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Rats with Low Brain Angiotensinogen Do Not Exhibit Insulin Resistance During Early Aging

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During aging increases in body weight, insulin resistance, and elevated systolic pressure contribute to the development of metabolic syndrome. Long-term systemic blockade of the renin–angiotensin system (RAS) with either an angiotensin (Ang) II type 1 (AT_1) receptor antagonist or angiotensin converting enzyme inhibitor improves insulin sensitivity and decreases risk of new onset (type II) diabetes. However, the role of the brain RAS in mediating development of insulin insensitivity during aging is not known. Therefore, we compared responses to an oral glucose load in transgenic rats with selective antisense suppression of brain angiotensinogen (ASrAogen); (mRen2)27 rats with high brain angiotensin II; and control Hannover Sprague–Dawley (SD) rats, at wk 16 and 68 of age. ASrAogen animals had lower body weight than either SD or (mRen2)27 rats at both ages ($p < 0.001$). The oral glucose tolerance test at 16 wk in (mRen2)27 animals revealed a higher glucose–insulin index (154,421 ± 11,231 units; $p < 0.05$) and a lower glucose–insulin index in ASrAogen rats (41,580 ± 10,923 units, $p < 0.05$) compared to SD rats (97,134 ± 19,822 units), suggesting insulin resistance in the (mRen2)27 and enhanced insulin sensitivity in the ASrAogen relative to SD rats. At 68 wk, the glucose–insulin index remained low in the ASrAogen rats as evidence of maintained insulin sensitivity during aging compared with either SD or (mRen2)27 ($p < 0.05$). SD animals do not differ from (mRen2)27 rats at 68 wk indicating the development of a state of relative insulin resistance with increased age in the SD rats. Moreover, there was a positive correlation ($r = 0.44$; $p < 0.05$) between body weight and the glucose–insulin index in SD, but not ASrAogen or (mRen2)27 rats. The relationships between insulin and leptin, insulin and glucose, and leptin and body weight observed in SD rats were absent in ASrAogen and (mRen2)27 rats. We conclude that the glial RAS plays a role in development of insulin resistance as well as influencing weight gain associated with early aging.

Key Words: Brain; renin–angiotensin system; insulin; aging; transgenics.

Introduction

Angiotensin converting enzyme (ACE) inhibitors *(1)* and angiotensin II type 1 (AT_1) receptor blockers *(2)* have recently been shown to attenuate development of insulin insensitivity. Obese Zucker rats treated with irbesartan had improved whole body insulin sensitivity, as well as an improvement in glucose uptake into the soleus and epitrochlearis muscle after 21 d of treatment *(3)*. This improvement was partially attributed to an increase in glucose transporter 4 (GLUT-4) protein levels in skeletal muscles. In Fisher 344 rats devoid of elevations in pressure during the aging process, long-term AT_1 blockade prevents the increase in insulin, leptin, and glucose and reduced body weight gain during early aging independent of differences in blood pressure *(4)*. Two recently published clinical trials, the Losartan Intervention for Endpoint Reduction in Hypertension Study (LIFE) and the VALUE trial, demonstrated reduced risk of new-onset diabetes with the AT_1 blockers, losartan and valsartan *(2,5)*. Similar results occurred in ALLHAT with an ACE inhibitor, lisinopril, compared to a diuretic, chlorthalidone, and a calcium channel blocker, amlodipine *(1)*. This suggests that the choice of antihypertensive agent, specifically interruption of the renin–angiotensin system, is important in the improvement in insulin sensitivity associated with hypertension, independent of blood pressure lowering per se.

Circulating angiotensin II influences insulin sensitivity via direct interference with insulin signaling pathway in many tissues in the periphery *(6)*. However, the role of the brain RAS in insulin and glucose metabolism is not well defined. Central actions of insulin may involve angiotensinergic pathways *(7)*. In addition, it is well known that angiotensin II receptors are associated with autonomic control centers within the brain and throughout the vagal sensory and motor systems, consistent with a major influence of the

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Table 1
Body Weight, Fasting Insulin, Fasting Glucose, Fasting Leptin, and Quicki Index for SD, ASrAogen, and (mRen2)27 at 16 and 68 wk

	Body weight (g)	Fasting insulin (ng/mL)	Fasting glucose (mmol/L)	Fasting leptin (ng/mL)	Quicki Index
SD					
16 wk (8)	488 ± 40	0.62 ± 0.48	5.13 ± 1.80	4.25 ± 1.0	0.64 ± 0.05
68 wk (13)	664 ± 54†	0.70 ± 0.39	5.82 ± 1.75	8.8 ± 2.8	0.57 ± 0.02
ASrAogen					
16 wk (10)	311 ± 21*	0.42 ± 0.36*	5.12 ± 1.30	1.2 ± 0.79*	0.74 ± 0.08
68 wk (10)	369 ± 24*	0.18 ± 0.29*	4.05 ± 1.58*	1.5 ± 0.8*	1.73 ± 0.30*†
(mRen2)27					
16 wk (8)	520 ± 68Φ	1.37 ± 0.51Φ	4.52 ± 1.60	3.67 ± 4.29Φ	0.50 ± 0.02*Φ
68 wk (5)	830 ± 126*Φ†	0.52 ± 0.40Φ	3.84 ± 0.95*	12.5 ± 7.8*	0.66 ± 0.07Φ†

