Westermeier F, Salomón C, González M, Pardo F, et al. (2012a) Insulin-increased L-arginine transport requires A_{2A} adenosine receptors activation in human umbilical vein endothelium. *PLoS One* 7: e41705.
- [69] Guzmán-Gutiérrez E, Salomón C, Pardo A, Leiva A, Sobrevia L. (2012b) Insulin reverses gestational diabetes-increased L-arginine transport involving A₁ and A_{2A} adenosine receptors activation in HUVEC. *J Mol Cell Cardiol* 53: S15-S16 (Abstract).
- [70] Hammermann R, Brunn G, Racké K. (2001) Analysis of the genomic organization of the human cationic amino acid transporters CAT-1, CAT-2 and CAT-4. *Amino Acids* 21: 211-219.
- [71] Han DH, Hansen PA, Nolte LA, Holloszy JO. (1998) Removal of adenosine decreases the responsiveness of muscle glucose transport to insulin and contractions. *Diabetes* 47: 1671-1675.
- [72] Hatzoglou M, Fernandez J, Yaman I, Closs E. (2004) Regulation of cationic amino acid transport: the story of the CAT-1 transporter. *Annu Rev Nutr* 24: 377-399.
- [73] Hiden U, Lang I, Ghaffari-Tabrizi N, Gauster M, Lang U, et al. (2009) Insulin action on the human placental endothelium in normal and diabetic pregnancy. *Curr Vasc Pharmacol* 7: 460-466.
- [74] Horovitz-Fried M, Brutman-Barazani T, Kesten D, Sampson SR. (2008) Insulin increases nuclear protein kinase Cdelta in L6 skeletal muscle cells. *Endocrinology* 149: 1718-1727.
- [75] Huang CJ, Tsai PS, Yang CH, Su TH, Stevens BR, et al. (2004) Pulmonary transcription of CAT-2 and CAT-2B but not CAT-1 and CAT-2A were upregulated in hemorrhagic shock rats. *Resuscitation* 63: 203-212.
- [76] Irie K, Tsukahara F, Fujii E, Uchida Y, Yoshioka T, He WR, et al. (1997) Cationic amino acid transporter-2 mRNA induction by tumor necrosis factor-alpha in vascular endothelial cells. *Eur J Pharmacol* 339: 289-293.

- [77] Jo M, Krause B, Casanello P, Sobrevia L. (2009) Differential reactivity to insulin in human umbilical and chorionic vessels from normal and gestational diabetic pregnancies. *DOHaD* 1:S307 (abstract).
- [78] Johansson GS, Arnqvist HJ. (2006) Insulin and IGF-I action on insulin receptors, IGF-I receptors, and hybrid insulin/IGF-I receptors in vascular smooth muscle cells. *Am J Physiol Endocrinol Metab* 291: 1124-1130.
- [79] Joost HG, Steinfelder HJ. (1982) Modulation of insulin sensitivity by adenosine. Effects on glucose transport, lipid synthesis, and insulin receptors of the adipocyte. *Mol Pharmacol* 22: 614-618.
- [80] Jung YD, Fan F, McConkey DJ, Jean ME, Liu W, et al. (2002) Role of P38 MAPK, AP-1, and NF-kappaB in interleukin-1beta-induced IL-8 expression in human vascular smooth muscle cells. *Cytokine* 18:206-213.
- [81] Ke RH, Xiong J, Liu Y, Ye ZR. (2009) Adenosine A2a receptor induced gliosis via Akt/NF-kappaB pathway in vitro. *Neurosci Res* 65: 280-285.
- [82] Kim JA, Montagnani M, Koh KK, Quon MJ. (2006) Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 113: 1888-1904.
- [83] Klinger M, Freissmuth M, Nanoff C. (2002) Adenosine receptors: G protein-mediated signalling and the role of accessory proteins. *Cell Signal* 2: 99-108.
- [84] Kolnes AJ, Ingvaldsen A, Bolling A, Stuenkel JT, Kreft M, et al. (2010) Caffeine and theophylline block insulin-stimulated glucose uptake and PKB phosphorylation in rat skeletal muscles. *Acta Physiol (Oxf)* 200: 65-74.
- [85] Kristensen C, Wiberg FC, Schäffer L, Andersen AS. (1998) Expression and characterization of a 70-kDa fragment of the insulin receptor that binds insulin. Minimizing ligand binding domain of the insulin receptor. *J Biol Chem* 273: 17780-17786.
- [86] Kuzuya T, Matsuda A. (1997) Classification of diabetes on the basis of etiologies versus degree of insulin deficiency. *Diabetes Care* 20: 219-220.
- [87] Laine H, Nuutila P, Luotolahti M, Meyer C, Elomaa T, et al. (2000) Insulin-induced increment of coronary flow reserve is not abolished by dexamethasone in healthy young men. *J Clin Endocrinol Metab* 85: 1868-1873.
- [88] Laine H, Sundell J, Nuutila P, Raitakari OT, Luotolahti M, et al. (2004) Insulin induced increase in coronary flow reserve is abolished by dexamethasone in young men with uncomplicated type 1 diabetes. *Heart* 90: 270-276.
- [89] Lam JK, Matsubara S, Mihara K, Zheng XL, Mooradian AD, et al. (2003) Insulin induction of apolipoprotein AI, role of Sp1. *Biochemistry* 42: 2680-2690.
- [90] Latham AM, Bruns AF, Kankanala J, Johnson AP, Fishwick CW, et al. (2012) Indolines and anilinothalazines differentially target VEGF-A- and basic fibroblast

