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Interferon-gamma induced adipose tissue inflammation is linked to endothelial dysfunction in type 2 diabetic mice

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Abstract

Interferon-gamma (IFN γ) has previously been associated with immuno-mediated inflammation in diet-induced obesity and type 1 diabetes. This study sought to define the role of IFN γ -induced adipose tissue inflammation in endothelial dysfunction in type 2 diabetes. We examined mesenteric adipose tissue (MAT) inflammation, and endothelial function of small mesenteric artery (SMA) in control mice (m Lepr^{db}), diabetic mice (Lepr^{db}), m Lepr^{db} treated with IFN γ , and Lepr^{db} treated with anti-IFN γ or anti-monocyte chemoattractant protein-1 (anti-MCP-1). mRNA and protein expression of IFN γ and MCP-1 were increased in MAT of Lepr^{db}, accompanied by increased T-lymphocyte and macrophage infiltration. Anti-IFN γ reduced MAT inflammatory cell infiltration and inflammatory cytokine expression in Lepr^{db}, while IFN γ treatment showed the opposite effects in m Lepr^{db}. Acetylcholine (ACh)-induced vasorelaxation of SMA was impaired in Lepr^{db} versus m Lepr^{db}, but sodium nitro-prusside (SNP)-induced vasorelaxation was comparable. Both anti-IFN γ and anti-MCP-1 improved endothelial function of Lepr^{db}, while IFN γ treatment impaired endothelial function of m Lepr^{db}. Superoxide production was higher in both MAT and SMA of Lepr^{db} mice, and anti-IFN γ reduced MAT and SMA superoxide production. Macrophage accumulation in the adventitia of SMA, and mRNA expression of MCP-1 in SMA were increased in Lepr^{db} and IFN γ -treated m Lepr^{db}, but reduced in anti-IFN γ treated Lepr^{db}. These findings suggest IFN γ has a key role in the regulation of visceral adipose tissue inflammatory response and endothelial dysfunction in type 2 diabetes.

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Conflict of interest None.

Keywords

Adipose; Diabetes; Endothelial function; Inflammation; Oxidative stress

Introduction

The onset of an obesity and type 2 diabetes pandemic has gained wide recognition [21]. Increased adiposity, especially abdominal adiposity, is associated with an increase in cardiovascular risk factors [39], although the mechanisms underlying these associations are not well understood. Accumulating evidence suggests that adipose tissue plays a critical role in the regulation of vascular function by releasing relaxing and contracting factors, pro-inflammatory cytokines/chemokines, hormones, and superoxide [7, 12, 14, 15, 25]. The pathogenic role of adipose tissue in type 2 diabetes is supported by clinical evidence that weight loss leads to improvement in glycemic control [11]. Thus, characterization of the biological and pathological functions of various adipose-derived factors will expand our understanding of the communication between the peri-vascular adipose and blood vessels and how adipose tissue affects vascular function through endocrine/paracrine actions in obese and type 2 diabetic patients [7, 52].

Obesity and type 2 diabetes are characterized by low-grade systemic inflammation, which is caused, at least in part, by inflamed adipose tissue [52]. Adipocytes are intrinsically capable of producing various pro-inflammatory cytokines/chemokines. Furthermore, adipose tissue inflammation may also be due to macrophage that has been shown to infiltrate adipose tissue [18]. Increased macrophage infiltration into adipose tissue is associated with insulin resistance and endothelial dysfunction in obese subjects [1, 17]. T lymphocyte infiltration in visceral adipose tissue occurs prior to the infiltration of macrophage and the development of insulin resistance in high-fat diet treated mice [22]. Together, this suggests that T lymphocyte infiltration may be a primary event that orchestrates the inflammation of adipose tissue [4]. Both T cells and natural killer (NK) cells express interferon-gamma (IFN γ) on activation [6, 30]. Obese IFN γ -deficient animals showed significantly reduced densities of inflammatory cells in adipose tissue, and better glucose tolerance than control animals when fed with the same diet [37]. Despite the established role of IFN γ in mediating the immune response in type 1 diabetes and diet-induced obesity [6, 37], the role of IFN γ in inducing adipose tissue inflammation in type 2 diabetes and the mechanisms of the crosstalk between IFN γ -induced adipose tissue inflammation/oxidative stress and vascular dysfunction have not been studied. Thus, the objective of this study is to test the hypothesis that IFN γ -induced adipose tissue inflammation/oxidative stress is linked to endothelial dysfunction in type 2 diabetes. We examined the role of IFN γ in adipose tissue inflammation/oxidative stress and its vascular pro-oxidant mechanisms putatively involved in the increased oxidative stress/reduced generation of NO in type 2 diabetic mice.

