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Review Article

Modulation of the action of insulin by angiotensin-(1–7)

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Abstract

The prevalence of Type 2 diabetes mellitus is predicted to increase dramatically over the coming years and the clinical implications and healthcare costs from this disease are overwhelming. In many cases, this pathological condition is linked to a cluster of metabolic disorders, such as obesity, systemic hypertension and dyslipidaemia, defined as the metabolic syndrome. Insulin resistance has been proposed as the key mediator of all of these features and contributes to the associated high cardiovascular morbidity and mortality. Although the molecular mechanisms behind insulin resistance are not completely understood, a negative cross-talk between AngII (angiotensin II) and the insulin signalling pathway has been the focus of great interest in the last decade. Indeed, substantial evidence has shown that anti-hypertensive drugs that block the RAS (renin–angiotensin system) may also act to prevent diabetes. Despite its long history, new components within the RAS continue to be discovered. Among them, Ang-(1–7) [angiotensin-(1–7)] has gained special attention as a counter-regulatory hormone opposing many of the AngII-related deleterious effects. Specifically, we and others have demonstrated that Ang-(1–7) improves the action of insulin and opposes the negative effect that AngII exerts at this level. In the present review, we provide evidence showing that insulin and Ang-(1–7) share a common intracellular signalling pathway. We also address the molecular mechanisms behind the beneficial effects of Ang-(1–7) on AngII-mediated insulin resistance. Finally, we discuss potential therapeutic approaches leading to modulation of the ACE2 (angiotensin-converting enzyme 2)/Ang-(1–7)/Mas receptor axis as a very attractive strategy in the therapy of the metabolic syndrome and diabetes-associated diseases.

Key words: angiotensin-(1–7), angiotensin-converting enzyme 2 (ACE2), insulin, Mas receptor, renin–angiotensin system (RAS)

INTRODUCTION

Diabetes mellitus is emerging as a worldwide epidemic, and the clinical implications and healthcare costs from the disease are overwhelming [1]. In many cases, this pathological condition is linked to obesity, systemic hypertension and hyperlipidaemia forming the so-called metabolic syndrome [2–4]. Insulin resistance is believed to be the pathogenic mediator of all these features and contributes to the associated high cardiovascular morbidity and mortality [3,5,6]. Resistance of the action of insulin is a state in which insulin-sensitive tissues (i.e. adipose tissue, liver and skeletal muscle) exhibit a failure to respond to normal circulating levels of insulin. To compensate for this inadequate response, pancreatic β-cells augment insulin production leading to hyperinsulinaemia [7]. Insulin resistance is associated with impaired insulin-mediated inhibition of gluconeogenesis, reduced skeletal muscle uptake of glucose, hyperglycaemia, inhibition of lypolisis and increased plasma levels of non-esterified ('free') fatty acids. Long-term resistance to the action of insulin and its consequent hypersecretion of insulin, eventually lead to a pancreatic β-cell failure, causes a pre-diabetic state and glucose intolerance, which can later progress to Type 2 diabetes [3,5–7].

The molecular mechanisms behind insulin resistance are not completely understood. However, it is clear that the cross-talk between insulin and other hormones, such as AngII (angiotensin II) [8–13], cortisol [14], adrenaline (epinephrine) [15], growth

Abbreviations: ACE, angiotensin-converting enzyme; Ang-(1–7), angiotensin-(1–7); AngI, angiotensin I; AngII, angiotensin II; ARB, angiotensin receptor blocker; AS160, Akt substrate of 160 kDa; AT_1R, AngII type 1 receptor; AT_2R, AngII type 2 receptor; EGF, epidermal growth factor; eNOS, endothelial NOS; ERK, extracellular-signal-regulated kinase; FOXO1, forkhead box O1; GLUT4, glucose transporter 4; GSK-3, glycogen synthase kinase-3; IKK, inhibitor of nuclear factor κB kinase; IR, insulin receptor; IRS, IR substrate; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; KO, knockout; LV, left ventricular; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; NOS, nitric oxide synthase; PDK1, phosphoinositide-dependent kinase; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PKC; protein kinase C; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; $PPAR\gamma$, peroxisome-proliferator-activated receptor γ; RAS, renin–angiotensin system; ROS, reactive oxygen species; SH2, Src homology 2; Shc, Src homology and collagen homology; STAT, signal transducer and activator of transcription; STZ, streptozotocin; VSMC, vascular smooth muscle cell.

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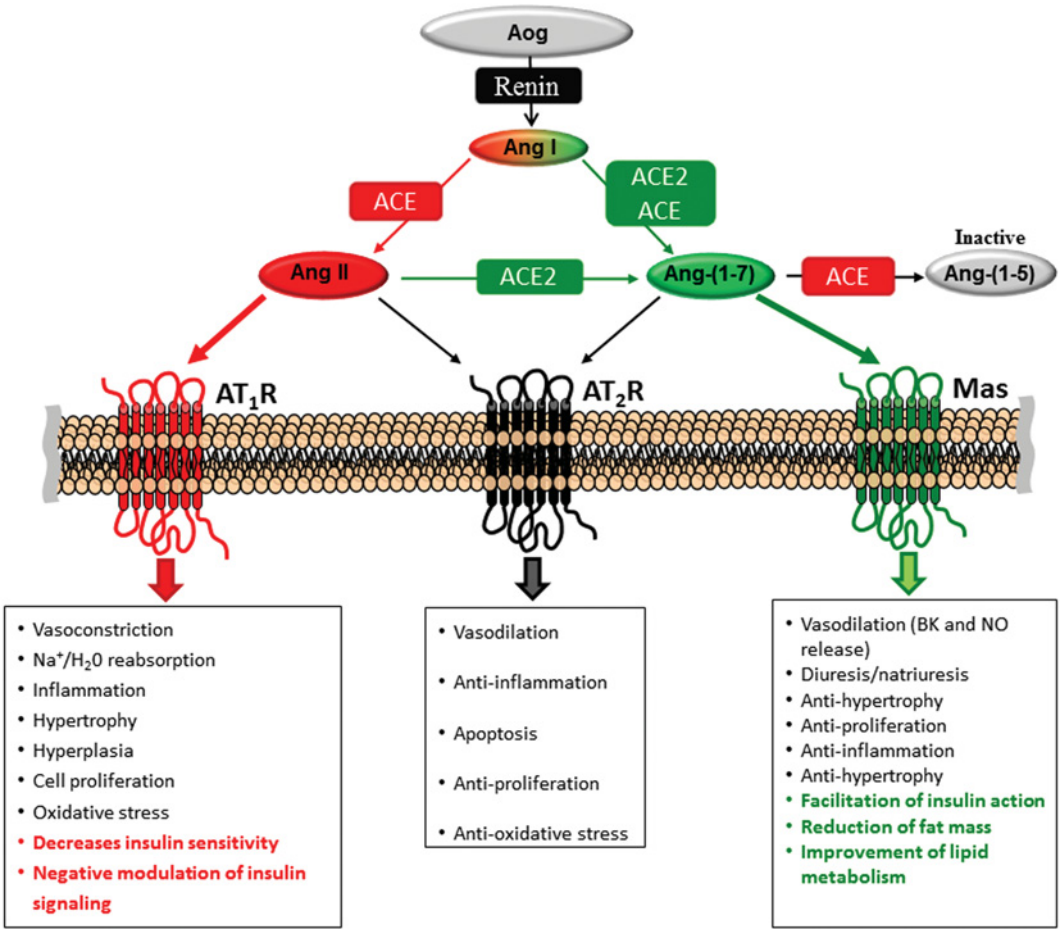


Figure 1 **Schematic diagram of the RAS**
Red: the pathway leading to AngII generation and its deleterious effect on insulin signaling via the AT_1R. Green: the pathway leading to Ang-(1–7) synthesis and its positive modulation of insulin actions via the Mas receptor. Aog, angiotensinogen; BK, bradykinin.

hormone [16], leptin [17] and adiponectin [18], can modulate the final outcome of the action of insulin. In particular, the participation of the RAS (renin–angiotensin system) in the modulation of the action of insulin has been the focus of great interest. Indeed, several studies demonstrating a close connection between insulin resistance and cardiovascular disease support the notion that alterations within the RAS could be related to dysregulation of the action of insulin [19–21]. Specifically, it has been demonstrated that AngII plays a critical role in the aetiology of insulin resistance [22,23]. The mechanism behind this deleterious effect appears to be related to a negative modulation exerted by AngII on several steps of the insulin-signalling cascade, including insulin-induced phosphorylation of the IR (insulin receptor), IRS-1 (IR substrate-1) and activation of Akt [also known as PKB (protein kinase B)] by PI3K (phosphoinositide 3-kinase) [8–13,24,25]. Accordingly, recent long-term large-scale clinical trials have shown that inhibition of ACE (angiotensin-converting enzyme) or selective blockade of the AT_1R (AngII type 1 receptor) improves glycaemic control in patients with diabetes and prevents new-onset of diabetes mellitus in patients without diabetes [26–28]. In line with these reports, several studies have shown that animal models of insulin resistance and/or Type 2 diabetes display enhanced insulin sensitivity [29–33] and an enhancement in the response to insulin at various steps in the insulin-signalling cascade [34,35] as a consequence of lower AngII formation or inhibition of its actions. These data clearly indicate that the signalling cross-talk between insulin and AngII has significant physiological relevance.

Despite its long history, new components and interactions between novel and established components of the RAS continue to be discovered. The RAS is classically conceived as a major regulator of body fluid and cardiovascular homoeostasis. It consists primarily of an enzymatic cascade through which angiotensin is sequentially converted into AngI (angiotensin I) and later the generation of AngII through the action of renin and the ACE respectively (Figure 1). AngII mediates its specific functions via type 1 and type 2 receptors, i.e. AT_1R and AT_2R (AngII type 2 receptor) (Figure 1). Most of the effects of AngII are mediated by the AT_1R, including vasoconstriction, and pro-inflammatory, pro-oxidative, proliferative and hypertrophic effects (Figure 1); the actions mediated by the AT_2R, in general, oppose those mediated by the AT_1R (Figure 1). By the action of ACE2, a homologue of ACE, Ang-(1–7) [angiotensin-(1–7)] is generated directly from

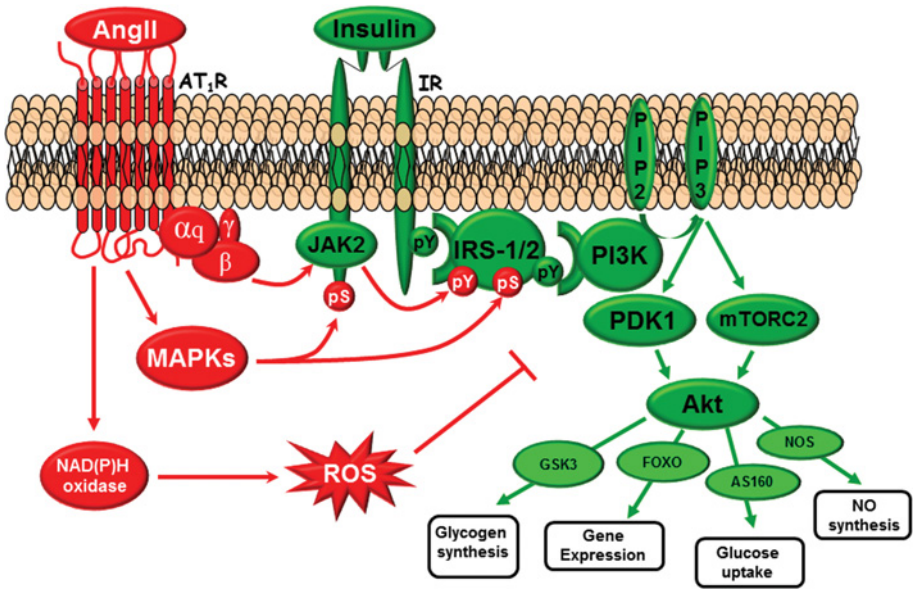


Figure 2 **Negative cross-talk between insulin and the AngII signalling pathway**
Proteins activated by insulin are shown in green and proteins activated by AngII are shown in red.

AngII and also indirectly from AngI (Figure 1). Ang-(1–7) binds to and activates the GPCR (G-protein-coupled receptor) Mas, through which Ang-(1–7) induces responses in opposition to those of AngII, including vasodilation, anti-hypertrophic effects and anti-proliferative properties [36–39] (Figure 1). Importantly, Ang-(1–7) seems to contribute to the anti-hypertensive effects of the blockade of the RAS [40–42]. Thus it is clear that the balance between the ACE/AngII/AT_1R axis and the ACE2/Ang-(1–7)/Mas receptor axis has great importance in the control of several body functions apart from the regulation of cardio-renal actions [39] (Figure 1). Given the major physiological relevance of this area, research on the ACE2/Ang-(1–7)/Mas receptor axis has expanded greatly over the last few years providing evidence for a metabolic role of the axis and supporting the concept that Ang-(1–7) participates in the maintenance of normoglycaemia. The circulating concentrations of Ang-(1–7) are within the picomolar range [43] and, interestingly, patients with Type 2 diabetes exhibit decreased circulating concentrations of Ang-(1–7) [44]. This is in good correlation with the observation that pregnancy tends to elevate the plasma level of Ang-(1–7), but women with gestational diabetes have lower levels of Ang-(1–7) compared with healthy pregnant women [45]. In general it appears that local levels of Ang-(1–7) correlate with ACE2 levels [46,47]. Administration of a high-fat diet to mice is associated with insulin resistance and results in reduced kidney ACE2 activity, increased levels of plasma AngII and decreased levels of plasma Ang-(1–7) [48].

In the present review, we will discuss recent evidence suggesting that the improvement in insulin resistance associated with different therapies might be mediated, at least in part, by Ang-(1–7) as a positive modulator of the action of insulin (Figure 1). We will focus on the negative cross-talk between the insulin-signalling pathway and AngII and the beneficial effects exerted by Ang-(1–7) at this level. Finally, we will discuss potential therapeutic applications of the ACE2/Ang-(1–7)/Mas receptor axis for the treatment of various alterations associated with Type 2 diabetes.

THE INSULIN SIGNALLING SYSTEM: BASIC CONCEPTS AND REGULATION

The IR is a tetrameric protein that consists of two α-subunits and two β-subunits. Insulin binding to the α-subunit leads to activation of the kinase activity residing in the β-subunit, followed by autophosphorylation of tyrosine residues in several regions of the cytoplasmic portion of the β-subunit and a further increase in its kinase activity [49]. The IR kinase phosphorylates the tyrosine residues of several cytosolic proteins [49,50]. Many insulin responses require the phosphorylation of IRS-1 and IRS-2 (Figure 2). Additional IR substrates include isoforms of the transforming protein Shc2 (Src homology and collagen homology 2), APS (adaptor protein with pleckstrin homology and src), c-Cbl (the proto-oncogene Casitas b-lineage lymphoma) and STAT5b (signal transducer and activator of transcription 5b) [50,51]. When tyrosine-phosphorylated, IRS proteins bind several SH2 (Src homology 2)-domain-containing proteins, which further propagate downstream signals. Some of these SH2-domain-containing proteins are adaptor proteins, such as the p85 regulatory subunit of PI3K and Grb2 (growth factor receptor-bound protein 2) [50]. Others have intrinsic enzymatic activity, such as the phosphotyrosine phosphatase SHP-2 (Src homology 2 domain-containing protein tyrosine phosphatase 2) and the cytoplasmic tyrosine kinases Fyn and JAK2 (Janus kinase 2) [50–52]. Substrate binding to these SH2 proteins can regulate their activities and/or subcellular location. Collectively, those molecules orchestrate the numerous insulin-mediated physiological responses.