growth factor-mediated responses in primary human endothelial cells. *Br J Pharmacol* 165: 245-259.

- [91] Leibovich SJ, Chen JF, Pinhal-Enfield G, Belem PC, Elson G, et al. (2002) Synergistic up-regulation of vascular endothelial growth factor expression in murine macrophages by adenosine A(2A) receptor agonists and endotoxin. *Am J Pathol* 160: 2231-2244.
- [92] Leiva A, Pardo F, Ramírez MA, Farías M, Casanello P, et al. (2011) Fetoplacental vascular endothelial dysfunction as an early phenomenon in the programming of human adult diseases in subjects born from gestational diabetes mellitus or obesity in pregnancy. *Exp Diabetes Res* 2011:349286.
- [93] Lindsay RS, Westgate JA, Beattie J, Pattison NS, Gamble G, et al. (2007) Inverse changes in fetal insulin-like growth factor (IGF)-1 and IGF binding protein-1 in association with higher birth weight in maternal diabetes. *Clin Endocrinol* 66: 322-328.
- [94] Liu SH, Shan LM, Wang H. (2002) Pharmacological characteristics of novel putative purinoceptors in vascular endothelium. *Cell Biol Int* 26:963-969.
- [95] Madi L, Cohen S, Ochayin A, Bar-Yehuda S, Barer F, et al. (2007) Overexpression of A3 adenosine receptor in peripheral blood mononuclear cells in rheumatoid arthritis: involvement of nuclear factor-kappaB in mediating receptor level. *J Rheumatol* 34: 20-26.
- [96] Maguire MH, Szabó I, Valkó IE, Finley BE, Bennett TL. (1998) Simultaneous measurement of adenosine and hypoxanthine in human umbilical cord plasma using reversed-phase high-performance liquid chromatography with photodiode-array detection and on-line validation of peak purity. *J Chromatogr B Biomed Sci Appl* 707: 33-41.
- [97] Marzioni D, Tamagnone L, Capparuccia L, Marchini C, Amici A, et al. (2004) Restricted innervation of uterus and placenta during pregnancy: evidence for a role of the repelling signal Semaphorin 3A. *Dev Dyn* 231: 839-848.
- [98] Mate A, Vázquez CM, Leiva A, Sobrevía L. (2012) New therapeutic approaches to treating hypertension in pregnancy. *Drug Discov Today* 17: 1307-1315.
- [99] Metzger BE, Buchanan TA, Coustan DR, de Leiva A, Dunger DB, et al. (2007) Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes Care* 30: S251-S260.
- [100] Metzger BE, Coustan DR. (1998) Summary and recommendations of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. The Organizing Committee. *Diabetes Care* 21: B161-B167.
- [101] Mohan S, Hamuro M, Koyoma K, Sorescu GP, Jo H, et al. (2003) High glucose induced NF-kappaB DNA-binding activity in HAEC is maintained under low shear

stress but inhibited under high shear stress: role of nitric oxide. *Atherosclerosis* 171: 225-234.

