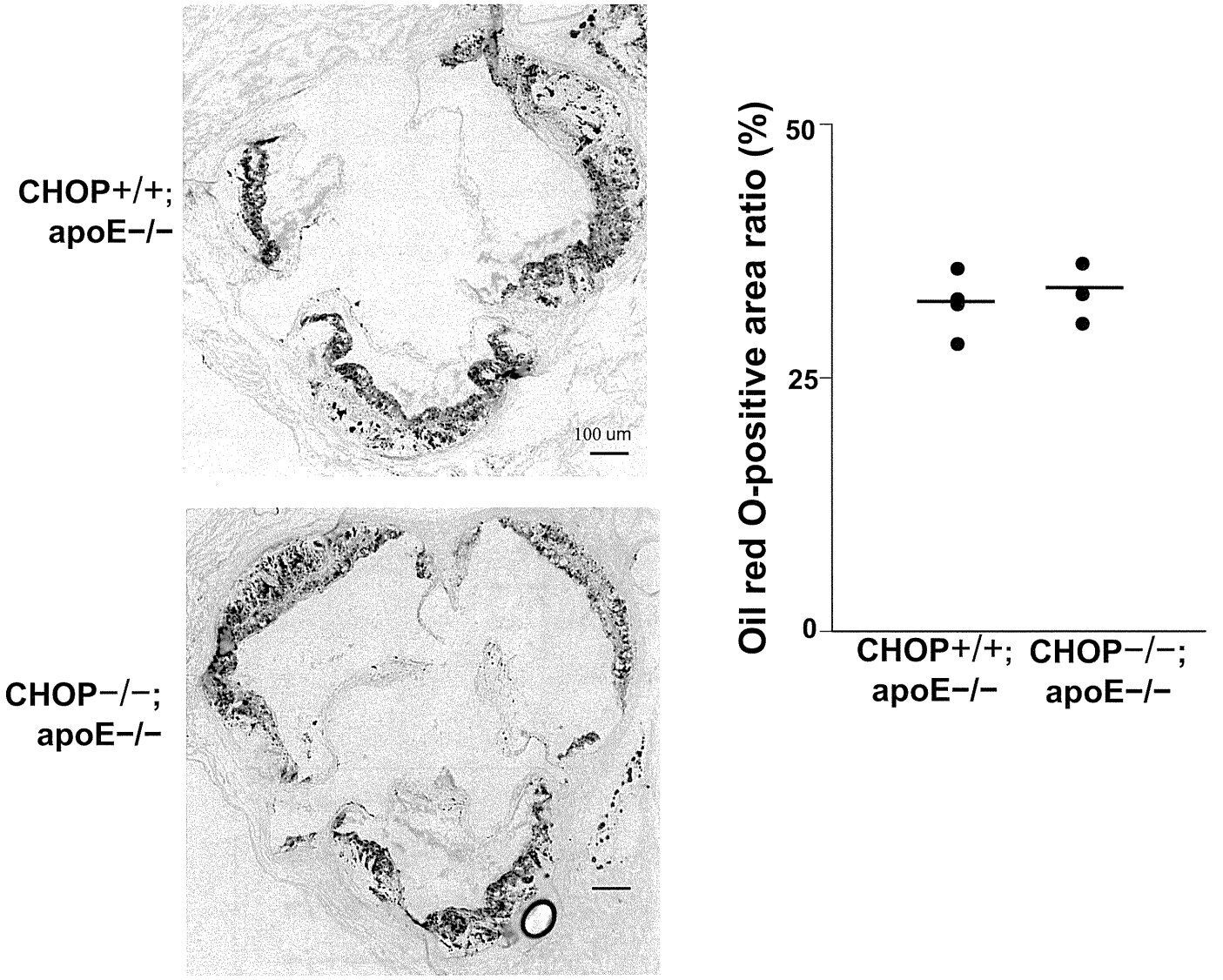
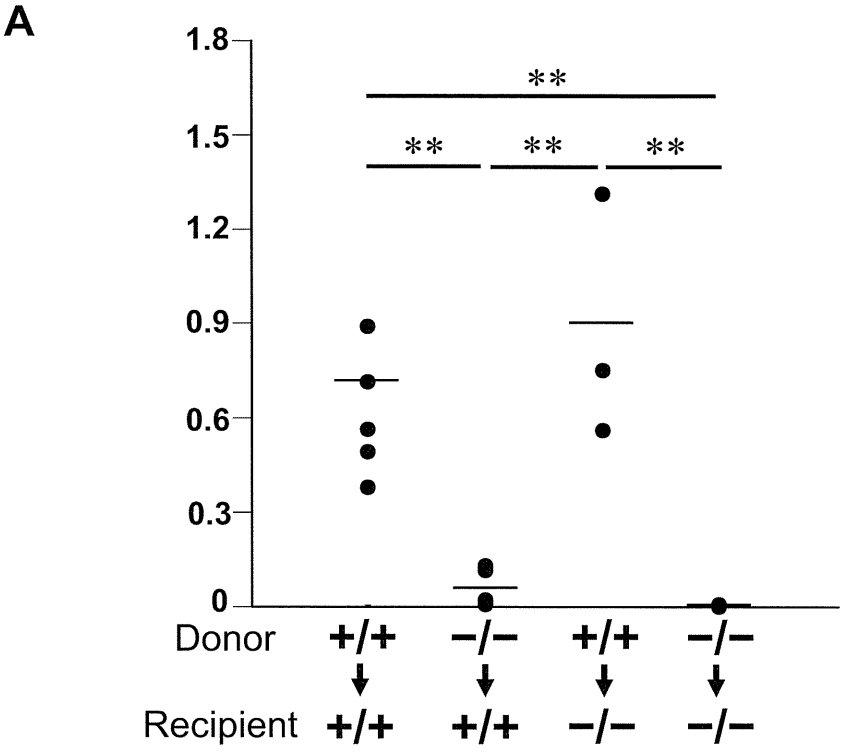
# Figure VIII