Methods

Animal models and treatment

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 (Bar Harbor, Maine) and maintained on a normal rodent chow diet. 12–16 week-old, male, 20–35 g m Lepr^{db}, and 40–60 g Lepr^{db} mice were used in this study. m Lepr^{db} was treated with murine recombinant IFN γ (R&D, Cat# 485-MI-100/CF, 330 μ g/kg/day, i.p. injection, 5 days) [20]. Lepr^{db} was treated with neutralizing antibody to IFN γ (anti-IFN γ ; R&D, Cat#AF-485-NA,

250 µg/kg/day, i.p. injection, 4 days) or neutralizing antibody to MCP-1 (anti-MCP-1, Biovision, Cat#5225-100, 200 µg/kg/day, i.p. injection, 3 days) [35].

mRNA expression by real time polymerase chain reaction

Total RNA was extracted from mesenteric adipose tissue (MAT) or small mesenteric artery (SMA) samples using RNeasy Lipid Tissue Mini Kit (Qiagen) or RNeasy Plus Micro Kit (Qiagen), respectively. cDNA was amplified with the use of qRT-PCR Kit with SYBR® Green (Invitrogen). The primer sets were designed by Primer3 software [37, 41, 50]. Quantification was performed using the $2^{-\Delta\Delta CT}$ method ($\Delta\Delta CT = C_{T, \text{target}} - C_{T, \beta\text{-actin}}$) [34]. Results were presented as fold change of transcripts for target normalized to internal control (β -actin), compared with m Lepr^{db} (defined as 1.0 fold).

Protein expression by western blot analyses

Protein expression in MAT or SMA samples was detected using IFN γ primary antibody (Millipore, Cat#MAB1152, 1:500), MCP-1 primary antibody (Abcam, Cat#ab8101, 1:100), or nitrotyrosine primary antibody (Abcam, Cat#ab7048, 1:500). Signals were visualized by enhanced chemiluminescence (ECL, Santa Cruz), scanned densitometrically using Fuji LAS3000 and quantified with Multigauge software (Fujifilm).

Immunohistochemistry

MAT was fixed in 10% Z-fix, and embedded in paraffin. 5 µm sections were stained for rabbit anti-mouse CD3 (Abcam, Cat#ab16669, 1:200), rat anti-mouse F4/80 (Abcam, Cat#ab6640, 1:200), or rat anti-mouse Mac-3 (BD Bioscience, Cat#550292, 1:800), then incubated with appropriate biotinylated secondary antibodies followed by incubation with avidin–biotin complex (Vector). The reaction was visualized with 3-amino-9 ethyl carbazole (DAKO). Sections were counterstained with Gill's hematoxylin solution (Sigma) [44].

Quantification of immunohistochemical staining data

The microscope (Leica CME) was set to 10 $\times$ magnification and positive staining of macrophages accumulated in the adventitia of SMA was observed in consecutive fields of the entire section. The percentage of macrophage-positive SMA over the total number of SMA being counted for each sample was calculated and statistically analyzed.

Functional assessment of small mesenteric arteries

Mesenteric arteries (first order branches) with internal diameter of 200–250 µm were cut into 2 mm long rings and mounted on Myograph 610 M (A & D Instrument). The passive tension-internal circumference was determined by stretching to achieve an internal circumference equivalent to 60–70% of that of the blood vessel under a transmural pressure of 100 mmHg. A cumulative dose–response curve was obtained by adding acetylcholine (ACh, 1 nmol/L–10 µmol/L) and sodium nitroprusside (SNP, 1 nmol/L–10 µmol/L). Relaxation at each concentration was measured and expressed as the percentage of force generated in response to 1 µmol/L phenylephrine (PE) [32, 40]. NO availability and ROS production were evaluated by ACh concentration–response curve repeated after incubation with the NO synthase inhibitor N-Nitro-L-arginine methyl ester (L-NAME, 100 µmol/L, 20 min) and the anti-oxidant and superoxide dismutase mimetic TEM-POL (3 mmol/L, 60 min), respectively.