A total of eight mammalian PI3Ks have been identified and grouped into three classes (I, II and III) according to their

sequence homology and *in vitro* substrate specificity [53]; the PI3Ks that are involved in the action of insulin belong to class I. PI3K is necessary for many, if not all, of the actions of insulin, including stimulation of glucose transport, activation of glycogen synthase and inhibition of hepatic gluconeogenesis [50]. Engagement of PI3K by the IRS proteins activates this lipid kinase at the plasma membrane, where its substrate, PIP_2 (phosphatidylinositol 4,5-bisphosphate), is abundant, stimulating the production of the key lipid second messenger PIP_3 (phosphatidylinositol 3,4,5-trisphosphate). PIP_3 then binds the PH (pleckstrin homology) domain of the serine/threonine kinase Akt, allowing two other kinases, PDK1 (phosphoinositide-dependent kinase 1) and mTORC2 [mTOR (mammalian target of rapamycin) complex 2], to phosphorylate and activate Akt/PKB [50] (Figure 2). Akt/PKB is a major effector of the insulin response, and its downstream substrates directly mediate many of the metabolic effects of insulin [54,55]. Well-established Akt substrates include GSK-3 (glycogen synthase kinase-3), which participates in the regulation of glycogen synthesis, the Rab GTPase-activating protein AS160 (Akt substrate of 160 kDa), involved in regulation of glucose transport, the small GTPase Rheb, involved in activation of TSC1–2 (tuberous sclerosis complex 1–2), regulation of mTOR and protein synthesis, FOXO1 (forkhead box O1) transcription factors, involved in regulation of expression of gluconeogenic and other genes, NOS (nitric oxide synthase), with a crucial role in the vasodilating effects of insulin, and BAD (Bcl-2/Bcl-xL-antagonist) causing cell death [54,55].

With regards to JAK2, insulin induces the tyrosine phosphorylation of this cytosolic tyrosine kinase and promotes its association with the IR, IRS proteins, members of the STAT family and Shc [52,56–62]. In response to insulin administration *in vivo*, JAK2 associates with the IR and becomes tyrosine-phosphorylated in insulin-sensitive tissues [61,62]. *In vitro*, tyrosine phosphorylation elicited by the IR or by JAK2 occurs at different sites in IRS-1 [52] (Figure 2). The physiological meaning of this phenomenon is not completely understood. Moreover, it has been reported that JAK2 participates in the insulin-stimulated mitogenic pathway, but not in its metabolic pathway [63]. However, in a state of insulin resistance, heightened JAK2 activation may attenuate insulin-induced Akt activation [63]. In this manner, JAK2 acts as an inhibitor of insulin signal transduction at the level of Akt, negatively effecting glucose metabolism.

Activation of the Ras/Raf/MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase]/ERK pathway is another major mechanism of insulin action and results in the activation of several MAPKs [64,65]. Various lines of observation indicate that both the IRS and the SHC family of proteins participate in this pathway. Moreover, in contrast with the final cellular events triggered by the PI3K/Akt signalling module, activation of MAPKs by insulin has a direct role in the growth-promoting effects of this hormone, and appears to be relatively dispensable in insulin-mediated metabolic regulation [50].

As stated above, tyrosine phosphorylation serves to propagate the signal emanating from the IR. However, in the last two decades, several mechanisms that control insulin signalling have been described. Among them, serine and threonine phosphorylation of the IR and its substrates (IRS proteins) has emerged as a key negative regulator of insulin signalling propagation [51,66] (Figure 2). There is evidence that serine phosphorylation of the IR impairs its autophosphorylation of tyrosine residues after insulin binding, and probably contributes to the impaired insulin signalling related to the metabolic syndrome [51,66]. Many protein kinases have been suggested as potential mediators of the serine/threonine phosphorylation of the IR. Cell-based and isolated protein studies have demonstrated that PKC (protein kinase C) mediates the phosphorylation of the IR at Ser^{967} and Ser^{968} of the juxtamembrane region [67], Ser^{994}, Ser^{1035} and Ser^{1037} in the catalytic domain [68,69], and Ser^{1288}, Ser^{1305}, Ser^{1306}, Ser^{1321}, Ser^{1327} and Thr^{1348} in the C-terminus [67,70,71]. It is not clear whether these phosphorylation sites are actually involved in the regulation of activation of the IR. However, increased PKC activity is thought to play a significant role in several models of human insulin resistance [72]. For instance, PKCβ activity is increased in the muscle of obese insulin-resistant subjects [73], whereas inhibition of PKC activity can reverse the impaired insulin-mediated glucose uptake in muscle strips obtained from obese subjects [74]. Indeed, lipid infusion in rats increases serine phosphorylation of the IR and reduces insulin-stimulated tyrosine phosphosphorylation of the IR in a PKC-dependent manner [72,75]. JNK (c-Jun N-terminal kinase) and MAPKs have also been suggested as putative kinases involved in the serine phosphorylation of the IR. These enzymes are also activated by lipid accumulation and are associated with impaired insulin signalling [72]. In support of this, the decrease in autophosphorylation of the IR induced by lipid incubation in 3T3-L1 adipocytes is prevented by blocking JNK expression [76]. In addition, we have demonstrated that obese Zucker rats displayed an increased level of phosphorylation of the IR at Ser^{994} that was reduced after chronic treatment with the AT_1R blocker irbesartan [77]. These data propose that AngII-induced serine phosphorylation of the IR could be a mechanism behind the negative cross-talk between the insulin and AngII signalling pathways (Figure 2).

Unlike the scant information regarding serine/threonine phosphorylation of the IR, the influence of this kind of phosphorylation in the co-ordination of IRS function has been widely studied. A number of different mechanisms appear to be involved in the tyrosine dephosphorylation of the IRS proteins, their dissociation from the IR, and their intracellular localization and eventual degradation. Specifically, most of the information regarding the serine phosphorylation events taking place on the IRS proteins is available for IRS-1, whereas much less is known about the regulation of IRS-2 [51]. Potential inhibitory serine/threonine residues have been suggested near tyrosine residues in PI3K-binding motifs or proximal to the IRS-1 PTB (phosphotyrosine-binding domain) [78]. One of the major sites of regulatory phosphorylation within IRS-1 is Ser^{307} (human Ser^{312}) that was identified in CHO (Chinese-hamster ovary) cells treated with a JNK agonist [79]. Together with JNK, the serine/threonine kinase IKKβ (inhibitor of nuclear factor κB kinase β) has also been suggested as a potential kinase for Ser^{307} [80]. As proof of this, many factors implicated in the development of insulin resistance, such as TNFα (tumour necrosis factor α), non-esterified fatty acids and serine phosphatase inhibitors, are able to activate the

IKK complex and JNK [80,81]. Besides JNK and IKKβ, to date several serine/threonine kinases have been reported to be involved in the phosphorylation of this particular site; many of them are activated by insulin itself. In cultured cells, insulin stimulates phosphorylation of IRS-1 at Ser^{307} via PI3K [82,83]. In addition, mTOR can mediate this insulin-stimulated Ser^{307} phosphorylation [84]. In line with these observations, we have shown that insulin-resistant rats exhibit enhanced phosphorylation of IRS-1 at Ser^{307} in adipose tissue and skeletal muscle in association with enhanced activation of mTOR and JNK [85]. In addition to Ser^{307}, Ser^{612} has emerged as a potential site of phosphorylation and regulation of IRS-1 function, with ERK1/2 being the main kinases involved in the process [86]. Consistent with a pathological effect of ERK-dependent IRS-1 phosphorylation, Ser^{632} (human Ser^{636}) phosphorylation is elevated in muscle cells from individuals with Type 2 diabetes, and this is reduced by chemical inhibition of ERK1/2 activity [87].

SIGNALLING MOLECULES ENGAGED BY ANG-(1–7)

Our current knowledge of the signalling pathways that are regulated by Ang-(1–7) is limited and originates from both *in vivo* and *in vitro* studies that have primarily used Western blot analysis as a tool [88–92]. In addition, time-resolved quantitative phosphoproteomics using human endothelial cells has shed light on Ang-(1–7) signalling [93]. Both approaches have generated consistent results showing that insulin and Ang-(1–7) share signalling events, implying a cross-talk between these two signalling systems. Given that Ang-(1–7) can improve insulin sensitivity, this signalling overlap could be implicated in this metabolic role of Ang-(1–7). The main findings of these studies are discussed below.

Node JAK/STAT

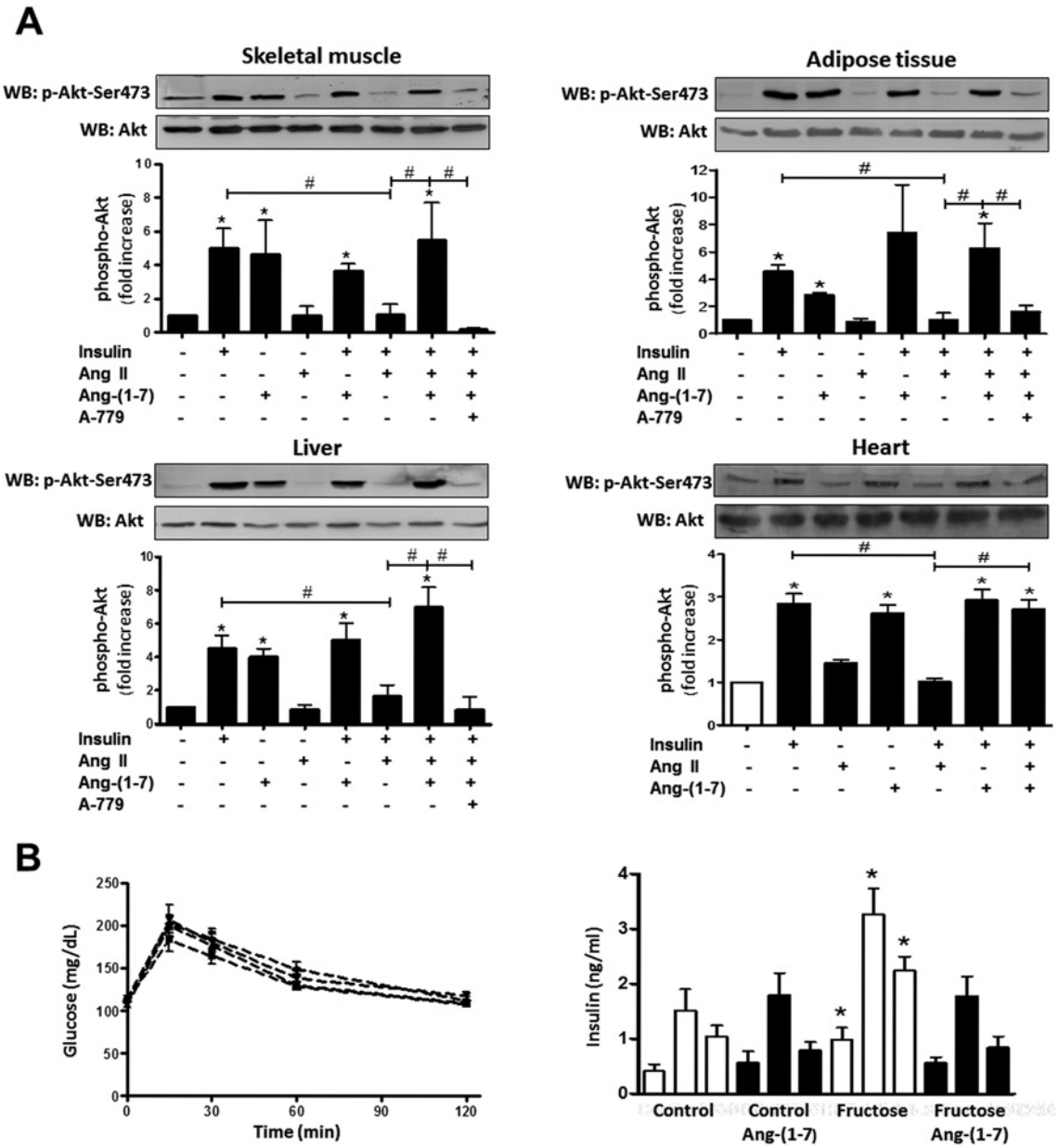
The cytosolic tyrosine kinase JAK2 becomes activated and associates with the AT_1R in response to AngII [94,95]. JAK2 activation by AngII leads to the phosphorylation of STAT1 and STAT3 [96]. We have demonstrated that Ang-(1–7) stimulates the activity of JAK2 in the rat heart *in vivo*, and that this activation proceeds through a mechanism that involves the AT_1R since it is abolished by losartan and it is not prevented by either Mas or AT_2R antagonists [89]. In line with these findings, we have shown that Ang-(1–7) stimulates the phosphorylation of STAT3 and STAT5 in the rat heart by an AT_1R-mediated mechanism [90]. The pattern of activation of these signalling molecules was both time- and concentration-dependent, and similar to that initiated by AngII [89,90]. The participation of the JAK/STAT signalling pathway in the signalling pathways of Ang-(1–7) is intriguing. Activation of the JAK/STAT pathway is critical for the development of AngII-induced hypertension by mediating its effects on renal sodium excretory capability, but the physiological control of blood pressure by AngII appears not to require JAK2 activation [97,98]. Moreover, activation of the JAK/STAT cascade can stimulate excessive proliferation and growth of VSMCs (vascular smooth muscle cells) and glomerular mesangial cells, contributing to the accelerated atherosclerosis and nephropathy observed in the diabetic state [99]. A potential explanation for this observation could be that, at the doses used, Ang-(1–7) could have activated JAK2 through the AT_1R or AT_2R, although it is not clear whether this could happen under physiological conditions. In any event, the participation of Ang-(1–7) as a stimulator of the JAK/STAT pathway contradicts its anti-atherogenic and anti-diabetic effects and deserves further exploration.

