
Universiteit
Leiden
The Netherlands

Studies on the pathophysiological aspects of the metabolic syndrome in transgenic mice

Hu, L.

Citation
Hu, L. (2009, February 25). *Studies on the pathophysiological aspects of the metabolic syndrome in transgenic mice*. Retrieved from https://hdl.handle.net/1887/13520

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/13520

Note: To cite this publication please use the final published version (if applicable).

Chapter 2

The clearance of plasma PAI-1 is not affected in insulin resistant mice

L. Hu[1], J.F.P. Berbée[1], B.J.M. van Vlijmen[2], L.M. Havekes[1,3], J.T. Tamsma[1]

[1]Department of Endocrinology and Metabolic Diseases, General Internal Medicine; Leiden University Medical Center
[2]Einthoven Laborator for Experimental Vascular Medicine and department of Haemostasis and Thrombosis, Leiden University Medical Center;
[3]TNO-Quality of Life, Gaubius Laboratory, Leiden, The Netherlands

Submitted for publication

Summary

Increased plasma PAI-1 levels are observed in insulin resistance human subjects. It is thought that increased plasma PAI-1 levels can predict the incident of insulin resistance. This is supported by several rodent mouse models. Mice lacking PAI-1 do not develop insulin resistance. However, others showed that PAI-1-deficient mice have more adipose tissue and a worsened metabolic profile. Therefore, it is not fully understood how PAI-1 is involved in insulin resistance. In the present study we investigated 1) the plasma PAI-1 levels in diet-induced insulin resistant mice in time and 2) the contribution of the clearance of PAI-1 to the increased plasma PAI-1 levels in insulin resistant mice. We found that plasma PAI-1 levels increase in diet-induced insulin resistance and that these increased plasma PAI-1 levels follow rather than precede insulin resistance. Insulin resistance was already present after 4 weeks of high fat diet, whereas increased plasma PAI-1 levels were observed only after 12 weeks of high fat diet. Furthermore, we showed that the clearance of PAI-1 does not contribute to the increased plasma PAI-1 levels in both diet-induced and genetically insulin resistance mice. Taken together, our data support the concept that PAI-1 is not causally involved in the development of insulin resistance.

Introduction

Plasminogen activator inhibitor-1 (PAI-1) is the main physiological inhibitor of tissue-type plasminogen activator (t-PA). Increased plasma PAI-1 levels are associated with decreased fibrinolysis.[1] Several epidemiological studies have shown strong association between increased plasma PAI-1 levels on one hand, and obesity and insulin resistance on the other.[2-5] Progression of PAI-1 plasma levels in addition to initial high plasma levels are associated with incident of diabetes.[6] However, the exact mechanism of increased PAI-1 insulin resistance are incompletely understood.

The expression of PAI-1 in adipose tissues is positively correlated with obesity in human and rodents,[7-11] suggesting a possible role in the development of obesity and insulin resistance. However, animal studies on the role of PAI-1 in insulin resistance show contradicting data. Disruption of the *pai-1 gene* in ob/ob mice reduces adiposity and improves the metabolic profile as determined by glucose and insulin tolerance test.[12] Additionally, PAI-1-deficient mice do not develop diet-induced obesity and insulin resistance. Administration of synthetic PAI-1 inhibitor induces higher insulin sensitivity in WT mice.[13,14] These studies suggest that PAI-1 may not merely increase in response to obesity and insulin resistance, but may have direct causal role in obesity and insulin resistance. In contrast to these studies, others showed that PAI-1-deficient mice develop more adipose tissue.[15] In agreement with this, transgenic mice overexpressing PAI-1 have a lower body weight, lower adipose tissue mass, intraperitoneal fat and an improved metabolic profile.[16] Taken together, it is still not apparent how PAI-1 is involved in obesity and insulin resistance.

PAI-1 is known to be synthesized by various tissues including liver and adipose tissue. Increased plasma PAI-1 levels can result from increased expression from the adipose tissue. Increased mRNA expression of PAI-1 is positively correlated with obesity in human and rodents.[7,8,11] Alternatively, increased plasma PAI-1 can result from decreased plasma PAI-1 clearance. We previously showed that increased plasma PAI-1 levels result from decreased clearance in mice overexpressing receptor-associated protein, the low-density lipoprotein receptor gene family.