*$p < 0.05$ vs SD; Φ$p < 0.05$ vs AsrAogen; †$p < 0.05$ vs 16 wk. Number of animals is in parentheses.

peptide on parasympathetic and sympathetic nervous system function *(8–10)*. These same autonomic pathways are involved in the responses to insulin, leptin, and other peptide transmitters regulating ingestive behaviors and energy metabolism *(11–13)*. Despite the above observations, evidence for a direct role of angiotensin II at brain sites on autonomic control on energy metabolism is lacking.

Kinnick et al. showed that the (mRen2)27 transgenic hypertensive rats, with high brain and modestly elevated blood levels of angiotensin II, are insulin resistant at an early age (6 wk) *(14)*. Subsequent studies confirmed these findings *(15)*. Rats with low brain angiotensinogen due to a GFAP promoter linked angiotensinogen antisense (ASrAogen) have lower non-fasting insulin at 16 wk compared to the (mRen2)27 and at 68 wk compared to both the (mRen2)27 rats and the control strain Hannover Sprague–Dawley (SD) rats *(16)*. Despite documented increases in brain tissue angiotensin II in the (mRen2)27 animals *(17,18)* and lower brain angiotensinogen and angiotensin I in the ASrAogen animals *(19–21)* relative to the SD control rats, levels of angiotensin II in plasma of young rats of all three strains are similar *(22, 23)*. Plasma angiotensin II is lower in SD rats at 68 wk than 16 wk of age, consistent with previous studies in older rats of other strains *(24)*, although values remain similar among the ASrAogen, SD, and (mRen2)27 rats at this age *(23)*. There is no information on insulin and glucose responses to an oral glucose in the ASrAogen rats, and studies of age-related changes in insulin and glucose metabolism in these three strains of rats is lacking. Therefore, in this study, we characterized the dynamic regulation of insulin in the (mRen2)27, ASrAogen, and SD rats at 16 and 68 wk of age, time points with previously documented differences in non-fasting insulin levels among strains, using the oral glucose tolerance test.

Results

There was a difference in body weight among the three groups of animals (Table 1). The ASrAogen animals had significantly lower body weights compared with either SD or (mRen2)27 animals at both ages. The SD and (mRen2)27 animals had similar body weights at 16 wk but body weight was significantly lower at 68 wk of age in SD compared with (mRen2)27 rats. Both SD and (mRen2)27 rats showed an increase in body weight with age. There was no significant increase in body weight in the ASrAogen animals.

At 16 wk of age, (mRen2)27 animals had higher fasting serum insulin compared to both SD and ASrAogen animals ($p < 0.01$) with no differences in fasting serum glucose levels among groups (Table 1). At 68 wk, both SD and (mRen2)27 animals had higher fasting insulin levels compared to ASrAogen animals ($p < 0.05$), and the SD animals had higher fasting glucose compared to both other groups of animals ($p < 0.001$) (Table 1). During the oral glucose tolerance test, there was a significant effect of genotype for serum insulin levels in both the young ($F = 30.54, 2; p < 0.001$) and old ($F = 46.10, 2; p < 0.001$) rats. There was also a significant effect of genotype on serum glucose when challenged with an oral glucose load for both younger ($F = 15.21, 2; p < 0.001$) and older animals ($F = 55.78, 2; p < 0.001$). A peak increase in insulin, defined by an increase above baseline and the highest point in the curve, occurred at 15 min for both SD and ASrAogen rats, but was absent in (mRen2)27 rats at 16 wk of age. At 68 wk, the insulin peak again occurred at 15 min for the SD, but was absent in both ASrAogen and (mRen2)27 rats (Figs. 1A,B). The peak increase in glucose at 16 wk occurred in the ASrAogen animals at approx 15 min, and in the SD and (mRen2)27 rats glucose levels increase until 30 min and then plateau. At 68 wk, there was a similar trend with the peaks in glucose occurring around 30 min and then reaching a plateau in each of the strains (Figs. 2A,B).

