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Cell Metabolism
Article

p53-Induced Adipose Tissue Inflammation Is Critically Involved in the Development of Insulin Resistance in Heart Failure

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SUMMARY

Several clinical studies have shown that insulin resistance is prevalent among patients with heart failure, but the underlying mechanisms have not been fully elucidated. Here, we report a mechanism of insulin resistance associated with heart failure that involves upregulation of p53 in adipose tissue. We found that pressure overload markedly upregulated p53 expression in adipose tissue along with an increase of adipose tissue inflammation. Chronic pressure overload accelerated lipolysis in adipose tissue. In the presence of pressure overload, inhibition of lipolysis by sympathetic denervation significantly downregulated adipose p53 expression and inflammation, thereby improving insulin resistance. Likewise, disruption of p53 activation in adipose tissue attenuated inflammation and improved insulin resistance but also ameliorated cardiac dysfunction induced by chronic pressure overload. These results indicate that chronic pressure overload upregulates adipose tissue p53 by promoting lipolysis via the sympathetic nervous system, leading to an inflammatory response of adipose tissue and insulin resistance.

INTRODUCTION

The p53 tumor suppressor pathway coordinates DNA repair, cell-cycle arrest, apoptosis, and senescence to preserve genomic stability and prevent oncogenesis. Activation of p53 is driven by a wide variety of stress signals that have the potential to promote tumor formation, such as DNA damage, telomere shortening, oxidative stress, and oncogene activation (Harris and Levine, 2005; Meek, 2009; Vousden and Prives, 2009). Recently, the contribution of p53 to many undesirable aspects of aging and age-associated diseases, such as cardiovascular and metabolic disorders, has been recognized (Royds and Iacopetta, 2006; Vousden and Lane, 2007). It has been reported that aging is associated with an increase of the p53-mediated transcriptional activity (Edwards et al., 2007) and that slight constitutive overactivation of p53 is associated with premature aging in mice (Maier et al., 2004; Tyner et al., 2002). Activation of p53 has also been observed in aged vessels and failing hearts and has been implicated in atherosclerosis and heart failure (Minamino and Komuro, 2007, 2008; Sano et al., 2007). Recent findings have indicated a role of p53 in determining the response of cells to nutrient stress and in regulating metabolism (Vousden and Ryan, 2009). It has also been demonstrated that excessive calorie intake induces p53-induced inflammation in adipose tissue, leading to insulin resistance and diabetes in mice (Minamino et al., 2009).

A close link between heart failure and diabetes has long been recognized in the clinical setting (Ashrafian et al., 2007; Lopaschuk et al., 2007; Witteles and Fowler, 2008). Many mechanisms have been suggested to explain the increased incidence of heart failure in diabetic patients, including the hypertrophic influence of insulin, the adverse effects of hyperglycemia, increased oxidative stress, and hyperactivity of neurohumoral systems, such as the renin-angiotensin-aldosterone system and the adrenergic system. Recently, increasing attention has been paid to insulin resistance as a distinct cause of cardiac dysfunction and heart failure in diabetic patients. A study of Swedish patients without prior cardiac dysfunction found that insulin resistance predicted the subsequent onset of heart failure independently of established risk factors (Ingelsson et al., 2005). In another clinical study, the plasma level of proinsulin (a marker of insulin resistance) was found to be higher in patients who subsequently developed heart failure than in control patients 20 years before the actual diagnosis of heart failure (Arnlöv et al., 2001). These findings indicate that insulin resistance precedes heart failure rather than being a consequence of it. Evidence has emerged that myocardial insulin resistance is central to altered metabolism in the failing heart and may play a crucial role in the development of heart failure (Ashrafian et al., 2007; Lopaschuk et al., 2007; Witteles and Fowler, 2008). The adaptive response of the failing heart involves a complex series of enzymatic shifts and changes in the regulation of transcriptional factors, which result in an increase of glucose metabolism and a decrease of fatty acid metabolism

to maximize the efficacy of energy production (Neubauer, 2007). Insulin resistance of the myocardium inhibits these adaptive responses, leading to increased reliance on fatty acid metabolism. This increases oxygen consumption and decreases cardiac function, raising the potential for lipotoxicity in the heart (Sharma et al., 2007; Young et al., 2002). Another line of evidence indicates that insulin signaling is upregulated in the failing heart and that excessive cardiac insulin signaling exacerbates systolic dysfunction (Shimizu et al., 2010).

