Improvement of Insulin Sensitivity by Antagonism of the Renin-Angiotensin System

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The reduced capacity of insulin to stimulate glucose transport into skeletal muscle, termed insulin resistance, is a primary defect leading to the development of pre-diabetes and overt type 2 diabetes. While the etiology of this skeletal muscle insulin resistance is multifactorial, there is accumulating evidence that one contributor is overactivity of the renin-angiotensin system (RAS). Angiotensin II (ANG II) produced from this system can act on ANG II type 1 receptors both in the vascular endothelium and in myocytes, with an enhancement of the intracellular production of reactive oxygen species (ROS). Evidence from animal model and cultured skeletal muscle cell line studies indicates ANG II can induce insulin resistance. Chronic ANG II infusion into an insulin-sensitive rat produces a markedly insulin-resistant state that is associated with a negative impact of ROS on the skeletal muscle glucose transport system. ANG II treatment of L6 myocytes causes impaired IRS-1-dependent insulin signaling that is accompanied by augmentation of NADPH oxidase-mediated ROS production. Further critical evidence has been obtained from the TG(mREN2)27 rat, a model of RAS overactivity and insulin resistance. The TG(mREN2)27 rat displays whole-body and skeletal muscle insulin resistance that is associated with local oxidative stress and a significant reduction in the functionality of the IR/IRS-1-dependent insulin signaling. Treatment with a selective ANG II type 1 receptor antagonist leads to improvements in whole-body insulin sensitivity, enhanced insulin-stimulated glucose transport in muscle, and reduced local oxidative stress. In addition, exercise training of TG(mREN2)27 rats enhances whole-body and skeletal muscle insulin action. However, these metabolic improvements elicited by antagonism of ANG II action or exercise training are independent of upregulation of IR/IRS-1-dependent signaling. Collectively, these findings support targeting the RAS in the design of interventions to improve metabolic and
cardiovascular function in conditions of insulin resistance associated with pre-diabetes and type 2 diabetes.

Key words: insulin resistance, skeletal muscle, glucose transport, angiotensin II

INSULIN RESISTANCE AND THE CARDIOMETABOLIC SYNDROME

Insulin resistance of skeletal muscle glucose transport represents a major defect in the normal maintenance of euglycemia (73) and is often accompanied by a variety of metabolic and cardiovascular abnormalities, including hypertension, obesity, dyslipidemia, type 2 diabetes, and atherosclerosis (13, 53, 54). This clustering of atherogenic risk factors in the same individual has been called “syndrome X” (53, 54), the “insulin resistance syndrome” (13), or more recently the “cardiometabolic syndrome” (21). These individuals have a greatly elevated risk of developing cardiovascular disease, the leading cause of death in Western society. While still controversial, an increasing body of evidence is consistent with the concept that insulin resistance may be a unifying factor for the development of the “cardiometabolic syndrome” and its associated metabolic and cardiovascular defects (49). This review will initially describe the normal regulation of the skeletal muscle glucose transport system before providing a brief overview of various signaling defects that can contribute to an insulin-resistant state. The specific contribution of the renin-angiotensin system (RAS) to the etiology of skeletal muscle insulin resistance will be covered next. This discussion will include a more detailed description of how the RAS, specifically angiotensin II (ANG II), can be linked with defective insulin action in skeletal muscle and will introduce various animal models that have provided critical information on the deleterious effects of RAS overactivity in this tissue. This will allow a transition to the
coverage of interventions, such as selective antagonism of the RAS and exercise training, that are associated with amelioration of certain insulin-resistant states.