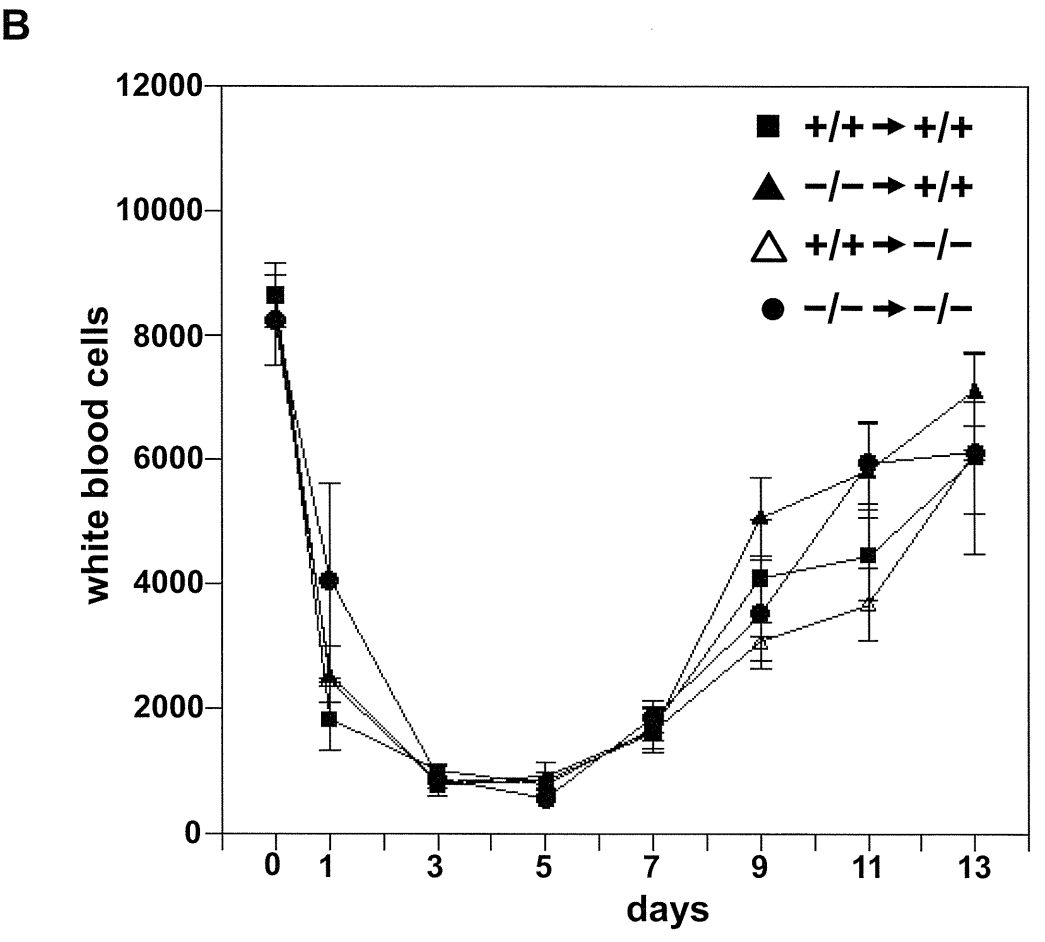




# Figure IX

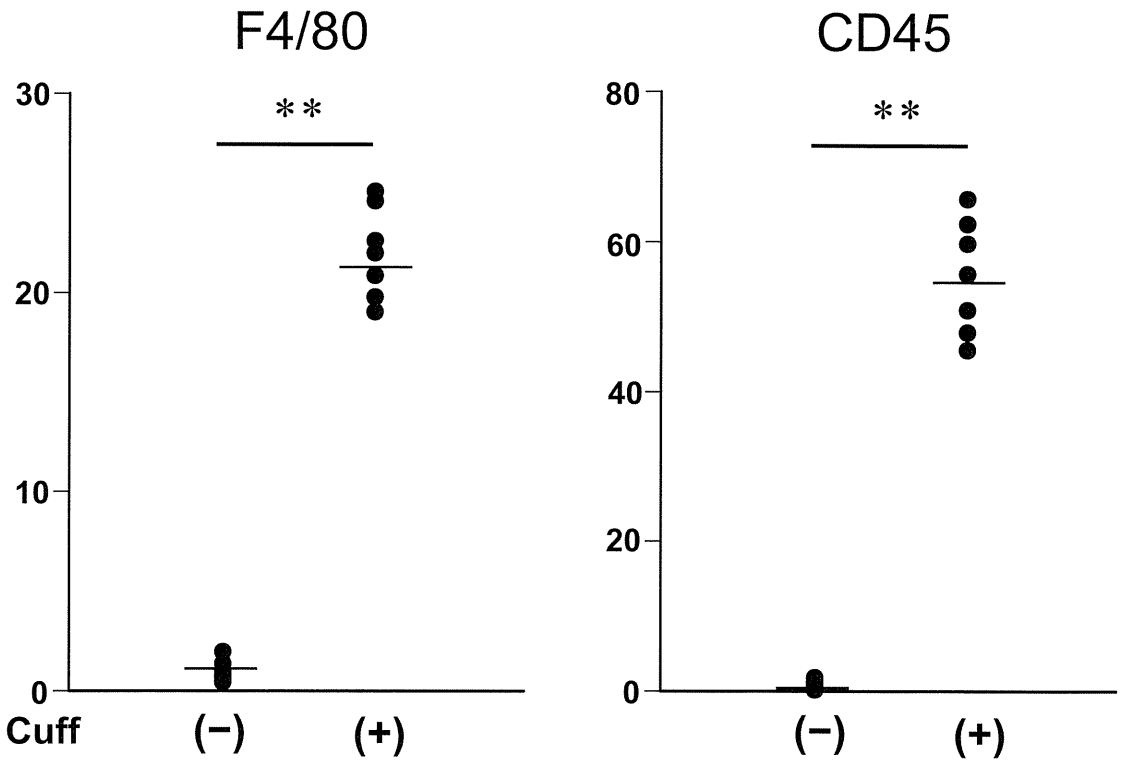




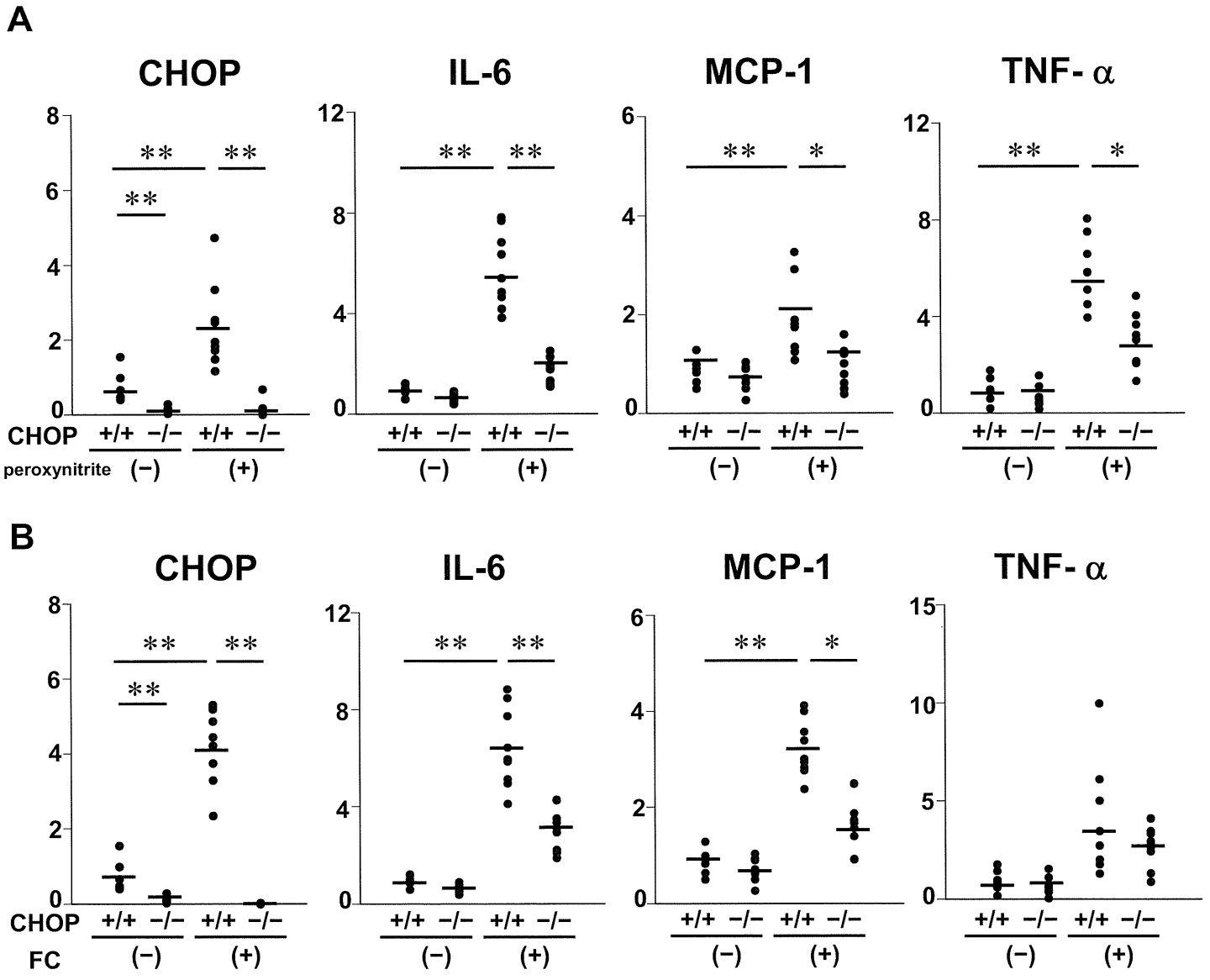
# Figure X





# Figure XI





**Supplemental Material**

**Supplemental Figure Legends**

**Figure I CHOP deficiency caused no gross phenotypic differences**

CHOP+/+ and CHOP-/- ($n$=5 per group) mice were fed normal chow and subjected to measurement of body weight (A), blood glucose (B), blood pressure (C), plasma levels of lipid parameters (D) such as total cholesterol (left), triglyceride (middle) and non-esterified fatty acids (NEFA) (right), and plasma levels of adipokines (E), such as adiponectin (left), MCP-1 (middle) and TNF-α (right), at 11 weeks of age. Data are presented as means ± SE.*$P < 0.05$ by the unpaired $t$ test.

**Figure II Arterial CHOP expression was up-regulated by cuff injury**

Arterial expression of CHOP in cuff-injured and uninjured arteries of CHOP+/+ and CHOP-/- ($n$=7 per group) mice 7 days after cuff placement were quantified by RT-PCR. Data are presented as means ± SE. **$P < 0.01$ by one-way ANOVA.

**Figure III Cuff injury-induced neointimal SMC proliferation was suppressed in CHOP deficient mice**

Expressions of α-smooth-muscle isoform of actin (SMA) in cuff-injured and uninjured arteries 3 weeks after cuff placement in CHOP+/+ and CHOP-/- ($n$=7 per group) mice were quantified by RT-PCR adjusted with GAPDH (A). Immunological stainings against SMA (B) and PCNA (C; upper panel) were prepared from cuff injured femoral arteries 3 weeks after placement in CHOP+/+ (left) and CHOP-/- (right) mice. The relative amounts of PCNA positive cells in neointimal lesions ($n$=14) were presented as the ratio to CHOP +/+ mice ($n$=9) (C; lower panel). *$P < 0.05$, **$P < 0.01$ by the

unpaired *t* test or one-way ANOVA.

**Figure IV CHOP deficiency caused no gross phenotypic differences in apoE-/- mice**

CHOP+/+;apoE-/- ($n$=18) and CHOP-/-;apoE-/- ($n$=13) mice were fed 1.25% high cholesterol chow starting at 8 weeks of age and subjected to measurement of body weight (A), blood glucose (B), blood pressure (C), plasma levels of lipid parameters (D) such as total cholesterol (left), triglyceride (middle), and non-esterified fatty acids (NEFA) (right). Data are presented as means ± SE.

**Figure V Aortic root cross-sections from 20-week-old CHOP+/+;apoE-/- and CHOP-/-;apoE-/- mice**

Aortic root cross-sections were prepared from 20-week-old CHOP+/+;apoE-/- ($n$=5) and CHOP-/-;apoE-/- ($n$=5) mice and stained with hematoxylin and eosin. Representative histological findings are shown in the left panels. Atherosclerosis was evaluated as plaque areas, which were expressed as percentages of the total lumen areas in CHOP+/+;apoE-/- ($n$=5) and CHOP-/-;apoE-/- ($n$=5) mice. Data are presented as means ± SE. $^{**}P < 0.01$ by the unpaired *t* test.

**Figure VI Immunohistological findings of aortic plaque lesions from 20-week-old CHOP+/+;apoE-/- and CHOP-/-;apoE-/- mice**

Aortic sections from 20-week-old CHOP+/+;apoE-/- (upper panels) and CHOP-/-;apoE-/- (lower panels) mice fed 1.25% high cholesterol chow were immunostained with antibodies against MOMA-2 (left), SMA (middle) or oxidized LDL (right) (A). A series of immunological staining with control antibodies, i.e. rat IgG2b as

a control for MOMA-2 (left), mouse IgG2a as a control for SMA (middle), rabbit immunoglobulin as a control for oxidized LDL (right), were performed in the corresponding aortic samples (B). Magnifications of upper and lower panels are x40 and x200, respectively. Representative immunostaining findings from all samples ($n$=7 per group) are shown.

**Figure VII Protein expressions of Bip and SR-AI in the aorta**

Tissue protein extracts of whole aorta samples from 24-week-old CHOP+/+;apoE-/-, CHOP-/-;apoE-/- mice were subjected to immunoblotting with antibodies to Bip (upper panels), SR-AI (middle panels) and GAPDH as a loading control (lower panel).

**Figure VIII Aortic root cross-sections from 14-week-old CHOP+/+;apoE-/- and CHOP-/-;apoE-/- mice**

Aortic root cross-sections were prepared from 14-week-old CHOP+/+;apoE-/- and CHOP-/-;apoE-/- mice and stained with oil red O. Representative histological findings are shown in the left panels. Atherosclerosis was evaluated as oil red O-stained areas, which were expressed as percentages of the total lumen areas in CHOP+/+;apoE-/- ($n$=3) and CHOP-/-;apoE-/- ($n$=3) mice. Data are presented as means ± SE.

**Figure IX Reconstitution of BM cells 2weeks after BMT**

Bone marrow cell expressions of CHOP 2 weeks after BMT were quantified by RT-PCR in CHOP+/+ to CHOP+/+ ($n$=5), CHOP-/- to CHOP+/+ ($n$=5), CHOP +/+ to CHOP-/- ($n$=3) and CHOP-/- to CHOP-/- ($n$=3) BMT mice (A). Time course of peripheral WBC counts from the day before BMT to 13 days after BMT (B). Data are presented as means

± SE. $^{**}P < 0.01$ by one-way ANOVA.

**Figure X Macrophage and hematopoietic cell markers before and after cuff placement**

Arterial expressions of F4/80 and Ptprc (CD45) before (2 weeks after BMT) ($n$=6), and 3 weeks after ($n$=8) cuff placement were quantified by RT-PCR in CHOP+/+ to CHOP+/+ BMT mice. Data are presented as means ± SE. $^{**}P < 0.01$ by the unpaired $t$ test.

**Figure XI**

**CHOP deficiency suppressed peroxynitrite- and free cholesterol-induced inflammatory responses of macrophages.**

mRNA expressions of CHOP and inflammatory cytokines in peritoneal macrophages from CHOP+/+ ($n$=9) and CHOP-/- ($n$=9) mice in response to peroxynitrite (A) and free cholesterol (B) treatments, were evaluated by RT-PCR. Peritoneal macrophages from CHOP+/+ ($n$=5) and CHOP-/- ($n$=6) mice without these ER stress-inducing agents were also subjected to analyses of mRNA expressions. $^{*}P < 0.05$, $^{**}P < 0.01$ by the one-way ANOVA.

