

The Effect of Leptin, Tumor Necrosis Factor- α (TNF- α), and Nitric Oxide (NO) Production on Insulin Resistance in Otsuka Long-Evans Fatty Rats

HIROSHI IWAI, YASUHIRO OHNO AND NORIHIKO AOKI

Department of Endocrinology, Metabolism, and Diabetology, Kinki University School of Medicine, Osaka 589-8511, Japan

Abstract. Adipocytokines and nitric oxide (NO) play important roles in type 2 diabetes; however, the regulatory mechanism has not been fully clarified. To investigate the role of adipocytokines and NO production on insulin resistance in type 2 diabetes, the LETO rats and the OLETF rats were fed a control diet or a high-fat diet for 4 weeks. After 4 weeks the blood levels of leptin, tumor necrosis factor- α (TNF- α), and NO were measured. As an indicator of insulin resistance, the homeostasis model assessment for insulin resistance (HOMA-R) was applied. Food intake in high-fat diet group rats was lower than in control diet group rats. The high fat diet increased body weight (BW), but did not significantly affect the HOMA-R and blood pressure (BP). Leptin and TNF- α levels were significantly higher in the OLETF rats than in the LETO rats, while NO levels did not change between the two groups. The high-fat diet elevated blood leptin levels, but not TNF- α and NO levels. The HOMA-R in the OLETF rats was correlated with leptin, but not with BP, BW, TNF- α or NO. NO showed an inverse correlation with BP. In conclusion, leptin, TNF- α , and NO may each regulate insulin sensitivity through their own unique pathways. The elucidation of the regulatory mechanism of adipocytokines and NO may give a clue to clarify the pathophysiology of insulin resistance.

Key words: Adipocytokine, NO, Insulin resistance, Type 2 diabetes mellitus, OLETF rats

(*Endocrine Journal* 50: 673–680, 2003)

GLUCOSE intolerance, obesity, dyslipidemia, and hypertension are found in the same individuals, and they are risk factors for coronary heart disease [1]. Based on these findings, a metabolic syndrome called syndrome X has been proposed [2], in which insulin resistance is believed to play a central role. Syndrome X is also called visceral fat obesity syndrome, suggesting that adipocytes may play an important role in insulin resistance.

To date, adipocytes are known to produce such biological effectors as free fatty acid (FFA) [3, 4], leptin [5–9], tumor necrosis factor- α (TNF- α) [10–13], adiponectin [14, 15], plasminogen activator inhibitor-1

(PAI-1) [16], and resistin [17].

Leptin is an adipocyte-specific cytokine that regulates feeding behavior and energy expenditure [6]. Leptin is associated with insulin resistance [18] and hypertension [19, 20]. Its production is regulated by hormones and cytokines such as insulin [21], glucocorticoid [22], TNF- α [23], interleukin-1 (IL-1) and transforming growth factor- β (TGF- β) [22, 24]. TNF- α secreted by mature adipocytes aggravates insulin resistance [12]. Adiponectin reverses insulin resistance by decreasing triglyceride content in muscle and liver in obese mice [14, 15]. Resistin is an adipocytokine inducing insulin resistance [17]. Drugs including β -adrenergic agonists [22] and peroxisome proliferator-activated receptor- γ (PPAR- γ) ligands [25] regulate adipocytokine production. TNF- α gene expression is inhibited by PPAR- γ ligands [26]. Although the role of adipocytokine on insulin sensitivity and the regulatory mechanism of adipocytokine production have been gradually clarified, the details of these issues still

Received: April 2, 2002

Accepted: July 10, 2003

Correspondence to: Dr. Hiroshi IWAI, Department of Endocrinology, Metabolism, and Diabetology, Kinki University School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan

remain obscure.

NO participates in the pathogenesis of many diseases via its cytotoxicity and its effects on nerve conduction, vasodilatation, and the immune system [29]. Glucose and advanced glycation end products decrease NO activity *in vitro* [30, 31]. An increase in NO production observed in rats following exercise training has been known to improve insulin resistance and leptin resistance in diabetes mellitus and obesity [32]. Urinary NO excretion decreases in parallel with the progression of insulin resistance in human type 2 diabetes [33]. Therefore, NO is suggested to be related to the pathogenesis of diabetes. Previous studies have demonstrated that adipose tissue is a central site for nitric oxide (NO) production in rats treated with lipopolysaccharide [27, 28].

