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Etiology and Pathophysiology

The renin-angiotensin system: a link between obesity, inflammation and insulin resistance

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Summary

The renin-angiotensin system (RAS) is classically known for its role in regulation of blood pressure, fluid and electrolyte balance. Recently, several local RASs in organs such as brain, heart, pancreas and adipose tissue have also been identified. Evidence from clinical trials suggests that in addition to anti-hypertensive effects, pharmacological inhibition of RAS also provides protection against the development of type-2 diabetes. Moreover, animal models with targeted inactivation of RAS genes exhibit improved insulin sensitivity and are protected from high-fat diet-induced obesity and insulin resistance. Because there is evidence for RAS overactivation in obesity, it is possible that RAS is a link between obesity and insulin resistance. This review summarizes the evidence and mechanistic insights on the associations between RAS, obesity and insulin resistance, with special emphasis on the role of adipose tissue RAS in the pathogenesis of metabolic derangements in obesity.

Keywords: Adipose tissue, insulin resistance, renin-angiotensin system.

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Introduction

The renin-angiotensin system (RAS) is traditionally known for its role in regulation of blood pressure, fluid and electrolyte balance (1). Angiotensinogen (Agt), the main precursor peptide of RAS, undergoes enzymatic cleavage by renin and angiotensin-converting enzyme (ACE) to form angiotensin II (Ang II), the main effector peptide of this system (2). Ang II exerts its physiological effects via two G-protein coupled receptors, viz. Ang II type 1 (AT_1) and type 2 (AT_2) receptors. In addition to the systemic RAS, several local ones also exist in organs such as brain, pancreas, heart and adipose tissue (3,4). Because Ang II increases blood pressure through AT_1, ACE inhibitors (ACEI) and AT_1 blockers (ARB) are clinically used as anti-hypertensive agents (4,5). Currently, RAS inhibition is also the first line treatment in diabetic nephropathy (5).

Evidence for the role of systemic and local RAS in hypertension, renal function and cardiovascular disease has been previously reviewed [RAS and cardiovascular disease (5); adipose RAS and cardiovascular disease (6); brain RAS and hypertension (7); endocrine and paracrine RAS (8); adipose RAS and metabolic diseases (9)]. Interestingly, epidemiological studies have shown that patients on ACEI or ARB have a lower risk of developing type-2 diabetes compared to ones treated with other anti-hypertensive medications (10). Subsequent randomized controlled trials have also shown that RAS blockade improves glycemic control (11) and lowers the risk of developing type-2 diabetes (12). Because there is evidence for RAS overactivation in obesity, and because RAS blockade improves insulin resistance, it is possible that RAS is implicated in the pathogenesis of insulin resistance in obesity. Evidence for this hypothesis, with specific emphasis on the role of adipose RAS on the pathogenesis of insulin resistance, is reviewed in this paper.

Components of the renin-angiotensin system

Components of the classical RAS are well characterized. The common precursor of all bioactive angiotensin peptides is Agt (Fig. 1). It is mainly secreted by the liver in lean individuals. Adipose tissue is another important source of Agt, especially in obese individuals (13). Agt is cleaved by the enzyme renin to form angiotensin I (Ang I). Renin is mainly produced by the kidneys, and its secretion is the main rate limiting step in the regulation of systemic RAS (2). Renin can also bind to the recently identified renin/pro-renin receptor, and increase the catalytic efficiency of Ang I formation (14).

