Inflammatory Induction of Human Resistin Causes Insulin Resistance in Endotoxemic Mice

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OBJECTIVE—Although adipocyte-derived murine resistin links insulin resistance to obesity, the role of human resistin, predominantly expressed in mononuclear cells and induced by inflammatory signals, remains unclear. Given the mounting evidence that obesity and type 2 diabetes are inflammatory diseases, we sought to determine the relationship between inflammatory increases in human resistin and insulin resistance.

RESEARCH DESIGN AND METHODS—To investigate the role of human resistin on glucose homeostasis in inflammatory states, we generated mice lacking murine resistin but transgenic for a bacterial artificial chromosome containing human resistin (BAC-Retn), whose expression was similar to that in humans. The metabolic and molecular phenotypes of BAC-Retn mice were assessed after acute and chronic endotoxemia (i.e., exposure to inflammatory lipopolysaccharide).

RESULTS—We found that BAC-Retn mice have circulating resistin levels within the normal human range, and similar to humans, lipopolysaccharide markedly increased serum resistin levels. Acute endotoxemia caused hypoglycemia in mice lacking murine resistin, and this was attenuated in BAC-Retn mice. In addition, BAC-Retn mice developed severe hepatic insulin resistance under chronic endotoxemia, accompanied by increased inflammatory responses in liver and skeletal muscle.

CONCLUSIONS—These results strongly support the role of human resistin in the development of insulin resistance in inflammation. Thus, human resistin may link insulin resistance to inflammatory diseases such as obesity, type 2 diabetes, and atherosclerosis.

The prevalence of obesity has increased worldwide, and therefore, the burden of obesity on health is progressively growing (1). Obesity is associated with insulin resistance and an increased risk of type 2 diabetes. Recently, it has become clear that obesity promotes a state of chronic, low-grade inflammation in white adipose tissue (WAT), which leads to insulin resistance. Adipocytes and macrophages in WAT both are involved in the development of insulin resistance in obesity through the release of free fatty acids (FFAs), proinflammatory cytokines, and adipokines (2–4).

Resistin, predominantly expressed in adipocytes, mediates insulin resistance in rodents. Circulating levels of resistin are increased in obesity, and treatment with recombinant resistin or transgenic overexpression of resistin induces hepatic insulin resistance in mice (5–8). Furthermore, deletion of resistin enhances insulin sensitivity in livers of mice fed a high-fat diet, and in muscle and WAT in leptin-deficient ob/ob mice (9,10). In contrast, resistin is mainly expressed in mononuclear cells, including macrophages in humans (11,12). Human resistin is induced in response to various inflammatory stimuli such as lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), or interleukin (IL)-6, and resistin itself induces proinflammatory cytokines, suggesting a role for resistin in inflammation in humans (13–16). Moreover, circulating resistin levels correlate with inflammatory markers in subjects with type 2 diabetes, coronary atherosclerosis, chronic kidney disease, rheumatoid arthritis, sepsis, and experimental endotoxemia (14,17–21).

Because it is widely accepted that obesity and type 2 diabetes are associated with chronic inflammation in WAT, and resistin is produced by macrophages in inflammation, hyperresistinemia might be a contributing factor to these pathophysiologic states in humans. However, studies relating resistin in humans to obesity, insulin resistance, or type 2 diabetes have shown conflicting results (22–28). Therefore, it remains uncertain whether the role of adipocyte-derived murine resistin in glucose homeostasis translates directly to the biology of macrophage-derived human resistin.

We have previously demonstrated that transgenic expression of human resistin in resistin-knockout (Rko) mice fed a high-fat diet results in insulin resistance in muscle and adipose tissue, indicating that macrophage-derived human resistin exacerbates insulin resistance (29). However, macrophage human resistin expression in that model was constitutive and not induced by inflammatory stimuli. Here we investigated the metabolic effects of human resistin induced by inflammation using a novel transgenic mouse that express human resistin from its human genomic elements (BAC-Retn mice). In contrast to the previous macrophage-specific humanized resistin mice, circulating resistin levels in this new BAC-Retn mouse are similar to those in healthy humans but increase in response to inflammatory signals such as LPS. Under experimental endotoxemia, BAC-Retn mice showed increased inflammation and hepatic insulin resistance. Thus, with a humanized expression pattern, human resistin contributes to the development of insulin resistance in inflammation.