Measurement of superoxide using electron paramagnetic resonance spectroscopy

Measurement of superoxide using electron paramagnetic resonance spectroscopy (EPR) was performed as previously described [13, 49]. In brief, a 10% MAT or SMA tissue

homogenate containing 2 mmol/L CPH (1-hydrox-3-carboxy-pyrrolidine) was prepared in a 50 mmol/L phosphate buffer with 0.01 mmol/L EDTA and were incubated for 30 min at 37 °C and frozen quickly in liquid nitrogen for measurement.

Data analysis

All data were presented as mean $\pm$ 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 Fisher's least significant difference test. Significance was accepted at $P < 0.05$. Expanded materials and methods are provided in the Online Supplement.

Results

Role of IFN γ in affecting body weight, visceral adiposity, glucose level and insulin sensitivity

Lepr^{db} showed significantly higher body weight than m Lepr^{db}. Abdominal girth, mesenteric bed weight and MAT adiposite size were increased in Lepr^{db} versus m Lepr^{db}. Fasting glucose level, insulin level and homeostasis model assessment of insulin resistance (HOMA-IR) were elevated in Lepr^{db} mice compared with control mice. Insulin tolerance tests show impaired insulin sensitivity in diabetic mice versus control mice. Anti-IFN γ and IFN γ treatment exerted no effects on the above parameters (Supplementary Table 1 and Supplementary Fig. 1).

Role of IFN γ in adipose tissue inflammation in type 2 diabetic mice

IFN γ mRNA and protein expression were higher in MAT of Lepr^{db} versus m Lepr^{db}. Anti-IFN γ reduced mRNA and protein expression of IFN γ in Lepr^{db}. IFN γ treatment to m Lepr^{db} increased mRNA expression of IFN γ , but did not significantly affect protein expression of IFN γ in MAT. MCP-1 mRNA and protein expression were also higher in MAT of diabetic mice versus control mice. Anti-MCP-1 treatment reduced both mRNA and protein expression of MCP-1 in Lepr^{db}. Although anti-IFN γ treatment ameliorated MCP-1 expression, anti-MCP-1 did not significantly affect mRNA and protein expression of IFN γ (Fig. 1). In addition to IFN γ and MCP-1, we also examined the mRNA expression of other adipose-derived factors in MAT of control and diabetic mice. The results indicate there were no differences in MAT mRNA expression of adiponectin, interleukin-10 (IL-10), RANTES, IL-6, and monokine induced by gamma-interferon (MIG) between control and diabetic mice (Supplemental Fig. 2).

We examined the infiltration of CD3 positive T lymphocytes, F4/80 and Mac-3 positive macrophages in MAT of m Lepr^{db}, Lepr^{db}, Lepr^{db} treated with anti-IFN γ or anti-MCP-1, and m Lepr^{db} treated with IFN γ using immunohistochemical staining. Multiple macrophages and lymphocytes fused around dead adipocytes to form crown-like structures (CLS), which were prevalent in MAT of Lepr^{db} (Fig. 2a). mRNA expression of CD3, CD4, CD8, and CD68 was increased in MAT of Lepr^{db} versus control mice. Anti-IFN γ reduced mRNA expression of T lymphocyte and macrophage markers, while IFN γ increased the mRNA levels of CD3, CD4, CD8, and CD68 in m Lepr^{db} mice. Anti-MCP-1 reduced macrophage infiltration, but did not significantly affect MAT T lymphocyte infiltration in Lepr^{db} mice (Fig. 2b–e).

The expression of macrophage marker CD68 in periaortic adipose tissue (PAT) and interscapular brown adipose tissue (BAT) was relatively lower compared with that in MAT in all groups, and there were no statistical differences in PAT and BAT CD68 expression between control and diabetic mice. However, CD68 expression in epididymal white adipose

tissue (EWAT) and inguinal subcutaneous adipose tissue (SCAT) was significantly higher in diabetic mice and IFN γ -treated control mice, but anti-IFN γ treatment to diabetic mice only minimally reduced the CD68 expression in EWAT and SCAT (Fig. 2f).

