Energy Metabolism in Human Renin-Gene Transgenic Rats
Does Renin Contribute to Obesity?

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Abstract—Renin initiates angiotensin II formation and has no other known functions. We observed that transgenic rats (TGR) overexpressing the human renin gene (hREN) developed moderate obesity with increased body fat mass and glucose intolerance compared with nontransgenic Sprague-Dawley (SD) rats. The metabolic changes were not reversed by an angiotensin-converting enzyme inhibitor, a direct renin inhibitor, or by (pro)renin receptor blocker treatment. The obese phenotype in TGR(hREN) originated from higher food intake, which was partly compensated by increases in resting energy expenditure, total thermogenesis (postprandial and exercise activity), and lipid oxidation during the first 8 weeks of life. Once established, the difference in body weight between TGR(hREN) and SD rats remained constant over time. When restricted to the caloric intake of SD, TGR(hREN) developed an even lower body weight than nontransgenic controls. We did not observe significant changes in the cocaine and amphetamine-regulated transcript, pro-opiomelanocortin, both anorexigenic, or neuropeptide Y, orexigenic, mRNA levels in TGR(hREN) versus SD controls. However, the mRNA level of the agouti-related peptide, orexigenic, was significantly reduced in TGR(hREN) versus SD controls at the end of the study, which indicates a compensatory mechanism. We suggest that the human renin transgene initiates a process leading to increased and early appetite, obesity, and metabolic changes not related to angiotensin II. The mechanisms are independent of any currently known renin-related effects. (Hypertension. 2009;53:516-523.)

Key Words: renin-angiotensin system ■ obesity ■ metabolic syndrome ■ ACE inhibitors ■ renin inhibitors ■ (pro)renin receptor ■ handle region peptide

Obesity is largely responsible for the metabolic syndrome that clusters dyslipidemia, insulin resistance, glucose intolerance/type 2 diabetes mellitus, and hypertension.1–3 The renin-angiotensin system is fundamental to blood pressure regulation, volume control, and salt balance through the action of angiotensin (Ang) II.4 Renin is an aspartyl protease that cleaves angiotensinogen, leading to Ang I and subsequently to Ang II formation via the Ang-converting enzyme (ACE). Several renin/prorenin animal models feature metabolic alterations.5–7 Mice solely harboring the human renin gene developed obesity.7 Rats overexpressing the mouse Ren-2 gene exhibit insulin resistance.5,6,8 However, this model is not obese, probably because of the severe hypertension and its sequelae.5 Takahashi et al9 reported recently that renin-deficient mice (Ren1c−/−) were lean, insulin sensitive, and resistant to diet-induced obesity. Nguyen et al10 cloned a novel (pro)renin receptor [(P)RR]. The receptor is expressed in a various tissues, including adipocytes.11 The (P)RR promotes direct renin actions independent of Ang II.12,13 The binding is specific for renin and prorenin, and the receptor enhances renin catalytic activity and unmasks receptor-bound prorenin catalytic activity.10 The binding of (pro)renin to the receptor also triggers intracellular signaling via the mitogen-activated protein kinase extracellular signal–regulated kinase 1/2 pathway.10,12,13 The relevance of the (P)RR and its function in humans are unknown. Ichihara et al14,15 blocked the (P)RR with a handle region decoy peptide (HRP) and ameliorated diabetic nephropathy in diabetic rats, as well as in Ang II type 1 receptor–deficient diabetic mice. Their studies await confirmation. We observed that rats harboring the human renin gene [TGR(hREN)] were consistently heavier than Sprague-Dawley (SD) rats, although they differ from SD solely by virtue of the human transgene. We investigated the phenomenon and found that TGR(hREN) develop obesity and metabolic alterations through an Ang II–independent mechanism.

