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Alternative renin-angiotensin system pathways in adipose tissue and their role in the pathogenesis of obesity

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Adipose tissue expresses all the renin-angiotensin system (RAS) components that play an important role in the adipogenesis, lipid and glucose metabolism regulation in an auto/paracrine manner. The classical RAS has been found to be over-activated during the adipose tissue enlargement, thus elevated generation of angiotensin II (Ang II) may contribute to the obesity pathogenesis. The contemporary view on the RAS has become more complex with the discovery of alternative pathways, including angiotensin-converting enzyme 2 (ACE2)/angiotensin (Ang)-(1-7)/Mas receptor, (pro)renin receptor, as well as angiotensin IV(Ang IV)/AT4 receptor. Ang-(1-7) *via* Mas receptor counteracts with most of the deleterious effects of the Ang II-mediated by AT1 receptor implying its beneficial role in the glucose and lipid metabolism, oxidative stress, inflammation, and insulin resistance. Pro(renin) receptor may play a role (at least partial) in the pathogenesis of the obesity by increasing the local production of Ang II in adipose tissue as well as triggering signal transduction independently of Ang II. In this review, modulation of alternative RAS pathways in adipose tissue during obesity is discussed and the involvement of Ang-(1-7), (pro)renin and AT4 receptors in the regulation of adipose tissue homeostasis and insulin resistance is summarized.

Key words: angiotensin-(1-7), Mas receptor, (pro)renin receptor, angiotensin IV, adipose tissue, obesity, insulin resistance

Over the past decade, substantial progress has been achieved regarding the adipose tissue metabolic function knowledge. The adipose tissue is now considered to be an active endocrine organ playing significant role in the regulation of the body metabolism homeostasis.

Positive energy balance leads to the adipose tissue expansion that is associated with adipocytes hyperplasia and hypertrophia and significantly affects the overall cell biology and tissue homeostasis (Kloting and Bluher 2014). Adipose tissue enlargement is accompanied by severe dysfunctions of adipocyte with impaired glucose and fatty acid metabolism. The obesity-associated pro-inflammatory state and oxidative stress, as response to severe hypoxia, increase the macrophage infiltration into the adipose tissue inducing further inflammation and expression of inflammatory mediators including tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) and may cause dysregulation of the adipokines [adiponectin, leptin, resistin, angiotensin II (Ang II)]. The increased reactive oxygen species (ROS) secretion into peripheral blood from adipose tissue is involved in the induction of insulin resistance in skeletal muscle and adipose tissue and obesity-associated vascular diseases (Wang et al. 2007; Netzer et al. 2015).

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It has been shown that the rennin-angiotensin system (RAS) is complicated and not all the physiological and pathophysiological roles of the Ang II are completely understood. Many of the other angiotensin peptides have also physiological effects. In addition to the classical RAS components, several new peptides, with interesting biological activity, have recently been discovered. The important one is angiotensin (Ang)-(1-7) produced through the Ang II degradation by angiotensin-converting enzyme 2 (ACE2). It has a counter-regulatory role by opposing many actions of Ang II on AT_1 receptors (Donoghue et al. 2000; Tipnis et al. 2000). Discovering the G-protein coupled Mas, as a functional receptor (MasR) for Ang-(1-7), has clearly established the ACE2/Ang-(1-7)/MasR axis as an active pathway of the RAS and opened new possibilities of RAS components interactions (Santos et al. 2003). Moreover, the Mas-related G-protein–coupled receptor (MrgD), as a second receptor for Ang-(1-7), has been identified (Tetzner et al. 2016). To the contemporary view on the RAS has also contributed with novel receptors such as pro(renin) receptor (PRR) and angiotensin type 4 (AT_4) receptor identified as a transmembrane insulin regulated aminopeptidase (IRAP) playing a potential role in the regulation of glucose homeostasis (Kalupahana and Moustaid-Moussa 2012).

This review is aimed to bring recent knowledge about the role of the alternative pathways of RAS in the regulation of the adipose tissue metabolism during obesity development, focusing (i) on the ACE2/Ang-(1-7)/MasR pathway; (ii) on the (pro)renin receptor as binding site for renin or pro(renin); (iii), and on IRAP/AT_4 receptor as binding site for Ang IV (Figure 1).