To clarify the regulatory mechanism of leptin, TNF- α , and NO in insulin resistance, we investigated the effects of a high-fat diet on their production in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a type 2 diabetic model with polyphagia, obesity, and insulin resistance [34].

Materials and Methods

Experimental protocol

Male OLETF rats (diabetes strain) and the controls, Long-Evans Tokushima Otsuka (LETO) rats (Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd. Tokushima, Japan), were randomly divided into 2 groups: a control diet group (C group: C-L for LETO rats ($n = 12$), C-O for OLETF rats ($n = 12$)) and a high-fat diet group (H group: H-L for LETO rats ($n = 12$), H-O for OLETF rats ($n = 12$)). The rats at the age of 16 weeks were individually housed and observed for 4 weeks. The C group rats were fed a control powder diet (CE-2; Clea Japan, Tokyo, Japan) and the H group rats were fed a high-fat powder diet consisting of the control diet mixed with beef tallow at a ratio of 1 : 1. A control diet contained 25.4 g protein, 50.18 g carbohydrate, 4.43 g fat, 4.13 g fiber, and others 15.86 g/100 g. A high-fat diet contained 14.95 g protein, 25.09 g carbohydrate, 42.61 g fat, 2.07 g fiber, and others 15.28 g/100 g. At the end of the experiment, blood was drawn from the inferior vena cava under ether anesthesia in rats fasted for more than 16 hours and all rats were killed by exsan-

guination. Mesenteric fat pad (MES), retroperitoneal fat pad (RETRO), and epididymal fat pad (EPI) were surgically removed from 3 rats in each group and weighed as visceral fat mass. The experiments were performed in accordance with the guidelines for Animal Experiments of the Kinki University School of Medicine. Daily food intake was measured at the age of 20 weeks. Daily caloric intake was calculated from the daily food intake as 342.2 cal per 100 g of the control diet or as 543.7 cal per 100 g of the high-fat diet. Body weight (BW) and systolic blood pressure (BP) of rats were measured at the ages of 16 and 20 weeks. Systolic BP was measured 3 times by the tail-cuff method (Softron, BP-98A) and the average of 3 measurements was used for analysis.

Measurement

Fasting plasma glucose levels were measured using the hexokinase method (Wako, Osaka, Japan). Plasma TNF- α levels were measured by an enzyme-linked immunosorbent assay (ELISA) using Rat TNF- α Ultra Sensitive[®] (Biosource, Camarillo, CA, USA), with a lower limit of 2.3 pg/ml. Serum insulin levels were measured by a radioimmunoassay kit (Amersham Pharmacia Biotech, Tokyo, Japan). Serum leptin levels were measured by an ELISA kit (Amersham Pharmacia Biotech, Tokyo, Japan) with a lower limit of 0.2 ng/ml. HOMA-R was calculated as an indicator of insulin resistance [35–37] according to the formula: $\text{HOMA-R} = \text{Fasting glucose (mM)} \times \text{Fasting insulin } (\mu\text{U/ml}) / 22.5$

After deproteinization of plasma with Ultrafree[®] (Millipore, Bedford, MA, USA), plasma NO levels were measured as nitrite/nitrate using the ozone chemiluminescence method (FES-450, Scholalor Pec) [38].

Statistical analysis

Statistical analyses were performed using Statview 4.5 software (Abacus Concepts, Berkeley, CA). Student's unpaired *t* test or the analysis of variance (ANOVA) plus Fisher's protected least significant difference (PLSD) were performed for the significance of differences between groups. Linear relationships between variables of interest were assessed using Pearson correlation analysis. A value of $P < 0.05$ was regarded as statistical significance. Data are shown as mean \pm SEM.

Results

Adipose tissue weight

Food intake, caloric intake, body weight, and systolic blood pressure

Since food intake in H-O was not suppressed, caloric intake was significantly (47%) higher in H-O than in C-O ($p < 0.0001$). Although food intake was significantly lower in H-L than in C-L ($p < 0.01$), caloric intake was significantly (26%) higher in H-L than in C-L ($p < 0.05$) (Table 1).