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Methods

Animals
Experiments were conducted in age and body weight (BW)-matched 5-week-old male untreated TGR(hREN) that are described in detail (animal facility: Max-Delbrück Center). The TGR(hREN) line was generated on an SD genetic background.16,17 Age-matched nontransgenic SD rats served as controls. In treatment protocol 1, we compared untreated TGR(hREN) with ACE inhibitor (cilazapril [Cila])-treated TGR(hREN) (10 mg/kg per day in the diet for 48 weeks; n = 13) and SD rats (n = 15). To test the hypothesis regarding whether aspartyl protease activity of renin or the recently cloned (P)RR is involved in the pathogenesis, we performed protocol 2. We compared untreated TGR(hREN) with human direct renin inhibitor (aliskiren)-treated TGR(hREN); the drug was given SC 3 mg/kg per day by minipump infusion for 16 weeks (n = 15) or horseradish peroxidase (HRP), 3.6 μg/kg per day (n = 15), given in the same manner. Because our TGR(hREN) express human and rat (pro)renin, we used a combination treatment with 2 decapetides, NH2-RILLKPKMPSV-COOH, as the “handle region” of rat prorenin and NH2-RIFLKRMPSI-COOH, which include the human handle region sequence. Local authorities approved the studies, and we followed the American Physiological Society guidelines for animal care. We measured blood pressure from weeks 7 to 20 by radiotelemetry. Serum and plasma were collected from overnight (16-hour) fasted rats at week 16 and after sacrifice at the end of the study. We used an ELISA (LINCO Research) to measure insulin. Serum triglycerides and glucose were determined by an automated clinical method. For glucose tolerance testing, rats were fasted overnight. Blood was drawn from the tail at baseline and 30, 60, 90, 120, 150, and 180 minutes after glucose injection (2 g/kg IP). Blood glucose was determined by a clinical glucose-o-meter (One Touch Ultra, Johnson&Johnson, Inc). At week 20, the body composition, including the fat content, was measured by MRI (Texas Instruments). In protocol 1, all of the rats were killed at 48 weeks and in protocol 2, at 21 weeks.

Energy Metabolism
To assess changes in energy balance, we estimated the net energy intake and energy expenditure at the ages of 6, 8, 10, 12, and 18 weeks, respectively. We determined net energy intake of TGR(hREN) (n = 11) and SD rats (n = 6) by subtracting energy content in feces from energy content of food consumed. Rats were placed individually in metabolic cages for 2 consecutive days at the ages indicated above. These cages allowed exact determination of the food and water amounts consumed and amounts of feces produced. Feces were dried and energy content determined by means of an adiabatic bomb calorimeter (IKA-Calorimeter C 5000, IKA-Werke GmbH & Co KG). Likewise, total energy content in food was measured in duplicate samples. Rats were fed a commercial standard chow diet (V1530, SSNIFF GmbH) containing (weight%) carbohydrates 41.2, fat 3.3, protein 19.0, fiber 4.9, ash 6.4, and water 12.3 with a total (bomb calorimetry) and metabolizable (manufacturer’s information) energy content of 16.7 and 12.3 kJ/g, respectively.

Oxygen consumption and carbon dioxide production of SD rats (n = 6) and TGR(hREN) (n = 11) were measured by using an open-circuit calorimeter (gas analyzers: Magnos 16 and Uras 14 for oxygen consumption and carbon dioxide production, respectively, Hartmann and Braun) to calculate total energy expenditure (TEE) and respiratory quotient (RQ = carbon dioxide production/oxygen consumption), as described by Wiedmer et al.18 Within the calorimeter, rats had free access to food and water. Rats of the 2 groups were measured in single cages for 2 consecutive days at the ages indicated above. Oxygen consumption and carbon dioxide production were measured in 6-minute intervals over 23 hours, from 9 AM to 8 AM the next day. RQ and TEE were calculated according to Aust et al.19 The mean of the 10 lowest TEE values (kilojoules per minute) was accepted to calculate resting energy expenditure (REE; kilojoules per day). The difference between TEE and REE values was used to calculate total thermogenesis (TTH; kilojoules per day), which implied postprandial thermogenesis, as well as nonexercise and exercise activity thermogenesis.