Local RAS in adipose tissue

The RAS belongs to one of the oldest hormone systems. Originally, it was only considered as a cardiovascular system. The classical pathway of RAS with the major biologically active hormone Ang II has been well clarified (Figure 1). The main physiological effects of Ang II mediated by AT_1 receptor lead to body fluid volume increase and blood pressure (Lavoie and Sigmund 2003). It has been found that the RAS is present in not only the circulating system, but also the peripheral tissue. RAS has been detected in different organs with multiple and specific functions (Danser 1996).

The expression of all the RAS components, including new alternative pathways, has been demonstrated in human and rodent adipose tissue (Jones et al. 1997; Karlsson et al. 1998; Engeli et al. 1999; Pinterova et al. 2000; Gembardt et al. 2005; Achard et al. 2007; Santos et al. 2010). Adipocyte-produced angiotensinogen contributes to the circulating RAS, but the effect of the obesity on the angiotensinogen production is still inconsistent. The most of the studies have shown an increased adipocyte production of angiotensinogen during chronic energy excess in rodent obesity models and obese humans, whereas others have shown no change or reduction in its production (Kalupahana and Moustaid-Moussa 2012).

Classical *versus* alternative RAS pathways in pathogenesis of obesity

RAS and preadipocytes differentiation

The adipogenesis is a process of preadipocytes differentiation into the mature adipocytes (Cristancho and Lazar 2011). Adipogenesis has been extensively studied *in vitro* with the aim to determine the participation of adipocytes in the pathogenesis of insulin resistance to reveal the appropriate therapeutic and preventive strategies for metabolic disorders (Fonseca-Alaniz et al. 2007). During the preadipocyte differentiation, the transcription of key factors including CCAAT-enhancer-binding proteins (C/EBPs), peroxisome proliferator-activated receptor gamma (PPAR-γ), and adipocyte determination and differentiation-dependent factor 1/sterol regulatory element binding protein-1 (ADD1/SREBP-1), are activated (Armani et al. 2010) and the expression of adipocyte markers including fatty acid binding protein 4 (FABP4), insulin-regulated glucose transporter 4 (GLUT4), leptin and adiponectin increased (Rosen and MacDougald 2006; Lefterova and Lazar 2009). The studies on the models of the PPAR-γ expression suppression or stimulation have confirmed that the PPAR-γ is the master regulator in the adipogenesis (Farmer 2006).

Adipogenesis has been suggested to modulate the obesity and the onset of the obesity-related adverse metabolic consequences, such as metabolic syndrome. Experimental studies have revealed that RAS expressed in the adipose tissue is implicated in the regulation of the adipocyte formation and supported a role for Ang II as a negative regulator of adipogenesis (Schling and Loffler 2001; Janke et al. 2002; Sharma et al. 2002; Matsushita et al. 2006; Mogi et al. 2006; Brucher et al. 2007; Thatcher et al. 2009). Inhibitory effect of Ang II on the adipocyte formation has been supported by observations that AT_1 receptor blockers