Node IRS proteins

Ang-(1–7) is capable of stimulating the phosphorylation of IRS-1 in the rat heart [89]. We have demonstrated that this stimulating effect of Ang-(1–7) is not mediated by the IR kinase, but instead involves the AT_1R through a mechanism that requires participation of JAK2 [89]. Both the time and concentration necessary to attain the maximum stimulatory response were similar to those described previously for AngII [100,101]. The demonstration that IRS-1 is used by Ang-(1–7) reinforces the previously postulated concept that the IRS proteins serve as convergence sites for the signal transduction of several hormones and cytokines [50,102,103]. Using systematic binding analysis in HEK (human embryonic kidney)-293 cells stably transfected with the AT_1R, it was shown that Ang-(1–7) exhibits minimal binding of the AT_1R (at 1 μM) [104]. Although this study employed an artificial system, this confirms previous studies that have shown that Ang-(1–7) binds to the AT_1R with a very low affinity [105–107]; however, it should be noted that, in the literature, binding data are not always matched with functional data. There are several reports showing that some of the effects of Ang-(1–7) appear to be mediated by the AT_1R [108–112]. This apparent mismatch could be attributed to tissue heterogeneity in the AT_1R, AT_2R and Mas receptor and/or differential receptor subtype expression. In addition, interaction of the AT_1R with the Mas receptor could be relevant for Ang-(1–7) signal transduction and action. A functional interaction has been described between the AT_1R and the Mas receptor [113]. The Mas receptor can hetero-oligomerize with the AT_1R and, by so doing, inhibit the actions of AngII. [114]. Moreover, at high doses, Ang-(1–7) has been shown to down-regulate the AT_1R [115,116]. Thus our observation that Ang-(1–7) recruits JAK2, STAT3, STAT5 and IRS-1 in the rat heart through an AT_1R-mediated mechanism can be included among these atypical observations and suggests that Ang-(1–7) can use the AT_1R to transmit some of its signal to the inside of the cell.

Node PI3K/Akt

The serine/threonine kinase Akt appears to have a central role in Ang-(1–7) signalling. Phosphorylation of Akt at the regulatory sites Ser^{473} and Thr^{308} is stimulated by Ang-(1–7) in human endothelial cells *in vitro* [88], as well as in the rat heart [89], liver, skeletal muscle and adipose tissue [91] *in vivo* (Figure 3A). Indirect evidence for the participation of PI3K in Ang-(1–7)-induced Akt activation was provided through the use of the inhibitor of class I PI3Ks wortmannin [88,89]. Since then, the participation of the PI3K/Akt pathway in the action of Ang-(1–7) has been consistently reproduced in different experimental settings, particularly in cardiomyocytes [117]. Time-resolved quantitative



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Parameters	Saline	Ang-(1–7)	A-779	Ang-(1–7) + A-779
Body weight (g)	381 ± 11	385 ± 2	404 ± 18	399 ± 14
SBP (mm Hg)	136 ± 10	116 ± 7*	135 ± 7	132 ± 5
Glucose (mg/dl)	104 ± 7	108 ± 8	111 ± 4	109 ± 8
Insulin (ng/ml)	4.2 ± 0.8	1.3 ± 0.3*	5.2 ± 1.4	4.7 ± 0.9
Triglycerides (mg/dl)	84 ± 8	22 ± 9*	102 ± 17	84 ± 12
HOMA score	27.5 ± 5.8	8.2 ± 1.7*	35.6 ± 10.9	32.1 ± 5.7

Figure 3 **Results from our laboratory demonstrating a positive modulation of insulin signalling by Ang-(1–7)**
(**A**) Acute *in vivo* administration of Ang-(1–7) induced the phosphorylation of Akt at Ser^{473} (p-Akt-Ser473) in the skeletal muscle, adipose tissue, liver and heart. Insulin-induced Akt was impaired when co-injected with AngII. This impairment was reversed by the presence of Ang-(1–7) and the Mas receptor-specific antagonist A-779 abolished this beneficial effect. $^{*}P < 0.05$ compared with the control group (saline-injected animals); $^{\#}P < 0.05$. WB, Western blot. (**B**) Ang-(1–7) reverses insulin resistance in fructose-fed rats. Control and fructose-fed rats (Fructose) were treated with Ang-(1–7) or saline. Although glucose tolerance was unaltered by the high-fructose diet, circulating insulin concentrations at the baseline and 15 min and 120 min after glucose administration were significantly higher in fructose-fed rats compared with the

phosphoproteomics analysis corroborated these findings, situating Akt in a central place in the action of Ang-(1–7) [93]. The participation of Ang-(1–7) as a modulator of lipid metabolism in adipose tissue has recently been evaluated and Ang-(1–7) was found to increase glycerol release from primary adipocytes in a dose-dependent manner [118]. This lipolytic effect of Ang-(1–7) appeared to be mediated by a Mas receptor/PI3K-dependent mechanism [118]. Interestingly, additional effects of Ang-(1–7) on adipose tissue have recently been reported, indicating that it promotes formation of small insulin-sensitive newly differentiated adipocytes [119]. This induction of adipogenesis by Ang-(1–7) proceeds via activation of PI3K/Akt [119]. Recently, it was shown that in patients with diabetes both the survival and proliferation of $CD34^+$ cells is enhanced by Ang-(1–7) in a Mas/PI3K/Akt-dependent manner [120]. Additional reports have shown that engagement of Akt by Ang-(1–7) appears to be essential for other actions exerted by this hormone, such as the maintenance of cardiomyocyte function [117] and stimulation of atrial natriuretic peptide secretion [121].

Aside from class I PI3K, phosphoproteomics analysis identified PI3KC2A, a class II PI3K, as a target of Ang-(1–7) [93]. These enzymes are insensitive to PI3K inhibitors and have been shown to have a major role in the action of insulin by stimulating insulin secretion [122]. Interestingly, it was found previously that PI3KC2A contributes to maximal insulin-induced translocation of GLUT4 (glucose transporter 4) to the plasma membrane and subsequent glucose uptake [123]. Moreover, another report identified signal transduction via PI3KC2A as a novel pathway whereby insulin activates Akt and thus identifies PI3KC2A as a potential pharmacological target in Type 2 diabetes [124]. Thus the engagement of PI3KC2A by Ang-(1–7) is in good agreement with the insulin-sensitizing effect of this heptapeptide.

Akt has several cytosolic substrates, including NOS, GSK-3, AS160 and FOXO1 [54], and the information available so far suggests that all of these substrates are phosphorylated by Ang-(1–7). Ang-(1–7) activates eNOS (endothelial NOS) leading to vasodilation. This process is triggered by the Mas receptor [125] and requires activation of Akt [88,117]. We have demonstrated that Ang-(1–7) is able to induce the phosphorylation of GSK-3β in the liver, adipose tissue and skeletal muscle as well as that of AS160 in skeletal muscle and adipose tissue of the rat *in vivo* [91,92]. The proline-directed serine/threonine kinase GSK-3β, being one of the principal downstream targets of Akt, regulates a wide range of cellular processes, including glycogen metabolism, gene transcription, protein translation and cell apoptosis [126]. Under basal conditions, GSK-3β is highly active and inhibits glycogen synthesis by phosphorylation of glycogen synthase [127]. Insulin-activated Akt phosphorylates GSK-3β at the inhibitory site Ser^9, leading to stimulation of glycogen synthesis and protein synthesis [50]. AS160 is a Rab GTPase-activating protein and an important target of Akt [128]. It regulates insulin-stimulated GLUT4 trafficking [129] and glucose uptake [130]. The importance of AS160 in insulin-mediated glucose transport has been highlighted recently by the phenotype of AS160-KO (knockout) mice that display whole-body insulin resistance [131]. As a corollary of the discovery that Ang-(1–7) is able to induce the phosphorylation of Akt and AS160, a recent study has shown that Ang-(1–7) stimulates glucose transport in adipocytes [132]. Interestingly, this was associated with a decrease in NADPH oxidase expression and oxidative stress [132]. Some studies suggest that oxidative stress is able to impair PI3K and Akt insulin-signalling steps in cultured insulin-sensitive cell lines, such as 3T3-L1 adipocytes and L6 myocytes [133,134]. These results suggest a causal relationship between the Ang-(1–7)-mediated negative regulation of NADPH oxidase [with a resulting decrease in ROS (reactive oxygen species)] and an increase in glucose uptake. Thus the observed Ang-(1–7)-induced phosphorylation of GSK-3β and AS160 is correlated well with the modulation of the action of insulin in terms of glucose metabolism.

FOXO1 is a transcription factor that regulates genes controlling many hepatic functions, including glucose production and lipid metabolism [135–137]. Under physiological conditions insulin inactivates FOXO1 by Akt-stimulated phosphorylation. This inactivation is required for adaptive nutrient homoeostasis during periods of fasting and feeding [138]. During fasting, insulin levels fall and FOXO1 becomes dephosphorylated and translocates to the nucleus which stimulates catabolic processes to maintain glucose homoeostasis. In the fed state, insulin levels increase leading to phosphorylation and inactivation of FOXO1 [138]. It has been shown recently that Ang-(1–7) stimulates the dephosphorylation of FOXO1 and consequently enhances its activity in human endothelial cells [93]. These findings suggest that Ang-(1–7) could be a fine-tuning regulator of insulin action through modulating the equilibrium between Akt and FOXO1 activation.

ANG-(1–7) COUNTERACTS THE NEGATIVE EFFECTS OF ANGII ON THE ACTION OF INSULIN: POTENTIAL MOLECULAR MECHANISMS INVOLVED

There is ample evidence which indicates that Ang-(1–7) is able to counteract many of the haemodynamic and non-haemodynamic actions of AngII. The evidence accumulated over the last few years suggests that Ang-(1–7) acts as regulator of both glucose

control group, suggesting a state of insulin resistance. Administration of Ang-(1–7) normalized the response to glucose, thus enhancing insulin sensitivity. $^*P < 0.05$ compared with the corresponding values in all other groups. (**C**) Table showing the metabolic parameters of fructose-fed rats chronically treated with saline, Ang-(1–7), A-779 or a mixture of Ang-(1–7)and A-779. Chronic administration of Ang-(1–7) reduced systolic blood pressure (SBP) and baseline plasma insulin and triacylglycerols in fructose-fed animals. In addition, concomitant administration of a Mas receptor blocker (A-779) blunted the beneficial effects exerted by Ang-(1–7). $^*P < 0.05$ compared with the saline-treated mice. HOMA, homoeostatic model assessment. The upper left-hand, upper right-hand and lower left-hand panels in (**A**), and (**C**) were reprinted from Regulatory Peptides, 177 (1–3), Muñoz M. C, Giani J. F, Burghi V., Mayer M. A, Carranza A., Taira C.A and Dominici F. P, The Mas receptor mediates modulation of insulin signaling by angiotensin-(1–7)., 1–11, Copyright (2012), with permission from Elsevier. Copyright © 2012 Elsevier. The lower right-hand panel in (**A**) was reproduced from [89] with permission from The American Physiological Society. (**B**) was reproduced from [85] with permission from The American Physiological Society.

and lipid metabolism [36,37,39,139]. In this section we will discuss recent findings that show that the counteraction of the actions of AngII exerted by Ang-(1–7) also extends to the arena of metabolism. The first evidence originated from an *in vivo* analysis that revealed that in the rat heart Ang-(1–7) counteracts the negative effects of AngII [89]. Strikingly, the AngII-induced inhibition of insulin-stimulated Akt phosphorylation was no longer evident when Ang-(1–7) was co-injected with AngII [83]. Later, we found that this counteracting effect was detected in the main insulin-target tissues, namely the liver, skeletal muscle and adipose tissue [86] (Figure 3A). It was found that Ang-(1–7) not only counteracted the AngII-mediated inhibition of insulin-stimulated Akt phosphorylation, but also improved the phosphorylation of other insulin-related intracellular proteins, such as AS160 and GSK-3β [86]. In a recent report, this counteraction of the inhibitory effects of AngII on insulin-induced Akt activation was confirmed using both human umbilical vein endothelial cells [140] and isolated rat soleus muscle [141]. Importantly, in skeletal muscle, Ang-(1–7) was shown to ameliorate the inhibitory effect of AngII on glucose transport activity [141]. Although the negative effects of AngII on insulin signalling are mediated by the AT_1R, the beneficial and counteracting effect of Ang-(1–7) on the actions of AngII at this level appear to be mediated by its specific receptor Mas [92,140,141] (Figure 3A).

AngII has been proposed as one of the most important inhibitors of the insulin signalling pathway. Evidence of this is the tight association that exists between the development of Type 2 diabetes and hypertension [142]. Indeed, the observation that antagonists of the AT_1R and ACE inhibitors not only reduce blood pressure, but also ameliorate insulin resistance is proof of the negative influence that AngII exerts over insulin signalling [19]. AngII inhibits insulin signalling at multiple levels [8–13,24,25]. For instance, AngII augments the phosphorylation of serine residues of the IR in aortic smooth muscle cells, leading to a significant reduction in insulin-mediated PI3K activation. [8,9]. However, serine phosphorylation of IRS-1 appears as one of the most important mechanisms of the AngII-mediated attenuation of insulin signalling. This negative effect of AngII is mediated by the AT_1R with important participation of ROS generation [143] (Figure 2). In fact, AngII has been shown to decrease insulin sensitivity in skeletal muscle and reduce glucose transport through ROS generation. A pivotal role has been assigned for NADPH oxidase in these events [144–146]. AngII impairs eNOS activation through ERK1/2-mediated phosphorylation of IRS-1 at Ser^{612} (human Ser^{616}) and JNK1/2-mediated Ser^{307} phosphorylation [24]. Alternatively, studies suggest that AngII induces the activation of p38 MAPK [147,148]. This enzyme has been suggested as a potential candidate for mediating indirect IRS-1 serine phosphorylation [149]. In VSMCs, AngII has been shown to decrease IRS-1 protein levels via Src, PDK1 and ROS-mediated phosphorylation of IRS-1 at Ser^{307} and the its subsequent proteasome-dependent degradation [150]. This evidence indicates that AngII-induced generation of ROS through an AT_1R/NADPH oxidase-dependent mechanism is linked to serine phosphorylation of the IRS proteins and acts a major contributing factor to AngII-induced insulin resistance. Finally, it has recently been shown that rapamycin, an inhibitor of mTOR, attenuates the AngII-stimulated phosphorylation of p70S6K (p70 S6 kinase) and phosphorylation of IRS-1 (at Ser^{636} or Ser^{639}) and blocks the ability of AngII to impair insulin-stimulated phosphorylation of eNOS and NO production [25]. In addition to mTOR and JNK, AngII-induced ERK1/2 activation has been shown to inhibit insulin-dependent glucose uptake through serine phophorylation of IRS-1 in RASMCs (rat aortic smooth muscle cells) [151]. In the vasculature, AngII has been shown to inhibit Akt phosphorylation specifically through PKC-α activation [152].