RESEARCH DESIGN AND METHODS

Cell culture and transfections. The BAC-Retn construct that included the human resistin gene plus 20 kb upstream and 4 kb downstream DNA regions was engineered and transfected into mouse macrophage RAW264 cells by
electroporation according to the manufacturer’s instructions (Lonza, Allendale, NJ). Primary mouse macrophages were obtained from male BAC-Retn mice by peritoneal lavage 3 days after an intraperitoneal injection with 1 mL of sterile 3% thioglycollate. RAW.264 cells or primary mouse macrophages were stimulated with LPS (Escherichia coli O 111:B4; Sigma-Aldrich, St. Louis, MO). For acute endotoxemia experiments, equivalent volumes of LPS (0.2 mg/kg body wt) or normal saline (0.9%) were intraperitoneally injected to both mice housed four or five per cage under an ambient temperature of 22°C with 12 h light/dark cycle (lights on at 0700 h). Regular chow (LabDiet, Richmond, IN) and water were provided ad libitum. All protocols for animal use and euthanasia were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania School of Medicine and were in accordance with National Institutes of Health guidelines.

RESULTS

Generation of mice that express human resistin with human-like inducible regulation. We generated a BAC that contains the human resistin gene plus all the DNA elements, we engineered a BAC as described previously (30). The engineered BAC contained the whole human resistin gene, including 21,300 bp upstream and 4,248 bp downstream of the human resistin start site. The fusion construct containing human resistin and the 25,548 bp surrounding it was then used to produce the BAC-transgenic (BAC-Retn) mice that express human resistin without expressing murine resistin (Retn+/BACRh). Retn+/− littersmates were used as controls in this study.

Animals used in all experiments were age-matched (10- to 12-week-old) male mice housed four or five per cage under an ambient temperature of 22°C with 12 h light/dark cycle (lights on at 0700 h). Regular chow (LabDiet, Richmond, IN) and water were provided ad libitum. All protocols for animal use and euthanasia were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania School of Medicine and were in accordance with National Institutes of Health guidelines.

LPS administration. Mice were weighed to determine the dose of LPS (Escherichia coli O 111:B4; Sigma-Aldrich, St. Louis, MO). For acute endotoxemia experiments, equivalent volumes of LPS (0.2 mg/kg body wt) or normal saline (0.9%) were intraperitoneally injected to both Rko and BAC-Retn mice. Blood was collected before and at 1, 5, and 6 h after injection. For chronic endotoxemia experiments, mice were implanted subcutaneously with an osmotic minipump (Alzet model 1002; BrainTeer Scientific Inc., Braintree, MA) as previously described (31). The pumps were filled with normal saline or LPS to infuse 2 mg/kg/day for 2 weeks.

Total RNA extraction and quantitative PCR. Mice were killed and their tissues isolated and immediately placed in liquid nitrogen for later use. Total RNA from tissues was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. RNA integrity was determined with ultraviolet spectrophotometry. Reverse transcription was performed with Sprint RT complete-Oligo(dT)18 (Clontech Laboratories, Mountain View, CA) according to the manufacturer’s instructions. Expression of genes was analyzed using real-time (TaqMan) quantitative PCR (ABI Prism; Applied Biosystems, Foster City, CA) as previously described (29). The level of mRNA expression was normalized to 36B4.