European Heart Journal Advance Access published August 8, 2011

European Heart Journal
doi:10.1093/eurheartj/ehr265

BASIC SCIENCE

# Hepatic peroxisome proliferator-activated receptor-γ–fat-specific protein 27 pathway contributes to obesity-related hypertension via afferent vagal signals

**Kenji Uno[1,2], Tetsuya Yamada[1], Yasushi Ishigaki[2], Junta Imai[2], Yutaka Hasegawa[2], Junhong Gao[1], Keizo Kaneko[2], Kimihiko Matsusue[3], Tomomi Yamazaki[4], Yoshitomo Oka[2], and Hideki Katagiri[1]***

[1]Department of Metabolic Diseases, Center for Metabolic Diseases, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan; [2]Division of Molecular Metabolism and Diabetes, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan; [3]Faculty of Pharmaceutical Science, Fukuoka University, Fukuoka 814-0180, Japan; and [4]Nutritional Science Program, National Institute of Health and Nutrition, Tokyo 162-8636, Japan

*Received 26 October 2010; revised 17 June 2011; accepted 7 July 2011*

**Aims** Obesity is commonly associated with hypertension. Increased sympathetic tonus in obese subjects contributes to the underlying mechanism. However, the precise mechanisms whereby obesity induces this sympathetic activation remain unclear. Hepatic peroxisome proliferator-activated receptor (PPAR)-γ2 expression, which is reportedly upregulated during obesity development, affects sympathetic activation via hepatic vagal afferents. Herein, we report involvement of this neuronal relay in obesity-related hypertension.

**Methods and results** Peroxisome proliferator-activated receptor-γ and a direct PPARγ target, fat-specific protein 27 (Fsp27), were adenovirally overexpressed or knocked down in the liver, in combination with surgical dissection or pharmacological deafferentation of the hepatic vagus. Adenoviral PPARγ2 expression in the liver raised blood pressure (BP) in wild-type but not in β1/β2/β3 adrenergic receptor-deficient mice. In addition, knockdown of endogenous PPARγ in the liver lowered BP in murine obesity models. Either surgical dissection or pharmacological deafferentation of the hepatic vagus markedly blunted BP elevation in mice with diet-induced and genetically-induced obesity. In contrast, BP was not elevated in other models of hepatic steatosis, DGAT1 and DGAT2 overexpressions, in which PPARγ is not upregulated in the liver. Thus, hepatic PPARγ upregulation associated with obesity is involved in BP elevation during obesity development. Furthermore, hepatic expression of Fsp27 raised BP and the effect was blocked by hepatic vagotomy. Hepatic Fsp27 is actually upregulated in murine obesity models and its knockdown reversed BP elevation.

**Conclusion** The hepatic PPARγ–Fsp27 pathway plays important roles in the development of obesity-related hypertension via afferent vagal signals from the liver.

**Keywords** Obesity • Hypertension • Neuronal signals • PPARγ • Fsp27

## Introduction

The worldwide prevalence of obesity is increasing at an alarming rate, with major adverse consequences including atherosclerotic morbidity, i.e. coronary heart disease and central vascular disease.[1] In particular, visceral obesity is prone to be associated with hypertension, glucose intolerance and dyslipidaemia, collectively termed the metabolic syndrome, the combination of which increases the risk for cardiovascular morbidities. Numerous mechanisms have been proposed to underlie obesity-related

* Corresponding author. Tel: +81 22 717 8228, Fax: +81 22 717 8228, Email: katagiri@med.tohoku.ac.jp

hypertension. These include increased sodium reabsorption, activation of the renin–angiotensin–aldosterone system, oxidative and inflammatory stress, endothelial dysfunction and decreased sensitivity to natriuretic peptides.[2,3] Adiposity-induced insulin resistance and the resulting hyperinsulinaemia also reportedly contribute to these mechanisms.[4] In addition, adiposity impairs adipocyte function and alters the adipokine profile, which is also involved in blood pressure (BP) elevation.[5] In particular, there is a strong correlation between plasma leptin concentrations and hypertension in human subjects.[6,7] Thiazolidindione treatment, which promotes adipocyte differentiation, reportedly lowers BP.[8,9] In addition to these humoral mechanisms, it is well known that sympathetic tonus is increased in obese subjects,[10] especially those with visceral adiposity.[11] Such sympathetic activation enhances cardiac output, renal sodium reabsorption, and vasoconstriction as well as potentiating the renin–angiotensin–aldosterone system, leading to the development of hypertension.[12] However, the precise mechanisms whereby obesity induces this sympathetic activation remain unclear.

Obesity is thought to be a disturbed state of energy metabolism homeostasis. To maintain metabolic homeostasis in the whole body, communication among organs/tissues, allowing coordinated metabolic regulation, appears to be essential. In addition to humoral factors, including insulin and adipokines, the autonomic nervous system is now increasingly being recognized as an important component of inter-organ metabolic communication.[1]

In particular, afferent neural signals originating in the liver play important roles in regulating energy[13] and glucose[14] homeostasis. We reported that adenoviral expression of peroxisome proliferator-activated receptor-γ (PPARγ) in the liver enhances both systemic energy expenditure and lipolysis in adipose tissue, via sympathetic activation mediated by the afferent vagus originating in the liver.[13] Although expression of PPARγ, a transcription factor which activates genes involved in lipid storage and production, is very low in the liver when compared with that in adipose tissues,[15] hepatic expression of PPARγ, especially PPARγ2,[16] is upregulated in a number of murine obesity models as well as in obese human subjects.[17] Therefore, this inter-organ communication system via the neuronal relay, consisting of vagal afferents and sympathetic efferents, likely has a role in preventing obesity development by increasing energy expenditure and lipolysis. Herein, we report that this neuronal relay, which may act as a protective mechanism against obesity development, ironically contributes to obesity-related hypertension, a major feature of the metabolic syndrome, via sympathetic activation.

# Methods

Due to space requirements, some methodological details have been placed online.

## Animals

Animal studies were conducted in accordance with Tohoku University institutional guidelines. Male C57BL/6N, KK, and KK-Ay mice were purchased from Japan Clea (Tokyo, Japan). Male Zucker diabetic fatty (ZDF) and Zucker lean rats were purchased from Japan SLC (Shizuoka, Japan). Diet-induced obese (DIO) mice were obtained by 10-week feeding of a high-fat diet (32% safflower oil, 33.1% casein, 17.6% sucrose, and 5.6% cellulose[13]) beginning at 5 weeks of age. β1/β2/β3 adrenergic receptor triple knockout (β-less) mice[18] were provided by Dr B. B. Lowell.

## Administration of recombinant adenovirus

Recombinant adenoviruses bearing murine PPARγ2 cDNA,[13] the bacterial β-galactosidase gene,[13] DGAT1, DGAT2,[19] or fat-specific protein 27 (Fsp27)[20] cDNA, and for RNA interference, recombinant adenovirus encoding short-hairpin RNA for PPARγ, scramble (AdK7-H1-scramble),[13] or FSP27[20] were injected into mice (see Supplementary material online for details).

## Blood analysis

Blood glucose was assayed with Antsense II (Horiba Industry, Kyoto, Japan). Serum insulin and leptin were determined with enzyme-linked immunosorbent assay kits (Morinaga Institute of Biological Science, Yokohama, Japan).

## Blood pressure (BP) measurement

Systolic blood pressure (SBP) was usually determined by the standard tail-cuff noninvasive measurement system[21] using a model MK-2000 sphygmomanometer for mice and rats (Muromachi Kikai, Tokyo, Japan) according to the manufacturer's instructions. The tail-cuff system is commonly used for measuring SBPs in conscious animals without operative intra-arterial catheterization, and the results reportedly correspond to those obtained with telemetry.[22] At least six readings were obtained for each experiment, and a mean value was assigned to each individual mouse and rat.

## Dissection of hepatic branch of the vagus

Selective hepatic vagotomy was performed as previously described[13] (see Supplementary material online for details).

## Selective hepatic vagal afferent blockade by perivagal application of capsaicin

Selective blockade of hepatic vagal afferent was performed by perivagal application of capsaicin.[13] The hepatic branch of the vagal trunk was isolated from surrounding tissues using paraffin films, then loosely tied with a cotton string with or without being immersed in capsaicin (Sigma Chemical Co, St Louis, MO, USA) dissolved in olive oil (5% wt/vol) (see Supplementary material online for details).

## Hepatic triglyceride content

Frozen livers were homogenized and triglycerides were extracted with $CHCl_3$:$CH_3OH$ (2:1, v:v), dried and resuspended in 2-propanol.[13] Triglyceride contents were measured using Lipidos liquid (TOYOBO, Osaka, Japan).

## Immunoblotting and histological analysis

Immunoblotting was performed as previously described[13] using anti-PPARγ antibody (Cell Signaling Technology, Danvers, MA, USA). Hepatic vagal nerve immunohistochemistry was performed as previously described[13] (see Supplementary material online for details).

### Quantitative reverse transcriptase–polymerase chain reaction-based gene expression

Quantitative reverse transcriptase–polymerase chain reaction (RT–PCR) was performed as previously described[13] (see Supplementary material online for details).

### Statistical analysis

All data are expressed as means ± SD. All statistical analyses were performed with Ekuseru-Toukei 2006 and 2010 statistical software (Social Survey Research Information Co., Ltd., Tokyo, Japan). Normality was tested with the Kolmogorov–Smirnov test. When data were normally distributed, the statistical significance of differences was assessed with the unpaired *t* test. The Mann–Whitney *U* test was applied when data were not normally distributed. In all analyses, values of $P < 0.05$ were accepted as statistically significant, and all tests were two-sided.

## Results

### Hepatic peroxisome proliferator-activated receptor-γ overexpression raises blood pressure via the neuronal relay

First, to examine whether hepatic PPARγ2 expression raises BP, we over-expressed PPARγ2 selectively in the livers of C57BL/6 mice fed a normal chow diet (PPARγ2-mice) by systemically infusing a recombinant adenovirus. Recombinant adenovirus infusion through the tail vein resulted in selective transgene expression in the liver (see Supplementary material online, *Figure S1A*) without increased expression in other peripheral tissues, as reported previously.[13,14] Mice given the lacZ adenovirus were used as controls. Hepatic expression of PPARγ2 in normal chow-fed lean mice caused hepatomegaly (*Figure 1A*) due to marked hepatic steatosis (*Figure 1B*), while reducing adipose tissue with suppression of weight gain (see Supplementary material online, *Figure S1B*). In addition, hepatic PPARγ2 expression decreased fasting blood glucose (FBG) (see Supplementary material online, *Figure S1C*), insulin and leptin levels (*Table 1A*). These findings are compatible with the observations in mice with diet-induced obesity.[13] Under these conditions, SBP was significantly elevated in PPARγ2 mice (*Figure 1C*). Thus, PPARγ2 overexpression in the liver raises BP via mechanisms independent of hyperinsulinaemia and hyperleptinaemia. In addition, hepatic PPARγ2 expression increased renal renin and adipose angiotensinogen expressions (see Supplementary material online, *Figure S1D–E*). Since these genes are known to be upregulated by sympathetic stimulation,[3,23,24] these results suggest that hepatic PPARγ2 expression increases sympathetic tonus to the kidney as well as adipose tissue, possibly contributing to BP elevation.

