Interferon regulatory factor 7 deficiency prevents diet-induced obesity and insulin resistance

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1Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, China; 2Cardiovascular Research Institute, Wuhan University, Wuhan, China; 3National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; 4Department of Cardiology, Institute of Cardiovascular Disease, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; 5Sanford Burnham Medical Research Institute, Cancer Center, La Jolla, California; and 6College of life sciences, Wuhan University, Wuhan, Peoples Republic of China

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Wang X, Zhang R, Zhang S, Deng S, Jiang D, Zhong J, Yang L, Wang T, Hong S, Guo S, She Z, Zhang X, Li H. Interferon regulatory factor 7 deficiency prevents diet-induced obesity and insulin resistance. Am J Physiol Endocrinol Metab 305:E485–E495, 2013. First published May 20, 2013; doi:10.1152/ajpendo.00505.2012.—Obesity-related inflammation has been implicated in the pathogenesis of insulin resistance and type 2 diabetes. In this study, we addressed the potential role of interferon regulatory factor 7 (IRF7), a master regulator of type I interferon-dependent immune responses, in the regulation of energy metabolism. The expression levels of IRF7 were increased in white adipose tissue, liver tissue, and gastrocnemius muscle of both diet-induced obese mice and ob/ob mice compared with their lean counterparts. After feeding a high-fat diet (HFD) for 24 wk, IRF7 knockout (KO) mice showed less weight gain and adiposity than wild-type controls. KO of IRF7 improved glucose and lipid homeostasis and insulin sensitivity. Additionally, KO of IRF7 ameliorated diet-induced hepatic steatosis. Next, we assessed the inflammatory state of the IRF7 KO mice on the HFD. These mice showed less macrophage infiltration into multiple organs and were protected from local and systemic inflammation. This study demonstrates a role for IRF7 in diet-induced alterations in energy metabolism and insulin sensitivity. Our results also suggest that IRF7 is involved in the etiology of metabolic abnormalities, which suggests a new strategy for treating obesity and type 2 diabetes.

type 2 diabetes; adiposity; fatty liver; inflammation

UNDER NORMAL PHYSIOLOGICAL conditions, organisms have a fine-tuned regulatory network to maintain metabolic homeostasis. However, energy imbalance develops with caloric excess and compromised regulatory functions that accompany aging. This imbalance leads to obesity, nonalcoholic fatty liver diseases (NAFLD), metabolic syndrome, and type 2 diabetes, which pose great challenges to public health (5, 45a). Obesity is now recognized as a chronic low-grade inflammatory state (19). Inflammatory mediators and cytokines are overexpressed in the adipose and other tissues in the obese state (45). Infiltration of immune cells and proinflammatory M1 polarization of macrophages are also associated with obesity (24). Activation of inflammatory signaling pathways, including c-Jun NH2-terminal kinase (JNK)/activator protein 1 (AP1) and IKK/NF-κB pathways, blunts insulin activity (13). Loss of IkB kinase ε (IKKe, also known as IKKi), a target gene of NF-κB, has also been reported to improve the energy balance in diet-induced obese mice (7, 33). In addition to systemic inflammation, ectopic lipid accumulation and endoplasmic reticulum (ER) stress were also proposed to explain the pathogenesis of insulin resistance (12, 34). However, the underlying mechanism of obesity-related metabolic disorders is still not completely understood.

Interferon (IFN) α and IFNβ, collectively known as type I IFNs, are the major mediators of the host immune response against viral infections (14, 31). IFN regulatory factors (IRF) are a family of transcription factors that consists of nine members (IRF-1 to IRF-9) in mammals (36). Most IRFs are involved in innate immunity and defense against pathogens (1, 17). IRF7 was first identified as a factor that binds to the Ebna1 Q promoter of the Epstein-Barr virus during latent infection (46). Thereafter, IRF7 was found to be a master regulator of type I IFN-dependent immune responses (18). Type I IFNs can induce the expression of IRF7 in various cell types (40). IRF7, mainly located in the cytoplasm, can be phosphorylated upon viral infection and Toll-like receptor-dependent signaling. It then translocates to the nucleus to activate the transcription of type I IFNs, creating a positive feedback loop (40). IRF7 was reported to suppress breast cancer metastasis by the activation of tumor immune surveillance mechanisms (2). The IRF family members can regulate hematopoietic cell differentiation and proliferation, which implicates these transcription factors in development and oncogenesis (36).

