t

Normal Plasma von Willebrand Factor and Thrombomodulin Levels in Type 2 Diabetic Patients with & without Retinopathy and No Effect of Metabolic Control on These Parameters

RETİNOPATİSİ OLAN VE OLMAYAN TİP 2 DIABETES MELLİTUS'LU HASTALARDA PLASMA VON WILLEBRAND FAKTÖR VE TROMBOMODUÜN DÜZEYLERİ VE BU PARAMETRELER ÜZERİNE METABOLİK KONTROLÜN ETKİSİ

Mustafa Kemal BALCI*, Ayşen TİMURAĞAOĞLU**, Hasan ALTUNBAŞ*,
Ümit KARAYALÇIN*, İhsan KARADOĞAN**, Levent ÜNDAR**

Dcpts. of *Division of Endocrinology and Metabolism, ** Division of Hematology,
Medical School of Akdeniz University, Antalya, TURKEY

Summary

Increased von Willebrand factor (vWF) and thrombomodulin (TM) levels were reported in diabetic patients and this may be associated with endothelial cell dysfunction, abnormal platelet adhesiveness and aggregation. In this study, we determined plasma TM and vWF concentrations in Type 2 diabetic patients with and without retinopathy and investigated the effects of metabolic regulation on these parameters.

TM and vWF levels were determined in 26 diabetic patients (13 female, 13 male; age 52.6±8.9years; the duration of diabetes 7.9±6.7 years). Twelve patients had background retinopathy and 14 patients had no retinopathy. Sixteen patients had good metabolic control (HbAlc<8 %) after 3 months of antihyperglycemic therapy. The control group consisted of 23 sex, age matched healthy subjects. Plasma TM and vWF concentrations were measured by EL1SA.

No statistically significant differences could be found in vWF and TM levels between control and diabetics. Good metabolic control had no effect on vWF and TM levels. There is no any statistically significant effect of retinopathy on vWF and TM levels. There was no correlation between vWF, TM, HbAlc levels and duration of diabetes mellitus.

This study showed that TM and vWF levels weren't different between control and diabetic patients with or without retinopathy, and there was not any effect of metabolic control on TM and vWF levels. Discordant results between our research and others may be due to age, duration of diabetes mellitus, degree of microvascular complications, different therapies, serum lipid levels, mono-unsaturated fat and carbohydrate intake, smoking and HLA antigens, that may all affect vlVF and TM levels.

Key Words: Type 2 diabetes mellitus,
Von Willebrand Factor, Thrombomodulin,
Retinopathy, Metabolic control

T Klin J Med Res 1999, 17:144-149

Received: April 19, 1999

Correspondance: Mustafa Kcmal BALCI
Dept. of Division of Endocrinology and
Metabolism, Medical School of
Akdcniz University, Antalya, TURKEY

Özet

Diabetik hastalarda von Willebrand faktör (vWF) ve trombomodulin (TM] düzeylerinde artışlar bildirilmiştir. Bu durum endolel hücre disfonksiyonu, anormal platelet adhezivitesi ve agregasyonu ile ilişkili olabilir. Biz bu çalışmada retinopatisi olan ve olmayan Tip 2 Diabetes mellitus'lu hastalarda plasma TM ve vWF düzeylerini ve metabolik kontrolün bu parametreler üzerine etkisini araştırdık.

TM ve vWF düzeyleri 26 diabetik hastada değerlendirildi (13 kadın, 13 erkek; yaş 52.6±8.9 yıl; diabet süresi 7.9±6.7 yıl). On iki hastada zemin diabetik retinopati mevcuttu. On dört hastada retinopati tespit edilmedi. Üç aylık antihiperglisemik tedavi sonrası iyi metabolik kontrol (HbAlc< %8) 16 hastada sağlandı. Kontrol grubu olarak 23 cins ve yaş uyumlu sağlıklı kişi alındı. Plasma TM ve vWF düzeyleri ELISA ile ölçüldü.

Kontrol grubu ve hastalar arasında plasma \>WF ve TM seviyeleri arasında anlamlı fark yoktu. İyi metabolik kontrol vWF ve TM düzeylerini etkilemedi. Retinopatisi olan ve olmayan hastalar arasında vWF ve TM düzeyleri farklı değildi. VWF, TM düzeyleri ile HbA 1c ve diabet süresi arasında korelasyon bulunmadı.