The area under the curve (AUC) for insulin at 16 and 68 wk in ASrAogen animals was lower compared to either SD or (mRen2)27 animals over the 120 min time course of the study (Fig. 3A) with a decline in the AUC for insulin with age. There was no difference in the AUC for insulin between

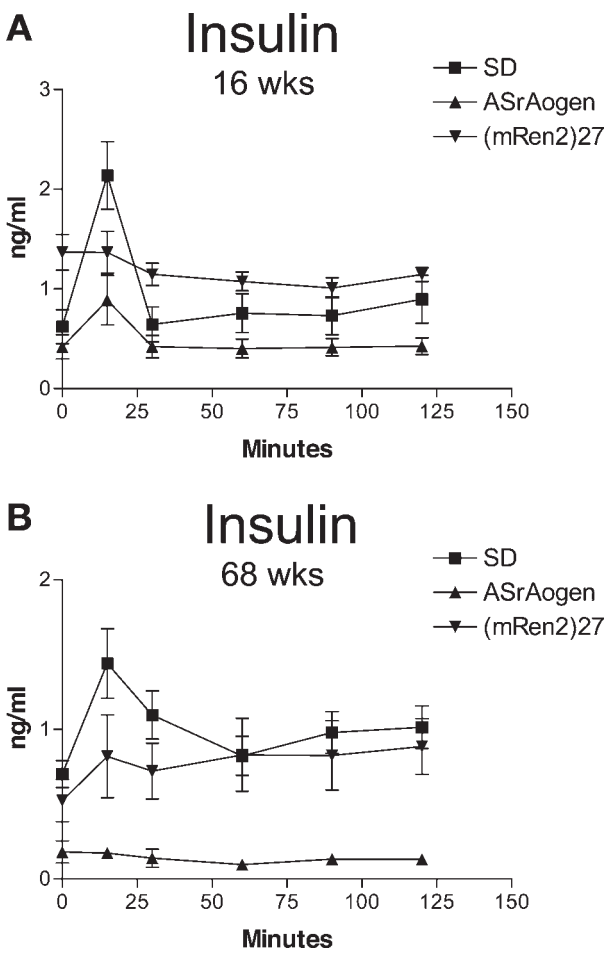


Fig. 1. Serum Insulin levels during the oral glucose tolerance test for both 16 (**A**) and 68 wk (**B**) of age in the SD, ASrAogen, and (mRen2)27 rats taken at 0, 15, 30, 60, 90, and 120 min post-gavage of 1 mg/kg glucose. ASrAogen: 16 wk $n = 10$, 68 wk $n = 10$; SD: 16 wk $n = 8$, 68 wk $n = 13$; (mRen2)27: 16 wk $n = 8$, 68 wk $n = 5$.

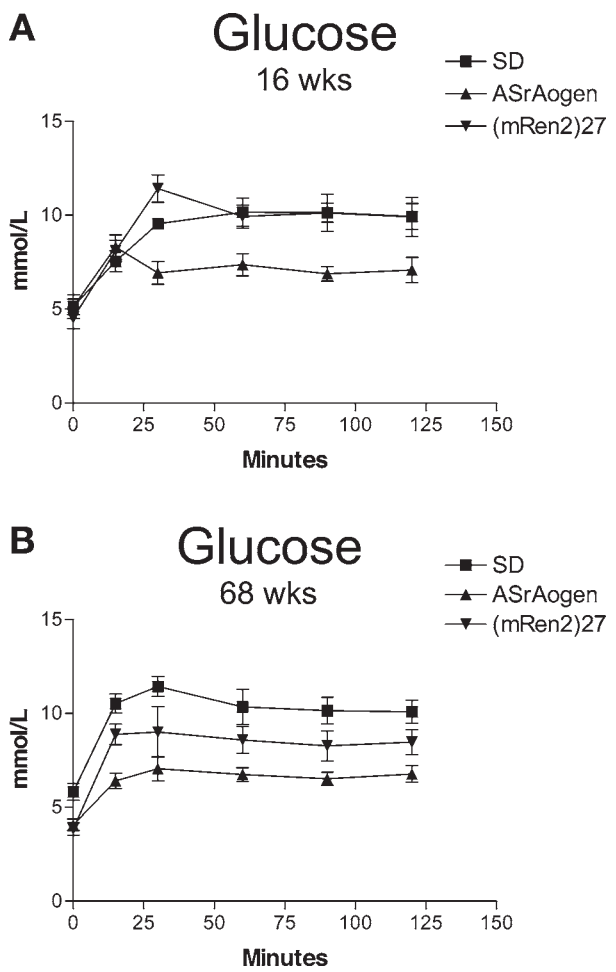


Fig. 2. Serum glucose levels during the oral glucose tolerance test taken at 0, 15, 30, 60, 90, and 120 min post-gavage of 1 mg/kg glucose for 16 (**A**) and 68 wk (**B**) of age in the SD, ASrAogen, and (mRen2)27 rats. ASrAogen: 16 wk $n = 10$, 68 wk $n = 10$; SD: 16 wk $n = 8$, 68 wk $n = 13$; (mRen2)27: 16 wk $n = 8$, 68 wk $n = 5$.