Moreover, there is increasing evidence that heart failure reciprocally augments the risk of insulin resistance and clinical diabetes (Ashrafian et al., 2007). Insulin resistance and abnormal glucose metabolism are very common in heart failure patients, being identified in 43% of these patients, and such abnormalities are associated with decreased cardiac function (Suskin et al., 2000). Surprisingly, the link between heart failure and insulin resistance grows stronger when patients with ischemic heart disease are excluded (Witteles and Fowler, 2008). Heart failure also predicts the development of type 2 diabetes in a graded way (Tenenbaum et al., 2003). Although the above mentioned clinical evidence supports a role of insulin resistance in the occurrence of heart failure, evidence for the reciprocal statement that heart failure promotes insulin resistance is largely associative. Moreover, the role of heart failure in the promotion of insulin resistance has been demonstrated by only a few animal studies (Nikolaidis et al., 2004; Shimizu et al., 2010) and the underlying mechanisms are largely speculative.

Here, we studied the role of heart failure in the development of insulin resistance and sought to elucidate the molecular mechanisms involved. We found that insulin resistance developed in two murine models of heart failure, a chronic pressure overload model and a myocardial infarction model. Heart failure markedly upregulated p53 expression in adipose tissue in association with increased inflammation of adipose tissue. Heart failure accelerated lipolysis in adipose tissue, whereas inhibition of lipolysis by sympathetic denervation or treatment with a lipase inhibitor significantly downregulated adipose tissue p53 expression and inflammation, thereby improving insulin resistance. Likewise, disruption of p53 activation in adipose tissue not only ameliorated inflammation in this tissue and improved insulin resistance but also improved cardiac dysfunction associated with heart failure. We conclude that heart failure upregulates p53 in adipose tissue by promoting lipolysis via activation of the sympathetic nervous system, leading to an inflammatory response of adipose tissue and insulin resistance. Our results indicate that inhibition of p53-induced adipose inflammation is a potential target for treating metabolic abnormalities and systolic dysfunction in patients with heart failure.

RESULTS

Pressure Overload Induces Adipose Tissue Inflammation and Insulin Resistance

To examine the effect of cardiac pressure overload on glucose homeostasis, we produced transverse aortic constriction (TAC) in 11-week-old mice. In this mouse model, systolic cardiac function deteriorated significantly along with left ventricular (LV) dilatation 2–6 weeks after surgery (Figure S1A available online). The insulin tolerance test (ITT) and the glucose tolerance test (GTT) showed that insulin sensitivity and glucose tolerance were impaired at 4–6 weeks after TAC (Figure 1A) without any change of food intake (Figure S1B). In patients with metabolic disorders, the recruitment of inflammatory macrophages to adipose tissue has been shown to increase the production of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and chemokine (C–C motif) ligand 2 (CCL2), also known as monocyte chemoattractant protein-1 (MCP-1), leading to the development of systemic insulin resistance (Hotamisligil et al., 1993; Kamei et al., 2006; Weisberg et al., 2003). Therefore, we investigated whether pressure overload provokes adipose tissue inflammation. Examination of hematoxylin- and eosin-stained sections demonstrated the infiltration of mononuclear cells into visceral fat, with most of these cells being identified as macrophages by immunofluorescent staining for Mac3 (Figure 1B). Consistent with these results, expression of a marker for macrophages (Egf-like module containing, mucin-like, hormone receptor-like 1; EMR1) and production of proinflammatory cytokines were significantly upregulated in the adipose tissue of TAC mice along with a decrease of adiponectin (Figure 1C) compared with sham-operated mice. Treatment of TAC mice with a neutralizing antibody for Tnf-α significantly improved insulin resistance and glucose intolerance, suggesting a crucial role in the upregulation of proinflammatory cytokines in the development of metabolic abnormalities during heart failure (Figure S1C).

Pressure Overload Increases Lipolysis and Induces p53-Dependent Inflammation in Adipose Tissue during Heart Failure

Computed tomography (CT) showed a significant decrease of visceral fat after the creation of pressure overload (Figure 1D). It is well accepted that sympathetic activity increases with heart failure (Floras, 2009), and norepinephrine regulates lipolysis in adipose tissue. We found that the norepinephrine levels of plasma and adipose tissue increased significantly and plasma fatty acid levels were markedly elevated in TAC mice compared with sham-operated mice, suggesting acceleration of lipolysis via the sympathetic nervous system in response to pressure overload (Figure 1E). It has been reported that exposure to an excess of fatty acids leads to p53 activation in various cells (Zeng et al., 2008) and that p53 is crucially involved in the regulation of adipose tissue inflammation in obese animals (Minamino et al., 2009). Therefore, we hypothesized that chronic pressure overload promotes lipolysis and the resultant increase of fatty acids leads to p53-induced inflammation in adipose tissue.

