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

Hanrui Zhang, Yong Wang, Jing Zhang, Barry J. Potter, James R. Sowers, Cuihua Zhang

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-γ–mediated adipose tissue inflammation/oxidative stress and improves endothelial function in type 2 diabetic mice.

Methods and Results—Control mice (m Leprdb) and diabetic mice (Leprdb) were treated with either sham surgery or improved gastric bypass surgery and then were evaluated at 5, 10, 20, and 30 days to assess postsurgical effects. Surgery reduced body weight, abdominal adiposity, blood glucose level, and food intake in Leprdb. 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 interferon-γ and other inflammatory cytokines in the mesenteric adipose tissue (MAT) of Leprdb mice. Furthermore, surgery improved endothelium-dependent, but not endothelium-independent, vasorelaxation in small mesenteric arteries (SMA) of Leprdb mice. The improvement in endothelial function was largely attenuated by nitric oxide synthase inhibitor (L-NAME) incubation. Interferon-γ treatment increased the mRNA expression of tumor necrosis factor-α in the MAT of control mice and incubation of SMA with tumor necrosis factor-α 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 Leprdb mice.

Conclusion—The amelioration of adipose tissue inflammation and the improvement of endothelial function may represent important mechanisms that result in cardiovascular benefits after bariatric surgery. (Arterioscler Thromb Vasc Biol. 2011;31:2063-2069.)

Key Words: cytokines ▪ diabetes mellitus ▪ endothelium ▪ surgery ▪ vascular biology

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 it 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.
MAT was higher in Leprdb plus sham vs IGBS. Data shown results show that CD3-positive T-lymphocyte infiltration in assessed 20 days after either sham surgery or IGBS. The mark cytokine, IFN-γ, increased in MAT of Leprdb plus sham. IGBS significantly reduced CD3 mRNA levels in MAT. The mRNA expression of CD3 increased in MAT of Leprdb plus sham. IGBS decreased the mRNA and protein expression of IFN-γ. Data represent mean±SEM (n=4–12 mice). *P<0.05 compared with m Leprdb plus sham surgery; #P<0.05 compared with Leprdb plus sham surgery.

visceral adipose tissue inflammation/oxidative stress and endothelial dysfunction in type 2 diabetic mice.

Subjects and 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 Leprdb) and diabetic (Leprdb) mice were assessed 20 days after either sham surgery or IGBS. Leprdb mice were assessed 5, 10, 20, and 30 days after IGBS (P5, P10, P20, and P30), or 20 days after sham surgery. The m Leprdb were assessed 20 days after either sham surgery or IGBS. The results show that CD3-positive T-lymphocyte infiltration in MAT was higher in Leprdb plus sham vs IGBS. Data shown are representative of 4 separate experiments. The mRNA expression of CD3 increased in MAT of Leprdb plus sham. IGBS significantly reduced CD3 mRNA levels in MAT. The mRNA (C) and protein (D) expression of T-lymphocyte hallmark cytokine, IFN-γ, increased in MAT of Leprdb plus sham. IGBS decreased the mRNA and protein expression of IFN-γ. Data represent mean±SEM (n=4–12 mice). *P<0.05 compared with m Leprdb plus sham surgery; #P<0.05 compared with Leprdb plus sham surgery.

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 available online at http://atvb.ahajournals.org). Mice were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneal 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 2 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 Leprdb and Leprdb mice were treated with either sham surgery or IGBS. Leprdb mice were assessed 5, 10, 20, and 30 days after IGBS (P5, P10, P20, and P30) or 20 days after sham surgery. The m Leprdb mice were assessed 20 days after either sham surgery or IGBS.

Experimental Design
IFN-γ, MCP-1, and nitrotyrosine protein expression were determined by Western blotting. The mRNA expressions of CD3, CD68, IFN-γ, monocyte chemotactant protein-1 (MCP-1), and so on, were examined by quantitative reverse-transcription polymerase chain reaction. Immunohistochemistry was used to examine mesenteric adipose tissue (MAT) accumulation of CD3-positive T-lymphocytes, Mac-3, or F4/80-positive macrophages. 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 can be found in the online data supplement.

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 1-way ANOVA for other data. Intergroup differences were tested with Fisher’s least significant difference test inequality. Significance was accepted at $P<0.05$.

Results

Bariatric Surgery Reduced Body Weight and 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 after 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 days 10, 20, and 30 after 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% to 25% in diabetic mice after 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 after surgery, and the blood glucose level continued to decrease at 10 and 20 days after surgery. Within 20 days after surgery, glucose had a parallel evolution to weight, abdominal girth, and fat mass; however, at 30 days after surgery, we noted a slight but nonsignificant 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, B). IFN-$\gamma$ is the hallmark cytokine of T-lymphocytes. The mRNA and protein expression of IFN-$\gamma$ were elevated in the MAT of diabetic mice. Bariatric surgery reduced MAT expression of IFN-$\gamma$ in diabetic mice but not in control mice (Figure 1C, D).

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, B, 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, D). Additionally, the mRNA expression of other macrophage-derived inflammatory cytokines, such as TNF-$\alpha$, macrophage inflammatory protein-1-alpha (MIP-1$\alpha$), and MIP-1$\beta$, were also increased in diabetic mice. Bariatric surgery inhibited MAT TNF-$\alpha$, MIP-1$\alpha$, and MIP-1$\beta$ mRNA expression (Supplemental Figure IV).

The Effects of Bariatric Surgery on SMA Endothelial Function

Acetylcholine-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-induced endothelium-independent vasorelaxation and phenylephrine-induced vascular constriction were comparable among groups (Supplemental Figures V and 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 nondiabetic control mice (Supplemental Figure VII).

