Original Paper

Raised Plasma Urotensin II in Type 2 Diabetes Patients Is Associated With the Metabolic Syndrome Phenotype

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Urotensin II (UII) exerts multiple effects on the cardiovascular system, acts as a diabetogenic agent, and may also contribute to the development of the metabolic syndrome (MetS). The aim of this study was to determine circulating UII in patients with type 2 diabetes mellitus (T2DM) and its relationship with MetS. A total of 360 consecutive patients with T2DM were included. MetS presence/absence (MetS [+]/[−]) was defined according to American Heart Association/National Heart, Lung and Blood Institute criteria. Plasma concentrations of UII were determined by radioimmunoassay. UII levels were significantly higher in MetS (+) than in MetS (−) T2DM patients (0.97 pg/mL [0.93–1.01], n=294 vs 0.82 pg/mL [0.75–0.88] pg/mL, n=66, respectively; P<.001*). Multiple logistic regression analysis showed that UII was significantly associated with MetS (+) (odds ratio, 6.41 [95% confidence interval, 1.21–16.04];* P=.02*). UII plasma concentrations are significantly higher in T2DM patients presenting with MetS. Therefore, circulating UII may participate in the worsening course of some T2DM patients and may provide novel therapeutic perspectives.* J Clin Hypertens (Greenwich). *2010;12:653–660.* ©*2010 Wiley Periodicals, Inc.*

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Urotensin II (UII) is a cyclic undecapeptide first isolated from the teleost fish *Gillichthys mirabiliss* where it participates in osmoregulation[1] and for which a human isoform of UII was identified in 1998.[2] UII is considered as the most potent vasoconstrictor identified so far in mammals.[3] The G protein–coupled receptor UT, a human homologue of the rat orphan receptor GPR14, was identified as the UII endogenous receptor.[3]

UII and its receptors are largely distributed throughout the cardiovascular system and UII has emerged as a contributor to cardiovascular physiopathology.[4–6] Recently, Chen and colleagues[7] have reported that UII is secreted from the heart and multiple other tissues into the circulation. Furthermore, these authors have observed an increase of UII immunoreactivity in persons with acute coronary syndrome. UII concentrations are also reported to be increased in heart failure and related to disease severity.[8,9] In other recent studies, the role of the UII system in the physiopathology of diabetes and the metabolic syndrome (MetS) have been suggested on the basis of its potential contribution to the development of hyperglycemia, insulin resistance, essential hypertension, and pro-inflammatory state.[10]

The rapid increase in type 2 diabetes mellitus (T2DM) and MetS prevalence is alarming, affecting

all age groups across most ethnogeographic boundaries.[12–15] T2DM and MetS are associated with higher risk for developing cardiovascular complications and microangiopathy,[16,17] as a result of overlapping occurrence of truncal fat distribution, overweight, hypertension, atherogenic dyslipidemia, systemic inflammation, insulin resistance, a procoagulant/hypofibrinolytic state, and hyperglycemia in the subset of patients with impaired fasting glucose.[14,18] Circulating biomarkers are increasingly used for patient risk stratification, cardiometabolic risk estimation and to support primary and secondary prevention initiatives.[19–21]

The aim of the present study was to determine the circulating UII levels in a population of T2DM patients and to evaluate the relationship of UII with MetS.

MATERIALS AND METHODS

Study Design

We studied 360 consecutive T2DM outpatients followed at the Cliniques Universitaires St-Luc in Brussels. T2DM was defined according to the Experts Committee criteria.[17] Mean age (1 SD) was 68 (11) years, sex ratio (male:female) was 67:33, and known diabetes duration was 16 (9) years. Hypertension was considered in patients treated with antihypertensive drugs and/or in patients with previously diagnosed hypertension (blood pressure [BP] >140/90 mm/Hg). MetS was defined according to the 2005 American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) statement criteria, with 1–5/5 discrete items scoring: MetS (−) for 1 to 2/5 score(s); MetS (+) for 3 to 5/5 scores.[14] Briefly, the 5 criteria considered were: elevated waist circumference (>102 cm in men and >88 cm in women), elevated triglyceride levels (>150 mg/dL or on drug treatment for elevated triglycerides), reduced high-density lipoprotein (HDL-C) level (<40 mg/dL in men and <50 mg/dL in women or on drug treatment for reduced HDL-C), elevated BP (>130 mm Hg systolic BP or >85 mm Hg diastolic BP or on antihypertensive drug treatment in a patient with history of hypertension), and elevated fasting glucose (>100 mg/dL or on drug treatment for elevated glucose). Each patient gave informed consent, and the protocol was approved by the local institutional review board.