REGULATION AND DYSREGULATION OF THE GLUCOSE TRANSPORT PROCESS

Activation of the glucose transport system in skeletal muscle is a highly regulated process that can be acutely stimulated by insulin via the sequential engagement of a series of intracellular proteins (for reviews, see refs. 22, 61, 73). The initial step involves insulin binding to the insulin receptor (IR), which stimulates the tyrosine kinase activity of IR ß-subunits. The activated IR then phosphorylates IR substrates (IRS; IRS-1 and IRS-2 in skeletal muscle) on tyrosine moieties, and these tyrosine-phosphorylated IRS molecules then interact with the SH2 domains of the p85 regulatory subunit of phosphotidylinositol-3-kinase (PI3-kinase), thereby activating the p110 catalytic subunit of this enzyme. The activated p110 subunit produces phosphoinositide moieties, which can subsequently activate 3-phosphoinositide-dependent kinases (PDK). Akt is one downstream target of PDK, and this serine/threonine kinase appear to be critical in the regulation of glucose transport (20, 33, 67), although a limited number of studies do not support this concept (32). While Akt has several substrates, including atypical protein kinase C isoforms and glycogen synthase kinase-3, a newly discovered molecule, AS160, has emerged as an additional distal step important in the activation of glucose transport in muscle (7). The activation of these steps ultimately results in the translocation of the glucose transporter protein isoform GLUT-4 to the sarcolemmal membrane, where glucose transport takes place via a facilitative diffusion process.

Glucose transport into muscle can also be stimulated by an insulin-independent mechanism activated by contractions (reviewed in ref. 28) or hypoxia (10). The translocation of
GLUT-4 to the plasma membrane can be elicited in response to contractions (17, 19) and hypoxia (10). However, much less is currently understood regarding the intracellular signaling mechanisms responsible for this contraction-dependent pathway. There is evidence in the literature indicating roles of 5’-adenosine-monophosphate-activated protein kinase (AMP kinase), an enzyme sensitive to decreases in cellular energy charge (36; reviewed in refs. 56 and 69), and several Ca²⁺-dependent mechanisms, including calcineurin and the Ca²⁺- and calmodulin-dependent protein kinase (70).

In skeletal muscle from subjects with the “cardiometabolic syndrome” and overt type 2 diabetes, insulin-stimulated tyrosine phosphorylation of IR and IRS-1 and activation of PI3-kinase and Akt are reduced (3, 18, 34, 35), leading to defects in GLUT-4 translocation to the sarcolemmal membrane (57, 72). A role of enhanced serine phosphorylation of IR and IRS-1 in the multifactorial development of this insulin resistance is supported by numerous lines of evidence (reviewed in ref. 62). The protein expression and functionality of IR and IRS-1 can be negatively regulated by serine/threonine phosphorylation (1, 2, 5, 6, 51, 52, 62, 66, 71). Serine/threonine kinases that can mediate this negative phosphorylation of IR and IRS-1 include Akt (41), atypical protein kinase C (PKC) isoforms (65), glycogen synthase kinase-3 (GSK3) (14, 25), p70S6 kinase (43), and the mitogen-activated protein kinases (MAPK) (12, 46). Moreover, serine phosphorylation of IR is associated with decreased tyrosine phosphorylation of IRS-1 and a subsequently diminished activation of PI3-kinase (6, 66), and serine phosphorylation of IRS-1 causes enhanced degradation of this protein in cultured cell lines (51, 52). Finally, the role of increased activity of protein phosphatases in the diminution of the functionality of insulin signaling elements and in the induction of insulin resistance should be recognized (61).
EVIDENCE FOR A ROLE OF THE RENIN-ANGIOTENSIN SYSTEM IN THE ETIOLOGY OF INSULIN RESISTANCE

Effect of the renin-angiotensin system on whole-body and skeletal muscle insulin action

The RAS ultimately regulates the local levels of the peptide hormones bradykinin and angiotensin II (Fig. 1), a process critical for normal regulation of blood flow and blood pressure (reviewed in ref. 23). Bradykinin, a powerful vasodilator and modulator of several hormone actions, including insulin (11, 44, 47), can be degraded by angiotensin-converting enzyme (ACE, an enzyme identical to kininase II (15)). In addition, this same enzyme converts angiotensin I (derived from angiotensinogen via the action of renin) to ANG II. ANG II is a powerful vasoconstricting hormone and growth factor with specific negative effects on insulin action, as described below. The local levels of bradykinin and ANG II can be modulated by specific inhibitors of ACE (Fig. 1), and ACE inhibitors are a widely used antihypertensive intervention with insulin-sensitizing actions (23). The metabolic effects of bradykinin appear to be mediated either via direct modulation of IRS-1-dependent insulin signaling or through the production and action of nitric oxide (Fig. 2) (see ref. 23). Moreover, selective antagonists of ANG II receptors (referred to as ANG II receptor blockers, or ARBs) will also have specific cardiovascular and metabolic actions (Fig. 1). The modulation of metabolic functions facilitated by ARBs is described below.

