Regulation of tissue factor gene expression in obesity
Fahumiya Samad, Manjula Pandey, and David J. Loskutoff

Altered expression of proteins of the fibrinolytic and coagulation cascades in obesity may contribute to the cardiovascular risk associated with this condition. In spite of this, the zymogenic nature of some of the molecules and the presence of variable amounts of activators, inhibitors, and cofactors that alter their activity may make it difficult to accurately monitor changes in the activities of these proteins in tissues where they are synthesized. Thus, as a first approach to determine whether tissue factor (TF) expression is altered in obesity, this study examined changes in TF mRNA in various tissues from lean and obese (ob/ob and db/db) mice. TF gene expression was elevated in the brain, lung, kidney, heart, liver, and adipose tissues of both ob/ob and db/db mice compared with their lean counterparts. In situ hybridization analysis indicated that TF mRNA was elevated in bronchial epithelial cells in the lung, in myocytes in the heart, and in adventitial cells lining the arteries including the aortic wall. Obesity is associated with insulin resistance and hyperinsulinemia, and administration of insulin to lean mice induced TF mRNA in the kidney, brain, lung, and adipose tissue. These observations suggest that the hyperinsulinemia associated with insulin-resistant states, such as obesity and noninsulin-dependent diabetes mellitus, may induce local TF gene expression in multiple tissues. The elevated TF may contribute to the increased risk of atherothrombotic disease that accompanies these conditions.

© 2001 by The American Society of Hematology

Introduction

Obesity and related noninsulin-dependent diabetes mellitus (NIDDM) are among the most common health problems in industrialized societies and are associated with an increased incidence of thrombosis and accelerated atherosclerosis. Interestingly, a number of clinical studies have demonstrated dysregulation of both the coagulation and fibrinolytic systems in obesity/NIDDM, which suggests that these changes may contribute to the cardiovascular complications in these disorders. In this regard, several studies have shown an increase in tissue factor (TF)-mediated coagulation and/or in factor VII activity or antigen in obese patients and those with NIDDM. TF is the major cellular initiator of the coagulation cascade and also serves as a cell-surface receptor for the activation of factor VII. Activation of the coagulation cascade by aberrant expression of TF may promote thrombosis in patients with a variety of clinical disorders. These disorders include Gram-negative sepsis and atherosclerosis, as well as adult respiratory distress syndrome, systemic lupus erythematosus, Crohn disease, rheumatoid arthritis, and various forms of cancer. TF is expressed in human atherosclerotic plaques and may play a significant role in the thrombotic complications associated with plaque rupture. These observations suggest that the increase in TF in obesity and NIDDM could promote the development of a hypercoagulable state and thereby contribute to the cardiovascular complications associated with these conditions. Interestingly, a number of recent reports have demonstrated TF activity in plasma. The origin of this activity and its biologic significance remain to be established.

We previously demonstrated that levels of plasminogen activator inhibitor-1 (PAI-1) and TF gene expression were elevated in adipose tissues of genetically obese (ob/ob) mice. These mice cannot produce leptin, and, as a consequence, they experience early-onset obesity, insulin resistance, and hyperinsulinemia. In this report, we used ob/ob mice together with obese db/db mice (which lack the leptin receptor) to determine whether TF was also elevated in other tissues of the obese mice. Because of the strong correlation between obesity and hyperinsulinemia, we also asked whether these effects were mediated by insulin. Our results demonstrate that TF mRNA is significantly elevated in the brain, lung, kidney, liver, and heart of both ob/ob and db/db mice. Moreover, we show that insulin can contribute to the increase in TF gene expression in some of these tissues. The coordinated increase in TF and PAI-1 in obesity would thus be expected to increase coagulation and impair fibrinolysis, thereby promoting a state that favors thrombosis.