One of the mechanisms by which Ang-(1–7) counteracts AngII-mediated insulin resistance appears to be the reduction in serine phosphorylation of IRS-1 (Figure 4). In the animal model of insulin resistance generated by a high-fructose diet in rats, we have demonstrated that Ang-(1–7) improves insulin signalling in the liver, skeletal muscle and adipose tissue [85]. This improvement was associated with a significant reduction in the phosphorylation of IRS-1 at Ser^{307} together with less activation of the kinases mTOR and JNK in skeletal muscle and adipose tissue [85]. In addition, Ang-(1–7) has been shown to counteract AngII signalling, leading to ERK1/2 and PKC activation both in proximal tubular cells and VSMCs [153–155], as well as in the rat heart [90] (Figure 2). Additional evidence for this mechanism was recently provided using human umbilical vein endothelial cells [140]. Accordingly, it was demonstrated that AngII induces the phosphorylation of IRS-1 at Ser^{616} that was associated with an impairment of insulin signalling [140]. Importantly, this impairment was restored by Ang-(1–7) concomitant with a reduction in phosphorylation of IRS at Ser^{616} [140]. Thus a reduction in serine phosphorylation of IRS-1 through a mechanism that involves inhibition of JNK, mTOR, and, potentially, ERK1/2 and PKC appears to play a key role in the counteraction of AngII-mediated inhibition of insulin signalling induced by Ang-(1–7). This improvement in insulin signalling may also involve suppression of oxidative stress as was shown previously [126] (Figure 4).

Although indirectly, the vasodilation induced by Ang-(1–7) could be considered as a possible contributing factor to its facilitating effect on the action of insulin (Figure 4). The delivery of insulin to the peripherical tissues is an important factor in the execution of its actions [156–158]. Thus Ang-(1–7) could potentiate the action of insulin both by enhancing vasodilation and counteracting the vasoconstrictive effects of AngII. This would result in enhanced insulin delivery to target tissues (Figure 4). In fact, a recent study has shown that blockade of the AT_1R with losartan increases muscle insulin delivery and counteracts the negative effects on insulin sensitivity associated with infusion of lipids [159].

THERAPEUTIC IMPLICATIONS

Previous reports have indicated that the modulation of ACE2/Ang-(1–7)/Mas receptor axis is a very attractive target in the therapy of the metabolic syndrome and diabetes-associated diseases affecting the heart and the kidney. These findings are summarized in Table 1. We found that chronic infusion of Ang-(1–7) ameliorates insulin resistance by reducing the levels of

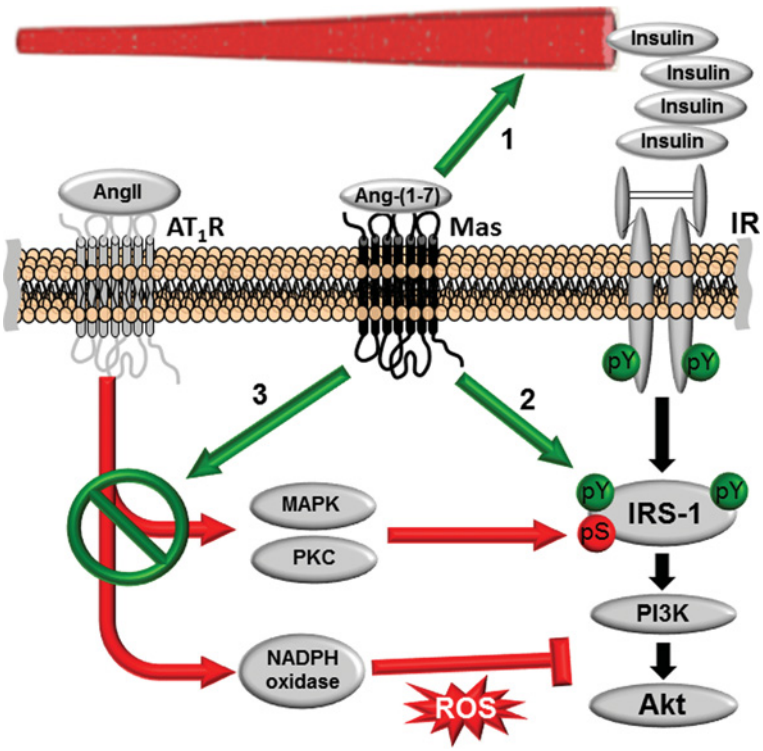

Figure 4 **Potential mechanisms involved in the modulation of the action of insulin by Ang-(1–7) through its specific receptor Mas**

1: Vasodilator effects increase the blood flow facilitating insulin delivery to peripherical tissues. 2: Direct stimulation of IRS-1 tyrosine phosphorylation. 3: Inhibition of the negative cross-talk between AngII and insulin signalling (serine/threonine kinases involved in serine phosphorylation of IRS-1 and production of ROS).

triacylglycerols and improving insulin sensitivity in fructose-fed rats [85] (Figures 3B and 3C). In a recent study these findings were confirmed and it was also demonstrated that Ang-(1–7) treatment prevents the metabolic-syndrome alteration, reduces ectopic lipid accumulation in the liver and diminishes the volume of epididymal adipocytes as well as the total body mass induced by fructose overload [160]. In agreement with these reports, transgenic rats overexpressing Ang-(1–7) display enhanced glucose tolerance and improved insulin sensitivity concomitant with enhanced insulin-stimulated glucose uptake in adipocytes and reduced triglyceridaemia, cholesterolaemia and abdominal fat mass [161]. Further efforts in characterizing the phenotype of these animals demonstrated the presence of reduced hepatic gluconeogenesis [162], as well as decreased susceptibility to the development of inflammation in adipose tissue [163]. This improvement in glucose and lipid metabolism induced by Ang-(1–7) correlates well with its capability for stimulating insulin signalling [88,89,91,92] and glucose transport [132]. These promising pre-clinical studies suggest that modulation of the ACE2/Ang-(1–7)/Mas receptor axis could improve haemodynamic and metabolic diseases in humans. Several meta-analyses have underscored the positive effects of ARBs (angiotensin receptor blockers) and ACE inhibitors on insulin sensitivity and the progression to Type 2 diabetes [164,165]. Since the ACE2/Ang-(1–7)/Mas receptor axis naturally counterbalances the effects of classical RAS components, it is reasonable to believe that part of the positive effects of ARBs and ACE inhibitors on metabolic diseases could be mediated by overactivation of the Ang-(1–7) pathway. Indeed, a recent study has suggested that the beneficial effects of olmesartan, an ARB, on vascular remodelling are mediated via activation of the ACE2/Ang-(1–7)/Mas receptor axis [166].

A role for Ang-(1–7) in metabolism is also provided by manipulation of ACE2. Accordingly, ACE2-KO mice display normal insulin sensitivity when fed on a standard diet. However, AngII infusion or administration of an HFHS (high-fat high-sucrose) diet resulted in accentuated glucose intolerance and greater impairment of insulin sensitivity in these animals [167]. Importantly, this negative effect was blunted by administration of Ang-(1–7) [167]. In *db/db* mice, overexpression of ACE2 in the pancreas significantly improved fasting glycaemia, enhanced intraperitoneal glucose tolerance, increased islet insulin content and β-cell proliferation, and reduced β-cell apoptosis [168]. These findings identified ACE2 as a novel target for the prevention of β-cell dysfunction and apoptosis, both hallmarks of the onset of Type 2 diabetes. Moreover, a recent study has determined that maintenance of a normal endogenous ACE2 compensatory activity in the pancreas appears critical to avoid the β-cell dysfunction induced by an overactive RAS [169]. In AngII-infused mice exhibiting hyperglycaemia, hyperinsulinaemia and impaired glucose-stimulated insulin secretion from pancreatic islets, treatment with adenovirus expressing human ACE2 restored pancreatic ACE2 expression and prevented AngII-mediated elevated glycaemia, thus improving β-cell function [169].

Table 1 Interventions in the ACE2/Ang-(1–7)/Mas receptor axis leading to improvement of metabolic syndrome, diabetes-associated pathologies and obesity
HDL, high-density lipoprotein; NF-κB, nuclear factor κB.

Pathology	Experimental approach	Effect
Metabolic syndrome	Chronic infusion of Ang-(1–7) into fructose-fed rats	Reduced serum triacylglycerols, improved glucose tolerance and insulin sensitivity [85], and reduced hepatosteatosis, epididymal adipocyte volume and total fat mass [160]
	Ang-(1–7) overexpression in transgenic rats	Enhanced glucose tolerance, insulin sensitivity and insulin-stimulated glucose uptake in adipocytes, reduced triglyceridaemia, cholesterolaemia and abdominal fat mass [161], down-regulation of hepatic gluconeogenesis [162], and resistance to high-fat diet with transgenic rats showing increased HDL-cholesterol levels, decreased abdominal fat mass and lower body mass compared with the wild-type animals [163]
	Acute Ang-(1–7) stimulation in rats	Activation of the insulin signalling-related proteins IRS-1, Akt, GSK3 and AS160 via the receptor Mas [88,89,91–93]
	Ang-(1–7) treatment of adipocytes	Improved glucose uptake both in basal and insulin-stimulated states via the Mas receptor and reduced ROS production [132]
	Deletion of ACE2 in mice	Normal insulin sensitivity when fed on a standard diet, but, increased susceptibility to AngII infusion or when fed on a high-fat and high-sucrose diet in terms of the development of glucose intolerance and impairment of insulin sensitivity; this negative effect was eradicated by Ang-(1–7) [167]
	Overexpression of ACE2 in the pancreas of *db/db* mice	Improved fasting glycaemia, enhanced intraperitoneal glucose tolerance, increased islet insulin content and β-cell proliferation, and reduced β-cell apoptosis; effect eliminated by A-779 [168]
	Overexpression of ACE2 in AngII-infused mice	AngII-infused mice exhibited hyperglycaemia, hyperinsulinaemia and impaired glucose-stimulated insulin secretion from pancreatic islets, decreased ACE2 expression and activity, increased AT_1R expression, and increased oxidative stress in the pancreas; adenovirus treatment encoding human ACE2 restored pancreatic ACE2 expression, improved β-cell function and restored glucose homoeostasis [169]
	Mas-KO mice	Dyslipidaemia, increased levels of insulin, leptin and abdominal fat mass with normal body mass, glucose intolerance, reduced insulin sensitivity and insulin-stimulated glucose uptake by adipocytes and decreased GLUT4 in adipose tissue [171]
	Adipocytes from Mas receptor-knockout male mice	Altered response of adipocytes to insulin action; effect related to decreased expression of $PPAR\gamma$ [172]
Diabetes-induced cardiovascular dysfunction	Chronic administration of Ang-(1–7) or AVE-0991 on diabetic rats	Prevention of diabetes-induced abnormal vascular responsiveness to noradrenaline, endothelin-1, AngII, carbachol and histamine in the mesenteric bed, isolated carotid and renal arteries [173]; increased LV-developed pressure and serum nitrite/nitrate and decreased LV mass/body mass and LV collagen content [174]; AVE-0991 rescued cardiac function under diabetic conditions as indicated by a normalization of blood pressure and contractility parameters [175]; and Ang-(1–7) inhibited EGF receptor transactivation via a Mas receptor/Src-dependent pathway, indicating that activation of the EGF receptor is a key player in mediating diabetes-induced vascular dysfunction [177]
	Chronic infusion of XNT (ACE2 activator) on STZ-induced diabetic rats	Improved endothelium-dependent vasorelaxation of aortic rings; effect blocked by A-779 [176]
	Deletion of ACE2 in diabetic Akita mice	Increased plasma and tissue AngII, impaired systolic and diastolic function, increased activation of NADPH oxidase, and greater oxidative stress [178]
	Chronic infusion of Ang-(1–7) into fructose-fed rats	Reduced blood pressure, heart-to-body mass ratio, myocyte diameter and LV fibrosis [179]
Diabetic nephropathy	Chronic infusion of Ang-(1–7) on STZ-treated spontaneously hypertensive rats	Reduction of renal NADPH oxidase activity and proteinuria, and attenuation of diabetes-induced increase in the renal vascular responsiveness to endothelin-1 [180]
	Chronic infusion of Ang-(1–7) into STZ-induced diabetic rats	Attenuated proteinuria and renal collagen content and improved endothelial functions without preventing tubular damage; effects blocked by A-779 [181]
	Chronic infusion of Ang-(1–7) into KK-A^y/Ta diabetic mice	Improved mesangial expansion, attenuated AngII-mediated NADPH oxidase activation and ROS production in glomeruli and mesangial cells, and attenuated AngII-induced NF-κB and MAPK signalling in mesangial cells [182]
	Chronic infusion of Ang-(1–7) into Zucker diabetic fatty rats	Reduced proteinuria and systolic blood pressure, restored creatinine clearance, and decreased renal fibrosis, oxidative stress and inflammatory cytokines [184]

Table 1 Continued

Pathology	Experimental approach	Effect
	Deletion of ACE2 in mice	Exaggerated diabetic nephropathy induced by STZ administration [185]
	Overexpression of ACE2 in podocytes of diabetic mice	Prevented albuminuria, attenuated the increase in the mesangial area and the decrease in glomerular area, and restored nephrin expression [186]
	Inhibition of ACE2 in diabetic rats	Exacerbation of diabetes-induced increase in cardiac and renal NADPH activity [187]
Diabetic retinopathy	Ang-(1–7) treatment of endothelial progenitor cells ($CD34^+$) from patients with diabetes	Restored migration and NO bioavailability/cGMP production via Mas receptor and decreased NADPH oxidase activity, and enhanced survival and proliferation of $CD34^+$ cells [188]
Diabetic wounds	NorLeu(3)-Ang-(1–7) [analogue of Ang-(1–7)] treatment of diabetic wounds	Induced progenitor cell proliferation and accelerated vascularization, collagen deposition and re-epithelialization [189]
Obesity	Administration of an oral formulation of Ang-(1–7)	Prevention of obesity, hepatic inflammation and hepatic steatosis induced by a high-fat diet, and improvement in lipid metabolism [191,192]

It is worth mentioning that ACE inhibitors fail to completely block AngII formation since many other alternative proteases can still produce AngII and generate hyperglycaemia in the presence of ACE inhibitors [170]. ACE2, on the other hand, acts directly on AngII, irrespective of the pathways involved in the production of this octapeptide. Thus, by ensuring depletion of AngII in the body, ACE2 therapy also increases the possibility of Ang-(1–7) emerging as a more efficient treatment than ACE inhibition in combating AngII-mediated hyperglycaemia [47,168,169]. The safety and tolerability, as well as the pharmacokinetics and pharmacodynamics of intravenous administration of recombinant soluble human ACE2 (known as APN01), is under evaluation currently [Safety and Tolerability Study of APN01 (Recombinant Human Angiotensin Converting Enzyme 2); ClinicalTrials.gov Identifier: NCT00886353].

Confirmatory evidence of a role for the ACE2/Ang-(1–7)/Mas receptor axis in glucose and lipid homoeostasis was provided by the characterization of the phenotype of Mas-KO mice [171]. Mas-KO mice displayed dyslipidaemia and increased levels of insulin, leptin and abdominal fat mass. In addition, these mice show glucose intolerance, reduced insulin sensitivity, insulin-stimulated glucose uptake in adipocytes and decreased GLUT4 expression in adipose tissue [171]. An additional study determined that adipocytes from Mas-KO mice exhibit a blunted response to insulin in terms of a decrease in glycerol release, suggesting that the lack of Ang-(1–7) action through the Mas receptor alters the response of adipocytes to insulin action. These effects could be related to the decreased expression of PPARγ (peroxisome-proliferator-activated receptor γ) in the adipocytes of Mas-KO mice [172].

