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Bariatric Surgery Reduces Visceral Adipose Inflammation and Improves Endothelial Function in Type 2 Diabetic Mice

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Abstract

Objective—Bariatric surgery is emerging as an effective method to alleviate a multitude of medical conditions associated with morbid obesity and type 2 diabetes. However, little is known about the effects and mechanisms of bariatric surgery on visceral fat inflammation and endothelial dysfunction in type 2 diabetes. We hypothesize that bariatric surgery ameliorates interferon-gamma (IFNγ-mediated adipose tissue inflammation/oxidative stress and improves endothelial function in type 2 diabetic mice.

Methods and Results—Control mice (m Lepr^{db}) and diabetic mice (Lepr^{db}) were treated with either sham surgery or Improved Gastric Bypass Surgery (IGBS) and then evaluated at 5, 10, 20, and 30 days to assess post-surgical effects. Surgery reduced body weight, abdominal adiposity, blood glucose level, and food intake in Lepr^{db}. The surgery-induced decrease in visceral adiposity was accompanied by amelioration of T-lymphocytes and macrophage infiltration, as well as reduction in the expression of IFNγ and other inflammatory cytokines in the mesenteric adipose tissue (MAT) of Lepr^{db} mice. Furthermore, surgery improved endothelium-dependent, but not endothelium-independent vasorelaxation in small mesenteric arteries (SMA) of Lepr^{db} mice. The improvement in endothelial function was largely attenuated by nitric oxide synthase inhibitor (L-NAME) incubation. IFNγ treatment increased the mRNA expression of tumor necrosis factor-alpha (TNFα) in the MAT of control mice, and incubation of SMA of control mice with TNFα caused impairment of endothelial function. Superoxide production in MAT/SMA and nitrotyrosine protein level in SMA were elevated in diabetic mice. Surgery reduced MAT/SMA oxidative stress in Lepr^{db} mice.

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None.

Conclusions—The amelioration of adipose tissue inflammation and the improvement of endothelial function may represent important mechanisms that result in cardiovascular benefits following bariatric surgery.

Keywords

Diabetes; Adipose; Inflammation; Endothelial Function; Interferon-gamma

Obesity and diabetes are becoming pandemic and pose a major risk for a number of comorbidities including cardiovascular diseases.[1] Morbid obesity remains largely refractory to diet, exercise and medication, but generally responds well to bariatric surgery.[2-7] Bariatric surgery demonstrates the most encouraging results in the treatment of patients with morbid obesity and type 2 diabetes by effectively reducing body weight and profoundly improving insulin sensitivity.[8-14] Moreover, a substantial majority of obese patients with diabetes, hypertension, and other cardiovascular complications experience complete resolution or improvement.[3] Importantly, endothelium-dependent vasodilatory function was enhanced after gastric bypass surgery in morbidly obese patients with type 2 diabetes,[11, 15] but the mechanism by which bariatric surgery improves endothelial function in morbidly obese and diabetic patients has yet to be clearly elucidated.

Macrophage infiltration and chemoattractant gene expression were reduced in white adipose tissue of morbidly obese subjects after gastric bypass surgery-induced weight loss.[16] Among various cytokines produced by activated macrophages, tumor necrosis factor-alpha (TNFα) is a key proinflammatory cytokine involved in the pathogenesis and progression of cardiovascular dysfunction[17] by stimulating vascular oxidative stress,[18] enhancing endothelial permeability,[19] promoting inflammation,[18] and potentiating vasoconstriction.[20] As a hallmark cytokine of T-lymphocyte, interferon-gamma (IFNγ) plays a critical role in the regulation of adipose tissue inflammation and enhances the production of various inflammatory cytokines, including TNFα, in cultured adipose tissue.[21] Within this context, the purpose of this study was to examine the effects of bariatric surgery on IFNγ-induced visceral adipose tissue inflammation/oxidative stress and endothelial dysfunction in type 2 diabetic mice.

Methods

Animals

The procedures followed were in accordance with approved guidelines set by the Animal Care Committee at the University of Missouri. Heterozygote control mice (m $Lepr^{db}$) (Background Strain: C57BLKS/J), and homozygote type 2 diabetic mice ($Lepr^{db}$) (Background Strain: C57BLKS/J) were purchased from Jackson Laboratory and maintained on a normal rodent chow diet. Male, 20-35g m $Lepr^{db}$, 40-60 g $Lepr^{db}$ mice were used in this study. m $Lepr^{db}$ was treated with murine recombinant IFNγ (R&D, 330 μg/kg/day, i.p. injection, 5 days) at the age of 12 to 16 weeks.[22]

Improved Gastric Bypass Surgery

Improved gastric bypass surgery (IGBS) was performed using a modified surgical method that mimics the traditional Roux-en-Y gastric bypass surgery[23] (Supplemental Figure I). Mice were anesthetized with sodium pentobarbital (50 mg/kg i.p. injection). The stomach and small intestine were exposed from the abdominal cavity, 15 cm away from Treitz ligament, and prepared for anastomosis. The small intestine and large curve of the stomach were anastomosed with 6-0 silk suture side to side. The pylorus was separated and the two parts of the pylorus were dissected and closed. In the sham surgery, the abdominal cavity

was opened, but no further surgical procedures were performed. At age 12 weeks, m $Lepr^{db}$ and $Lepr^{db}$ mice were treated with either sham surgery or IGBS. $Lepr^{db}$ mice were assessed 5, 10, 20, and 30 days post IGBS (P5, P10, P20, and P30), or 20 days after sham surgery. m $Lepr^{db}$ mice were assessed 20 days after either sham surgery or IGBS.