SD and (mRen2)27 animals at either age and no age-related change in either strain. The ASrAogen animals also had the lowest AUC for glucose at both 16 and 68 wk of age relative to the two other strains of rats. There was no difference in glucose AUC between the SD and (mRen2)27 rats at 16 wk, but there was a significant difference between the two at 68 wk (Fig. 3B) with no age-related changes. There was a main effect for genotype for the glucose–insulin index, a measure of insulin sensitivity ($F = 14.22, 2$; $p < 0.0001$). The index was lower in the ASrAogen rats compared with both the SD and (mRen2)27 rats at both ages, consistent with relatively greater insulin sensitivity in the ASrAogen rats. The SD rats had a lower glucose–insulin index at 16 wk compared to the (mRen2)27 rats, but the index was similar between these two groups at 68 wk of age (Figs. 4A,B). There were no significant age-related changes in the glucose–insulin index within strains. Using the quantitative insulin-sensitivity check index (Quicki) method, which has been shown to be highly correlated with the euglycemic clamp approach *(25)*, gave similar results as the glucose–insulin index calculated from the oral glucose tolerance test. At 16 wk the (mRen2)27 rats had a lower Quicki index, which indicates relative insulin resistance compared to both the SD and ASrAogen animals. At 68 wk, both the SD and (mRen2)27 animals had a lower Quicki index compared to the ASrAogen animals suggesting at this later age that the ASrAogen rats were relatively more insulin sensitive than the other two groups of animals. There were no significant age-related changes in the SD animals in terms of the Quicki index. However, the Quicki index improved with age in the ASrAogen rats, whereas the (mRen2)27 rats had a further decline in the Quicki index with age.

The ASrAogen animals had lower fasting serum leptin levels compared to both the SD and (mRen2)27 animals at 16 ($p < 0.05$) and 68 ($p < 0.001$) wk of age (Table 1). SD rats had lower fasting serum leptin compared to (mRen2)27 animals at 68 wk of age ($p < 0.05$), and there were no age-related differences among the three groups. The AUC for

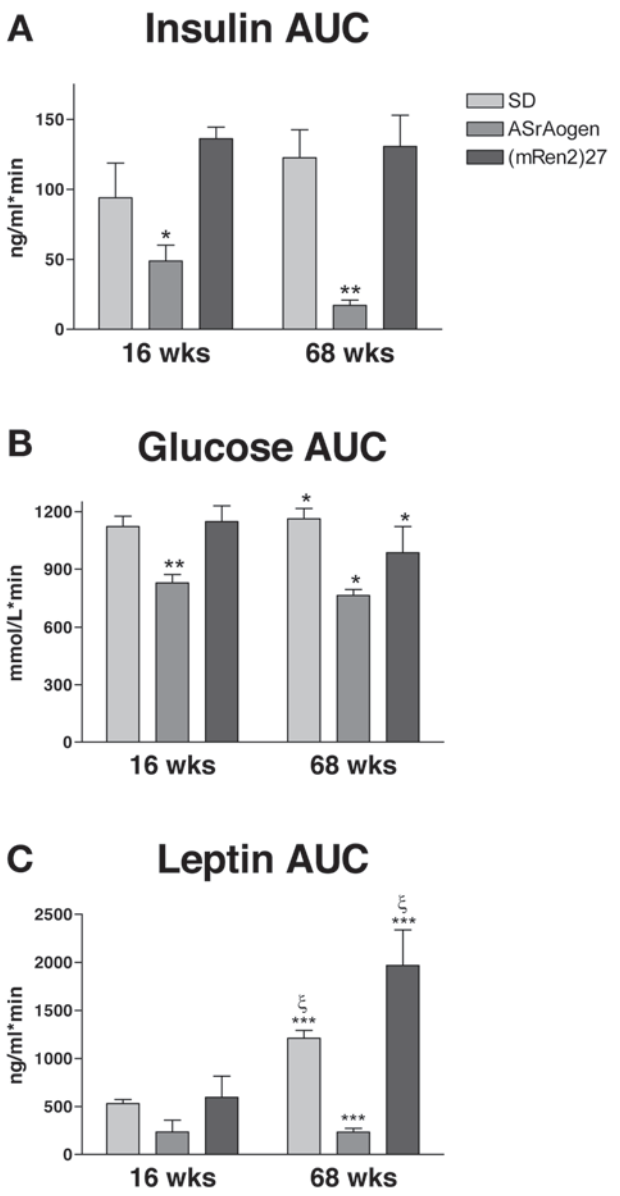


Fig. 3. Area under the curve (AUC) for insulin (**A**), glucose (**B**), and leptin (**C**) levels calculated from the oral glucose tolerance test at 16 and 68 wk of age in the SD, ASrAogen, and (mRen2)27 rats. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs other genotypes at same age. $^{\xi}p < 0.05$ vs 16 wk value in same genotype. ASrAogen: 16 wk $n = 10$, 68 wk $n = 10$; SD: 16 wk $n = 8$, 68 wk $n = 13$; (mRen2)27: 16 wk $n = 8$, 68 wk $n = 5$.