Gene Expression in Brain and In Situ Hybridization
We performed in situ hybridization and TaqMan RT-PCR for human renin and for rat (P)RR. Cocaine and amphetamine-regulated transcript, pro-opiomelanocortin, neuropeptide Y, and agouti-related peptide (AgRP) gene expression were determined in brain hypothalamus tissue after mRNA had been extracted, as described earlier.13,15

Human Adipocyte Differentiation Assay
Human primary preadipocytes were isolated and differentiated as described previously.20 Adipogenesis was quantified at day 12 by Oil Red O staining.20 Human recombinant renin (10 and 100 ng/mL) and aliskiren (1 μmol/L) were added to the differentiation medium for the complete period of the experiment (n = 4 for each substance and concentration). The amount of accumulated triglycerides in immature adipocytes was compared between renin- or aliskiren-treated cells with control cells that were only stimulated with the adiogenic mixture.

Statistics
Data are presented as means±SEMs. Significant differences in mean values were tested by ANOVA, BW by repeated-measures ANOVA, and the posthoc Bonferroni’s t test. Area under the curve was calculated for glucose tolerance and analyzed by ANOVA and posthoc Bonferroni’s multiple t test. A value of P<0.05 was considered significant. The data were analyzed with Statview.

Results
TGR(hREN) Develop Obesity and Metabolic Alterations
At age 6 weeks, BW of TGR(hREN) and nontransgenic SD rats was 156±4 and 134±8 g, respectively (P value not significant; TGR(hREN) versus SD; Figure 1A). Within the following weeks, BW increased significantly in both groups and reached 547±7 and 510±11 g for TGR(hREN) and SD rats (P<0.05), respectively, at age 20 weeks. At ages 12, 14, 15, 18, and 20 weeks, BW was significantly higher in TGR(hREN) versus SD rats (P<0.05 or less). We also studied BW in female rats to test for sex differences. At week 21, female TGR(hREN) had greater BW than female SD rats (316±2 versus 279±9 g; P<0.01; Figure S1, please see http://hyper.ahajournals.org). Food intake was 397±8 and 300±15 kJ/d for male TGR(hREN) and SD rats, respectively, at age 6 weeks (P<0.001, TGR(hREN) versus SD; Figure 1B) and increased within the following 4 weeks to 506±7 and 453±16 kJ/d for TGR(hREN) and SD rats, respectively (P<0.01, TGR(hREN) versus SD). Thereafter, food intake remained at these levels in both groups and was significantly higher in TGR(hREN) versus SD rats at almost all of the time points until the end of testing. Higher food intake on the basis of food consumption was also observed in our pharmacological studies with Cila, aliskiren, and HRP. At age 6 weeks, net energy intake was 360±9 and 310±10 kJ/d for TGR(hREN) and SD rats, respectively (P<0.01, TGR(hREN) versus SD; Figure 2A). Within the following weeks, net energy intake increased in both groups and reached 387±8 and 337±14 kJ/d for TGR(hREN) and SD rats, respectively (P<0.05, TGR(hREN) versus SD), at age 8 weeks. Then, until age 12 weeks, net energy intake remained almost unchanged in SD rats but decreased to the level of SD rats in TGR(hREN). At
age 18 weeks, net energy intake was significantly lower in TGR(hREN) versus SD rats.

Interestingly, fecal loss of energy was ≈15 kJ higher in TGR(hREN) versus SD rats between the ages of 6 and 10 weeks (P<0.01; Figure 2B). Thereafter, the difference became smaller, and at the end of the study fecal loss of energy was even smaller in TGR(hREN) versus SD rats. These results clearly indicate that TGR(hREN) at least partially compensate their higher food consumption with a higher loss of energy via their feces.

Water intake was 33±1 g in SD rats at the beginning of the study and remained at that level until the end (Figure 2C). In TGR(hREN), water intake was similar to SD rats at the age 6 weeks. However, during the following weeks, it increased significantly up to 46±1 g at age 10 weeks (P<0.001, TGR(hREN) versus SD rats), followed by a decrease down to the initial values at age 18 weeks.