At the start of the experiment body weight did not differ between C and H group in either OLETF or LETO rats, while body weight in H-O was significantly heavier than C-O at the age of 20 weeks. Food intake, caloric intake, and BW were significantly higher in C-O compared to C-L at the age of 20 weeks ($p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively).

Systolic BP was significantly higher in C-O than in C-L ($p < 0.001$), but no significant differences were observed in systolic BP between C and H groups in either rat strain (Table 1).

Fat pad weight in C-O was heavier than that in C-L. In LETO rats, there were no differences between C and H groups in each of the fat pad weights (Table 2). In OLETF rats there were no significant differences between C and H groups in fat pad weight of MES, EPI, RETRO and total WAT (Table 2).

Glucose, insulin levels, and HOMA-R

Fasting blood glucose levels were significantly higher in C-O than in C-L ($p < 0.001$), but they did not differ between C and H groups in each rat strain (Table 1). Mean levels of fasting blood insulin were significantly higher in C-O than in C-L ($p < 0.05$), but were not significantly higher in H groups than in C groups in each rat strain.

HOMA-R was significantly higher in C-O than in C-L ($p < 0.05$) and significantly higher in H-O than in H-L ($p < 0.0001$). Although HOMA-R was approximately 2 fold higher in H-L than in C-L and approximately 1.5 fold higher in H-O than in C-O, the differences between C-L and H-L and between C-O and H-O were not significant (Fig. 1A).

Table 1. Food intake, caloric intake, body weight, blood pressure, glucose, and insulin in each group at the age of 20 weeks.

	(n)	food intake (g/day)	caloric intake (cal/day)	body weight (g)	systolic blood pressure (mmHg)	glucose (mM)	insulin (μ U/ml)
C-L	(12)	22.7 \pm 0.6	78 \pm 2	466 \pm 9	106 \pm 3	8.1 \pm 0.4	27.5 \pm 5.4
H-L	(12)	17.9 \pm 1.6 ^b	98 \pm 8 ^a	497 \pm 11	111 \pm 5	9.2 \pm 0.5	44.5 \pm 6.3
C-O	(12)	29.1 \pm 1.5 ^c	100 \pm 5 ^c	573 \pm 20 ^c	128 \pm 3 ^c	12.0 \pm 0.5 ^c	90.5 \pm 26.4 ^a
H-O	(12)	27.0 \pm 1.4 ^f	147 \pm 7 ^{d, f}	658 \pm 8 ^{d, f}	127 \pm 4 ^e	12.0 \pm 0.4 ^f	148.6 \pm 37.5 ^e

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ vs C-L, ^d $p < 0.0001$ vs C-O, ^e $p < 0.05$, ^f $p < 0.001$ vs H-L.

Blood pressure was measured in 7-8 rats in each group. Data are shown as mean \pm SEM.

C-L, a control diet group of LETO rats; H-L, a high-fat diet group of LETO rats; C-O, a control diet group of OLETF rats; H-O, a high-fat diet group of OLETF rats.

Table 2. Fat pads weight in each group at the age of 20 weeks.

	n	MES (g)	EPI (g)	RETRO (g)	total WAT (g)
C-L	(3)	0.43 \pm 0.15	1.39 \pm 0.14	4.22 \pm 0.13	6.05 \pm 0.31
H-L	(3)	1.09 \pm 0.11	2.16 \pm 0.44	6.68 \pm 0.75	9.92 \pm 1.27
C-O	(3)	2.44 \pm 0.29 ^a	3.57 \pm 0.55	19.03 \pm 4.23 ^a	25.04 \pm 4.96 ^a
H-O	(3)	3.26 \pm 0.53 ^d	5.02 \pm 0.80 ^d	27.03 \pm 2.36 ^e	35.32 \pm 3.68 ^e

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ vs C-L, ^d $p < 0.05$, ^e $p < 0.01$ vs H-L. Data are shown as mean \pm SEM.

