Decrements in the Thrombin Activatable Fibrinolysis Inhibitor (TAFI) Levels in Association with Orlistat Treatment in Obesity

Gulay Sain Guven, MD,* Alpaslan Kılıçaslan, MD,* S. Gul Oz, MD,* Ibrahim C. Haznedaroglu, Prof MD,† Serafettin Kirazli,† Dilek Aslan, MD, MSc,‡ and Tümay Sözen, Prof MD*

*Department of Internal Medicine, Sections of *General Internal Medicine and †Hematology, and ‡Department of Public Health, Hacettepe University Medical School, Ankara, Turkey*

Summary: Obesity and its associated metabolic complications can impair the physiologic regulation of fibrinolysis, leading to a hypercoagulable state. We aimed to assess circulating thrombin activatable fibrinolysis inhibitor (TAFI) levels in obese female patients and to test the effects of orlistat-induced weight loss on basal TAFI concentrations. Obese female outpatients age 18 and older, with a body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters) of at least 30, were included into the study. Thirteen nonobese (median BMI, 22.60 kg/m^2) age-matched females were taken as controls. Plasma TAFI levels were measured before orlistat administration and after 6 months of orlistat treatment in the obese group and only one measurement was done in the control group. Twenty-seven obese patients were recruited into the study. The median TAFI level of the control group was 124.00; this value was significantly lower than the basal TAFI level of the obese group ($p < 0.001$). TAFI levels after orlistat therapy were statistically significantly lower than basal TAFI levels ($p < 0.001$) in the obese group. Hemostatic abnormalities including TAFI alterations represent a link between obesity and vascular thrombosis. Effective interventions should be considered in improving the obesity-associated prothrombotic risk profile.

Key Words: Obesity—Thrombosis—TAFI.

Obesity and its associated metabolic complications can impair physiologic regulation of fibrinolysis, leading to a hypercoagulable state. Therefore, morbid obesity is considered as an acquired prothrombotic condition resulting in a fibrinolytic deficit that can cause vascular complications (1,2). Identification of obese patient populations with an impaired fibrinolytic state is an important step toward the prevention of thrombotic events (2). Dietary restriction, lifestyle modification, and pharmacological agents, such as orlistat, are often used for the prevention and treatment of "the acquired prethrombotic state" of obesity. Thrombin activatable fibrinolysis inhibitor (TAFI) is a recently identified independent risk factor of thrombosis (3). TAFI attenuates fibrinolysis by delaying the lysis of clots mediated by all the fibrinolysis activators (4).

The aim of this study was to assess circulating TAFI levels in obese patients and to test the effects of orlistat-induced weight loss on basal TAFI concentrations. Orlistat, an inhibitor of gastrointestinal lipase, is used in the treatment of obesity by significantly reducing the absorption of dietary fat. Elucidation of the critical regulatory molecule of fibrinolysis, that is, TAFI, in morbid obese patients could help better understand the pathophysiology of the enigmatic prethrombotic state (2) of obesity. Furthermore, alterations of hemostatic molecules including TAFI after orlistat administration may be of assistance for improvement of the pharmacological management of obesity.

Address correspondence and reprint requests to Gulay Sain Guven, MD, Hacettepe University Medical School, Department of Internal Medicine, Sıhhıye, Ankara 06100, Turkey; e-mail: gsain@tr.net.

Clinical and Applied Thrombosis/Hemostasis
Vol. 12, No. 3, July 2006 364-368
DOI: 10.1177/1076029606291403

PATIENTS AND METHODS

Obese female outpatients age 18 and older, with a body mass index (5) (BMI, calculated as weight in kilograms divided by the square of height in meter) of at least 30, were included into the study. Thirteen nonobese (median BMI, 22.60 kg/m^2) age-matched females were taken as controls. Obese subjects had received consultation on dietary restriction and lifestyle modification; however, all remained obese for at least 6 months before recruitment to the study. All subjects gave written informed consent. Patients with diabetes mellitus, psychiatric or neurological disorders, a history or the presence of malignancy, significant history of cardiovascular complications (stroke, ischemic heart disease, congestive heart failure, renal impairment with a 1.5 mg/dL), and higher plasma creatinine levels were not included in the study. Likewise, subjects who had pregnancy, lactation, childbearing potential with inadequate contraceptive measures, alcohol or other substance abuse problems, previous gastrointestinal surgery for weight reduction, or who used oral contraceptives or anticoagulant therapy, were also excluded from the study.