Next, to examine whether vagal afferent signals originating in the liver are the mechanism whereby hepatic PPARγ2 expression raises BP, we surgically dissected the hepatic vagus. Seven days after selective hepatic vagotomy or sham-operation, we administered recombinant adenovirus encoding LacZ or PPARγ2. Liver weights were similarly increased by PPARγ2 overexpression in sham-operated and hepatically vagotomized mice (*Figure 1D*). In contrast, while hepatic PPARγ2 significantly raised SBP in sham-operated mice, selective hepatic vagotomy completely blocked SBP elevation (*Figure 1E*). We next administered PPARγ2 adenovirus to β-less mice.[18] Although adenoviral expression of hepatic PPARγ2 also increased liver weight in β-less mice to an extent similar to that in wild-type controls (*Figure 1F*), SBP was unaffected in β-less mice (*Figure 1G*). Thus, the hepatic PPARγ-modulated neuronal pathway, consisting of afferent vagal and efferent sympathetic nerves, mediates BP elevation.

### Knockdown of peroxisome proliferator-activated receptor-γ expression in the liver lowers blood pressure in murine obesity models

Does this system physiologically contribute to obesity-related hypertension? To answer this question, we knocked down hepatic PPARγ expression in murine obesity models. At 8 weeks of age, hepatic PPARγ expression was markedly enhanced in genetically obese KK-Ay mice when compared with lean control KK mice (*Figure 2A*). Administration of recombinant adenovirus expressing shRNA for PPARγ substantially decreased endogenous PPARγ expression in the livers of KK-Ay mice (*Figure 2A*). On Day 3 after adenoviral administration, the knockdown of hepatic PPARγ expression in KK-Ay mice significantly lowered BP, while exerting no effects on BP in KK mice (*Figure 2B*). Note that, at this time point, neither liver weights (*Figure 2C*) nor hepatic triglyceride contents (*Figure 2D*) had yet decreased significantly. Nor were body weights (see Supplementary material online, *Figure S2*), plasma insulin, or leptin levels (*Table 1B*) significantly affected by PPARγ–shRNA administration. Findings were similar in another murine obesity model mice with diet-induced obesity. High fat loading of C57BL/6 mice enhanced endogenous PPARγ expression in the liver (*Figure 2E*) and raised BP (*Figure 2F*). Without significant decreases in liver weights (*Figure 2G*) and hepatic triglyceride contents (*Figure 2H*), hepatic PPARγ knock-down (*Figure 2E*) significantly lowered BP in DIO mice, but not in normal chow-fed lean controls (*Figure 2F*). Thus, upregulation of endogenous PPARγ in the liver during obesity development, rather than hepatic lipid accumulation *per se*, contributes to the development of obesity-related hypertension.

### Blockade of hepatic vagal afferents inhibits blood pressure elevation during obesity development

Next, to investigate whether signals mediated by the hepatic vagus are involved in the development of obesity-related hypertension, we performed selective hepatic vagotomy in KK-Ay mice at 8 weeks of age, followed by BP monitoring. Selective hepatic vagotomy did not affect endogenous PPARγ expression in the liver in either KK or KK-Ay mice (*Figure 3A*). Systolic BP rose steadily in sham-operated KK-Ay mice, but hepatic vagotomy blocked this BP elevation (*Figure 3B*), with no changes in plasma insulin or leptin levels (*Table 1C*). In contrast, in KK mice, SBP was unaffected by hepatic vagotomy (*Figure 3B*). Thus, selective hepatic vagotomy

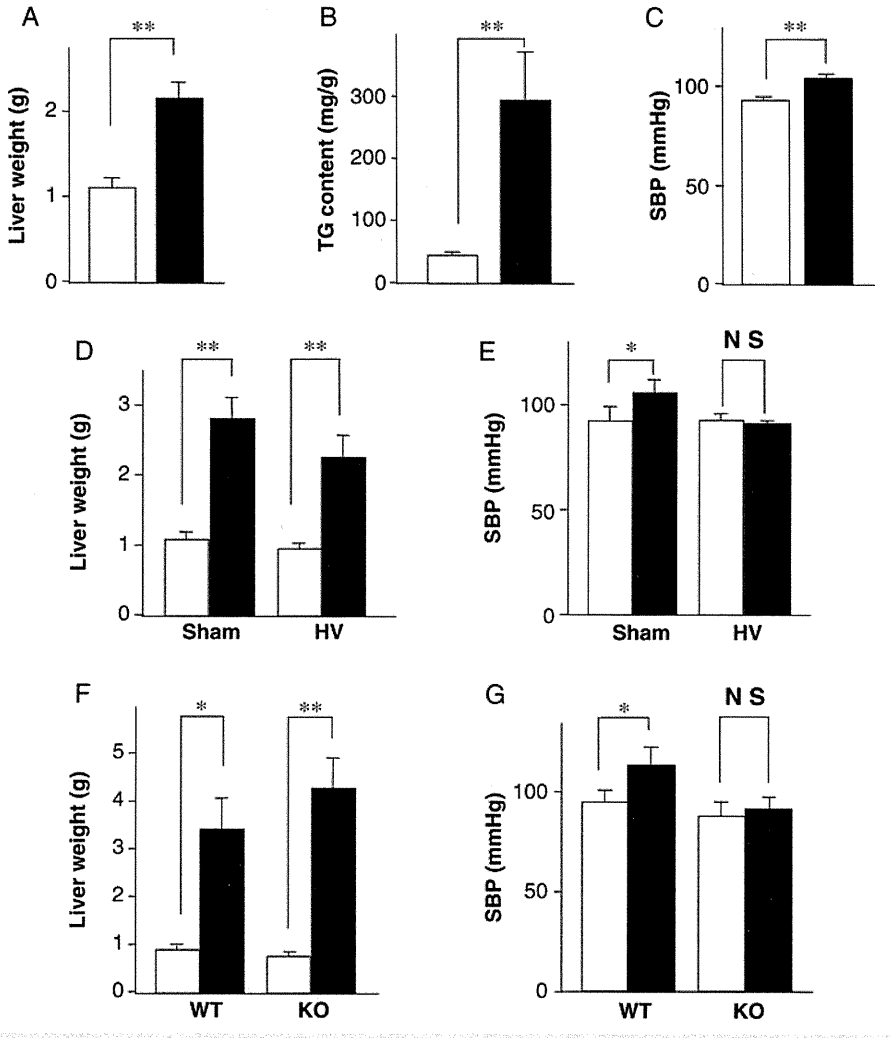



**Figure 1** Hepatic peroxisome proliferator-activated receptor (PPAR)-γ2 expression raises systolic blood pressure (SBP), and this elevation is inhibited by hepatic vagotomy. (*A–E*) PPARγ2 (black bars) or LacZ (white bars) adenovirus was administered to C57BL/6 mice. Liver weights (*A*), hepatic triglyceride contents (*B*), and SBP (*C*) of LacZ and PPARγ2 mice were measured on Day 7 after adenovirus administration ($n = 5–7$). (*D* and *E*) Mice were subjected to sham operation (sham) or hepatic vagotomy (HV) 7 days prior to adenoviral administration. Liver weight (*D*) and SBP (*E*) were measured in these mice 7 days after adenovirus administration ($n = 5–8$). (*F* and *G*) LacZ (white bars) or PPARγ2 (black bars) adenovirus was administered to 8-week-old β-less (KO) and wild-type control (WT) mice. Liver weight (*F*) and SBP (*G*) were measured 7 days after adenovirus administration ($n = 4–5$). Data are presented as means ± SD. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with LacZ mice. NS, not significant.

protected KK-Ay mice from the progression of hypertension associated with obesity.

Hepatic vagotomy involves dissection of both afferent and efferent vagal branches innervating the liver. Therefore, to confirm the involvement of afferent vagal signals originating in the liver, we applied a specific afferent neurotoxin, capsaicin, to the hepatic branch of the vagus in 8-week-old KK and KK-Ay mice. As reported previously,[13] expression of calcitonin gene-related peptide, a sensory neuropeptide, was markedly decreased in the capsaicin-treated vagal nerve, while immunoreactivity for S100 proteins was similar in vehicle- and capsaicin- treated nerves (see Supplementary material online, *Figure S3*), indicating selective deafferentation of the vagal nerve by capsaicin treatment. Perivagal capsaicin treatment did not affect endogenous PPARγ expression in the livers of either KK or KK-Ay mice (*Figure 3C*). Under these conditions, blockade of afferent vagal signals from the liver prevented SBP elevation during obesity development in KK-Ay mice, but had no effect on BP in KK mice (*Figure 3D*). Thus, hepatic PPARγ upregulation associated with obesity is involved in the development of hypertension via the afferent vagal pathway from the liver.

To examine whether this neuronal pathway involves leptin signalling, capsaicin was applied perivagally in a leptin receptor-mutant animal obesity model, ZDF rats, which develop a syndrome with obesity and hypertension.[25] In ZDF rats, endogenous PPARγ expression in the liver is enhanced when compared with that in

**Table 1 Fasting plasma insulin and leptin concentrations in the experimental models used in this study**

**(A)**

| Adenovirus | LacZ | PPARγ2 | *P*-value |
|---|---|---|---|
| Insulin (ng/mL) | 0.60 ± 0.20 | 0.38 ± 0.15 | 0.049 |
| Leptin (ng/mL) | 2.98 ± 0.94 | 0.61 ± 0.36 | <0.001 |

**(B)**

| | KK | | | KK-Ay | | |
|---|---|---|---|---|---|---|
| **Adenovirus** | **H1-scramble** | **H1-PPARγ** | ***P*-value** | **H1-scramble** | **H1-PPARγ** | ***P*-value** |
| Insulin (ng/mL) | 0.77 ± 0.24 | 0.80 ± 0.64 | NS | 2.90 ± 0.79 | 1.92 ± 0.79 | NS |
| Leptin (ng/mL) | 1.64 ± 0.75 | 1.64 ± 0.32 | NS | 23.52 ± 3.08 | 20.26 ± 6.57 | NS |

**(C)**

| | KK | | | KK-Ay | | |
|---|---|---|---|---|---|---|
| **Operation** | **Sham** | **HV** | ***P*-value** | **Sham** | **HV** | ***P*-value** |
| Insulin (ng/mL) | 0.89 ± 0.24 | 0.79 ± 0.37 | NS | 5.25 ± 1.58 | 5.91 ± 2.14 | NS |
| Leptin (ng/mL) | 4.99 ± 1.93 | 3.69 ± 1.23 | NS | 42.00 ± 5.33 | 37.41 ± 8.79 | NS |

**(D)**

| | ZDF | | |
|---|---|---|---|
| **Treatment** | **veh** | **cap** | ***P*-value** |
| Insulin (ng/mL) | 4.31 ± 1.11 | 3.62 ± 0.29 | NS |
| Leptin (ng/mL) | 73.74 ± 8.12 | 74.76 ± 10.46 | NS |

control Zucker lean rats (*Figure 3E*). Again, as obesity progressed, BP rose in ZDF rats, but this elevation was blocked (*Figure 3F*) by capsaicin-mediated selective deafferentation of the hepatic vagus (see Supplementary material online, *Figure S4*), with no changes in plasma insulin or leptin levels (*Table 1D*). These findings indicate that afferent signals of the hepatic vagus contribute to the development of obesity-related hypertension in a fashion independent of leptin signalling.