Experimental Design

IFNγ, MCP-1 and nitrotyrosine protein expression were determined by western blotting. mRNA expression of CD3, CD68, IFNγ, MCP-1 etc. was examined by quantitative RT-PCR. Immunohistochemistry was used to examine mesenteric adipose tissue (MAT) accumulation of CD3 positive T-lymphocytes, Mac-3 or F4/80 positive macrophages. EPR (Electron Paramagnetic Resonance) spectroscopy was used to determine the superoxide production in both MAT and small mesenteric arteries (SMA). Isolated SMA responses were studied using wire Myograph. The expanded Methods section in the Online Data Supplement can be found at http://atvb.ahajournals.org.

Data Analysis

All data were presented as mean±SEM except as specifically stated. Statistical comparisons were performed with 2-way ANOVA for vasomotor responses under various treatments, and with one-way ANOVA for other data. Intergroup differences were tested with LSD inequality. Significance was accepted at $P < 0.05$.

Results

Bariatric surgery reduced body weight, adiposity, and improved glycemic control

The effects of bariatric surgery on weight loss and glycemic control were examined. We note that 5, 10, 20, and 30 days post surgery in mice are equivalent to 0.5, 1, 2, and 3 years after surgery in human beings. Our results revealed rapid weight loss and decrease in body fat mass by day 5 after surgery, and the body weight and body fat mass continued to decrease at day 10, 20, and 30 following surgery (Supplemental Table I). Surgery also reduced abdominal adiposity by decreasing abdominal girth, mesenteric bed weight and MAT adipocyte size in diabetic mice (Supplemental Table II). The food intake decreased by 15%-25% in diabetic mice following surgery (Supplemental Table I). Adiponectin level was lower in the serum of $Lepr^{db}$ mice, and surgery increased serum adiponectin levels (Supplemental Figure II).

Bariatric surgery also exerted profound effects on glycemic control and metabolism. IGBS significantly decreased blood glucose level as early as 5 days post surgery, and the blood glucose level continued to decrease at 10 and 20 days post surgery. Within 20 days following surgery, glucose had a parallel evolution to weight, abdominal girth and fat mass although at 30 days after surgery, we noted a slight, but non-significant increase in glucose level (Supplemental Table I).

The Effects of Bariatric Surgery on Adipose Tissue Inflammatory Cell Infiltration and Inflammatory Cytokine Expression

CD3 is the marker of T-lymphocytes. The CD3 positive T-lymphocyte infiltration was increased in the MAT of diabetic mice. The mRNA expression of CD3 was also higher in the MAT of diabetic mice. Bariatric surgery reduced MAT CD3 positive T-lymphocytes infiltration as well as CD3 mRNA expression (Figure 1A and 1B). IFNγ is the hallmark cytokine of T-lympohcytes. The mRNA and protein expression of IFNγ were elevated in the MAT of diabetic mice. Bariatric surgery reduced MAT expression of IFNγ in diabetic mice but not in control mice (Figure 1C and 1D).

Mac-3, CD68 and F4/80 are the markers of macrophages. The Mac-3 or F4/80 positive macrophage infiltration and CD68 mRNA expression were higher in MAT of diabetic mice vs. control mice. Bariatric surgery reduced macrophage accumulation in MAT of diabetic mice (Figure 2A and 2B, and Supplemental Figure III). MCP-1 is mainly produced by macrophages. The mRNA and protein expression of MCP-1 were higher in diabetic mice vs. control mice. Bariatric surgery ameliorated the mRNA and protein expression of MCP-1 in MAT of diabetic mice (Figure 2C and 2D). Additionally, the mRNA expression of other macrophage-derived inflammatory cytokines, such as TNFα, macrophage inflammatory protein-1-alpha (MIP-1α) and MIP-1β, were also increased in diabetic mice. Bariatric surgery inhibited MAT TNFα, MIP-1α and MIP-1β mRNA expression (Supplemental Figure IV).

The Effects of Bariatric Surgery on SMA Endothelial Function

Acetylcholine (ACh)-induced endothelium-dependent vasorelaxation was impaired in SMA of diabetic mice vs. control mice. Bariatric surgery improved endothelial function of diabetic mice (Figure 3). Sodium nitroprusside (SNP)-induced endothelium-independent vasorelaxation and phenylephrine (PE)-induced vasoconstriction were comparable among groups (Supplemental Figure V and Supplemental Figure VI). Nitric oxide synthase inhibitor (L-NAME) incubation largely attenuated the surgery-induced improvement of endothelial function in diabetic mice (Figure 4). Despite the profound effects of bariatric surgery on improving endothelial function of diabetic mice, bariatric surgery affected neither the endothelium-dependent nor the endothelium-independent vasorelaxation in non-diabetic control mice (Supplemental Figure VII).

m Lepr^{db} mice treated with recombinant IFNγ showed significantly increased TNFα mRNA expression in MAT (Figure 5A). Incubation of SMA with 10 ng/ml of recombinant TNFα impaired endothelial function of SMA in m Lepr^{db} mice (Figure 5B).