Materials and methods

Animals and tissue preparation

Adult male obese mice (C57BL/6J ob/ob, weight 49 ± 2.3 g; C57BL/KsJ db/db, weight 46 ± 1.9 g) at 3 months of age and their lean littermates (C57BL/6J/+ and C57BL/KsJ+/+, weight 18.1 ± 1.1 g) were obtained from Jackson Labs (Bar Harbor, ME). For insulin experiments, lean mice (C57BL/6J+/+) were injected intraperitoneally with 10 U regular human insulin (Humulin R; Eli Lilly, Indianapolis, IN), and the controls were injected with an equivalent volume of saline alone. At the conclusion of each experiment, mice were anesthetized by metofane (Pitman-Moore, Research Institute, 10550 North Torrey Pines Rd, VB-3, La Jolla, CA 92037; e-mail: loskutoff@scripps.edu.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2001 by The American Society of Hematology

From the Department of Vascular Biology, Scripps Research Institute, La Jolla, CA.

Submitted April 17, 2001; accepted August 1, 2001.

Supported by grants from the National Institutes of Health (HL 47819) and Novartis Pharmaceuticals.

Reprints: David J. Loskutoff, Department of Vascular Biology, The Scripps Institute, 10550 North Torrey Pines Rd, VB-3, La Jolla, CA 92037; e-mail: loskutoff@scripps.edu.
Mundelein, IL), and various tissues were removed and processed either for
in situ hybridization analysis or for the isolation of total RNA as described
previously.32

Quantitative reverse transcriptase–polymerase chain reaction
The concentration of TF mRNA in tissues was determined by quantitative
reverse transcriptase–polymerase chain reaction (RT-PCR) using a competi-
tor cRNA containing upstream and downstream primers for TF and β-actin
(internal control), as described previously.33-35 After reverse transcrip-
tion (using 10⁶ molecules of cRNA for TF and 10⁷ molecules for β-actin, as
determined in preliminary experiments) and PCR using ²⁹P-end–labeled 5’
primers, 20 μL of the PCR products was subjected to electrophoresis on
2.5% agarose gels. The appropriate bands corresponding to the internal
standard cRNA product and the target mRNA product were excised from
the gel, and the incorporated radioactivity was quantified using a scintilla-
tion counter. A standard curve for the internal control cRNA was con-
structed and used to determine the specific activity of the target mRNA, as
described previously.33-35 Variations in sample loading were assessed by
comparison with β-actin mRNA.

Riboprobe preparation and in situ hybridization
A subclone containing 821 bp of the mouse TF cDNA (nucleotides
229-1049) cloned into the vector pGEM-3Z10 was used to prepare a
riboprobe for in situ hybridization.37 This vector was linearized and used as
a template for in vitro transcription of radiolabeled antisense or sense
riboprobes with the use of SP6 or T7 RNA polymerase, respectively, in the
presence of [³⁵S]UTP (greater than 1200 Ci/mmol [³⁷ TBq/mmol]; Amer-
sham, Arlington Heights, IL). Both sense and antisense probes were
routinely labeled to specific activities between 0.5 and 2 × 10⁶ cpm/mg
RNA. In situ hybridization was performed as described previously.37 Slides
were exposed in the dark at 4°C for 4 to 12 weeks and then developed and
stained with hematoxylin and eosin.

Statistical analysis
Statistical comparison of results was performed using the unpaired Student
t test.

Results
TF mRNA levels in tissues from lean and obese mice
We previously demonstrated that TF gene expression was elevated in
adipose tissues of obese (ob/ob) mice.29 To determine whether it
was also elevated in other tissues, we compared the levels of TF
mRNA in a variety of tissues from lean and ob/ob mice (Figure 1).
Tissues were removed from 3-month-old mice, and total RNA was
prepared and analyzed for TF mRNA by quantitative RT-PCR. TF
mRNA levels were elevated in all of the tissues examined from the
ob/ob mice when compared with their lean counterparts (Figure
1A). For example, in the 3-month-old ob/ob mice, TF mRNA was
increased by approximately 4-fold in the brain (P < .04, n = 6)
and lung (P < .0001, n = 6), by 3-fold in the heart (P < .0001,
n = 6), and by 2-fold in the kidney (P < .02, n = 6) and liver
(P < .001, n = 6). We next determined whether TF mRNA was
elevated in tissues of the db/db mouse, a different model of genetic
obesity. Again, TF mRNA levels were elevated in all of the tissues
examined (Figure 1B), including the brain (4.7-fold; P < .003,
n = 6), the lung (5-fold; P < .002, n = 6), the heart (5-fold;
P < .0007, n = 6), the kidney (6-fold; P < .001, n = 6), the
adipose tissue (3.5-fold; P < .03, n = 6), and the liver (3.7-fold;
P < .01, n = 6). These results indicate that elevated TF mRNA is
not unique to the ob/ob mouse.