- [102] Moller DE, Yokota A, White MF, Pazianos AG, Flier JS. (1989) A naturally occurring mutation of insulin receptor alanine 1134 impairs tyrosine kinase function and is associated with dominantly inherited insulin resistance. *J Biol Chem* 265: 14979–14985.
- [103] Montecinos VP, Aguayo C, Flores C, Wyatt AW, Pearson JD, et al. (2000) Regulation of adenosine transport by D-glucose in human fetal endothelial cells: involvement of nitric oxide, protein kinase C and mitogen-activated protein kinase. *J Physiol* 529: 777-790.
- [104] Montesinos MC, Shaw JP, Yee H, Shamamian P, Cronstein BN. (2004). Adenosine A(2A) receptor activation promotes wound neovascularization by stimulating angiogenesis and vasculogenesis. *Am J Pathol* 164: 1887-1892.
- [105] Morello S, Sorrentino R, Porta A, Forte G, Popolo A, et al. (2009) CI-IB-MECA enhances TRAIL-induced apoptosis via the modulation of NF-kappaB signalling pathway in thyroid cancer cells. *J Cell Physiol* 221: 378-386.
- [106] Morisset AS, St-Yves A, Veillette J, Weisnagel SJ, Tchernof A, et al. (2010) Prevention of gestational diabetes mellitus: a review of studies on weight management. *Diabetes Metab Res Rev* 26: 17-25.
- [107] Mosthaf L, Vogt B, Häring HU, Ullrich A. (1991) Altered expression of insulin receptor types A and B in the skeletal muscle of non-insulin-dependent diabetes mellitus patients. *Proc Natl Acad Sci U S A* 88: 4728-4730.
- [108] Mounier C, Posner BI. (2006) Transcriptional regulation by insulin: from the receptor to the gene. *Can J Physiol Pharmacol* 84: 713-724.
- [109] Mundell S, Kelly E. (2010) Adenosine receptor desensitization and trafficking. *Biochim Biophys Acta* 1808: 1319-1328.
- [110] Muniyappa R, Montagnani M, Koh KK, Quon MJ. (2007) Cardiovascular actions of insulin. *Endocr Rev* 28: 463-491.
- [111] Muñoz G, San Martín R, Farías M, Cea L, Casanello P, et al. (2006) Insulin restores glucose–inhibition of adenosine transport by increasing the expression and activity of the equilibrative nucleoside transporter 2 in human umbilical vein endothelium. *J Cell Physiol* 209: 826-835.
- [112] Murao K, Wada Y, Nakamura T, Taylor AH, Mooradian AD, et al. (1998) Effects of glucose and insulin on rat apolipoprotein A-I gene expression. *J Biol Chem* 273: 18959-18965.
- [113] Nakao S, Ogtata Y, Shimizu E, Yamazaki M, Furuyama S, et al. (2002) Tumor necrosis factor alpha (TNF-alpha)-induced prostaglandin E2 release is mediated by the

activation of cyclooxygenase-2 (COX-2) transcription via NFkappaB in human gingival fibroblasts. *Mol Cell Biochem* 238: 11-18.