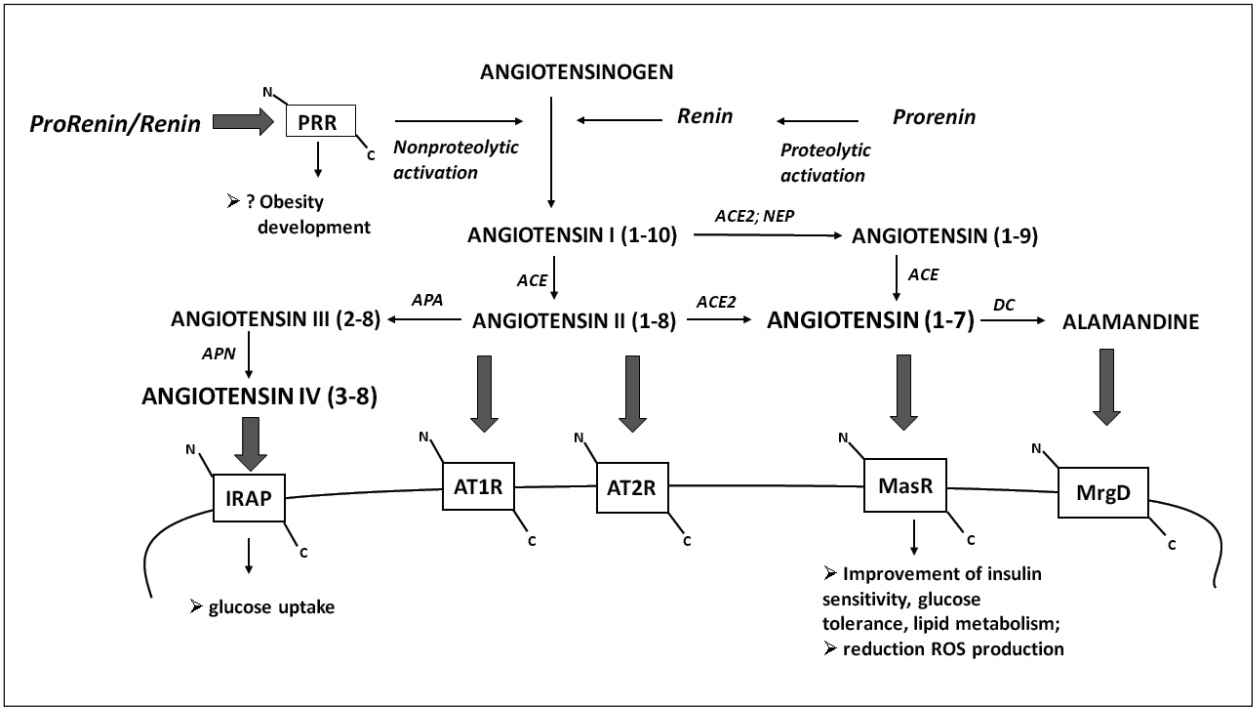

Figure 1. Schematic renin-angiotensin cascade showing the involvement of alternative components in the regulation of adipose tissue metabolism. PRR – (pro)renin receptor; ACE – angiotensin converting enzyme; ACE2 – angiotensin converting enzyme 2; NEP – neutral endopeptidase; APA – aminopeptidase A; APN – aminopeptidase N; DC – descarboxylase; IRAP – insulin regulated aminopeptidase; AT1R – angiotensin type 1 receptor; AT2R – angiotensin type 2 receptor; MasR – Mas receptor; MgprD – Mas related G-protein-coupled receptor, member D

may improve the preadipocyte differentiation (Fujimoto et al. 2004; Furuhashi et al. 2004; Zorad et al. 2006; Fuentes et al. 2010). Reduced adipocyte formation by Ang II may lead to predominance of large dysfunctional adipocytes characterized by insulin-resistant state and chronic inflammation. AT_1 receptor blockers restore small-differentiated adipocytes and it seems that the mechanism of their action is dependent on PPAR-γ. Small differentiated adipocytes produce less TNF-α and more beneficial adipokines such as adiponectin (Lenz and Fornoni 2008).

AT_1 receptor and MasR are co-expressed in adipocytes and we could hypothesize considering the Ang II inhibitory effects on adipogenesis that ACE2/Ang-(1-7)/MasR axis may counterbalance anti-adipogenic effect of Ang II, thus may promote preadipocyte differentiation. So far, the reports dealing with the involvement of Ang-(1-7) in adipocyte formation are almost missing. Only few studies have demonstrated that ACE2 and MasR expression steadily increase the overtime during the 3T3-L1 differentiation and have a positive regulation of Ang-(1-7)/MasR signaling to accelerate adipogenesis, particularly, in the early stage (Gupte et al. 2008; Than et al. 2013). Moreover, the authors have revealed the adipogenic effect of Ang-(1-7) through activation of MasR as well as the counteracting interplays between Ang-(1-7)/MasR and Ang II/AT_1R signaling on the adipogenesis. The mechanism by which Ang-(1-7) promoted differentiation of human and 3T3-L1 preadipocytes included the activation of phosphatidylinositol-3-kinase (PI3K) and the inhibition of PPAR-γ phosphorylation since Ser-112 phosphorylation of this nuclear receptor inactivates its adipogenic activity (Than et al. 2013). However, more studies are needed to support the potential beneficial effects of Ang-(1-7) on the preadipocyte differentiation.

