Diet-related obesity is a major metabolic disorder. Excessive fat mass is associated with type 2 diabetes, hepatic steatosis, and arteriosclerosis. Dysregulation of lipid metabolism and adipose tissue function contributes to diet-induced obesity. Here, we report that β-arrestin-1 knock-out mice are susceptible to diet-induced obesity. Knock-out of the gene encoding β-arrestin-1 caused increased fat mass accumulation and decreased whole-body insulin sensitivity in mice fed a high-fat diet. In β-arrestin-1 knock-out mice, we observed disrupted food intake and energy expenditure and increased macrophage infiltration in white adipose tissue. At the molecular level, β-arrestin-1 deficiency affected the expression of many lipid metabolic genes and inflammatory genes in adipose tissue. Consistently, transgenic overexpression of β-arrestin-1 repressed diet-induced obesity and improved glucose tolerance and systemic insulin sensitivity. Thus, our findings reveal that β-arrestin-1 plays a role in metabolism regulation.

The prevalence of obesity is increasing dramatically in many regions and among many ethnicities and has become a serious public health concern. Obesity is regarded as the major risk factor for insulin resistance, type 2 diabetes, and various cardiovascular diseases (1, 2). An abnormal accumulation of adipose tissue, the infiltration of macrophages into adipose tissue, and enhanced systemic inflammation are the major pathological features of obesity. Furthermore, numerous studies have revealed that adipose tissue modulates whole-body glucose and lipid homeostasis by controlling lipid turnover in the body (3). Obesity-induced inflammation is considered a potential link between obesity and its related pathologies, such as insulin resistance, cardiovascular diseases, type 2 diabetes, and other immune disorders (4). However, the cellular and molecular mechanisms underlying obesity are extremely complex and not yet fully understood.

β-Arrestins (β-arrestin-1 and β-arrestin-2) are traditionally regarded as terminators of G protein-coupled receptor signaling and regulators of receptor desensitization following stimulation (5, 6). Studies have demonstrated that β-arrestins regulate diverse signaling pathways in addition to G protein-coupled receptors by serving as multiple-function binding partners for various protein complexes (7–10). Our recent study demonstrated that β-arrestin-2, which is located primarily in the cytoplasm, functions as an indispensable scaffold that links Akt and Src to the insulin receptor following insulin stimulation, and a deficiency of β-arrestin-2 contributes to insulin resistance (11). Previous studies have shown that β-arrestin-1 mediates insulin receptor substrate-1 and glucagon-like peptide-1 signaling in β-cells. In this study, we report that a deficiency of β-arrestin-1 contributes to diet-induced obesity.

EXPERIMENTAL PROCEDURES

Animals—β-Arrestin-1 knock-out (βarr1-ko) and βarr2-ko mice were provided by Dr. Robert J. Lefkowitz (Duke University Medical Center, Durham, NC). β-Arrestin-1 transgenic (βarr1-tg) and βarr2-tg mice were generated as described (12). All other mice were from the Shanghai Laboratory Animal Center, Chinese Academy of Sciences. Animal experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were fed a regular diet (Formulab 5008 and Labdiet 5053) or a high-fat diet (55% fat calories; Harlan Teklad 93075) and had free access to water and food.

Metabolic Parameters—We measured food intake, mean activity, oxygen consumption, and carbon dioxide production in metabolic chambers (Columbus CLAMS-8). We analyzed the fat mass and lean mass by NMR spectroscopy (Bruker minispec™). Hematoxylin and Eosin Staining—Adipose tissue samples were fixed overnight in 4% paraformaldehyde. Paraffin embedding, sectioning, and hematoxylin and eosin staining were performed according to standard protocols. Macrophages in the epididymal fat pads were visualized by anti-F4/80 antibody (eBioscience) immunostaining and quantified as described previously (13).

3 The abbreviations used are: βarr1-ko, β-arrestin-1 knock-out; βarr1-tg, β-arrestin-1 transgenic; GTT, glucose tolerance test; ITT, insulin tolerance test; HFD, high-fat diet; RD, regular diet; TG, triglyceride; WAT, white adipose tissue.
RESULTS