Role of IFN γ in endothelial dysfunction in type 2 diabetic mice

Endothelium-dependent vasorelaxation of SMA to ACh was impaired in Lepr^{db} versus m Lepr^{db} (Fig. 3). Anti-IFN γ and anti-MCP-1 improved endothelial function of Lepr^{db}, but IFN γ impaired ACh-induced vasorelaxation of control mice (Fig. 3a, c). The endothelium-independent vasorelaxation to sodium nitroprusside (SNP) and vaso-constriction to PE were identical among all groups (Fig. 3b, d and Supplemental Fig. 3).

TEMPOL incubation restored the endothelial function of Lepr^{db} back to the level of control mice (Fig. 4a). L-NAME incubation abolished the difference in endothelium-dependent vasorelaxation between control and diabetic mice (Fig. 4b).

The link between adipose tissue inflammation/oxidative stress and endothelial dysfunction in type 2 diabetic mice

The accumulation of Mac-3 positive macrophages in the adventitia of SMA vascular wall was higher in diabetic mice versus control mice. Anti-IFN γ reduced macrophage accumulation in SMA of Lepr^{db} mice, but IFN γ showed opposite effects in control mice (Fig. 5a, b). Moreover, the mRNA expression of MCP-1 was higher in the SMA of diabetic mice. Anti-IFN γ reduced mRNA expression of MCP-1 in SMA of Lepr^{db} mice, but IFN γ treatment to control mice increased SMA MCP-1 expression (Fig. 5c). However, the mRNA expression of VCAM-1, ICAM-1 and E-selectin in SMA was not different between control and diabetic mice (Supplements Fig. 4). Serum level of MCP-1 was comparable in diabetic and control mice (Fig. 5d). Serum level of inflammatory cytokine tumor necrosis factor- α (TNF α) was elevated in diabetic mice and control mice treated with IFN γ (Fig. 5e).

Superoxide production was elevated in both MAT and SMA of diabetic mice. Anti-IFN γ and anti-MCP-1 ameliorated both MAT and SMA superoxide production, while IFN γ increased MAT/SMA superoxide levels (Fig. 6a, b). Moreover, nitrotyrosine protein expression was higher in SMA of diabetic mice. Both anti-IFN γ and anti-MCP-1 decreased nitrotyrosine levels in SMA of Lepr^{db}, but IFN γ treatment to m Lepr^{db} increased SMA nitrotyrosine protein expression (Fig. 6c).

Discussion

Endothelial dysfunction is an important factor in the pathogenesis of cardiovascular disorders and has gained increasing attention in the study of obesity and diabetes-associated vascular complications [3, 16, 31]. Central adiposity, particularly a greater amount of intra-abdominal or visceral fat, contributes to chronic subclinical inflammation, which is linked to endothelial dysfunction and atherosclerosis [43]. The study of the paracrine/endocrine effects of inflamed adipose tissue on the regulation of vascular function will further enhance our understanding of the mechanisms involved in diabetes-associated vascular complications. The major findings in this study are: (1) IFN γ expression is increased in MAT of type 2 diabetic mice; (2) IFN γ induces visceral adipose tissue inflammation/oxidative stress in control mice; (3) IFN γ increases macrophage accumulation and MCP-1 expression in the vascular wall and impairs endothelial function of SMA; (4) Anti-IFN γ ameliorates MAT inflammation/oxidative stress and improves SMA endothelial function in type 2 diabetic mice. We posit that IFN γ is a mechanism for the generation of inflammation and oxidative stress derived from adipose tissue and plays an important role in the regulation of endothelial function.

T lymphocyte/macrophage infiltration and adipose tissue inflammation in type 2 diabetic mice

Obesity is associated with inflammatory cell accumulation in the adipose tissue [46]. Diet-induced increases in adiposity promote macrophage infiltration into white adipose tissue and the extent of macrophage accumulation correlates with the degree of adiposity [8]. In addition to macrophages, T lymphocytes have recently been identified as a key player in adipose tissue inflammation by orchestrating the evolution of the inflammatory cascade [29]. In patients with type 2 diabetes, lymphocyte content in adipose tissue biopsies is significantly correlated with waist circumference, a marker of central adiposity, and insulin resistance [22]. Our results suggest that the increased IFN γ expression in MAT in Lepr^{db} was accompanied by enhanced T lymphocyte/macrophage infiltration.