An increasing body of evidence supports a role of ANG II in the multifactorial etiology of skeletal muscle insulin resistance. Initial investigations demonstrated that the direct infusion of ANG II into the interstitial space of canine skeletal muscle induces insulin resistance of muscle glucose uptake that is independent of alterations in blood flow (55). In addition, chronic infusion of ANG II in the rat is associated with a diminution of whole-body glucose disposal and
reduced skeletal muscle and adipocyte glucose uptake, possibly due to increased ROS production (50). In L6 myocytes, ANG II treatment causes a diminution of IRS-1-dependent insulin signaling and insulin-stimulated GLUT-4 translocation that is associated with the activation of NADPH oxidase and ROS production (68). Some these effects of ANG II to reduce insulin signaling may be related to the ANG II-associated enhancement of PTP-1B activity (42). It should be noted that discrepancies in the literature do exist, as some investigations have reported that acute ANG II infusion actually increases insulin sensitivity and glucose utilization in fat cells (30) and in human subjects with normal insulin sensitivity and with type 2 diabetes (8, 16, 45).

Further evidence supporting a role of ANG II in the etiology of insulin resistance comes from investigations using the TG(mREN2)27 rat, a monogenetic model of both hypertension and insulin resistance. The TG(mREN2)27 rat harbors the mouse Ren-2 renin gene (48) and develops severe cardiovascular defects, such as hypertension, left ventricular hypertrophy, and cardiac failure (37-39, 58). These animals are characterized by locally-elevated tissue ANG II levels (4, 9, 39) and by whole body and skeletal muscle insulin resistance (4, 29, 31, 40, 63). For example, compared to non-transgenic, normotensive Sprague-Dawley controls, the TG(mREN2)27 rat exhibits an exaggerated insulin response and decreased whole-body insulin sensitivity during an oral glucose challenge (29, 31). Additionally, insulin-stimulated glucose transport activity is substantially reduced in isolated skeletal muscles from TG(mREN2)27 rats (4, 31, 63), likely due to an impairment of the IR/IRS-1-dependent insulin signaling pathway (63) (Fig. 3). Finally, local production of ROS from skeletal muscle is increased in the TG(mREN2)27 rat (4), and this may also contribute to these signaling defects and impaired insulin action on muscle glucose transport.
Selective antagonism of angiotensin II receptors: metabolic effects

The critical role of ANG II in the etiology of insulin resistance has been addressed using selective inhibitors of ANG II action at cellular AT₁ receptors (ARBs; Fig. 1). The chronic administration of ARBs elicits significant improvements of whole-body insulin action in several distinct insulin-resistant rodent models, including the obese Zucker rat (24), the spontaneously hypertensive rat (59), and the fructose-fed rats (26). Interestingly, our research group has also shown that acute (1 hr) treatment of the obese Zucker rat with the ARB irbesartan enhances insulin-stimulated glucose transport activity in type I skeletal muscle of the obese Zucker rat (24), and that chronic (21 days) treatment of this animal model of insulin resistance with irbesartan leads to increases in insulin action that are associated with upregulation of GLUT-4 protein levels in skeletal muscle and myocardium (24). These latter results support the contention that ANG II antagonizes the signaling factors responsible for maintaining and expanding the protein expression of GLUT-4 in muscle (Fig. 2).

In the TG(mREN2)27 rat, administration of ARBs is associated with a significant reduction in systolic blood pressure (64). Moreover, this selective antagonism of AT₁-subtype receptors in the TG(mREN2)27 rat leads to significant improvements in whole-body glucose tolerance and insulin sensitivity (4, 64) and insulin-stimulated glucose transport activity in isolated skeletal muscle (4, 64) (Fig. 4). The increased insulin action on glucose transport activity in ARB-treated TG(mREN2)27 rats is not accompanied by an increased capacity of the IR/IRS-1-dependent insulin signaling pathway (Fig. 4) (64), but is related to a decrease in local production of ROS from skeletal muscle of these animals (4).

The results from studies using animal models and skeletal muscle cell lines on the contribution of the RAS, and specifically ANG II, to the development of insulin resistance have
been translated into viable treatments of insulin-resistant human populations at-risk for cardiovascular disease. In general, treatment with ACE inhibitors or ARBs can substantially reduce the risk of conversion from a pre-diabetic state to an overt type 2 diabetic condition (these clinical trials are summarized in refs. 23 and 60).