Several recent lines of evidence suggest the involvement of IRFs in metabolism. Our previous work determined that IRF9 interacts with peroxisome proliferator-activated receptor (PPAR)-α to attenuate NAFLD (42). IRF3 was reported to regulate metabolism-related nuclear receptors, such as liver X receptor (LXR) and retinoid X receptor α (4, 8). Another group found that IRFs regulate adipogenesis and lipid metabolism in adipocytes (10, 11). However, the systemic effects of IRFs on metabolism are largely unknown. In this study, we aimed to identify the metabolic roles of IRF7 in obesity and provide a new perspective on metabolic disorder treatment.

MATERIALS AND METHODS

Animals and diets. C57BL/6 [wild-type (WT)] mice and IRF7 knockout (KO) mice (C57BL/6 background, kindly provided by Dr. Tadatsugu Taniguchi, University of Tokyo, Japan) were housed in a 12:12-h light-dark cycle with free access to water and a standard
Fig. 1. Interferon regulatory factor 7 (IRF7) expression in the insulin target organs. A: representative immunofluorescence expression of IRF7 (red) in the insulin target organs: white adipose tissue (WAT), liver tissue, skeletal muscle (gastrocnemius muscle) in C57BL/6 mice [wild-type (WT)] and ob/ob mice. WT mice were on the normal chow diet (NC) or high-fat diet (HFD) feeding for 24 wk. ob/ob mice and their lean controls (WT on normal chow) were fed with standard rodent chow diet for 9 wk. Each group contained four sections for staining analysis. Arrows indicate positive cells in WAT. Scale bars stand for 25 μm (WAT), 25 μm (liver), and 50 μm (skeletal muscle). B and C: relative quantification of IRF7-positive cells in WAT, liver tissue, and skeletal muscle. Data are presented as means ± SE; n = 4 mice. *P < 0.05 and ***P < 0.001 vs. WT NC; ##P < 0.01 and ###P < 0.001 vs. lean. D and E: mRNA levels of IRF7 in WAT, liver, and skeletal muscle tissue were determined through real-time PCR. ***P < 0.001 vs. WT NC; ###P < 0.001 vs. lean; n = 9–12/group. F: protein expression of IRF7 in WAT, liver, and skeletal muscle tissue was detected by Western blotting. Quantification of protein expression levels was normalized to the GAPDH loading control in WT mice with NC or HFD (G) and lean mice and ob/ob mice (H). Values represent means ± SE; n = 3/group. **P < 0.01 vs. WT NC; ##P < 0.01 vs. lean.
rodent diet before the study was initiated. Only 8-wk-old male mice were used for experiments. The mice were randomly divided into two groups: an HFD group fed ad libitum with a high fat-diet (60% kcal fat, D12492; Research Diets) and a NC group with a normal chow diet (10% kcal fat, D12450B; Research Diets) for the following 24 wk. Nine-week-old female ob/ob mice were obtained from the Jackson Laboratory (stock number: 000632). 

Metabolic studies. Food intake was recorded weekly. Body weight and fasting glucose levels were measured every 4 wk. All protocols were approved by the Animal Care and Use Committee of the Renmin Hospital of Wuhan University.