## Blood pressure is not elevated in murine models of hepatic steatosis without hepatic peroxisome proliferator-activated receptor-γ upregulation

Taking the aforementioned results together, hepatic upregulation of PPARγ, which induces hepatic steatosis, is likely to contribute to the development of obesity-related hypertension via afferent vagal signals. This prompted us to question whether hepatic triglyceride accumulation or hepatic PPARγ upregulation causes hypertension. To answer this question, we examined other models of hepatic steatosis without hepatic upregulation of PPARγ. Acyl-CoA:diacylglycerol acyltransferase (DGAT) is a membrane-bound enzyme that catalyses the last step in the synthesis of triglycerides. DGAT1 and DGAT2 are unrelated proteins that exhibit DGAT activity. Adenoviral expressions of these enzymes, particularly DGAT2, in the liver reportedly result in hepatic steatosis.[19] Indeed, adenoviral DGAT1 or DGAT2 expression in the liver induced hepatomegaly (*Figure 4A*) with hepatic triglyceride accumulation (*Figure 4B*). In particular, hepatic triglyceride contents in DGAT2 mice were similar to those in PPARγ2 mice (*Figure 1B*). However, hepatic PPARγ2 expression was not affected by hepatic expressions of these DGAT enzymes (*Figure 4C*). In contrast to PPARγ2 overexpression, neither DGAT1 nor DGAT2 overexpression in the liver significantly raised BP (*Figure 4D*). White adipose tissue (WAT) weights were not reduced in DGAT1 or DGAT2 mice (see Supplementary material online, *Figure S5*). These findings collectively suggest that DGAT-induced hepatic steatosis has minimal effects on sympathetic tonus.

## Fat-specific protein 27 upregulation in the liver contributes to obesity-related hypertension downstream from peroxisome proliferator-activated receptor-γ

What molecules function downstream from PPARγ in the liver? It was recently reported that Fsp27 functions as a direct target gene of PPARγ in the liver and plays a major role in obesity-related hepatic steatosis.[20] In fact, adenovirus-mediated expression of PPARγ2 in the liver markedly enhanced hepatic Fsp27 expression (see Supplementary material online, *Figure S6A*). Therefore, we

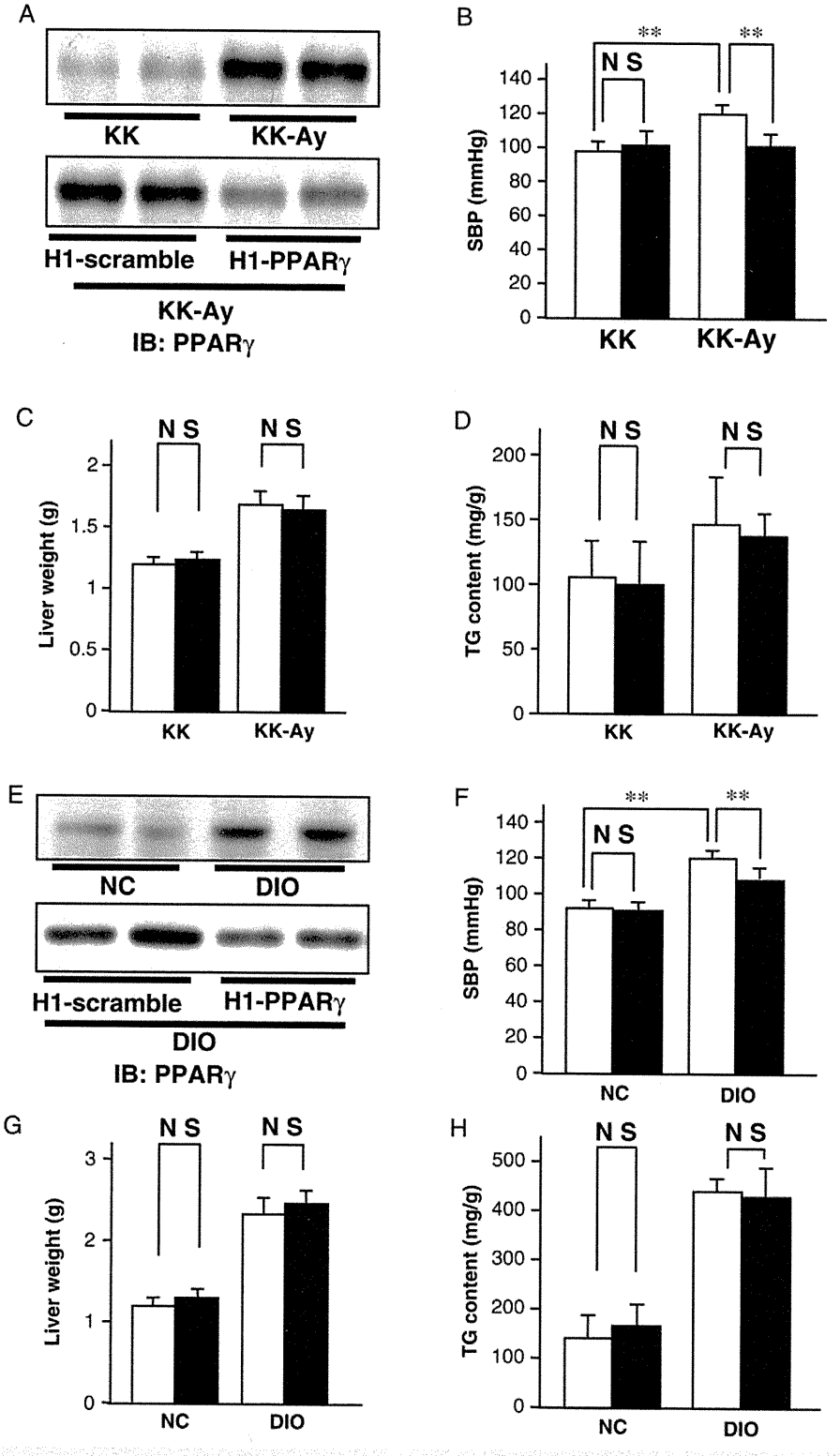



**Figure 2** Knockdown of peroxisome proliferator-activated receptor (PPAR)-γ expression in the liver lowers blood pressure (BP) in murine obesity models. Recombinant adenovirus expressing shRNA for PPARγ (H1-PPARγ: black bars) or control scramble (H1-scramble: white bars) was administered to 8-week-old KK or KK-Ay mice ($n = 5–6$) (*A–D*) and C57BL/6 mice fed normal chow (NC) or with high fat diet-induced obesity (DIO) ($n = 4–5$) (*E–H*), followed by measurement of systolic blood pressures (SBPs) (B and F), liver weights (C and G), and hepatic triglyceride contents (D and H) on Day 3 after adenoviral administration. Liver extracts were subjected to immunoblotting with anti-PPARγ antibody (A and E). Data are presented as means ± SD. $^{**}P < 0.01$; NS, not significant.