The study was performed in a prospective cohort design. After obtaining written informed consent from all of the obese female patients, eligible subjects underwent a comprehensive medical assessment including documentation of detailed history, physical examination, anthropometric assessment, and measurement of essential laboratory variables. All of the subjects were given orlistat capsules, 120 mg three times daily, with appropriate information and warnings about adverse effects. The subjects were asked to maintain their usual diet and physical exercise. Lipid soluble vitamins were not supplemented, as the study design was planned for only 6 months. Plasma TAFI levels were measured before orlistat administration and after 6 months of orlistat treatment in the obese group, and only one measurement was done in the control group. Venous blood samples were obtained by venipuncture of the large antecubital veins of the studied patients without stasis. The samples were then centrifuged immediately; plasma was separated and stored at –80°C until measurement of TAFI. Fasting plasma total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride levels were measured at baseline and at the end of the study. TAFI levels were quantified by enzyme-linked immunosorbent assay (ELISA) method via using a commercially available assay. The computer program SPSS v10.0 was used to analyze the data. A Wilcoxon sample nonparametric test was used for comparison of TAFI levels before and after orlistat treatment. A p value below 0.05 was considered statistically significant.

RESULTS

Twenty-seven obese patients were recruited into the study. The mean age of the participants was 45.2±9.1 years. Four of 27 patients (14.8%) were smokers. None of the subjects were on a strict vegetarian diet. Seven women (25.9%) were on a regular exercise agenda. None of the subjects had another chronic disease and 17 (63%) of them mentioned a family history of obesity.

Essential clinical and laboratory parameters of the study subjects, together with TAFI levels before and after 6 months of orlistat treatment, are depicted in Table 1. TAFI levels after orlistat therapy were statistically significantly lower than the basal TAFI levels ($p < 0.001$) in obese group (Fig. 1). Median TAFI level of the control group was 124.00; this value was significantly lower than the basal TAFI level of obese group ($p < 0.001$).

Adverse events of orlistat during the study period were uncommon, apart from the effects on the gastrointestinal tract. Most gastrointestinal tract events were of mild-to-moderate intensity and occurred early during treatment. No subject withdrew from the study because of adverse effects to the gastrointestinal tract.

DISCUSSION

In this study, orlistat treatment without the confounding factors of a hypocaloric diet significantly decreased plasma TAFI concentrations in obese patients. TAFI relates to the regulation of both fibrinolytic and proinflammatory substances. Activated TAFI plays a pivotal role in various critical interactions at the cross talk between coagulation, fibrinolysis, and inflammation (6). Patients with high TAFI levels had significantly higher levels of coagulation factors and a high risk of thrombosis has been detected among them (3). TAFI is related to endothelial injury (7). Furthermore, TAFI is critically located at the delicate balance between clot formation

TABLE 1. Essential Clinical and Laboratory Parameters of the Study Subjects Together with the Thrombin Activatable Fibrinolysis Inhibitor (TAFI) Levels Before and After Six Months of the Orlistat Treatment

	Baseline	After the 6 Months of Orlistat Therapy	p Value
Anthropometrical assessment			
Weight	94.85(±16.77)	87.56(±16.68)	<0.001*
Body mass index	38.71(±6.14)	35.75(±6.37)	<0.0001*
Waist circumference	104.81(±12.10)	99.67(±12.29)	0.001*
Hip circumference	123.41(±12.67)	116.56(±13.16)	<0.001*
Metabolic profiles			
Fasting plasma glucose	90.15(±10.78)	86.96(±9.59)	0.124
Triglyceride	200.81(±40.62)	182.70(±24.18)	0.082
LDL-cholesterol	111.38(±37.17)	98.11(±23.49)	0.046*
HDL-cholesterol	58.52(±14.49)	56.59(±15.08)	0.647
VLDL-cholesterol	27.04(±12.28)	24.52(±17.01)	0.178
ALP	213.04(±60.29)	85.56(±40.87)	0.000*
ALT	21.48(±10.43)	20.89(±10.45)	0.477
GGT	20.67(±8.97)	17.85(±6.33)	0.089
TAFI	194.48(±24.50)	168.81(±31.74)	<0.001*

*Statistically significant changes after orlistat.