Immunohistochemical staining generally showed T lymphocytes in clusters with macrophages, which formed CLS around adipocytes. Anti-IFN γ reduced MAT IFN γ and MCP-1 expression, as well as the T lymphocyte and macrophage infiltration. Anti-MCP-1 reduced macrophage infiltration, but did not significantly affect IFN γ expression and T lymphocyte infiltration (Figs. 1, 2). Thus, although T lymphocytes are quantitatively less prominent than macrophages [37], T cells may decisively affect the infiltration of macrophages, and the expression of macrophage-derived chemokines, in the visceral adipose tissue of type 2 diabetic mice.

Adipose tissue inflammation and endothelial dysfunction in type 2 diabetic mice

Growing evidence suggests a link between adipose tissue inflammation and endothelial dysfunction [1, 26]. The production of inflammatory cytokines and hormones by adipose tissue is of particular interest, since their local secretion by peri-vascular adipose deposits may provide a novel mechanistic link between obesity and diabetes-associated vascular complications [5, 7, 19]. The secretory products from human adipocytes stimulated pro-inflammatory cytokine secretion by human umbilical venous endothelial cells (HUVECs), suggesting that endothelial cell secretion was significantly tilted towards a pro-inflammatory pattern by adipocyte-derived factors [9, 38]. Moreover, macrophages stimulated with IFN γ activate nuclear factor-kappa B (NF- κ B) and induce MCP-1 gene expression in HUVECs, which posits to a possible interaction between adipose inflammatory cells and vascular cells [36]. Mesenteric vasculature regulates total blood flow to the gut and mediates transport of nutrients in the circulation [24]. The mesenteric bed is surrounded by a variable volume of peri-vascular mesenteric adipose tissue, which plays an important role in sustaining an inflammation cascade in obesity and type 2 diabetes [14]. Changes in the fat may have consequences for the regulation of mesenteric artery tone and mesenteric endothelial function [12]. Thus, SMA is an ideal model to study the possible local effects of MAT inflammation on vascular function.

Oxidative stress and inflammation are known to be the key mechanisms in the pathogenesis of vascular dysfunction in atherosclerosis, myocardial ischemia/reperfusion, and diabetes [2, 42, 45, 48]. Many adipose-derived cytokines, such as IL-6, TNF α , and MCP-1 profoundly stimulate ROS production and reduce NO bioavailability [23, 33, 52, 53]. Our results show that ACh-induced vasorelaxation was impaired in SMA of Lepr^{db}. This impairment was completely restored by SOD mimetic, TEMPOL, incubation (Fig. 4a). L-NAME incubation abolished the difference in endothelium-dependent vasorelaxation between control and diabetic mice (Fig. 4b), supporting the view that the impairment of endothelial function in Lepr^{db} was mediated by ROS and NO bioavailability. Furthermore, SMA superoxide production and nitrotyrosine protein expression were higher in Lepr^{db} versus m Lepr^{db}. Anti-IFN γ and anti-MCP-1 reduced SMA superoxide and nitrotyrosine expression, while IFN γ showed the opposite effects in m Lepr^{db} (Fig. 6).