C-L, a control diet group for LETO rat; H-L, a high-fat diet group for LETO rat; C-O, a control diet group for OLETF rat; H-O, a high-fat diet group for OLETF rat. MES, mesenteric fat pads; EPI, epididymal fat pads; RETRO, retroperitoneal fat pads; WAT, white adipose tissue.

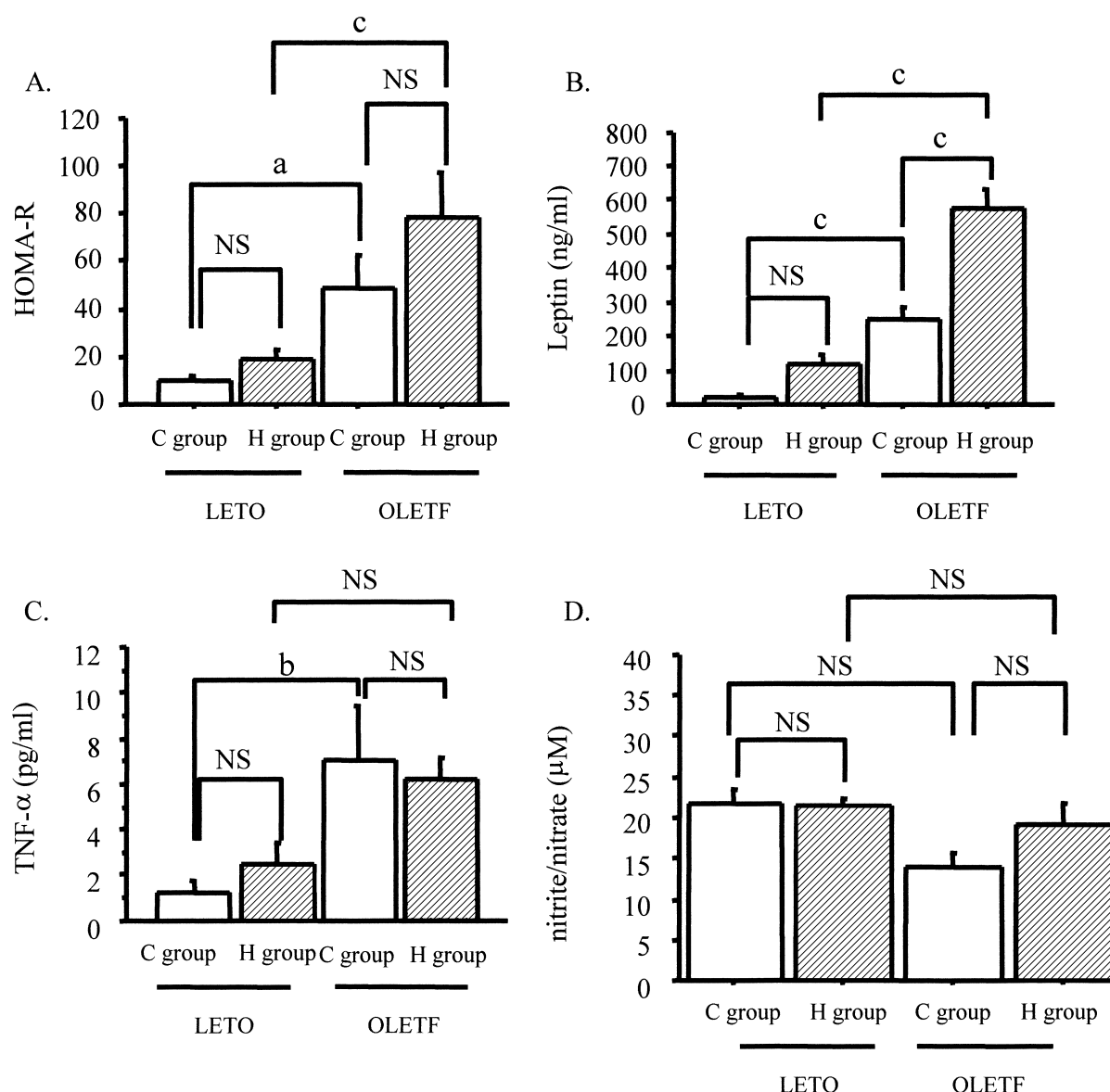


Fig. 1. Comparisons of the HOMA-R and blood levels of adipocytokines and NO (nitrite/nitrate). The number of subject in each group is 10 in A-C and 4-5 in D. Data are shown as mean \pm SEM. a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$.