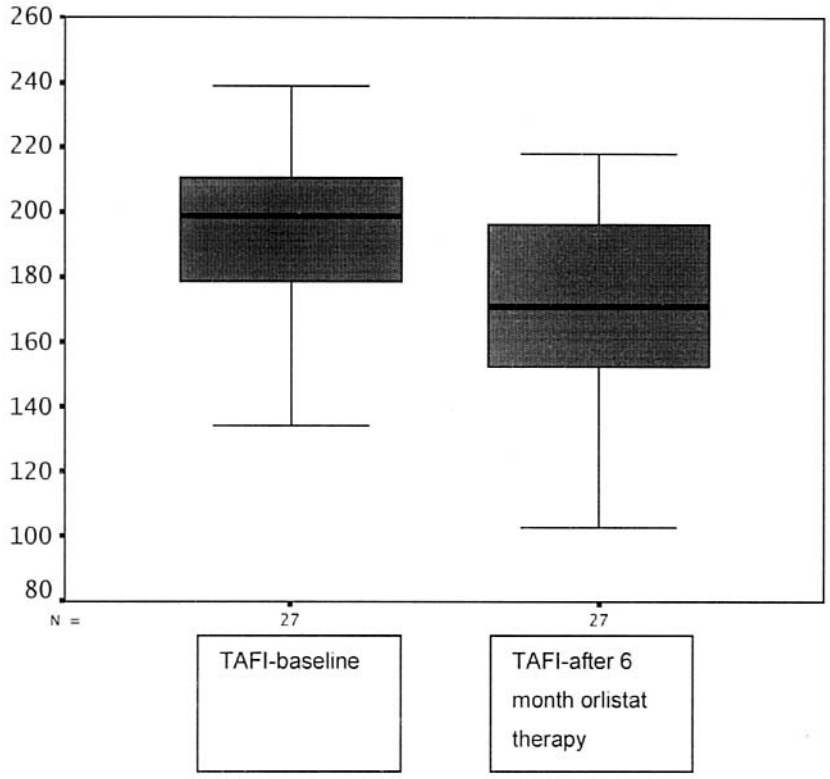

FIG. 1. TAFI levels: Baseline and after 6-month orlistat therapy in obese female patients.

and fibrinolysis, which determines clot stability (8). Therefore, the significant effect of orlistat on TAFI together with weight loss seems to be related to therapy-related hemostatic improvement in obese patients.

Short-term weight loss itself can affect fibrinolysis; the maintenance of modest weight loss is associated with long-term benefits of plasminogen activator inhibitor-1 (PAI-1) in obese women (9). Weight loss was also shown to alter PAI-1 and tissue-type plasminogen activator antigen (tPA) in obese children (10). Anthropometrical measures including weight and BMI of the obese patients have also improved during the 6 months of orlistat treatment in our study. Thus, decreased TAFI may also be associated with significant weight loss. Imbalance of the fibrinolytic potential is encountered primarily in coronary risk (11). Increased incidence of cardiovascular diseases in obese subjects has been attributed to impaired fibrinolysis. BMI may be a major determinant of an increased PAI-1 level in obesity. During weight reduction with a hypocaloric diet, the decrements in PAI-1 are directly or indirectly related to changes in adipose tissue (12). In our study, however, none of the studied subjects had been instructed to take the hypocaloric diet. Orlistat administration could decrease plasma TAFI either as a "cause" or as an "effect." Nevertheless, since decreased TAFI is associated with improved fibrinolysis (3,4,6,8,13–16), clinicopathological advantage is evident regarding occlusive vascular events during a complicated clinical course of obesity.