The m Lepr$^{db}$ mice treated with recombinant IFN-$\gamma$ showed significantly increased TNF-$\alpha$ mRNA expression in MAT (Figure 5A). Incubation of SMA with 10 ng/mL of recombinant TNF-$\alpha$ impaired endothelial function of SMA in the 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, B). Furthermore, the nitrotyrosine protein expres-
Discussion

Bariatric surgical procedures have increased exponentially in the United States and animal models are increasingly being used in the study of bariatric surgery to examine the underlying mechanisms of the therapeutic effects. 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 to establish the 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 that bariatric surgery leads to rapid weight loss, reduces whole body and abdominal adiposity, and improves glycemic control; bariatric surgery serves as an effective anti-inflammatory strategy by ameliorating IFN-α-mediated adipose tissue inflammation; and bariatric surgery reverses endothelial dysfunction by improving nitric oxide availability and inhibiting vascular oxidative stress.

Obesity-related chronic inflammation is implicated in the pathogenesis of type 2 diabetes. 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, IL-6, serum amyloid A, and leptin, but increased the circulating level of adiponectin. Bariatric surgery also reduced subcutaneous adipose tissue macrophage attraction and gene expression of inflammatory proteins.
cytokines, such as TNF-α and IL-6.\textsuperscript{16,29} Compared with subcutaneous fat, visceral fat showed a higher transcript level of IFN-γ and a broader leukocytosis that included macrophages, T cells, and natural killer cells.\textsuperscript{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 IFN-γ, MCP-1, TNF-α, 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.\textsuperscript{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 in overweight adults,\textsuperscript{10,35} suggesting the link between visceral adiposity and vascular dysfunction.\textsuperscript{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.\textsuperscript{34} As an important adipose-derived proinflammatory mediator, TNF-α plays a key role in endothelial dysfunction associated with ischemia reperfusion injury,\textsuperscript{38,39} obesity,\textsuperscript{40} and diabetes.\textsuperscript{41} In type 2 diabetic mice, increase in TNF-α and TNF-α receptor 1 expression induced activation and production of superoxide via NAD(P)H oxidase or the mitochondria respiratory chain, leading to endothelial dysfunction in coronary microcirculation and aortas.\textsuperscript{42–44} Our results suggest that IFN-γ treatment significantly increased the mRNA expression of TNF-α in the MAT of nonobese control mice. Recombinant TNF-α incubation impaired the endothelial function of SMA in control mice, suggesting the potential role of the IFN-γ–induced MAT proinflammatory 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 Nitric Oxide Availability

In morbidly obese patients, bariatric surgery rapidly improved endothelial function.\textsuperscript{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.\textsuperscript{47,48} Our study shows that bariatric surgery remarkably improved the endothelium-dependent vasorelaxation of SMA without affecting endothelium-independent vasorelaxation and phenylephrine-induced vasoconstriction (Figure 3, Supplemental Figures V, VI). The superoxide level and nitrotyrosine protein expression in the SMA were elevated in diabetic mice but were 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 nitric oxide availability (Figure 4).

Although we observed that the SMA endothelial function of Lepr\textsuperscript{db} at 5, 10, and 20 days after surgery was completely restored to the level of nonobese control mice, in Lepr\textsuperscript{db} at 30 days post surgery, this procedure only partially improved endothelial function (Figure 3). Moreover, the protein expression of IFN-γ and MCP-1 in diabetic mice at 30 days after surgery slightly returned toward the level observed in the Lepr\textsuperscript{db} plus sham surgery group, even though there was no significant body weight regain or hyperglycemia. We postulate that an early indicator of relapse after surgery 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 likely is not the determinant of endothelial dysfunction.\textsuperscript{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 months old) when subjected to the surgery procedure. However, because the lifespan of Lepr\textsuperscript{db} mice is up to 10 months, our protocol will potentially allow the observation of long-term effects of bariatric surgical procedures. We found that the endothelial function of Lepr\textsuperscript{db} at 90 days after surgery was slightly impaired compared with Lepr\textsuperscript{db} at 30 days after surgery (although still better than Lepr\textsuperscript{db} plus 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. The long-term follow-up study of patients undergoing bariatric surgery showed that body weight reached the lowest point at \( \approx \)2 years and there was a significant increase in body mass index from the nadir to 5 years, and from 5 years to 10 years after surgery.\textsuperscript{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. Because of 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 and whole body and abdominal adiposity, and improves glycemic control in type 2 diabetic mice. Bariatric surgery ameliorates IFN-γ-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 after bariatric surgery.

Sources of Funding
This study was supported by grants from NIH (RO1-HL077566 and RO1-HL085119 to C.Z., RO1-DK085495 to J.Y. and C.Z. and RO1-HL073101 to J.S. and C.Z.) and American Heart Association Predoctoral Fellowship (10PRE4300043 to H.Z.).

Disclosure
None.

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Arterioscler Thromb Vasc Biol. 2011;31:2063-2069; originally published online June 16, 2011; doi: 10.1161/ATVBAHA.111.225870

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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Supplemental Material

Supplemental Methods

Animal Models

The procedures followed were in accordance with approved guidelines set by the Animal Care Committee at the University of Missouri. Heterozygote control mice (mLepr<sup>db</sup>) (Background Strain: C57BLKS/J), and homozygote type 2 diabetic mice (Lepr<sup>db</sup>) (Background Strain: C57BLKS/J) were purchased from Jackson Laboratory and maintained on a normal rodent chow diet. Male, 20-35 g mLepr<sup>db</sup>, and 40-60 g Lepr<sup>db</sup> mice were used in this study. At the age of 12 to 16 weeks, m Lepr<sup>db</sup> mice were treated with murine recombinant IFNγ (R&D, 330 µg/kg/day, i.p. injection, 5 days).<sup>1</sup>

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<sup>2</sup> (Supplemental Figure I). Food was restricted for 16 hours before surgery. The skin was prepared after anesthesia. 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. The abdominal cavity was washed with warm 0.9% saline and the abdominal cavity was closed. Penicillin G (50,000 U/kg) was injected subcutaneously after surgery to prevent infection. The animals were fed a normal diet during recovery. In the sham surgery, the abdominal cavity was opened, but no further surgical procedures were performed. The cage was cleaned daily to reduce risk of infection and body
weight was measured every week until euthanasia. At age 12 weeks, m Lepr\textsuperscript{db} and Lepr\textsuperscript{db} mice were treated with either sham surgery or IGBS. Lepr\textsuperscript{db} mice were assessed at 5, 10, 20, and 30 days post IGBS (P5, P10, P20, and P30), or 20 days after sham surgery. m Lepr\textsuperscript{db} mice were assessed 20 days after either sham surgery or IGBS.