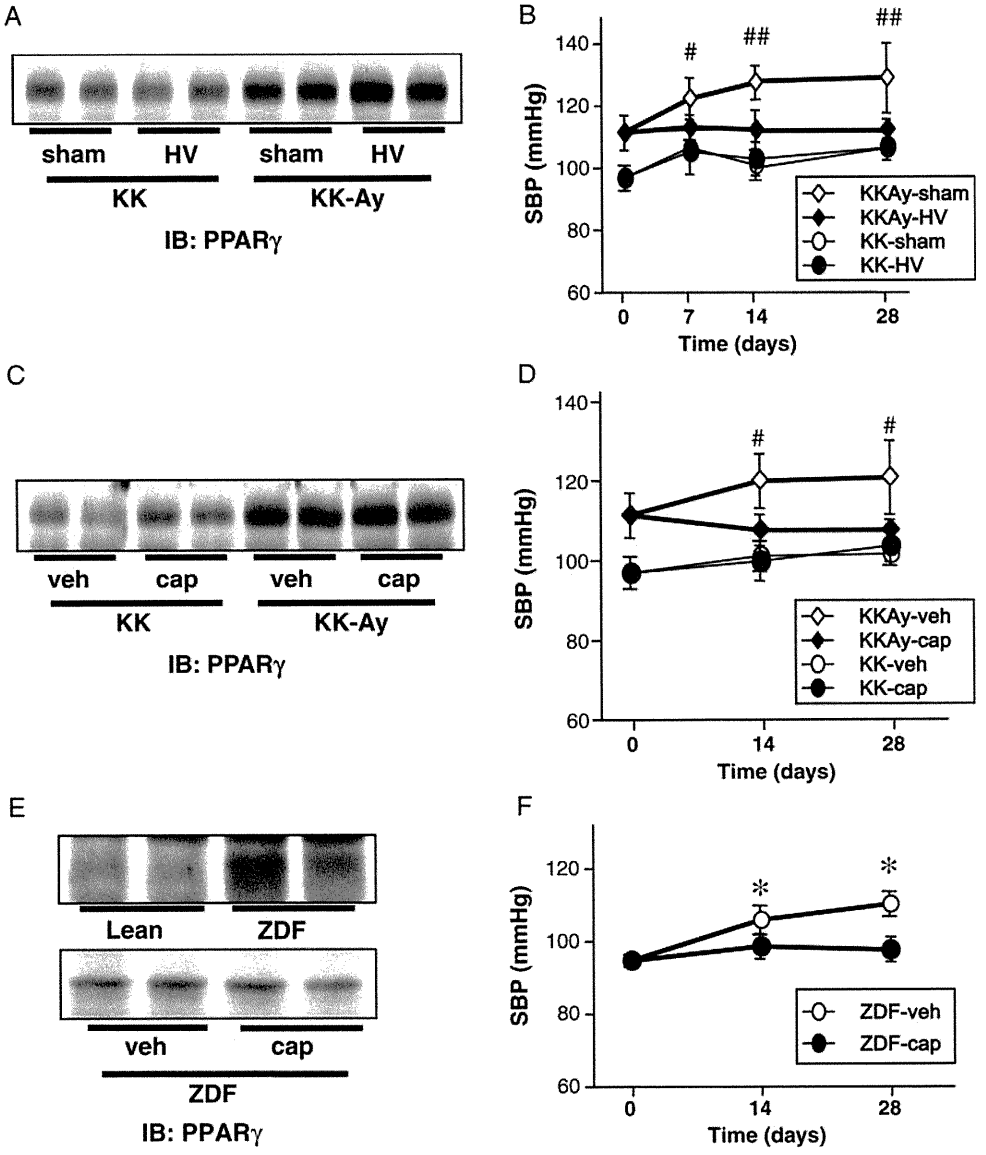



**Figure 3** Surgical dissection or pharmacological deafferentation of the hepatic vagus blocks blood pressure (BP) elevation during obesity development. (*A* and *B*) KK and KK-Ay mice at 8 weeks of age were subjected to hepatic vagotomy (HV) or sham operation (sham). (B) Systolic BP (SBP) was monitored for 28 days after these operations. Open circles and squares and closed circles and squares indicate sham operated and KK and KK-Ay mice and hepatically vagotomized-KK and KK-Ay mice ($n = 5–6$), respectively. $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ indicate KK-Ay (sham) vs. KK-Ay (HV) mice. (*C* and *D*) Vehicle (veh) or capsaicin (cap) was applied to the hepatic vagus of 8-week-old KK and KK-Ay mice. (D) SBP was measured on Days 14 and 28 after these operations. Open circles and squares indicate vehicle-treated KK and KK-Ay mice and closed circles and squares indicate capsaicin-treated KK and KK-Ay mice ($n = 4–6$), respectively. $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ indicate vehicle- vs. capsaicin-treated KK-Ay mice. (*E* and *F*) Vehicle (veh) or capsaicin (cap) was applied to the hepatic vagus in 6-week-old Zucker fatty diabetic (ZDF) rats. (*F*) SBP was measured on Days 14 and 28 after these operations. Open and closed circles indicate vehicle- and capsaicin-treated ZDF rats ($n = 4$), respectively. $^{*}P < 0.05$ indicate vehicle- vs. capsaicin-treated ZDF rats. Liver extracts were subjected to immunoblotting with anti-PPARγ antibody (A, C, E). Data are presented as means ± SD.

examined the role of Fsp27 in obesity-related hypertension. First, Fsp27 was overexpressed in the liver by recombinant adenovirus infusion. Similar to the case of hepatic PPARγ2 expression, hepatic Fsp27 expression increased liver weights (*Figure 5A*) with marked steatosis (*Figure 5B*). Consistent with this, hepatic expressions of sterol regulatory element binding protein-1c (see Supplementary material online, *Figure S6B*) and fatty acid synthase (see Supplementary material online, *Figure S6C*) were enhanced in Fsp27-mice. In contrast, WAT weights were reduced (see Supplementary material online, *Figure S6D*) and uncoupling protein-1 expression in brown adipose tissue was significantly upregulated (see Supplementary material online, *Figure S6E*).

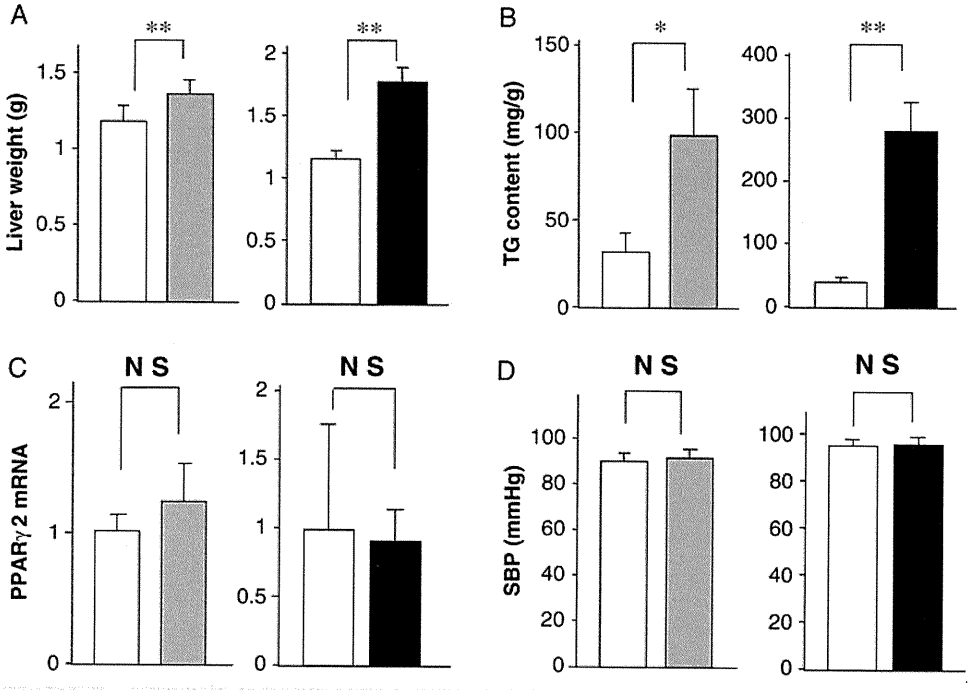



**Figure 4** Hepatic steatosis itself does not raise blood pressure (BP) in murine models of hepatic steatosis without hepatic peroxisome proliferator-activated receptor (PPAR)-γ upregulation. DGAT1 (gray bars), DGAT2 (black bars), or LacZ (white bars) adenovirus was administered to C57BL/6 mice. Liver weights (*A*), hepatic triglyceride contents (*B*), hepatic expressions of PPARγ2 mRNA (*C*), and SBPs (*D*) of LacZ-, DGAT1- and DGAT2-mice were measured on Day 7 after adenovirus administration ($n = 4–6$). Data are presented as means ± SD. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to LacZ mice. NS, not significant.

In addition, hepatic Fsp27 expression increased renal renin expression (see Supplementary material online, *Figure S6F*). These findings, taken together, suggest enhanced sympathetic tonus in Fsp27 mice. Furthermore, hepatic Fsp27 expression significantly lowered FBG (see Supplementary material online, *Figure S6G*). In addition to these metabolic phenotypes, SBP was significantly elevated in Fsp27 mice (*Figure 5C*). Hepatic vagotomy prior to adenoviral administration did not affect liver weights (*Figure 5D*) but did block the BP elevation induced by hepatic Fsp27 expression (*Figure 5E*). These phenotypic features observed in Fsp27 mice, including not only local (in the liver) but also remote effects, are very similar to those induced by hepatic PPARγ2 expression. Therefore, Fsp27 upregulation is likely to mediate the metabolic effects induced by hepatic PPARγ2 expression.

Next, we examined the involvement of hepatic Fsp27 in the development of obesity-related hypertension. In both KK-Ay and DIO mice, hepatic expression of Fsp27 was upregulated when compared with their respective lean controls (*Figure 5F*). Administration of recombinant adenovirus expressing shRNA for Fsp27 decreased endogenous Fsp27 expression in the liver, especially in the murine obesity models (see Supplementary material online, *Figure S7*). Under these conditions, Fsp27 knockdown in the liver significantly lowered SBP in both KK-Ay and DIO mice, but not in their lean counterparts (*Figure 5G*). These findings were similar to the observations in mice with hepatic knockdown of PPARγ (*Figure 2B* and *F*). Thus, hepatic upregulation of Fsp27, a downstream target of PPARγ, is involved in the development of obesity-related hypertension.

## Discussion

The first important finding in this study is that hepatic PPARγ upregulation, which is associated with obesity development,[16,17] contributes to obesity-related hypertension via the neuronal relay originating in the liver. This neuronal relay system was shown to enhance systemic energy expenditure and decrease peripheral adiposity and thus appears to function as an anti-obesity mechanism counteracting excess energy accumulation.[13] This, in turn, leads to hypertension development under conditions of chronic excess energy intake. Thus, the endogenous anti-obesity mechanism appears, ironically, to cause pathological states associated with obesity.

This concept might be somewhat contradictory to the effects of thiazolidinediones on obese subjects. Thiazolidindione treatment of mice as well as human subjects with obesity reportedly increases adiposity as well as lowering BP,[8,9] while hepatic PPARγ2 expression in the present study decreased adiposity and raised BP. These inconsistencies raise the possibility that unbalanced overexpression of PPARγ2 is characterized by a relative lack of its coactivators, leading to non-physiological effects. However, this possibility is quite unlikely for the following reasons. First, hepatic PPARγ2 expression increased hepatic lipid

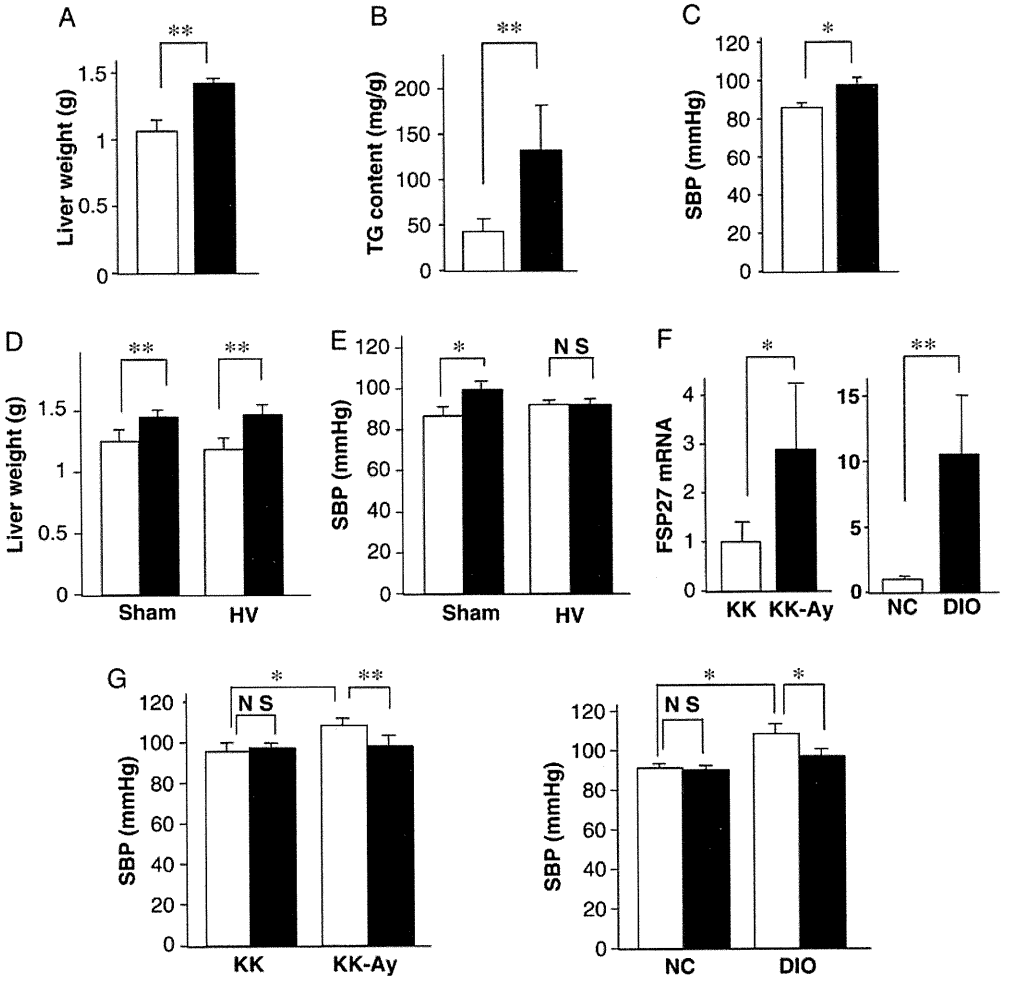



**Figure 5** Hepatic fat-specific protein 27 (Fsp27) expression is involved in obesity-related hypertension. (*A–C*) Fsp27 (black bars) or LacZ (white bars) adenovirus was administered to C57BL/6 mice. Liver weights (A), hepatic triglyceride contents (B), and systolic blood pressures (SBPs) (C) of LacZ and Fsp27 mice were measured on Day 7 after adenovirus administration ($n = 5–7$). (*D* and *E*) Mice were subjected to sham operation (sham) or hepatic vagotomy (HV) 7 days prior to adenoviral administration. Liver weights (D) and SBPs (E) of LacZ and Fsp27 mice were measured on Day 7 after adenovirus administration ($n = 4–6$). (*F*) Expression of Fsp27 mRNA was measured by quantitative reverse transcriptase–polymerase chain reaction (RT–PCR) in 8-week-old KK or KK-Ay mice and C57BL/6 mice fed normal chow (NC) or with high fat diet-induced obesity (DIO) ($n = 5–6$). (*G*) Recombinant adenovirus expressing shRNA for Fsp27 (black bars) or control scramble (white bars) was administered to KK or KK-Ay mice ($n = 5$) and NC or DIO mice ($n = 4$). SBP was measured on Day 3 after adenoviral administration. Data are presented as means ± SD. $^{**}P < 0.01$ and $^{*}P < 0.05$, compared with LacZ mice. NS, not significant.