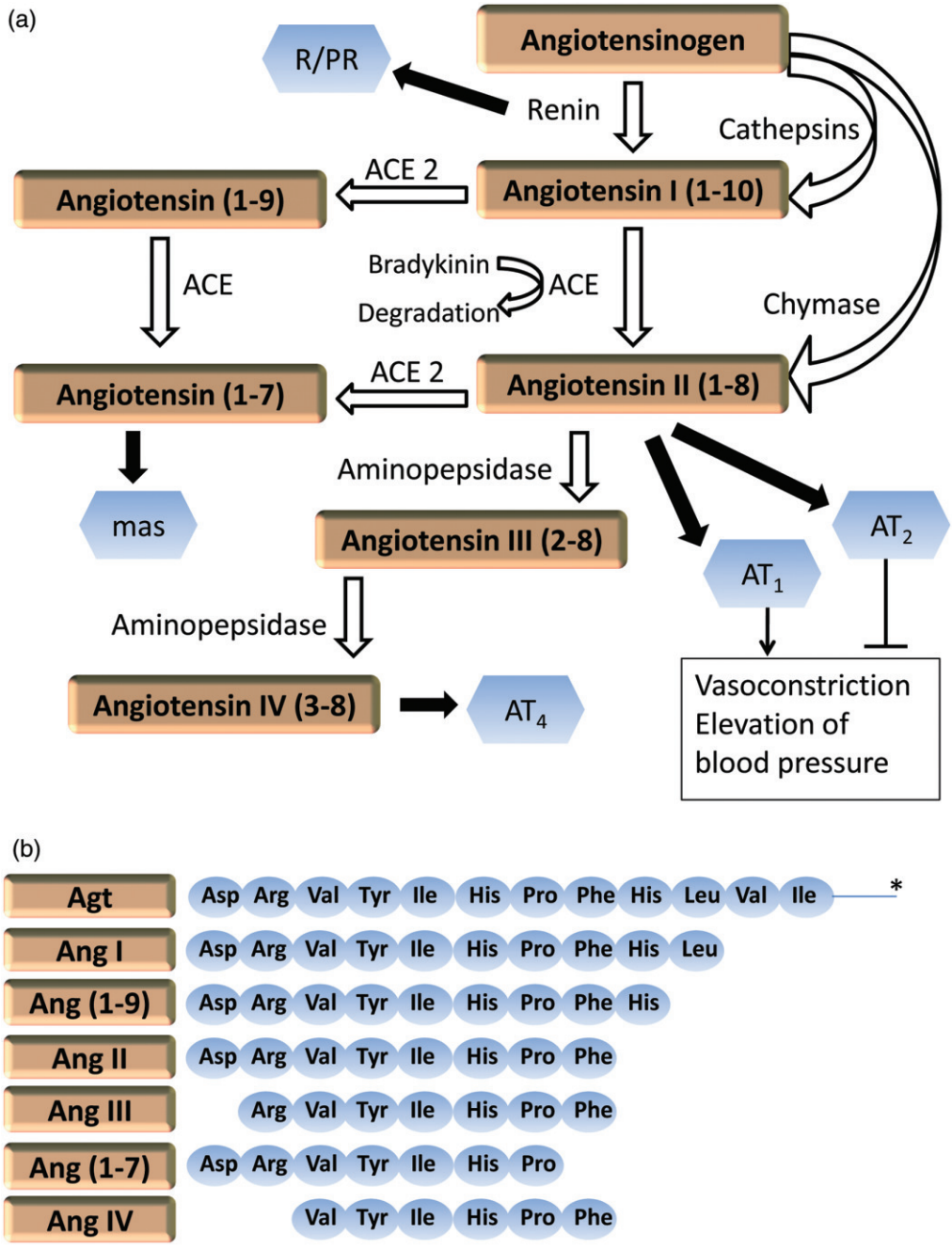

Figure 1 Components of the renin-angiotensin system. Components of the renin-angiotensin system (a) and amino acid sequences of human angiotensins (b) are shown. Angiotensinogen (Agt) is cleaved by renin and angiotensin-converting enzyme (ACE) to form angiotensin (Ang) I and II, respectively. Ang II acts via Ang II type 1 (AT_1) or type 2 (AT_2) receptors to exert its physiological actions. Ang I and II can also be cleaved by ACE2 to form Ang 1-9 and 1-7, which in turn can act on the mas receptor. Ang II is degraded to Ang III and IV. The latter can act on the AT_4 receptor. Renin can also act on the renin/pro-renin receptor (R/PR). * First 12 amino acids of Agt are shown.

Ang I is subsequently cleaved by ACE, present mainly in the vascular endothelium of the lungs, to produce Ang II. Alternatively, Ang II can also be formed by the action of cathepsins and chymase, especially in local RAS (1) (Fig. 1).

Ang II is the main effector peptide of the RAS, which exerts its effect via AT_1 or AT_2 (1). Stimulation of AT_1 induces vasoconstriction and aldosterone secretion from the adrenal cortex, resulting in increased blood pressure and sodium and water retention. Stimulation of AT_2 generally exerts blood pressure lowering effects (1). However, because of paucity of AT_2, AT_1 effects typically predominate. Ang I and Ang II can be cleaved to angiotensin 1-9 and 1-7, respectively, by the action of recently discovered enzyme ACE2 (1) (Fig. 1). Mas receptor has been identified as the angiotensin 1-7 receptor (15). Ang II is subsequently degraded by aminopeptidases to produce angiotensin III and IV. Angiotensin IV acts on the AT_4 receptor (1). Most components of the systemic RAS are also found in the adipose tissue (3,16). Additionally, Agt can be cleaved by cathepsins and chymase to produce Ang II, bypassing the renin-ACE axis in the adipose tissue (16). Thus, Agt production is a key regulatory step in the adipose RAS.

Renin-angiotensin system overactivation in obesity

Obesity is associated with overactivation of both systemic and adipose RAS in humans and animals (Table 1). In humans, obesity is associated with increases in plasma Agt (17), renin (18), ACE and Ang II (17) (Table 1). The increase in plasma renin levels in obesity is likely secondary to the increased sympathetic tone present in obese individuals (19). Moreover, the elevation of plasma Ang II following beta-adrenergic stimulation is also greater in obese than lean individuals (20). Adipose tissue renin, ACE and AT_1 expression are also increased in obesity (21). Most, but not all studies show that adipose Agt expression is also higher in obese humans (20) (Table 1). Moreover, weight loss leads to reductions in plasma Agt, renin, ACE and adipose Agt levels (17). Animal studies, in contrast, show that the direction of change in RAS components in obesity is strain dependent (Table 1). Similar to humans, most diet-induced obese rodent models show overexpression of both systemic and adipose RAS components (13,22). When genetic models of obesity are considered, ob/ob and db/db mice show activation of systemic and adipose RAS (23), while the obese (fa/fa) Zucker rat, Wistar fatty rat and viable yellow mouse exhibit lower expression of systemic and adipose RAS components compared to lean littermates (3,24,25) (Table 1).

While there are discrepancies on adipose Agt expression in animal models of obesity, these studies consistently report no change in hepatic Agt production in obese compared to lean animals (26,27) (Table 1). Considering that plasma Agt levels are increased in obesity, with relatively unchanged hepatic Agt production, this emphasizes the potential contribution of adipose-derived Agt to systemic

Table 1 Association of RAS components with obesity

Subjects	RAS component	Association with obesity		
		Positive	No association	Negative
Humans	Plasma Agt	(17,116–120)	(20,121)	
	Plasma renin	(17,18,122,123)		
	Plasma ACE	(17,120)		
	Plasma Ang II	(17)	(20)	
	Plasma Ang II (sympathetic stimulated)	(20)		
	Adipose Agt	(20,124–126)	(95,121)	(17,21)
	Adipose AT_1	(21,121)		
	Adipose renin	(21)		
	Adipose ACE	(21)		
Animals				
Rats	Plasma Agt	DIO-SD (22)		ZF (24)
	Plasma Ang II	DIO-SD (22), SF-Wistar (127)		
	Plasma renin	DIO-SD (128)		ZF (129,130)
	Adipose Agt	ZF (26), DIO-SD (22), FF-SD (131)	FF-SD (132)	Wistar fatty (25)
	Adipose AT_1	FF-SD (132)		ZF (133)
	Hepatic Agt		ZF (26), DIO-SD (22)	
Mice	Plasma Agt	DIO-B6 (13)		
	Adipose Agt	Ob/ob, Db/db (23), DIO-B6 (13,27)		Viable yellow (3)
	Hepatic Agt		Viable yellow (3), DIO-B6 (13,27)	
Dogs	Plasma renin	(134)		

ACE, angiotensin-converting enzyme; B6, C57BL/6J; DIO, diet-induced obese; FF, fructose-fed; RAS, renin-angiotensin system; SD, Sprague-Dawley; SF, sucrose-fed; ZF, Zucker fatty.