The Effects of Bariatric Surgery on MAT/SMA oxidative stress

The superoxide level was elevated in both MAT and SMA of diabetic mice. Bariatric surgery reduced superoxide production in diabetic mice without affecting that in control mice (Figure 6A and 6B). Furthermore, the nitrotyrosine protein expression in SMA was higher in diabetic mice vs. control mice. Bariatric surgery decreased SMA nitrotyrosine protein expression (Figure 6C).

Discussion

Bariatric surgical procedures have increased exponentially in the United States[24] and animal models are increasingly being used in the study of bariatric surgery in order to examine the underlying mechanisms of the therapeutic effects.[25] However, no studies to date have examined the effects of bariatric surgery in the type 2 diabetic murine model. We modified the work of Troy et al.[23] to establish the Improved Gastric Bypass Surgery (IGBS) method in murine model of type 2 diabetes; this allows the study of mechanisms responsible for the therapeutic effects of bariatric surgery in morbid obesity and type 2 diabetes. The major findings in this study are: 1) Bariatric surgery leads to rapid weight loss, reduces whole body and abdominal adiposity, and improves glycemic control; 2) Bariatric surgery serves as an effective anti-inflammatory strategy by ameliorating IFNγ-mediated adipose tissue inflammation; and 3) Bariatric surgery reverses endothelial dysfunction by improving NO availability and inhibiting vascular oxidative stress.

Bariatric surgery serves as a successful anti-inflammatory strategy

Obesity-related chronic inflammation is implicated in the pathogenesis of type 2 diabetes.[26] Previous studies demonstrated that long-term weight loss after bariatric surgery is accompanied by a decreased proinflammatory state. Bariatric surgery decreased circulating levels of c-reactive protein (CRP),[27-30] IL-6,[29] serum amyloid A (SAA),[31-32] and leptin,[8] but increased the circulating level of adiponectin.[8] Bariatric surgery also reduced subcutaneous adipose tissue macrophage attraction, and gene expression of inflammatory cytokines, such as TNFα and IL-6.[16, 29] Compared with subcutaneous fat, visceral fat showed a higher transcript level of IFNγ and a broader leukocytosis that included macrophages, T cells and natural killer (NK) cells.[33] Our murine model of IGBS allowed us to examine the effects of bariatric surgery on the inflammatory status of MAT. Our results showed that bariatric surgery reduced T-lymphocyte and macrophage infiltration, as well as the expression of IFNγ, MCP-1, TNFα, MIP-1α and MIP-1β in MAT of diabetic mice. Thus, surgery-induced weight loss is accompanied by reduced adipose tissue inflammation, and bariatric surgery serves as a successful anti-inflammatory strategy in type 2 diabetes.

The association between adipose tissue inflammation and endothelial dysfunction

Increased adipose tissue inflammation in type 2 diabetes reflects the positive association between cardiovascular diseases and diabetes.[34] An abdominal fat pattern, as determined by an increased waist-to-hip ratio and visceral fat diameter, was the sole significant predictor of flow-mediated vasodilation (FMD) in overweight adults,[10, 35] suggesting the link between visceral adiposity and vascular dysfunction.[36-37] The mechanisms whereby excessive visceral fat depot leads to deterioration of vascular health are complex. Adipose tissue-derived inflammatory cytokines may serve as mechanisms linking adipose tissue inflammation and endothelial dysfunction.[34] As an important adipose-derived proinflammatory mediator, TNFα plays a key role in endothelial dysfunction associated with ischemia reperfusion injury,[38-39] obesity[40] and diabetes.[41] In type 2 diabetic mice, increase in TNFα and TNFα receptor 1 (TNFR1) expression induced activation and production of superoxide via NAD(P)H oxidase and/or the mitochondria respiratory chain, leading to endothelial dysfunction in coronary microcirculation and aortas.[42-44] Our results suggest that IFNγ treatment significantly increased the mRNA expression of TNFα in the MAT of non-diabetic control mice. Recombinant TNFα incubation impaired the endothelial function of SMA in control mice, suggesting the potential role of the IFNγ-induced MAT pro-inflammatory status in the regulation of SMA endothelial function. Moreover, the superoxide level in the MAT of diabetic mice was significantly higher, but bariatric surgery reduced MAT superoxide production. Thus, visceral obesity-associated alterations of the vasculature are likely a consequence of perturbation of the normal physiological balance of adipose-derived inflammatory cytokines and oxidative stress, and bariatric surgery can reverse the alteration.

Bariatric Surgery Improves Endothelial Function by Inhibiting Oxidative Stress and Increasing NO Availability

In morbidly obese patients, bariatric surgery rapidly improved endothelial function.[45-46] The mechanisms of bariatric surgery-induced amelioration of endothelial dysfunction are not clearly elucidated, but some studies suggest that reduction in circulating level of markers of endothelial activation and oxidative stress may serve as mechanisms.[47-48] Our study shows that bariatric surgery remarkably improved the endothelium-dependent vasorelaxation of SMA without affecting endothelium-independent vasorelaxation and PE-induced vasoconstriction (Figure 3, Supplemental Figure V and Figure VI). The superoxide level and nitrotyrosine protein expression in the SMA were elevated in diabetic mice, but reversed by bariatric surgery (Figure 6). Although bariatric surgery improved endothelium-dependent vasorelaxation of SMA in diabetic mice, the improvement was largely attenuated by

incubating the vessels with nitric oxide synthase inhibitor, L-NAME, suggesting that bariatric surgery improves endothelial function by improving NO availability (Figure 4).