Thrombosis is a critical component of cardiovascular disease. Although obesity is associated with increased cardiovascular risk, the mechanism has not been fully explained. Association between obesity and a prothrombotic state has recently been examined in 3230 subjects without a history of cardiovascular disease in the Framingham Offspring Study (2). Obesity was assessed by BMI and waist-to-hip ratio. BMI was directly associated with fibrinolytic parameters. The association between increased BMI and waist-to-hip ratio and prothrombotic factors and impaired fibrinolysis suggested that obesity is a risk factor whose effect may be mediated by a prothrombotic state (2). Our novel findings on TAFI and orlistat treatment shed further light on this large study. Hence, the association between hypofibrinolysis and obesity-related thrombotic events should be researched in further studies not only for identification of the pathogenesis but also for a better clinical management of obese patients. TAFI may serve as a promising drug target (17) in obesity. Hemostatic abnormalities including TAFI alterations represent a link between obesity and vascular thrombosis. Effective interventions should be considered in improving the obesity-associated prothrombotic risk profile (18). Development of pharmacological antithrombotic strategies seems to be rational, in light of our present results and previous studies that focused on interactions of the fibrinolytic system and obesity-associated metabolic abnormalities (5,10,18–33).

REFERENCES

1. Fareed J, Hoppensteadt DA, Jeske WP, et al. Acquired defects of fibrinolysis associated with thrombosis. *Semin Thromb Hemost* 1999;25:367.
2. Rosito GA, D'Agostino RB, Massaro J, et al. Association between obesity and a prothrombotic state: The Framingham Offspring Study. *Thromb Haemost* 2004;91:683.
3. Eichinger S, Schonauer V, Weltermann A, et al. Thrombin-activatable fibrinolysis inhibitor and the risk for recurrent venous thromboembolism. *Blood* 2004;103:3773.
4. Guimaraes AH, Rijken DC. Thrombin activatable fibrinolysis inhibitor (TAFI) affects fibrinolysis in a plasminogen activator concentration-dependent manner. Study of seven plasminogen activators in an internal clot lysis model. *Thromb Haemost* 2004;91:473.
5. Bowles LK, Cooper JA, Howarth DJ, et al. Associations of haemostatic variables with body mass index: A community-based study. *Blood Coagul Fibrinolysis* 2003;14:569.
6. Bajzar L, Jain N, Wang P, Walker JB. Thrombin activatable fibrinolysis inhibitor: Not just an inhibitor of fibrinolysis. *Crit Care Med* 2004;32:S320.
7. Malyszko J, Malyszko JS, Hryszko T, Mysliwiec M. Thrombin activatable fibrinolysis inhibitor (TAFI) and markers of endothelial cell injury in dialyzed patients with diabetic nephropathy. *Thromb Haemost* 2004;91:480.
8. Bouma BN, Meijers JC. New insights into factors affecting clot stability: A role for thrombin activatable fibrinolysis inhibitor (TAFI; plasma procarboxypeptidase B, plasma procarboxypeptidase U, procarboxypeptidase R). *Semin Hematol* 2004;41:13.
9. Rissanen P, Vahtera E, Krusius T, et al. Weight change and blood coagulability and fibrinolysis in healthy obese women. *Int J Obes Relat Metab Disord* 2001;25:212.
10. Sudi KM, Gallistl S, Trobinger M, et al. The influence of weight loss on fibrinolytic and metabolic parameters in obese children and adolescents. *J Pediatr Endocrinol Metab*. 2001;14:85.
11. Juhan-Vague I, Morange P, Christine AM. Fibrinolytic function and coronary risk. *Curr Cardiol Rep* 1999;1:119.
12. Mavri A, Stegnar M, Krebs M, et al. Impact of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women. *Arterioscler Thromb Vasc Biol* 1999; 19:1582.