At age 6 weeks, TEE was 226±7 and 169±6 kJ/d (P<0.001, TGR(hREN) versus SD; Figure 3A), REE was 177±5 and 133±5 kJ/d (P<0.05, TGR(hREN) versus SD; Figure 3B), and TTh was 46±2 and 34±3 kJ/d (P value not significant, TGR(hREN) versus SD; Figure 3C) for TGR(hREN) and SD rats, respectively. Within the following weeks, TEE and REE increased in both groups to 333±8 and 287±8 kJ/d and 228±7 and 210±4 kJ/d for TGR(hREN) and SD rats, respectively. TTh increased to 98±2 and 73±4 kJ/d (P<0.05, TGR(hREN) versus SD) for TGR(hREN) and SD rats at age 18 weeks, respectively. Over the entire experimental period, TTh was generally significantly higher in TGR(hREN) versus SD rats.

Mean daily RQ was 0.95±0.003 and 1.03±0.003 for TGR(hREN) and SD rats (P<0.001, TGR(hREN) versus SD rats; Figure 4A) at age 6 weeks, respectively. This observation indicates a significantly higher lipid oxidation rate of TGR(hREN) versus SD rats. In SD rats, 24-hour RQ de-
creased within the following weeks to 0.93±0.003 at age 12 weeks but increased again to 0.98±0.05 at age 18 weeks. In TGR(hREN) rats, 24-hour RQ showed an undulating pattern within the following weeks, increasing to 0.99±0.005 at age 8 weeks (P value not significant, TGR(hREN) versus SD), decreasing to 0.94±0.007 at age 10 weeks (P value not significant, TGR(hREN) versus SD), increasing again to 0.98±0.007 at age 12 weeks (P<0.001, TGR(hREN) versus SD), and ending at 0.96±0.009 at age 18 weeks (P value not significant, TGR(hREN) versus SD). Resting RQ was 0.93±0.013 and 1.02±0.004 for TGR(hREN) and SD rats (P<0.001, TGR(hREN) versus SD rats; Figure 4B), respectively, at age 6 weeks, also indicating a significantly higher lipid oxidation rate of TGR(hREN) versus SD rats at rest. Within the following weeks, resting RQ values of TGR(hREN) and SD rats changed in the same manner as described for the 24-hour RQ values.

The arcuate nucleus and hypothalamic neuronal network play an important role in the regulation of BW, energy homeostasis, and feeding behavior in rodents. To test these pathways in our animals, we performed in situ hybridization of the rat (P)RR. Our results clearly showed that the human transgene was expressed in the hypothalamus but was not detectable in nontransgenic SD brains (Figure S2A and S2B). In addition, the rat (P)RR was expressed in transgenic and SD rats in a similar manner (Figure S2C). The results were confirmed by RT-PCR (Figure S2B and S2D). We next studied orexigenic and anorexigenic neuropeptide expression in the hypothalamus. At week 21, we observed no significant change in the mRNA levels of cocaine and amphetamine-regulated transcript, pro-opiomelanocortin, or neuropeptide Y in TGR(hREN) rats (Figure S3). However, the AgRP mRNA level was significant reduced in TGR(hREN) compared with SD controls (Figure S3).
**TGR(hREN) Are Normotensive Despite High Serum Leptin and Fat Inflammation**

Because obesity and metabolic alterations are often associated with hypertension, we measured blood pressure radiotlemetrically. Both transgenic and nontransgenic rats were normotensive (104/8 mm Hg, respectively; Figure S4A). Because of higher energy intake in TGR(hREN), we measured leptin. TGR(hREN) had significantly higher serum leptin levels compared with SD rats (1408 versus 597 pg/mL; \( P < 0.0001 \); Figure S4B). Consistent with previous findings that obesity is associated with increased accumulation of T cells in adipose tissue, which have been shown to inhibit preadipocyte differentiation, TGR(hREN) had significantly increased numbers of CD3* cells in epididymal fat tissue compared with SD rats (\( P < 0.02 \); Figure S4C).