In the current study, we investigated the plasma PAI-1 levels during the development of insulin resistance. Additionally, we studied the contribution of the clearance of PAI-1 in insulin resistance. For this purpose, we used both diet-induced and genetically insulin resistant mouse models. Here, we show that the plasma PAI-1 levels increase in insulin resistance. Moreover, the increased plasma PAI-1 levels follow rather than precede insulin resistance. Furthermore, we also showed that the increased plasma PAI-1 levels are not due to delayed clearance in both diet-induced and genetically obese insulin resistant mice. Our data do not support the concept that PAI-1 has a direct causal role in insulin resistance. Plasma PAI-1 levels merely increase in response to insulin resistance

Material and Methods

Animals and diet

Twelve weeks old male wild-type C57Bl/6 mice (Charles River, Maastricht, The Netherlands) were housed in a temperature and humidity-controlled room on a 12:12-h light-dark cycle. Mice were fed a high fat diet (45 energy%, HFD) or a control diet (10 energy%, control) with fat derived from palm oil (Hope Farms, Woerden, The Netherlands). Male db/db mice (Charles River, Maastricht, The Netherlands) and their respective C57Bl/6 control mice were fed regular chow diet. Mice had free access to water.

All animal experiments were approved by the Animal Ethics Committee from the Leiden University Medical Center, Leiden, The Netherlands.

Blood sampling and analysis

For glucose and insulin measurements, blood was collected in EDTA-coated vials by tail bleeding. For PAI-1 antigen measurements, blood samples were obtained collected in vials containing 1/10 volume of 3.2% (w/v) citrate. Plasma was prepared by centrifugation (8000x*g* for 10 minutes at 4°C), snap-frozen and stored at -80°C prior to analysis. Mouse plasma PAI-1 antigen (Innovative Research, CA) was determined by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions. Plasma glucose was determined using commercially available kits (Instruchemie, Delftzijl, The Netherlands). Insulin was determined by a mouse insulin ELISA (Mercodia, Uppsula, Sweden). Exogenous PAI-1 decay experiments were performed as previously described.[17] In short, mice received a bolus of 1 μg/mouse purified latent murine PAI-1 (Innovative research, CA) via the tail vein. Values are expressed as percentage of PAI-1 remaining in the circulation, with the amount of PAI-1 present at 1 minute after injection considered as 100%. An one phase exponential fit was used to calculate the half-lifes (t1/2).

Hyperinsulinemic euglycemic clamp experiments

Hyperinsulinemic euglycemic clamps experiments were performed as described.[18] Mice were fasted overnight with food withdrawn at 5 p.m. the day prior to the experiments. Mice were anesthetised with 6.25 mg/kg acepromazine (Alfasan, Woerden, The Netherlands), 6.25 mg/kg midazolam (Roche, Mijdrecht, The Netherlands) and 0.31 mg/kg fentanyl (Janssen-Cilag, Tilburg, The Netherlands). Basal glucose turnover was determined by a continuous infusion of ^{14}C-glucose (GE Healthcare, Little Chalfont, U.K.) for 60 minutes. Subsequently, insulin was administered for 90 minutes to attain steady state circulating insulin levels of ~4 ng/ml. A 12.5% D-glucose solution was used to maintain euglycemia as determined at 10 min intervals via tail bleeding with a hand glucose monitor (Accu-chek, Sensor Comfort, Roche Diagnostics GmbH, Mannheim, Germany). Blood samples (60 μl) were taken during

the basal period (after 50 and 60 min) and during the hyperinsulinemic period (after 70, 80, and 90 min) to determine plasma concentrations of glucose and insulin.

Statistical analysis

Data are analysed by means of the Mann-Whitney *U* test. $P < 0.05$ was regarded as statistically significant.

Results

Body weight, plasma glucose and insulin levels in mice on a high fat diet

Male C57B1/6 mice were fed a high fat diet (HF) to induce insulin resistance. The body weight was determined at baseline, and at 4 and 12 weeks after high fat diet (HFD) or control diet. A significant increase in body weight in the HFD group was already observed from 4 weeks of HFD on (Figure 1). To confirm insulin resistance, we performed hyperinsulinemic euglycemic clamp analyses.[18] The glucose infusion rate (GIR) was significantly lower in mice fed the HFD after 4 and 12 weeks as compared to mice fed the control diet, confirming rapid onset (*i.e.* 4 weeks) of insulin resistance (Figure 2).