Bu çalışma plasma vWF ve TM düzeylerinin retinopatisi olan ve olmayan diabetik hastalar ile diabetik hastalar ve kontroller arasında farklı olmadığını; ayrıca metabolik kontrolün bu parametreler üzerine etkisinin olmadığını göstermiştir. Bizim çalışmamız ve literatürdeki diğer çalışmaların sonuçlarının uyuşmaması hastalar arasında yaş, diabet süresi, milcrovasküler komplikasyonların derecesi, tedaviler, lipid düzeyleri, mono-ıuısatüre yağ ve karbohidrat alımı, sigara içimi, HLA antijenleri gibi plasma vWF ve TM düzeylerini etkileyebilen faktörlerin olması ile açıklanabilir.

Anahtar Kelimeler: Tip 2 Diabetes Mellitus,
von VVillcbrand Faktör, Trombomodulin,
Retinopati, Metabolik kontrol

T Klin Araştırma 1999, 17:144-149

The most important part of the vascular system is endothelium, which regulates the function of the vascular smooth muscle and platelets. Endothelial dysfunction may be associated with multiple alterations of coagulation in diabetic patients (1).

Von Willebrand factor (vWF) is a complex glycoprotein synthesized by vascular endothelial ,ells and megakaryocytes. It is deposited in these cells, platelet alfa granules and subendothelium (2).

Thrombomodulin (TM), the membrane glycoprotein existing on the vascular endothelial cell surface, plays an important role as a cofactor in the (hrombin-catalyzcd activation of protein C (3). In addition, TM is found to inhibit the procoagulant activities of thrombin and also inhibits factor Xa activity in the prothrombinase complex (4). Thus, TM converts thrombin from a procoagulant protease to an anticoagulant and acts as a regulator of intravascular coagulation.

Increased vWF and TM levels were reported in diabetic patients that may be associated with endothelial cell dysfunction, abnormal platelet adhesiveness and aggregation (5-12). In this study, we determined plasma TM and vWF concentrations in diabetic patients with and without retinopathy and investigated the effects of metabolic regulation on these parameters.

Subjects and Methods

Plasma antigenic TM and vWF levels were detennined in 26 diabetic patients with poor metabolic control (HbA1O 8%) (Table 1). The mean age was 52.6±8.9 years and the duration of diabetes was 7.9±6.7 years (mean±SD). Thirteen patients were female, 13 were male. The patients did not suffer from major cardiovascular, hepatic and renal disease or malignancy; did not have a history of thromboembolism and had not taken any drug that could affect hemostatic parameters. Four patients were on diet only, 3 on insulin, and 19 on oral antidiabetics. Fundoscopic examination of all patients were made by an ophtalmologist. Twelve patients had background retinopathy (R +) and 14 patients had no retinopathy (R -). Sixteen patiens had good metabolic control (HbA1c < 8%) after 3 months of antihyperglycemic therapy. The control group consisted of 23 sex, age and body mass index matched healthy subjects. The design of the study was explained and informed consent was obtained from all subjects.

Table 1. Clinical characteristics and laboratory data of the control group and diabetic patients

	Type 2 Diabetic Patients	Control Group
n	26	23
Sex (M/F)	13/13	13/10
Age (year)	52.6 ± 8.9	47.5 ± 11.9
BMI(kg/m²)*	26 ±4.5	26.2 ±3.3
Duration of Diabetes (year)	7.9 ±6.7	-
HbA1c (%)**	12.2 ±2.1	5.4 ±0.6
Retinopathy (+/-)	12/14	-

**BMI =Body Mass Index , ** p<0.0001 patients vs controls. All results are given as mean±SD*

Study Design

Baseline blood samples were obtained from diabetic patients who had poor glycaemic control. Then, therapeutic regimens of the patients were reviewed and diet, physical activity, oral antidiabetics or insulin doses were adjusted according to weekly fasting and postprandial 2 hours blood glucose levels. Oral antidiabetic agent was started to 4 patients on diet, and conventional insulin treatment was switch to oral andiabetics on 2 patients. After three months, good glycemic control (HbA_{1c} <8%) was achieved in 16 patients; whereas 5 patients remained in poor glycaemic control ($HbA|_c$ >8%). Five patients dropped out from the study.