Owing to the demonstrated role of Ang-(1–7) as a facilitator of the action of insulin and as a negative regulator of the action of AngII, several studies have analysed its beneficial effects on pathologies associated with Type 2 diabetes. An important area of research is the treatment of diabetes-induced cardiovascular dysfunction. In this regard, it has been demonstrated that chronic administration of Ang-(1–7), or its synthetic analogue AVE-0991, prevented the diabetes-induced abnormal vascular responsiveness to noradrenaline, endothelin-1, AngII, carbachol and histamine in the mesenteric bed, isolated carotid and renal arteries of rats made diabetic by treatment with STZ (streptozotocin) [173]. Further studies in STZ-treated rats demonstrated that treatment with Ang-(1–7) increases LV (left ventricular)-developed pressure and serum nitrite/nitrate, and decreases the LV mass/body mass ratio and LV collagen content in diabetic animals [174]. In agreement with these studies, treatment with AVE-0991 has been shown to rescue cardiac function under diabetic conditions as indicated by a normalization of blood pressure and contractility parameters [175]. More recently, it was shown that chronic infusion of XNT {1-[(2-dimethylamino) ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl) sulfonyl oxy]-9H-xanthene-9-one; an ACE2 activator} into STZ-induced diabetic rats improves the endothelium-dependent vasorelaxation of aortic rings. This effect was blocked by the Mas receptor antagonist A-779 [176]. In general these studies imply a cardioprotective role for Ang-(1–7) under hyperglycaemic conditions and point to new therapeutic strategies using Ang-(1–7) agonists to treat the cardiovascular complications associated with diabetes mellitus. A potential mechanism involved in these beneficial effects of Ang-(1–7) could be related to its ability to inhibit EGF (epidermal growth factor) receptor transactivation via a Mas receptor-dependent pathway [177]. In addition, an indirect role for this cardioprotective role of Ang-(1–7) has been demonstrated by deletion of the *Ace2* gene in diabetic Akita mice.These animals displayed increased plasma and tissue AngII, impaired systolic and diastolic function, and increased NADPH oxidase activity and greater oxidative stress in the heart [178]. A protective effect for Ang-(1–7) on cardiac function has also been demonstrated in fructose-fed rats, where chronic infusion of Ang-(1–7) reduced blood pressure, heart-to-body mass ratio, myocyte diameter and LV fibrosis. These beneficial effects were associated with a reduced activity of growth-promoting signalling molecules pathways (i.e. ERK1/2, JNK1/2 and p38 MAPK) in the heart. [179].

Diabetic nephropathy is another major complication of Type 2 diabetes and is a main cause of end-stage renal disease. In line with its beneficial effect on the action of insulin, Ang-(1–7) has been shown to have a renoprotective effect in both diabetic and insulin-resistant rats. In diabetic spontaneously hypertensive rats, chronic infusion of Ang-(1–7) decreased the elevated levels of renal NADPH oxidase activity, reduced proteinuria

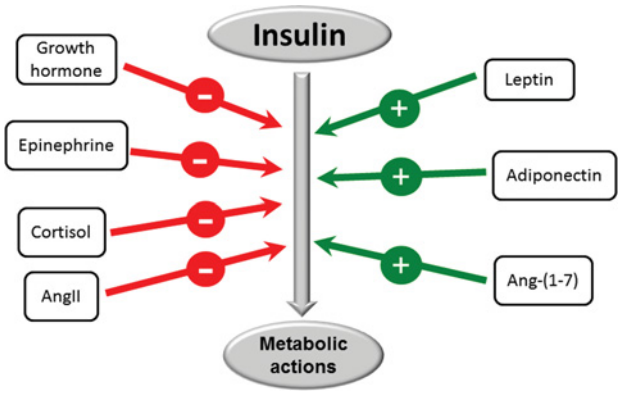

Figure 5 Modulation of insulin-mediated metabolic actions by different hormones

and attenuated the diabetes-induced increase in renal vascular responsiveness to endothelin-1 [180]. In STZ-treated diabetic rats, chronic infusion of Ang-(1–7) attenuated proteinuria and renal collagen content. This correlated with an improvement in endothelial function. Such beneficial effects were blocked by A-779 [181]. A study using KK-A^{y}/Ta mice demonstrated that chronic infusion of Ang-(1–7) improves mesangial expansion and attenuates AngII-mediated NADPH oxidase activation and ROS production in glomeruli and mesangial cells [182]. Ang-(1–7)-induced attenuation of renal NADPH oxidase has also been shown to correlate with the preservation of PPARγ and catalase activity [183]. We have demonstrated that treatment of Zucker diabetic rats with Ang-(1–7) reduces proteinuria and systolic blood pressure, restores creatinine clearance, and decreases renal fibrosis, oxidative stress and inflammatory cytokines [184]. Highlighting the role of Ang-(1–7), ACE2-KO mice displayed accelerated time-dependent glomerular and tubulointerstitial damage when treated with STZ [185]. In good agreement with this finding, overexpression of ACE2 in the podocytes of diabetic mice was recently shown to prevent microalbuminuria, attenuate the increase in mesangial area, decrease the glomerular area and restore nephrin expression [186]. Confirmation of the beneficial role of endogenous Ang-(1–7) in the cardiovascular system was provided by a study where chronic treatment with the ACE2 inhibitor DX600 exacerbated the diabetes-induced increase in cardiac and renal NADPH activity [187]. Finally, modulation of the ACE2/Ang-(1–7)/Mas receptor axis appears to be an attractive therapeutic target in the treatment of diabetic retinopathy [188] and also has a potential application as an accelerator of vascularization and re-epithelization [189].

In view of the great therapeutic effect of the modulation of the ACE2/Ang-(1–7)/Mas receptor axis, a formulation of Ang-(1–7) bound in a cavity in the oligosaccharide HPβCD23 (hydroxypropyl β-cyclodextrin 23) that protects the peptide, when orally administrated, during passage through the gastrointestinal tract has been developed [190]. Recently, this oral formulation of Ang-(1–7) has been shown to counteract the deleterious effects of a high-fat diet [191,192], supporting its potential application in the treatment of the metabolic syndrome and diabetic complications.

CONCLUSIONS AND PERSPECTIVES

The overall knowledge of the physiological and pathophysiological role of Ang-(1–7) has advanced greatly in the last decade. In particular, it has become clear that Ang-(1–7) counteracts both the cardiovascular and non-cardiovascular actions of AngII. Recent findings have shown that this counteraction applies to the modulation of the action of insulin in glucose and lipid metabolism. Potential mechanisms by which this beneficial effect is exerted include direct activation of insulin signalling, inhibition of the negative actions of AngII and increased delivery of insulin to the target tissues. Thus Ang-(1–7) should be considered as a member of the group of hormones that can modulate the action of insulin, in this case positively (Figure 5). The implications of these findings are beginning to be revealed, and make modulation of the ACE2/Ang-(1–7)/Mas receptor axis a very attractive therapeutic option in the treatment of the metabolic syndrome and pathologies associated with Type 2 diabetes.

REFERENCES

1 Scully, T. (2012) Diabetes in numbers. Nature **485**, S2–S3
2 Grundy, S. M., Brewer, Jr, H. B., Cleeman, J. I., Smith, Jr, S. C. and Lenfant, C. (2004) Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation **109**, 433–438
3 Biddinger, S. B. and Kahn, C. R. (2006) From mice to men: insights into the insulin resistance syndromes. Annu. Rev. Physiol. **68**, 123–158
4 Reaven, G. M. (2011) The metabolic syndrome: time to get off the merry-go-round? J. Intern. Med. **269**, 127–136
5 DeFronzo, R. A. (1997) Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis. Neth. J. Med. **50**, 191–197
6 Matthaei, S., Stumvoll, M., Kellerer, M. and Haring, H. U. (2000) Pathophysiology and pharmacological treatment of insulin resistance. Endocr. Rev. **21**, 585–618
7 Rask-Madsen, C. and Kahn, C. R. (2012) Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. Arterioscler. Thromb. Vasc. Biol. **32**, 2052–2059
8 Velloso, L. A., Folli, F., Sun, X. J., White, M. F., Saad, M. J. and Kahn, C. R. (1996) Cross-talk between the insulin and angiotensin signaling systems. Proc. Natl. Acad. Sci. U.S.A. **93**, 12490–12495
9 Folli, F., Kahn, C. R., Hansen, H., Bouchie, J. L. and Feener, E. P. (1997) Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. J. Clin. Invest. **100**, 2158–2169
10 Calegari, V. C., Alves, M., Picardi, P. K., Inoue, R. Y., Franchini, K. G., Saad, M. J. and Velloso, L. A. (2003) Suppressor of cytokine signaling-3 provides a novel interface in the cross-talk between angiotensin II and insulin signaling systems. Endocrinology **146**, 579–588
11 Carvalheira, J. B., Calegari, V. C., Zecchin, H. G., Nadruz, Jr, W., Guimaraes, R. B., Ribeiro, E. B., Franchini, K. G., Velloso, L. A. and Saad, M. J. (2005) The cross-talk between angiotensin and insulin differentially affects phosphatidylinositol 3-kinase and mitogen-activated protein kinase-mediated signaling in rat heart: implications for insulin resistance. Endocrinology **144**, 5604–5614

12 Velloso, L. A., Folli, F., Perego, L. and Saad, M. J. (2006) The multi-faceted cross-talk between the insulin and angiotensin II signaling systems. Diabetes Metab. Res. Rev. **22**, 98–107
13 Muscogiuri, G., Chavez, A. O., Gastaldelli, A., Perego, L., Tripathy, D., Saad, M. J., Velloso, L. and Folli, F. (2008) The crosstalk between insulin and renin-angiotensin-aldosterone signaling systems and its effect on glucose metabolism and diabetes prevention. Curr. Vasc. Pharmacol. **6**, 301–312
14 Rizza, R., Mandarino, L. J. and Gerich, J. E. (1982) Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor defect of insulin action. J. Clin. Endocrinol. Metab. **54**, 131–138
15 Deibert, D. C. and DeFronzo, R. A. (1980) Epinephrine-induced insulin resistance in man. J. Clin. Invest. **65**, 717–721
16 Houssay, B. A. (1936) The hypophysis and metabolism. N. Engl. J. Med. **214**, 961–986
17 Coppari, R. and Bjørbæk, C. (2012) Leptin revisited: its mechanism of action and potential for treating diabetes. Nat. Rev. Drug. Discov. **11**, 692–708
18 Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N. et al. (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat. Med. **7**, 941–946
19 Henriksen, E. J. (2007) Improvement of insulin sensitivity by antagonism of the renin–angiotensin system. Am. J. Physiol. Regul. Integr. Comp. Physiol. **293**, R974–R980
20 Perkins, J. M. and Davis, S. N. (2008) The renin–angiotensin aldosterone system: a pivotal role in insulin sensitivity and glycemic control. Curr. Opin. Endocrinol. Diabetes Obes. **15**, 147–152
21 Putnam, K., Shoemaker, R., Yiannikouris, F. and Cassis, L. A. (2012) The renin–angiotensin system: a target of and contributor to dyslipidemias, altered glucose homeostasis, and hypertension of the metabolic syndrome Am. J. Physiol. Heart Circ. Physiol. **302**, H1219–H1230
22 Rao, R. H. (1994) Effects of angiotensin II on insulin sensitivity and fasting glucose metabolism in rats. Am. J. Hypertens. **7**, 655–660
23 Richey, J. M., Ader, M., Moore, D. and Bergman, R. N. (1999) Angiotensin II induces insulin resistance independent of changes in interstitial insulin. Am. J. Physiol. **277**, E920–E926
24 Andreozzi, F., Laratta, E., Sciacqua, A., Perticone, F. and Sesti, G. (2004) Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser^{312} and Ser^{616} in human umbilical vein endothelial cells. Circ. Res. **94**, 1211–1218
25 Kim, J. A., Jang, H. J., Martinez-Lemus, L. A. and Sowers, J. R. (2012) Activation of mTOR/p70S6 kinase by ANG II inhibits insulin-stimulated endothelial nitric oxide synthase and vasodilation. Am. J. Physiol. Endocrinol. Metab. **302**, E201–E208
26 Scheen, A. J. (2004) Renin-angiotensin system inhibition prevents type 2 diabetes mellitus. A meta-analysis of randomized clinical trials. Diabetes Metab. **30**, 487–496
27 Jandeleit-Dahm, K. A., Tikellis, C., Reid, C. M, Johnston, C. I. and Cooper, ME. (2005) Why blockade of the renin-angiotensin system reduces the incidence of new-onset diabetes J. Hypertens. **23**, 463–473
28 Prabhakar, S. (2013) Inhibition of renin–angiotensin system: implications for diabetes control and prevention J. Invest. Med. **61**, 551–557
29 Furuhashi, M., Ura, N., Takizawa, H., Yoshida, D., Moniwa, N., Murakami, H., Higashiura, K. and Shimamoto, K. (2004) Blockade of the renin-angiotensin system decreases adipocyte size with improvement in insulin sensitivity. J. Hypertens. **22**, 1977–1982
30 Henriksen, E. J., Jacob, S., Kinnick, T. R., Teachey, M. K. and Krekler, M. (2001) Selective angiotensin II receptor antagonism reduces insulin resistance in obese Zucker rats. Hypertension **38**, 884–890
31 Reference deleted
32 Higashiura, K., Ura, N., Takada, T., Li, Y., Torii, T., Togashi, N., Takada, M., Takizawa, H. and Shimamoto, K. (2000) The effects of an angiotensin-converting enzyme inhibitor and an angiotensin II receptor antagonist on insulin resistance in fructose-fed rats. Am. J. Hypertens. **13**, 290–297
33 Muñoz, M. C., Argentino, D. P., Dominici, F. P., Turyn, D. and Toblli, J. E. (2006) Irbersartan restores the in vivo insulin signaling pathway leading to Akt activation in obese Zucker rats. J. Hypertens. **24**, 1607–1617
34 Nawano, M., Anai, M., Funaki, M., Kobayashi, H., Kanda, A., Fukushima, Y., Inukai, K., Ogihara, T., Sakoda, H., Onishi, Y. et al. (1999) Imidapril, an angiotensin-converting enzyme inhibitor, improves insulin sensitivity by enhancing signal transduction via insulin receptor substrate proteins and improving vascular resistance in the Zucker fatty rat. Metabolism **48**, 1248–1255
35 Carvalho, C. R., Thirone, A. C., Gontijo, J. A., Velloso, L. A. and Saad, M. J. (1997) Effect of captopril, losartan, and bradykinin on early steps of insulin action. Diabetes **46**, 1950–1957
36 Iusuf, D., Henning, R. H., van Gilst, W. H. and Roks, A. J. (2008) Angiotensin-(1–7): pharmacological properties and pharmacotherapeutic perspectives. Eur. J. Pharmacol. **585**, 303–312
37 Ferrario, C. M., Ahmad, S., Joyner, J. and Varagic, J. (2010) Advances in the renin angiotensin system focus on angiotensin-converting enzyme 2 and angiotensin-(1–7). Adv. Pharmacol. **59**, 197–233
38 Zimmerman, D. and Burns, K. D. (2012) Angiotensin-(1–7) in kidney disease: a review of the controversies. Clin. Sci. **123**, 333–346
39 Passos-Silva, D. G., Verano-Braga, T. and Santos, RA. (2013) Angiotensin-(1–7): beyond the cardio-renal actions. Clin. Sci. **124**, 443–456
40 Iyer, S. N., Ferrario, C. M. and Chappell, M. C. (1988) Angiotensin-(1–7) contributes to the antihypertensive effects of blockade of the renin–angiotensin system. Hypertension **31**, 356–361
41 Yamada, K., Iyer, S. N., Chappell, M. C., Brosnihan, K. B., Fukuhara, M. and Ferrario, C. M. (1999) Differential response of angiotensin peptides in the urine of hypertensive animals. Regul. Pept. **80**, 57–66
42 Chappell, M. C., Gomez, M. N., Pirro, N. T. and Ferrario, C. M. (2000) Release of angiotensin-(1–7) from the rat hindlimb: influence of angiotensin-converting enzyme inhibition. Hypertension **35**, 348–352
43 Nussberger, J., Brunner, D. B., Nyfeler, J. A., Linder, L. and Brunner, H. R. (2001) Measurement of immunoreactive angiotensin-(1–7) heptapeptide in human blood. Clin. Chem. **47**, 726–729
44 Hao, P. P., Chen, Y. G., Liu, Y. P., Zhang, M. X., Yang, J. M., Gao, F., Zhang, Y. and Zhang, C. (2013) Association of plasma angiotensin-(1–7) level and left ventricular function in patients with type 2 diabetes mellitus. PLoS ONE **8**, e62788
45 Nogueira, A. I., Souza, Santos, R. A., Simões, E., Silva, A. C., Cabral, A. C., Vieira, R. L., Drumond, T. C., Machado, L. J., Freire, C. M. and Ribeiro-Oliveira, Jr, A. (2007) The pregnancy-induced increase of plasma angiotensin-(1–7) is blunted in gestational diabetes. Regul. Pept. **141**, 55–60
46 Neves, L. A., Stovall, K., Joyner, J., Valdés, G., Gallagher, P. E., Ferrario, C. M., Merrill, D. C. and Brosnihan, K. B. (2008) ACE2 and ANG-(1–7) in the rat uterus during early and late gestation. Am. J. Physiol. Regul. Integr. Comp. Physiol. **294**, R151–R161
47 Chhabra, K. H., Chodavarapu, H. and Lazartigues, E. (2013) Angiotensin converting enzyme 2: A new important player in the regulation of glycemia. IUBMB Life **65**, 731–738