RAS and metabolism of adipose tissue

ACE/Ang II/AT_1R axis. The obesity is associated with over activation of both systemic and adipose RASs in humans and animals (Kalupahana and Moustaid-Moussa 2012). An interaction of Ang II

with insulin signaling cascades in insulin-sensitive tissues including skeletal muscle and liver has been shown (Zhou et al. 2012), supporting a direct role for Ang II in the development of insulin resistance (Kwon and Pessin 2013; Makki et al. 2013). Although adipose tissue glucose uptake accounts for only a small part of the body, the mechanism of insulin action in this tissue is of a high importance, since the adipose RAS plays an important role in the pathogenesis of the obesity and insulin resistance (Olivares-Reyes et al. 2009). However, the molecular effect of Ang II on insulin signal transduction is controversial, since there is evidence that Ang II treatment may lead to enhanced insulin signaling through AT_1R on adipocytes (Ogihara et al. 2002; Juan et al. 2005). On the other hand, Ang II can inhibit the adipose tissue insulin-signaling cascade and impair the GLUT4 translocation and glucose transport into the cells, indirectly. Several studies have demonstrated that Ang II may mediate insulin resistance by oxidative stress increase *via* activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the ROS production as well as by stimulating the inflammatory pathways and dysregulating of the secretion of chemokines (Harrison et al. 2003; Skurk et al. 2004; Blendea et al. 2005; Lastra et al. 2009). The angiotensinogen gene silencing decreased the expression of pro-inflammatory markers, such as IL-6, TNF-α, and MCP-1 in adipocyte cell culture (Carroll et al. 2013). In the genetically obese mice or mice with diet-induced obesity (DIO), the AT_1 receptor blockade reduced the formation of the ROS in adipose tissue (Kurata et al. 2006). Ang II inhibits the secretion of adiponectin and the secretion of this anti-inflammatory hormone is increased after RAS blockers treatment (Furuhashi et al. 2004; Zorad et al. 2006).

Another mechanism underlying the effect of Ang II on insulin sensitivity is that Ang II markedly inhibits adipogenic differentiation and the blockade of the RAS prevents adipocyte hypertrophy by promoting differentiation of preadipocytes and formation of new insulin sensitive adipocytes (Sharma et al. 2002).

ACE2/Ang-(1-7)/MasR axis. The ACE2/Ang-(1-7)/MasR axis is active in many organs, including cardiovascular system, kidneys, and adipose tissue and has been found to be implicated in vasorelaxation, antiproliferative, anti-inflammatory, and anti-fibrotic effects (Montezano et al. 2015).

The metabolic effects of Ang-(1-7) have been studied in various animal models and *in vitro* experiments, as summarized in Table 1. Santos and colleagues (2008) have found out that genetic deletion of MasR in mice may have devasting effect on the lipid and glucose metabolism in adipose tissue, leading ultimately to a metabolic syndrome-like state (Santos et al. 2008). Furthermore, the data obtained on MasR knockout mice have shown that the lack of Ang-(1-7) action through MasR may impair the response of adipocytes to the antilipolytic effect of insulin. The authors have suggested the involvement of a decrease in the adipose PPAR-γ expression, its target enzymes fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC) (Mario et al. 2012).