Adipocytokines and NO (nitrite/nitrate)

As shown in Fig. 1B, blood leptin levels were significantly higher in C-O than in C-L ($p < 0.001$). In OLETF rats, blood leptin levels were significantly higher in H group than in C group ($p < 0.001$), while there was no difference in leptin levels between the C and H groups in LETO rats. Blood leptin levels were significantly higher in H-O than in H-L ($p < 0.001$).

Blood TNF- α levels were significantly higher in C-O than in C-L ($p < 0.01$), but they did not signifi-

cantly differ between the C and H groups in each rat strain (Fig. 1C).

There were no significant differences in blood NO (nitrite/nitrate) levels among all groups (Fig. 1D).

Relationship between HOMA-R, adipocytokines and other factors

Forty-eight rats (12 rats in C-L, 12 rats in H-L, 12 rats in C-O, 12 rats in H-O) were evaluated altogether. HOMA-R in LETO rats, OLETF rats and all rats

Table 3. Relationship between HOMA-R and adipocytokines or the other factors.

	Total			OLETF rats			LETO rats		
	n	r	p	n	r	p	n	r	p
Leptin	48	0.702	<0.0001 ^c	24	0.554	0.005 ^c	24	0.681	0.0002 ^c
TNF- α	48	0.359	0.0121 ^a	24	0.145	0.4958	24	0.399	0.0534
NO	18	0.019	0.941	9	0.65	0.0579	9	0.069	0.8594
Blood pressure	30	0.586	0.0007 ^c	16	0.329	0.2142	14	0.055	0.8511
Body weight	48	0.553	0.0001 ^c	24	0.279	0.1867	24	0.383	0.0644

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$

showed significant positive correlations with leptin ($p = 0.0002$, $r = 0.681$, $p = 0.005$, $r = 0.554$, and $p < 0.0001$, $r = 0.702$, respectively). In all rats, HOMA-R was significantly correlated with TNF- α , BP or BW ($p = 0.0121$, $r = 0.359$, $p = 0.0007$, $r = 0.586$, and $p = 0.0001$, $r = 0.553$, respectively), not with NO (nitrite/nitrate) (Table 3).

Systolic blood pressure showed an inverse correlation with blood NO (nitrite/nitrate) levels in OLETF and LETO rats ($p < 0.0001$, $r = -0.650$ and $p < 0.05$, $r = -0.221$, respectively).

Discussion

Diabetes mellitus in the OLETF rats is characterized by polygene and visceral fat accumulation and hypertriglyceridemia [39–40]. Moreover, hypertension is observed at the age of 20 weeks as shown in Table 1 and myocardial fibrosis becomes apparent at the age of 30 weeks, just as seen in human type 2 diabetes [41]. These findings suggest that the OLETF rats are useful not only as a type 2 diabetic model, but also as a model of syndrome X as well. Based on the reports above, to investigate the role of adipocytokines and NO production on insulin resistance in type 2 diabetes, we examined blood adipocytokine levels in the rats fed the high-fat diet. We expected that the high-fat diet might enhance obesity, and aggravate insulin resistance and hypertension, and increase the production of leptin and TNF- α , and decrease NO production in the OLETF rats.

Feeding behavior is suppressed by the elevation of peripheral blood leptin levels through the hypothalamus [6, 7]. In the LETO rats fed the high-fat diet, the food intake was decreased and the increase of caloric intake and BW was limited to 26% and 7%, respectively. On the other hand, in the OLETF rats

fed the high-fat diet, the food intake did not decrease and caloric intake was increased by 47%. Consequently, body weight was increased by 15%. These results indicated that abnormal feeding behavior in the OLETF rats enhanced obesity and excessive visceral fat accumulation [42].