**Body Weight, Abdominal Girth, Whole Body Fat Mass and Lean Mass Analysis**

Body weight was determined using an electronic balance. Abdominal girth was measured with the use of a soft ruler. Whole body fat mass was determined using dual energy X-ray absorptiometry analysis (DEXA) methods. Briefly, a PIXImus small animal densitometer (GE Medical Systems; Waukesha, WI) was used to carry out DEXA. Mice were anesthetized with 2% isoflurane and subjected to 5 min DEXA scans at the beginning and end of the study. Analyses were carried out per manufacturer’s instructions.\textsuperscript{3}

**Mesenteric Bed Weight and Adipocyte Size**

The mesentery bed was rapidly separated from the intestine, and wet weight was determined. Freshly isolated mesenteric adipose tissue (MAT) was fixed in Z-fix, dehydrated and embedded in paraffin. The paraffin-embedded tissue was sectioned at 5 μm thickness, stained with hematoxylin and eosin, and then examined under a microscope (Olympus IX81, USA). The average size of adipocyte cells from each mouse was determined by analyzing 30-50 randomly-selected adipocytes from 2 sections using the Image J Software.\textsuperscript{4}

**Serum Concentration of Adiponectin**

Serum adiponectin level was determined by a commercially available ELISA kit (Millipore). Values were expressed as micrograms per milliliter.

**Western Blotting**
10 mg of MAT, or 4-6 branches of small mesenteric arteries (SMA) were homogenized in lysis buffer (Cellytic™ MT Mammalian Tissue Lysis/Extraction Reagent, Sigma). Protein concentration was assessed using BCA™ Protein Assay Kit (Pierce), and equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes (Biorad). Protein expression was detected using IFN\(\gamma\) primary antibody (Millipore), monocyte chemoattractant protein-1 primary antibody (MCP-1) (Abcam), or nitrotyrosine primary antibody (Abcam), Horseradish peroxidase-conjugated secondary antibodies were used. Signals were visualized by enhanced chemiluminescence (ECL, Santa Cruz), scanned densitometrically using Fuji LAS3000 and quantified with Multigauge software (Fujifilm). The relative amounts of protein expression were quantified to those of the corresponding mLepr\(^{db}\) control, which was set to a value of 1.0.\(^5\)

**Quantitative RT-PCR**

Quantitative real-time PCR was done as previously reported with modification.\(^6\) Briefly, total RNA was extracted from 10 mg of MAT using RNeasy Lipid Tissue Mini Kit (Qiagen), and 1.0 \(\mu\)g total RNA was processed directly to cDNA synthesis using the SuperScript™ III Reverse Transcriptase (Invitrogen). cDNA was amplified with the use of qRT-PCR Kit with SYBR® Green (Invitrogen). The primer sets for specific amplification of CD3, IFN\(\gamma\), CD68, MCP-1, TNF\(\alpha\), MIP-1\(\alpha\) and MIP-1\(\beta\) were designed by Primer3 software. Efficiency of the PCR reaction was determined using a dilution series of a standard MAT sample. The housekeeping gene \(\beta\)-actin was used for internal normalization. The mean threshold cycle (\(C_T\)) values for both the target (CD3, IFN\(\gamma\), CD68 or MCP-1 etc.) and the internal control (\(\beta\)-actin) genes were determined. Data
were calculated by $2^{-\Delta\Delta CT}$ method ($\Delta\Delta CT = C_{T, \text{target}} - C_{T, \beta-\text{actin}}$). Results was presented as fold change of transcripts for target normalized to internal control ($\beta$-actin), compared with mLepGdb (defined as 1.0 fold).

**Immunohistochemistry**

Analysis of inflammatory cells in MAT by Immunohistochemistry was done as previously reported. Briefly, freshly isolated MAT was fixed in Z-fix, and embedded in paraffin. 5 µm sections were stained for rabbit anti-mouse CD3 (Abcam), rat anti-mouse Mac-3 (BD Biosciences), and rat anti-mouse F4/80 (Abcam), then incubated with appropriate biotinylated secondary antibodies followed by incubation with avidin-biotin complex (Vector). The reaction was visualized with 3-amino-9ethyl carbazole (DAKO). Sections were counterstained with Gill’s hematoxylin solution (Sigma).

**Functional Assessment of Small Mesenteric Arteries**

Isolated small mesenteric artery responses were studied using wire myograph as previously reported. Briefly, first order of main branch of mesenteric arteries with internal diameter of 200 µm to 250 µm were cut into 2 mm long rings and mounted on Myograph 610M (A&D Instrument). The passive tension-internal circumference was determined by stretching to achieve an internal circumference equivalent to 60% to 70% of that of the blood vessel under a transmural pressure of 100 mmHg. Vessels were maintained in Physiological Saline Solution (PSS) bubbled with 95% $O_2$-5% $CO_2$ at 37 ºC for the remainder of the experiment. PSS contained 118.99 mM NaCl, 4.69 mM KCl, 1.18 mM KH$_2$PO$_4$, 1.17 mM MgSO$_4$$\cdot$7H$_2$O, 2.50 mM CaCl$_2$$\cdot$2H$_2$O, 14.9 mM NaHCO$_3$, 5.5 mM D-Glucose, and 0.03 mM EDTA. After an equilibration period of 45 min, vessels were precontracted with 1 µmol/L phenylephrine (PE). A cumulative dose-
response curve was obtained by adding acetylcholine (ACh, 1 nmol/L to 10 µmol/L) and sodium nitroprusside (SNP, 1 nmol/L to 10 µmol/L). Relaxation at each concentration was measured and expressed as the percentage of force generated in response to PE.\(^9\)-\(^{10}\) NO availability was evaluated by ACh concentration-response curve repeated after incubation with the NO synthase inhibitor N-Nitro-L-arginine methyl ester (L-NAME, 100 µM, 20 min). PE-induced vasoconstriction was evaluated by cumulative addition of PE (1 nmol/L to 10 µmol/L). The contraction induced by PE was normalized to the maximal force of contraction induced by 120 mM of KCl.\(^{10}\)