- [114] Németh ZH, Bleich D, Csóka B, Pacher P, Mabley JG, et al. (2007) Adenosine receptor activation ameliorates type 1 diabetes. *FASEB J* 21: 2379-2388.
- [115] Nitert MD, Chisalita SI, Olsson K, Bornfeldt KE, Arnqvist HJ. (2005) IGF-I/insulin hybrid receptors in human endothelial cells. *Mol Cell Endocrinol* 229: 31-37.
- [116] Nold JL, Georgieff MK. (2004) Infants of diabetic mothers. *Pediatr Clin North Am* 51: 619-637.
- [117] Norgren S, Li LS, Luthman H. (1994) Regulation of human insulin receptor RNA splicing in HepG2 cells: effects of glucocorticoid and low glucose concentration. *Biochem Biophys Res Commun* 199: 277-284.
- [118] Olanrewaju HA, Mustafa SJ. (2000). Adenosine A(2A) and A(2B) receptors mediated nitric oxide production in coronary artery endothelial cells. *Gen Pharmacol* 35: 171-177.
- [119] Olsson RA, Pearson JD. (1990) Cardiovascular purinoceptors. *Physiol Rev* 70: 761-845.
- [120] Pandini G, Frasca F, Mineo R, Sciacca L, Vigneri R, et al. (2002) Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. *J Biol Chem* 277: 39684-39695.
- [121] Pandolfi A, Di Pietro N. (2010) High glucose, nitric oxide, and adenosine: a vicious circle in chronic hyperglycaemia? *Cardiovasc Res* 86: 9-11.
- [122] Pardo F, Arroyo P, Salomón C, Westermeier F, Guzmán-Gutiérrez E, et al. (2012) Gestational Diabetes Mellitus and the Role of Adenosine in the Human Placental Endothelium and Central Nervous System. *J Diabetes Metab* S2:010. doi: 10.4172/2155-6156.S2-010
- [123] Pawelczyk T, Sakowicz-Burkiewicz M, Kocbuch K, Szutowicz A. (2005) Differential effect of insulin and elevated glucose level on adenosine handling in rat T lymphocytes. *J Cell Biochem* 96: 1296-1310.
- [124] Phillips DI. (1998) Birth weight and the future development of diabetes. A review of the evidence. *Diabetes Care* 21: 150-155.
- [125] Pietryga M, Brazert J, Wender-Ozegowska E, Dubiel M, Gudmundsson S. (2006) Placental Doppler velocimetry in gestational diabetes mellitus. *J Perinat Med* 34: 108-110.
- [126] Puebla C, Farías M, González M, Vecchiola A, Aguayo C, et al. (2008) High D-glucose reduces SLC29A1 promoter activity and adenosine transport involving specific protein 1 in human umbilical vein endothelium. *J Cell Physiol* 215: 645-656.

- [127] Ray CJ, Marshall JM. (2006) The cellular mechanisms by which adenosine evokes release of nitric oxide from rat aortic endothelium. *J Physiol* 570: 85-96.
- [128] Ribé D, Sawbridge D, Thakur S, Hussey M, Ledent C, et al. (2008) Adenosine A2A receptor signaling regulation of cardiac NADPH oxidase activity. *Free Radic Biol Med* 44: 1433-1442.
- [129] Sala R, Rotoli BM, Colla E, Visigalli R, Parolari A, et al. (2002) Two-way arginine transport in human endothelial cells: TNF-alpha stimulation is restricted to system y(+). *Am J Physiol Cell Physiol* 282: C134-C143.
- [130] Salomón C, Westermeier F, Puebla C, Arroyo P, Guzmán-Gutiérrez E, et al. (2012) Gestational diabetes reduces adenosine transport in human placental microvascular endothelium, an effect reversed by insulin. *PLoS One* 7: e40578.
- [131] Samson SL, Wong NC. (2002) Role of Sp1 in insulin regulation of gene expression. *J Mol Endocrinol* 29: 265-279.
- [132] San Martin R, Sobrevia L. (2006) Gestational diabetes and the adenosine/L-arginine/nitric oxide (ALANO) pathway in human umbilical vein endothelium. *Placenta* 27: 1-10.
- [133] Sands WA, Martin AF, Strong EW, Palmer TM. (2004) Specific inhibition of nuclear factor-kappaB-dependent inflammatory responses by cell type-specific mechanisms upon A2A adenosine receptor gene transfer. *Mol Pharmacol* 66: 1147-1159.
- [134] Savkur R, Philips V, Cooper H. (2001) Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. *Nat Genet* 29: 40-47.
- [135] Schulte G, Fredholm BB. (2003) The G(s)-coupled adenosine A (2B) receptor recruits divergent pathways to regulate ERK1/2 and p38. *Exp Cell Res* 290: 168-176.
- [136] Sciacca L, Cassarino MF, Genua M, Pandini G, Le Moli R, et al. (2010) Insulin analogues differently activate insulin receptor isoforms and post-receptor signalling. *Diabetologia* 53: 1743-1753.
- [137] Sciacca L, Prisco M, Wu A, Belfiore A, Vigneri R, et al. (2003) Signaling differences from the A and B isoforms of the insulin receptor (IR) in 32D cells in the presence or absence of IR substrate-1. *Endocrinology* 144: 2650-2658.
- [138] Seino S, Seino M, Nishi S, Bell GI. (1989) Structure of the human insulin receptor gene and characterization of its promoter. *Proc Natl Acad Sci U S A* 86: 114-118.
- [139] Sen S, Talukdar I, Liu Y, Tam J, Reddy S, et al. (2010) Muscleblind-like 1 (Mbnl1) promotes insulin receptor exon 11 inclusion via binding to a downstream evolutionarily conserved intronic enhancer. *J Biol Chem* 285: 25426-25437.