**Role of Ang II for the Obese Phenotype**

To investigate the role of Ang II in the development of obesity, we treated TGR(hREN) with the ACE inhibitor Cila at a dose that inhibits Ang II formation. Cila did not affect BW (Figure 5A). TGR(hREN) were indeed fatter than SD rats, as measured by magnetic resonance (88±2 versus 69±3 g, respectively; \( P < 0.05 \); Figure 5B). Food intake (28±1 versus 21±1 g/d, respectively; \( P < 0.05 \); Figure 5C) was greater in untreated TGR(hREN) compared with SD rats. Cila did not influence food intake (Figure 5C) or body fat content (Figure 5B). Serum triglyceride levels were significantly increased in TGR(hREN) compared with SD rats and Cila-treated TGR(hREN) (168±8 versus 66±5 versus 78±4 mg/dL, respectively; \( P < 0.05 \); Figure 5D). A glucose tolerance test was impaired in untreated TGR(hREN) compared with SD rats. Cila did not improve glucose intolerance (Figure 5E). We next analyzed serum insulin levels (Figure 5F) and found that untreated TGR(hREN), Cila-treated TGR(hREN), and SD rats had similar insulin levels (1.6±0.3 versus 1.7±0.3 versus 1.2±0.1 ng/mL, respectively). Untreated and Cila-treated TGR(hREN) tended to be higher than SD rats.

**Pair Feeding Prevented Obesity**

We next addressed the question of whether caloric restriction would affect the development of obesity. When untreated TGR(hREN) were restricted to the caloric intake of SD rats by pair feeding, BW of TGR(hREN) was reduced, slightly below the SD BW (Figure 6). Feeding TGR(hREN) a high-fat diet did not aggravate the obese phenotype (data not shown).

**Direct Renin Inhibition and (P)RR Blockade**

We next tested the hypothesis that renin, because of its aspartyl protease activity, might cleave another substrate, which might promote obesity in our transgenic model. Therefore, we treated TGR(hREN) with aliskiren. Aliskiren had no effect on BW (Figure 7A). Furthermore, both groups had similar food intake (29±1 versus 29±1 g/d, respectively; Figure 7B). Glucose intolerance was not improved by aliskiren (Figure 7C). However, aliskiren treatment lowered serum triglyceride levels compared with untreated TGR(hREN) (125±7 versus 148±9 mg/dL, respectively; \( P < 0.05 \); Figure 7D). Recently, blockade of the (P)RR with HRP was claimed to ameliorated diabetic nephropathy.\(^{14,15}\)
Therefore, we tested the hypothesis that \((P)RR\) activation initiates signal transduction leading to obesity and metabolic alterations. Chronic HRP treatment had no effect on BW (Figure S5A). In addition, both groups had similar food intake (30\(\pm\)1 versus 31\(\pm\)1 g/d, respectively; Figure S5B). Glucose intolerance (Figure S5C) and hypertriglyceridemia were not improved by HRP treatment (Figure S5D).

Treatment of human preadipocytes with an adipogenic combination of insulin, cortisol, triiodothyronine, and isobutylmethylxanthine resulted in adipogenic differentiation and resulted in triglyceride accumulation in \(65\%\) of the preadipocytes. Adding human renin, with or without aliskiren, had no effect on preadipocyte differentiation as revealed by Oil Red O staining (Figure S6).

**Discussion**

Our study shows that human renin affects the metabolism of the TGR(hREN) so that the animal develops relative obesity and metabolic alterations in an age-dependent manner. Although BW did not differ between TGR(hREN) and SD rats in early life (<10 weeks), food intake was significantly higher in TGR(hREN) versus SD rats. However, the higher food intake in TGR(hREN) was almost completely compensated by a loss of energy in the feces and a higher TEE. Interestingly, the higher TEE in TGR(hREN) was mainly because of a higher REE at age 6 weeks and a higher total (postprandial and exercise activity) thermogenesis at age 8 weeks. These results are in agreement with a similar observation in humans by Leibel et al.\(^{21}\) At age 6 weeks, the higher energy expenditure of TGR(hREN) was accompanied by a higher lipid oxidation rate, as indicated by significantly lower RQ values, a measure to combat obesity. However, once set, the difference in BW of \(~35\) g between TGR(hREN) and SD rats remained quite stable until the end of the study. Interestingly, until this BW difference was set, water intake and TTh were significantly higher in TGR(hREN) versus SD rats. The increased water intake during weeks 8 to 12 particularly interests us because we showed recently that water drinking elicits a thermogenic response in fasting men and women because of sympathetic activation by stimulation of osmosensitive afferent neurons that probably reside in the liver.\(^{22,23}\)