Table 2 Effects of RAS manipulation in humans and animals

Manipulation	Body weight/adiposity	Insulin sensitivity	Adipocyte size	Adipocyte number	Reference
Hyperactive states					
Ang II infusion (acute – humans)	ND	+	ND	ND	(56,58,135,136)
Ang II infusion (chronic – rodents)	–	–	ND	ND	(32,33,55,59,137)
Renin overexpression	–	–	ND	ND	(60,63)
Ang 1–7 infusion (rodents)	ND	+	ND	ND	(138)
Adipose Agt overexpression	+	–	+	–	(28,64,104)
Adipose Agt overexpression + AT_2 knockout	–	ND	–	ND	(104)
Hypoactive states					
Renin knockout	–	+	–	ND	(37)
Agt knockout	–	ND	–	+	(28,34)
ACE knockout	–	+	ND	ND	(38)
AT_1 knockout	–	+	–	ND	(39,139)
AT_2 knockout	–	+	–	+	(40)
Mas genetic deletion	+	–	ND	ND	(41)

+ positive association; – negative association.
ACE, angiotensin-converting enzyme; ND, not determined; RAS, renin-angiotensin system.

levels of this precursor. Indeed, adipose tissue may contribute up to 30% of plasma Agt level in obesity (28). However, because the rate limiting step in the activation of systemic RAS is renal renin release, rather than Agt secretion, the relative contribution of adipose tissue-derived Agt to systemic RAS overactivation is debatable. It should, however, be pointed out that in transgenic mice overexpressing Agt in adipose tissue, overproduction of Agt by only about 20% is able to drive both adipocyte hypertrophy, hyperinsulinemia and high blood pressure (28), indicating an adipose Agt-dependent activation of systemic RAS. Indeed, we previously reported that overexpression of Agt in adipose tissue was associated with increased protein expression of both renal AT_1 and renal Agt (29,30). Adipose Agt-driven activation of renal RAS may be responsible for high blood pressure of the aP2-Agt mice (28). Further, sole re-expression of Agt in adipose tissue of Agt-KO mice normalized renal gene expression that was altered by systemic Agt inactivation (29,31).

Overall, there is evidence for both systemic and adipose RAS overactivation in obese humans. Discrepancies in regulation of RAS components in obese animal models demonstrate the complexity of RAS-obesity interactions, and highlight the importance of selecting the appropriate animal model and dietary conditions to study the role of systemic and adipose RAS in obesity.

Effects of renin-angiotensin system manipulations on body weight and adiposity

While obesity is the result of a chronic imbalance between energy intake and expenditure, its causes can be complex. There is evidence that RAS manipulations can impact body weight, adiposity and obesity via impacting energy intake, expenditure or both. Surprisingly, systemic RAS overactivation via chronic Ang II infusions or renin overproduction induces weight loss, rather than weight gain, in rodents (32) (Table 2). This is attributed to initial reduction in energy intake and subsequent increase in energy expenditure (32,33). In contrast, adipose-specific RAS overactivation via increased expression of Agt leads to increased adiposity (28). Moreover, mice with global Agt deficiency exhibit lower adiposity compared to wild-type mice (34), while adipose-specific Agt overexpression in these mice leads to increased adiposity (28). Thus, it appears that while systemic RAS overactivation negatively affects body weight, adipose-specific RAS overactivation leads to excessive body weight and adiposity. This highlights the importance of paracrine/autocrine actions of Ang II in adipose tissue in the regulation of adiposity. The higher adiposity observed in aP2-Agt mice is possibly related to reduced energy expenditure, since aP2-Agt mice exhibit reduced locomotor activity, with no difference in energy intake, when compared to Wt counterparts (28). Detailed studies on energy balance in these mice are warranted. Moreover, the importance of adipose RAS should be further confirmed in animal models specifically lacking RAS components in adipose tissue.

Similar to findings in Agt-deficient mice, pharmacological RAS blockade via ACEI or ARB reduces adiposity in rodents (35,36), but not in humans. RAS blockade via deletion of other RAS genes such as renin (37), ACE (38), AT_1 (39) or AT_2 (40) all protect rodents from diet-induced obesity (Table 2), suggesting a role of RAS in the development of obesity. Furthermore, deficiency of mas receptor increases adiposity in rodents, suggesting a potential beneficial role of the Ang 1-7-mas axis on adiposity (41).

Taken together, these findings suggest a role for RAS, especially adipose RAS, in positively regulating adiposity.

Renin-angiotensin system and insulin resistance

Insulin resistance is a state in which insulin-sensitive tissues (liver, skeletal muscle and adipose tissue) exhibit an inadequate response to normal circulating levels of insulin (42). Insulin binding to its receptor in these tissues normally results in activation of a cascade of intracellular signalling events, which leads to tissue-specific responses. For example, insulin inhibits hepatic glucose production, promotes skeletal muscle glucose uptake and inhibits lipolysis in adipose tissue. Therefore, insulin resistance results in impairments in insulin-mediated suppression of hepatic glucose production, skeletal muscle glucose disposal and inhibition of lipolysis, leading to relative hyperglycaemia and increased plasma levels of free fatty acids (43). Insulin-resistant individuals initially maintain normoglycaemia through insulin hypersecretion. However, long-term insulin resistance and hypersecretion of insulin eventually result in pancreatic β-cell failure, giving rise to pre-diabetes and glucose intolerance, which can later progresses to frank hyperglycaemia and type-2 diabetes (44).