The profound MAT oxidative stress and macrophage accumulation in the vascular wall of SMA may serve as link between MAT inflammation and SMA endothelial dysfunction. In Lepr^{db} mice, the superoxide production is elevated in both MAT and SMA. By normalizing the superoxide level of MAT/SMA to the protein content, the superoxide production is quantitatively more prominent in MAT than in SMA (Fig. 6a, b). Anti- $\text{IFN}\gamma$ reduced MAT superoxide production in Lepr^{db} , but $\text{IFN}\gamma$ increased MAT oxidative stress in m Lepr^{db} mice (Fig. 6a). Moreover, macrophage accumulation in the adventitia of mesenteric vessels was significantly higher in Lepr^{db} and m Lepr^{db} treated with $\text{IFN}\gamma$. Lepr^{db} treated with anti- $\text{IFN}\gamma$ showed reduced macrophage accumulation in SMA. mRNA expression of MCP-1 was also elevated in the SMA of diabetic mice and anti- $\text{IFN}\gamma$ treatment attenuated the MCP-1 expression in SMA (Fig. 5c). Importantly, compared with the remarkable impairment of SMA endothelial function following $\text{IFN}\gamma$ systemic in vivo administration, in vitro incubation of the adipose-free vessel with a pathological concentration of murine recombinant $\text{IFN}\gamma$ (1.6 or 16 ng/ml for 1 h, or 16 ng/ml for 4 h) did not significantly affect ACh-dependent SMA vasorelaxation (Supplemental Fig. 5). This suggests that the role of $\text{IFN}\gamma$ in the impairment of vascular function occurs indirectly following $\text{IFN}\gamma$ -induced adipose tissue inflammation.

Separating the local versus systemic effects of adipose tissue on the regulation of vascular function is also important. Although adipose tissue is considered as a potential systemic source of inflammation, only a limited number of adipose-derived cytokines are released into the circulation in sufficient amounts to account for obesity-associated increased systemic levels [28]. The trivial contribution of adipose-derived MCP-1 [10], $\text{TNF}\alpha$ [27], and PAI-1 [47] to the circulating concentrations has been demonstrated by measuring the arteriovenous difference over the inferior epigastric vein [28]. In fact, although MCP-1 expression was increased in Lepr^{db} MAT, the serum level of MCP-1 was not statistically different between control and diabetic mice (Fig. 5d). The serum level of $\text{TNF}\alpha$ was higher in diabetic mice and $\text{IFN}\gamma$ -treated control mice (Fig. 5e). However, as a chronic disease model, the circulating concentration of $\text{TNF}\alpha$ in the pg/ml range is unlikely to cause endothelial dysfunction in type 2 diabetic mice [51]. Therefore, mesenteric adipose tissue exhibiting a pro-inflammatory phenotype may primarily exert local effects by promoting inflammatory cell accumulation and the release of inflammatory cytokines, thereby enhancing vascular inflammation/oxidative stress and attenuating endothelial function.

In conclusion, our study suggests that $\text{IFN}\gamma$ -induced adipose tissue inflammation and oxidative stress is linked to endothelial dysfunction in type 2 diabetes. These findings help define the linkage between adipose tissue inflammation and vascular dysfunction and highlight the therapeutic potential of anti- $\text{IFN}\gamma$ for alleviating diabetes-related vascular complications, which may result in better remediation strategies in the longer term.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

The mRNA and Protein Expression of IFN γ and MCP-1 in MAT by qRT-PCR and western blot mRNA and protein expression of IFN γ (**a, b**) and MCP-1 (**c, d**) were increased in mesenteric adipose tissue (MAT) of Lepr^{db} versus m Lepr^{db}. Treatment of anti-IFN γ reduced IFN γ and MCP-1 expression in Lepr^{db}, but treatment by recombinant IFN γ show opposite effects in control mice. Anti-MCP-1 reduced the expression of MCP-1, but not IFN γ in MAT of Lepr^{db} mice. Results in various groups were normalized to those of the corresponding m Lepr^{db} control, which were set to a value of 1.0. Data were shown as mean $\pm$ SEM. $n = 4$ separate experiments. * $p < 0.05$ versus m Lepr^{db} mice, # $p < 0.05$ versus Lepr^{db} mice

Fig. 2.