Fig. 2. IRF7 knockout mice displayed decreased weight gain and adiposity on a HFD. A: the comparison of body weight gains in WT or irf7⁻/⁻ (KO) mice on the 24-wk NC or HFD feeding; n = 15–22/group. ###P < 0.001 vs. WT NC; *P < 0.05, **P < 0.01, and ***P < 0.001 vs. WT HFD. B: representative macroscopic images of the mice (top) and their visceral fat accumulation (middle and bottom). C: quantification of visceral fat weights and the ratio to body weight; n = 15–31 in each group. ##P < 0.01 vs. WT NC; *P < 0.05 vs. WT HFD. D: histological analysis [hematoxylin and eosin (H&E) staining] of WAT from WT and KO mice in the NC or HFD group. Scale bars represent 100 μm. E: real-time PCR analysis indicated the genes encoding adipogenesis in the adipose tissue of the WT HFD group and the KO HFD group. The mRNA levels of genes that participate in fatty acid synthesis, uptake, lipogenesis, lipolysis, and fatty acid oxidation were also examined by real-time PCR in adipose tissue. All values are expressed as means ± SE; n = 6–12/group. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. WT HFD.
mice were fasted for 6 h (8:00 A.M. to 2:00 P.M.) before the serum glucose test. Blood samples were obtained from the tail tip blood vessels. Blood glucose level was detected using a glucometer (One Touch Ultra Easy; Life Scan). Levels of representative metabolites and insulin in the serum were tested every 8 wk. The mice were anesthetized by inhalation of diethyl ether, and blood was collected by orbital puncture. The collected blood was centrifuged at 4,000 rpm for 30 min at 4°C and then stored at −80°C for future analysis. The concentration of triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL)-cholesterol (C), low-density lipo-
Western blot. Proteins were extracted from epididymal adipose tissues (200 mg for each sample), liver tissue (60 mg for each sample), and gastrocnemius muscle tissue (150 mg for each sample) using RIPA lysis buffer (RIPA (720 µl) contains 20 µl phenylmethylsulfonyl fluoride (P7626; Sigma), 100 µl Complete (04693124001; Roche), 100 µl Phosstop (04906837001; Roche), 50 µl NaF, and 10 µl Na3VO4. RIPA (100 ml, pH = 7.5) contains 0.7882 g Tris-HCl, 0.8766 g NaCl, 1 ml Nonidet P-40, 0.5 g sodium deoxycholate, 0.1 g SDS, and 0.0292 g EDTA). The Western blots were processed as previously described (9, 23). The protein levels were quantified and normalized to the loading control GAPDH. Antibodies used in this study were obtained from Cell Signaling Technology (Danvers, MA), Santa Cruz Biotechnology, Bioworld Technology (Minneapolis, MN), Millipore, and Abcam.

RNA isolation and real-time PCR. Total RNA was extracted from epididymal adipose tissues, liver tissue, and gastrocnemius muscle tissue with TRIZol reagent (Roche, Mannheim, Germany). cDNA was synthesized with a Transcriptor First Stand cDNA Synthesis Kit (Roche). Quantitative real-time PCR was performed with a Light Cycler 480 SYBR Green I Master (Roche) using the Light Cycler 480 real-time PCR system according to the manufacturer’s instructions (Roche). The expression levels of the target genes were normalized to β-actin.

Statistical analysis. The data are expressed as means ± SE. All statistical analyses were performed by two-tailed Student’s t-test or one-way ANOVA followed by Fisher’s least-significant difference procedure for post hoc testing. A P value <0.05 was considered significant.