**Impact of exercise training in conditions of RAS overactivity**

Interesting insights into the role of RAS overactivity in the etiology of insulin resistance can be derived from exercise training studies using the TG(mREN2)27 rat. These hypertensive rats will run voluntarily in exercise wheels, reaching a plateau of ~7 km/day after 3-4 weeks of training (Fig. 5A) (31, 40). The expected increases in whole-body oxidative capacity (Fig. 5B), whole-body insulin sensitivity (Fig. 5C), and insulin-stimulated skeletal muscle glucose transport activity (Fig. 5D and Fig. 6) are elicited by this training regimen in the TG(mREN2)27 rat. However, this increased capacity for insulin-dependent glucose transport activity in skeletal muscle of the TG(mREN2)27 rat is not associated with any enhancements of insulin signaling functionality (Fig. 6) (40). This disconnect between glucose transport activation and insulin signaling capacity is similar to that noted in skeletal muscle of ARB-treated TG(mREN2)27 rats (Fig. 4 ) (64).

In addition, the normal increase in muscle protein expression of GLUT-4 and mitochondrial enzymes elicited in response to voluntary exercise training in non-transgenic Sprague-Dawley rats is not observed in exercise-trained skeletal muscle of TG(mREN2)27 rats (Fig. 7; refs. 27, 40, and Henriksen EJ and TR Kinnick, unpublished data). One can speculate that the regimen of exercise training by the TG(mREN2)27 rats enhanced the capacity for the insulin-mediated translocation of the existing pool of GLUT-4 transporters. One interpretation
of these observations is that the elevated ANG II levels of the TG(mREN2)27 rat, perhaps mediated by the activation of NADPH oxidase-derived oxidant stress, prevent the intracellular signaling mechanisms that normally facilitate the increases in GLUT-4 and mitochondrial enzyme biogenesis in response to endurance exercise training. An effective approach for testing this hypothesis would involve the use of ARBs and/or free radical scavengers in conjunction with the exercise training by the TG(mREN2)27 rats.

SUMMARY AND PERSPECTIVES

The results reviewed in this document support the concept that modulation of critical components of the RAS can have an important impact on whole-body and skeletal muscle glucose metabolism (summarized in Fig. 2). Interventions that bring about an enhancement of bradykinin levels and action in skeletal muscle, acting directly through the BK2 receptor system or via increases in nitric oxide, can potentiate the IRS-1-dependent insulin signaling pathway, ultimately leading to increased GLUT-4 translocation and glucose transport at the sarcolemmal membrane. This appears to be a critical action of ACE inhibitors to increase insulin sensitivity. Moreover, the deleterious action of ANG II, acting via its AT1 receptors and the NADPH oxidase-mediated production of reactive oxygen species (ROS), to antagonize multiple steps of insulin signaling and glucose transport can be overcome using selective antagonists of these AT1 receptors. This ANG II receptor antagonism, via a reduction in intracellular ROS production, may ameliorate the blunted augmentation of GLUT-4 and mitochondrial enzyme biogenesis mediated by ANG II. The antagonism of ANG II receptors may be a critical intervention for allowing the normal adaptive response of increased GLUT-4 and mitochondrial biogenesis following exercise training in conditions of locally elevated ANG II production and action, as
has been described in skeletal muscle of TG(mREN2)27 rats. However, the evidence supporting this concept is still limited, and this hypothesis needs to be rigorously tested in both appropriate animal models (such as the TG(mREN2)27 rat or in the ANG II-infused rat) and in human clinical trials to verify its validity.

In closing, the various results discussed in this review support the approach of targeting the RAS in the design of interventions to improve not only cardiovascular function, but also the regulation of skeletal muscle glucose metabolism in conditions characterized by components of the “cardiometabolic syndrome”. This would be an especially important approach for those individuals with insulin resistance associated with pre-diabetes and type 2 diabetes.

**GRANTS**

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REFERENCES


52. Potashnik R, Bloch-Damti A, and Bashan N. IRS1 degradation and increased serine phosphorylation cannot predict the degree of metabolic insulin resistance induced by


protein kinase B in insulin-induced glucose transport, glycogen synthesis, and protein


70. **Wright DC, Hucker KA, Holloszy JO and Han DH.** Ca\(^{2+}\) and AMPK both mediate stimulation of glucose transport by muscle contractions. *Diabetes* 53: 330-335, 2004.