In rat model, that mimics human metabolic syndrome with insulin resistance state and dyslipidemia, generated by high-fructose diet, administration of Ang-(1-7) improved the insulin signaling in the adipose tissue through the activation of the insulin receptor/insulin receptor substrate-1/PI3K/protein kinase B (IR/IRS-1/PI3K/Akt) pathway *via* MasR-dependent mechanism. This improvement was associated with a significant decrease in IRS-1 serine phosphorylation and activation of other proteins, including Akt substrate of 160 kDa (AS160) and glycogen synthase kinase 3β (GSK-3β) (Giani et al. 2009; Munoz et al. 2012). Furthermore, long-term Ang-(1-7) treatment in rats fed with high-fructose or high-fat diets may lead to reduction of the total fat mass, adipocyte size, adipose inflammation, and superoxide production by NADPH (Marcus et al. 2013; Santos et al. 2013). In a novel transgenic rat model of inducible insulin resistance, the administration of a newly developed Ang-(1-7) nano-formulation exerts a positive effect on the insulin signaling by decreasing the serine phosphorylation of IRS-1 and increasing Akt phosphorylation in adipose tissue (Santos et al. 2014). Recent study have shown that transgenic rats with increased plasma Ang-(1-7), caused by over expression of an Ang-(1-7)-releasing fusion protein, had a significantly reduced adipose tissue mass associated with augmented action of Akt and increased expression of FABP4 and adiponectin in this tissue and decreased plasma triacylglycerides (Santos et al. 2010). Moreover, chronic high circulating Ang-(1-7) level in this transgenic rats fed with high-fat diet protect against the adipose metabolic stress decreasing pro-inflammatory state (Santos et al. 2012).

In vitro studies have revealed that Ang-(1-7) may protect against an oxidative stress *via* MasR by decreasing ROS production and increasing adiponectin production in fully differentiated 3T3-L1 adipocytes. Additionally, Ang-(1-7) has been found to improve the glucose uptake into primary cultured mice adipocytes under both, the basal and insulin-stimulated states (Liu et al. 2012).

Table 1
The interventions in the ACE2/Ang-(1-7)/MasR axis in regard to insulin, glucose and lipid metabolism and obesity development

Research Design		Effects of treatmentor model		Ref.
Model	**Treatment**	**Adipose**	**Systemic**	
Rats High-fructose diet	Ang-(1-7) by Alzet pumps 2 weeks	↑ activation of insulin receptor signalling pathway	↓ TAG, ↓ insulin, ↓ insulin resistant state	(Giani et al. 2009)
Rats High-fructose diet	Ang-(1-7) by Alzet pumps 24 weeks	↓ fat mass, ↓ fat cell size, ↓ NADPH activity, ↓ macrophage infiltration	↓ TAG, ↓ glucose, ↑ insulin sensitivity	(Marcus et al. 2013)
Rats High-fructose diet	Ang-(1-7) by Alzet pumps 2 weeks	↑ insulin-stimulated phosphorylation of Akt, AS160; ↑ insulin-stimulated phosphorylation of GSK-3β	↓ insulin, ↓ TAG	(Munoz et al. 2012)
Rats High-fat diet	Oral administration of Ang-(1-7) 8 weeks	↓ abdominal fat mass	↓ insulin, ↓ glucose, ↓ TAG, ↓ total cholesterol ↑ insulin sensitivity	(Santos et al. 2013)
Transgenic rats (model of insulin resistance)	Oral nano-formulation of Ang-(1-7)	↑ activation of insulin receptor signalling pathway	↓ glucose, ↑ insulin sensitivity	(Santos et al. 2014)
Transgenic rats Standard diet	Expressing an Ang-(1-7)-releasing fusion protein	↓ adiposity, **X** fat cell size, ↑ FABP4 mRNA, adiponectin mRNA, ↑ activation of insulin receptor signalling pathway	↑ adiponectin, ↓ TAG, ↑ insulin sensitivity	(Santos et al. 2010)
Transgenic rats High-fat diet	Expressing an Ang-(1-7)-releasing fusion protein	↓ adiposity, ↓ COX2 mRNA, ↓ IL-1β mRNA	↑ HDL cholesterol **X** insulin sensitivity	(Santos et al. 2012)
3T3-L1 adipocytes	Exogenous Ang-(1-7) treatment 24 h	↓ ROS production, ↑ glucose uptake, adiponectin mRNA		(Liu et al. 2012)
Human subcutaneous pre-adipocytes	Gene silencing of MasR Exogenous Ang-(1-7) treatment	↑ adypocytic differentiation ↑ FABP4, PPARγ and FAS mRNA		(Than et al. 2013)
ACE2 knockout mice High-calorie diet	Ang-(1-7) by Alzet pumps 2 weeks		↑ insulin sensitivity	(Takeda et al. 2013)
Mice Standard diet	Diminazene aceturate (DIZE) (ACE2 activator) 4 weeks	↓ adiposity ↑ ACE2 mRNA, ¯ ACE mRNA ↓ FAS and ACC mRNA	↓ TAG and cholesterol **X** insulin sensitivity	(de Macedo et al. 2015)
MasR knockout mice Standard diet		↓ PPARγ and ACC mRNA, ↓ FAS protein	↑ NEFA	(Mario et al. 2012)
MasR knockout mice Standard diet		↑ adipose fat mass, ↓ glucose uptake, ↓ GLUT 4 protein,	↑ glucose, leptin and insulin, ↑ total cholesterol and TAG, ↓ insulin sensitivity	(Santos et al. 2008)
Mice High-fat diet		↑ ACE2 and ADAM17 mRNA, **X** ACE2 protein and activity	↑ ACE2 activity	(Gupte et al. 2008)