Although we observed that the SMA endothelial function of $Lepr^{db}$ at 5, 10, and 20 days after surgery was completely restored to the level of non-diabetic control mice, In $Lepr^{db}$ at 30 days, this procedure only partially improved endothelial function (Figure 3). Moreover, the protein expression of IFNγ and MCP-1 in diabetic mice at 30 days post surgery slightly returned towards the level observed in the $Lepr^{db}$+Sham surgery group even though there was no significant body weight regain or hyperglycemia. We postulate that an early indicator of post-surgery relapse may be characterized by the partial restoration of adipose tissue inflammation and endothelial dysfunction that precedes a regain of body weight and increased incidence of hyperglycemia over the long term after surgery in type 2 diabetic mice. Thus, weight is likely not the determinant of endothelial dysfunction.[49-50] The inflammatory milieu that was rapidly corrected by surgery is linked to endothelial dysfunction in diabetes.

One caveat to this study is that the mice were fairly young (3 month old) when subjected to the surgery procedure. However, since the lifespan of $Lepr^{db}$ mice is up to 10 month, our protocol will potentially allow the observation of long-term effects by bariatric surgical procedures. We found that the endothelial function of $Lepr^{db}$ at 90 days after surgery was slightly impaired compared with $Lepr^{db}$ at 30 days after surgery (although still better than $Lepr^{db}$+Sham surgery), with a slight increase in body weight and blood glucose level (unpublished data), which highlights the need to examine the long-term effects of bariatric surgery. Indeed, the long-term follow-up study of patients undergoing bariatric surgery showed that body weight reached the lowest point at approximately 2 years and there was a significant increase in BMI from the nadir to 5 years and from 5 years to 10 years post-surgery.[51] Thus, although bariatric surgery is a favorable option in the treatment of diabetic patients with severe obesity, discerning the benefits over time requires further evaluation. Due to the difficulties in conducting long-term follow-up studies in human subjects treated with bariatric surgery over time, our study using type 2 diabetic mice can explore a wider spectrum of interest more quickly and definitely to evaluate and refine the most relevant protocols that may be translatable to clinical studies.

Conclusion

In summary, bariatric surgery reduces body weight, whole body and abdominal adiposity, and improves glycemic control in type 2 diabetic mice. Bariatric surgery ameliorates IFNγ-mediated MAT inflammation/oxidative stress and improves SMA endothelial function in type 2 diabetes. We posit that the vascular benefits of bariatric surgery are chiefly derived from a surgery-induced reduction in adipose tissue inflammation. These data demonstrate that the amelioration of adipose tissue inflammation and the improvement of endothelial function may represent important mechanisms that result in cardiovascular benefits following bariatric surgery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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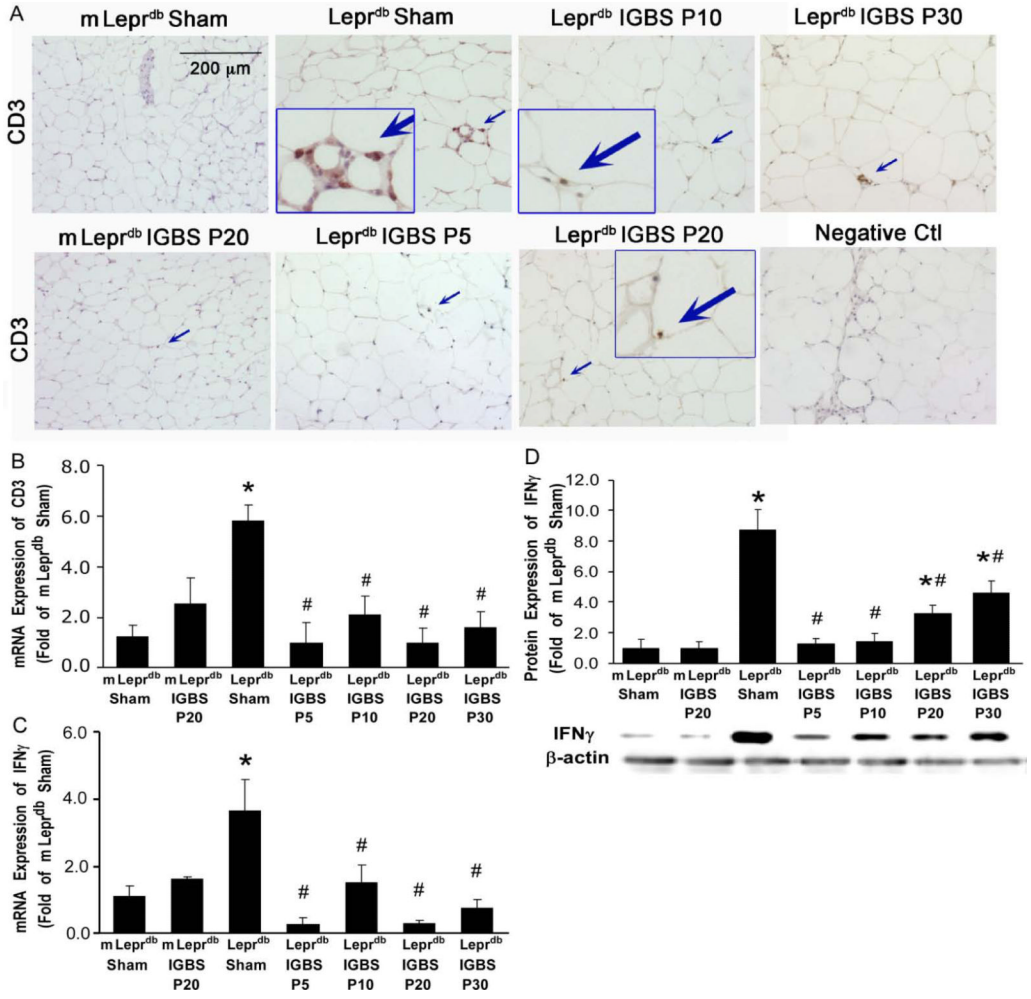