Plasma PAI-1 levels and clearance in diet-induced insulin resistant mice

Plasma PAI-1 levels were measured in time to study when plasma PAI-1 levels will increase during the development of insulin resistance. Plasma PAI-1 levels were not affected until 12 weeks of HFD feeding. In HFD-induced insulin resistant mice, plasma PAI-1 levels were similar between the control and HFD groups after 4 and 8 weeks of diet (Figure 3). However, after 12 weeks of HFD plasma PAI-1 levels were significantly increased as compared to the control diet (Figure 3). This increase was still present after 16 weeks of HFD. We next examined whether altered clearance of PAI-1 contributed to the observed increased plasma PAI-1 levels under prolonged HFD feeding conditions. Plasma PAI-1 clearance of intravenously administered purified murine PAI-1 were studied in diet-induced insulin resistant mice after 4 and 16 weeks of HFD or control diet. Not surprisingly, the plasma PAI-1 decay after 4 weeks of diet was not different between the control and HF diet groups, since plasma PAI-1 levels were similar between the groups (Figure 4). The half-lives at 4 weeks of diet were 12.9 ± 4.7 and 9.8 ± 3.0 minutes for control and HF group, respectively. However, after 16 weeks of HFD, the decay was also not affected by HFD as compared to control diet (Figure 4). The half-lives were 8.5 ± 1.6 and 7.9 ± 1.4 for HFD and control diet, respectively. Taken together, the increase of plasma PAI-1 levels followed the development of insulin resistance and this increase was not the consequence of decreased plasma clearance.

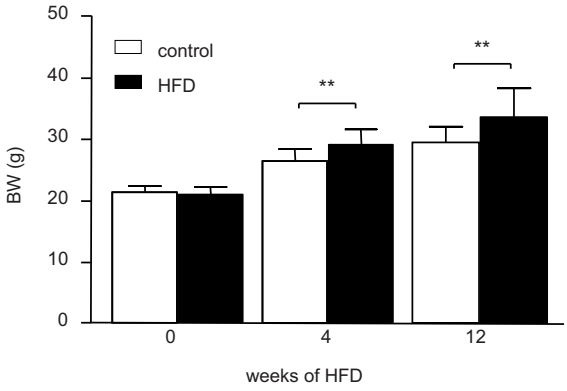

Figure 1 **Body weight**
Body weight of twelve weeks old male C57Bl/6 mice (Charles River, Maastricht, The Netherlands) at base line, 4 and 12 weeks of HFD (45 energy%) or control diet (10 energy%). The HFD and the control diet group are depicted by black and white bars, respectively. **P < 0.01, significantly different from the control group.

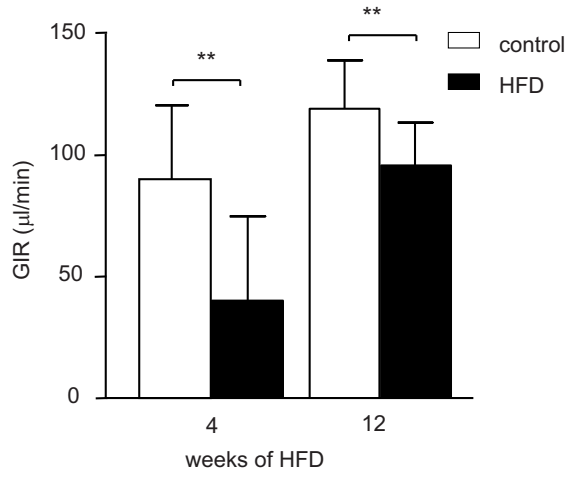

Figure 2 **Glucose infusion rate**
Glucose infusion rate as measured by hyperinsulinemic euglycemic clamp after 4 and 12 weeks of diet. GIR: glucose infusion rate. . **P < 0.01, significantly different from the control group.