**Measurement of Superoxide Using Electron Paramagnetic Resonance Spectroscopy**

Measurement of superoxide using Electron Paramagnetic Resonance Spectroscopy (EPR) was performed as previously described from our laboratory.\(^5\),\(^{11}\) EPR spectra was determined from the MAT and SMA homogenates. In brief, a 10% tissue homogenate was prepared in a 50 mmol/L phosphate buffer containing 0.01 mmol/L EDTA. The homogenate was then subjected to low-speed centrifugation (1,000 g) for 10 min to remove unbroken cells and debris. The supernatants containing 2 mmol/L CPH (1-hydrox-3-carboxypyrrolidine) were incubated for 30 min at 37°C and frozen quickly in liquid nitrogen. Superoxide quantification from the EPR spectra was determined by double integration of the peaks, with reference to a standard curve generated from horse radish peroxidase generation of the anion from standard solutions of hydrogen peroxide using p-acetamidophenol as the co-substrate normalized by protein concentration.

**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.
Supplemental Results

Table I. Basic Parameters

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<th>m Lepr&lt;sup&gt;ab&lt;/sup&gt; Sham</th>
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<td>10.2±0.3**</td>
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<td>Lean Mass, Before, g</td>
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<td>20.8±1.2</td>
<td>16.4±0.9*</td>
<td>16.0±1.7*</td>
<td>17.3±1.9*</td>
<td>15.7±1.3*</td>
<td>17.0±1.9*</td>
</tr>
<tr>
<td>Lean Mass, After, g</td>
<td>22.5±1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.2±2.3</td>
<td>16.1±1.4*</td>
<td>15.4±1.7*</td>
<td>15.3±1.4*</td>
<td>16.5±1.0*</td>
<td>17.0±1.3*</td>
</tr>
<tr>
<td>Fat Mass, Before, g</td>
<td>6.2±1.2</td>
<td>5.1±1.3</td>
<td>31.9±2.0*</td>
<td>34.1±1.9**</td>
<td>34.2±0.9**</td>
<td>33.6±2.3**</td>
<td>34.6±1.2**</td>
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<td>Fat Mass, After, g</td>
<td>4.3±0.9</td>
<td>2.7±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.1±3.0*</td>
<td>28.0±3.5**&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.8±2.0**&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.7±2.0**&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.5±1.1**&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blood Glucose, Before, mg/dl</td>
<td>109±12</td>
<td>117±15</td>
<td>450±35*</td>
<td>462±100*</td>
<td>517±49*</td>
<td>504±46*</td>
<td>465±50*</td>
</tr>
<tr>
<td>Blood Glucose, After, mg/dl</td>
<td>106±11</td>
<td>78±13</td>
<td>405±57*</td>
<td>215±78**&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162±55**&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133±45**&lt;sup&gt;a&lt;/sup&gt;</td>
<td>189±71**&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Food Intake, Before, g</td>
<td>3.9±0.4</td>
<td>4.2±0.3</td>
<td>6.4±0.4*</td>
<td>6.4±0.4*</td>
<td>6.1±0.3*</td>
<td>6.3±0.5*</td>
<td>6.4±0.5*</td>
</tr>
<tr>
<td>Food Intake, After, g</td>
<td>4.2±0.3</td>
<td>4.0±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7±0.3*</td>
<td>4.8±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
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Table II. Mesenteric Bed Weight and Adipocyte Size

<table>
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<tr>
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<th>Mesenteric Bed Weight, g</th>
<th>Adipocyte Size, μm&lt;sup&gt;2&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>m Lepr&lt;sup&gt;ab&lt;/sup&gt; Sham</td>
<td>0.161±0.009</td>
<td>943.432±69.026</td>
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<tr>
<td>m Lepr&lt;sup&gt;ab&lt;/sup&gt; IGBS P20</td>
<td>0.134±0.016</td>
<td>952.223±26.997</td>
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<tr>
<td>Lepr&lt;sup&gt;ab&lt;/sup&gt; Sham</td>
<td>1.490±0.061*</td>
<td>5189.787±344.191*</td>
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<tr>
<td>Lepr&lt;sup&gt;ab&lt;/sup&gt; IGBS P5</td>
<td>1.269±0.068**</td>
<td>4885.988±301.041*</td>
</tr>
<tr>
<td>Lepr&lt;sup&gt;ab&lt;/sup&gt; IGBS P10</td>
<td>1.230±0.038**</td>
<td>4266.563±161.064**</td>
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<tr>
<td>Lepr&lt;sup&gt;ab&lt;/sup&gt; IGBS P20</td>
<td>1.252±0.044**</td>
<td>4192.925±244.800**</td>
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<tr>
<td>Lepr&lt;sup&gt;ab&lt;/sup&gt; IGBS P30</td>
<td>1.267±0.061**</td>
<td>4168.630±212.424**</td>
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Table III. Primer Sequences Used for Real-Time RT-PCR