Measurement of serum and tissue metabolites. Blood glucose measurements were obtained from tail vein blood using the OneTouch Ultra Glucometer (Lifescan, Milpitas, CA). Serum was harvested by tail vein blood or cardiac puncture, spun at 4°C for 30 min, and assayed immediately or stored at −20°C. Serum alanine aminotransferase (ALT), TGs, cholesterol, β-hydroxybutyryc acid, and FFAs levels were measured using colorimetric assays (Stahlosi Bio Laboratory, Boerne, TX, Wako Pure Chemical Industries, Osaka, Japan). Serum mouse insulin, resistin, leptin, high-molecular-weight adiponectin, and human resistin levels were measured by ELISA (Crystal Chem Inc., Downers Grove, IL, Millipore Corporation, Billerica, MA). Serum TNF-α and IL1β levels were measured with Luminex-100 multiplex immunoassay (Millipore). Lipids were extracted from liver for TGs and cholesterol measurements, as previously described (35). For diacylglycerol and ceramides levels, 100-μg samples of liver tissue were submitted to the Mouse Metabolic Phenotyping Center at Yale Medical School (New Haven, CT).

Glucose tolerance test. On the 8th day after the minipump subcutaneous implantation, glucose tolerance testing was performed after an 8-h fast. Blood glucose concentrations were measured before and at 15, 30, 60, 90, and 120 min after an injection of glucose (1 g/kg, 20% glucose solution). Tail blood samples collected before the glucose injection were used to measure basal levels of mouse insulin and human resistin.

Hyperinsulinemic-euglycemic clamp. Hyperinsulinemic-euglycemic clamp analysis was performed as described previously (29). Clamp studies were performed at 6 h after LPS (0.2 mg/kg) or saline intraperitoneal injection, and also at 10 days after the minipump subcutaneous implantation. Mice were fasted for 4 h, placed in restrainers, and administered a bolus injection of 5 μCI of [3-3H]glucose, followed by continuous intravenous infusion at 0.05 μCi/min. Baseline glucose kinetics was measured for 60 min, followed by hyperinsulinemic clamp for 120 min. A priming dose of regular insulin (16 μU/kg, Humulin; Eli Lilly, Indianapolis, IN) was given intravenously, followed by continuous infusion at 2.5 μU/kg/min. Tail blood glucose was measured by glucometer at 10-min intervals, and blood glucose was maintained at 120–140 mg/dL by a variable infusion rate of 20% glucose. 2-Deoxy-D-[1-14C]glucose (10 μCi) was injected 45 min before the end of the clamp, and blood samples were collected to estimate glucose uptake. The mice were killed, and liver, perigonadal WAT, and gastrocnemius muscle were excised, frozen immediately in liquid nitrogen, and stored at −80°C for subsequent analysis of glucose uptake and other analyses.

Statistics. Data are presented as means ± SEM. Changes of various parameters were analyzed with one-way ANOVA, followed by post hoc Tukey multiple comparison test or unpaired Student t test using Prism software (GraphPad Software, San Diego, CA). Values of P < 0.05 were considered as statistically significant.

Generation of mice that express human resistin with human-like inducible regulation. We generated a BAC that contains the human resistin gene plus all the DNA elements up to 21,300 bp upstream and 4,248 bp downstream relative to the human resistin start site (Fig. 1A). Human resistin was basally expressed and markedly induced by LPS treatment of RAW.264 murine macrophages transfected with this BAC fusion construct (Fig. 1B and C). To better understand the role of human resistin in the pathophysiology of inflammation and metabolism, we then derived transgenic mice on the C57BL/6 J Rko background using the BAC fusion construct (BAC-Retn mice). The expression pattern of human resistin in BAC-Retn mice was similar to that seen in humans, with the highest expression seen in bone marrow (Fig. 1D) (11). Moreover, human resistin expression was dramatically induced in peritoneal macrophages isolated from BAC-Retn mice (Fig. 1E).

LPS-induced hypoglycemia is less severe in BAC-Retn mice. In the mice fed normal chow, there were no appreciable differences between Rko and BAC-Retn mice in serum levels of TGs, FFAs, ketone, adiponectin, or leptin. LPS increased serum alanine aminotransferase (ALT), FFA, and leptin levels in both groups and decreased serum total and high-molecular-weight adiponectin levels (Table 1). Circulating levels of human resistin increased about fivefold from baseline within 6 h after LPS injection in BAC-Retn mice, which was consistent with the robust increase in human resistin expression and secretion observed in blood mononuclear cells (Fig. 2A and B). As has been previously described (35), LPS injection caused hypoglycemia acutely. Although LPS induced similar increases in serum TNF-α and IL1β levels in Rko and BAC-Retn mice (Fig. 2C and D), hypoglycemia was less severe in BAC-Retn compared with Rko mice (100 ± 6 vs. 77 ± 6 mg/dL, P < 0.05; Fig. 2E). Serum insulin levels were not different between groups (P = 0.32), suggesting human resistin attenuated hypoglycemia in acute endotoxemia via insulin resistance (Fig. 2F).