Consistent with this concept, we found that p53 expression was upregulated in the adipose tissue of TAC mice at 2–4 weeks after surgery and the change was sustained until 6 weeks (Figures 2A and S2A). To further investigate the role of adipose tissue p53 in the response to pressure overload, we performed TAC in adipocyte-specific p53 knockout (adipo-p53 KO) mice. The pressure overload-induced increase of p53 expression was attenuated in adipo-p53 KO mice compared with littermate controls (Figure S2B). Production of proinflammatory cytokines as well as cyclin-dependent kinase inhibitor 1A (*Cdkn1a*) expression was also decreased in adipo-p53 KO mice, along with a decline in the infiltration of macrophages into visceral fat

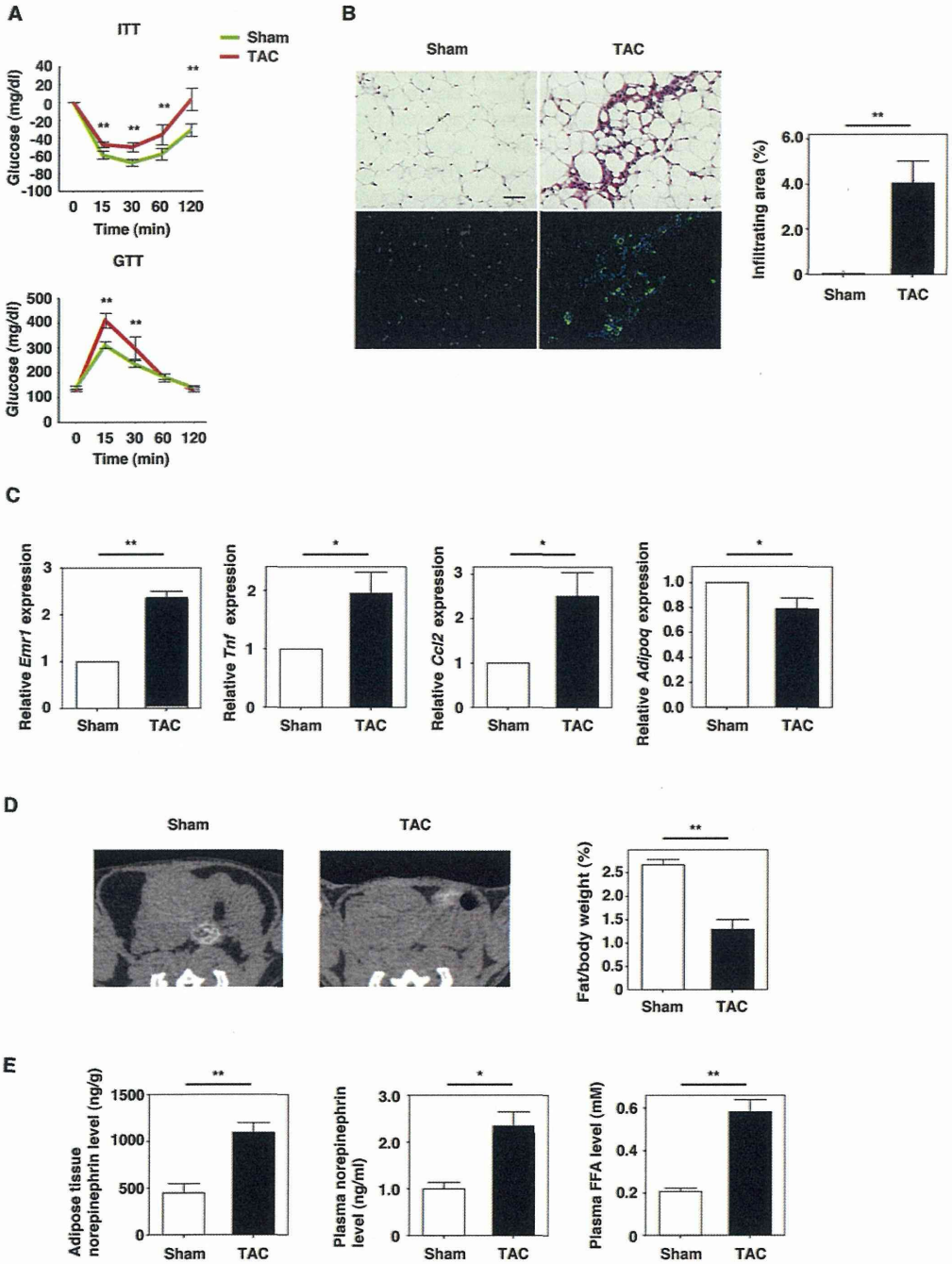


Figure 1. Pressure Overload Induces Systemic Insulin Resistance and Adipose Tissue Lipolysis and Inflammation

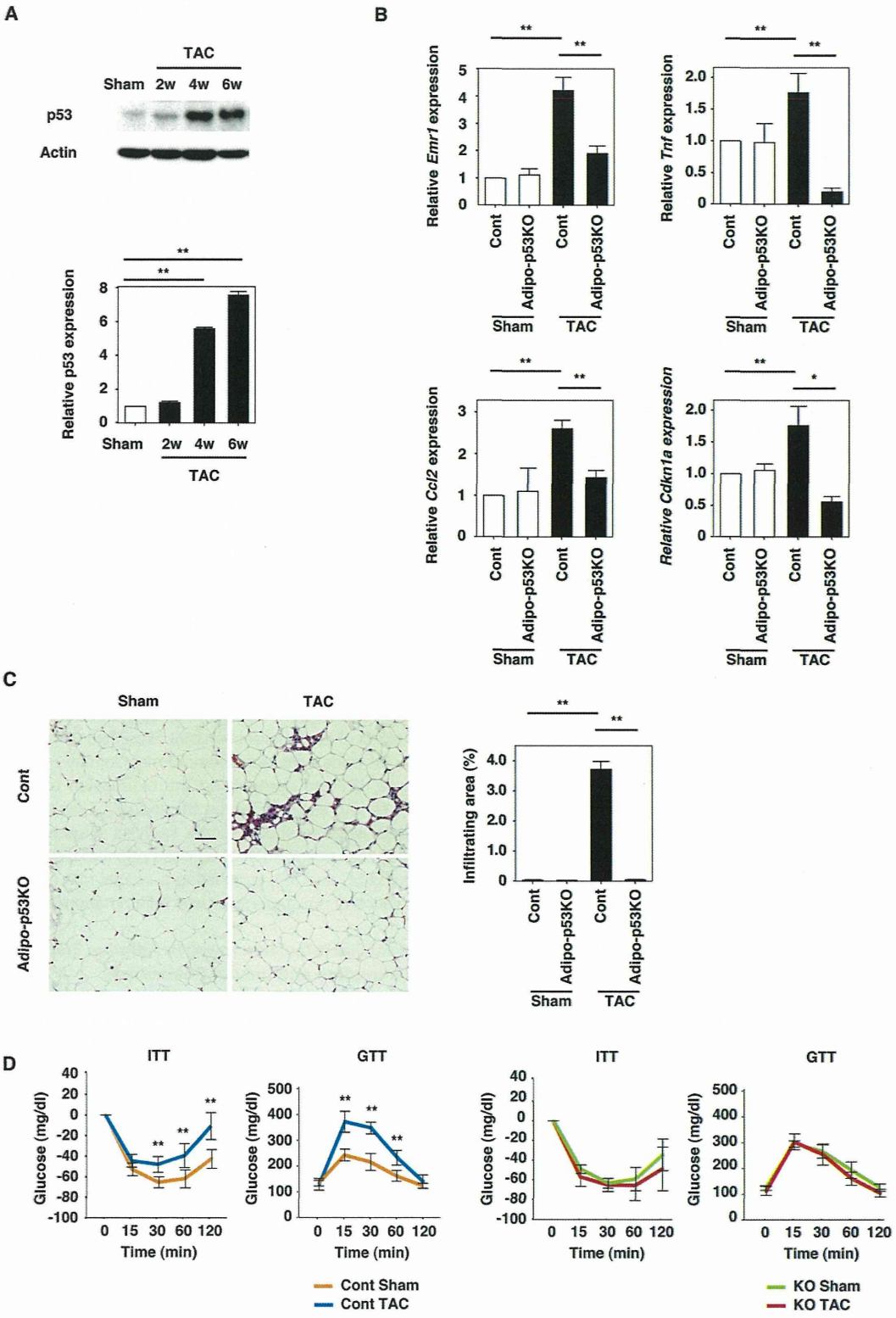
(A) *Insulin tolerance test* (ITT) *and glucose tolerance test* (GTT) *in mice at 6 weeks after sham operation* (Sham) *or* TAC ($n = 30$).