Analytical Methods

All lipid values were obtained in the fasting state. Plasma lipids and creatinine were measured using conventional methods. Triglycerides and high-sensitivity C-reactive protein (hs-CRP) were measured using colorimetric and turbidimetric methods on a Beckman Coulter LX20 analyzer (Beckman Coulter Inc, Fullerton, CA). Glycated hemoglobin A_{1c} (HbA_{1c}) was determined by ion-exchange HPLC. Creatinine clearance (CrCl) was estimated using Cockcroft and Gault's formula. Fasting total homocysteine was measured on heparinized plasma. Microalbuminuria was assessed by immunonephelometry (defined as 20 mg/L–200 mg/L [random sample] and/or 30–300 mg/24 h).

For neurohormonal assaying, venous blood samples were collected in chilled tubes containing EDTA 3.0 $mM.L^{-1}$ and benzamidine 9.0 $mM.L^{-1}$. Plasma was carefully separated and frozen at −80°C before assay. Plasma UII levels were measured by radioimmunoassay (RIA), after Sep-Pack C18 cartridge (Waters, Milford, MA) extraction as previously reported.[8] Briefly, 5 mL of plasma recovered from blood collected on EDTA and benzamidine was mixed with 2 g of guanidine hydrochloride. This mix was eluted on Sep-Pack previously activated by 3-mL acetonitrile with 0.1% of trifluoroacetic acid. The eluates were lyophilized under vacuum using a speedvack centrifuge. Pellets were dissolved in 0.5 mL of assay buffer. UII RIA was based on commercially available antibody and standards (RAS H4768 and H4768.0001, respectively, Bachem, Torrance, CA). The tracers were iodinated in our laboratory and purified by RP-HPLC. In this UII RIA, the samples displaced the tracers parallel to standards curves. The RIA characteristics were (mean ± 1 SD): zero binding, 35%±4% (n=12); standard curve 50% effective tracer displacement concentration, 37±6 pg/mL; detection limit (10% tracer displacement), 6±1 pg/mL. Cross-reactivity with human brain natriuretic peptide (BNP), human Big endothelin-1 (Big ET-1), and N-terminal pro-atrial natriuretic peptide (NT-proANP) was <0.01%. Plasma levels of BNP, Big-ET-1, and NT-proANP were measured on the same extracts with specific RIAs. BNP RIA was based on commercially available antibody and standards (RAS 9086 and H9060.0500, respectively). Big-ET-1 and NT-proANP RIAs were based on homemade antisera, generated by immunization of rabbits with Big-ET 122–38 and NT-proANP 68–98 fragments coupled to KLH.[22] Synthetic Big-ET 122–38 and NT-proANP 68–98 peptides were used as standards.

Statistical Analysis

Statistical analysis was performed using the MedCalc package (Medcalc Software, Mariakerke, Belgium). Results are expressed as mean ± 1 SD, as

Table I. Patient Characteristics According to MetS Phenotype

	Absence vs Presence of MetS			Comparison Within MetS Categories			
	MetS (−)	MetS (+)	*P* Value	MetS 3/5	MetS 4/5	MetS 5/5	*P* Value
No.	66	294	–	92	104	98	–
Age, y	68 (11)	68 (11)	NS	71 (10)	68 (12)	66 (11)	<.001
Male:female ratio, %	80:20	64:36	<.002	67:33	61:39	63:37	NS
Smoking: never/former/current, %	49/22/29	49/22/30	NS	49/22/29	52/20/28	45/23/32	NS
BMI, kg/m^2	25.2 (3.3)	30.6 (5.2)	<.0001	27.9 (3.4)	31.2 (5.4)	32.7 (5.3)	<.0001
Waist circumference, cm	93 (9)	109 (13)	<.0001	103 (11)	109 (13)	114 (12)	<.0001
Fat mass, %[d]	27.7 (8.0)	34.0 (7.2)	<.0001	32.1 (6.8)	33.8 (7.8)	35.9 (6.5)	<.0001
Hypertension, %	45	78	<.0001	73	76	84	<.01
Systolic blood pressure, mm Hg	130 (16)	141 (18)	<.0001	140 (20)	140 (17)	143 (18)	NS
Diastolic blood pressure, mm Hg	76 (9)	79 (11)	NS	78 (12)	78 (10)	80 (11)	NS
Fasting insulinemia, pmol/mL	76 (63)	122 (76)	<.0001	103 (73)	119 (76)	139 (78)	<.003
ACEI/ARB, %	39/55	50/23	<.001/<.02	51/21	49/22	52/26	NS/NS
CCB, %	9	30	<.005	28	28	33	NS
β-Blocker, %	30	49	<.03	50	52	45	NS
Diuretic, %	15	34	<.001	37	30	37	NS
Low-dose aspirin, %	49	63	NS	66	63	60	NS
Statin/fibrate, %	36/2	48/20	<.0005/<.05	50/3	46/21	48/31	<.0005/<.0001

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; MetS, metabolic syndrome; NS, not significant.

medians (interquartile range), or as proportions (%). When appropriate, data were *log* transformed prior to statistical analysis. Differences between respective means from unmatched data were assessed by Student *t* or Welsch tests for parametric or nonparametric data distributions and by Bonferroni tests. Differences between respective proportions were assessed by Fisher exact test or Chi-square test for trend across MetS categories. The associations between UII plasma concentrations and baseline characteristics were determined by Spearman correlation coefficient. Associations between the presence of MetS and baseline patient characteristics were first analyzed by simple logistic regression analysis and then by multivariate analysis in which age and significant factors disclosed during the univariate analysis were entered. Differences were considered statistically significant at $P<.05$. Differences were considered statistically significant at $P<.05$.