48 Gupte, M., Thatcher, S. E., Boustany-Kari, C. M., Shoemaker, R., Yiannikouris, F., Zhang, X., Karounos, M. and Cassis, L. A. (2012) Angiotensin converting enzyme 2 contributes to sex differences in the development of obesity hypertension in C57BL/6 mice. Arterioscler. Thromb. Vasc. Biol. **32**, 1392–1399

49 Hubbard, S. R. (2013) The insulin receptor: both a prototypical and atypical receptor tyrosine kinase. Cold Spring Harb. Perspect. Biol. **5**, a008946

50 Taniguchi, C. M., Emanuelli, B. and Kahn, C. R. (2006) Critical nodes in signalling pathways: insights into insulin action. Nat. Rev. Mol. Cell Biol. **7**, 85–96

51 Copps, K. D. and White, M. F. (2012) Regulation of insulin sensitivity by serine/threonine phosphorylation of insulin receptor substrate proteins IRS1 and IRS2. Diabetologia **55**, 2565–2582

52 Giorgetti-Peraldi, S., Peyrade, F., Baron, V. and Van Obberghen, E. (1995) Involvement of Janus kinases in the insulin signaling pathway. Eur. J. Biochem. **234**, 656–660

53 Cantley, L. C. (2002) The phosphoinositide 3-kinase pathway. Science **296**, 1655–1657

54 Manning, B. D. and Cantley, L. C. (2007) AKT/PKB signaling: navigating downstream. Cell **129**, 1261–1274

55 Vasudevan, K. M. and Garraway, L. A. (2010) AKT signaling in physiology and disease. Curr. Top. Microbiol. Immunol. **347**, 105–133

56 Maegawa., H., Kashiwagi., A., Fujita, T., Ugi, S., Hasegawa, M., Obata, T., Nishio, Y., Kojima, H., Hidaka, H. and Kikkawa, R. (1996) SHPTP2 serves adapter protein linking between Janus kinase 2 and insulin receptor substrates. Biochem. Biophys. Res. Commun. **228**, 122–127

57 Chen, J., Sadowski, H. B., Kohanski, R. A. and Wang, L. H. (1997) Stat5 is a physiological substrate of the insulin receptor. Proc. Natl. Acad. Sci. U.S.A. **94**, 2295–2300

58 Gual, P., Baron, V., Lequoy, V. and Van Obberghen, E. (1998) Interaction of Janus kinases JAK-1 and JAK-2 with the insulin receptor and the insulin-like growth factor-1 receptor. Endocrinology **139**, 884–893

59 Campos, S. P., Wang, Y. and Baumann, H. (1996) Insulin modulates STAT3 protein activation and gene transcription in hepatic cells. J. Biol. Chem. **271**, 24418–24424

60 Sawka-Verhelle, D., Tartare-Deckert, S., Decaux, J. F., Girard, J. and Van Obberghen, E. (2000) Stat 5B, activated by insulin in a Jak-independent fashion, plays a role in glucokinase gene transcription. Endocrinology **141**, 1977–1988

61 Saad, M. J. A., Carvalho, C. R. O., Thirone, A. C. P. and Velloso, L. A. (1996) Insulin induces tyrosine phosphorylation of JAK2 in insulin-sensitive tissues of the intact rat. J. Biol. Chem. **271**, 22100–22104

62 Velloso, L. A., Carvalho, C. R., Rojas, F. A., Folli, F. and Saad, M. J. (1998) Insulin signalling in heart involves insulin receptor substrates-1 and -2, activation of phosphatidylinositol 3-kinase and the JAK 2-growth related pathway. Cardiovasc. Res. **40**, 96–102

63 Thirone, A. C., JeBailey, L., Bilan, P. J. and Klip, A. (2006) Opposite effect of JAK2 on insulin-dependent activation of mitogen-activated protein kinases and Akt in muscle cells: possible target to ameliorate insulin resistance. Diabetes **55**, 942–951

64 Avruch, J. (2007) MAP kinase pathways: the first twenty years. Biochem. Biophys. Acta **1773**, 1150–1160

65 Siddle, K. (2011) Signalling by insulin and IGF receptors: supporting acts and new players. J. Mol. Endocrinol. **47**, R1–R10

66 Youngren, J. F. (2007) Regulation of insulin receptor function. Cell. Mol. Life Sci. **64**, 873–891

67 Feener, E. P., Backer, J. M., King, G. L., Wilden, P. A., Sun, X. J., Kahn, C. R. and White, M. F. (1993) Insulin stimulates serine and tyrosine phosphorylation in the juxtamembrane region of the insulin receptor. J. Biol. Chem. **268**, 11256–11264

68 Coba, M. P., Muñoz, M. C., Dominici, F. P., Toblli, J. E., Pena, C., Bartke, A. and Turyn, D. (2004) Increased *in vivo* phosphorylation of insulin receptor at serine 994 in the liver of obese insulin-resistant Zucker rats. J. Endocrinol. **182**, 433–444

69 Liu, F. and Roth, R. A. (1994) Identification of serines-1035/1037 in the kinase domain of the insulin receptor as protein kinase C α mediated phosphorylation sites. FEBS Lett. **352**, 389–392

70 Ahn, J., Donner, D. B. and Rosen, O. M. (1993) Interaction of the human insulin receptor tyrosine kinase from the baculo-virus expression system with protein kinase C in a cell-free system. J. Biol. Chem. **268**, 7571–7576

71 Al Hasani, H., Eisermann, B., Tennagels, N., Magg, C., Passlack, W., Koenen, M., Muller-Wieland, D., Meyer, H. E. and Klein, H. W. (1997) Identification of Ser-1275 and Ser-1309 as autophosphorylation sites of the insulin receptor. FEBS Lett. **400**, 65–70

72 Schmitz-Peiffer, C. (2002) Protein kinase C and lipid-induced insulin resistance in skeletal muscle. Ann. N.Y. Acad. Sci. **967**, 146–157

73 Itani, S. I., Zhou, Q., Pories, W. J., MacDonald, K. G. and Dohm, G. L. (2000) Involvement of protein kinase C in human skeletal muscle insulin resistance and obesity. Diabetes **49**, 1353–1358

74 Cortright, R. N., Azevedo, Jr, J. L., Zhou, Q., Sinha, M., Pories, W. J., Itani, S. I. and Dohm, G. L. (2000) Protein kinase C modulates insulin action in human skeletal muscle. Am. J. Physiol. Endocrinol. Metab. **278**, E553–E562

75 Pillay, T. S., Xiao, S., Keranen, L. and Olefsky, J. M. (2004) Regulation of the insulin receptor by protein kinase C isoenzymes: preferential interaction with β isoenzymes and interaction with the catalytic domain of βII. Cell. Signal. **16**, 97–104

76 Nguyen, M. T., Satoh, H., Favelyukis, S., Babendure, J. L., Imamura, T., Sbodio, J. I., Zalevsky, J., Dahiyat, B. I., Chi, N. W. and Olefsky, J. M. (2005) JNK and tumor necrosis factor-α mediate free fatty acid-induced insulin resistance in 3T3-L1 adipocytes. J. Biol. Chem. **280**, 35361–35371

77 Muñoz, M. C., Giani, J. F., Mayer, M. A., Toblli, J. E., Turyn, D. and Dominici, F. P. (2009) TANK-binding kinase 1 mediates phosphorylation of insulin receptor at serine residue 994: a potential link between inflammation and insulin resistance. J. Endocrinol. **201**, 185–197

78 Mothe, I. and Van Obberghen, E. (1996) Phosphorylation of insulin receptor substrate-1 on multiple serine residues, 612, 632, 662, and 731, modulates insulin action. J. Biol. Chem. **271**, 11222–11227

79 Aguirre, V., Uchida, T., Yenush, L., Davis, R. and White, M. F. (2000) The c-Jun NH2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser^{307}. J. Biol. Chem. **275**, 9047–9054

80 Gao, Z., Hwang, D., Bataille, F., Lefevre, M, York, D., Quon, M. J. and Ye, J. (2002) Serine phosphorylation of insulin receptor substrate 1 by inhibitor B kinase complex. J. Biol. Chem. **277**, 48115–48121

81 Solinas, G. and Karin, M. (2010) JNK1 and IKKβ: molecular links between obesity and metabolic dysfunction. FASEB J. **24**, 2596–2611

82 Rui, L., Aguirre, V., Kim, J. K., Shulman, G. I., Lee, A., Corbould, A., Dunaif, A. and White, M. F. (2001) Insulin/IGF-1 and TNF-α stimulate phosphorylation of IRS-1 at inhibitory Ser^{307} via distinct pathways. J. Clin. Invest. **107**, 181–189

83 Aguirre, V., Werner, E. D., Giraud, J., Lee, Y. H., Shoelson, S. E. and White, M. F. (2002) Phosphorylation of Ser^{307} in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J. Biol. Chem. **277**, 1531–1537