Insulin resistance in BAC-Retn mice in acute endotoxemia. To examine the role of human resistin in glucose homeostasis in acute endotoxemia, we performed hyperinsulinemic-euglycemic clamp studies after LPS or saline injection. Compared with saline injection, LPS acutely suppressed the basal rate of hepatic glucose production in Rko mice by 61%, and in BAC-Retn mice by 40% (Supplementary Fig. 1A). The glucose infusion rate required to maintain euglycemia was 31% lower in BAC-Retn
than in Rko mice, indicating that the BAC-Retn mice were more insulin resistant during acute endotoxemia (Fig. 3A). Hepatic insulin resistance in acute endotoxemia was manifested by a higher rate of glucose production (HGP) in BAC-Retn mice compared with Rko mice (Fig. 3B). In support of this result, both glucose-6-phosphatase (G6pase) and PEPCK expression levels were slightly higher in BAC-Retn mice than in Rko mice under clamp studies in acute endotoxemia, although the differences did not reach statistical significance (Supplementary Fig. 1B and C). Although the whole-body rate of glucose disposal (Rd) was not different between both groups, insulin-stimulated glucose uptake in WAT was 46% lower in BAC-Retn mice (Fig. 3C–E). Impaired glucose uptake in WAT in BAC-Retn mice was also consistent with significantly higher serum FFA levels compared with Rko mice, indicating less suppression of lipolysis by insulin during hyperinsulinemic clamp studies (0.13 ± 0.03 vs. 0.07 ± 0.03 mEq/L, P < 0.05).

To understand the possible mechanism of hepatic insulin resistance, we next measured lipids content in liver. Compared with Rko mice, BAC-Retn mice had a 24% higher total ceramides content in liver (Fig. 3F) during acute endotoxemia, with most ceramide species being elevated (Supplementary Fig. 1D). Moreover, there was a significant correlation between hepatic ceramide content and HGP during hyperinsulinemic clamp studies (r = 0.77, P < 0.01; Fig. 3G). However, there were no differences in hepatic diacylglycerol and TG levels between Rko and BAC-Retn mice (Supplementary Fig. 1E and F). Despite the changes in hepatic ceramide content, there were no differences in the expression of several genes involved in pathways of ceramide generation and salvage between Rko and BAC-Retn mice (Supplementary Fig. 1G) (34).

**Impaired glucose tolerance in BAC-Retn mice in chronic endotoxemia.** BAC-Retn mice were studied under chronic experimental endotoxemia to mimic the chronic inflammation of obesity and type 2 diabetes. Body weight and blood glucose of chronic LPS-infused mice significantly decreased initially, similar to the effect of acute endotoxemia described above, but recovered later (Supplementary Fig. 2A and B). During chronic endotoxemia, glucose tolerance in BAC-Retn mice was significantly impaired relative to the Rko mice (Fig. 4A), while circulating resistin levels rose approximately fourfold in these mice (Fig. 4B). Serum insulin, TNF-α and IL1β levels were not different in both groups, suggesting that human resistin is responsible for glucose intolerance in chronic endotoxemia (Fig. 4C, Supplementary Fig. 2C and D).
TABLE 1
Serum metabolic profile 6 h after LPS administration