(B) Hematoxylin and eosin staining of adipose tissues of mice at 6 weeks after sham operation (Sham) or TAC (upper panel). In the lower panel, the infiltration of macrophages was evaluated by immunofluorescent staining for Mac3 (green). Nuclei were stained with Hoechst dye (blue). Scale bar, 50 μm. The right graph indicates the quantitative data on the infiltration of macrophages ($n = 5$).

(C) Real-time PCR assessing the expression of *Emr1*, *Tnf* (Tnfα), *Ccl2* (MCP1), and *Adipoq* (Adiponectin) levels in adipose tissues of mice at 6 weeks after sham operation (Sham) or TAC ($n = 10$).

(D) CT analysis of mice at 6 weeks after sham operation (Sham) or TAC. The graph shows the ratio of visceral fat tissue weight estimated by CT to whole body weight ($n = 7$).

(E) Norepinephrine level in adipose tissue (left) and plasma (middle), and plasma free fatty acid (FFA) level (right) of mice at 6 weeks after sham operation (Sham) or TAC ($n = 10$). Data are shown as the means ± S.E.M. *$p < 0.05$, **$p < 0.01$.


A
TAC
Sham 2w 4w 6w
p53
Actin
Relative p53 expression
Sham 2w 4w 6w
TAC
B
Relative Emr1 expression
Relative Tnf expression
Relative Ccl2 expression
Relative Cdkn1a expression
Cont
Adipo-p53KO
Sham
TAC
C
Sham
TAC
Cont
Adipo-p53KO
Infiltrating area (%)
D
ITT
GTT
Glucose (mg/dl)
Time (min)
Cont Sham
Cont TAC
KO Sham
KO TAC

(Figures 2B and 2C). Consequently, adipo-p53 KO mice showed improved insulin sensitivity and glucose tolerance after induction of pressure overload compared with littermate controls (Figure 2D) without any change of food intake (Figure S2C). These results suggest that p53 has a critical role in the regulation of adipose tissue inflammation and insulin resistance during pressure overload. In contrast, a decrease of fat mass and an increase of plasma free fatty acids were observed to a similar extent in both adipo-p53 KO and control mice after TAC (Figures S2D–S2F), suggesting that pressure overload accelerates lipolysis in a p53-independent manner.

Pressure Overload Promotes Lipolysis via the Sympathetic Nervous System

We inhibited sympathetic activity in epididymal fat tissue by surgical denervation and then performed TAC. As a result, surgical denervation effectively inhibited an increase of the norepinephrine level of adipose tissue and attenuated lipolysis after the onset of pressure overload (Figures S3A and S3B and data not shown). Histological examination of adipose tissue showed that infiltration of inflammatory cells after TAC was attenuated by denervation (Figures S3C and S3D). Likewise, disruption of the sympathetic efferent nerves significantly reduced pressure overload-induced upregulation of *Emr1*, a proinflammatory cytokine expression in adipose tissue (Figure 3A), and this reduction was associated with significant improvement of insulin resistance and glucose tolerance in TAC mice (Figure 3B). Surgical denervation attenuated pressure overload-induced upregulation of p53 and *Cdkn1a* expression in adipose tissue (Figures 3A and 3C). We also pharmacologically inhibited the sympathetic activity in adipose tissue by injecting guanethidine directly into epididymal fat and then performed TAC. As a result, pharmacological denervation also significantly inhibited lipolysis (Figures S3A and S3B) and attenuated upregulation of p53 and *Cdkn1a* expression and inflammation in adipose tissues (Figures S3C, S3D, S4A and S4B). Mice treated with guanethidine showed better insulin sensitivity and glucose tolerance after creation of pressure overload (Figure S4C), indicating that pressure overload-induced activation of the sympathetic nervous system accelerates lipolysis and, thus, leads to adipose tissue inflammation and insulin resistance in TAC mice.