Abbreviation: Ang-(1-7) – angiotensin (1-7); MasR – Mas receptor; ACE – angiotensin converting enzyme; TAG – triacyglycerol; NADPH – nicotinamide adenine dinucleotide phosphate; Akt – protein kinase B; AS160 – Akt substrate of 160 kDa; GSK-3β – glycogen synthase kinase 3β; FABP4 – fatty acid-binding protein 4; COX2 – cyclooxygenase 2; IL-1β – interleukin 1β; HDL – high density lipoprotein; ROS – reactive oxygen species; PPARγ – peroxisome proliferator-activated receptor γ; FAS – fatty acid synthase; ACC – acetyl-CoA carboxylase; NEFA – non-esterified fatty acids; GLUT4 – glucose transporter type 4; ADAM17 – disintegrin and metalloproteinase domain-containing protein 17.
Explanatory notes: ↑ - increase, ↓ - decrease, X - without alteration.

The role of the endogenous ACE2 in maintaining the insulin sensitivity and metabolic profile has been studied using ACE2 knockout mice (Takeda et al. 2013) or by the administration of ACE2 activator, diminazene aceturate (DIZE) (de Macedo et al. 2015). The results have revealed that ACE2 protects against high-calorie diet-induced insulin resistance in mice (Takeda et al. 2013). Activation of ACE2 by oral DIZE treatment in mice improved the plasma lipid profile, decreased the gene transcription of adipogenesis-related proteins (FAS, ACC), lowered the body weight, and reduced adipose tissue mass (de Macedo et al. 2015). Gupte et al. (2008) have demonstrated that ACE2 expression in adipocytes is dysregulated in high-fat diet fed mice compared with the low-fat diet fed controls.

Taken together, these results may imply a beneficial role of ACE2/Ang-(1-7)/MasR axis in the glucose and lipid metabolisms, oxidative stress, and insulin resistance as opposed to the ACE/AngII/AT_1R axis, which is activated in obesity-associated metabolic diseases. Moreover, these results may indicate that in addition to ACE inhibitors and AT_1R blockers, MasR agonists and ACE2 activators may represent new drugs for the treatment of metabolic syndrome.

Ang IV/AT_4 receptor axis. Angiotensin IV (Ang IV) has attracted a lot of attention, due to its unique role in the memory and learning enhancing and regulation of insulin signaling in the brain (Gard 2008; Wong et al. 2011). IRAP (EC 3.4.11.3.) has been identified as a placental leucin aminopeptidase/oxytocinase (Rogi et al. 1996) and also as the specific binding site for Ang IV in the brain AT_4 receptor (Albiston et al. 2001). In insulin-responsive cells IRAP accompanies GLUT4 in specialized storage vesicles (GSV) cycling within intracellular compartments at the basal state (Keller et al. 1995; Ross et al. 1996). However, IRAP has been found in a range of tissues independently of GLUT4 expression (Chai et al. 2004).