In situ hybridization experiments were performed to determine the
cellular distribution of TF mRNA within various tissues from lean
and ob/ob mice (Figures 2, 3). TF mRNA was detected in
bronchial epithelial cells in the lungs of lean mice (Figure 2A), and
this signal was markedly elevated in the same cells in lungs
from obese mice (Figure 2B). A weak but cell-specific signal for TF
mRNA was observed in cardiomyocytes in the heart of lean mice
(Figure 2C). In heart tissue from ob/ob mice, a larger proportion of
the myocytes expressed TF mRNA, and the intensity of this signal
was increased as well (Figure 2D). TF expression was observed in
adventitial cells lining the aorta (Figure 2E) and other arteries
(Figure 3A) of lean mice, and this signal for TF was also elevated in
vessels from obese mice (Figure 2F, Figure 3B). It is well
established that TF is expressed in adventitial fibroblasts surround-
ing blood vessels under normal and pathologic conditions. For
example, in 1989, Wilcox et al22 reported that TF mRNA was
expressed in fibroblastic cells in the adventitia surrounding
normal vessels. Since then, several other investigators have con-
firmed the expression of TF mRNA and antigen in fibroblastic cells
in vascular adventitia.18,19,38-43 The TF-positive adventitial cells
observed in this study (Figure 2F, Figure 3B) are thus, in all
likelihood, stromal fibroblasts of the vascular adventitia. The fact
that these cells did not stain with the smooth muscle cell–specific
marker α-actin (Dako, Carpenteria, CA; data not shown) and the
macrophage marker F4/80 (Bachem, Philadelphia, PA; data not
shown), 2 other cell types likely to be found in this location,
supports this hypothesis. It should be noted that we did not observe
TF expression in large-vessel endothelial cells in any of the tissues
examined from either lean or obese mice (Figure 2E,F, arrows;
In the liver, hepatocytes did not express TF in the lean or obese mice (Figure 2G,H, arrows). However, patches of inflammatory/Kupffer cells in the obese liver appeared to express TF mRNA (Figure 2H, arrowheads).

Regulation of TF mRNA by insulin in vivo

Insulin is increased in the plasma of obese insulin-resistant ob/ob and db/db mice because of the compensatory hyperinsulinemia that usually accompanies insulin resistance in these models. In previous studies, we demonstrated that intraperitoneal administration of insulin to lean or ob/ob mice increased PAI-1 expression in the plasma and adipose tissues. We therefore hypothesized that the elevated insulin might also induce TF gene expression in these mice. To begin to test this hypothesis, we determined the effect of exogenously administered insulin on TF gene expression in lean mice. A variety of tissues were removed 3, 6, and 24 hours after...
intrapertoneal administration of 10 U insulin, and total RNA was prepared and analyzed for TF mRNA by quantitative RT-PCR (Figure 4). Insulin induced TF mRNA in the kidney (2.5-fold; \( P < .004, n = 3 \)), lung (3-fold; \( P < .02, n = 3 \)), brain (2.5-fold; \( P < .05, n = 3 \)), and adipose tissues (2-fold; \( P < .04, n = 3 \); data not shown). Insulin did not induce TF mRNA in the heart, and TF expression in the liver decreased 3-fold after insulin treatment (data not shown). In situ hybridization analysis failed to detect specific cellular signals for TF mRNA in the kidney, lung, or brain of insulin-treated lean mice (data not shown). In the lung, however, insulin increased TF mRNA in the bronchiolar epithelial cells (Figure 5B). This pattern of TF expression in the lungs from insulin-treated lean mice was similar to the pattern of expression observed in the lungs from obese mice (Figure 2B). Taken together, these results are consistent with the hypothesis that the hyperinsulinemia associated with obesity may, in part, be responsible for the local elevation of TF mRNA observed in some tissues of the obese mice.