| Variable                        | Rko-Saline | BAC-Retn-Saline | Rko-LPS | BAC-Retn-LPS |
|---------------------------------|------------|-----------------|---------|-------------|
| Weight (g)                      | 29.4 ± 0.6 | 29.6 ± 0.8      | 29.5 ± 0.7 | 30.1 ± 0.5   |
| Triglyceride (mg/dL)            | 40.3 ± 5.2 | 32.6 ± 1.8      | 38.0 ± 3.0 | 55.9 ± 6.7†*|
| Cholesterol (mg/dL)             | 66.6 ± 5.3 | 67.5 ± 2.6      | 66.4 ± 2.5 | 72.1 ± 2.6   |
| FFAs (mEq/L)                    | 0.90 ± 0.05| 0.85 ± 0.04     | 1.10 ± 0.05‡| 1.22 ± 0.13* |
| Ketone (mg/dL)                  | 3.3 ± 0.5  | 5.5 ± 0.8       | 8.4 ± 1.0‡ | 7.2 ± 0.6    |
| Alanine aminotransferase (U/L)  | 12 ± 1     | 14 ± 1          | 25 ± 2*   | 44 ± 3*§     |
| Adiponectin (µg/mL)             | 25.7 ± 0.9 | 26.3 ± 2.0      | 21.3 ± 1.3‡| 20.2 ± 1.5‡  |
| HMW adiponectin (µg/mL)         | 3.70 ± 0.13| 3.45 ± 0.36     | 2.45 ± 0.28‡| 2.25 ± 0.17‡ |
| Leptin (ng/mL)                  | 5.7 ± 1.0  | 4.8 ± 1.1       | 10.9 ± 1.7‡| 10.6 ± 1.4‡  |

LPS (0.2 mg/kg) or normal saline (0.9%) was intraperitoneally injected in 12-week-old, normal-chow-fed Rko and BAC-Retn mice (n = 5–7 per group). Data are presented as means ± SEM. BAC-Retn, BAC-transgenic; HMW, high-molecular-weight. *P < 0.01 vs. saline; †P < 0.05; ‡P < 0.005; §P < 0.01 vs. Rko.

To explore the role of resistin on inflammation in chronic endotoxemia, we next performed gene-expression analysis. BAC- Retn mice had increased expression of proinflammatory cytokines as well as macrophage markers such as monocyte chemotactic protein-1 (MCP1) and F4/80 in liver and skeletal muscle (Fig. 4D and E), indicating resistin exacerbated inflammation and promoted tissue infiltration of macrophages in chronic endotoxemia but not in WAT (Supplementary Fig. 3). Although serum levels of TGs, FFA, and total and high-molecular-weight adiponectin decreased in chronic endotoxemia, there were no differences between the Rko and BAC- Retn mice (Table 2).

Hepatic insulin resistance in BAC- Retn mice in chronic endotoxemia. To determine the mechanism underlying the effect of humanized resistin induction on glucose homeostasis in chronic endotoxemia, we performed hyperinsulinemic-euglycemic clamp studies. The glucose infusion rate required to maintain euglycemia was significantly lower in BAC- Retn than in Rko mice (Fig. 5A). Although Rd was not different, HGP was 60% higher in BAC- Retn mice, indicating that human resistin induced severe hepatic insulin resistance in chronic endotoxemia (Fig. 5B and C). To understand the mechanisms of hepatic insulin resistance, we examined gene-expression analysis in liver under clamp studies. TNF-α expression was approximately threefold higher in liver of BAC- Retn mice, suggesting resistin exacerbates inflammation in liver (Fig. 5D). Expression levels of G6pase and PEPCK were also significantly higher in BAC- Retn mice compared with Rko mice (fourfold and twofold, respectively; Fig. 5E and F), which is compatible with a higher rate of HGP in chronic endotoxemia. Measurements of lipids content in liver showed no differences in hepatic TG and total ceramides content between Rko and BAC- Retn mice (Supplementary Fig. 4A and B).

DISCUSSION

Murine resistin is secreted by adipocytes, whereas human resistin is secreted by mononuclear cells. Although genetic and pharmacologic studies strongly support links between resistin, obesity, and insulin resistance in mice, the role of human resistin as a modulator of metabolism in inflammatory states is not well understood. The present finding that induction of human resistin alters glucose metabolism in endotoxemia clearly links human resistin to inflammation-induced insulin resistance. Therefore, this study provides much needed insight into the pathophysiological role of human resistin in various inflammatory states associated with insulin resistance, ranging from sepsis to type 2 diabetes and atherosclerosis. In this respect, it is noteworthy that several epidemiologic studies have recently showed close relationships between resistin and type 2 diabetes, cardiovascular disease, and inflammatory markers (17,18,21,35).