Figures.

**Fig. 1.** The renin-angiotensin system and its effects on the regulation of skeletal muscle blood flow and glucose uptake. The impact of the inhibition of angiotensin converting enzyme and the antagonism of angiotensin II action to modulate blood flow and glucose uptake in skeletal muscle are also depicted. ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker.

**Fig. 2.** The modulation of the insulin signaling pathway and GLUT-4 biogenesis by angiotensin II (ANG II) and bradykinin in skeletal muscle cells. Insulin acts through a series of intracellular signaling steps to stimulate the translocation of GLUT-4-containing vesicles to the sarcolemma, where glucose transport takes place via a facilitative diffusion process. Flux through the insulin signaling pathway can be potentiated by bradykinin (BK) and bradykinin-mediated nitric oxide (NO). In contrast, ANG II, acting via its AT1 receptors and the NADPH oxidase-mediated production of reactive oxygen species (ROS), can antagonize multiple steps of insulin signaling and glucose transport. Moreover, ANG II may also antagonize the normal adaptive response of muscle to augment GLUT-4 (and mitochondrial enzyme) biogenesis following chronically enhanced muscle activity.

**Fig. 3.** Decreased ability of insulin to activate glucose transport, tyrosine phosphorylation of the insulin receptor β-subunit, and ser\textsuperscript{473} phosphorylation of Akt in isolated skeletal muscle from the TG(mREN2)27 (TG) rat compared to the non-transgenic Sprague-Dawley (SD) rat. Units for glucose transport are pmol 2-deoxyglucose/mg muscle/20 min, whereas units for insulin receptor
and Akt phosphorylation are relative units. * p<0.05, TG vs. SD for same incubation condition. Data are adapted from ref. 63.

**Fig. 4.** Effect of chronic treatment of TG(mREN2)27 rats with the angiotensin II receptor (AT1-subtype) receptor antagonist irbesartan on insulin-stimulated glucose transport, tyrosine phosphorylation of the insulin receptor β-subunit, and ser<sup>473</sup> phosphorylation of Akt in isolated skeletal muscle. TG(mREN2)27 rats were treated daily with either vehicle (TG Veh) or 50 mg/kg irbesartan (TG Irb) for three weeks. Units for glucose transport are pmol 2-deoxyglucose/mg muscle/20 min, whereas units for insulin receptor and Akt phosphorylation are relative units. * p<0.05, TG vs. SD for same incubation condition. Data are adapted from ref. 64.

**Fig. 5.** Responses for average daily running activity (A), whole-body oxidative capacity (B) and insulin sensitivity (C), and insulin-mediated glucose transport activity in skeletal muscle (D) in TG(mREN2)27 rats given free access to running wheels. Measurements were made 8-10 hours after the last bout of exercise. * p<0.05, trained vs. sedentary. Data are adapted from refs. 31 and 40.

**Fig. 6.** Effect of voluntary exercise training by TG(mREN2)27 rats on insulin-stimulated glucose transport, tyrosine phosphorylation of insulin receptor β-subunit, and ser<sup>473</sup> phosphorylation of Akt in isolated skeletal muscle. TG(mREN2)27 rats were allowed to remain sedentary (TG Sed) or had free access to running wheels (TG Exer) for five weeks. Measurements were made 8-10 hours after the last bout of exercise. Units for glucose transport are pmol 2-deoxyglucose/mg muscle/20 min, whereas units for insulin receptor and Akt
phosphorylation are relative units. ND, not detectable. * p<0.05, trained vs. sedentary for same incubation condition. Data are adapted from ref. 40.

**Fig. 7.** Effect of voluntary exercise training by Sprague-Dawley or TG(mREN2)27 rats on GLUT-4 protein level or citrate synthase activity in skeletal muscle. Animals were allowed to remain sedentary (Sed) or had free access to running wheels (Exer) for five weeks. Measurements were made 8-10 hours after the last bout of exercise. * p<0.05, trained vs. sedentary. Data are adapted from refs. 27 and 40, and from Henriksen EJ and Kinnick TR (unpublished results).
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