IRAP is an essential regulator of the glucose uptake into the insulin-responsive cells. Adipose and muscle cells have a specialized mechanism to retain GSV intracellularly in the absence of insulin. IRAP possesses of three domains: extracellular catalytic, transmembrane, and N-terminal cytoplasmic that interacts with three proteins – tankyrase (Chi and Lodish 2000), acyl-CoA dehydrogenase (ACD) (Katagiri et al. 2002), and formins (Tojo et al. 2003). The interaction of these proteins with IRAP appears to be involved in vesicular retention at the basal state and their trafficking to the cell surface upon insulin stimulation (Keller et al. 1995; Chai et al. 2004; Keller 2004). Thus, in response to insulin, GSV are translocated to plasma membrane where GLUT4 facilitates the glucose uptake into the cells and catalytic domain of IRAP gets from vesicle lumen to extracellular cell surface (Kandror et al. 1994; Bryant et al. 2002). IRAP belongs to the M1 family of Zn-dependent metallopeptidases and it is considered as a major protein in the GSV (Jordens et al. 2010). Catalytic domain of IRAP is responsible for the extracellular breakdown of peptide substrates such oxytocin, vasopressin, lys-bradykinin, and somatostatin (Yamahara et al. 2000; Chai et al. 2004; Fernando et al. 2005; Wallis et al. 2007).

IRAP, as a regulator of the glucose uptake, is suggested to play a role in the obesity pathogenesis. Insulin resistance is associated with an abnormal subcellular distribution and impaired translocation of GSV to plasma membrane. GLUT4 and IRAP are accumulated in high-density membrane fraction in basal state and insulin is not able to stimulate their trafficking (Sinha et al. 1991; Garvey et al. 1998; Maianu et al. 2001; Keller 2004). *In vitro* studies, using 3T3-L1 cells, have determined that cytosolic tail of IRAP is likely to be associated with AS160 at the basal state and dissociates in the response of insulin enabling the GSV to translocate to the cell surface (Larance et al. 2005; Peck et al. 2006).

The role of IRAP in the regulation of metabolism has been confirmed by the study of Niwa and co-workers (2015) who have found that the IRAP-knockout mice may be protected from the development of high-fat diet-induced obesity. Glucose and insulin tolerance tests have revealed that the glucose disposal and the hypoglycemic effect of insulin are pronounced in IRAP-knockout mice after a high-fat diet (Niwa et al. 2015).

We may only hypothesize from the previous data that besides the effect of IRAP on the modulation of the glucose uptake by increasing the GLUT4 trafficking, another possible mechanism might be involved. It has been shown that endogenous IRAP substrate, oxytocin, regulates food intake, adipose tissue homeostasis and adipogenesis (Eckertova et al. 2011; Altirriba et al. 2015; Blevins and Baskin 2015) and decreased plasma oxytocin level has been found in obese mice, rats, and humans (Morton et al. 2012; Gajdosechova et al. 2014; Qian et al. 2014; Plante et al. 2015). Moreover, oxytocin or oxytocin receptor deficient mice develop obesity and impaired glucose tolerance (Takayanagi et al. 2008; Camerino 2009). We have previously shown that obesity-associated reduction in plasma oxytocin levels is due to increased peripheral peptide degradation by adipose tissue and liver, rather than changes in hormone synthesis

(Gajdosechova et al. 2014). Thus, inhibition of oxytocinase activity of IRAP may prolong the beneficial peptide action on the adipose tissue metabolism and insulin sensitivity. The inhibitors of IRAP activity or compounds that could attract IRAP and GSV trafficking to the cell membrane in order to facilitate glucose transport may represent a therapeutic potential for the treatment of resistant obesity (Amri 2016).

The concept of Ang IV as an inhibitor of IRAP and its positive central effects on the learning and memory have stimulated the development of new selective inhibitors of IRAP (Albiston et al. 2008; Albiston et al. 2011; Mountford et al. 2014). However, the impact of IRAP/AT_4R axis on the energy metabolism has been hardly even elucidated and there is need for further studies that will open a view into the IRAP complex physiological effects.