**Discussion**

Thrombotic episodes associated with various diseases, including atherosclerosis, septic shock, and cancer, are often correlated with increased expression of TF.\(^{17,21-23}\) Obese/NIDDM patients are at a higher risk for developing atherothrombotic disease,\(^ {1,2}\) and several studies have documented abnormalities in the coagulation system in these patients, including increases in the plasma concentrations of factor VII.\(^ {8}\) Although factor VII increases in the plasma of obese individuals, little information is available about whether TF, the cellular receptor for factor VII and the primary initiator of the coagulation cascade,\(^ {16-19}\) is also elevated. In previous studies, we demonstrated that the ob/ob mouse is a potentially useful model of human obesity because it provided novel insights into the elevation and abnormal regulation of PAI-1 gene expression in this condition.\(^ {24}\) Moreover, when compared with lean mice, genetically obese mice have higher levels of TF gene expression in their adipose tissues.\(^ {25}\) In the experiments described in the present study, we used the same model system (ie, ob/ob mice) as well as an additional model of murine obesity (db/db mice) to investigate whether TF gene expression in obese mice was altered in other tissues besides the fat. Because hyperinsulinemia is associated with obesity and appears to be an independent risk factor for cardiovascular disease,\(^ {3,46-48}\) we also investigated the effects of insulin on TF activity in plasma and on TF gene expression in tissues.

Our results demonstrate that TF mRNA is elevated in several tissues of obese mice compared with their lean counterparts, including the brain, lung, kidney, heart, adipose tissue, and liver (Figure 1A,B). In situ hybridization demonstrated elevated TF expression in extravascular cells in most of these tissues (Figure 2). For example, elevated TF mRNA was observed in the bronchiolar epithelial cells of the lung, in myocytes of the heart, in adventitial cells (probably stromal fibroblasts) of blood vessels, in tubular epithelial cells of the kidney (data not shown), and in astrocytes of the brain (data not shown). The increased expression of TF mRNA in tissues from obese mice appears to result from increased synthesis by the same cells that constitutively produce it in lean mice.\(^ {18,19,36,39,41,42}\) Many studies have demonstrated the extravascular activation of the TF-dependent coagulation pathway.\(^ {49-54}\) Thus, the increase in TF expression by extravascular cells in many tissues of the obese mice could conceivably promote a local hypercoagulable state in these tissues and thereby promote local fibrin deposition. Recent studies have demonstrated the presence of circulating and potentially active TF in the blood of healthy subjects, and this plasma TF may be involved in thrombus propagation at the site of vascular injury.\(^ {24}\) Whether elevated TF mRNA observed in tissues of obese mice in this study actually leads to elevated TF activity in the blood remains to be determined. An increase in plasma TF antigen and activity has been observed in a number of disease states, such as myocardial infarction,\(^ {25}\) unstable angina,\(^ {26}\) and sickle cell disease.\(^ {52}\) Plasma TF activity also was observed in patients with diabetes mellitus, with the concentrations being significantly higher in patients with retinopathy or nephropathy than in patients with no complications.\(^ {55}\) Finally, hypercoagulable states as a result of shedding of TF-rich microvesicles from cell surfaces have been demonstrated in cancer\(^ {56}\) and disseminated intravascular coagulation,\(^ {57,58}\) as well as in collagen disease, diabetic microangiopathy, and chronic renal failure.\(^ {59}\)