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<th>Name</th>
<th>Accession #</th>
<th>Sequence</th>
<th>Target Length</th>
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<tr>
<td>IFNγ s</td>
<td>NM_008337</td>
<td>ACTGGCATAAGGATGGTGAC</td>
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<tr>
<td>IFNγ as</td>
<td>NM_008337</td>
<td>GACCTGTTGTTGGTTTGAACCT</td>
<td>212</td>
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<tr>
<td>MCP-1 s</td>
<td>NM_011333</td>
<td>AGCACCAGCCACTCTCATAGCC</td>
<td>184</td>
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<tr>
<td>MCP-1 as</td>
<td>NM_011333</td>
<td>TCATTGGGATCATCTTGTG</td>
<td>184</td>
</tr>
<tr>
<td>CD3 s</td>
<td>NM_007648</td>
<td>TCCCAACCCAGCCTATGAG</td>
<td>95</td>
</tr>
<tr>
<td>CD3 as</td>
<td>NM_007648</td>
<td>GCGATGTCTCTCTATCTGTCA</td>
<td>95</td>
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<tr>
<td>CD68 s</td>
<td>NM_009853</td>
<td>TACCCCAATTCCAGGTGAAG</td>
<td>191</td>
</tr>
<tr>
<td>CD68 as</td>
<td>NM_009853</td>
<td>CTCGGGCTCTGATGTAGGTC</td>
<td>191</td>
</tr>
<tr>
<td>TNFα s</td>
<td>NM_013693</td>
<td>GTCCCCAAAGGATGAGAAG</td>
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<td>TNFα as</td>
<td>NM_013693</td>
<td>CACTTGGGTTGTTATGCTACGA</td>
<td>134</td>
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<tr>
<td>MIP-1α s</td>
<td>NM_011337</td>
<td>GTGTAGAGCAGGGGCTTGAG</td>
<td>99</td>
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<td>MIP-1α as</td>
<td>NM_011337</td>
<td>AGAGTCCCTCAGTGAGCTA</td>
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<tr>
<td>MIP-1β s</td>
<td>NM_013652</td>
<td>CCTGAACAAAGAAGGCAGAC</td>
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<td>GAGGAGGCCCTCCTGAGT</td>
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<tr>
<td>β-actin s</td>
<td>NM_007393</td>
<td>GCTCTTTCCAGCCTCTCTT</td>
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<tr>
<td>β-actin as</td>
<td>NM_007393</td>
<td>CTTCTGACATCCTGTCAGCAA</td>
<td>168</td>
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Figure I.

Figure II.

Serum Level of Adiponectin (µg/ml)

<table>
<thead>
<tr>
<th></th>
<th>m Lepr&lt;sup&gt;db&lt;/sup&gt; Sham</th>
<th>Lepr&lt;sup&gt;db&lt;/sup&gt; Sham</th>
<th>Lepr&lt;sup&gt;db&lt;/sup&gt; IGBS P20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>6</td>
<td>15</td>
</tr>
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</table>

* Indicates significant difference from control group.
Figure III.

![Image of staining results]

Figure IV.

![Graphs showing mRNA expression of TNFα, IFNγ, and IL-1β]
Figure V.

A. % of Maximal Relaxation vs. Sodium Nitroprusside (log mol/L)

B. % of Maximal Relaxation vs. Sodium Nitroprusside (log mol/L)

C. % of Maximal Relaxation vs. Sodium Nitroprusside (log mol/L)

D. % of Maximal Relaxation vs. Sodium Nitroprusside (log mol/L)

Figure VI.

A. % of Contraction by 120 mM KCl vs. Phenylephrine (log mol/L)

B. % of Contraction by 120 mM KCl vs. Phenylephrine (log mol/L)

C. % of Contraction by 120 mM KCl vs. Phenylephrine (log mol/L)

D. % of Contraction by 120 mM KCl vs. Phenylephrine (log mol/L)
Figure VII.
**Table I. Basic Parameters**

The body weight, abdominal girth, whole body fat mass and lean mass, blood glucose level, and food intake were examined before and after Improved Gastric Bypass Surgery (IGBS). IGBS decreased body weight, abdominal girth, whole body fat mass, blood glucose level and food intake in diabetic mice (Lepr\textsuperscript{db}) without remarkably affecting the lean mass. IGBS slightly reduced the body weight, fat mass and food intake in non-diabetic control mice (m Lepr\textsuperscript{db}), but did not significantly affect other parameters including abdominal girth, lean mass, and blood glucose level. Data represent mean±SEM. n=6 mice per group. * P<0.05 compared with m Lepr\textsuperscript{db}+Sham surgery; # P<0.05 compared with Lepr\textsuperscript{db}+Sham surgery; & P<0.05 compared with animals prior to IGBS.

**Table II. Mesenteric Bed Weight and Adipocyte Size**

IGBS decreased mesenteric bed weight and adipocyte size of MAT in Lepr\textsuperscript{db} mice, but not in m Lepr\textsuperscript{db} mice. Data represent mean±SEM. n=6-12 mice. *P<0.05 compared with m Lepr\textsuperscript{db}+Sham surgery; # P<0.05 compared with Lepr\textsuperscript{db}+Sham surgery.

**Table III. Primer Sequences Used for Real-Time RT-PCR**

**Figure Legends**

**Figure I.** Schematic figure showing the surgery procedure of improved gastric bypass surgery (IGBS). IGBS is a modified method from the traditional Roux-en-Y gastric bypass surgery. The small intestine and large curve of the stomach was anastomosed. The pylorus was separated and the two parts of the pylorus were dissected and closed. After IGBS, food
bypassed the pylorus and part of the small intestine and entered the distal part of the small intestine.

**Figure II.** IGBS increased the serum level of adiponectin in diabetic mice. Serum level of adiponectin was reduced in Lepr\textsuperscript{db} vs. m Lepr\textsuperscript{db} control. IGBS significantly increased the level of serum adiponectin at 20 days post surgery (IGBS P20). Data represent mean±SEM. n=4-6 mice. *P<0.05 compared with m Lepr\textsuperscript{db}+Sham surgery; # P<0.05 compared with Lepr\textsuperscript{db}+Sham surgery.