Inflammatory cell infiltration in adipose tissue. **a** Immunohistochemical staining of T-lymphocyte marker CD3, macrophage marker F4/80 and Mac-3 in MAT. T-lymphocyte and macrophage accumulation was increased in the MAT of diabetic mice. Anti-IFN γ reduced T-lymphocyte/macrophage infiltration, and treatment of m Lepr^{db} mice with IFN γ increased MAT T-lymphocyte/macrophage infiltration. $n = 4$ separate experiments. mRNA expression of T cell markers, CD3 (**b**), CD4 (**c**), CD8 (**d**), and macrophage marker CD68 (**e**) were higher in MAT of Lepr^{db} mice. Anti-IFN γ reduced the mRNA expression of T cell and macrophage markers in diabetic mice, while IFN γ treatment increased their levels in control mice. Anti-MCP-1 reduced CD68 mRNA expression without significantly affecting the level of T cell markers. $n = 4$ separate experiments. Data were shown as mean $\pm$ SEM. * $p < 0.05$ versus m Lepr^{db}; # $p < 0.05$ versus Lepr^{db}. **f** mRNA expression of CD68 in periaortic adipose tissue (PAT), interscapular brown adipose tissue (BAT), epididymal white adipose tissue (EWAT), and inguinal subcutaneous adipose tissue (SCAT) was examined by quantitative RT-PCR and was normalized to the CD68 level in MAT of m Lepr^{db} control mice. $n = 4-8$ separate experiments. Data were shown as mean $\pm$ SEM. CD68 expression in each adipose tissue depot expressed as: * $p < 0.05$ versus m Lepr^{db}; # $p < 0.05$ versus Lepr^{db}.

Fig. 3. Endothelium-dependent and Endothelium-independent vasorelaxation of SMA. **a, c** ACh-induced endothelium-dependent vasorelaxation of SMA was impaired in Lepr^{db}. Anti-IFN γ and anti-MCP-1 improved endothelial function, but treatment of control mice with IFN γ impaired endothelial function. **b, d** SNP-induced endothelium-independent vasorelaxation was not different among groups. Data were shown as mean $\pm$ SEM. $n = 6-13$ rings from 6-8 mice. * $p < 0.05$ versus m Lepr^{db} mice, # $p < 0.05$ versus Lepr^{db} mice

Fig. 4.

The role of superoxide anion and nitric oxide (NO) in the regulation of endothelial function of SMA. **a** TEMPOL incubation (3 mmol/L, 60 min) restored impaired endothelial function in Lepr^{db}. $n = 8$ rings from 6 mice. **b** Endothelium-dependent vasorelaxation was identical between control and diabetic mice after L-NAME incubation (100 μ mol/L, 20 min). $n = 11$ –16 rings from 7–10 mice. Data were shown as mean $\pm$ SEM. * $p < 0.05$ versus m Lepr^{db} mice, # $p < 0.05$ versus Lepr^{db} mice

Fig. 5.

The Accumulation of Macrophages and the mRNA Expression of MCP-1 in the Vascular Wall of SMA, and the Serum Level of MCP-1 and TNF α **a** Mac-3 positive macrophage accumulation in the vascular wall of SMA was increased in Lepr^{db} and m Lepr^{db} treated with IFN γ . Anti-IFN γ reduced macrophage accumulation in SMA vascular wall. **b** The percentage of SMA with Mac-3 positive macrophage accumulation was quantified. $n = 4$ separate experiments. **c** mRNA expression of MCP-1 in SMA was higher in diabetic mice versus control mice. Anti-IFN γ reduced MCP-1 expression in SMA of diabetic mice. $n = 8$ separate experiments. **d** Serum level of MCP-1 was not different among m Lepr^{db}, Lepr^{db}, and m Lepr^{db} + IFN γ . **e** Serum level of TNF α was higher in Lepr^{db} and m Lepr^{db} + IFN γ versus m Lepr^{db}. $n = 4-6$ mice. Data were shown as mean $\pm$ SEM. * $p < 0.05$ versus m Lepr^{db} mice, # $p < 0.05$ versus Lepr^{db} mice

Fig. 6.

Oxidative stress in MAT and SMA **a, b** Superoxide levels measured by EPR were increased in MAT and SMA of Lepr^{db} compared with m Lepr^{db} control mice. Anti-IFN γ reduced MAT/SMA superoxide level in Lepr^{db}, but IFN γ treatment increased MAT/SMA superoxide production in m Lepr^{db}. Data represent mean $\pm$ SEM. $n = 6-12$ mice. * $p < 0.05$ versus m Lepr^{db} mice, # $p < 0.05$ versus Lepr^{db} mice. **c** Nitrotyrosine (N-Tyr) expression measured by Western Blotting was higher in SMA of diabetic mice. Anti-IFN γ and anti-MCP-1 reduced SMA expression of N-Tyr, while IFN γ showed the opposite effects in control mice SMA. Data represent blots from three separate experiments