Figure 1.

Improved Gastric Bypass Surgery (IGBS) reduced T-lymphocyte infiltration and IFNγ expression in mesenteric adipose tissue (MAT) of diabetic mice. A, Immunohistochemical staining was performed in control (m Lepr^{db}) and diabetic (Lepr^{db}) mice treated with either sham surgery or IGBS. Lepr^{db} mice were assessed 5, 10, 20, and 30 days post IGBS (P5, P10, P20, and P30), or 20 days after sham surgery. m Lepr^{db} mice were assessed 20 days after either sham surgery or IGBS. The results show that CD3 positive T-lymphocyte infiltration in MAT was higher in Lepr^{db}+Sham versus IGBS. Data shown are representative of 4 separate experiments. B, mRNA expression of CD3 increased in MAT of Lepr^{db}+Sham. IGBS significantly reduced CD3 mRNA levels in MAT. The mRNA (C) and protein (D) expression of T-lymphocyte hallmark cytokine, IFNγ, increased in MAT of Lepr^{db}+Sham. IGBS decreased the mRNA and protein expression of IFNγ. Data represent mean±SEM, n=4-12 mice. *P<0.05 compared with m Lepr^{db}+Sham surgery; $^{\#}$P<0.05 compared with Lepr^{db}+Sham surgery.

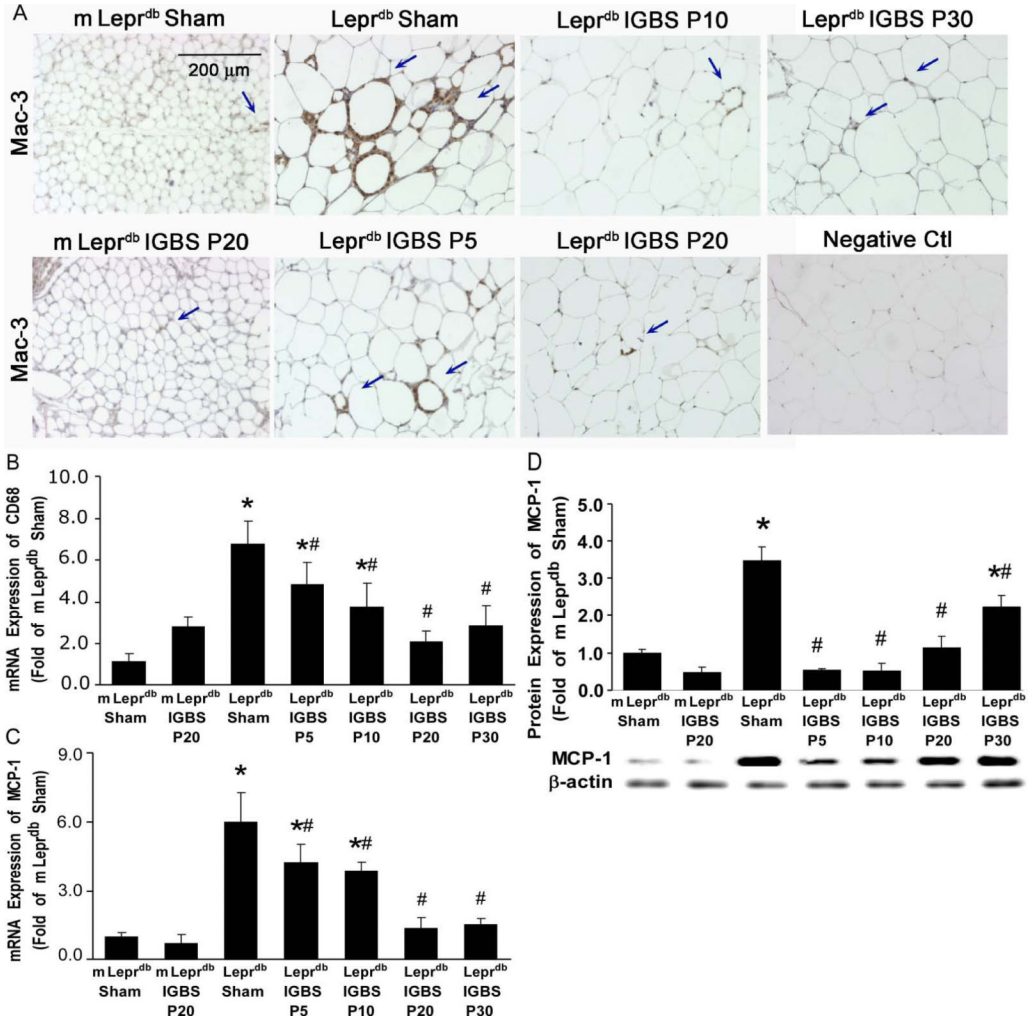

Figure 2.