accumulation, suggesting activation of physiological pathways downstream from PPARγ2. In addition, loss of function of PPARγ reversed these phenotypes: i.e. liver-specific knockout of PPARγ reportedly increased peripheral adiposity in murine models of obesity[26] and liver-selective knockdown reversed obesity-related hypertension (*Figure 2B* and *F*). Furthermore, and most importantly, hepatic expression and knockdown of Fsp27, a physiological target of PPARγ,[20] exerted effects similar to those of PPARγ2. Therefore, activation of the PPARγ–Fsp27 pathway in the liver is likely to induce sympathetic activation, leading to reduced peripheral adiposity and elevated BP. Systemic administration of thiazolidinediones activates PPARγ not only in the liver but also other organs/tissues throughout the body, especially adipose tissue, where PPARγ expression is markedly higher than that in the liver. In contrast, liver-selective expression and knockdown, as performed in this study, enabled us to examine the specific role of hepatic PPARγ and revealed its importance in the development of obesity-related hypertension.

In mammals, visceral innervation is generally recognized as being based on a dual nerve branch structure, involving both parasympathetic (vagal) and sympathetic nerves. Furthermore, each nerve branch consists of both afferent and efferent neurons. The sympathetic afferents from the aorta[27] or abdominal viscera[28] reportedly play important roles in BP regulation. Splanchnic (sympathetic) afferents from the liver are also involved in insulin hypersecretion during obesity development.[14] In this study, selective

pharmacological deafferentation of the hepatic vagus blocked BP elevation during obesity development in both KK-Ay mice and ZDF rats. In addition to the sympathetic afferent signals, therefore, vagal afferents from the liver modulate BP, contributing to the development of obesity-related hypertension. Infusion of fatty acids into the portal vein reportedly activates hepatic vagal function[29] and the efferent sympathetic nervous system,[30] as well as raising BP.[31] Intraperitoneal dexamethasone administration has also been shown to raise BP via a mechanism involving PPARα and vagal afferents.[21] Thus, neuronal signals, mediated by the afferent vagal nerve from the liver, are involved in determining BP in various settings.

There are numerous reports on the relationship between obesity and sympathetic activation. Several pathological states combining to produce the metabolic syndrome have been shown to be associated with increased adrenergic drive.[32] Humoral factors, including insulin and leptin, have been proposed to be involved in the development of obesity-related disorders. Insulin resistance and the resultant hyperinsulinaemia reportedly contribute to pathological phenotypes of the metabolic syndrome.[4] Hyperleptinaemia is also associated with hypertension[6,7] and renal sympathetic activation[33] in human subjects. Despite resistance to leptin's anorexigenic effect in obese states, the sympathoexcitatory effect of leptin is reportedly preserved,[34] a state termed selective leptin resistance. Thus, sympathetic activation in response to hyperleptinaemia may contribute to hypertension development in patients afflicted with the metabolic syndrome. However, in the present study, adenoviral overexpression of PPARγ in the liver raised BP despite marked decrements in both plasma insulin and leptin levels. Furthermore, dissection or deafferentation of the hepatic vagus did not decrease fasting plasma insulin or leptin levels but did block hypertension development in obesity models. In particular, in leptin receptor mutant ZDF rats, deafferentation of the hepatic vagus blocked BP elevation during obesity development, confirming that this effect is independent of leptin signalling. Thus, in addition to these humoral factors, the present study revealed the importance of the neuronal mechanism underlying obesity-related hypertension; an afferent nerve pathway from the liver which mediates pressor signals from the periphery to the brain.

As we reported previously,[13] hepatic PPARγ2 expression enhances systemic metabolic rates and lipolysis in adipose tissue. These effects were blocked by administration of a pan-β adrenergic blocker, bupranorol, suggesting involvement of sympathetic activation. The results obtained using β-less mice in this study further support the hypothesis that hepatic PPARγ2 expression enhances sympathetic tonus, leading to BP elevation. Since sympathetic activities were not measured directly, however, we cannot rule out the possibility that other, including renal and vascular, potential mechanisms are also involved in BP elevation induced by hepatic PPARγ2 expression. As described above, various mechanisms have been proposed to underlie obesity-induced hypertension, including, hyperinsulinaemia, hyperleptinaemia, and activation of the renin–angiotensin system. Oxidative and inflammatory stress, endothelial dysfunction, and decreased sensitivity to natriuretic peptides are also reportedly involved in hypertension development associated with obesity.[2,3] Therefore, it was quite unexpected that BP elevation was almost completely blocked by either hepatic PPARγ2 knockdown or hepatic vagotomy. These findings suggest the contributions of various mechanisms to differ among stages of obesity. We used young rodents and measured BP during obesity development rather than after the establishment of obesity, indicating that the neuronal mechanism originating in hepatic PPARγ2 expression is likely to play important roles in BP elevation, at least, during the course of obesity development. All results presented herein were obtained from animal obesity models, such as KK-Ay and DIO mice as well as ZDF rats. Animal studies have limitations and further examinations are, of course, required to conclude whether this neuronal mechanism is also relevant to human obese subjects.

Hepatic PPARγ expression induced both hepatic lipid accumulation and BP elevation. In search of the target downstream from PPARγ in the liver, therefore, we first examined the possibility that hepatic lipid accumulation *per se* raises BP. However, BP was not elevated in other models of hepatic steatosis, DGAT1 and DGAT2 overexpressions, in which PPARγ is not upregulated in the liver. In addition, hepatic knockdown of PPARγ in KK-Ay and DIO mice lowered BP prior to hepatic triglyceride reduction. These findings together suggest that hepatic PPARγ upregulation, rather than hepatic lipid accumulation, contributes to the development of obesity-related hypertension. Then we examined the role of Fsp27, a direct target of PPARγ.[20] Fsp27 is a member of the Cide family of proteins, also known as Cidec in humans.[35] Fat-specific protein 27 is expressed mainly in WAT but high fat feeding increases hepatic Fsp27 expression.[36] Fat-specific protein 27 deficiency inhibited lipid accumulation in the liver during high fat feeding,[37] indicating that Fsp27 mediates hepatic lipid accumulation downstream from PPARγ.[20] In the present study, overexpression of Fsp27 in the liver also resulted in remote metabolic phenotypes, including adipose tissue reduction, blood glucose lowering, and BP elevation, all of which were blocked by hepatic vagotomy. In addition, hepatic Fsp27 is upregulated in murine obesity models and its knockdown reversed the BP elevation associated with obesity development. These findings indicate that the hepatic PPARγ–Fsp27 pathway contributes to the development of obesity-related hypertension.

In conclusion, afferent vagal signals triggered by the hepatic PPARγ–Fsp27 pathway are likely to play an important role in the development of obesity-related hypertension. Thus, the liver may sense metabolic states and transmit neuronal signals modulating energy and glucose metabolism to maintain metabolic homeostasis. However, these signals may also have adverse impacts due to sympathetic overflow[13] and hyperinsulinaemia[14] during obesity development. Therefore, neuronal signals from the liver may contribute to the development of various pathological phenotypes characteristic of the metabolic syndrome.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

## Acknowledgements

We thank Drs B. B. Lowell, F. J. Gonzalez, and H. Mizuguchi for generous gifts of β-less mice, Fsp27 adenoviruses, and PPARγ

shRNA adenoviruses, respectively, and Drs M. Nakazato and Y. Minokoshi for technical advice and helpful discussions.

## Funding

This work was supported by Grants-in-Aid for Scientific Research, to H.K. (B2, 20390252) and Y.O. (A2, 19209034), a Research Fellowship to K.U. (19007896) from the Ministry of Education, Science, Sports and Culture of Japan, and a Grant-in-Aid for Scientific Research (H19-genome-005) to Y.O. from the Ministry of Health, Labor and Welfare of Japan. This work was also supported by the Global-COE Program for 'Network Medicine' to Y.O. and H.K. from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