84 Carlson, C. J., White, M. F. and Rondinone, C. M. (2004) Mammalian target of rapamycin regulates IRS-1 serine 307 phosphorylation. Biochem. Biophys. Res. Commun. **316**, 533–539
85 Giani, J. F., Mayer, M. A., Muñoz, M. C., Silberman, E., Höcht, C., Taira, C., Gironacci, M. M., Turyn, D. and Dominici, F. P. (2009) Chronic infusion of angiotensin-(1–7) improves insulin resistance and hypertension induced by a high-fructose diet in rats. Am. J. Physiol. Endocrinol. Metab. **296**, E262–E271
86 Andreozzi, F., Laratta, E., Sciacqua, A., Perticone, F. and Sesti, G. (2004) Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser^{312} and Ser^{616} in human umbilical vein endothelial cells. Circ. Res. **94**, 1211–1218
87 Bouzakri, K., Roques, M., Gual, P., Espinosa, S., Guebre-Egziabher, F., Riou, J. P., Laville, M., Le Marchand-Brustel, Y., Tanti, J. F. and Vidal, H. (2003) Reduced activation of phosphatidylinositol-3 kinase and increased serine 636 phosphorylation of insulin receptor substrate-1 in primary culture of skeletal muscle cells from patients with type 2 diabetes. Diabetes **52**, 1319–1325
88 Sampaio, W. O., Souza dos Santos, R. A., Faria-Silva, R., Mata Machado, L. T., Schiffrin, E. L. and Touyz, R. M. (2007) Angiotensin-(1–7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. Hypertension **49**, 185–192
89 Giani, J. F., Gironacci, M. M., Muñoz, M. C., Peña, C., Turyn, D. and Dominici, F. P. (2007) Angiotensin-(1–7) stimulates the phosphorylation of JAK2, IRS-1 and Akt in rat heart *in vivo*: role of the AT1 and Mas receptors. Am. J. Physiol. Heart Circ. Physiol. **293**, H1154–H1163
90 Giani, J. F., Gironacci, M. M., Muñoz, M. C., Turyn, D. and Dominici, F. P. (2008) Angiotensin-(1–7) has a dual role on growth-promoting signalling pathways in rat heart *in vivo* by stimulating STAT3 and STAT5a/b phosphorylation and inhibiting angiotensin II-stimulated ERK1/2 and Rho kinase activity. Exp. Physiol. **93**, 570–578
91 Muñoz, M. C., Giani, J. F. and Dominici, F. P. (2010) Angiotensin-(1–7) stimulates the phosphorylation of Akt in rat extracardiac tissues *in vivo* via receptor Mas. Regul. Pept. **161**, 1–7
92 Muñoz, M. C., Giani, J. F., Burghi, V., Mayer, M. A., Carranza, A., Taira, C. A. and Dominici, F. P. (2012) The Mas receptor mediates modulation of insulin signaling by angiotensin-(1–7). Regul. Pept. **177**, 1–11
93 Verano-Braga, T., Schwämmle, V., Sylvester, M., Passos-Silva, D. G., Peluso, A. A., Etelvino, G. M., Santos, R. A. and Roepstorff, P. (2012) Time-resolved quantitative phosphoproteomics: new insights into angiotensin-(1–7) signaling networks in human endothelial cells. J. Proteome Res. **11**, 3370–3381
94 Marrero, M. B., Schieffer, B., Paxton, W. G., Heerdt, L., Berk, B. C., Delafontaine, P. and Bernstein, K. E. (1995) Direct stimulation of Jak/STAT pathway by the angiotensin II AT1 receptor. Nature **375**, 247–250
95 Kodama, H., Fukuda, K., Pan, J., Makino, S., Sano, M., Takahashi, T., Hori, S. and Ogawa, S. (1998) Biphasic activation of the JAK/STAT pathway by angiotensin II in rat cardiomyocytes. Circ. Res. **82**, 244–250
96 McWhinney, C. D., Hunt, R. A., Conrad, K. M., Dostal, D. E. and Baker, K. M. (1997) The type I angiotensin II receptor couples to Stat1 and Stat3 activation through Jak2 kinase in neonatal rat cardiac myocytes. J. Mol. Cell. Cardiol. **29**, 2513–2524
97 Guilluy, C., Brégeon, J., Toumaniantz, G., Rolli-Derkinderen, M., Retailleau, K., Loufrani, L., Henrion, D., Scalbert, E., Bril, A., Torres, R. M. et al. (2010) The Rho exchange factor Arhgef1 mediates the effects of angiotensin II on vascular tone and blood pressure. Nat. Med. **16**, 183–190
98 Banes-Berceli, A. K., Al-Azawi, H., Proctor, D., Qu, H., Femminineo, D., Hill-Pyror, C., Webb, R. C. and Brands, M. W. (2011) Angiotensin II utilizes Janus kinase 2 in hypertension, but not in the physiological control of blood pressure, during low-salt intake. Am. J. Physiol. Regul. Integr. Comp. Physiol. **301**, R1169–R1176
99 Marrero, M. B., Fulton, D., Stepp, D. and Stern, D. M. (2005) Angiotensin II-induced signaling pathways in diabetes. Curr. Diabetes Rev. **1**, 197–202
100 Saad, M. J., Velloso, L. A. and Carvalho, C. R. (1995) Angiotensin II induces tyrosine phosphorylation of insulin receptor substrate 1 and its association with phosphatidylinositol 3-kinase in rat heart. Biochem. J. **310**, 741–744
101 Ali, M. S., Schieffer, B., Delafontaine, P., Bernstein, K. E., Ling, B. N. and Marrero, M. B. (1997) Angiotensin II stimulates tyrosine phosphorylation and activation of insulin receptor substrate 1 and protein-tyrosine phosphatase 1D in vascular smooth muscle cells. J. Biol. Chem. **272**, 12373–12379
102 White, M. F. (1997) The insulin signalling system and the IRS proteins. Diabetologia **40**, S2–S17
103 Shaw, L. M. (2011) The insulin receptor substrate (IRS) proteins: at the intersection of metabolism and cancer. Cell Cycle **10**, 1750–1756
104 Bosnyak, S., Jones, E. S., Christopoulos, A., Aguilar, M. I., Thomas, W. G. and Widdop, R. E. (2011) Relative affinity of angiotensin peptides and novel ligands at AT_1 and AT_2 receptors. Clin. Sci. **121**, 297–303
105 Rowe, B. P., Saylor, D. L., Speth, R. C. and Absher, D. R. (1995) Angiotensin-(1–7) binding at angiotensin II receptors in the rat brain. Regul. Pept. **56**, 139–146
106 Handa, R. K., Ferrario, C. M. and Strandhoy, J. W. (1996) Renal actions of angiotensin-(1–7): *in vivo* and *in vitro* studies. Am. J. Physiol. **270**, F141–F147
107 Tallant, E. A., Lu, X., Weiss, R. B., Chappell, M. C. and Ferrario, CM. (1997) Bovine aortic endothelial cells contain an angiotensin-(1–7) receptor. Hypertension **29**, 388–393
108 Pfeilschifter, J., Huwiler, A., Merriweather, C. and Briner, V. A. (1992) Angiotensin II stimulation of phospholipase D in rat renal mesangial cells is mediated by the AT_1 receptor subtype. Eur. J. Pharmacol. **225**, 57–62
109 Osei, S. Y., Ahima, R. S., Minkes, R. K., Weaver, J. P., Khosla, M. C. and Kadowitz, P. J. (1993) Differential responses to angiotensin-(1–7) in the feline mesenteric and hindquarters vascular beds. Eur. J. Pharmacol. **234**, 35–42
110 Garcia, N. H. and Garvin, J. L. (1994) Angiotensin 1–7 has a biphasic effect on fluid absorption in the proximal straight tubule. J. Am. Soc. Nephrol. **5**, 1133–1138
111 Oudot, A., Vergely, C., Ecarnot-Laubriet, A. and Rochette, L. (2005) Pharmacological concentration of angiotensin-(1–7) activates NADPH oxidase after ischemia-reperfusion in rat heart through AT_1 receptor stimulation. Regul. Pept. **127**, 101–110
112 Lara, L. S., De Carvalho, T., Leão-Ferreira, L. R., Lopes, A. G. and Caruso-Neves, C. (2005) Modulation of the $(Na^+ + K^+)$ATPase activity by angiotensin-(1–7) in MDCK cells. Regul. Pept. **129**, 221–226
113 Von Bohlen, O., Halbach, U., Walther, T., Bader, M. and Albrecht, D. (2000) Interaction between Mas and the angiotensin AT_1 receptor in the amygdala. J. Neurophysiol. **83**, 2012–2021
114 Kostenis, E., Milligan, G, Christopoulos, A., Sanchez-Ferrer, C. F., Heringer-Walther, S., Sexton, P. M., Gembardt, F., Kellett, E., Martini, L., Vanderheyden, P. et al. (2005) G-protein-coupled receptor Mas is a physiological antagonist of the angiotensin II type 1 receptor. Circulation **111**, 1806–1813
115 Clark, M. A., Tallant, E. A. and Diz, D. I. (2001) Downregulation of the AT_{1A} receptor by pharmacologic concentrations of angiotensin-(1–7). J. Cardiovasc. Pharmacol. **37**, 437–448

116 Clark, M. A., Tallant, E. A., Tommasi, E., Bosch, S. and Diz, D. I. (2003) Angiotensin-(1–7) reduces renal angiotensin II receptors through a cyclooxygenase-dependent mechanism. J. Cardiovasc. Pharmacol. **41**, 276–283

117 Dias-Peixoto, M. F., Santos, R. A., Gomes, E. R., Alves, M. N., Almeida, P. W., Greco, L., Rosa, M., Fauler, B., Bader, M., Alenina, N. and Guatimosim, S. (2008) Molecular mechanisms involved in the angiotensin-(1–7)/Mas signaling pathway in cardiomyocytes. Hypertension **52**, 542–548

118 Oh, Y. B., Kim, J. H., Park, B. M., Park, B. H. and Kim, S. H. (2012) Captopril intake decreases body weight gain via angiotensin-(1–7). Peptides **37**, 79–85

119 Than, A., Leow, M. K. and Chen, P. (2013) Control of adipogenesis by the autocrine interplays between angiotensin (1–7)/mas receptor and angiotensin II/AT_1 receptor signaling pathways. J. Biol. Chem. **288**, 15520–15531

120 Jarajapu, Y. P., Bhatwadekar, A. D., Caballero, S., Hazra, S., Shenoy, V., Medina, R., Kent, D., Stitt, A. W., Thut, C., Finney, E. M. et al. (2013) Activation of the ACE2/angiotensin-(1–7)/Mas receptor axis enhances the reparative function of dysfunctional diabetic endothelial progenitors. Diabetes **62**, 1258–1269

121 Shah, A., Gul, R., Yuan, K., Gao, S., Oh, Y. B., Kim, U. H. and Kim, S. H. (2010) Angiotensin-(1–7) stimulates high atrial pacing-induced ANP secretion via Mas/PI3-kinase/Akt axis and Na^+/H^+ exchanger. Am. J. Physiol. Heart Circ. Physiol. **298**, H1365–H1374

122 Dominguez, V., Raimondi, C., Somanath, S., Bugliani, M., Loder, M. K., Edling, C. E., Divecha, N., da Silva-Xavier, G., Marselli, L., Persaud, S. J. et al. (2011) Class II phosphoinositide 3-kinase regulates exocytosis of insulin granules in pancreatic beta cells. J. Biol. Chem. **286**, 4216–4225

123 Falasca, M., Hughes, W. E., Dominguez, V., Sala, G., Fostira, F., Fang, M. Q., Cazzolli, R., Shepherd, P. R., James, D. E. and Maffucci, T. (2007) The role of phosphoinositide 3-kinase C2α in insulin signaling. J. Biol. Chem. **282**, 28226–28236

124 Leibiger, B., Moede, T., Uhles, S., Barker, C. J., Creveaux, M., Domin, J., Berggren, P. O. and Leibiger, I. B. (2010) Insulin-feedback via PI3K-C2α activated PKBα/Akt1 is required for glucose-stimulated insulin secretion. FASEB J. **24**, 1824–1837

125 Xu, P., Costa-Goncalves, A. C., Todiras, M., Rabelo, L. A., Sampaio, W. O., Moura, M. M., Santos, S. S., Luft, F. C., Bader, M., Gross, V. et al. (2008) Endothelial dysfunction and elevated blood pressure in MAS gene-deleted mice. Hypertension **51**, 574–580

126 Rayasam, G. V., Tulasi, V. K., Sodhi, R., Davis, J. A. and Ray, A. (2009) Glycogen synthase kinase 3: more than a namesake. Br. J. Pharmacol. **156**, 885–898

127 Sutherland, C., Leighton, I. A. and Cohen, P. (1993) Inactivation of glycogen synthase kinase-3β by phosphorylation: new kinase connections in insulin and growth-factor signalling. Biochem. J. **296**, 15–19

128 Kane, S., Sano, H., Liu, S. C., Asara, J. M., Lane, W. S., Garner, C. C. and Lienhard, G. E. (2002) A method to identify serine kinase substrates. Akt phosphorylates a novel adipocyte protein with a Rab GTPase-activating protein (GAP) domain. J. Biol. Chem. **277**, 22115–22118

129 Sano, H., Kane, S., Sano, E., Mîinea, C. P., Asara, J. M., Lane, W. S., Garner, C. W. and Lienhard, G. E. (2003) Insulin-stimulated phosphorylation of a Rab GTPase-activating protein regulates GLUT4 translocation. J. Biol. Chem. **278**, 14599–14602

130 Kramer, H. F., Witczak, C. A., Taylor, E. B., Fujii, N., Hirshman, M. F. and Goodyear, L. J. (2006) AS160 regulates insulin- and contraction-stimulated glucose uptake in mouse skeletal muscle. J. Biol. Chem. **281**, 31478–31485

131 Wang, H. Y., Ducommun, S., Quan, C., Xie, B., Li, M., Wasserman, D. H., Sakamoto, K., Mackintosh, C. and Chen, S. (2013) AS160 deficiency causes whole-body insulin resistance via composite effects in multiple tissues. Biochem. J. **449**, 479–489

132 Liu, C., Lv, X. H., Li, H. X., Cao, X., Zhang, F., Wang, L., Yu, M. and Yang, J. K. (2012) Angiotensin-(1–7) suppresses oxidative stress and improves glucose uptake via Mas receptor in adipocytes. Acta Diabetol. **49**, 291–299

133 Tirosh, A., Potashnik, R., Bashan, N. and Rudich, A. (1999) Oxidative stress disrupts insulin-induced cellular redistribution of insulin receptor substrate-1 and phosphatidylinositol 3-kinase in 3T3-L1 adipocytes. A putative cellular mechanism for impaired protein kinase B activation and GLUT4 translocation. J. Biol. Chem. **274**, 10595–10602

134 Blair, A. S., Hajduch, E., Litherland, G. J. and Hundal, H. S. (1999) A putative cellular mechanism for impaired protein kinase B activation and GLUT4 translocation. Regulation of glucose transport and glycogen synthesis in L6 muscle cells during oxidative stress. Evidence for cross-talk between the insulin and SAPK2/p38 mitogen-activated protein kinase signaling pathways. J. Biol Chem. **274**, 36293–36299

135 Accili, D. and Arden, K. C. (2004) FOXOs at the crossroads of cellular metabolism, differentiation, and transformation. Cell **117**, 421–426

136 Matsumoto, M., Han, S., Kitamura, T. and Accili, D. (2006) Dual role of transcription factor FOXO1 in controlling hepatic insulin sensitivity and lipid metabolism. J. Clin. Invest. **116**, 2464–2472

137 Puig, O. and Tjian, R. (2006) Nutrient availability and growth: regulation of insulin signaling by dFOXO/FOXO1. Cell Cycle **5**, 503–505

138 Dong, X. C., Copps, K. D., Guo, S., Li, Y., Kollipara, R., DePinho, R. A. and White, M. F. (2008) Inactivation of hepatic FOXO1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. Cell Metab. **8**, 65–76

139 Santos, R. A. S., Ferreira, A. J., Verano-Braga, T. and Bader, M. (2013) Angiotensin-converting enzyme 2, angiotensin-(1–7) and Mas: new players of the renin–angiotensin system. J. Endocrinol. **216**, R1–R17

140 Tassone, E. J., Sciacqua, A., Andreozzi, F., Presta, I., Perticone, M., Carnevale, D., Casaburo, M., Hribal, M. L., Sesti, G. and Perticone, F. (2013) Angiotensin (1–7) counteracts the negative effect of angiotensin II on insulin signalling in HUVECs. Cardiovasc. Res. **99**, 129–136

141 Prasannarong, M., Santos, F. R. and Henriksen, E. J. (2012) ANG-(1–7) reduces ANG II-induced insulin resistance by enhancing Akt phosphorylation via a Mas receptor-dependent mechanism in rat skeletal muscle. Biochem. Biophys. Res. Commun. **426**, 369–373

142 Olivares-Reyes, J. A., Arellano-Plancarte, A. and Ramon Castillo-Hernandez, J. (2009) Angiotensin II and the development of insulin resistance: implications for diabetes. Mol. Cell. Endocrinol. **302**, 128–139

143 Huisamen, B., Pêrel, S. J., Friedrich, S. O., Salie, R., Strijdom, H. and Lochner, A. (2011) ANG II type I receptor antagonism improved nitric oxide production and enhanced eNOS and PKB/Akt expression in hearts from a rat model of insulin resistance. Mol. Cell. Biochem. **349**, 21–31

144 Nakayama, M., Inoguchi, T., Sonta, T., Maeda, Y., Sasaki, S., Sawada, F., Tsubouchi, H., Sonoda, N., Kobayashi, K., Sumimoto, H. and Nawata, H. (2005) Increased expression of NAD(P)H oxidase in islets of animal models of Type 2 diabetes and its improvement by an AT_1 receptor antagonist. Biochem. Biophys. Res. Commun. **332**, 927–933

145 Wei, Y., Sowers, J. R., Clark, S. E., Li, W., Ferrario, C. M. and Stump, C. S. (2008) Angiotensin II-induced skeletal muscle insulin resistance mediated by NF-κB activation via NADPH oxidase Am. J. Physiol. Endocrinol. Metab. **294**, E345–E351