IGBS reduced macrophage infiltration and MCP-1 expression in MAT of diabetic mice. A, Immunohistochemical staining shows that Mac-3 positive macrophage infiltration in MAT was higher in Lepr^{db}+Sham versus IGBS. Data shown are representative of 4 separate experiments. B, mRNA expression of CD68 was increased in MAT of Lepr^{db}+Sham. IGBS significantly reduced CD68 mRNA levels in MAT. The mRNA (C) and protein (D) expression of MCP-1 were increased in MAT of Lepr^{db}+Sham, and were reduced by IGBS. Data represent mean±SEM, n=4-12 mice. *P<0.05 compared with m Lepr^{db}+Sham surgery; # P<0.05 compared with Lepr^{db}+Sham surgery.

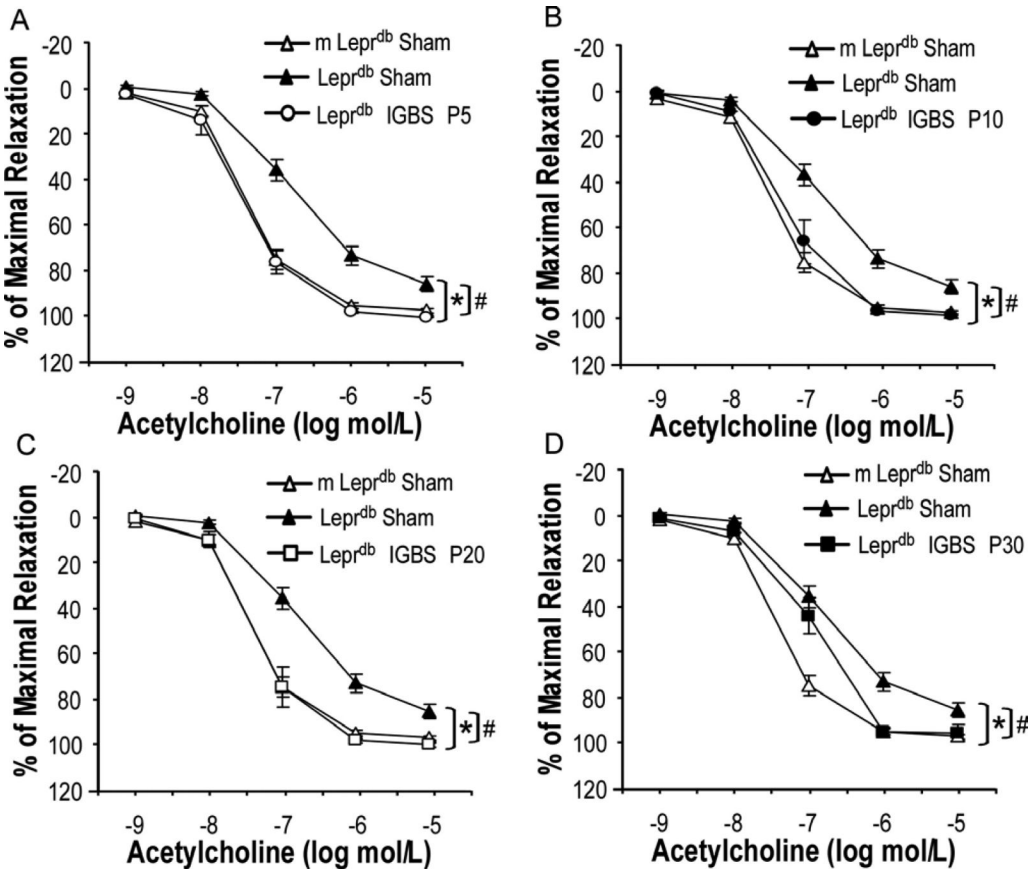

Figure 3.

IGBS improved endothelium-dependent vasorelaxation to acetylcholine (ACh) in small mesentery arteries (SMA) of Lepr^{db} mice. Data represent mean±SEM. n=6-31 rings from 4-18 mice (1 or 2 rings per mouse). *P<0.05 compared with m Lepr^{db}+Sham surgery; # P<0.05 compared with Lepr^{db}+Sham surgery.