Experiments were performed to identify mechanisms that may contribute to the elevated levels of TF mRNA in tissues of the obese mice. The ob/ob and db/db mice are insulin resistant and hyperinsulinemic,\(^ {44}\) and both of these features appear to represent

![Figure 4. Induction of TF mRNA expression in lean mice by insulin. Male lean mice (C57BL/6J \(^ {+/+}\) ) 6 to 8 weeks old were injected intraperitoneally with 10 U human insulin (Himulin) or saline, and various tissues were removed 3, 6, and 24 hours later. Total RNA was prepared and analyzed for TF gene expression by quantitative RT-PCR. \( n = 3, \text{ mean } \pm \text{ SD.} \)](image)

**Figure 4.** Induction of TF mRNA expression in lean mice by insulin. Male lean mice (C57BL/6J \(^ {+/+}\) ) 6 to 8 weeks old were injected intraperitoneally with 10 U human insulin (Himulin) or saline, and various tissues were removed 3, 6, and 24 hours later. Total RNA was prepared and analyzed for TF gene expression by quantitative RT-PCR. \( n = 3, \text{ mean } \pm \text{ SD.} \)

![Figure 5. Effect of insulin on the cellular distribution of TF mRNA in the lungs of lean mice. In situ hybridization was performed on paraffin sections from the lungs of untreated (A) and insulin-treated (Himulin, 10 U; 6 hours) (B) lean (C57BL/6J) mice. Slides were exposed for 4 weeks at 4°C and stained with hematoxylin and eosin. Arrowheads indicate positive cells. Original magnification is \( \times 400 \) for both sections.](image)
important risk factors for cardiovascular disease.3,46-48 This hyper-
insulinemia may promote atherosclerosis by a number of mech-
nisms. For example, high insulin levels may stimulate mitogenic
signaling pathways leading to the proliferation of vascular endothe-
rial and smooth muscle cells.62,63 Moreover, insulin regulates
lipoprotein metabolism60,63 and stimulates the synthesis of endothe-
lin and PAI-1,60,64 both of which are atherogenic molecules. In
this study, we therefore asked whether insulin could also induce TF
expression in various tissues from lean mice. Intraperitoneal
injection of 10 U regular insulin into lean mice increased plasma
TF mRNA expression in several tissues, including kidney, lung, brain,
and adipose tissue (Figure 4). However, except in the lung, we were
unable to detect insulin-mediated increases in TF mRNA in these
tissues by using in situ hybridization. A possible explanation for
this failure may be that in these tissues, TF mRNA is widely
distributed and thus diluted below the detection threshold of the in
situ technique. According to this idea, TF mRNA would still be
detectable by the more sensitive PCR assay. In the lung, we
observed an increase in TF in patches of bronchiolar epithelial cells
(Figure 5). Induction of TF by insulin in tissues such as the kidney
may create a prothrombotic milieu, thus contributing to the diabetic
nephropathy and glomerulosclerosis often associated with obesity
and NIDDM. In this regard, recent human studies have demon-
strated that hyperinsulinemic patients have a reduced capacity to
detect TFPI, the inhibitor of tissue factor pathway inhibitor (TFPI),
the inhibitor of coagulation and fibrinolytic cascades. Our data clearly
indicate that TF mRNA expression is elevated in several
tissues of obese mice when compared with those from lean mice
and that this expression may be regulated by insulin in some
tissues. These changes in TF expression together with elevated
PAI-1 levels in obesity28 may simultaneously compromise normal
fibrin clearance mechanisms and lead to a procoagulant state.
These observations thus raise the possibility that increased coagula-
tion and impaired fibrinolysis may contribute to the increased
cardiovascular risk associated with conditions such as obesity
and NIDDM.