**Figure III.** Immunohistochemical staining of F4/80 positive macrophage. F4/80 positive macrophage infiltration in MAT was higher in Lepr\textsuperscript{db}+Sham surgery. IGBS reduced MAT macrophage infiltration. Data shown were representative of 4 separate experiments.

**Figure IV.** mRNA expression of TNF\textalpha, MIP-1\alpha, and MIP-1\beta by quantitative RT-PCR. IGBS decreased mRNA expression of TNF\textalpha (A), MIP-1\alpha (B), and MIP-1\beta (C) in MAT of Lepr\textsuperscript{db} mice. Data represent mean±SEM, n=6-8 mice. *P<0.05 compared with m Lepr\textsuperscript{db}+Sham surgery; # P<0.05 compared with Lepr\textsuperscript{db}+Sham surgery. MIP, macrophage inflammatory protein.

**Figure V.** IGBS did not affect endothelium-independent vasorelaxation. Sodium nitroprusside (SNP)-induced endothelium-independent vasorelaxation of SMA was not statistically different among all groups. Data represent mean±SEM. n=4-18 mice.

**Figure VI.** IGBS did not affect vasoconstriction to phenylephrine. Phenylephrine (PE)-induced vasoconstriction of SMA was similar among all groups. Data represent mean±SEM. n=4-18 mice.

**Figure VII.** The effects of IGBS on m Lepr\textsuperscript{db} control mice. IGBS did not affect ACh (A) and SNP (C)-induced vasorelaxation, or PE-induced vasoconstriction (D) of SMA in m
Lepr\textsuperscript{db} control mice. Data represent mean±SEM. n=4-18 mice. *P<0.05 compared with m
Lepr\textsuperscript{db}+Sham surgery; # P<0.05 compared with Lepr\textsuperscript{db}+Sham surgery.
Reference


배리아트릭 수술이 제2형 당뇨생쥐의 내장지방염증을 줄이고 혈관 내피기능을 호전시킨다.

김 재현 교수
삼성서울병원 내분비대사내과

Summary

목적
배리아트릭 수술이 제2형 당뇨병에서 내장지방염증과 혈관 내피기능이상에 미치는 정량화된 기전은 잘 알려져 있지 않다. 이번 연구는 제2형 당뇨병에서 배리아트릭 수술이 인터페론-γ에 의한 지방염증과 혈관내피 기능에 미치는 영향을 분석하고자 하였다.

방법 및 결과
당뇨병쥐(Lep^{ob/-})의 정상생쥐를 위우회술(gastric bypass) 또는 모의수술(sham operation)을 시행하고 수술 후 30일간 변화를 관찰하였다. 위우회술 후 당뇨생쥐에서 체중, 복부비만, 혈당, 섭취량이 감소하였다. 장간막 지방세포에서 인터페론-γ 및 다른 염증 사이토카인 발현과 산화 스트레스가 감소하였다. 또한 혈관 내피세포의 염증 및 혈관화학반응이 감소하였다. 당뇨생쥐에서는 산화 스트레스가 증가하였고, 위우회술 후에는 다시 감소하였다.

결론
배리아트릭 수술 이후 지방세포 염증 감소와 혈관 내피기능 개선을 통해 심혈관 시스템에 좋은 효과를 보이는 것으로 여겨진다.
중증 비만 및 동반된 당뇨병은 식사, 운동, 약물치료에 잘 반응하지 않지만 배리아트릭 수술 후에는 효과적으로 체중이 감소한다. 배리아트릭 수술 이후 체중이 감소하고 인슐린 감수성이 개선되며, 고혈압 및 다른 심혈관 합병증도 개선된다. 그러나 당뇨가 있는 중증비만 환자에서 배리아트릭 수술 이후 혈관내피의존 혈관확장능력이 개선되는 것과 보고되었지만 정확한 메커니즘에 대해서는 알려진 바 없었다. 중증비만환자에서 배리아트릭 수술 이후 체중이 감소하면 대식세포 침윤과 화학유인물질 유전자 발현이 지방조직에서 감소한다. 활성화된 대식세포에서 대표적으로 분비되는 염증유발 사이토카인인 TNF-α는 산화 스트레스를 증가시키고, 혈관 내피 투과도를 높이고 염증을 늘리고 혈관수축을 유발하여 심혈관 기능이상을 일으킨다. 한편, T-임파구의 대표적 사이토카인인 인터페론-γ는 지방조직의 염증을 조절하고 TNF-α를 포함한 다양한 염증성 사이토카인 발현을 증가시킨다. 본 연구는 배리아트릭 수술이 인터페론-γ에 의한 내장지방조직의 염증과 산화 스트레스 그리고 혈관 내피기능이상에 미치는 영향을 제12형 당뇨생쥐에서 알아보았다. 배리아트릭 수술은 제12형 당뇨생쥐에서 체중을 줄이면서, 복부지방이 감소하고, 혈당 조절이 개선되고, 인터페론-γ에 의한 지방조직 염증을 줄이고, 혈관 산화 스트레스를 줄이고 산화질소 합성을 통한 혈관 내피 기능저하인 혈관내피기능이상의 회복시켰다. 임상적으로 배리아트릭 수술은 혈당 조절이 개선되고, 인터페론-γ에 의한 지방조직 염증은 줄어도, 혈관 산화 스트레스는 줄이고 산화질소 합성을 통한 혈관 내피 기능저하인 혈관내피기능이상의 회복시켰다. 임상적으로 배리아트릭 수술을 통해 혈당 조절이 개선되는 것이 보고되어, 배리아트릭 수술은 당뇨 치료목적으로 위우회술의 효과가 주목을 받고 있으며, 이 분야에서도 지방조직 염증개선 및 혈관 기능이상이 회복되는지 그리고 그 기전이 제12형 당뇨 환자에서 독립적인 주요 인자인 연구가 필요하다.