Blood Collection and Laboratory Methods

All blood samples were obtained between 8 a.m. and 10 a.m. after an overnight fasting. Blood samples were obtained with a 19 gauge needle from an antecubital vein without using a tourniquet and the first 5 ml of blood was not used for assay of TM and vWF to overcome the confounding effect of veinpuncture-induced coagulation activation. Blood samples were drawn into plastic tubes containing 3.8% sodium citrate. The tubes were immediately placed in an ice bath and centrifuged at 2500 g at 4°C for 15 minutes. The supernatant platelet poor plasma was aliquated and stored at -80°C.

TM and vWF concentrations in plasma were measured by ELISA (Asserachrom Thrombomodulin and Asserachrom vWF, Diagnostica Stago, Asnieres, France, respectively).

HbA_k. was measured by ion-exchange microcolumn method (Eagle Diagnostics Glycohemoglobin. Eagle Diagnostics, DeSoto, Texas, USA).

Statistical Methods

All results normally distrubuted. So, Student's t test and paired sampled t test was used for comparison of the groups. Pearson correlation was performed for correlation parameters, p values <0.05 were considered statistically significant. All values were given as mean ± standart deviation.

Results

Table 1 shows the clinical characteristics and laboratory data of the diabetic patients and control groups. No statistically significant differences could be found in vWF and TM levels between control and diabetic groups (Table 2). Good metabolic control ($HbA_{ıc}$ <8%) had no effect on vWF and TM levels in 16 diabetic patients (p>0.05) (Table 3). No statistically significant difference could be demonstrated between VWF and TM levels between patients with good and poor metabolic control (p>0.05) (Table 4). At the follow-up investigation baseline vWF and TM levels were not statistically different between R(-) and R(+) groups (p>0.05) (Table 5). There was no correlation between vWF, TM, HbA1c levels and duration of diabetes mellitus.

Discussion

In diabetes mellitus, microvascular dysfunction and coagulation system abnormalities result in high morbidity and mortality (13,14). Vascular endothelium is intimately involved in the maintenance of blood fluidity, modulation of the coagulation and fibrinolytic systems, regulation of vascular permeability and vessel tone, and synthesis of glycosaminoglycans, von Willebrand factor, thrombomodulin, growth factors, and subcndothelial matrix components. Disturbance of endothelial cell function represents a central position in the pathogenesis of atherosclerosis and diabetic vascular disease,

Table 2. Baseline plasma vWF and TM levels of the type 2 diabetic patients and control group

	Diabetic Patients	Control Group
TM (ng/mL)*	42.8±16.9	50.6±27.0
VWF (%)*	1.11±0.19	1.05±0.19

**p>0.05, patients vs controls. Results are given as mean±SD*

Table 3. Baseline and after 3 months plasma vWF and TM levels of patients with good metabolic control (n=16)

	Baseline	After 3 months
TM (ng/mL)*	48.25±18.61	48.40±17.86
VWF (%)*	1.11±0.17	1.U0.15
$HbA_{ıc}$ (%)**	12.1±2.8	6.7±0.8

**p>0.05, **p< 0.0001, baseline vs after 3 months. Results are given as mean±SD*

Table 4. Plasma vWF and TM levels in patients with good ($HbA_{ıc}$<8%) and POOR ($HbA_{ıc}$>8 %) metabolic control at the end of 3. months

	POOR (n=5)	GOOD (n=16)
TM (ng/mL)*	34.80±6.88	48.40±17.86
VWF (%)*	1.1liO.21	1.10±0.15
$HbA\|_c$ (%)**	9.0±0.6	6.7± 0.8

**p>0.05, **p<0.0001 poor vs good metabolic control. Results are given as mean±SD*

Table 5. Baseline TM and vWF levels in patients with (R +) and without (R -) retinopathy

	R(+)(n=12)	R(-)(0=14)
TM (ng/mL)*	52.5±24.55	39.84±10.17
VWF (%)*	1.15±0.17	1.08=1=0.18
$HbA_{ıc}$(%)*	11.2=1=1 5	12.7*3.2

**p > 0.05, retinopathy (+) vs (-) patients. Results are given as mean±SD*

nd lTia y ^ecausec^ non-enzymatic protein glytion or action of lipid peroxides. Parameters that show microvascular and macrovascular diseases (15) could be shown early before the process. New arat rieters that show early microvascular dysfunction may be useful to switch to more appropriate therapy f°r diabetes mellitus. Some authors suggest]ia i an increase of TM and vWF levels may reflect E,ly endothelial damage.