13. Bruni F, Pasqui AL, Pastorelli M, et al. Effect of atorvastatin on different fibrinolyis mechanisms in hypercholesterolemic subjects. *Int J Cardiol* 2004;95:269.
14. Guimaraes AH, van Tilburg NH, Vos HL, et al. Association between thrombin activatable fibrinolysis inhibitor genotype and levels in plasma: Comparison of different assays. *Br J Haematol* 2004;124:659.
15. Schneider M, Brufatto N, Neill E, Nesheim M. Activated thrombin-activatable fibrinolysis inhibitor reduces the ability of high molecular weight fibrin degradation products to protect plasmin from antiplasmin. *J Biol Chem* 2004;279:13340.
16. Watanabe T, Minakami H, Sakata Y, et al. Changes in activity of plasma thrombin activatable fibrinolysis inhibitor in pregnancy. *Gynecol Obstet Invest* 2004;58:19.
17. Zirlik A. TAFI: A promising drug target? *Thromb Haemost* 2004;91:420.
18. De Pergola G, Pannacciulli N. Coagulation and fibrinolysis abnormalities in obesity. *J Endocrinol Invest* 2002;25:899.
19. Turkoglu C, Duman BS, Gunay D, et al. Effect of abdominal obesity on insulin resistance and the components of the metabolic syndrome: Evidence supporting obesity as the central feature. *Obes Surg* 2003;13:699.
20. Aso Y, Matsumoto S, Fujiwara Y, et al. Impaired fibrinolytic compensation for hypercoagulability in obese patients with type 2 diabetes: Association with increased plasminogen activator inhibitor-1. *Metabolism* 2002; 51:471.
21. Hori Y, Gabazza EC, Yano Y, et al. Insulin resistance is associated with increased circulating level of thrombin-activatable fibrinolysis inhibitor in type 2 diabetic patients. *J Clin Endocrinol Metab* 2002;87:660.
22. Juhan-Vague I, Morange PE, Alessi MC. The insulin resistance syndrome: Implications for thrombosis and cardiovascular disease. *Pathophysiol Haemost Thromb* 2002; 32:269.
23. Kohler HP. Insulin resistance syndrome: Interaction with coagulation and fibrinolysis. *Swiss Med Wkly* 2002; 132:241.
24. Lijnen HR, Maquoi E, Demeulemeester D, et al. Modulation of fibrinolytic and gelatinolytic activity during adipose tissue development in a mouse model of nutritionally induced obesity. *Thromb Haemost* 2002;88:345.
25. Mertens I, Van Gaal LF. Obesity, haemostasis and the fibrinolytic system. *Obes Rev* 2002;3:85.
26. Cohn G, Valdes G, Capuzzi DM. Pathophysiology and treatment of the dyslipidemia of insulin resistance. *Curr Cardiol Rep* 2001;3:416.
27. Mutch NJ, Wilson HM, Booth NA. Plasminogen activator inhibitor-1 and haemostasis in obesity. *Proc Nutr Soc* 2001;60:341.
28. Juhan-Vague I, Alessi MC, Morange PE. Hypofibrinolysis and increased PAI-1 are linked to atherothrombosis via insulin resistance and obesity. *Ann Med* 2000;32(Suppl 1):78.
29. Meigs JB, Mittleman MA, Nathan DM, et al. Hyperinsulinemia, hyperglycemia, and impaired hemostasis: The Framingham Offspring Study. *JAMA* 2000;283:221.
30. Riccardi G, Rivellese AA. Dietary treatment of the metabolic syndrome—the optimal diet. *Br J Nutr* 2000;83 (Suppl 1):S143.
31. de Leiva A. What are the benefits of moderate weight loss? *Exp Clin Endocrinol Diabetes*. 1998;106(Suppl 2):10.
32. Marckmann P, Toubro S, Astrup A. Sustained improvement in blood lipids, coagulation, and fibrinolysis after major weight loss in obese subjects. *Eur J Clin Nutr* 1998;52:329.
33. Juhan-Vague I, Vague P. Hypofibrinolysis and insulin-resistance. *Diabete Metab* 1991;17:96.