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

Hanrui Zhang, Yong Wang, Jing Zhang, Barry J. Potter, James R. Sowers, Cuihua Zhang

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-γ-mediated adipose tissue inflammation/oxidative stress and improves endothelial function in type 2 diabetic mice.

Methods and Results—Control mice (m Leprdb) and diabetic mice (Leprdb) were treated with either sham surgery or improved gastric bypass surgery and then were evaluated at 5, 10, 20, and 30 days to assess postsurgical effects. Surgery reduced body weight, abdominal adiposity, blood glucose level, and food intake in Leprdb. 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 interferon-γ and other inflammatory cytokines in the mesenteric adipose tissue (MAT) of Leprdb mice. Furthermore, surgery improved endothelium-dependent, but not endothelium-independent, vasorelaxation in small mesenteric arteries (SMA) of Leprdb mice. The improvement in endothelial function was largely attenuated by nitric oxide synthase inhibitor (L-NAME) incubation. Interferon-γ treatment increased the mRNA expression of tumor necrosis factor-α in the MAT of control mice and incubation of SMA of control mice with tumor necrosis factor-α 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 Leprdb mice.

Conclusion—The amelioration of adipose tissue inflammation and the improvement of endothelial function may represent important mechanisms that result in cardiovascular benefits after bariatric surgery. (Arterioscler Thromb Vasc Biol. 2011;31:2063-2069.)

Key Words: cytokines ■ diabetes mellitus ■ endothelium ■ surgery ■ vascular biology

Obesity and diabetes are becoming pandemic and pose a major risk for a number of comorbidities, including cardiovascular diseases. Morbid obesity remains largely refractory to diet, exercise, and medication, but it generally responds well to bariatric surgery. 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. Moreover, a substantial majority of obese patients with diabetes, hypertension, and other cardiovascular complications experience complete resolution or improvement. Importantly, endothelium-dependent vasodilatory function was enhanced after gastric bypass surgery in morbidly obese patients with type 2 diabetes, 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. Among various cytokines produced by activated macrophages, tumor necrosis factor-α (TNF-α) is a key proinflammatory cytokine involved in the pathogenesis and progression of cardiovascular dysfunction by stimulating vascular oxidative stress, enhancing endothelial permeability, promoting inflammation, and potentiating vasoconstriction. As a hallmark cytokine of T-lymphocyte, interferon-gamma (IFN-γ) plays a critical role in the regulation of adipose tissue inflammation and enhances the production of various inflammatory cytokines, including TNF-α, in cultured adipose tissue. Within this context, the purpose of this study was to examine the effects of bariatric surgery on IFN-γ–induced...
The m Leprdb were treated with murine recombinant INF-γ and INF-γ expression in mesenteric adipose tissue (MAT) of diabetic mice. A, Immunohistochemical staining was performed in control (m Leprdb) and diabetic (Leprdb) mice treated with either sham surgery or IGBS. Leprdb mice were assessed 5, 10, 20, and 30 days after IGBS (P5, P10, P20, and P30), or 20 days after sham surgery. The m Leprdb 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 Leprdb plus sham vs IGBS. Data shown are representative of 4 separate experiments. B, The mRNA expression of CD3 increased in MAT of Leprdb plus sham. IGBS significantly reduced CD3 mRNA levels in MAT. The mRNA (C) and protein (D) expression of T-lymphocyte hallmark cytokine, INF-γ, increased in MAT of Leprdb plus sham. IGBS decreased the mRNA and protein expression of INF-γ. Data represent mean ± SEM (n=4–12 mice). *P<0.05 compared with m Leprdb plus sham surgery; **P<0.05 compared with Leprdb plus sham surgery.

Visceral adipose tissue inflammation/oxidative stress and endothelial dysfunction in type 2 diabetic mice.

Subjects and 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 Leprdb, background strain, C57BLKS/J) and homozygote type 2 diabetic mice (Leprdb, background strain, C57BLKS/J) were purchased from Jackson Laboratory (Bar Harbor, ME) and maintained on a normal rodent chow diet. Male, 20- to 35-gm m Leprdb and 40- to 60-gm Leprdb mice were used in this study. The m Leprdb were treated with murine recombinant INF-γ (330 μg/kg/d, intraperitoneal injection, 5 days; R&D) at the age of 12 to 16 weeks.

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 surgery23 (Supplemental Figure I available online at http://atvb.ahajournals.org). Mice were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneal injection). The

Experimental Design
INF-γ, MCP-1, and nitrotyrosine protein expression were determined by Western blots. The mRNA expressions of CD3, CD68, INF-γ, monocyte chemoattractant protein-1 (MCP-1), and so on, were examined by quantitative reverse-transcription polymerase chain reaction. Immunohistochemistry was used to examine mesenteric adipose tissue (MAT) accumulation of CD3-positive T-lymphocytes, Mac-3, or F4/80-positive macrophages. Electron paramagnetic resonance spectroscopy was used to determine the superoxide production in both MAT and small mesenteric arteries. Isolated SMA responses were studied using wire Myograph. Figures V and VI). Nitric oxide synthase inhibitor (L-NAME) incubation largely attenuated the surgery-induced improvement in MAT/SMA (Supplemental Figure II). The superoxide level was elevated in both MAT and SMA of diabetic mice vs control mice. Bariatric surgery reduced superoxide production in both MAT and small mesenteric arteries.

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 1-way ANOVA for other data. Intergroup differences were tested with Fisher’s least significant difference test inequality. Significance was accepted at P<0.05.

**Results**

**Bariatric Surgery Reduced Body Weight and 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 after 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 days 10, 20, and 30 after 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% to 25% in diabetic mice after surgery (Supplemental Table I). Adiponectin level was lower in the serum of Leprdb 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 after surgery, and the blood glucose level continued to decrease at 10 and 20 days after surgery. Within 20 days after surgery, glucose had a parallel evolution to weight, abdominal girth, and fat mass; however, at 30 days after surgery, we noted a slight but nonsignificant 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-lymphocytes 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, B). IFN-γ is the hallmark cytokine of T-lymphocytes. The mRNA and protein expression of IFN-γ were elevated in the MAT of diabetic mice. Bariatric surgery reduced MAT expression of IFN-γ in diabetic mice but not in control mice (Figure 1C, D).