Although TM and vWF levels were found to 5c elevated in diabetics in most papers (5,14,16-**?5** 15,26-28), there were a few studies which found no diff erence °f these parameters in diabetics and controls (29). Also some papers report that the degree of retinopathy, neuropathy, nephropathy and metabolic regulation may (1,30-45), or may not (20,46-48) affect TM and vWF levels. In Type I diabetic patients Jensen and co-workers (49) found elevated levels of vWF only in patients with microalbuminuria and frank albuminuria, but not in the nornioalbuminuric group.

This study showed that TM and vWF levels weren't different between controls and diabetic patients; and there was not any effect of metabolic control and background retinopathy on TM and vWF. Plasma TM concentration did not correlate with vWF level.

Discordant results between our research and others may be due to age, mono-unsaturatcd fat and carbohydrate intake; duration of diabetes mellitus, degree of microvascular complications, different therapies, serum lipid levels, smoking and HLA antigens, that may all affect vWF and TM levels (50,51).

REFERENCES.

1. Bloomgarden ZT. Microvascular function. Diabetes Care 1995; 18(1): 144-8.
2. Porta M., La Seiva M., Molinatti P.A. von Willebrand factor and endothelial abnormalities in diabetic microangiopathy. Diabetes Care 1991; 14(suppl 1): 167-72.
3. Salem HH, Maruyama I, Islii H, Majerus PW. Isolation and characterization of thrombomodulin from human placenta, j Biol Chem 1984; 259: 12246-51.
4. Thompson EA, Salem HH. Inhibition by human thrombomodulin of factor Xa-mediatcd cleavage of prothrombin. J Clin Invest 1986; 78: 13-7.
5. Coller BS, Frank RN, Milton RC, Gralnick HR. Plasma cofactors of platelet function: Correlation with diabetic retinopathy and hemoglobins Ala-c. Ann Tnt Med 1978; 88:311-6.
6. Colwell JA, Winocour PD, Halusbka PV. Do platelets have anything to do with diabetic microvascular disease ? Diabetes 1983; 32 (Suppl 2): 14-9.
7. Collier A, Rumley A, Rumley AG, Paterson JR, Leach JP, Lowe GD, Small M. Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. Diabetes 1992; 41: 909-13.
8. Maruyama I, Shinmyozu K. Soluble thrombomodulin: as a marker of endothelial injury. Rinsho-Byori 1994; 42(6): 563-8.
9. Tani N, Hada K, Kitami A, Nakano M, Takahashi H, Ito S, Sato I, Shibata A. Effects of acetyl salicylic acid and cilostazol administration on scrum thrombomodulin concentration in diabetic patients. Thromb Res 1993; 69(1): 153-8.
10. Takahashi H, Ito S, Hanano M, Wada EC, Niwano H, Seki Y, Shibata A. Circulating thrombomodulin as a novel endothelial cell marker: comparison of its behavior with von Willebrand factor and tissue-type plasminogen activator. Am JHematol 1992; 41(1): 32-9.
11. Glassman AB. Platelet abnormalities in diabetes mellitus. Aim Clin Lab Sci 1993; 23(1) 47-50.
12. Mustard JF, Packham MA. Platelets and diabetes mellitus. NEJM 1984; 311(10): 665-6.
13. Husted S.E., Nielsen HK, Bak JF et al. Antithrombin III activity, von Willebrand fator antigen and platelet function in young diabetic patients treated with multiple insulin injections versus insulin pump treatment. E J Clin Invest 1989; 19: 90-4.
14. Knobl P, Schcmthaner G, Shnack C, et al. Haemostatic abnormalities persist despite glycaemic improvement by insulin therapy in lean type 2 diabetic patients. Throm Haemost 1994; 71(6): 692-7.
15. Matsuoka TA, Yamasaki Y, Watarai et al. Serum thrombomodulin as a marker of diabetic macroangiopathy. Diabetes Care 1995; 18(5): 731.
16. Jensen. T. Increased plasma concentration of von Willebrand factor in insulin dependent diabetics with incipient nephropathy BMJ 1989; 298: 27-8.
17. Kn6bl P, Schcmthaner G, Schnack C, Pietschmann P, Griesmacher A, Prager R, Miiller M. Thrombogenic factors are related to urinary albumin excretion rate in Type 1 (insulin-dependent) and Type 2 (noninsulin-dcpcndent) diabetic patients. Diabetologia 1993; 36: 1045-50.
18. 1washima Y, Sato T, Watanabe K, et al. Elevation of plasma thrombomodulin level in diabetic patients with early diabetic nephropathy. Diabetes 1990; 39: 983-8.
19. el Khawand C, Jamart J, Donckier J, et al. Hemostasis variables in type I diabetic patients without demnostrable vascular complications. Diabetes Care 1993; 16(8): 1137-45.