- [140] Sesti G, Federici M, Lauro D, Sbraccia P, Lauro R. (2001) Molecular mechanism of insulin resistance in type 2 diabetes mellitus: role of the insulin receptor variant forms. *Diabetes Metab Res Rev* 17: 363-373.
- [141] Shen J, Halenda SP, Sturek M, Wilden PA. (2005) Cell-signaling evidence for adenosine stimulation of coronary smooth muscle proliferation via the A1 adenosine receptor. *Circ Res* 97: 574-582.
- [142] Shepherd PR. (2004) Secrets of insulin and IGF-1 regulation of insulin secretion revealed. *Biochem J* 377: e1-e2.
- [143] Sheu ML, Chao KF, Sung YJ, Lin WW, Lin-Shiau SY, et al. (2005) Activation of phosphoinositide 3-kinase in response to inflammation and nitric oxide leads to the up-regulation of cyclooxygenase-2 expression and subsequent cell proliferation in mesangial cells. *Cell Signal* 17: 975-984.
- [144] Shryock JC, Snowdy S, Baraldi PG, Cacciari B, Spalluto G, et al. (1998) A2A-adenosine receptor reserve for coronary vasodilation. *Circulation* 98: 711-718.
- [145] Simmons WW, Closs EI, Cunningham JM, Smith TW, Kelly RA. (1996) Cytokines and insulin induce cationic amino acid transporter (CAT) expression in cardiac myocytes. Regulation of L-arginine transport and no production by CAT-1, CAT-2A, and CAT-2B. *J Biol Chem* 271: 11694-702.
- [146] Sobrevia L, Abarzúa F, Nien JK, Salomón C, Westermeier F, et al. (2011) Review: Differential placental macrovascular and microvascular endothelial dysfunction in gestational diabetes. *Placenta* 32: S159-S164.
- [147] Sobrevia L, Cesare P, Yudilevich DL, Mann GE. (1995) Diabetes-induced activation of system y⁺ and nitric oxide synthase in human endothelial cells: association with membrane hyperpolarization. *J Physiol* 489:183-192.
- [148] Sobrevia L, Mann GE. (1997) Dysfunction of the endothelial L-arginine-nitric oxide signalling pathway in diabetes and hyperglycaemia. *Exp Physiol* 82: 1-30.
- [149] Sobrevia L, Puebla C, Farías M, Casanello P. (2009). Role of equilibrative nucleoside transporters in fetal endothelial dysfunction in gestational diabetes. In: *Membrane Transporters and Receptors in Disease*. Eds. L Sobrevia, P Casanello. Ed. Research Signpost, India. Chapter 1, pp 1-25.
- [150] Sobrevia L, Yudilevich DL, Mann GE. (1998) Elevated D-glucose induces insulin insensitivity in human umbilical endothelial cells isolated from gestational diabetic pregnancies. *J Physiol* 506: 219-230.
- [151] Sobrevia L, González M. (2009) A Role for insulin on L-arginine transport in fetal endothelial dysfunction in hyperglycaemia. *Curr Vasc Pharmacol* 7: 467-474.