In our study, we obtained the following results: 1. High-fat diet increased body weight and enhanced leptin production, especially in the OLETF rats, while it did not affect production of TNF- α and NO. 2. High-fat diet did not significantly change HOMA-R and blood pressure. 3. HOMA-R in the OLETF rats was correlated with leptin, but not with TNF- α or NO. 4. NO showed an inverse correlation with systolic blood pressure.

Leptin regulates feeding behavior and energy expenditure and is regulated by fat tissue volume and hormones and cytokines [6, 21–24]. As blood leptin levels in rats are followed by the increase of body weight, the excessive visceral fat accumulation is considered to be one of the causes of elevated leptin levels. TNF- α is an inflammatory cytokine produced mainly by monocytes and macrophages [43]. As TNF- α produced from adipocytes is thought to be an important molecule for insulin resistance, TNF- α in blood is regarded as an insulin resistance marker [11, 13]. Blood TNF- α levels were higher in the OLETF rats than in the LETO rats and were not enhanced by the high fat diet. Elevation of blood TNF- α levels in the OLETF rats may be the result of excessive visceral fat accumulation, not caused by the high-fat diet. This idea corresponds to previous reports that blood TNF- α levels are high in obese rats [12, 44]. NO is known as a blood pressure regulating factor [45]. NO bioavailability has been reported to decrease in rat model of hypertension induced by a high-fat diet [46]. In our study, however, the high-fat diet did not lower NO levels and did not increase blood pressure. The differ-

ence in NO production between our results and the previous report may be due to rat strain or the period of experiment.

Insulin resistance was expected to be enhanced by the change of leptin, TNF- α and NO [12, 18, 32, 33]. To evaluate insulin resistance, we used the HOMA-R, which is well correlated with M-values obtained by euglycemic hyperinsulinemic clamp [35–37]. We demonstrated that leptin played an important role in the regulation of insulin sensitivity. Some reports that leptin regulates insulin sensitivity support our results [14, 47]. We also showed that NO was inversely correlated with systolic blood pressure. These results support the hypothesis that hypertension is caused by decreased NO production in the presence of insulin resistance and hyperinsulinemia [48] and is enhanced by the sympathetic nerve activation induced by elevated leptin levels under decreased NO production

[19, 20]. The reports that leptin is involved in regulation of glucose uptake or blood pressure via NO [9, 19, 20] suggest the importance in the reciprocal correlation of leptin and NO in syndrome X.

In conclusion, leptin, TNF- α , and NO may each regulate insulin sensitivity through their own unique pathways. The elucidation of the regulatory mechanism of adipocytokines and NO may give a new clue to clarify the pathophysiology of insulin resistance.

Acknowledgments

We would like to express our thanks to Dr. Yoshitaka Nishimura, Dr. Hideaki Higashino, and Dr. Aritomo Suzuki of the Department of Pharmacology, Kinki University School of Medicine, for their cooperation in the nitric oxide measurement.

References

1. Matsuzawa Y, Funahashi T, Nakamura T (1999) Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. *Ann NY Acad Sci* 892: 146–154.
2. Reaven GM (1988) Role of insulin resistance in human disease. *Diabetes* 37: 1595–1607.
3. Shulman GI (2000) Cellular mechanisms of insulin resistance. *J Clin Invest* 106: 171–176.
4. Borden G (1997) Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46: 3–10.
5. Friedman JM (2000) Obesity in the new millennium. *Nature* 404: 632–634.
6. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269: 543–546.
7. Niimi M, Sato M, Yokote R, Tada S, Takahara J (1999) Effects of central and peripheral injection of leptin on food intake and on brain Fos expression in the Otsuka Long-Evans Tokushima Fatty rat with hyperleptinaemia. *J Neuroendocrinol* 11: 605–611.
8. Kim Y-B, Uotani S, Pierroz DD, Flier JS, Kahn BB (2000) *In vivo* administration of leptin activates signal transduction directly in insulin-sensitive tissues: overlapping but distinct pathways from insulin. *Endocrinology* 141: 2328–2339.
9. Shiuchi T, Nakagami H, Iwai M, Takeda Y, Cui T-X, Chen R, Minokoshi Y, Horiuchi M (2001) Involvement of bradykinin and nitric oxide in leptin-mediated glucose uptake in skeletal muscle. *Endocrinology* 142: 608–612.
10. Hotamisligil GS (1999) The role of TNF α and TNF receptors in obesity and insulin resistance. *J Intern Med* 245: 621–625.
11. Peraldi P, Spiegelman B (1998) TNF- α and insulin resistance: summary and future prospects. *Mol Cell Biochem* 182: 169–175.
12. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science* 259: 87–91.
13. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF- α - and obesity-induced insulin resistance. *Science* 271: 665–668.
14. Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K, Ide T, Kubota N, Terauchi Y, Tobe K, Miki H, Tsuchida A, Akanuma Y, Nagai R, Kimura S, Kadowaki T (2001) The mechanisms by which both heterozygous peroxisome proliferator-activated receptor γ (PPAR γ) deficiency and PPAR γ agonist improve insulin resistance. *J Biol Chem* 276: 41245–41254.
15. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M,