Plasma PAI-1 levels and clearance in genetically insulin resistant mice

To investigate whether elevated plasma PAI-1 levels were related to genetically insulin resistance, we measured plasma PAI-1 levels and plasma PAI-1 clearance in db/db mice. The body weight of db/db mice was significantly higher as compared to wild-type C57B6/J mice (48.5 ± 3.0 vs. 22.9 ± 1.3, $P < 0.0001$). Plasma PAI-1 levels were about 5-fold higher in the genetically insulin resistant db/db mice as compared to wild-type C57B6/J mice consistent with previous findings (Figure 5A).[19]

Similar as in the diet-induced obese insulin resistance mice, plasma PAI-1 clearance was similar between the db/db and wild-type C57B6/J mice (Figure 5B). The plasma PAI-1 half-lives were 10.7 ± 5.5 and 9.1 ± 2.4 minutes for db/db and wild-type C57B6/J mice, respectively ($P = 0.56$).

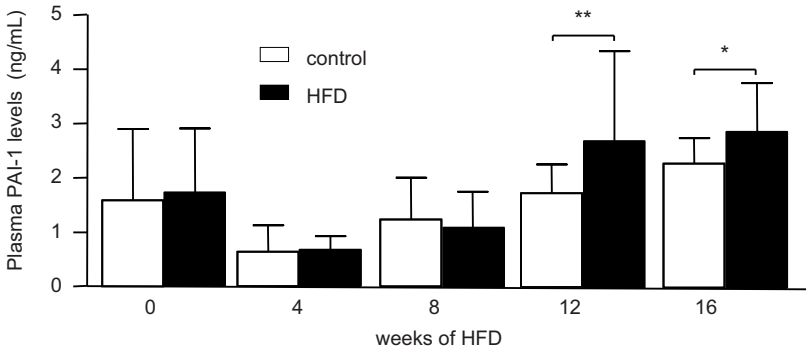

Figure 3 **Plasma PAI-1 levels in time**
Plasma PAI-1 levels at t = 0, 4, 8, 12 and weeks of diet. *P < 0.05, **P < 0.01, significantly different from the control group.

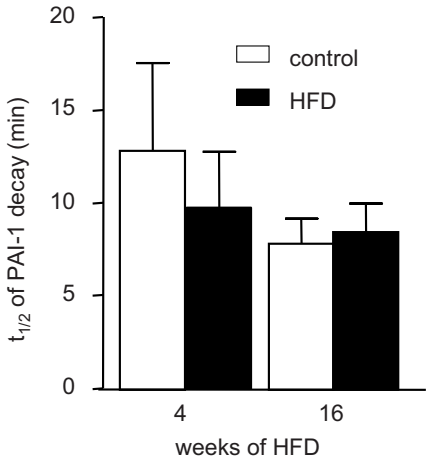

Figure 4 **Plasma PAI-1 clearance in diet-induced insulin resistant mice**
Plasma PAI-1 half-lives of diet-induced insulin resistant mice. A one-exponential fit was used to calculate the half-lives, considering the amount of PAI-1 present at 1 minute after injection as 100%.

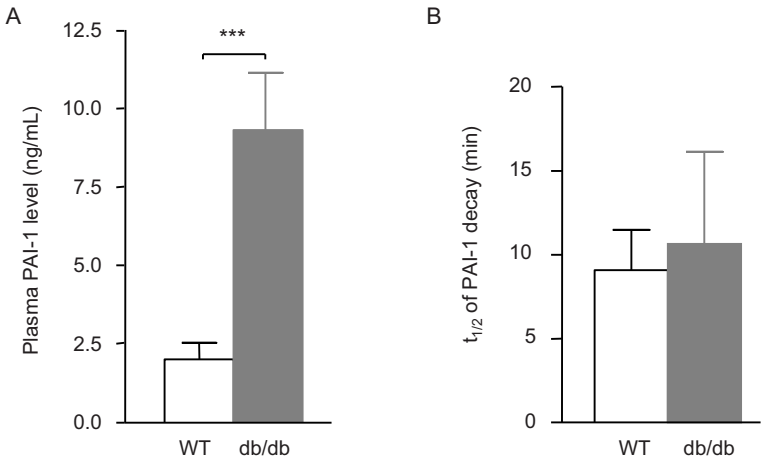

Figure 5 **Plasma PAI-1 levels and clearance in genetically insulin resistant db/db mice**
(A) Plasma PAI-1 levels of genetically insulin resistant 12-weeks old db/db mice (grey bars) and control wild-type (WT) C57Bl/6 mice (white bars). (B) Plasma PAI-1 half-lives of db/db and WT mice. ***P < 0.001, significantly different from control WT C57Bl/6J mice.