**β-Arrestin-1 Ameliorates Diet-induced Obesity**—β-Arrestin-1 and β-arrestin-2 are known as cytosolic signaling mediators and scaffold proteins. To investigate the physiological role of β-arrestin-1 in metabolism, we used transgenic mice overexpressing human β-arrestin-1 (βarr1-tg) and βarr1-ko mice. We fed βarr1-tg and βarr1-ko mice and their wild-type littermates a high-fat (55% fat) diet (HFD) to generate diet-induced obese mice (16). There was a 2-fold increase in the expression of β-arrestin-1 in βarr1-tg mice compared with non-transgenic mice (data not shown). βarr1-tg mice developed normally, and the body lengths of all mice were similar. Although the total body weights did not differ at weaning, at 4 weeks of age, βarr1-tg mice fed a HFD began to gain weight at a slower rate than their wild-type littermates (Fig. 1A). This difference in body weight increased as the mice aged, and after 14 weeks of HFD treatment, βarr1-tg mice weighed 33.6 ± 1.3 g (n = 10), whereas their wild-type littermates weighed 36.9 ± 0.9 g (n = 10; p < 0.05). βarr1-ko mice were viable and fertile. After 14 weeks of HFD treatment, βarr1-ko mice gained ~21.6 ± 0.7 g, whereas their wild-type littermates gained 17.3 ± 0.8 g. This discrepancy in weight gain was also observed in βarr1-tg and βarr1-ko mice that were fed a regular diet (RD), albeit to a lesser extent (Fig. 1, A and B). The body lengths of these mice were similar (Fig. 1C). It is noteworthy that the weight gain in βarr1-tg mice that were fed a HFD did not differ from that in mice fed a RD. These results suggest that elevation of β-arrestin-1 expression slows the development of obesity.

Obese mice show abnormal blood lipid profiles (17). We monitored blood triglyceride (TG) and free fatty acid levels in βarr1-tg, βarr1-ko, and wild-type mice fed a HFD or RD. Compared with their wild-type littermates, βarr1-tg mice fed a HFD showed markedly lower levels of TGs and free fatty acids, whereas βarr1-ko mice showed significantly higher lipid levels (Fig. 1D). Leptin has been demonstrated to reduce the plasma TG content, and the development of leptin resistance and elevated blood leptin levels are commonly observed in obese animals and patients (17). We found that following HFD treatment, βarr1-tg mice showed lower levels of leptin, whereas
β-Arrestin-1 Represses Diet-induced Obesity

Figure 2. βarr1-ko mice show HFD-induced insulin resistance. A, glucose levels during GTTs (1 g/kg) or ITTs (1 g/kg) in βarr1-tg mice (Tg) and wild-type littermates (WT) fed a RD (n = 10/group). B, glucose levels during GTTs (1 g/kg) or ITTs (1 g/kg) in βarr1-ko mice (KO) and wild-type littermates fed a RD (n = 10/group). C, resting plasma glucose levels of βarr1-tg, βarr1-ko, and wild-type mice fed either a RD or HFD (n = 10/group). D, insulin levels of βarr1-tg, βarr1-ko, and wild-type mice fed either a RD or HFD following overnight fasting (n = 10/group). E, glucose levels during GTTs (1.5 g/kg) in βarr1-tg mice and wild-type littermates fed a HFD (n = 10/group). F, insulin levels during GTTs (1.5 g/kg) in βarr1-tg mice and wild-type littermates fed a HFD (n = 10/group). G, glucose levels during ITTs (1.5 g/kg) in βarr1-tg mice and wild-type littermates fed a HFD (n = 10/group). H, glucose levels during ITTs (1.5 g/kg) in βarr1-ko mice and wild-type littermates fed a HFD (n = 10/group). I, glucose levels during ITTs (1.5 g/kg) in βarr1-ko mice and wild-type littermates fed a HFD (n = 10/group). Data are presented as means ± S.E. *, p < 0.05.

βarr1-ko mice showed higher leptin levels (Fig. 1E). Together, these results indicate that βarr1-ko mice are susceptible and that βarr1-tg mice are resistant to diet-induced obesity.

β-Arrestin-1 Improves Systemic Insulin Sensitivity in HFD-treated Mice—Obesity is known to engender insulin resistance and non-insulin-dependent diabetes mellitus (18–20). Diet-induced obese mice show deficient systemic insulin sensitivity and glucose homeostasis (17, 21). As we reported previously, we did not find any evidence that β-arrestin-1 affects systemic insulin sensitivity (22). No change was observed in GTTs and ITTs using βarr1-ko, βarr1-tg, and wild-type mice that were fed a RD (Fig. 2, A and B). However, after 14 weeks of HFD treatment, βarr1-tg mice had lower blood glucose and insulin levels than their wild-type littermates, whereas βarr1-ko mice showed higher blood glucose and insulin levels (Fig. 2, C and D). This result indicates that HFD-treated βarr1-ko mice have a higher demand for insulin accompanied by a defect in glucose homeostasis, whereas βarr1-tg mice are resistant to the development of obesity-associated insulin resistance.