Ang II does not appear to be responsible for the metabolic alterations, as tested by Ang II generation inhibition. The ACE inhibitor Cila, the Ang receptor blocker losartan (D.N.M., unpublished data, 2008), and the human renin inhibitor aliskiren were unable to influence BW, glucose intolerance, and food intake. In contrast, serum triglyceride levels were Ang II dependent because they were partially reduced by both inhibitors. We found no evidence that renin might have cleaved an unknown substrate, which would be involved in our phenotype. Chronic \((P)RR\) blockade with the HRP did not affect BW gain, food intake, or metabolic changes.

Uehara et al\(^7\) made similar observations to those reported here, namely that male mice transgenic for hREN developed...
obesity after 15 weeks of age, resulting in a BW that was 2 times higher than that of age-matched wild-type mice at 60 weeks of age. Obese hREN mice also exhibited hyperglycemia, hyperinsulinemia, hyperleptinemia, and hyperlipidemia, as well as an increase in fat mass and liver, heart, and kidney size.24 Interestingly, the BW increase depended on the level of human renin in that study, because heterozygous mice developed a less pronounced phenotype.7 However, possible changes in energy expenditure were not reported. The phenotypic similarity of the 2 transgenic models clearly speaks against the chance that the human transgene was integrated twice in a locus, leading to the same metabolic changes.

Takahashi et al9 showed recently that 3- to 5-month-old mice lacking renin (Ren1c–/–) are lean, insulin sensitive, and resistant to diet-induced obesity without changes in food intake and physical activity. The lean phenotype was likely attributed to a higher resting and TEE, a higher lipid oxidation rate, and gastrointestinal loss of dietary fat. Takahashi et al9 observed that the metabolic changes were reversed by Ang II administration in their model. Our TGR(hREN) developed an obese phenotype between ages 3 and 5 months, most likely because of increased food intake. The results of the 3 renin-dependent models indicate that alterations in the renin level initiate a process that takes several months, leading to changes in BW with metabolic changes. Obviously, renin plays a distinct role within the complex network for regulating energy intake and energy expenditure. When renin is absent, as in Ren1c–/– mice, development of obesity is prevented by prior to an increased resting and TEE and impaired nutrient absorption. When renin is overexpressed, as in TGR(hREN), food intake is increased a priori, which favors the development of obesity, possibly by an increased BW set point that can be sensed and counteracted only partly by increasing resting and TEE and lipid oxidation, at least in early life. To our knowledge, such adjustments have not been observed earlier in rat or mouse obesity models. Interestingly, Ren1c–/– had similar triglyceride levels compared with controls, whereas TGR(hREN) and hREN transgenic mice had significantly higher triglyceride levels compared with controls. Finally, we have no evidence for any gastrointestinal fat losses in our model.

Our treatment attempts with ACE inhibition, a direct renin inhibitor, and (P)RR blockade did not influence appetite or BW in TGR(hREN). Inhibition of Ang II generation improved carbohydrate and lipid metabolism to some extent, whereas rat (P)RR blockade was not effective. Our results are unlike the state-of-affairs in Ren1c–/– mice, where Ang II treatment reversed the lean phenotype and some of the metabolic changes.9 However, Ang II treatment did not increase the food intake in their model, and the Ang II–induced mode of action remains unclear in their study. Results from large clinical trials with renin-Ang system blockers might have predicted some of the effects that we observed.25 Reductions in triglyceride concentrations have also been described in animal models of diet-induced obesity. Araki et al25 described improved glucose tolerance, lowered lipid values, and reduced BW after treating such a model with telmisartan and attributed these effects to an increased expression of uncoupling protein 1. Takahashi et al9 found increased plasma adiponectin levels in their Ren1c–/– mice, which are known to increase the expression of uncoupling proteins.26