**Conflict of interest:** none declared.

## References

1. Katagiri H, Yamada T, Oka Y. Adiposity and cardiovascular disorders: disturbance of the regulatory system consisting of humoral and neuronal signals. *Circ Res* 2007;**101**:27–39.
2. Haffner S, Taegtmeyer H. Epidemic obesity and the metabolic syndrome. *Circulation* 2003;**108**:1541–1545.
3. Sarzani R, Salvi F, Dessi-Fulgheri P, Rappelli A. Renin-angiotensin system, natriuretic peptides, obesity, metabolic syndrome, and hypertension: an integrated view in humans. *J Hypertens* 2008;**26**:831–843.
4. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities—the role of insulin resistance and the sympathoadrenal system. *N Engl J Med* 1996;**334**:374–381.
5. Bays HE, Gonzalez-Campoy JM, Bray GA, Kitabchi AE, Bergman DA, Schorr AB, Rodbard HW, Henry RR. Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. *Expert Rev Cardiovasc Ther* 2008;**6**:343–368.
6. Rosmond R, Chagnon YC, Holm G, Chagnon M, Perusse L, Lindell K, Carlsson B, Bouchard C, Bjorntorp P. Hypertension in obesity and the leptin receptor gene locus. *J Clin Endocrinol Metab* 2000;**85**:3126–3131.
7. Shankar A, Xiao J. Positive relationship between plasma leptin level and hypertension. *Hypertension* 2010;**56**:623–628.
8. Walker AB, Chattington PD, Buckingham RE, Williams G. The thiazolidinedione rosiglitazone (BRL-49653) lowers blood pressure and protects against impairment of endothelial function in Zucker fatty rats. *Diabetes* 1999;**48**:1448–1453.
9. Raji A, Seely EW, Bekins SA, Williams GH, Simonson DC. Rosiglitazone improves insulin sensitivity and lowers blood pressure in hypertensive patients. *Diabetes Care* 2003;**26**:172–178.
10. Morse SA, Zhang R, Thakur V, Reisin E. Hypertension and the metabolic syndrome. *Am J Med Sci* 2005;**330**:303–310.
11. Grassi G, Dell'Oro R, Facchini A, Quarti Trevano F, Bolla GB, Mancia G. Effect of central and peripheral body fat distribution on sympathetic and baroreflex function in obese normotensives. *J Hypertens* 2004;**22**:2363–2369.
12. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci* 2006;**7**:335–346.
13. Uno K, Katagiri H, Yamada T, Ishigaki Y, Ogihara T, Imai J, Hasegawa Y, Gao J, Kaneko K, Iwasaki H, Ishihara H, Sasano H, Inukai K, Mizuguchi H, Asano T, Shiota M, Nakazato M, Oka Y. Neuronal pathway from the liver modulates energy expenditure and systemic insulin sensitivity. *Science* 2006;**312**:1656–1659.
14. Imai J, Katagiri H, Yamada T, Ishigaki Y, Suzuki T, Kudo H, Uno K, Hasegawa Y, Gao J, Kaneko K, Ishihara H, Niijima A, Nakazato M, Asano T, Minokoshi Y, Oka Y. Regulation of pancreatic beta cell mass by neuronal signals from the liver. *Science* 2008;**322**:1250–1254.
15. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J. The organization, promoter analysis, and expression of the human PPARgamma gene. *J Biol Chem* 1997;**272**:18779–18789.
16. Rahimian R, Masih-Khan E, Lo M, van Breemen C, McManus BM, Dube GP. Hepatic over-expression of peroxisome proliferator activated receptor gamma2 in the ob/ob mouse model of non-insulin dependent diabetes mellitus. *Mol Cell Biochem* 2001;**224**:29–37.
17. Westerbacka J, Kolak M, Kiviluoto T, Arkkila P, Siren J, Hamsten A, Fisher RM, Yki-Jarvinen H. Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes* 2007;**56**:2759–2765.
18. Bachman ES, Dhillon H, Zhang CY, Cinti S, Bianco AC, Kobilka BK, Lowell BB. betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 2002;**297**:843–845.
19. Yamazaki T, Sasaki E, Kakinuma C, Yano T, Miura S, Ezaki O. Increased very low density lipoprotein secretion and gonadal fat mass in mice overexpressing liver DGAT1. *J Biol Chem* 2005;**280**:21506–21514.
20. Matsusue K, Kusakabe T, Noguchi T, Takiguchi S, Suzuki T, Yamano S, Gonzalez FJ. Hepatic steatosis in leptin-deficient mice is promoted by the PPARgamma target gene Fsp27. *Cell Metab* 2008;**7**:302–311.
21. Bernal-Mizrachi C, Xiaozhong L, Yin L, Knutsen RH, Howard MJ, Arends JJ, Desantis P, Coleman T, Semenkovich CF. An afferent vagal nerve pathway links hepatic PPARalpha activation to glucocorticoid-induced insulin resistance and hypertension. *Cell Metab* 2007;**5**:91–102.
22. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, Wang R. H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 2008;**322**:587–590.
23. Kim SM, Chen L, Faulhaber-Walter R, Oppermann M, Huang Y, Mizel D, Briggs JP, Schnermann J. Regulation of renin secretion and expression in mice deficient in beta1- and beta2-adrenergic receptors. *Hypertension* 2007;**50**:103–109.
24. Serazin V, Dieudonne MN, Morot M, de Mazancourt P, Giudicelli Y. cAMP-positive regulation of angiotensinogen gene expression and protein secretion in rat adipose tissue. *Am J Physiol Endocrinol Metab* 2004;**286**:E434–E438.
25. Fujiwara K, Hayashi K, Matsuda H, Kubota E, Honda M, Ozawa Y, Saruta T. Altered pressure-natriuresis in obese Zucker rats. *Hypertension* 1999;**33**:1470–1475.
26. Matsusue K, Haluzik M, Lambert G, Yim SH, Gavrilova O, Ward JM, Brewer B Jr, Reitman ML, Gonzalez FJ. Liver-specific disruption of PPARgamma in leptin-deficient mice improves fatty liver but aggravates diabetic phenotypes. *J Clin Invest* 2003;**111**:737–747.
27. Pagani M, Pizzinelli P, Bergamaschi M, Malliani A. A positive feedback sympathetic pressor reflex during stretch of the thoracic aorta in conscious dogs. *Circ Res* 1982;**50**:125–132.
28. Fu LW, Longhurst JC. Reflex pressor response to arterial phenylbiguanide: role of abdominal sympathetic visceral afferents. *Am J Physiol* 1998;**275**(Pt 2):H2025–H2035.
29. Randich A, Spraggins DS, Cox JE, Meller ST, Kelm GR. Jejunal or portal vein infusions of lipids increase hepatic vagal afferent activity. *Neuroreport* 2001;**12**:3101–3105.
30. Benthem L, Keizer K, Wiegman CH, de Boer SF, Strubbe JH, Steffens AB, Kuipers F, Scheurink AJ. Excess portal venous long-chain fatty acids induce syndrome X via HPA axis and sympathetic activation. *Am J Physiol Endocrinol Metab* 2000;**279**:E1286–E1293.
31. Grekin RJ, Vollmer AP, Sider RS. Pressor effects of portal venous oleate infusion. A proposed mechanism for obesity hypertension. *Hypertension* 1995;**26**:193–198.
32. Grassi G. Sympathetic overdrive and cardiovascular risk in the metabolic syndrome. *Hypertens Res* 2006;**29**:839–847.
33. Eikelis N, Schlaich M, Aggarwal A, Kaye D, Esler M. Interactions between leptin and the human sympathetic nervous system. *Hypertension* 2003;**41**:1072–1079.
34. Correia ML, Haynes WG, Rahmouni K, Morgan DA, Sivitz WI, Mark AL. The concept of selective leptin resistance: evidence from agouti yellow obese mice. *Diabetes* 2002;**51**:439–442.
35. Gong J, Sun Z, Li P. CIDE proteins and metabolic disorders. *Curr Opin Lipidol* 2009;**20**:121–126.
36. Guillen N, Navarro MA, Arnal C, Noone E, Arbones-Mainar JM, Acin S, Surra JC, Muniesa P, Roche HM, Osada J. Microarray analysis of hepatic gene expression identifies new genes involved in steatotic liver. *Physiol Genomics* 2009;**37**:187–198.
37. Nishino N, Tamori Y, Tateya S, Kawaguchi T, Shibakusa T, Mizunoya W, Inoue K, Kitazawa R, Kitazawa S, Matsuki Y, Hiramatsu R, Masubuchi S, Omachi A, Kimura K, Saito M, Amo T, Ohta S, Yamaguchi T, Osumi T, Cheng J, Fujimoto T, Nakao H, Nakao K, Aiba A, Okamura H, Fushiki T, Kasuga M. FSP27 contributes to efficient energy storage in murine white adipocytes by promoting the formation of unilocular lipid droplets. *J Clin Invest* 2008;**118**:2808–2821.

ORIGINAL

Advance Publication
doi: 10.1507/endocrj. EJ11-0145

# Recurrent hypoglycemia during pregnancies in a woman with multiple autoantibodies including anti-insulin receptor antibody and anti-platelet antibody, whose serum lowered murine blood glucose levels and phosphorylated insulin receptor of CHO-IR cells

Makiko Toshihiro[1)], Hideki Katagiri[2)], Koujiro Kataoka[3)], Akimune Fukushima[4)], Toshie Segawa[5)], Takuya Fujiwara[5)], Isao Hikichi[6)], Noriko Takebe[1)] and Jo Satoh[1)]

[1)]*Division of Diabetes and Metabolism, Iwate Medical University School of Medicine, Japan*
[2)]*Department of Metabolic Diseases, Center for Metabolic Diseases, Tohoku University Graduate School of Medicine, Japan*
[3)]*Division of Gastroenterology and Hepatology, Iwate Medical University School of Medicine, Japan*
[4)]*Department of Obstetrics and Gynecology, Iwate Medical University School of Medicine, Japan*
[5)]*Division of Endocrinology, Department of Internal Medicine, Iwate Medical University School of Medicine, Japan*
[6)]*Department of Diabetes, Morioka City Hospital, Japan*

**Abstract.** We report a rare case of recurrent hypoglycemia in a pregnant woman during the period of pregnancies. She suffered from severe hypoglycemia and intrauterine fetal death during the first pregnancy. Thereafter, there was no hypoglycemia, and no obvious cause of hypoglycemia was found by close examinations. Two years later, at eight weeks into the second pregnancy, hypoglycemia recurred. The patient had multiple auto-antibodies including anti-insulin receptor antibody and anti-platelet antibody associated with decreased platelet count. She completed the pregnancy with continuous intravenous administration of glucose that prevented hypoglycemia and finally delivered a healthy baby by Caesarian section. Both the hypoglycemia and thrombocytopenia, and the auto-antibodies disappeared after the delivery. We analyzed the patient's serum as a possible cause of hypoglycemia. Administration of the serum lowered blood glucose levels of mice more strongly than control serum. In addition, the serum phosphorylated tyrosine of insulin receptor of *Chinese hamster ovary cells* overexpressing human insulin receptors (CHO-IR cells) in vitro. These results suggest that multiple auto-antibodies might have been induced by a trigger of pregnancy, although the precise mechanism was unclear, and the anti-insulin receptor antibody and anti-platelet antibody might have induced hypoglycemia and thrombocytopenia, respectively, during the pregnancy.

*Key words*: Hypoglycemia, Pregnancy, Anti-insulin receptor antibody, Anti-platelet antibody, Phosphorylation of insulin receptor

**HYPOGLYCEMIA** is induced by various causes [1]. We experienced a case of recurrent hypoglycemia in a pregnant woman during the period of pregnancy on two separate occasions. The patient had multiple autoantibodies including anti insulin receptor (IR) antibody and anti-platelet antibody associated with thrombocytopenia. After the delivery, the titers of autoantibodies were decreased, the hypoglycemia disappeared, and thrombocytopenia improved. We analyzed the patient's serum as a possible cause of hypoglycemia. Here we report the results that the serum lowered murine blood glucose levels and phosphorylated tyrosine of insulin receptor of CHO-IR cells.

Submitted Jul. 19, 2011; Accepted Aug. 24, 2011 as EJ11-0145
Released online in J-STAGE as advance publication Sep. 17, 2011

Correspondence to: Jo Satoh, Division of Diabetes and Metabolism, Department of Internal Medicine, Iwate Medical University School of Medicine, 19-1 Uchimaru, Morioka, Iwate 020-8505, Japan.
E-mail: jsatoh@iwate-med.ac.jp



## Case Report

The patient (a 32-year-old woman) has no particular family history and past history except acute pneumonia