We next performed GTTs and ITTs with HFD-treated βarr1-ko, βarr1-tg, and wild-type control mice. In wild-type mice, an intraperitoneal injection of 1.5 g of glucose/kg induced
a rapid increase in blood glucose levels with a gradual return to normal levels over 2 h. In contrast, the same injection in \( \text{bar} \) mice resulted in a lower glucose increase and a lower glucose response overall (Fig. 2E), suggesting improved glucose homeostasis in these HFD-treated transgenic mice. Insulin secretion in response to glucose loading during GTTs was also lower in \( \text{bar} \) mice, indicating improved insulin sensitivity in transgenic mice (Fig. 2F). This result was further confirmed by ITTs. In ITTs, an intraperitoneal injection of 1.5 units of insulin/kg induced a time-dependent reduction of blood glucose and a gradual return to nearly normal levels in both wild-type littermates and \( \text{bar} \) mice. However, the reduction in blood glucose was enhanced, and the hypoglycemic effect of insulin was more sustained in \( \text{bar} \) mice than in wild-type littermates (Fig. 2G). These results demonstrate that, compared with their wild-type littermates, HFD-treated \( \text{bar} \) mice had enhanced insulin sensitivity. The results of GTTs (1.5 g/kg) and ITTs (1.5 units/kg) in HFD-treated \( \text{bar} \) mice revealed that the insulin sensitivity of \( \text{bar} \) mice was compromised compared with wild-type littermates (Fig. 2, H–J). Taken together, these analyses indicate that a deficiency of \( \text{arrestin-1} \) contributes to diet-induced impairment of insulin sensitivity, whereas an elevation in the protein levels of \( \text{arrestin-1} \) improves systemic insulin sensitivity in HFD-treated mice.

To further substantiate the role of \( \text{arrestin-1} \) in modulating obesity-associated whole-body and tissue-specific insulin sensitivity and glucose metabolism, hyperinsulinemic-euglycemic clamp studies were performed. There was no significant difference in basal and clamped hepatic glucose production between HFD-treated \( \text{bar} \) mice and wild-type littermates (Fig. 3, A and B). In \( \text{bar} \) mice, however, both whole-body glucose disposal and glucose infusion rates were significantly decreased.
decreased (Fig. 3, C and D), indicating that HFD-fed βarr1-ko mice had an impaired insulin sensitivity.

β-Arrestin-1 Reduces Energy Expenditure in Mice—To further evaluate the effect of β-arrestin-1 on metabolic changes, we monitored changes in energy expenditure and animal activity by keeping RD- and HFD-treated mice in metabolic chambers. When βarr1-tg, βarr1-ko, and wild-type mice were fed a RD, all of the mice had similar mean activity levels, consumed a similar amount of food and oxygen, and had similar CO2 release (Fig. 4, A–D). Mice fed a HFD had similar mean activities, but βarr1-tg mice consumed significantly less food and expended more energy than their wild-type littermates (Fig. 4, E and F), whereas βarr1-ko mice consumed significantly more food and expended less energy than their wild-type littermates (Fig. 4, F–H).

We further determined the fat and lean masses of these mice using NMR spectroscopy. As shown in Fig. 5 (A and B), after 14 weeks of HFD treatment, the percent fat mass was 13.8 ± 2.3 g in βarr1-tg mice and 24.1 ± 2.5 g in wild-type mice but 27.4 ± 3.3 g in βarr1-ko mice (n = 10; p < 0.05). Various adipose depots contribute to whole-body fat mass, including visceral fat, subcutaneous fat, and gonadal fat pad. We found that the lower body weight gain of βarr1-tg mice can be ascribed largely to slower growth of gonadal fat pads, the major adipose depot (Fig. 5C).

Hematoxylin and eosin staining of epididymal white adipose tissue (WAT) from βarr1-tg, βarr1-ko, or wild-type mice fed a HFD or RD revealed that wild-type mice fed a HFD showed a marked increase in adipocyte size (mean diameter of 63.5 ± 3.8 μm). Such diet-induced hypertrophy was less pronounced in βarr1-tg mice (mean diameter of 45.4 ± 4.1 μm).
but was more severe in βarr1-ko mice (mean diameter of 75.1 ± 4.5 μm) (Fig. 5, D and E). In another type of adipose tissue, brown adipose tissue, the functions of which are different from those of WAT (23), we observed no difference in adipocyte size in mice fed either a HFD or RD (Fig. 5F). Hema-toxylin and eosin staining and TG levels of liver samples from βarr1-ko mice showed that hepatic steatosis was more severe in βarr1-ko mice than in wild-type mice fed a HFD, whereas βarr1-tg mice showed less hepatic steatosis compared with their wild-type littermates fed a HFD (Fig. 5, G and H). These data suggest that β-arrestin-1 might act in WAT to affect fat deposition and weight gain.