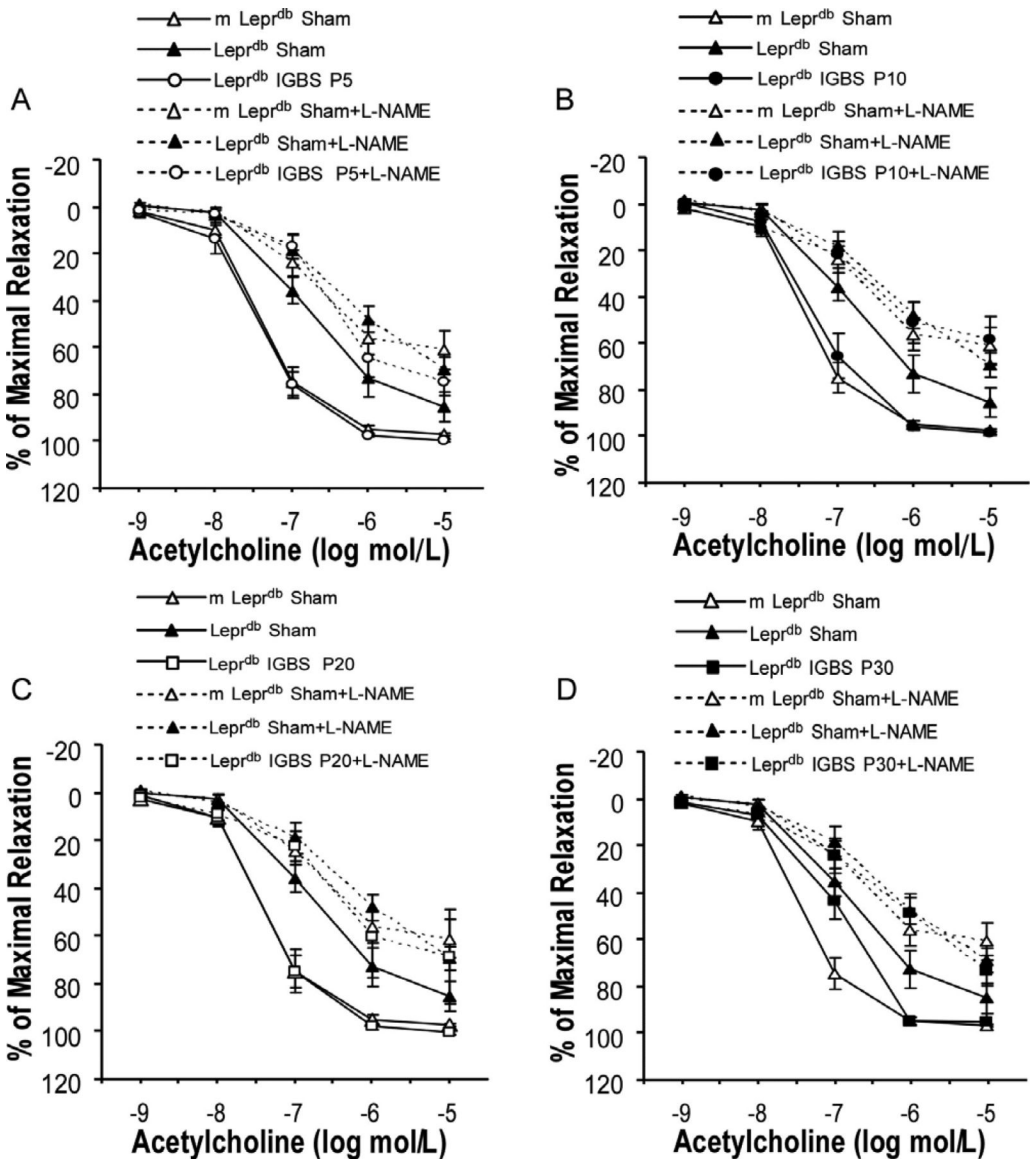

Figure 4.

Incubation with nitric oxide synthase inhibitor, L-NAME, largely attenuated the improvement of SMA endothelial function in surgery-treated diabetic mice. Data represent mean±SEM. n=6-31 rings from 4-18 mice (1 or 2 rings per mouse).

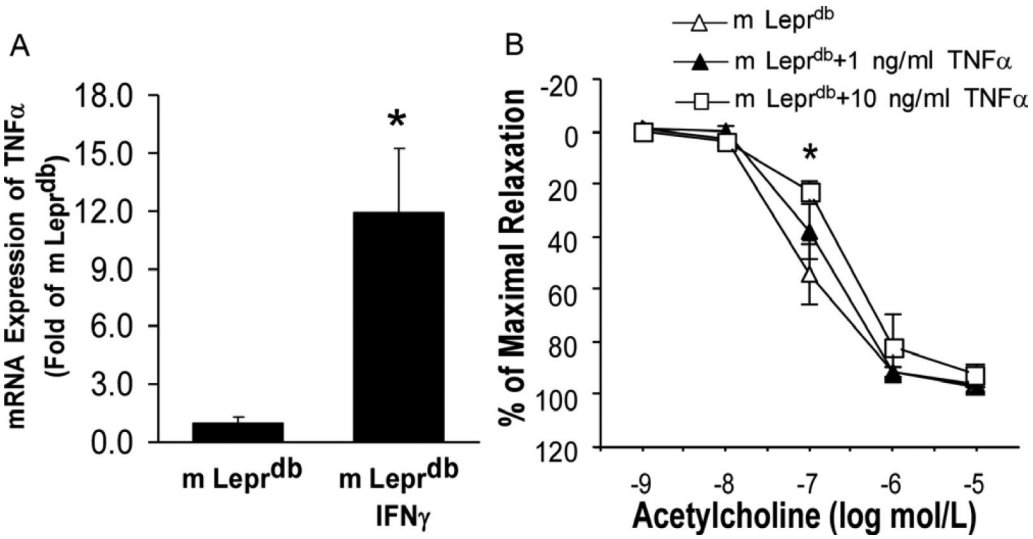

Figure 5.