Role of Lipolysis in the Regulation of Adipose p53 Expression and Inflammation

To examine the role of lipolysis in influencing adipose tissue expression of p53 and inflammation after TAC, we inhibited lipolysis by administering acipimox, a selective inhibitor of lipolysis, to mice with TAC. Treatment with acipimox markedly inhibited lipolysis and also reduced infiltration of inflammatory cells into adipose tissue during pressure overload (Figures S3A–S3D). Inhibition of lipolysis also significantly reduced pressure overload-induced upregulation of *Emr1* and proinflammatory cytokine production in adipose tissue (Figure 4A), along with significant improvement of insulin resistance and glucose intolerance in TAC mice (Figure 4B). Furthermore, treatment with acipimox attenuated pressure overload-induced upregulation of p53 and *Cdkn1a* expression in adipose tissue (Figures 4A and 4C), confirming a close relationship between lipolysis and p53 expression.

Next, we promoted lipolysis by administering isoproterenol to mice via an infusion pump. Treatment with isoproterenol significantly decreased the visceral fat mass and increased plasma fatty acid levels (Figures S5A–S5C) and increased p53 expression in adipose tissue (Figure 5A). Isoproterenol also induced adipose tissue inflammation (Figures 5B and 5C). To further investigate the role of lipolysis in the regulation of p53 expression and inflammation in adipose tissue, we tested the influence of deleting adipose triglyceride lipase (patatin-like phospholipase domain containing protein 2, encoded by *Pnpla2*; hereafter referred to as Atgl) on adipose tissue expression of p53. It has been reported that Atgl homozygous KO mice show massive accumulation of lipids in the heart, causing cardiac dysfunction and premature death (Haemmerle et al., 2006). When we generated TAC mice, we also noted that cardiac function was worse and LV enlargement was more marked in Atgl heterozygous KO mice compared with their littermates (Figure S5D). In fact, most of the KO mice died of heart failure within 4 weeks after TAC. Therefore, we utilized Atgl-deficient adipose tissue for ex vivo experiments. We cultured epididymal fat pad tissues from Atgl KO mice or wild-type littermates and examined the effect of isoproterenol on p53 expression. Treatment of wild-type fat pads with isoproterenol significantly induced lipolysis (Figure 5D) and upregulated the expression of both p53 and *Cdkn1a* expression (Figures 5E and 5F). Disruption of Atgl inhibited isoproterenol-induced lipolysis (Figure 5D) and prevented the upregulation of adipose p53 and *Cdkn1a* expression (Figures 5E and 5F), suggesting a crucial role of lipolysis in the regulation of p53 expression and inflammation in adipose tissue.

Myocardial Infarction Induces Adipose Tissue Inflammation and Insulin Resistance

To investigate whether myocardial infarction (MI) induced insulin resistance, we created MI in 11-week-old mice and assessed the animals 6 weeks after surgery. Insulin sensitivity and glucose tolerance were significantly impaired in MI mice compared with sham-operated mice (Figure S5E). Significant loss of fat tissue was also observed in MI mice (Figures S5F and S5G) and this was associated with upregulation of adipose

Figure 2. p53-Dependent Adipose Tissue Inflammation Provokes Systemic Insulin Resistance during Heart Failure

(A) Expression of p53 was examined in adipose tissues of mice by western blot analysis at indicated time points after sham operation (Sham) or TAC. Actin was used as an equal loading control. The graph indicates the quantitative data on p53 expression (n = 3).

(B) Real-time PCR assessing the expression of *Emr1*, *Tnf* (Tnfα), *Ccl2* (MCP1), and *Cdkn1a* (p21) levels in adipose tissue of adipocyte-specific p53-deficient mice (adipo-p53 KO) and littermate controls (Cont) at 6 weeks after sham operation or TAC procedure (n = 12).

(C) Hematoxylin and eosin staining of adipose tissues of adipocyte-specific p53-deficient mice (adipo-p53 KO) and littermate controls (Cont) at 6 weeks after sham operation (Sham) or TAC procedure. Scale bar, 50 μm. The right graph indicates the quantitative data on the infiltration of macrophages (n = 4).

(D) Insulin tolerance test (ITT) and glucose tolerance test (GTT) in adipocyte-specific p53-deficient mice (KO) and littermate controls (Cont) at 6 weeks after sham operation (Sham) or TAC procedure (n = 16). Data are shown as the means ± S.E.M. *$p < 0.05$, **$p < 0.01$.