References
1. Larson B. Obesity, fat distribution and cardiovas-
2. Björntorp P. Abdominal fat distribution and dis-
ease: an overview of epidemiological data. Ann
3. Juhan-Vague I, Alesi MC. PAI-1, obesity, insulin
resistance and risk of cardiovascular events. Thromb
4. Juhan-Vague I, Alesi MC, Vague P. Thrombo-
genic and fibrinolytic factors and cardiovascular
risk in non-insulin-dependent diabetes mellitus.
5. Ceriello A. Coagulation activation in diabetes mel-
litus: the role of hyperglycaemia and therapeutic
6. Juhan-Vague I, Vague P. Interepivalence between
carbohydrates, lipids, and the hemostatic system
in relation to the risk of thrombotic and cardiovas-
7. Schor K. Blood vessel wall interactions in diabe-
8. Yudkin JS. Abnormalities of coagulation and fibri-
nyolysis in insulin resistance. Diabetes Care. 1999;
9. Meade TW, Ruddock V, Sirting Y, Chakrabarti T,
Miller GJ. Fibrinolytic activity, clotting factors and
the hemostatic system. Thromb Haemost. 1996;
75:7S3-75.
10. Chan P, Lin TH, Pan WH, Lee YH. Thrombophilia
meningococcal infection related to an unfavor-
plasma tissue factor levels in acute myocardial
coagulation and fibrinolysis in obese NIDDM pa-
anism on disorders of coagulation and fibrinolysis
14. Mansfield MW, Heywood DM, Grant PJ. Sex dif-
fenes in coagulation and fibrinolysis in white
subjects with non-insulin-dependent diabetes
16:160-164.
15. Kario K, Matsuo T, Kobayashi H, Matsuo M,
Sakata T, Miyata T. Activation of tissue factor-
induced coagulation and endothelial cell dysfunc-
tion in non-insulin-dependent diabetic patients
with microalbuminuria. Arteriosclerosis Thromb Vasc
16. Mann KG, van't Veer C, Cawthorn K, Butenas S.
The role of the tissue factor pathway in initiation
of coagulation. Blood Coagul Fibrinolysis. 1998;5:
S3-S7.
17. Semeraro N, Colucci M. Tissue factor in health and
18. Camerer E, Kolsto AB, Prydz H. Cell biology of
tissue factor: the principal initiator of blood coag-
19. Carmeliet P, Collen D. Molecules in focus—tissue
20. Ostendor B, Flaggstad T. Increased tissue throm-
boplastin activity in monocytes of patients with
meningococcal infection related to an unfavor-
21. Tremoli E, Camera M, Toschi V, Coll S. Tissue
factor in atherosclerosis. Atherosclerosis. 1999;
144:273-283.
22. Wilcox JN, Smith KM, Schwartz SM, Gordon D.
Localization of tissue factor in the normal vessel
wall and in the atherosclerotic plaque. Proc Natl
23. Taubman MB, Fallon JT, Schacter AD, et al. Tis-
sue factor in the pathogenesis of atherosclerosis.
borne tissue factor: another view of thrombosis.
tissue factor pathway activity in diabetics with
antistreptolysin-titer. Thromb Haemost. 1996;
75:7S3-75.
tissue factor activity with tissue factor path-
way inhibitor and prognostic factors in patients with
un-
27. Key NS, Slunsgard A, Dandelot L, et al. Whole
blood tissue factor procoagulant activity is el-
evated in patients with sickle cell disease. Blood.
1998;91:4216-4223.
28. Samad F, Loskutoff DJ. Tissue distribution and
regulation of plasminogen activator inhibitor-1 in
29. Samad F, Pandey M, Loskutoff DJ. Tissue factor
gene expression in the adipose tissues of obese
mice. Proc Natl Acad Sci U S A. 1998;95:7591-
7596.
30. Spiegelman BM, Flier JS. Adipogenesis and obe-
sity: rounding out the big picture. Cell. 1996;87:
377-389.
L, Friedman JM. Positional cloning of the mouse
32. Samad F, Yamamoto K, Loskutoff DJ. Distribution
and regulation of plasminogen activator inhibi-
tor-1 in murine adipose tissue in vivo: induction
From www.bloodjournal.org by guest on October 3, 2017. For personal use only.
Regulation of tissue factor gene expression in obesity

Fahumiya Samad, Manjula Pandey and David J. Loskutoff