Both genetic and pharmacological studies support involvement of RAS in insulin resistance. Indeed, numerous genetic studies have shown associations between polymorphisms of RAS genes and glucose homeostasis. The DD genotype of the *ACE* I/D polymorphism is associated with glucose intolerance and insulin resistance in several adult (45,46) and infant (47) populations. Further, the *AGT* T174M and M235T polymorphisms are significantly associated with metabolic syndrome in aboriginal Canadians (48) and glycated haemoglobin in neonates (49), respectively. The plasma level of insulin sensitizer adiponectin is also associated with the *AT1* A1166C polymorphism in young women (50). Having multiple risk genotypes of RAS polymorphisms significantly increases the risk for type-2 diabetes (51).

Pharmacologically, the strongest clinical evidence for an association between systemic RAS and insulin resistance originates from clinical trials which have shown that RAS blockade reduces the risk for developing type-2 diabetes (1,52). For example, there was a 14% lower risk of developing type-2 diabetes for patients on ACEI vs. conventional treatment in the Captopril Primary Prevention Project (12). In the Heart Outcomes Prevention Evaluation trial, there was a 34% risk reduction in the Ramipril (ARB) group compared to the placebo control group (52). Pharmacological RAS blockade also improves insulin sensitivity in several rodent models of obesity or insulin resistance (53,54). Moreover, rodents with genetic deletions of renin (37), ACE (38), AT_1 (39) or AT_2 (40) show improvements in insulin sensitivity and/or resistance to high fat (HF) diet-induced insulin resistance (Table 2). Thus, RAS blockade is associated with improved insulin sensitivity.

Renin-angiotensin system overactivation and insulin resistance

Conversely, chronic overactivation of systemic RAS induces whole-body insulin resistance in rodents (55) (Table 2). However, acute RAS overactivation via short-term Ang II infusions increases glucose disposal, and improves insulin sensitivity in humans (56) and rodents (57). This latter phenomenon is attributed to acute haemodynamic adaptations in the form of redistribution of blood flow to skeletal muscle in response to increased Ang II levels (56,58). Because experimental chronic Ang II infusions are not feasible in human studies, animal models have been used to study the effects of chronic RAS overactivation. In these studies, chronic Ang II infusion induces skeletal muscle and hepatic insulin resistance, giving rise to whole-body insulin resistance (59). The TG(mREN2)27 rat, another model of chronic systemic RAS overactivation, also develops skeletal muscle and systemic insulin resistance (60). The insulin resistance in these animals is improved by either direct renin inhibition (61) or AT_1 blockade (62). Consistent with this observation, we have also shown that mice overexpressing renin in the liver (RenTg) develop glucose intolerance (63). Moreover, we recently demonstrated that adipose-specific RAS overactivation leads to glucose intolerance and systemic insulin resistance (64). Taken together this shows that chronic systemic or adipose RAS overactivation leads to systemic insulin resistance.

Mechanisms of Ang II-mediated insulin resistance

The mechanisms of Ang II-mediated skeletal muscle insulin resistance have been studied extensively. A summary is given in Fig. 2. Muscle glucose uptake depends upon glucose delivery, glucose transport across the cell membrane and glucose utilization (65). Of these processes, Ang II mainly impairs glucose transport and glucose utilization by the skeletal muscle (66) (Fig. 2). Ang II impairs glucose transport mainly via inhibition of insulin signalling. Specifically, Ang II abolishes the insulin-mediated tyrosine phosphorylation of insulin receptor substrate (IRS)-1, activation of protein kinase B/Akt and translocation of glucose transporter (Glut)-4 in L6 myocytes *in vitro,* in an NADPH a reduced NADP (nicotinamide adenine dinucleotide phosphate) oxidase, AT_1 and NF-kB-dependent manner (67,68). Similarly, defective insulin-stimulated phosphorylation of IRS-1, Akt and glycogen synthase kinase-3beta was

reported in isolated skeletal muscle of the TG(mREN2)27 rat (60). The Ang II-mediated inhibition of IRS-1 is also due to its inactivation by serine phosphorylation or by the action of protein tyrosine phosphatase-1B (69). It is likely that Ang II activates NADPH oxides via AT_1, which leads to increased production of reactive oxygen species (ROS). This activates the NF-kB pathway, which increases transcription of cytokines such as TNF-α and IL-6. These cytokines acting in a paracrine fashion increase suppressor of cytokine signalling 3 expression (70), which further inhibits insulin signalling (Fig. 2). In terms of glucose utilization, Ang II reduces skeletal muscle mitochondrial content in an AT_1- and AT_2-dependent manner in rodents both *in vitro and vivo* (71), an effect proposed to be mediated via ROS (71).

ACEI and ARB prevent these Ang II effects on insulin signalling and glucose utilization and improve skeletal muscle insulin sensitivity (72). An additional mechanism of ACEI-mediated improvement in insulin sensitivity is via prevention of the degradation of bradykinin, a potent vasodilator and potentiator of insulin signalling (72). This is explained by the ability of ACE to degrade bradykinin (Fig. 1), which is prevented by ACE inhibition.

Additional mechanisms contributing to Ang II-mediated insulin resistance include the ability of Ang II to increase hepatic glucose production (73), which can contribute to whole-body insulin resistance. However, the exact mechanism responsible for this is not known, although recent evidence suggests that Ang II might be implicated in the development of hepatic steatosis (74). Ang II's actions on the endocrine pancreas may also play a role in defective glucose homeostasis associated with RAS overexpression. Indeed, the endocrine pancreas expresses a local RAS which is involved in the regulation of glucose-stimulated insulin secretion, insulin synthesis and pancreatic blood flow (75). Further, RAS blockade improves islet morphology and function (76), and transfection of ACE2 into pancreatic islets of db/db mice also improves glycemic control (77).