We were not able to elucidate how the transgene product, namely, human renin, influenced food intake, BW, and other aspects of metabolism. Possibly renin or prorenin, which are both produced by the transgene, play a major role in regulating energy homeostasis by cleaving an as-yet-unknown alternative substrate not addressed by our inhibitors. However, no renin substrate other than angiotensinogen has been discovered. Peripheral substrate cleavage seems to be rather unlikely, because aliskiren did not affect BW and food intake. The role of the elusive (P)RR remains unclear. HRP was said to block this receptor. Whether HRP crosses the blood-brain barrier is unknown. HRP is a small peptide, and its central penetration has not been determined. A central action of the transgene remains possible. We did not observe significant changes in the mRNA levels of cocaine and amphetamine-regulated transcript and pro-opiomelanocortin (anorexigenic) or neuropeptide Y (orexigenic) in TGR(hREN) versus SD controls. However, the mRNA level of AgRP (orexigenic) was significantly reduced in TGR(hREN) versus SD controls. Because we measured AgRP gene expression at the end of our study (week 21), it is tempting to speculate that decreased AgRPs are a compensatory mechanism. The same was found in young SD rats treated with a high-fat diet.27

Perspectives
Renin seems to have a distinct effect on the complex network for regulating food intake and energy expenditure, independent of Ang II. Because the renin-Ang-aldosterone system is upregulated in obesity, higher renin levels might augment the maintenance of positive energy balance generating a vicious cycle. Such a cycle could make the reduction of food intake more difficult and interfere with normalizing BW. Renin might be an important link between obesity and related diseases, such as hypertension and diabetes mellitus within the metabolic syndrome framework. Our strategies reported here were not successful; nonetheless, renin would appear to be a target gene for obesity.

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Disclosures
F.C.L. has served as an advisor for Novartis. The remaining authors report no conflicts.
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**Early alterations in energy balance of rats transgenic for human renin.**

**A contribution to the development of obesity?**

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Results

Body Weight in female TGR(hREN) and SD rats

We also studied the body weight in female rats. At week 21, female TGR had a significantly increased body weight compared to female SD rat (316±4 vs. 279±9 g (p<0.01; Figure S1).

Human renin, (pro)renin receptor, orexigenic and anorexigenic neuropeptide expression

In rodents the arcuate (Arc) and hypothalamic neuronal network plays an important role in the regulation of body weight, energy homeostasis and feeding behaviour. Therefore, we performed in situ hybridization and TaqMan RT-PCR for human renin and for rat (pro)renin receptor [r(P)RR]. Our results clearly show that the human transgene is expressed in the hypothalamus, but was not detectable in non-transgenic SD brains (online supplement Figure S2A and B). In addition, the rat (P)RR is expressed in transgenic and SD rats in a similar manner (Figure S2C). The results were confirmed by RT-PCR (Figure S2B and D).

We next studied orexigenic and anorexigenic neuropeptide expression in the hypothalamus. We observed no significant change in the mRNA levels of amphetamine-regulated transcript (CART), pro-opiomelanocortin (POMC) or neuropeptide Y (NPY) in transgenic TGR(hREN) rats (Figure S3). However, the agouti related peptide (AgRP) mRNA level was significant reduced in TGR(hREN) compared to SD controls (Figure S3).

TGR(hREN) are normotensive despite high serum leptin and fat inflammation

Since obesity and metabolic alterations are often associated with hypertension, we measured blood pressure radio-telemetrically. Both transgenic and non-transgenic rats were normotensive (104±2 and 103±2 mm Hg, respectively, Figure S4A). Due to higher energy intake in TGR(hREN), we measured leptin. TGR(hREN) had significantly higher serum leptin levels compared to SD (1408±116 vs. 597±58 pg/ml; p<0.0001, Figure S4B). Consistent with previous findings that obesity is associated with increased accumulation of T cells in adipose tissue, which have been shown to inhibit preadipocyte differentiation, TGR(hREN) had significantly increased numbers of CD3+ cells in epididymal fat tissue compared to SD (p<0.02, Figure S4A).