146 Diamond-Stanic, M. K. and Henriksen, E. J. (2010) Direct inhibition by angiotensin II of insulin-dependent glucose transport activity in mammalian skeletal muscle involves a ROS-dependent mechanism. Arch. Physiol. Biochem. **116**, 88–95

147 Ushio-Fukai, M., Alexander, R. W., Akers, M. and Griendling, K. K. (1998) p38MAPK is a critical component of the oxidant stress (H_2O_2)-sensitive signaling pathways activated by angiotensin II in vascular smooth muscle. cells **273**, 15022–15029

148 Eguchi, S., Dempsey, P. J., Frank, G. D., Motley, E. D. and Inagami, T. (2001) Activation of MAPKs by angiotensin II in vascular smooth muscle cells. Metalloprotease-dependent EGF receptor activation is required for activation of ERK and p38 MAPK but not for JNK. J. Biol. Chem. **276**, 7957–7962

149 Hemi, R., Yochananov, Y., Barhod, E., Kasher-Meron, M., Karasik, A., Tirosh, A. and Kanety, H. (2011) p38 mitogen-activated protein kinase-dependent transactivation of ErbB receptor family: a novel common mechanism for stress-induced IRS-1 serine phosphorylation and insulin resistance. Diabetes **60**, 1134–1145

150 Taniyama, Y., Hitomi, H., Shah, A., Alexander, R. W. and Griendling, K. K. (2005) Mechanisms of reactive oxygen species-dependent downregulation of insulin receptor substrate-1 by angiotensin II. Arterioscler. Thromb. Vasc. Biol. **25**, 1142–1147

151 Izawa, Y., Yoshizumi, M., Fujita, Y., Ali, N., Kanematsu, Y., Ishizawa, K., Tsuchiya, K., Obata, T., Ebina, Y., Tomita, S. and Tamaki, T. (2005) ERK1/2 activation by angiotensin II inhibits insulin-induced glucose uptake in vascular smooth muscle cells. Exp. Cell. Res. **308**, 291–299

152 Motley, E. D., Eguchi, K., Gardner, C., Hicks, A. L., Reynolds, C. M., Frank, G. D., Mifune, M., Ohba, M. and Eguchi, S. (2003) Insulin-induced Akt activation is inhibited by angiotensin II in the vasculature through protein kinase C-α. Hypertension **41**, 775–780

153 Zhu, Z., Zhong, J., Zhu, S., Liu, D., Van Der Giet, M. and Tepel, M. (2002) Angiotensin-(1–7) inhibits angiotensin II-induced signal transduction. J. Cardiovasc. Pharmacol. **40**, 693–700

154 Su, Z., Zimpelmann, J. and Burns, K. D. (2006) Angiotensin-(1–7) inhibits angiotensin II-stimulated phosphorylation of MAP kinases in proximal tubular cells. Kidney Int. **69**, 2212–2218

155 Sampaio, W. O., Henrique de Castro, C., Santos, R. A., Schiffrin, E. L. and Touyz, R. M. (2007) Angiotensin-(1–7) counterregulates angiotensin II signaling in human endothelial cells. Hypertension **50**, 1093–1098

156 Yang, Y. J., Hope, I. D., Ader, M. and Bergman, R. N. (1989) Insulin transport across capillaries is rate limiting for insulin action in dogs. J. Clin. Invest. **84**, 1620–1628

157 Barrett, E., Eggleston, E., Inyard, A., Wang, H., Li, G., Chai, W. and Liu, Z. (2009) The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. Diabetologia **52**, 752–764

158 Barrett, E. J., Wang, H., Upchurch, C. T. and Liu, Z. (2011) Insulin regulates its own delivery to skeletal muscle by feed-forward actions on the vasculature. Am. J. Physiol. Endocrinol. Metab. **301**, E252–E263

159 Wang, N., Chai, W., Zhao, L., Tao, L., Cao, W. and Liu, Z. (2013) Losartan increases muscle insulin delivery and rescues insulin's metabolic action during lipid infusion via microvascular recruitment. Am. J. Physiol. Endocrinol. Metab. **304**, E538–E545

160 Marcus, Y., Shefer, G., Sasson, K., Kohen, F., Limor, R., Pappo, O., Nevo, N., Biton, I., Bach, M., Berkutzki, T. et al. (2013) Angiotensin-1–7 as means to prevent the metabolic syndrome: lessons from the fructose-fed rat model. Diabetes **62**, 1121–1130

161 Santos, S. H. S., Felix Braga, J., Guilhen Mario, E., Pôrto, L. C. J., Rodrigues-Machado, M. G., Murari, A., Botion, L. M., Alenina, N., Bader, M. and Santos, R. A. S. (2010) Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1–7). Arterioscler. Thromb. Vasc. Biol. **30**, 953–961

162 Bilman, V., Mares-Guia, L., Nadu, A. P., Bader, M., Campagnole-Santos, M. J., Santos, R. A. and Santos, S. H. (2012) Decreased hepatic gluconeogenesis in transgenic rats with increased circulating angiotensin-(1–7). Peptides **37**, 247–251

163 Santos, S. H., Fernandes, L. H., Pereira, C. S, Guimaraes, A. L., de Paula, A. M., Campagnole-Santos, M. J., Alvarez-Leite, J. I., Bader, M. and Santos, R. A (2012) Increased circulating angiotensin-(1–7) protects white adipose tissue against development of a proinflammatory state stimulated by a high-fat diet. Regul. Pept. **178**, 64–70

164 Abuissa, H., Jones, P. G., Marso, S. P. and O'Keefe, Jr, J. H. (2005) Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for prevention of type 2 diabetes. A meta-analysis of randomized clinical trials. J. Am. Coll. Cardiol. **46**, 821–826

165 Padwal, R. and Laupacis, A. (2004) Antihypertensive therapy and incidence of type 2 diabetes. A systematic review. Diabetes Care **27**, 247–255

166 Iwai, M., Nakaoka, H., Senba, I., Kanno, H., Moritani, T. and Horiuchi, M. (2012) Possible involvement of angiotensin-converting enzyme 2 and Mas activation in inhibitory effects of angiotensin II Type 1 receptor blockade on vascular remodeling. Hypertension **60**, 137–144

167 Takeda, M., Yamamoto, K., Takemura, Y., Takeshita, H., Hongyo, K., Kawai, T., Hanasaki-Yamamoto, H., Oguro, R., Takami, Y., Tatara, Y. et al. (2013) Loss of ACE2 exaggerates high-calorie diet-induced insulin resistance by reduction of GLUT4 in mice. Diabetes **62**, 223–233

168 Bindom, S. M., Hans, C. P., Xia, H., Boulares, A. H. and Lazartigues, E. (2010) Angiotensin I-converting enzyme type 2 (ACE2) gene therapy improves glycemic control in diabetic mice. Diabetes **59**, 2540–2548

169 Chhabra, K. H., Xia, H., Pedersen, K. B., Speth, R. C. and Lazartigues, E. (2013) Pancreatic angiotensin-converting enzyme 2 improves glycemia in angiotensin II-infused mice. Am. J. Physiol. Endocrinol. Metab. **304**, E874–E884

170 Park, S., Bivona, B. J., Ford, Jr, S. M., Hiroyuki Kobori, S. X., de Garavilla, L. and Harrison-Bernard, L. M. (2013) Direct evidence for intrarenal chymase-dependent angiotensin II formation on the diabetic renal microvasculature. Hypertension **61**, 465–471

171 Santos, S. H., Fernandes, L. R., Mario, E. G., Ferreira, A. V., Pôrto, L. C., Alvarez-Leite, J. I., Botion, L. M., Bader, M., Alenina, N. and Santos, R. A. (2008) Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism. Diabetes **57**, 340–347

172 Mario, E. G., Santos, S. H., Ferreira, A. V., Bader, M., Santos, R. A. and Botion, L. M. (2012) Angiotensin-(1–7) Mas-receptor deficiency decreases peroxisome proliferator-activated receptor γ expression in adipocytes. Peptides **33**, 174–177

173 Benter, I. F., Yousif, M. H. M, Cojocel, C., Al-Maghrebi, M. and Diz, D. I. (2007) Angiotensin-(1–7) prevents diabetes-induced cardiovascular dysfunction. Am. J. Physiol. Heart. Circ. Physiol. **292**, H666–H672

174 Singh, K., Singh, T. and Sharma, P. L. (2011) Beneficial effects of angiotensin-(1–7) in diabetic rats with cardiomyopathy. Ther. Adv. Cardiovasc. Dis. **5**, 159–167

175 Ebermann, L., Spillmann, F., Sidiropoulos, M., Escher, F., Heringer-Walther, S., Schultheiss, H. P., Tschope, C. and Walther, T. (2008) The angiotensin-(1–7) receptor agonist AVE0991 is cardioprotective in diabetic rats. Eur. J. Pharmacol. **590**, 276–280
176 Fraga-Silva, R. A., Costa-Fraga, F. P., Murça, T. M., Moraes, P. L., Martins Lima, A., Lautner, R. Q., Castro, C. H., Soares, C. M. A., Borges, C. L., Nadu, A. P. et al. (2013) Angiotensin-converting enzyme 2 activation improves endothelial function. Hypertension **61**, 1233–1238
177 Akhtar, S., Yousif, M. H., Dhaunsi, G. S., Chandrasekhar, B., Al-Farsi, O. and Benter, I. F. (2012) Angiotensin-(1–7) inhibits epidermal growth factor receptor transactivation via a Mas receptor-dependent pathway. Br. J. Pharmacol. **165**, 1390–1400
178 Patel, V. B., Bodiga, S., Basu, R., Das Subhash, K., Wang, W., Wang, Z., Lo, J., Grant, M. B., JiuChang, Z., Zamaneh, K. and Oudit, G. Y. (2012) Loss of angiotensin-converting enzyme-2 exacerbates diabetic cardiovascular complications and leads to systolic and vascular dysfunction: a critical role of the angiotensin II/AT1 receptor axis. Circ. Res. **110**, 1322–1335
179 Giani, J. F., Muñoz, M. C., Mayer, M. A., Veiras, L. C., Arranz, C., Taira, C. A., Turyn, D., Toblli, J. E. and Dominici, F. P. (2010) Angiotensin-(1–7) improves cardiac remodeling and inhibits growth-promoting pathways in the heart of fructose-fed rats. Am. J. Physiol. Heart Circ. Physiol. **298**, H1003–H1013
180 Benter, I. F., Yousif, M. H., Dhaunsi, G. S., Kaur, J., Chappell, M. C. and Diz, D. I. (2008) Angiotensin-(1–7) prevents activation of NADPH oxidase and renal vascular dysfunction in diabetic hypertensive rats. Am. J. Nephrol. **28**, 25–33
181 Singh, T., Singh, K. and Sharma, P. L. (2010) Ameliorative potential of angiotensin-1–7/Mas receptor axis in streptozotocin-induced diabetic nephropathy in rats. Methods Find. Exp. Clin. Pharmacol. **32**, 19–25
182 Moon, J-Y., Tanimoto, M., Gohda, T., Hagiwara, S., Yamazaki, T., Ohara, I., Murakoshi, M., Aoki, T., Ishikawa, T., Lee, S-H. et al. (2011) Attenuating effect of angiotensin-(1–7) on angiotensin II-mediated NAD(P)H oxidase activation in type 2 diabetic nephropathy of KK-A^y/Ta mice. Am. J. Physiol. Renal Physiol. **300**, F1271–F1282
183 Dhaunsi, G. S., Yousif, M. H., Akhtar, S., Chappell, M. C., Diz, D. I. and Benter, I. F. (2010) Angiotensin-(1–7) prevents diabetes-induced attenuation in PPAR-γ and catalase activities. Eur. J. Pharmacol. **638**, 108–114
184 Giani, J. F., Burghi, V., Veiras, L. C., Tomat, A., Muñoz, M. C., Cao, G., Turyn, D., Toblli, J. E. and Dominici, F. P. (2012) Angiotensin-(1–7) attenuates diabetic nephropathy in Zucker diabetic fatty rats. Am. J. Physiol. Renal Physiol. **302**, F1606–F1615
185 Shiota, A., Yamamoto, K., Ohishi, M., Tatara, Y., Ohnishi, M., Maekawa, Y., Iwamoto, Y., Takeda, M. and Rakugi, H. (2010) Loss of ACE2 accelerates time-dependent glomerular and tubulointerstitial damage in streptozotocin-induced diabetic mice. Hypertens. Res. **33**, 298–307
186 Nadarajah, R., Milagres, R., Dilauro, M., Gutsol, A., Xiao, F., Zimpelmann, J., Kennedy, C., Wysocki, J., Batlle, D. and Burns, K. D. (2012) Podocyte-specific overexpression of human angiotensin-converting enzyme 2 attenuates diabetic nephropathy in mice. Kidney Int. **82**, 292–303
187 Yousif, M. H., Dhaunsi, G. S., Makki, B. M., Qabazard, B. A., Akhtar, S. and Benter, I. F. (2012) Characterization of angiotensin-(1–7) effects on the cardiovascular system in an experimental model of type-1 diabetes. Pharmacol. Res. **66**, 269–275
188 Verma, A., Shan, Z., Lei, B., Yuan, L., Liu, X., Nakagawa, T., Grant, M. B., Lewin, A. S., Hauswirth, W. W., Raizada, M. K. and Li, Q. (2012) ACE2 and Ang-(1–7) confer protection against development of diabetic retinopathy. Mol. Ther. **20**, 28–36
189 Rodgers, K., Verco, S., Bolton, L. and Dizerega, G. (2011) Accelerated healing of diabetic wounds by NorLeu(3)-angiotensin (1–7). Expert Opin. Investig. Drugs **20**, 1575–1581
190 Lula, I., Denadai, A. L., Resende, J. M., Sousa, F. B., Lima, G. F., Pilo-Veloso, D., Heine, T., Duarte, H. A., Santos, R. A. S. and Sinisterra, R. D. (2007) Study of angiotensin-(1–7) vasoactive peptide and its β-cyclodextrin inclusion complexes: complete sequence-specific NMR assigments and structural studies. Peptides **28**, 2199–2210
191 Santos, S. H., Andrade, J. M., Fernandes, L. R., Sinisterra, R. D., Sousa, F. B., Feltenberger, J. D., Alvarez-Leite, J. I. and Santos, R. A. (2013) Oral angiotensin-(1–7) prevented obesity and hepatic inflammation by inhibition of resistin/TLR4/MAPK/NF-κB in rats fed with high-fat diet. Peptides **46**, 47–52
192 Feltenberger, J. D., Andrade, J. M., Paraíso, A., Barros, L. O., Filho, A. B., Sinisterra, R. D., Sousa, F. B., Guimarães, A. L., de Paula, A. M., Campagnole-Santos, M. J. et al. (2013) Oral formulation of angiotensin-(1–7) improves lipid metabolism and rrevents high-fat diet-induced hepatic steatosis and inflammation in mice. Hypertension **62**, 324–330

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