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, B, 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, D). Additionally, the mRNA expression of other macrophage-derived inflammatory cytokines, such as TNF-α, macrophage inflammatory protein-1-alpha (MIP-1α), and MIP-1β, were also increased in diabetic mice. Bariatric surgery inhibited MAT TNF-α, MIP-1α, and MIP-1β mRNA expression (Supplemental Figure IV).

**The Effects of Bariatric Surgery on SMA Endothelial Function**

Acetylcholine-induced endothelium-dependent vasorelaxation was impaired in SMA of diabetic mice vs control mice. Bariatric surgery improved endothelium function of diabetic mice (Figure 3). Sodium nitroprusside-induced endothelium-independent vasorelaxation and phenylephrine-induced vasoconstriction were comparable among groups (Supplemental Figures V and 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 nondiabetic control mice (Supplemental Figure VII).

The m Leprdb mice treated with recombinant IFN-γ showed significantly increased TNF-α mRNA expression in MAT (Figure 5A). Incubation of SMA with 10 ng/mL of recombinant TNF-α impaired endothelial function of SMA in the m Leprdb 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, B). Furthermore, the nitrotyrosine protein expres-
Expression (Figure 6B). The superoxide level and nitrotyrosine protein expression in SMA were significantly higher, but bariatric surgery reduced MAT superoxide level and nitrotyrosine expression of SMA in diabetic mice (Lepr db). Data represent mean±SEM (n=6–8 mice). *P<0.05 compared with m Lepr db plus sham surgery; #P<0.05 compared with Lepr db plus sham surgery. C, IGBS decreased protein expression of nitrotyrosine in SMA of diabetic mice. Data shown are representative of 3 separate experiments.

Discussion

Bariatric surgical procedures have increased exponentially in the United States and animal models are increasingly being used in the study of bariatric surgery to examine the underlying mechanisms of the therapeutic effects. 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 to establish the 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 that bariatric surgery leads to rapid weight loss, reduces whole body and abdominal adiposity, and improves glycemic control; bariatric surgery serves as an effective anti-inflammatory strategy by ameliorating IFN-γ-mediated adipose tissue inflammation; and bariatric surgery reverses endothelial dysfunction by improving nitric oxide 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. 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, IL-6, serum amyloid A, and leptin, but increased the circulating level of adiponectin. Bariatric surgery also reduced subcutaneous adipose tissue macrophage attraction and gene expression of inflammatory markers that may be translatable to clinical studies.
cytokines, such as TNF-α and IL-6. \( \text{compared with subcutaneous fat, visceral fat showed a higher transcript level of IFN-γ and a broader leukocytosis that included macrophages, T cells, and natural killer cells.} \] 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 IFN-γ, MCP-1, TNF-α, 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. 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 in overweight adults, suggesting the link between visceral adiposity and vascular dysfunction. 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. As an important adipose-derived proinflammatory mediator, TNF-α plays a key role in endothelial dysfunction associated with ischemia reperfusion injury, obesity, and diabetes. In type 2 diabetic mice, increase in TNF-α and TNF-α receptor 1 expression induced activation and production of superoxide via NAD(P)H oxidase or the mitochondria respiratory chain, leading to endothelial dysfunction in coronary microcirculation and aortas. Our results suggest that IFN-γ treatment significantly increased the mRNA expression of TNF-α in the MAT of nondiabetic control mice. Reombinant TNF-α incubation impaired the endothelial function of SMA in control mice, suggesting the potential role of the IFN-γ-induced MAT proinflammatory 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 Nitric Oxide Availability**

In morbidly obese patients, bariatric surgery rapidly improved endothelial function. 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. Our study shows that bariatric surgery remarkably improved the endothelium-dependent vasorelaxation of SMA without affecting endothelium-independent vasorelaxation and phenylephrine-induced vasoconstriction (Figure 3, Supplemental Figures V, VI). The superoxide level and nitrotyrosine protein expression in the SMA were elevated in diabetic mice but were 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 nitric oxide availability (Figure 4).

Although we observed that the SMA endothelial function of Leprdb at 5, 10, and 20 days after surgery was completely restored to the level of nondiabetic control mice, in Leprdb at 30 days post surgery, this procedure only partially improved endothelial function (Figure 3). Moreover, the protein expression of IFN-γ and MCP-1 in diabetic mice at 30 days after surgery slightly returned toward the level observed in the Leprdb plus sham surgery group, even though there was no significant body weight regain or hyperglycemia. We postulate that an early indicator of relapse after surgery 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 likely is not the determinant of endothelial dysfunction. 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 months old) when subjected to the surgery procedure. However, because the lifespan of Leprdb mice is up to 10 months, our protocol will potentially allow the observation of long-term effects of bariatric surgical procedures. We found that the endothelial function of Leprdb at 90 days after surgery was slightly impaired compared with Leprdb at 30 days after surgery (although still better than Leprdb plus 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. The long-term follow-up study of patients undergoing bariatric surgery showed that body weight reached the lowest point at 2 years and there was a significant increase in body mass index from the nadir to 5 years, and from 5 years to 10 years after surgery. 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. Because of 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 and whole body and abdominal adiposity, and improves glycem
creres in type 2 diabetic mice. Bariatric surgery ameliorates IFN-γ-mediated 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 reduc
tion 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 bene
cfits after bariatric surgery.

Sources of Funding
This study was supported by grants from NIH (RO1-HL077566 and RO1-HL085119 to C.Z., RO1-DK085495 to J.Y. and C.Z. and RO1-HL073101 to J.S. and C.Z.) and American Heart Association Predoctoral Fellowship (10PRE4300043 to H.Z.).

Disclosure
None.

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