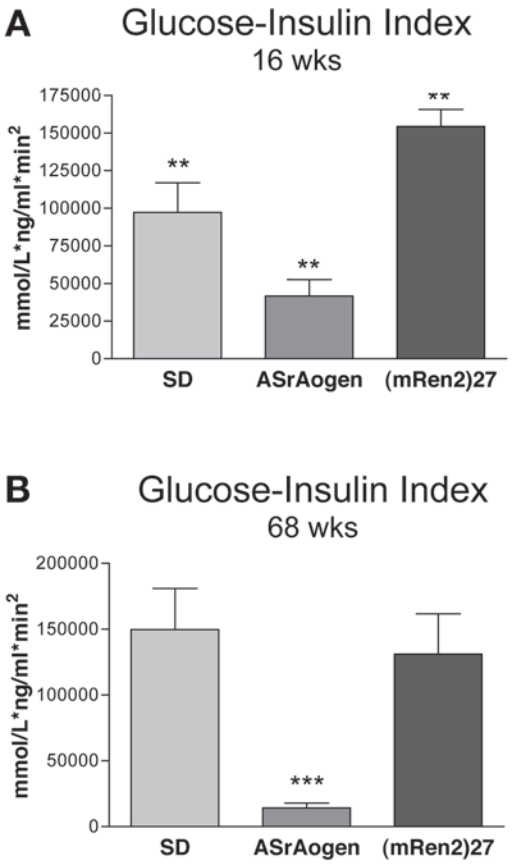


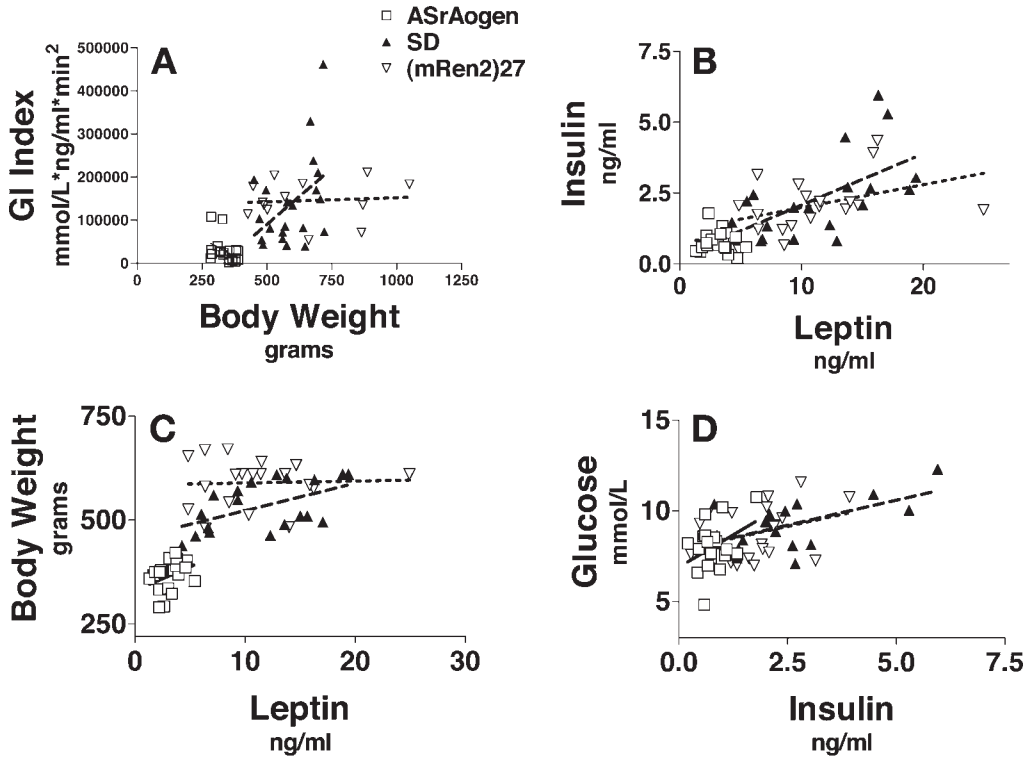
Fig. 4. The glucose–insulin (GI) index, a measure of whole body insulin sensitivity, calculated from the areas under the curve for insulin and glucose in the SD, ASrAogen, and (mRen2)27 rats at 16 (**A**) and 68 (**B**) wk of age. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs other genotypes at same age. ASrAogen: 16 wk $n = 10$, 68 wk $n = 10$; SD: 16 wk $n = 8$, 68 wk $n = 13$; (mRen2)27: 16 wk $n = 8$, 68 wk $n = 5$.