(Pro)renin/renin receptor (PPR) axis. The discovery of the PRR highlights the role of the cell surface in Ang II generation and opens new perspectives on the tissue RAS and prorenin or renin effects independent of Ang II (Nguyen et al. 2002). PRR is a single transmembrane domain receptor containing 350 amino acids that interacts with V-ATPase (Nguyen and Contrepas 2008). PRR specifically binds both the (pro)renin and renin. The binding of renin to PRR induces a 4-fold increase in the catalytic efficiency of angiotensinogen conversion to Ang I, while the binding of (pro)renin leads to non-enzymatic activation of this renin precursor (Nguyen et al. 2002). Moreover, binding of (pro)renin or renin to PRR triggers Ang II-independent intracellular signaling pathways associated with the activation of the mitogen-activated protein kinases (MAPK) and extracellular signal-regulated kinases 1 and 2 (ERK1/2) cascade, and the promyelocytic zinc finger transcription factor (PLZF). Activated PLZF suppresses PRR gene expression and stimulates p85α subunit of PI3K (Schefe et al. 2006; Nguyen and Contrepas 2008). In mesangial cells, the activation of ERK1/2 pathway increased transcription of transforming growth factor β1 (TGF-β1) and expression of molecules involved in fibrosis and inflammation (Huang et al. 2007).

PRR is expressed in many tissues including brain, adipose tissue, endothelial cells, vascular smooth muscle cells, skeletal muscle, and kidney (Danser and Deinum 2005). The expression of PRR in adipose tissue has been found in both subcutaneous and visceral fat depots in humans as well as rodents (Nagai et al. 2009; Achard et al. 2011; Tan et al. 2014) and the development of obesity has been found to be accompanied by PRR upregulation in adipose tissue (Achard et al. 2007; Achard et al. 2011; Tan et al. 2014).

Accordingly, in mice fed with high-fat/high-carbohydrate diet or in fructose-fed rats, inhibition of PRR using the handle region peptide (HRP), a PRR blocker, led to a reduction of visceral fat mass and adipocyte size with simultaneous improvement of the glucose tolerance, suggesting at least a partial role for PRR in the insulin resistance development (Nagai et al. 2009; Tan et al. 2014; Tan et al. 2016). Tan and co-workers (2014) have elucidated a potential mechanisms implicated in these observations as the stimulation of the adipogenesis, angiogenesis, and adiponectin production in subcutaneous adipose tissue accompanying by reduced expression of inflammatory markers in visceral fat depots after HRP treatment leading to the activation of healthy fat storage in subcutaneous adipose tissue and normalization of the plasma free acid and triglyceride levels (Tan et al. 2014; Tan et al. 2016).

In 3T3-L1 cells, knockdown of PRR by gene silencing significantly decreased mRNA abundance of PPAR-γ and FABP4, indicating an important role for PRR in adipogenesis and fatty acid storage in adipocytes (Wu et al. 2016). The specific deletion of PRR in adipocytes of male mice fed by a standard diet induced a marked reduction in all white adipose tissues without abnormal distribution of fat pads. Despite the lipodystrophy accompanied by hepatic steatosis, adipocyte-PRR-deficient mice had normal glucose tolerance and decreased fasting glucose level. Interestingly, high-fat–fed mice with adipocyte-PRR deficiency were resistant to diet-induced obesity and had improved glucose tolerance and fasting blood glucose when compared with wild type control (Wu et al. 2016). These data have pointed out the importance of the adipose tissue PRR role in the normal development of adipocytes, lipid, glucose, and insulin homeostasis.

These new and preliminary findings may indicate that renin and (pro)renin via PRR, by Ang II-dependent or -independent manner, may play an important role in the modulation of the adipose tissue homeostasis and functions, and that PRR may substantially contribute to the pathophysiology of obesity and insulin resistance.

Perspectives and conclusion

The research progress made over the past few decade has defined the role of the alternative RAS pathways in the regulation of the adipose tissue metabolism. In addition to cardiovascular system, the establishing the active and functional ACE2/Ang-(1-7)/MasR axis, as a counter regulatory pathway of

deleterious effects of Ang II in adipose tissue, opens a new perspective for the metabolic disorder pharmacotherapy. Demonstration of the importance of adipocyte (pro)renin receptor in the normal development of adipose tissue and its potential implication in obesity and insulin homeostasis may consider the (pro)renin receptor for a possible novel therapeutic target. Nevertheless, further investigation is necessary to identify the mechanisms by which the (pro) renin receptor regulates the adipose tissue and overall body metabolism.

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