RESULTS

IRF7 expression levels are increased in WAT, liver tissue, and skeletal muscle of obese mice. To investigate the involvement of IRF7 in energy metabolism, we began testing the expression levels of IRF7 in insulin-responsive organs of obese mice compared with normal controls. We used a HFD-induced obesity model and a genetic (ob/ob mice) obesity model for the study. We performed immunofluorescence to detect IRF7, which is mainly located in the nucleus. The mice that had consumed the HFD for 24 wk showed a higher proportion of IRF7-positive cells in WAT, liver tissue, and gastrocnemius muscle compared with the Chow diet controls (Fig. 1, A and B). Consistently, IRF7 expression levels in all three tissue types in ob/ob mice were also greatly higher than in lean mice (Fig. 1, A and C). Real-time PCR (Fig. 1, D and E) and immunoblotting (Fig. 1, F–H) results also determined that IRF7 expression was markedly increased in these tissues. Therefore, both dietary and genetic obese mice showed greatly increased IRF7 expression in insulin target organs. Posttranslational modifications may lead to the shutting of IRF7 between cytoplasm and nucleus (28). However, we observed that, at least in hepato-
IRF7 KO mice displayed decreased weight gain and adiposity on a HFD. After 24 wk of the HFD, although the food intake had no significant difference between these two genotypes [Supplemental Fig. S1A (Supplemental data for this article may be found on the American Journal of Physiology: Endocrinology and Metabolism website.)], the WT mice gained an average of 22 grams of body weight, whereas the IRF7 KO mice only gained 15 grams (Fig. 2, A and B). WAT weight was significantly lower in the IRF7 KO mice, most likely because of less lipid accumulation (Fig. 2, B and C). H&E staining showed smaller adipocytes in the IRF7 KO mice (Fig. 2D and Supplemental Fig. S1, B and C). To study how the expression of adipogenesis-encoding genes changed in WAT, real-time PCR was performed. The expression of anti-adipogenic genes [e.g., Pref-1, CyclinD1, CCAAT enhancer-binding homologous protein (CHOP) 10] was higher, whereas adipogenic gene expression (e.g., C/EBPβ, C/EBPδ, C/EBPα) was lower in the WAT of KO mice compared with that of WT mice (Fig. 2E). In accordance with this finding, the lipid metabolic profile of IRF7-deficient WAT showed a higher catabolic level and a lower anabolic level. Lower expression of genes involved in fatty acid (FA) synthesis [e.g., stearoyl-CoA desaturase 1 (SCD1)], FA uptake [e.g., cluster of differentiation 36 (CD36), fatty acid-binding proteins (FABPs), and fatty acid transporting protein 1 (FATP1)], and lipogenesis [e.g., diacyl eride acyltransferase (DGAT) and glycerol-3-phosphate acyltransferase (GPAT)] and higher levels of mRNA-encoding proteins that participate in lipolysis [e.g., hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL)] and fatty acid oxidation [e.g., PPARα, PPARβ, peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α), and medium-chain acyl-CoA dehydrogenase (MCAD)] were found in the WAT of IRF7 KO mice (Fig. 2E). All of the above results demonstrate a protection against weight gain and adiposity in IRF7-deficient mice. However, it seems paradoxical that PPARγ was markedly increased in the WAT of IRF7 KO mice, given that PPARγ promotes adipogenesis and lipid storage in adipocytes (22). A plausible explanation would be that the adipogenic function of PPARγ is a less important mechanism of the adiposity-relieving effect of IRF7 deficiency. PPARγ has a role in keeping global energy balance by attenuating insulin resistance and inflammation in multiple metabolic organs (39). This aspect of PPARγ may contribute to the protective role of IRF7 deficiency against adipocyte hypertrophy.

KO of IRF7 improves glucose and lipid homeostasis and insulin sensitivity. Because IRF7 KO mice were protected from diet-induced obesity, we investigated whether this gene might play a role in glucose and lipid homeostasis. After the HFD, the fasting blood glucose level increased greatly in the WT mice but only increased slightly in the IRF7 KO mice (Fig. 3A). Via real-time PCR, we found lower expression levels of genes encoding gluconeogenesis enzymes (e.g., phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) in the liver tissue of KO mice (Fig. 3B). The fasting serum insulin level was markedly higher in the HFD-fed WT mice than in the IRF7 KO mice (Fig. 3C). The lower levels shown by the HOMA-IR indicated that IRF7 deficiency alleviated insulin resistance (Fig. 3D). In addition to the decrease in blood glucose, the IRF7 KO mice displayed reduced fasting serum free fatty acid (FFA), TG, cholesterol, β-hydroxybutyrate, and LDL levels and increased serum HDL levels compared with the WT mice on the HFD (Table 1). Abnormal serum lipid levels usually accompany glucose deregulation. We performed intraperitoneal glucose and insulin tolerance tests after 23 and 24 wk of chow or the HFD. The HFD-fed WT mice were glucose intolerant, whereas the IRF7 KO mice maintained relatively normal glucose tolerance. Although there were no differences detected between the genotypes on a chow diet, the HFD-fed IRF7 KO mice were more sensitive to insulin stimulation compared with the WT controls (Fig. 3, E and F). Because IRF7 deficiency improves glucose and lipid regulation, this gene most likely influences insulin activity. Immunoblotting showed that the basal (with no exogenous insulin treated) phosphorylation levels of key nodes in the insulin-signaling pathway, such as insulin receptor substrate 1 and protein kinase B, were increased in the WAT, liver tissue, and gastrocnemius muscle of IRF7 KO mice (Fig. 3, G and H). IRF7 expression was induced by the chronic HFD in all insulin-responsive tissues, as previously suggested.