leptin was similar in the three groups at 16 wk as expected from previous studies *(3)*, but was significantly different among the three groups at 68 wk of age (Fig. 3C). The ASrAogen animals had the lowest AUC for leptin compared to the SD and (mRen2)27 animals at this later time point. The SD animals had a lower AUC for leptin at 68 wk compared to the (mRen2)27 animals. There was an age-related increase in the AUC for leptin in both SD and (mRen2) 27 rats with no increase in the AUC for leptin in ASrAogen animals with age.

We also examined the relationships between insulin and glucose, body weight and insulin resistance, body weight and leptin, and glucose and insulin in all three strains of rats using data from both ages to determine correlation coefficients. There was a significant positive correlation between the glucose–insulin index and body weight in the SD animals but not in either the ASrAogen or the (mRen2)27 animals as shown in Fig. 5A. In non-fasted animals, we also observed a relationship between insulin and leptin, body weight and leptin, and glucose and insulin in the SD rats, but there was no significant relationship observed in the ASrAogen or (mRen2)27 rats (Figs. 5B–D and table of correlation coefficients).

Discussion

In this study, we confirmed that the (mRen2)27 animals are relatively insulin insensitive compared with SD rats at 16 wk and remain insensitive at 68 wk of age. In addition, we now report that rats with selective underexpression of glial angiotensinogen (ASrAogen) are more insulin sensitive at both 16 and 68 wk relative to the SD or (mRen2)27



Correlation Coefficients

	SD	ASrAogen	(mRen2)27
GI vs. Body Weight	0.44*	-0.42	0.08
Insulin vs. Leptin	0.58*	0.10	0.40
Body Weight Vs. Leptin	0.53*	0.35	0.01
Glucose Vs. Insulin	0.63**	0.37	0.32
	*=p<0.05; **=p<.005		

Fig. 5. Relationships of metabolic parameters for the SD, ASrAogen, and (mRen2)27 animals. There is a positive correlation between the GI index and body weight (**A**), insulin and leptin (**B**), body weight and leptin (**C**) and glucose and insulin (**D**) using values from both ages in the analysis for SD rats but not for either the ASrAogen or (mRen2)27 rats. ASrAogen, $n = 20$; SD $n = 20–21$; (mRen2)27 $n = 13–19$. The actual correlation coefficient for each relationship is displayed in the table at the bottom of the figure.

animals as indicated by the glucose–insulin index. The SD animals become relatively insulin resistant at the 68 wk time point and there is no difference in glucose–insulin index between the SD or (mRen2)27 animals at this age. Finally, the normal relationships between insulin resistance and body weight, leptin and body weight, insulin and leptin, insulin and glucose in SD rats are absent in (mRen2)27 and ASrAogen rats, an important observation suggesting that the brain RAS is a primary factor in setting these relationships.

ACE inhibitors and AT_1 receptor blockers have been shown recently to improve insulin sensitivity in both humans and rat models. Zucker rats treated with irbesartan have improved insulin sensitivity as well as an increase in GLUT-4 protein compared to untreated controls *(3)*. Also, KK-Ay mice, a model for type 2 diabetes, treated with valsartan had a profile similar to the treated Zucker rats with improved insulin sensitivity and increased GLUT-4. Valsartan treatment also increased insulin-induced phosphorylation of insulin receptor substrate (IRS)-1 and coupling of IRS-1 with p85 subunit of phosphatidylinositol (PI) 3-kinase in skeletal muscle, as well as levels of these proteins *(26)*. It is reported that acute and chronic administration of an ACE inhibitor in patients decreases the daily glucose profile and improves insulin sensitivity in type 2 diabetic patients *(27)*. Most of the above actions were likely attributed to blockade of the actions of angiotensin II at sites in the periphery. Our findings in the ASrAogen rats argue for a contribution of the brain RAS, either directly or indirectly through regulation of neural or neurohumoral pathways, because these animals have similar levels of circulating angiotensin II compared to SD rats at all ages *(21,23)*.