- Froguel P, Kadowaki T (2001) The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7: 941–946.
16. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, Yamashita S, Miura M, Fukuda Y, Takemura K, Tokunaga K, Matsuzawa Y (1996) Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med* 2: 800–803.
 17. Steppan CM, Balley ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar M (2001) The hormone resistin links obesity to diabetes. *Nature* 409: 307–312.
 18. Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL (1999) Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 401: 73–76.
 19. Frühbeck G (1999) Pivotal role of nitric oxide in the control of blood pressure after leptin administration. *Diabetes* 48: 903–908.
 20. Kuo JJ, Jones OB, Hall JE (2001) Inhibition of NO synthesis enhances chronic cardiovascular and renal actions of leptin. *Hypertension* 37: 670–676.
 21. Kieffer TJ, Habener JF (2000) The adipoinular axis: effects of leptin on pancreatic β -cells. *Am J Physiol Endocrinol Metab* 278: E1–E14.
 22. Sliker LJ, Sloop KW, Surface PL, Kriauciunas A, LaQuier F, Manetta J, Bue-Valleskey J, Stephens TW (1996) Regulation of expression of *ob* mRNA and protein by glucocorticoids and cAMP. *J Biol Chem* 271: 5301–5304.
 23. Kirchgesner TG, Uysal KT, Wiesbrock SM, Mario MW, Hotamisligil GS (1997) Tumor necrosis factor- α contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. *J Clin Invest* 100: 2777–2782.
 24. Granowitz EV (1997) Transforming growth factor- β enhances and pro-inflammatory cytokines inhibit *OB* gene expression in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 240: 382–385.
 25. De Vos P, Lefebvre AM, Miller SG, Guerre-Millo M, Wong K, Saladin R, Hamann LG, Staels B, Briggs MR, Auwerx J (1996) Thiazolidinediones repress *ob* gene expression in rodents via activation of peroxisome proliferator-activated receptor γ . *J Clin Invest* 98: 1004–1009.
 26. Hofmann C, Lorenz K, Braithwaite SS, Colca JR, Palazuk BJ, Hotamisligil GS, Spiegelman BM (1994) Altered gene expression of tumor necrosis factor- α and its receptors during drug and dietary modulation of insulin resistance. *Endocrinology* 134: 264–270.
 27. Ribiere C, Jaubert AM, Gaudiot N, Sabourault D, Marcus ML, Boucher JL, Denis-Henriot D, Giudicelli Y (1996) White adipose tissue nitric oxide synthase: a potential source for NO production. *Biochem Biophys Res Commun* 222: 706–712.
 28. Kapur S, Marcotte B, Marette A (1999) Mechanism of adipose tissue iNOS induction in endotoxemia. *Am J Physiol* 276: E635–E641.
 29. Moncada S, Palmer RMJ, Higgs EA (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43: 109–142.
 30. Gupta S, Sussman I, McArthur CS, Tornheim K, Cohen RA, Ruderman NB (1992) Endothelium-dependent inhibition of $\text{Na}^+\text{-K}^+$ ATPase activity in rabbit aorta by hyperglycemia: possible role of endothelium-derived nitric oxide. *J Clin Invest* 90: 727–732.
 31. Bucala R, Tracey KJ, Cerami A (1991) Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest* 87: 432–438.
 32. Sakamoto S, Minami K, Niwa Y, Ohnaka M, Nakaya Y, Mizuno A, Kuwajima M, Shima K (1998) Effect of exercise training and food restriction on endothelium-dependent relaxation in the Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous NIDDM. *Diabetes* 47: 82–86.
 33. Kurioka S, Koshimura K, Murakami Y, Nishiki M, Kato Y (2000) Reverse correlation between urine nitric oxide metabolites and insulin resistance in patients with type 2 diabetes mellitus. *Endocr J* 47: 77–81.
 34. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T (1992) Spontaneous long-term hyperglycemic rat with diabetic complications: Otsuka Long-Evans Tokushima Fatty (OLETF) Strain. *Diabetes* 41: 1422–1428.
 35. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and β -cell function fasting plasma glucose and insulin concentration in man. *Diabetologia* 28: 412–419.
 36. Pickavance LC, Tadayyon M, Widdowson PS, Buckingham RE, Wilding JPH (1999) Therapeutic index for rosiglitazone in dietary obese rats: separation of efficacy and haemodilution. *Br J Pharmacol* 128: 1570–1576.
 37. Ikeda Y, Suehiro T, Nakamura T, Kumon Y, Hashimoto K (2001) Clinical significance of the insulin resistance index as assessed by homeostasis model assessment. *Endocr J* 48: 81–86.
 38. Palmer RMJ, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327: 524–526.
 39. Moralejo DH, Wei S, Wei K, Weksler-Zangen S, Koike G, Jacob HJ, Hirashima T, Kawano K, Sugiura K, Sasaki Y, Ogino T, Yamada T, Matsumoto K (1998) Identification of quantitative trait loci for non-insulin-