- [152] Solomon SS, Majumdar G, Martinez-Hernandez A, Raghow R. (2008) A critical role of Sp1 transcription factor in regulating gene expression in response to insulin and other hormones. *Life Sci* 83: 305-312.
- [153] Sonne MP, Højbjerg L, Alibegovic AC, Vaag A, Stallknecht B, et al. (2010) Diminished insulin-mediated forearm blood flow and muscle glucose uptake in young men with low birth weight. *J Vasc Res* 47: 139-147.
- [154] Srinivasan M, Herrero P, McGill JB, Bennik J, Heere B, et al. (2005) The Effects of Plasma Insulin and Glucose on Myocardial Blood Flow in Patients With Type 1 Diabetes Mellitus. *J Am Coll Cardiol* 46: 42-48.
- [155] Steinberg HO, Baron AD. (2002) Vascular function, insulin resistance and fatty acids. *Diabetologia* 45: 623-634.
- [156] Sullivan GW, Lee DD, Ross WG, DiVietro JA, Lappas CM, et al. (2004) Activation of A2A adenosine receptors inhibits expression of alpha 4/beta 1 integrin (very late antigen-4) on stimulated human neutrophils. *J Leukoc Biol* 75: 127-134.
- [157] Sundell J, Knuuti J. (2003) Insulin and myocardial blood flow. *Cardiovasc Res* 57: 312-319.
- [158] Sundell J, Laine H, Nuutila P, Rönnekaa T, Luotolahti M, et al. (2002) The effects of insulin and short-term hyperglycaemia on myocardial blood flow in young men with uncomplicated Type I diabetes. *Diabetologia* 45: 775-782.
- [159] Tchirikov M, Rybakowski C, Hüneke B, Schoder V, Schröder HJ. (2002) Umbilical vein blood volume flow rate and umbilical artery pulsatility as 'venous-arterial index' in the prediction of neonatal compromise. *Ultrasound Obstet Gynecol* 20: 580-585.
- [160] Thakur S, Du J, Hourani S, Ledent C, Li JM. (2010) Inactivation of adenosine A2A receptor attenuates basal and angiotensin II-induced ROS production by Nox2 in endothelial cells. *J Biol Chem* 285: 40104-40113.
- [161] Thorsøe KS, Schlein M, Steensgaard DB, Brandt J, Schluckebier G, et al. (2010) Kinetic evidence for the sequential association of insulin binding sites 1 and 2 to the insulin receptor and the influence of receptor isoform. *Biochemistry* 49: 6234-6246.
- [162] Timmerman KL, Lee JL, Dreyer HC, Dhanani S, Glynn EL, et al. (2010) Insulin stimulates human skeletal muscle protein synthesis via an indirect mechanism involving endothelial-dependent vasodilation and mammalian target of rapamycin complex 1 signaling. *J Clin Endocrinol Metab* 95: 3848-3857.
- [163] Tong BC, Barbul A. (2004) Cellular and physiological effects of arginine. *Mini Rev Med Chem* 4: 823-832.