20. Lambcrton RP, Goodman AD, Kassoff A, et al. von Willebrand factor (VIII R:Ag), fibroncctin, and insulin-like growth factors I and II in diabetic retinopathy and nephropathy. Diabetes 1984; 33: 125-9.

21. Takahashi H, Ito S, Hanano M et al. Circulating thrombomudulin as a novel endothelial cell marker: comparison of its behavior with von Willebrand factor and tissue type plasminogen activator. Am JHematol 1992; 41(1): 32-9.

22. Silveira AM, Sjoberg S, Blomback M, Ostman J. von Willebrand factor antigen in plasma and urine in patients with type I (insulin-dependent) diabetes mellifus with and without nephropathy. J Diabetes Complications 1992; 6(2): 89-95.

23. Kocrt M, Nowak Gottl U, Kreuz W, Gruttner HP, Komhuber B, Breddin HK. 15 parameters of coagulation and fibrinolysis in children with type I diabetes mellitus (onset period). Klin-Padiatr 1991; 203(6): 429-32.

24. Nowak-Gottl-U, Kreuz-WD, Besicr-E, Gruttner-HP, Krackhardt-B, Breddin-HK, Kornhubcr-B. Thrombocyte function in children with Type I diabetes mellitus. Cross-sectional study Monatsschr-Kinderheilkd. 1991; 139(12): 821-5,

25. Grudc G, Pagano G, Romagnoli R, et al. Thrombomodulin levels in insulin dependent diabetic patients with microalbuminuria. DiabctMed 1995; 12 (3): 258-60.

26. Grant MB, Fitzgerald C, Guay G and Lottenberg R. Fibrinolytic capacity following stimulation with desmopressin acetate in patients with diabetes mellitus. Metabolism 1989; 38(9): 901-7.

27. Fradc LJG, Calle H de la, Alava I et al. Diabetes mellitus as a hypcrcoagulable state: Its relationship with fibrin fragments and vascular damage. Thrombosis Research 1987; 47: 533-40.

28. Galajda P, Martinka E, Mokan M, Kubisz P. Endothelial markers in diabetes mellitus Thrombosis Research 1997; 85: 63-5.

29. Graves JM, Colwell JA, Nair RM, Sarji KE. The effect of oral glucose on von Willebrand factor activity in normal and diabetic subjects. Clin Endocrinol (Oxf) 1977; 6: 437-42 .

30. Janka HU, Standi E, Schramm W and Mehnert H. Platelet enzyme activities in diabetes mellitus in relation to endothelial damage. Diabetes 1983; 32 (suppl 2): 47-51.

31. Tanaka A, Ishii H, Hiraishi S, Kazama M, Maezawa H. Increased thrombomodulin values in plasma of diabetic men with microangiopathy. Clin Chem 1991; 37(2) 269-72.

32. Deguchi A, Kawamura K, Wada H, Murashima S, Deguchi K, Shirakawa S, Ishii H. Plasma thrombomodulin levels in elderly subjects. NipponRoncn-Igakkai-Zasshi 1991; 28(2): 205-9.

33. Stchouwer CD, Stroes ES, Hackeng WH, Mulder PG, Den Ottolander GJ. von Willebrand factor and development of diabetic nephropathy in IDDM. Diabetes 1991; 40(8): 971-6.

34. Pctty RG, Pottinger BE, Greenwood RM, Pearson JD, Mahler RF. Diabetes is associated with a high incidence of endothelial-binding antibodies which do not correlate with retinopathy, von Willebrand factor, angiotensin-convcrting enzyme or C-reactive protein. Diabetes Res 1991; 17(3): 115-23.