Unlike in skeletal muscle, Ang II does not induce insulin resistance in adipose tissue (57). Indeed, Ang II potentiates insulin-stimulated glucose uptake by adipocytes (57) via activation of insulin signalling molecules *in vitro* (78). However, adipose tissue RAS could be important in the pathogenesis of systemic insulin resistance for several reasons. First, adipose-derived Ang II contributes to systemic levels of these hormones (79). Next, paracrine effects of Ang II on adipose tissue alter the adipokine profile toward a pro-inflammatory phenotype, which can then lead to skeletal muscle insulin resistance. Indeed, recently

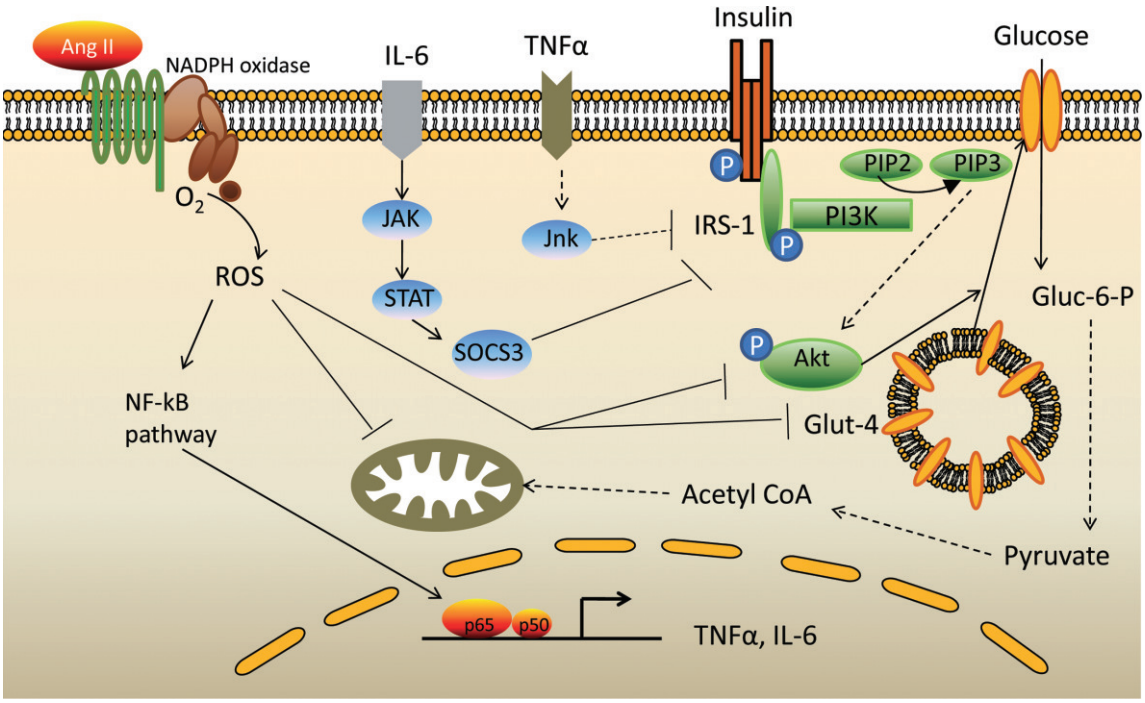

Figure 2 Mechanisms of Ang II-mediated skeletal muscle insulin resistance. Ang II activates NADPH a reduced NADP (nicotinamide adenine dinucleotide phosphate) oxidase via AT_1. This leads to generation of reactive oxygen species (ROS), which induce and activate nuclear translocation of NF-kB pathway. The latter mediates transcription of cytokines such as TNF-α and IL-6 and subsequent binding to their receptors. This binding induces serine kinases and SOCS3 expression, further inhibiting the tyrosine phosphorylation of IRS-1. This leads to deactivation of downstream insulin signalling and Glut-4 translocation, resulting in reduced glucose entry into the cell. ROS also inhibits mitochondrial biogenesis leading to reduced glucose utilization.

we showed that adipose-specific Agt overexpression leads to systemic insulin resistance at least in part due to reduced skeletal muscle and cardiac glucose uptake (64). Also, Ang II infusions reduce plasma adiponectin levels in an AT_1-dependent manner (80). Finally, similar to several pro-inflammatory cytokines, Agt is expressed higher in visceral compared to subcutaneous adipose tissue (81). Thus, Ang II's effects on insulin resistance are tissue specific. It induces insulin resistance in skeletal muscle and liver, while promoting a pro-inflammatory adipokine profile in the adipose tissue. Both these effects likely play a role in the development of Ang II-mediated systemic insulin resistance.

Obesity, adipose tissue inflammation and insulin resistance

White adipose tissue is the major site for storage of excess energy in the body. It is composed of adipocytes, an extracellular matrix, blood vessels, nerves and several other cell types including preadipocytes, stem cells and immune cells (82) (Fig. 3). These immune cells include alternatively activated macrophages (M2), classically activated macrophages (M1), T helper (Th) 1 and 2 cells, regulatory T cells (Treg) and effector T cells (83,84). In addition to storing energy, adipose tissue secretes numerous hormones, which have important homeostatic functions. These hormones are collectively known as adipokines. Obesity leads to adipocyte hypertrophy, changes in immune cell populations and dys-regulation of adipokine secretory patterns, shifting the balance of the latter toward a pro-inflammatory one (43). These changes in immune cell populations are characterized by increases in M1/M2 and Th1/Th2 ratios, a decrease in Treg number and increase in effector T cell number (82) (Fig. 3). Therefore, it is now established that obesity leads to a chronic low-grade inflammation in the adipose tissue, which is at least in part responsible for the pathogenesis of insulin resistance and metabolic syndrome (43). While the exact trigger for this inflammatory process is hitherto unknown, defective adipose tissue expansion due to inadequate adipogenic capacity, adipose tissue hypoxia and endoplasmic reticulum stress are implicated (85). There is emerging evidence that adipose tissue RAS could also