IRF7 KO mice are protected from diet-induced hepatic steatosis. Hepatic steatosis can be induced by a chronic HFD in WT mice. However, KO of IRF7 protects against hepatic steatosis development. Liver weight was significantly lower because of less lipid accumulation in the IRF7 KO mice (Fig. 4, A and B), which was confirmed by H&E and Oil red O staining (Fig. 4C). The content of liver TG, cholesterol, and FFA was greatly increased in the WT mice on the HFD. However, KO of IRF7 reduced

Table 1. Serum metabolism parameters vary during the initiation and after 24 wk of diet treatment both in WT and KO mice

<table>
<thead>
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<th>Parameters</th>
<th>Initiation of Diet Treatment</th>
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<th>24 wk of Diet Treatment</th>
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<td></td>
<td>NC (n = 8)</td>
<td>HFD (n = 6)</td>
<td>NC (n = 8)</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td>33.46 ± 0.45</td>
<td>31.46 ± 0.87</td>
<td>33.59 ± 0.48</td>
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<tr>
<td>Cholesterol, mg/dl</td>
<td>123.82 ± 2.25</td>
<td>131.95 ± 2.59</td>
<td>123.78 ± 3.50</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>97.25 ± 3.17</td>
<td>97.15 ± 3.26</td>
<td>97.34 ± 2.02</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>18.43 ± 0.49</td>
<td>16.19 ± 0.69</td>
<td>18.42 ± 0.60</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.82 ± 0.01</td>
<td>0.77 ± 0.02</td>
<td>0.82 ± 0.01</td>
</tr>
<tr>
<td>β-Hydroxybutyrate,</td>
<td>0.26 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.26 ± 0.01</td>
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<tr>
<td>mmol/l</td>
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Data are expressed as means ± SE; n, no. of mice. NC, normal chow; HFD, high-fat diet; WT, wild type; KO, knockout; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acid. ***P < 0.01 vs. WT NC (24 wk); **P < 0.01 vs. WT HFD (24 wk).
these indexes (Fig. 4D). We further examined lipid metabolism in the liver by real-time PCR. The results showed that mRNAs involved in cholesterol synthesis (e.g., HMGCR and LDLR) were lower and mRNAs involved in cholesterol export [e.g., LXR-α, ATP-binding cassette transporter A1, ATP-binding cassette transporter G (ABCG) 5, and ABCG8] were higher in the liver tissue of KO mice compared with that of WT controls fed a HFD for 24 wk (Fig. 4E). Additionally, higher levels of mRNA-encoding proteins involved in lipolysis (e.g., HSL and ATGL) and FA oxidation (e.g., PPARβ, PGC1α, MCAD, and uncoupling protein 2) and lower expression of genes involved in FA synthesis [e.g., acetyl-CoA carboxylase (ACC) α, fatty acid synthase (FAS), and SCD1], FA uptake (e.g., CD36), and lipogenesis (e.g., GPAT) were shown in the liver tissue of KO mice (Fig. 4E). AMP-activated protein kinase (AMPK) is an important cellular
energy sensor that promotes catabolism when the AMP-to-ATP ratio is high. Compromised AMPK signaling may contribute to metabolic disorders and aging (38). The IRF7 KO mice showed higher phosphorylation levels of AMPKα, AMPKβ, and ACC and lower levels of sterol regulatory element-binding protein 1c (a downstream gene associated with lipogenesis), which indicated a heightened activity of the AMPK signaling pathway (Supplemental Fig. S2, A and B). Taken together, these results reflect the importance of IRF7 in mediating obesity-induced hepatic steatosis.

The ER is the major site of protein processing and trafficking. When the ER receives an excessive influx of newly synthesized proteins, unfolded or misfolded proteins accumulate in the ER lumen, and certain signaling pathways are activated to trigger the unfolded protein response (UPR) (16). During obesity, not only the increase in protein synthesis but also the higher levels of glucose and FFAs trigger the development of ER stress, which further contributed to insulin resistance and diabetes (30). We examined the influence of IRF7 on ER stress signaling. Real-time PCR showed a ubiquitous decrease in the expression of mediators in ER stress signaling pathways (Supplemental Fig. S3A). Using immunoblotting, we found that the phosphorylation levels of PKR-like ER-localized eIF2α kinase, translation initiation factor 2α, and the expression of downstream gene CHOP were reduced in the liver tissue of KO mice (Supplemental Fig. S3, B and C). The levels of inositol-requiring gene 1α and phosphorylated JNK, as well as the level of X-box-binding protein 1 (XBp1) were decreased in the IRF7 KO mice (Supplemental Fig. S3, B and C). The protein chaperone glucose-regulated protein, 78 kDa, a UPR target, was also downregulated in the liver tissue of IRF7 KO mice (Supplemental Fig. S3, B and C). All of these findings indicate that ER stress was attenuated in the liver tissue of IRF7 KO mice.