- [164] Töpfer M, Burbiel CE, Müller CE, Knittel J, Verspohl EJ. (2008) Modulation of insulin release by adenosine A1 receptor agonists and antagonists in INS-1 cells: the possible contribution of 86Rb⁺ efflux and 45Ca²⁺ uptake. *Cell Biochem Funct* 26: 833-843.
- [165] Tsai PS, Chen CC, Tsai PS, Yang LC, Huang WY, et al. (2006) Heme oxygenase 1, nuclear factor E2-related factor 2, and nuclear factor kappaB are involved in hemin inhibition of type 2 cationic amino acid transporter expression and L-Arginine transport in stimulated macrophages. *Anesthesiology* 105: 1201-1210.
- [166] Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, et al. (1985) Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* 313: 756-761.
- [167] Vásquez G, Sanhueza F, Vásquez R, González M, San Martín R, et al. (2004) Role of adenosine transport in gestational diabetes-induced L-arginine transport and nitric oxide synthesis in human umbilical vein endothelium. *J Physiol* 560: 111-122.
- [168] Vásquez R, Farías M, Vega JL, Martin RS, Vecchiola A, et al. (2007) D-glucose stimulation of L-arginine transport and nitric oxide synthesis results from activation of mitogen-activated protein kinases p42/44 and Smad2 requiring functional type II TGF-beta receptors in human umbilical vein endothelium. *J Cell Physiol* 212: 626-632.
- [169] Vergauwen L, Hespel P, Richter EA. (1994) Adenosine receptors mediate synergistic stimulation of glucose uptake and transport by insulin and by contractions in rat skeletal muscle. *J Clin Invest* 93: 974-981.
- [170] Verrey F, Closs EI, Wagner CA, Palacin M, Endou H, et al. (2004) CATs and HATs: the SLC7 family of amino acid transporters. *Pflügers Arch* 447: 532-542.
- [171] Vina-Vilaseca A, Bender-Sigel J, Sorkina T, Closs EI, Sorkin A. (2011) Protein kinase C-dependent ubiquitination and clathrin-mediated endocytosis of the cationic amino acid transporter CAT-1. *J Biol Chem* 286: 8697-8706.
- [172] Visigalli R, Barilli A, Bussolati O, Sala R, Gazzola GC, et al. (2007) Rapamycin stimulates arginine influx through CAT2 transporters in human endothelial cells. *Biochim Biophys Acta* 1768: 1479-87.
- [173] von Mandach U, Lauth D, Huch R. (2003) Maternal and fetal nitric oxide production in normal and abnormal pregnancy. *J Matern Fetal Neonatal Med* 13: 22-27.
- [174] Webster JM, Heseltine L, Taylor R. (1996) In vitro effect of adenosine agonist GR79236 on the insulin sensitivity of glucose utilisation in rat soleus and human rectus abdominus muscle. *Biochim Biophys Acta* 1316: 109-113.
- [175] Westermeier F, Puebla C, Vega JL, Farías M, Escudero C, et al. (2009) Equilibrative Nucleoside Transporters in Fetal Endothelial Dysfunction in Diabetes Mellitus and Hyperglycaemia. *Curr Vasc Pharmacol* 7:435-449.
- [176] Westermeier F, Salomón C, González M, Puebla C, Guzmán-Gutiérrez E, et al. (2011) Insulin restores gestational diabetes mellitus-reduced adenosine transport involving

differential expression of insulin receptor isoforms in human umbilical vein endothelium. *Diabetes* 60: 1677-1687.

- [177] Westgate JA, Lindsay RS, Beattie J, Pattison NS, Gamble G, et al. (2006) Hyperinsulinemia in cord blood in mothers with type 2 diabetes and gestational diabetes mellitus in New Zealand. *Diabetes Care* 29: 1345-1350.
- [178] Wierstra I. (2008) Sp1: emerging roles--beyond constitutive activation of TATA-less housekeeping genes. *Biochem Biophys Res Commun* 372: 1-13.
- [179] Wu G. (2009) Amino acid: metabolism, function, and nutrition. *Amino acid* 37: 1-17.
- [180] Wyatt AW, Steinert JR, Wheeler-Jones CPD, Morgan AJ, Sugden D, et al. (2002) Early activation of the p42/p44 MAPK pathway mediates adenosine-induced nitric oxide production in human endothelial cells: a novel calcium insensitive mechanism. *FA-SEB J* 16:1584-1594.
- [181] Yan X, Zhu MJ, Xu W, Tong JF, Ford SP, et al. (2010) Up-regulation of Toll-like receptor 4/nuclear factor-kappaB signaling is associated with enhanced adipogenesis and insulin resistance in fetal skeletal muscle of obese sheep at late gestation. *Endocrinology* 151: 380-387.
- [182] Youngren JF. (2007) Regulation of insulin receptor function. *Cell. Mol. Life Sci* 64: 873-891.
- [183] Zeng G, Quon MJ. (1996) Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J Clin Invest* 98: 894-898.
- [184] Zhang WF, Hu DH, Xu CF, Lü GF, Dong ML, et al. (2010) Inhibitory effect of insulin on nuclear factor-kappa B nuclear translocation of vascular endothelial cells induced by burn serum. *Zhonghua Shao Shang Za Zhi* 26: 175-179.
- [185] Zhang WZ, Venardos K, Finch S, Kaye DM. (2008) Detrimental effect of oxidized LDL on endothelial arginine metabolism and transportation. *Int J Biochem Cell Biol* 40: 920-928.