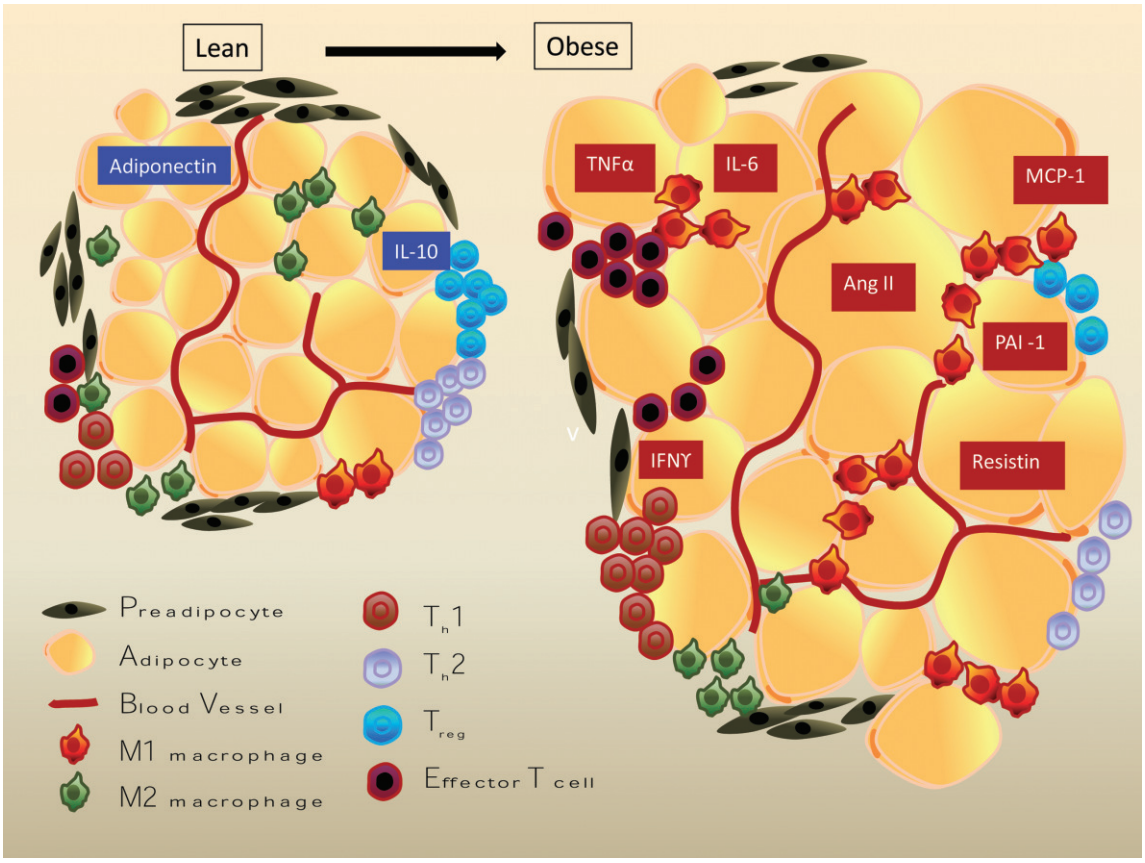

Figure 3 Changes in immune cell populations and adipokine secretory patterns in obesity. Lean adipose tissue contains a higher proportion of M2/M1 and Th2/Th1 cells, a higher number of Treg and lower number of effector T cells compared to obese adipose tissue. Obesity leads to changes in these cell ratios and adipokine secretory patterns. This is characterized by a shift from high adiponectin and IL-10 secretion in the lean state to high pro-inflammatory adipokine secretion in the obese adipose tissue.

potentially contribute to this inflammatory process in the adipose tissue, which is discussed below.

Role of adipose tissue renin-angiotensin system in regulating adipose tissue function

In the adipose tissue, Agt is synthesized and secreted by adipocytes. Most of the other RAS components necessary to produce Ang II are also present in the adipose tissue (86). Thus, the presence of an adipose RAS is well established. In addition to the classical regulatory steps, adipose RAS appears to be regulated at the level of Agt production, which is controlled by hormones such as insulin, androgens and dexamethasone, cytokines such as TNF-α (3,81) and nutrients such as glucose and fatty acids (87). Indeed, insulin (88) and cytokine (89) response elements have been reported in the Agt promoter.

Adipose renin-angiotensin system and lipid accumulation

Functionally, Ang II plays a role in energy sensing, as well as modulating fat mass expansion via its effect on adipogenesis, lipogenesis and lipolysis. In rodents, feeding increases adipose Agt expression, while fasting reduces it (23), suggesting a role of Agt in energy sensing, possibly via the hexosamine pathway (90,91). It is plausible that in a state of acute energy influx to the adipose tissue, Agt production leads to increased local Ang II levels, which in turn induces local vasoconstriction resulting in lower lipolytic rates (92). Conversely, in fasting conditions, due to lower local Ang II levels, vasodilatation occurs, leading to increased rates of lipolysis. These Ang II effects are mediated via AT_1 (93).

Ang II also increases lipogenesis via AT_2 (94). This is through induction of key lipogenic enzymes such as glycerol-3-phosphate dehydrogenase. Consistent with these effects, Ang II also potentiates insulin-stimulated glucose uptake by adipocytes (57). Therefore, unlike in skeletal muscle, Ang II appears to enhance insulin action in adipocytes *in vitro*. Taken together, the net paracrine effect of Ang II is to reduce lipolysis and promote lipogenesis, ultimately increasing lipid storage and inflammation in adipose tissue (Fig. 4). Detailed mechanisms of Ang II's effect on lipolysis and lipogenesis have been reviewed by Yvan-Charvet *et al*. (9).