IRF7 KO mice are protected from diet-induced inflammation. Because inflammation plays critical roles in metabolic dysfunction, we next sought to examine the inflammatory state of the IRF7 KO mice. The serum levels of leptin, resistin, and proinflammatory cytokines were lower, whereas the levels of adiponectin and anti-inflammatory cytokines were higher in the IRF7 KO mice compared with lean counterparts. MCP-1, monocyte chemotactic protein-1, and TNF-α were higher in WT NC mice (Fig. 5, A and B). We also performed immunofluorescence of molecular markers of pan leukocytes (CD45), neutrophils (7/4), and M1 (CD68) and M2 macrophages (CD206 and Arginase1) and then counted their numbers. The proportions of M1 cells and other proinflammatory cells were lower and M2 cells were higher in all of these tissues in the IRF7 KO mice than in the WT mice, which further confirmed an attenuated M1 polarization of macrophages in IRF7 KO mice (Supplemental Fig. S4, A-D). Furthermore, we confirmed the downregulation of the NF-κB signaling pathway by showing lower phosphorylation levels of IkBε and p65 (RelA, a subunit of NF-κB complex) in multiple organs of the KO mice (Fig. 5, C and D).

**DISCUSSION**

In the present study, IRF7, a master regulator of type I interferon-dependent immune responses (18), was shown to increase in multiple tissues in both dietary and genetic obese mice. Using IRF7 whole body KO mice, we found that IRF7 has a critical role in the development of obesity and diabetes. On the HFD, the WT mice presented compromised energy regulatory functions and a significantly obese phenotype. However, even chronically challenged with a HFD, the IRF7 KO mice still showed almost intact glucose and lipid homeostasis, resistance to diet-induced hepatic steatosis, less adiposity, and relatively normal physical appearance. Hepatic ER stress and local and systemic inflammation were also attenuated in the IRF7 KO mice compared with the WT mice. Therefore, targeting IRF7 may help to maintain energy balance and prevent metabolic diseases.

We found that the expression levels of IRF7 were higher in the WAT, liver tissue, and skeletal muscle of both diet-induced obese mice and ob/ob mice compared with lean counterparts. As a nutrient-overabundant state, obesity allows the upregulation of the mammalian target of the rapamycin (mTOR) pathway (47). The phosphatidylinositol 3-kinase/protein kinase B/mTOR pathway was reported to facilitate the activation of IRF7 and induce the expression of type I IFNs (3, 43). IKKe, a major kinase of IRF7 that will be discussed in detail later, was also reported to increase during obesity (7). Activation of protein kinase R (PKR) increases markedly during obesity and