Early stages of sepsis and experimental endotoxemia are associated with hypoglycemia through increased glucose disposal by macrophage-rich tissues as well as decreased glucose production in humans as well as animals (33,36–38). In line with previous reports, LPS administration acutely caused hypoglycemia in Rko and BAC- Retn mice, with similar robust increases in serum proinflammatory cytokines, which are known to cause hypoglycemia (33,36). LPS increased serum leptin levels and decreased adiponectin, which can be explained by cytokine responses (39–42). During acute LPS-induced hypoglycemia, however, the induction of human resistin increased hepatic glucose production and decreased glucose uptake, thereby reducing the severity of hypoglycemia. Intriguingly, mice lacking resistin are more prone to fasting-induced hypoglycemia than wild-type mice (9), suggesting similar functions of resistin across species, albeit in different physiologic settings. Thus the human-specific expression pattern of resistin facilitates its function as a glucose counterregulatory hormone to mitigate hypoglycemia during acute endotoxemia. Of note, the metabolic effects of chronic endotoxemia in wild-type mice (31) are likely through a resistin-independent mechanism because murine resistin is not induced by LPS (Supplementary Fig. 5A–C) (43,44), although it was recently reported to be induced by homocysteine, which is proinflammatory among other pleiotropic effects (45).

Animal studies on the acute effect of LPS on insulin sensitivity have yielded conflicting results (46,47). In the setting of acute endotoxemia, we found that insulin sensitivity was enhanced in liver as well as in WAT of Rko mice and that human resistin abrogated this insulin-sensitizing effect of LPS, inducing insulin resistance. Our finding that hepatic ceramide content correlated with hepatic insulin resistance is consistent with a previous report (48). Ceramide has been shown to induce insulin resistance by inhibiting insulin signaling. Interestingly, TNF-α can acutely stimulate the accumulation of ceramide and
various ceramide metabolites (49). Although there we did not detect differences in ceramide-related gene expression in liver of the BAC-Retn mice, the changes of hepatic ceramide content in acute endotoxemia could be due to changes in enzyme activity or to alterations in substrate flux.

During chronic endotoxemia, induction of human resistin promoted glucose intolerance and hepatic insulin resistance, suggesting its role as a link between innate immunity, inflammation, and insulin resistance. Epidemiologic studies provide strong evidence that chronic inflammation and obesity-induced insulin resistance are closely related (50–53). Experimental endotoxemia in humans, akin to states of infection and sepsis, also induces systemic insulin resistance as well as increased circulating resistin (16,37,54). Here we found that, in mice, chronic endotoxemia robustly induced inflammation in liver and

![FIG. 2. Changes in resistin, cytokines, and glucose in acute endotoxemia in BAC-Retn mice. Acute LPS (0.2 mg/kg) or normal saline (0.9%) intraperitoneal administration to Rko (□) and BAC-Retn (■) mice (n = 5–7 per group). A and B: Human resistin was induced in response to LPS in vivo in BAC-Retn mice. C and D: Serum TNF-α and IL1β levels increased similarly in Rko and BAC-Retn mice. E: LPS-induced hypoglycemia is less severe in BAC-Retn than in Rko mice at 6 h after LPS injection. F: Serum insulin levels in 6 h after LPS injection. PBMC, peripheral blood mononuclear cells. Data are presented with the SEM. *P < 0.05, **P < 0.01, ***P < 0.001 vs. saline; #P < 0.05 vs. Rko.
skeletal muscle and was exacerbated by induction of human resistin. Our finding that human resistin promotes insulin resistance by inducing TNF-α and upregulating G6pase and PEPCK in liver are consistent with previous reports (15,55,56).