Previous reports indicate that (mRen2)27 rats are insulin insensitive at 6 wk of age using the oral glucose tolerance test *(14)*. These animals also have impaired activation

of insulin signaling in the soleus muscle *(27)*. Whether this is due to increased blood pressure, or increased centrally mediated sympathetic outflow, both of which are present in the (mRen2)27 animals, is unknown. Increased circulating angiotensin II has been reported inconsistently in these animals *(17,28–30)*, and we found no differences among strains at 16 and 68 wk of age in a recent study *(23)*. Therefore, there may be a contribution of circulating angiotensin II to the impairments in insulin and glucose handling in these animals, but this is not unequivocal. There are also reports suggesting that (mRen2)27 animals have enhanced glucose disposal using an intravenous glucose tolerance test and euglycemic insulin clamp in spite of elevated baseline insulin levels *(31)*. Nonetheless, the authors did find that the animals had lower glucose uptake in isolated skeletal muscles, consistent with the above-mentioned studies. There is also consistency in the observation of elevated baseline insulin levels in the (mRen2)27 animals across the various studies. In the present study, we confirm the higher insulin levels and insulin resistance in the younger (mRen2)27 rats relative to SD controls and find that these animals remain insulin insensitive at 68 wk of age, but that there was no further decrease in the glucose–insulin index between the 16 and 68 wk time points studied. The SD animals show relative signs of insulin resistance at 68 wk of age with a glucose–insulin index and Quicki index that is not different from the (mRen2)27 animals at this later time point. The oral glucose tolerance test is a measure of both insulin response to glucose and glucose utilization. The euglycemic clamp, for which Quicki is an index *(25)*, is a measure of glucose utilization, which may account for the slight differences obtained with the two tests. The fact that the ASrAogen animals show no signs of developing insulin resistance at this later age in our studies is remarkable, because age is known to be an independent risk factor for developing insulin resistance *(32)*. This may be due to our early aging time point and impairment may be seen if these animals were allowed to age further. However, the data reveal that glial angiotensinogen is important in the age-related decrease in glucose utilization, because the ASrAogen animals are clearly protected from the decline in insulin sensitivity seen at this time point in the SD rats.

There is also evidence that an activated sympathetic nervous system will decrease insulin sensitivity *(33)*. Sympathetic and parasympathetic innervation of adipose tissue clearly contributes to overall body insulin and leptin metabolism *(34)*. Angiotensin II is well known to activate the sympathetic nervous system through actions in the brain *(35)*. Therefore, it is possible that the brain renin–angiotensin system is an important contributor to insulin resistance through this mechanism. In fact, there appears to be low sympathetic activity in the ASrAogen and high in the (mRen2)27, as evidenced by the resting heart rates and blood pressure levels *(16)* in the conscious state. Differences between the type of autonomic disturbance produced by elevation of the glial versus neuronal renin–angiotensin system components is reported *(36–38)*. And, long-standing differences in arterial pressure among the three strains of animals studied may contribute to hemodynamic and vascular changes that influence insulin resistance. Therefore, the fact that the increased brain renin in the (mRen2)27 and reduced glial-derived angiotensinogen do not produce exactly opposite effects in all variables studied is not surprising, especially in the older animals. In addition, high levels of corticosterone can cause insulin resistance *(39)* and elevated cortocosterone levels are involved in the development of hypertension in the (mRen2)27 rats *(40)*. However, corticosterone levels were not measured in the ASrAogen animals in this study. Therefore, further examination is required to determine the contribution of potential differences in corticosterone on insulin resistance in these models.

The difference in leptin levels among the three groups is not surprising because there are weight differences between the three groups and leptin is produced in the adipose tissue. However, even when corrected for body weight, there was lower leptin in the older ASrAogen rats relative to the other groups *(16)*. Because leptin is known to increase sympathetic activity *(41)*, the higher levels in the SD rats as they age may be another contributor to the higher sympathetic outflow and thus the insulin resistance and increase in systolic blood pressure *(16)* in these animals at the older age. Leptin cannot be the only contributor, because there is no difference in leptin AUC at 16 wk while insulin sensitivity and body weight differed among the three groups at that time point. Food intake was higher in ASrAogen animals at all ages compared to the SD and (mRen2)27 rats *(16)*, further suggesting that the difference in body weight is due to a difference in energy metabolism and unrelated to leptin levels in the circulation. However, it is possible that the lower body weight in the ASrAogen animals is a contributor to the enhanced insulin sensitivity in these animals.

A significant positive correlation between the glucose–insulin index and body weight, insulin and leptin, body weight and leptin, and glucose and insulin in the SD animals was observed using data from both young and old rats. The connection between body weight, insulin and leptin metabolism, and blood pressure is of considerable current interest in light of the growing concern over increases in the occurrence of the metabolic syndrome. We show that these relationships are not observed in either the ASrAogen or the (mRen2)27 animals. In fact, the values for the animals with the low glial angiotensinogen animals are clustered at the lower end of the curves, whereas the (mRen2)27 rats predominate at the upper end, especially for the relationship between glucose–insulin index or leptin and body weight. This would imply that the brain angiotensin levels in these two transgenic strains are a major factor influencing these relationships.