- dependent diabetes mellitus that interact with body weight in the Otsuka Long-Evans Tokushima Fatty rat. *Proc Assoc Am Physicians* 110: 545–558.
40. Ishikawa M, Koga K (1998) Measurement of abdominal fat by magnetic resonance imaging of OLETF rats, an animal model of NIDDM. *Magn Reson Imaging* 16: 45–53.
 41. Yagi K, Kim S, Wanibuchi H, Yamashita T, Yamamura Y, Iwao H (1999) Characteristics of diabetes cardiac and renal complications in the OLETF rat. In: Shima K (ed). *Obesity and NIDDM: Lessons from the OLETF Rat*. Elsevier; Netherlands, 141–148.
 42. Ishikawa M, Koga K (1998) Measurement of abdominal fat by magnetic resonance imaging of OLETF rats, an animal model of NIDDM. *Magnetic Resonance Imaging* 16: 45–53.
 43. Vilcek J, Lee TH (1991) Tumor Necrosis Factor: New insights into the molecular mechanisms of its multiple actions. *J Biol Chem* 266: 7313–7316.
 44. Morin CL, Eckel RH, Marcel T, Pagliassotti MJ (1997) High fat diets elevate adipose tissue-derived tumor necrosis factor- α activity. *Endocrinology* 138: 4665–4671.
 45. Furchgott RF (1983) Role of endothelium in responses of vascular smooth muscle. *Circ Res* 53: 557–573.
 46. Dobrian AD, Davies MJ, Schriver SD, Lauterio TJ, Prewitt RL (2001) Oxidative stress in a rat model of obesity-induced hypertension. *Hypertension* 37: 554–560.
 47. Yamauchi T, Waki H, Kamon J, Murakami K, Motojima K, Komeda K, Miki H, Kubota N, Terauchi Y, Tsuchida A, Tsuboyama-Kasaoka N, Yamauchi N, Ide T, Hori W, Kato S, Fukayama M, Akanuma Y, Ezaki O, Itai A, Nagai R, Kimura S, Tobe K, Kagechika H, Shudo K, Kadowaki T (2001) Inhibition of RXR and PPAR γ ameliorates diet-induced obesity and type 2 diabetes. *J Clin Invest* 108: 1001–1013.
 48. King GL, Wakasaki H (1999) Theoretical mechanisms by which hyperglycemia and insulin resistance could cause cardiovascular diseases in diabetes. *Diabetes Care* 22 (Suppl): C31.