Although hepatic ceramide accumulation can at least partly explain hepatic insulin resistance in acute endotoxemia, tissue inflammation exacerbated by resistin, not hepatic ceramide, seems to be a plausible mechanism for insulin resistance in chronic endotoxemia. Recently, regulatory T-cells have been demonstrated to contribute to macrophage recruitment and adipose inflammation in obesity, and resistin has been shown to indirectly enhance regulatory T-cells (57,58). Thus, T-cells, in addition to macrophages, may possibly be involved in the enhanced inflammation of the BAC-Retn mice. It remains unanswered

FIG. 3. Acute endotoxemia induces hepatic and adipose tissue insulin resistance in BAC-Retn mice. A–C: Hyperinsulinemic-euglycemic clamp analysis of BAC-Retn (■) vs. Rko (□) mice (n = 4 per group) in 6 h after LPS administration. D and E: Rate of insulin-stimulated glucose uptake in WAT (D) and skeletal muscle (E). F: Total ceramides content in liver. G: Correlation between hepatic ceramide content and hepatic glucose production (HGP) during the clamp study. Data are presented with the SEM. GIR, glucose infusion rate; Rd, whole-body rate of glucose disposal. *P < 0.05 vs. saline; #P < 0.05 vs. Rko.
how resistin functions in various tissues or cells, because the receptor for resistin has not yet been uncovered.

In summary, we have demonstrated that human resistin modulates glucose homeostasis under inflammation using a humanized resistin mouse model. Human resistin is induced in response to inflammation. Resistin attenuates endotoxemia-induced hypoglycemia by inducing insulin resistance in liver and WAT and promotes hepatic insulin resistance by exacerbating inflammatory responses in chronic endotoxemia. Increased inflammation is accompanied by increased infiltration of macrophages, which can, in turn, augment resistin induction. Thus, induction of resistin is expected to be a component of the pathophysiology of inflammation-induced insulin resistance in humans. The mouse model of humanized resistin expression reported here can be used to provide a better

**TABLE 2**

| Variable                        | Rko-Saline | BAC-Retn-Saline | Rko-LPS   | BAC-Retn-LPS |
|---------------------------------|------------|-----------------|-----------|--------------|
| Weight (g)                      | 28.0 ± 0.4 | 26.8 ± 0.8      | 26.2 ± 0.2| 25.8 ± 0.3   |
| Triglycerides (mg/dL)           | 85.7 ± 5.4 | 70.2 ± 1.5      | 64.0 ± 6.3*| 60.0 ± 2.8*  |
| Cholesterol (mg/dL)             | 80.8 ± 4.2 | 73.7 ± 1.2      | 72.3 ± 1.7| 62.9 ± 5.2   |
| FFAs (mEq/L)                    | 0.77 ± 0.07| 0.84 ± 0.08     | 0.41 ± 0.07| 0.47 ± 0.04*|
| Ketone (mg/dL)                  | 2.6 ± 0.3  | 3.2 ± 0.8       | 2.0 ± 0.3 | 1.9 ± 0.1*   |
| Alanine aminotransferase (U/L) | 7 ± 1      | 8 ± 1           | 6 ± 1     | 7 ± 2        |
| Adiponectin (μg/mL)             | 25.2 ± 1.4 | 25.5 ± 1.2      | 15.9 ± 1.6| 15.3 ± 1.2   |
| HMW adiponectin (μg/mL)         | 3.60 ± 0.19| 4.00 ± 0.99     | 1.57 ± 0.08| 1.59 ± 0.11*|

LPS (2 mg/kg/day) or normal saline (0.9%) was subcutaneously infused with a minipump to 10-week-old, normal-chow-fed Rko and BAC-Retn mice for 10 days (n = 5–7 per group). Data are presented as means ± SEM. BAC-Retn, BAC-transgenic; HMW, high-molecular-weight. *P < 0.05; †P < 0.01 vs. saline.
understanding and potential amelioration of the metabolic pathology of inflammatory states in humans.

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H.-K.P. designed experiments, researched data, and wrote the manuscript. M.Q. designed experiments, researched data, and edited the manuscript. E.R.B. researched data and edited the manuscript. R.S.A. and M.A.L. designed experiments, wrote and edited the manuscript, and obtained funding supporting the research.

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