IFNγ stimulated the expression of proinflammatory cytokine TNFα, which impaired endothelial function of SMA. A. mRNA expression of TNFα increased in the MAT of m Lepr^{db} mice treated with IFNγ. Data represent mean±SEM, n=6-8 mice. *P<0.05 compared with m Lepr^{db}. B, 1 ng/ml recombinant TNFα incubation (90 minutes) only slightly impaired endothelial function of m Lepr^{db} mice. 10 ng/ml TNFα incubation significantly impaired endothelial function. n=4-5 rings from 4-5 mice (1 ring per mouse). *P<0.05 compared with m Lepr^{db}.

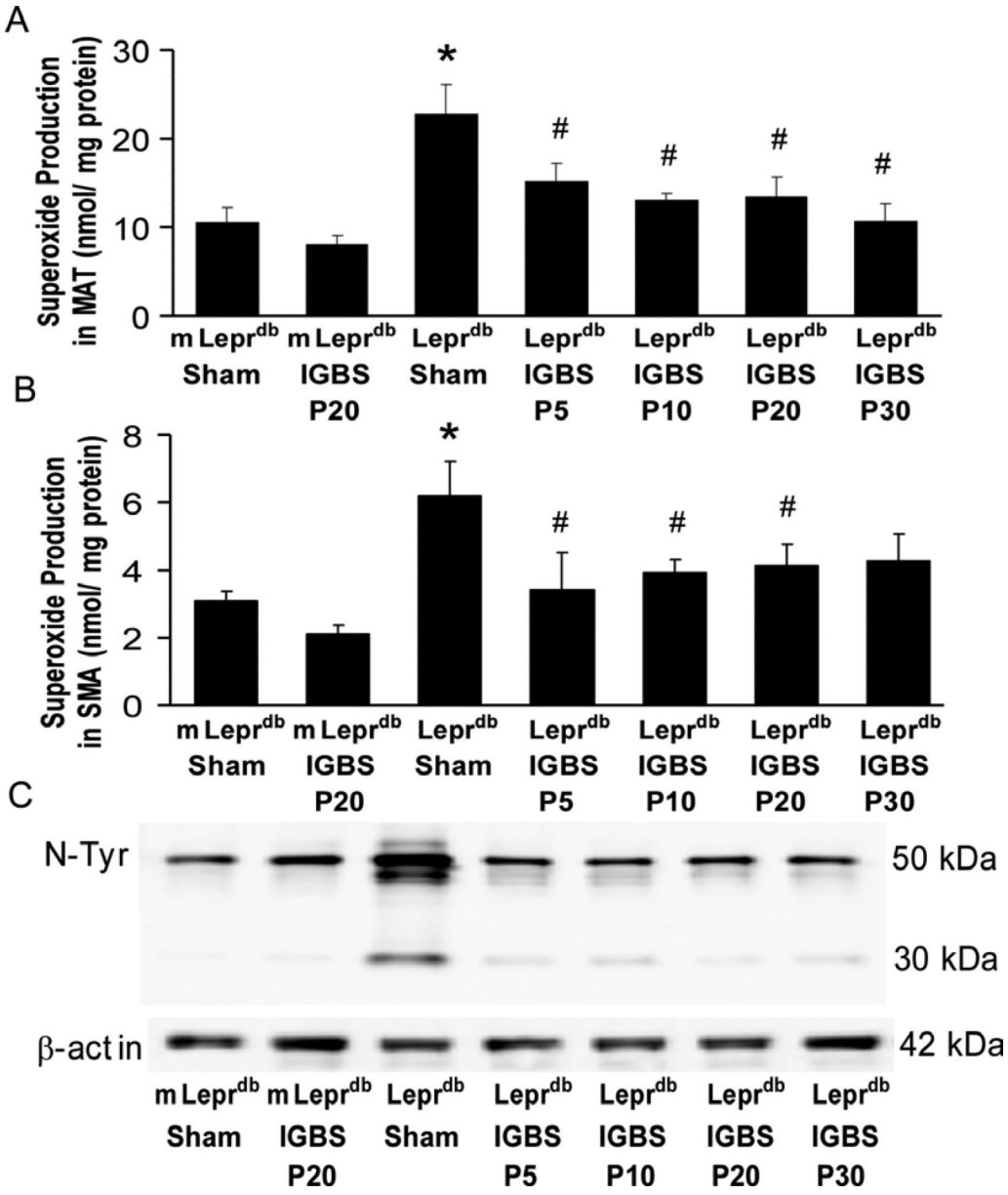

Figure 6.

IGBS ameliorated MAT/SMA oxidative stress. A and B, IGBS reduced superoxide level in MAT and SMA of Lepr^{db} mice. Data represent mean±SEM. n=6-8 mice. *P<0.05 compared with m Lepr^{db}+Sham surgery; # P<0.05 compared with Lepr^{db}+Sham surgery. C, IGBS decreased protein expression of nitrotyrosine in SMA of diabetic mice. Data shown are representative of 3 separate experiments.