<table>
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<tr>
<th>Parameter</th>
<th>NC WT (n = 8)</th>
<th>KO WT (n = 6)</th>
<th>HFD WT (n = 8)</th>
<th>KO HFD (n = 6)</th>
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<tr>
<td>Leptin, pg/ml</td>
<td>4,338.90 ± 99.57</td>
<td>4,175.80 ± 121.76</td>
<td>1,305.00 ± 231.28##</td>
<td>4,228.20 ± 127.37**</td>
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<tr>
<td>Resistin, pg/ml</td>
<td>1,128.50 ± 28.22</td>
<td>1,124.50 ± 39.07</td>
<td>1,366.80 ± 18.47##</td>
<td>1,228.00 ± 35.25**</td>
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<td>Adiponectin, μg/ml</td>
<td>1.13 ± 0.03</td>
<td>1.18 ± 0.03</td>
<td>0.58 ± 0.01##</td>
<td>1.45 ± 0.04**</td>
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<td>IL-1β, pg/ml</td>
<td>36.50 ± 1.03</td>
<td>35.58 ± 1.12</td>
<td>57.63 ± 1.55##</td>
<td>38.65 ± 1.27**</td>
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<tr>
<td>IL-4, pg/ml</td>
<td>17.64 ± 0.62</td>
<td>17.57 ± 0.69</td>
<td>27.52 ± 0.85##</td>
<td>20.66 ± 0.70**</td>
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<tr>
<td>IL-6, pg/ml</td>
<td>0.67 ± 0.02</td>
<td>0.74 ± 0.02</td>
<td>1.44 ± 0.04##</td>
<td>0.78 ± 0.03##</td>
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<td>TNF-α, pg/ml</td>
<td>2.23 ± 0.06</td>
<td>2.97 ± 0.09</td>
<td>4.14 ± 0.13##</td>
<td>3.28 ± 0.13##</td>
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<tr>
<td>MCP-1, pg/ml</td>
<td>16.73 ± 0.45</td>
<td>17.38 ± 0.52</td>
<td>33.53 ± 0.82##</td>
<td>20.39 ± 0.70**</td>
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<tr>
<td>IL-10, pg/ml</td>
<td>16.57 ± 0.39</td>
<td>14.42 ± 0.45</td>
<td>8.87 ± 0.22##</td>
<td>9.80 ± 0.34</td>
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Data are expressed as means ± SE; n, no. of mice. MCP-1, monocyte chemotactic protein-1. ##P < 0.01 vs. WT NC; **P < 0.01 vs. WT HFD.
coordinates the activity of JNK to regulate insulin action and metabolism (15, 27). IRF1 was reported to activate NF-κB in a PKR-dependent manner (21). Whether IRF7 also acts in a similar way needs more research to elucidate. The IRFs play crucial roles in innate immunity (17). In the early phase of viral infection, IRF3 and IRF7 induce a small amount of IFN-β. Secreted IFNs can bind and activate IFN receptors in an autocrine or paracrine manner. Upon binding IFNs, IFN receptors can induce additional IRF7 expression. Therefore, IRF7 and type I IFNs form a positive-feedback loop (25, 35), i.e., activation of IRF7 triggers additional IRF7 expression. Therefore, an elevated mTOR pathway, activation of IKKe, or other unidentified protein kinases may also contribute to the increase of IRF7 in obesity.

We next aimed to understand how IRF7 affects energy metabolism. By comparing HFD-fed KO mice with weight-matched WT controls, we found that the effect of IRF7 on insulin resistance is largely secondary to IRF7’s effect on weight gain, whereas the influence of IRF7 on lipid metabolism and systemic inflammation may refer to other mechanisms (data not shown). We used IRF7 KO mice and challenged them with a HFD for 24 wk. Weight gain and hypertrophy in visceral adipose tissue in IRF7 KO mice were not as significant as in the WT mice, although no significant difference in food intake exists between these two genotypes (Supplemental Fig. S1A). Hence, we proposed that the energy expenditure in the IRF7 KO mice was higher than in WT mice to dissipate the excessive energy ingested. This hypothesis was substantiated by

![Fig. 5. IRF7 knockout mice were protected from diet-induced inflammation. A: the mRNA levels of macrophage marker F4/80 and markers of proinflammatory M1-type macrophages were examined by real-time PCR; n = 6–12/group. B: the mRNA levels of markers of anti-inflammatory M2-type macrophages were examined by real-time PCR; n = 6–12/group. C: the NF-κB signaling activation was indicated by immunoblotting. D: quantification of protein expression changes is presented as means ± SE; n = 4/group. In A, B, and D, *P < 0.05, **P < 0.01, and ***P < 0.001 vs. WT HFD.](http://ajpendo.physiology.org/doi/abs/10.1152/ajpendo.00505.2012)
real-time PCR results of metabolic genes in WAT. The expression of fatty acid oxidation-related genes (e.g., PPARγ, PPARβ, and PGC1α) and lipolytic genes (e.g., HSL and ATGL) was markedly increased in IRF7-deficient WAT (Fig. 2E), whereas the levels of lipid anabolic genes (e.g., DGAT1, FATT4, FABP4, CD36, SCD1, and FAS) were significantly downregulated in IRF7 KO WAT (Fig. 2E). Altogether, IRF7 KO mice had a higher lipid catabolic level in WAT. Paradoxically, the major adipogenic factor PPARγ was upregulated in the fat of KO mice. In addition to its importance in adipogenesis, PPARγ also favors the uptake of lipids from circulation and other organs into the adipose tissue (22). In the present study, however, we found that the expression of genes involved in FA uptake, including CD36, FABPs, and FATP1, were lower in the WAT of IRF7 KO mice, which indicated a secondary role of PPARγ in the regulation of adipocyte metabolism in IRF7-deficient mice. The expression levels of HSL, ATGL, and fatty acid oxidative genes, such as PPARγ, PPARβ, and PGC1α, were higher in the WAT of IRF7 KO mice, therefore partially offsetting the adipogenic effect of PPARγ and contributing to a lean phenotype. In addition to the regulation of adipocyte metabolism, PPARγ functions to suppress inflammation [inhibition of NF-κB, AP1, and signal transducers and activators of transcription (STATs)], induce adiponectin, and improve insulin sensitivity (32). Accordingly, proinflammatory cytokines and resistin were lower and anti-inflammatory cytokines and adiponectin were higher in the serum of IRF7 KO mice compared with that of WT mice on a HFD. Additionally, lower macrophage infiltration and proinflammatory gene expression and higher insulin sensitivity were detected in multiple organs of the IRF7 KO mice. Therefore, systemic glucose and lipid homeostasis were preserved.