Chronic treatment with the ostensible (P)RR blocker HRP does not reduced obesity and metabolic alterations in TGR(hREN)

Recently, blockade of the (P)RR with HRP was claimed to ameliorated diabetic nephropathy. Therefore, we tested the hypothesis that (P)RR activation initiates signal transduction leading to obesity and metabolic alterations. Chronic HRP treatment had no effect on BW (Figure S5A). Additionally, both groups had similar food intake (30±1 vs. 31±1 g/day, respectively, Figure S5B). Glucose intolerance (Figure S5C) and hypertriglyceridemia was not improved by HRP treatment (Figure S5D).

Adipogenic differentiation is not influenced by renin or aliskiren

Treatment of human preadipocytes with an adipogenic combination of insulin, cortisol, triiodothyronine, and isobutylmethylxanthine resulted in adipogenic differentiation and resulted in triglyceride accumulation in approximately 65% of the preadipocytes. Adding human renin, with or without aliskiren, had no effect on preadipocyte differentiation as revealed by Oil Red O staining (Figure S6).
Figure S1. Female TGR(hREN) have a significantly increased body weight at week 21 compared to female SD. Data are means±SEM, n = 6-12 each group.
**Figure S2.** In situ hybridisation analysis of human renin using non-radio digoxigenin labelled riboprobe (panel A). The expression pattern shows a very defined distribution of hREN mRNA in the ventromedial hypothalamus (VMH), arcuate nucleus (Arc) and also in the lateral hypothalamus (LH). TaqMan RT-PCR in the hypothalamus detected a high expression signal in TGR(hREN) and no signal in SD (panel B). Panel C shows in situ hybridisation analysis of rat (pro)renin receptor [r(P)RR] using radio labelled anti-sense riboprobe (panel B) in the coronal brain section. (P)RR distribution was observed in the cerebaral cortex (cor), hippocampus (Hipp), striatum (CPu), ventro medial hypothalamus (VMH) and also in arcuate nucleus (Arc). TaqMan RT-PCR in the hypothalamus showed a similar (P)RR expression level in TGR(hREN) and SD (panel D). Data are means±SEM, n = 4 each group.
Figure S3. TaqMan RT-PCR analyses in the hypothalamus showed only a significant difference in Agouti-related protein gene (AgRP) expression, while neuro peptide Y (NPY), cocaine- and amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC) were not differentially expressed. Data are means±SEM, n = 4 each group.
Fig. S4

A  
**Blood Pressure**  

![Graph showing Blood Pressure comparison between SD and TGR(hREN) groups.](image)

B  
**Leptin**  

![Graph showing Leptin levels comparison between SD and TGR(hREN) groups.](image)

C  
**CD3**  

![Graph showing CD3 mRNA Expression comparison between SD and TGR(hREN) groups.](image)
Figure S4. (A) Mean arterial blood pressure (MAP) was not different between the groups. In contrast, (B) TGR(hREN) had 3-fold increased serum leptin levels and significantly increased CD3 mRNA expression (C) in epididymal fat tissue. Results are mean±SEM (* p< 0.05 vs. other group).

Fig. S5

Figure S5. (A) BW gain in TGR(hREN) was not influenced by HRP. (B) TGR(hREN) had a higher food intake compared to SD controls that was not influenced by HRP. (C) Glucose tolerance was impaired in all TGR(hREN) groups. (D) HRP treatment did not reduce serum triglyceride levels in TGR(hREN). Results are mean±SEM (* p< 0.05 vs. other groups).
**Figure S6.** Human renin did not affect lipid accumulation of human preadipocytes during adipogenesis nor did aliskiren. Results are mean±SEM.