Discussion

The aim of the current study was to investigate the plasma PAI-1 levels in the development of insulin resistant mice. In addition, the clearance of plasma PAI-1 levels was studied in diet-induced and genetically insulin resistant mice. We showed that the increase of plasma PAI-1 levels follows the insulin resistance rather than preceding insulin resistance. Furthermore, the increased plasma PAI-1 levels do not find its origin in delayed clearance.

Several epidemiological studies have shown that plasma PAI-1 can predict the development of diabetes independently from other known risk factors.[3,4] The progression of PAI-1 plasma levels in addition to initial high plasma levels is thought to be associated with incident diabetes.[6] PAI-1-deficient mice do not develop insulin resistance and have improved metabolic profiles, suggesting a causal relation between PAI-1 and insulin resistance.[13,14] However, our data do not support these previous findings. We showed that the increase of plasma PAI-1 levels follows insulin resistance rather than precede insulin resistance. Insulin resistance was already present as early as 4 weeks of HFD, whereas plasma PAI-1 levels increased only after 12 weeks of HFD. Lower insulin levels and higher glucose levels were observed in mice overexpressing PAI-1.[16] The PAI-1 deficient mouse model by Morange *et al.* [15] has improved metabolic profiles. These data together with our results suggest that PAI-1 does not have a causal role in the development of insulin resistance. Increased plasma PAI-1 levels may be of clinical relevance, but a pathophysiologically epiphenomenon

of the inflammatory setting of insulin resistance. Improvement of the insulin resistance and thereby the inflammatory setting may also result in decreased plasma PAI-1 levels.[24]

The increased plasma PAI-1 in insulin resistance is thought to be the result of increased expression by the adipose tissue.[20,21] However, decreased plasma PAI-1 clearance may also result in increased plasma PAI-1 levels. We now show that the clearance of PAI-1 in both genetically and diet-induced insulin resistant mice models, does not contribute to the plasma PAI-1 levels. Therefore, the increased plasma PAI-1 levels are most likely the result of increased expression. PAI-1 is expressed in several tissues, including the adipose tissue, the liver and the endothelium. The expression of PAI-1 in the liver was similar between insulin resistant and control mice (data not shown). Insulin resistance is associated with endothelial dysfunction.[22,23] The main physiological function of PAI-1 is the inhibition of fibrinolysis of a thrombus present in the blood vessel after endothelial damage. Therefore, the vascular endothelium might also be an important source of increased PAI-1 expression in insulin resistance in addition to the adipose tissue.

In conclusion, our data demonstrate that increased plasma PAI-1 levels follow insulin resistance rather than precede insulin resistance. This plasma PAI-1 elevation does not find its origin in delayed plasma clearance.