35. Kario K, Matsuo T, Kobayashi H, Matsuo M, Sakata T, Miyata T. Activation of tissue factor-induced coagulation and endothelial cell dysfunction in non-insulin-dependent diabetic patients with microalbuminuria. Arterioscler Thromb Vase Biol 1995; 15(8): 1114-20.

36. Morise T, Takeuchi Y, Kawano M, Koni I, Takeda R. Increased plasma levels of immunoreactive endothelin and von Willebrand factor in NIDDM patients. Diabetes Care 1995; 18(1): 87-9.

37. Julius U. Blood coagulation/fibrinolytic disorders in diabetic patients. Versichcrungsmedizinl 1995; 47(2): 60-3.

38. Morise T, Takeuchi Y, Kawano M. et all Increased plasma levels of immunoreactive endothelin and von Willebrand factor in NIDDM patients. Diabetes Care 1995; 18(1): 87-9.

39. Neri S, Bruno CM, Raciti C et al. Alteration of oxide reductive and hameostatic factors in type 2 diabetics. J Int Medicine 1994; 236: 495-500.

40. Yaqoob M, Patrick AW, McClelland P, Stevenson A, Mason H, White MC, Bell GM. Relationship between markers of endothelial dysfunction, oxidant injury and tubular damage in patients with insulin-dependent diabetes mellitus. Clin Sci Colch 1993; 85(5): 557-62.

41. Stehouwer CDA, Nauta JJP, Zeldenrus GC et al. Urinary albumin excretion, cardiovascular disease, and endothelial dysfunction in non-insulin-dmepcndent diabetes mellitus. Lancet 1992; 340: 319-23.

42. Collier A, Rumley A, Rumley AG, Paterson JR, Leach JP, Lowe GD, Small-M. Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. Diabetes 1992; 41(8): 909-13.

43. Shimano H, Takahashi K, Kawakami M, Gotoda T, Harada K, Shimada M, Yazaki Y, Yamada N. Elevated serum and urinary thrombomodulin levels in patients with non-insulin-dependent diabetes mellitus. Clin Chim Acta 1994; 225(2): 89-96.

44. Abayomi EA, Miller E, Paton RC. von Willebrand factor in diabetic retinopathy. Cent Afr J Med 1996; 42: 205-6.

45. Vukovich TC, Schcrnthaner G, Knobl PN, Hay U. The effect of near-normoglycaemic control on plasma factor VIII/von Willebrand factor and fibrin degradation products in insulin dependent diabetic patients. J Clin Endocrinol Metab 1989; 69: 84-9.

46. Stehouwer CD, Zellenrath P, Polak BC, Baarsma GS, Naula JJ, Donkcr AJ, den-Ottolander GJ. von Willebrand factor and early diabetic retinopathy: no evidence for a relationship in patients with type 1 (insulin-dependent) diabetes mellitus and normal urinary albumin excretion Diabetologia 1994; 35(6): 555-9.

47. Knobl P, Schcrnthaner G, Schnack C. et al. Thrombogenic factors are related to urinary albumin excretion rate in type I (insulin-dependent) and type II (non-insulin-dependent) diabetic patients. Diabetologia 1993; 36: 1045-50.

48. Yaqoob M, Patrick A W, McClelland P, Stevenson A, Mason H, White M C, Bell G M. Relationship between markers of endothelial dysfunction, oxidant injury and tubular damage in patients with insulin-dependent diabetes mellitus. Clin SciColch 1993; 85(5): 557-62.

49. Jensen T, Knudscn JB, Rasmussen BF, et al. Features of endothelial dysfunction in early diabetic nephropathy. Lancet 1989; 4: 461-3.

50. Thomsen C, Rasmussen OW, Ingerslev J, Hcrmansen K. Plasma levels of von Willebrand factor in non-insulin-dependent diabetes mellitus are influenced by dietary monounsaturated fatty acids. Thrombosis Research 1995; 77(4): 347-56.

51. Rasmussen O, Thomsen C, Ingerslev J, et al. Decrease in von Willebrand factor levels after a high-monounsaturated fat diet in non-insulin-dependent diabetic subjects. Metabolism 1994; 43(11): 1406-9.