While acute changes in energy availability modulate adipose RAS activity, the effects of chronic energy excess on it are inconsistent. While most studies report Agt overexpression in human obesity, some have reported no change or a negative association (Table 1). Because adipose Agt expression is acutely regulated by hormonal and nutritional signals, this could be a confounding factor when studying the chronic effect of obesity on adipose Agt expression. It is also possible that genetic factors, such as polymorphisms in RAS genes, also play a role in this discrepancy of results (95). This is supported by that fact that while adipose RAS is overactivated in most animal models of diet-induced obesity, it is down-regulated in some models with genetic forms of obesity (Table 1). Given that not all obese individuals develop metabolic derangements (96), it is possible that adipose RAS is also overexpressed in some, but not all, obese individuals. Gene–environment interactions could also play a role in this relationship (97). In this context, it is important to study the effects of adipose RAS overactivation on adipose tissue function and systemic insulin sensitivity, to elucidate its role in the pathogenesis of metabolic derangements in obesity.

Adipose tissue renin-angiotensin system and adipogenesis

Because a reduced adipogenic capacity is linked to adipose tissue inflammation and systemic insulin resistance in obesity (85), it is important to investigate the effects of adipose RAS on both adipogenesis and lipogenesis, and conversely on both adipose hyperplasia and hypertrophy. It is important to recognize that lipogenesis and adipogenesis are distinct processes. The former refers to storage of lipids in adipocytes, which is positively regulated by Ang II as described above. Adipogenesis refers to formation of new adipocytes, either from preadipocytes or other precursors such as mesenchymal stem cells (98). In a state of positive energy balance, adipose tissue expansion occurs as a result of both these processes. Adipogenesis leads to adipocyte hyperplasia, while lipogenesis leads to adipocyte hypertrophy. An inadequate adipogenic capacity, which is postulated to be linked to systemic insulin resistance, is characterized by adipocyte hypertrophy and a lower adipocyte number (98).

RAS blockade via either ACEI or ARB results in smaller adipocyte size in numerous rodent models of obesity (99–101). Furthermore, ARB also increases the number of small differentiated adipocytes in diabetic rats (36). Both these findings support the assertion that while RAS blockade inhibits lipogenesis, it also promotes adipogenesis *in vivo*. However, these findings are confounded by the fact that some ARB such as Losartan, activates PPARγ, an adipogenic transcription factor (102,103). Thus, the effects of ARB on adipogenesis could be attributed to indirect effects. Rodents with genetic deletion of renin, Agt, AT_1 or AT_2 also exhibit smaller adipocytes (34,37) (Table 2). Of these, mice lacking AT_2 have a relative increase in adipocyte number (40), suggesting an inhibitory effect of AT_2 on adipogenesis.

The only animal model available to study the paracrine effects of RAS overactivation on adipose tissue is the transgenic mouse model overexpressing Agt in the adipose tissue

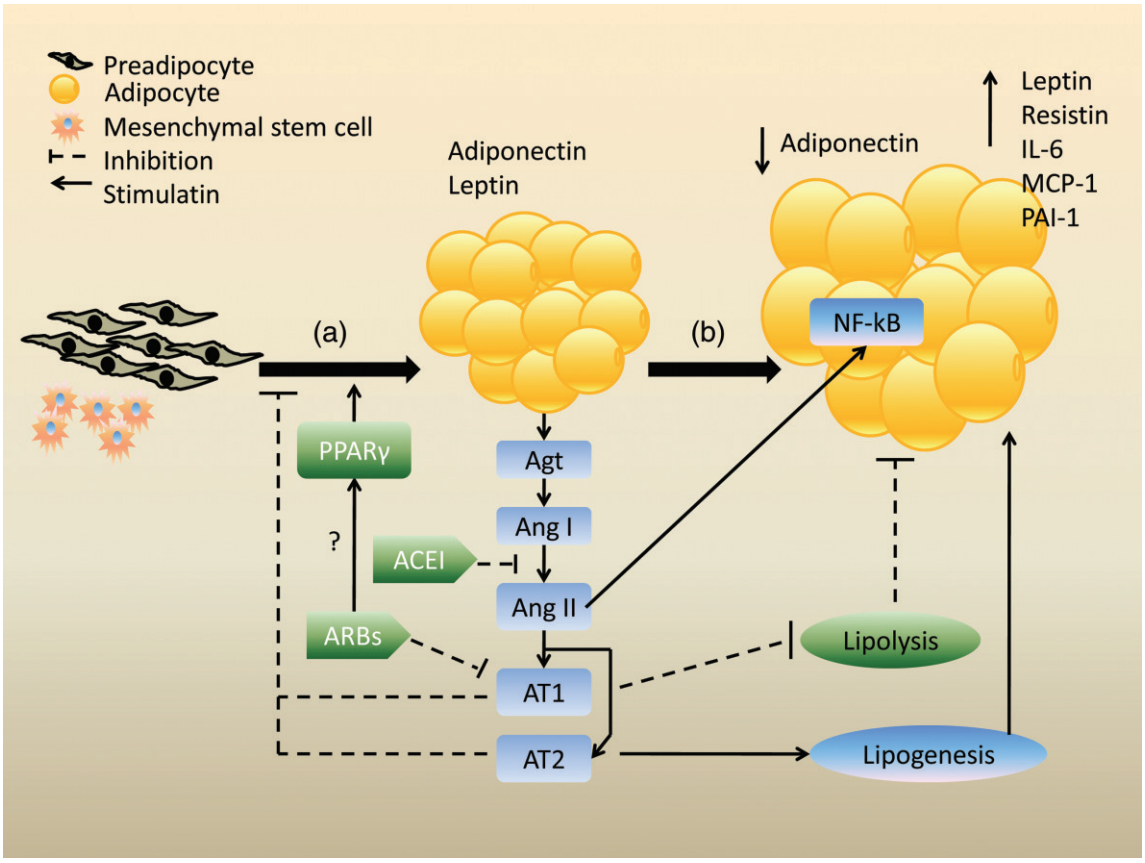

Figure 4 Effects of Ang II and RAS blockade on adipose tissue function. Adipose tissue expansion during positive energy balance involves a combination of adipocyte hypertrophy and hyperplasia. Adipogenesis via preadipocyte differentiation to adipocytes results in adipocyte hyperplasia (a). Mature adipocytes secrete Agt, which is converted to Ang II. Ang II acting on AT_1 and AT_2 inhibits preadipocyte differentiation. Stimulation of AT_2 also promotes lipogenesis, while activation of AT_1 inhibits lipolysis. Both processes promote adipocyte hypertrophy (b) which is associated with a pro-inflammatory adipokine profile.