References

1. Taeye BD, Smith LH, Vaughan DE. Plasminogen activator inhibitor-1: a common denominator in obesity, diabetes and cardiovascular disease. *Curr Opin Pharmacol* 2005;5:149-154.
2. Juhan-Vague I, Alessi MC, Vague P. Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. *Diabetologia* 1991;34:457-462.
3. Festa A, D'Agostino R, Jr., Tracy RP, Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 2002;51:1131-1137.
4. Kanaya AM, Wassel FC, Vittinghoff E, Harris TB, Park SW, Goodpaster BH, Tylavsky F, Cummings SR. Adipocytokines and incident diabetes mellitus in older adults: the independent effect of plasminogen activator inhibitor 1. *Arch Intern Med* 2006;166:350-356.
5. Meigs JB, O'Donnell CJ, Tofler GH, Benjamin EJ, Fox CS, Lipinska I, Nathan DM, Sullivan LM, D'Agostino RB, Wilson PW. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: the Framingham Offspring Study. *Diabetes* 2006;55:530-537.
6. Festa A, Williams K, Tracy RP, Wagenknecht LE, Haffner SM. Progression of plasminogen activator inhibitor-1 and fibrinogen levels in relation to incident type 2 diabetes. *Circulation* 2006;113:1753-1759.
7. Alessi MC, Bastelica D, Morange P, Berthet B, Leduc I, Verdier M, Geel O, Juhan-Vague I. Plasminogen activator inhibitor 1, transforming growth factor-beta1, and BMI are closely associated in human adipose tissue during morbid obesity. *Diabetes* 2000;49:1374-1380.
8. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, Yamashita S, Miura M, Fukuda Y, Takemura K, Tokunaga K, Matsuzawa Y. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med* 1996;2:800-803.
9. Landin K, Stigendal L, Eriksson E, Krotkiewski M, Risberg B, Tengborn L, Smith U. Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. *Metabolism* 1990;39:1044-1048.
10. Sawdey MS, Loskutoff DJ. Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo. Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta. *J Clin Invest* 1991;88:1346-1353.
11. Samad F, Yamamoto K, Loskutoff DJ. Distribution and regulation of plasminogen activator inhibitor-1 in murine adipose tissue in vivo. Induction by tumor necrosis factor-alpha and lipopolysaccharide. *J Clin Invest* 1996;97:37-46.
12. Schafer K, Fujisawa K, Konstantinides S, Loskutoff DJ. Disruption of the plasminogen activator inhibitor 1 gene reduces the adiposity and improves the metabolic profile of genetically obese and diabetic ob/ob mice. *FASEB J* 2001;15:1840-1842.
13. Ma LJ, Mao SL, Taylor KL, Kanjanabuch T, Guan Y, Zhang Y, Brown NJ, Swift LL, McGuinness OP, Wasserman DH, Vaughan DE, Fogo AB. Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. *Diabetes* 2004;53:336-346.
14. De Taeye BM, Novitskaya T, Gleaves L, Covington JW, Vaughan DE. Bone marrow plasminogen activator inhibitor-1 influences the development of obesity. *J Biol Chem* 2006;281:32796-32805.
15. Morange PE, Lijnen HR, Alessi MC, Kopp F, Collen D, Juhan-Vague I. Influence of PAI-1 on adipose tissue growth and metabolic parameters in a murine model of diet-induced obesity. *Arterioscler Thromb Vasc Biol* 2000;20:1150-1154.
16. Lijnen HR, Maquoi E, Morange P, Voros G, Van Hoef B, Kopp F, Collen D, Juhan-Vague I, Alessi MC. Nutritionally induced obesity is attenuated in transgenic mice overexpressing plasminogen activator inhibitor-1. *Arterioscler Thromb Vasc Biol* 2003;23:78-84.
17. Hu L, Bovenschen N, Havekes LM, Van Vlijmen BJ, Tamsma JT. Plasma plasminogen activator inhibitor-1 level is not regulated by the hepatic low-density lipoprotein receptor-related protein. *J Thromb Haemost* 2007;5:2301-2304.
18. Voshol PJ, Jong MC, Dahlmans VE, Kratky D, Levak-Frank S, Zechner R, Romijn JA, Havekes LM. In muscle-specific lipoprotein lipase-overexpressing mice, muscle triglyceride content is increased without inhibition of insulin-stimulated whole-body and muscle-specific glucose uptake. *Diabetes* 2001;50:2585-2590.

19. Samad F, Loskutoff DJ. The fat mouse: a powerful genetic model to study elevated plasminogen activator inhibitor 1 in obesity/NIDDM. *Thromb Haemost* 1997;78:652-655.
20. Alessi MC, Peiretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes* 1997;46:860-867.
21. Eriksson P, Reynisdottir S, Lonnqvist F, Stemme V, Hamsten A, Arner P. Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. *Diabetologia* 1998;41:65-71.
22. Duncan E, Crossey P, Walker S, Anilkumar N, Poston L, Douglas G, Ezzat V, Wheatcroft S, Shah AM, Kearney M. The effect of endothelium specific insulin resistance on endothelial function in vivo. *Diabetes* 2008.
23. Oudot A, Behr-Roussel D, Compagnie S, Caisey S, Le CO, Gorny D, Alexandre L, Giuliano F. Endothelial dysfunction in insulin resistant rats is associated with oxidative stress and cox pathway dysregulation. *Physiol Res* 2008.
24. Kruszynska YT, Yu JG, Olefsky JM, Sobel BE. Effects of troglitazone on blood concentrations of plasminogen activator inhibitor 1 in patients with type 2 diabetes and in lean and obese normal subjects. *Diabetes* 2000;49:633-639.