The NF-κB signaling pathway, which is activated in the fat and liver tissue in obesity, plays crucial roles in the progression of insulin resistance and metabolic disorders (26). Chiang et al. proposed that protein kinase IKKe, which is induced by activation of the NF-κB pathway and elevated in obesity, might largely mediate the metabolic effects of NF-κB (7). The authors found that a HFD increased IKKe expression in WAT and liver tissue, as previously mentioned. KO of IKKe helped to defend against the deleterious effects of a HFD, including obesity, insulin resistance, hepatic steatosis, macrophage infiltration in adipose tissue, and elevations in proinflammatory gene expression in fat and liver tissue (7). Recently, this group successfully improved obesity-related metabolic dysfunctions in mice using amlexanox, an inhibitor of the protein kinases TBK1 and IKKe (33). We noticed that the metabolic phenotypes of the IKKe KO mice and the IRF7 KO mice largely overlapped. We speculate that IRF7 may be responsible for the metabolic functions of IKKe, given that IKKe is a major kinase for IRF3/7 phosphorylation and activation (37). Unpublished data from our group shows that IRF3 deficiency aggravated obesity and energy imbalance, which contradicts the presentations of IKKe KO mice. IKKe was also reported to phosphorylate STAT1 and favors its association with STAT2 and IRF9 to form the interferon-stimulated gene factor 3 complex, which binds to interferon-stimulated response elements in the promoters of target genes (41). However, we also observed antidiabetic effects of IRF9 (42), which are opposite to the effects of IKKe and therefore indicate that the metabolic effects of IKKe (and perhaps, NF-κB) are at least in part mediated by IRF7 and unlikely to be mediated by IRF3 or IRF9.

Although their predominant function is in innate immune responses and oncogenesis, the IRF family members are currently thought to regulate energy metabolism as well. Eguchi et al. reported that IRFs regulate adipogenesis and further identified the anti-adipogenic properties of IRF4 (10, 11). Unlike IRF4, the expression of which is highly restricted to adipose tissue and immune cells, IRF7 is widely distributed. We confirmed the attenuation of diet-induced obesity, insulin resistance, ER stress, and inflammation in IRF7-deficient mice. Although the underlying mechanism is not fully understood, we propose that the metabolism-improving effects of IRF7 deficiency may be partly mediated by PPARγ. Our results reveal a multifaceted role of IRF7 in metabolism and propose a new link between immunity and metabolism. Future work may focus on the regulation of IRFs, as well as their regulatory functions and the interplay between different IRF family members. In summary, targeting IRF7 will hopefully become a new strategy for the treatment of obesity and related metabolic disorders.

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DISCLOSURES

No conflicts of interest are reported by the authors.

AUTHOR CONTRIBUTIONS

Xin-An Wang designed and performed the experiments, analyzed the data and wrote the manuscript; Ran Zhang wrote the manuscript; Shumin Zhang, Xin-An Wang, Slaney CY, Withana NP, Forster S, Cao Y, Loi S, Angewandt A, Micekta T, Mangan NE, Samarajiwa SA, de Weerd NA, Gott J, Argani P, Moller A, Smyth MJ, Anderson RL, Hertzog PJ, Parker BS. Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. Nat Med In press.

REFERENCES