Because our studies are in transgenic animals, the contribution of developmental differences to the results observed must be considered. However, at 16 wk, there is no difference in leptin AUC as well as no difference in resting serum leptin levels among the three strains of rats *(16)*. Also, urinary levels as well as plasma levels of the angiotensin peptides are comparable at 16 wk of age, in spite of differences in body weight and blood pressure among the three groups at this time *(16,23)*. While the (mRen2)27 rats exhibit impairments in insulin and glucose metabolism at the early time point, there is no difference in fasting and resting insulin levels at 16 wk between the SD and ASrAogen animals *(16)*. Only at the older time point were differences in the resting or stimulated serum insulin and leptin levels observed in the three strains of rats. Moreover, the phenotype seen in the ASrAogen rats across time in this and our previous studies in these animals mimics the profile seen in adult animals treated long-term with inhibitors of the renin–angiotensin system *(3,42)*. This is true in terms of maintenance of memory ability, lower blood pressure, and normal renal function *(4,42,43)*, in addition to the similar features of lower body weight and serum leptin levels and maintenance of normal or better insulin and glucose metabolism. Although not all of the above features have been studied, the body weight is lower in transgenic mice with complete angiotensinogen deficiency *(44)*. Thus, it is unlikely that developmental changes account for the entire pattern of differences among strains especially at the later time points.

The current studies reveal that animals with high levels of brain angiotensin II have lower glucose tolerance even at a young age, while animals with low brain angiotensinogen have indices of higher glucose utilization at 16 wk of age compared to the SD. With increased age, SD animals show relative insulin resistance with no change across time in the ASrAogen animals. While the hypertension, possibly elevated circulating levels of Ang II, and developing heart failure may complicate interpretation of the mechanisms involved in the alterations in metabolism in the (mRen2)27 animals, the data in the ASrAogen rats indicate that the glial renin–angiotensin system exerts an important influence on the development of insulin resistance and regulation of metabolism during aging.

Perspective

Recently, interest in the connection between angiotensin II and insulin resistance has emerged. This is in part due to recent clinical trials showing that new onset diabetes was reduced in patients treated with AT_1 receptor blockers *(2,5)* or an ACE inhibitor *(1)*. This is further supported in animal models treated long-term with RAS inhibitors (3). The current data contribute to this literature suggesting a link between the brain renin–angiotensin system and the effects observed with long-term systemic blockade of RAS. However, given the complex effects of Ang II directly on various transmitter systems, further studies are required to elucidate the precise mechanisms involved in this improvement. Nonetheless, the importance of brain Ang II as a key link in regulation of blood pressure, leptin, and insulin resistance and the relationships of those factors with both body weight and age is put forth as a working hypothesis.

Methods

Animals

Male rats from all three lines [Hannover SD, hemizygote (mRen2)27, or ASrAogen680 at 16 and 68 wk of age] were obtained from the colonies maintained in the Hypertension and Vascular Disease Center at Wake Forest University. The Hannover SD rats are the parent line for the two transgenic rat strains. All animals were bred and exposed to the same housing conditions (12:12 light:dark cycle) and provided *ad libitum* food and water. All experimental protocols were approved by the institutional animal care and use committee.

Oral Glucose Tolerance Test

Food was removed from animals between 1600 and 1700 the night before the procedure and all studies were performed between 8 AM and 11 AM the following day. On the day of the procedure, animals were given 1 mg/kg dextrose *(3)* by gavage in 1 mL of water. Venous blood (approx 0.3 mL) was taken from the tail vein at 0, 15, 30, 60, 90, and 120 min post-gavage and replaced with a flush of saline *(3)*.

Glucose, Insulin, and Leptin Analysis

Glucose was measured in the serum of each animal using a Freestyle glucose monitor as published *(16)*. Insulin and leptin were measured using radioimmunoassays specific for rat according to the manufacturer's protocol (Linco, Inc), as previously published *(16)*. The glucose–insulin index is the product of the area under the curve (AUC) for insulin times the AUC for glucose.

Quicki Method

The quantitative insulin-sensitivity check index (Quicki) was used as a secondary measure of insulin sensitivity. This measure uses the ratio of baseline fasting insulin and glucose values (taken from the 0 time point). Calculations were adapted from Chen et al. *(25)* using the units of ng/mL for insulin and mg/dL for glucose.

Statistics

Area under the curve was calculated using the trapezoid method. Two-way ANOVA was used to assess overall interactions across strain and ages. Subsequently, one-way ANOVA and Student–Newman–Keuls post-hoc tests were used to compare each genotype or age to each other. All analyses, including calculations of correlation coefficients,

were carried out using GraphPad Prism (version 3; Graphpad, Inc). $p < 0.05$ was used for significance and all values are reported as means ± standard error of the mean (SEM).

Acknowledgments

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