**β-Arrestin-1 Suppresses Macrophage Infiltration in Adipose Tissue**—The infiltration of macrophages into adipose tissue is one of the major pathological features of obesity. Adipose tissue macrophage infiltration was monitored by immunohistochemical staining with anti-F4/80 antibody. As shown in Fig. 5I, adipose tissue from βarr1-tg mice fed a HFD exhibited a 70% reduction in adipose tissue macrophage infiltration compared with adipose tissue from wild-type littermates. In contrast, there were more detectable macrophages in the adipose tissue of βarr1-ko mice. Adipose tissue macrophages produce inflammatory cytokines and contribute to obesity-related abnormal immune responses. We measured the serum levels of inflammatory cytokines in βarr1-tg, βarr1-ko, and wild-type mice fed either a HFD or RD. The serum levels of IL-6, TNF-α, and MCP-1 in wild-type and βarr1-ko mice fed either a RD or HFD elevated similarly. However, there was no significant difference in the levels of these cytokines in βarr1-ko mice compared with those in wild-type littermates (Fig. 5F). These results indicate that overexpression of the β-arrestin-1 gene prevents the obesity-related immune response induced by HFD treatment.

**Deficiency of β-Arrestin-1 Affects the Expression of Lipid Metabolic Genes and Inflammatory Genes**—We further measured the expression of genes related to adipocyte differentiation, lipid metabolism, and inflammation in the adipose tissue of wild-type and βarr1-ko mice fed a HFD. We found that FABP4 (fatty acid-binding protein 4; also called aP2 (adipocyte protein 2)) and genes associated with lipid metabolism, including the fatty acid transporter CD36, fatty acid synthase, and lipoprotein lipase, were significantly increased in the adipocyte fraction of WAT from βarr1-ko mice fed a HFD compared with their expression levels in white adipocytes from wild-type mice (Fig. 6A). These results are consistent with the increased fat mass and adipocyte size observed in these knockout mice.

We also measured the mRNA expression of inflammatory response genes in fat tissue. Consistent with a higher production of inflammatory mediators by adipose-resident macrophages, there was an increased expression of TNF and other macrophage-derived molecules, along with Nos2 and Mcp-1, in the WAT of βarr1-ko mice fed a HFD (Fig. 6A). This gene expression profile suggests that a higher production of inflammatory mediators might also contribute to insulin resistance in βarr1-ko mice fed a HFD. In mice fed a HFD, the expression of these genes was significantly lower in βarr1-tg mice compared with wild-type mice (Fig. 6B). Together, these data indicate that a deficiency of β-arrestin-1 increases the expression of adipogenic genes and inflammatory response genes.

**DISCUSSION**

β-Arrestin-1 and β-arrestin-2 are ubiquitously and abundantly expressed signaling proteins that have been extensively studied for their critical functions in neural and immune systems (12, 24–26). These studies demonstrated that mice deficient in either β-arrestin isoform appear normal but that, in the presence of various pathological challenges, these mice display phenotypes associated with related disorders, suggesting a link between β-arrestin malfunction and a predisposition toward many complex diseases. Combined with previous studies (11, 27, 28), our work establishes that β-arrestin-1 and β-arrestin-2 critically regulate whole-body metabolic reactions and energy balance in a distinct but coordinate manner. A deficiency in either β-arrestin isoform brings the pathogenesis of metabolic disorders, including obesity, insulin resistance, and diabetes. However, considering the versatile signaling pathways β-arrestin-1 regulates and mediates, we speculate that β-arrestin-1 may contribute to metabolic control by playing different regulatory roles. To discriminate between the different functions of β-arrestin-1, we are currently working on research with animals that have tissue-specific depletion of β-arrestin-1.

![Graph](https://example.com/graph.png)

**FIGURE 6. Transcription of adipogenesis and inflammation genes is down-regulated in βarr1-tg mice.** A and B, mRNA levels were examined by quantitative RT-PCR in WAT from βarr1-ko (KO; A) and βarr1-tg (Tg; B) mice and their wild-type littermates (WT) fed a HFD (n = 10/group). *p < 0.05 versus the corresponding control value.
**β-Arrestin-1 Represses Diet-induced Obesity**

Previous reports have shown that repression of adipogenesis in adipose tissue slows the pathogenesis of obesity in mice (29, 30). Inflammation in adipose tissue is another factor associated with obesity and obesity-induced insulin resistance (31). In this work, we compared the expression levels of adipogenesis- and inflammation-related genes in the adipose tissue of βarr1-1g and βarr1-ko mice with those of their wild-type littermates when fed a HFD. We found significant differences between the genotypes, indicating that the regulation of adipogenesis and the inflammatory response by β-arrestin-1 may be an underlying mechanism. Previous studies have shown that β-arrestin-1 mediates glucagon-like peptide-1 signaling, which induces insulin secretion in cultured β-cells (27). We have demonstrated here that insulin sensitivity is maintained in HFD-fed βarr1-1g mice compared with HFD-fed wild-type littermate control mice. This protection from diet-induced insulin resistance indicates that regulation of the G protein-coupled receptor signaling pathway by β-arrestin-1 may also play a role in whole-body metabolism. This hypothesis will require further investigation with tissue-specific βarr1-ko mice.

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