(aP2-Agt mice). These mice become moderately obese and develop large adipocytes (28). Their adipocyte number is also reduced compared to wild-type mice (104) (Table 2). Moreover, in Agt-deficient mice, adipose-specific Agt overexpression leads to reduced adipocyte number (28). Taken together, this suggests that increased local levels of Ang II inhibit adipogenesis *in vivo*. When mice with adipose-specific Agt overexpression are crossed with mice lacking the AT_2 gene, their adipocyte number and size becomes comparable to wild-type mice (104), demonstrating a critical role of AT_2 in mediating Ang II's inhibitory effects on adipogenesis. However, because their adipocyte number is still lower than AT_2 knockouts (with normal adipose Agt expression), AT_1 also seems to be, at least in part, involved in mediating Ang II's anti-adipogenic effects (104).

In vitro studies on Ang II's effects on adipogenesis are inconsistent; practical difficulties in dissociating adipogenesis from lipogenesis likely being a major reason. Another reason relates to the doses used, which in many cases are pharmacological amounts. Earlier studies show that Ang II increases murine preadipocyte differentiation via prostacyclin and AT_2-dependent manner (105). However, it was later shown that Ang II inhibits human mesenchymal stem cell differentiation into adipocytes in an AT_2-dependent manner (106). There is also evidence that Ang II inhibits differentiation of human (107–109) and 3T3-L1 (110) preadipocytes in an AT_1-dependent manner *in vitro*. While there is some evidence to suggest that Ang II exerts these effects via mitogen-activated protein kinase and extracellular signal-regulated kinase pathways (109), further studies are certainly warranted. Discrepancies in these *in vitro* studies relate in part to whether the doses of Ang II employed are physiological or not. Under physiological conditions, plasma Ang II concentration can vary between 1 and 100 pmol/L (111). This range and limited plasma volumes from animal studies make it often difficult to accurately measure circulating Ang II levels, especially in mice. Moreover, many *in vitro* studies have used an Ang II dose in the nmol/L to μmol/L range (6). While the local tissue levels of Ang II in the adipose tissue can be higher

than that of the plasma, it is important to use physiological doses (in the range of pmol/L to nmol/L) in these types of studies. Indeed, we have previously shown that responsiveness of cultured adipocytes to Ang II depends on doses used, such that doses in the pmol/L to nmol/L range increase lipogenic enzyme activities and expression, while μmol/L doses are not effective (94).

Overall, this evidence suggests that Ang II promotes lipogenesis and inhibits adipogenesis leading to an adipose tissue phenotype characterized by large adipocytes or adipocyte hypertrophy (Fig. 3). Whether this could be an important mechanism for insulin resistance in conditions of adipose RAS overactivation remains to be tested.

Renin-angiotensin system and adipose tissue inflammation

We and others have shown that high-saturated fat feeding leads to adipose tissue inflammation and systemic insulin resistance in rodents (112,113). Because high-fat feeding also up-regulates the adipose tissue RAS (27), it is possible that Ang II is a mediator of adipose tissue inflammation in obesity. Indeed, aP2-Agt mice express higher levels of inflammatory genes in the adipose tissue even on a low-fat diet (104). However, because these mice also have increased adiposity and adipocyte hypertrophy, it is unclear whether this is a direct effect of Ang II. *In vitro* studies show that Ang II increases pro-inflammatory cytokines IL-6 and IL-8 secretion from human adipocytes (114) and MCP-1 from preadipocytes (115) in an NF-kB-dependent manner. Recently, we showed that Ang II also increases MCP-1 and resistin secretion from 3T3-L1 adipocytes in an NF-kB and NADPH oxidase-dependent manner (64). Conversely, RAS blockade reduces MCP-1 expression and macrophage infiltration in HF diet-induced obese mice (36). Taken together this suggests that Ang II promotes adipose tissue inflammation in an NF-kB-dependent manner (Fig. 4).

Conclusion

Evidence from both human and animal studies strongly suggests that the systemic RAS is overactivated in obesity. Additionally, there is evidence to support the assertion that adipose tissue RAS is also activated in obese states. Conversely, RAS blockade via either ACEI or ARB leads to improvements in insulin sensitivity. Therefore, RAS blockade is a promising approach to alleviate metabolic derangements in obesity. Mechanistically, Ang II induces skeletal muscle insulin resistance in an NADPH oxidase and NF-kB-dependent manner. Ang II also promotes lipid deposition in adipose tissue via inhibiting lipolysis and promoting lipogenesis. Further, Ang II increases pro-inflammatory cytokine secretion from adipose tissue. Emerging evidence also suggests a role of Ang II in inhibiting adipogenesis. Whether Ang II-mediated deterioration of adipogenic capacity is important in the development of systemic insulin resistance remains to be elucidated. While there is evidence for a role of adipose tissue RAS in the pathogenesis of systemic insulin resistance, further research is certainly warranted. Adipose-specific knockout of Agt will be a good model to study these effects. Overall, both systemic and adipose tissue RAS are associated with obesity and insulin resistance and could be a potential causal link for the metabolic derangements in obesity.

Conflict of interest statement

None.

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