RESULTS

Patient Characteristics

Baseline characteristics of diabetic patients according to the presence or absence of a MetS phenotype are summarized in Table I. The laboratory values and neurohormonal markers according to MetS phenotype are illustrated in Table II. MetS was present in 82% of patients (n=294). Within MetS categories were as follows: 92 patients (26%) had 3 of 5 AHA/NHLBI criteria (3/5); 104 patients (29%) were classified as 4/5, and 98 patients (27%) as 5/5. The majority of patients in both MetS (+) and MetS (−) groups were men. Tobacco smoke exposure was not different between groups. Mean body mass index (BMI) was higher by 5.4 kg/m^2 in MetS (+); these patients had a mean +11 mm Hg higher systolic BP (both $P<.0001$). Fasting specific insulinemia was lower in MetS (−) than in MetS (+) patients ($P<.0001$), and there was a progressive gradient of insulinemia according to MetS categories ($P<.003$).

Except for low-dose aspirin, usage of commonly prescribed cardiovascular drug was significantly higher in MetS (+) patients. MetS (−) and MetS (+) patients did not differ with respect to degree of recent glucose control (reflected by HbA_{1c} levels), nor to kidney function estimated by glomerular filtration rate (Table II). On the other hand, MetS (+) patients had higher $_{hs}$CRP, homocystinemia, and albuminuria values ($P<.0001$).

UII Circulating Levels

Circulating UII concentrations were significantly higher in MetS (+) T2DM patients (0.97 [95% confidence interval (CI) 0.93–1.01], n=294 vs 0.82 [95%

Table II. Laboratory Values and Neurohormonal Markers According to MetS Phenotype

	Absence vs Presence of MetS			Comparison Within MetS Categories			
	MetS (−)	MetS (+)	*P* Value	MetS 3/5	MetS 4/5	MetS 5/5	*P* Value
No.	66	204	–	92	104	98	–
HbA_{1c}, %	7.53239 (1.4)	7.644 (1.5)	NS	7.4 (1.5)	7.7 (1.4)	7.8 (1.7)	<.03
GFR, mL/min	76 (25)	84 (37)	NS	76 (33)	86 (36)	89 (39)	<.003
hs-CRP, mg/dL	0.25 (0.27)	0.35 (0.34)	<.0001	0.26 (0.26)	0.4 (0.44)	0.37 (0.27)	<.0003
Homocysteine, μmol/L	10.0 (3.1)	15.0 (8.0)	<.0001	13.9 (5.7)	15.8 (6.9)	15.2 (10.1)	NS
Albuminuria, mg/L	34 (84)	85 (182)	<.0001	95 (176)	62 (135)	98 (223)	<.05
Cholesterol, mg/dL	190 (39)	191 (42)	NS	187 (39)	193 (45)	192 (40)	NS
LDL-C, mg/dL	112 (33)	112.4 (36)	NS	116 (34)	115 (35)	105 (36)	<.03
HDL-C, mg/dL	58 (15)	44 (13)	<.0001	52 (13)	45 (14)	36 (8)	<.0001
Cholesterol/HDL-C	3.44 (0.89)	4.70 (1.79)	<.0001	3.77 (1.06)	4.62 (1.64)	5.67 (2.00)	<.0001
Triglycerides, mg/dL	101 (58)	194 (180)	<.0001	118 (67)	190 (216)	269 (181)	<.0001
LDL-C/apoB	1.19 (0.40)	1.01 (0.28)	<.02	1.07 (0.25)	1.01 (0.25)	0.96 (0.33)	<.0001
Urotensin II, pg/mL	0.82 (0.28)	0.97 (0.32)	<.001	0.97 (0.28)	1.00 (0.34)	0.93 (0.33)	NS
NT-proANP, pg/mL	457 (278–710)	500 (290–813)	NS	655 (362–890)	453 (246–799)	422 (268–750)	<.05
BNP, pg/mL	9.7 (5.3–13.9)	10.4 (6.2–17.6)	NS	12.4 (7.7–20.1)	10.0 (5.7–16.3)	9.5 (6.2–17.2)	NS
Big ET-1, pg/mL	5.2 (4.30–6.00)	6.7 (5.20–9.00)	<.0001	6.5 (5.20–8.60)	6.8 (5.10–9.10)	6.8 (5.30–9.10)	NS

Abbreviations: apoB, apolipoprotein B; Big ET-1, big endothelin-1; BNP, brain natriuretic peptide; GFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MetS, metabolic syndrome; NS, not significant; NT-proANP, N-terminal pro-atrial natriuretic peptide.

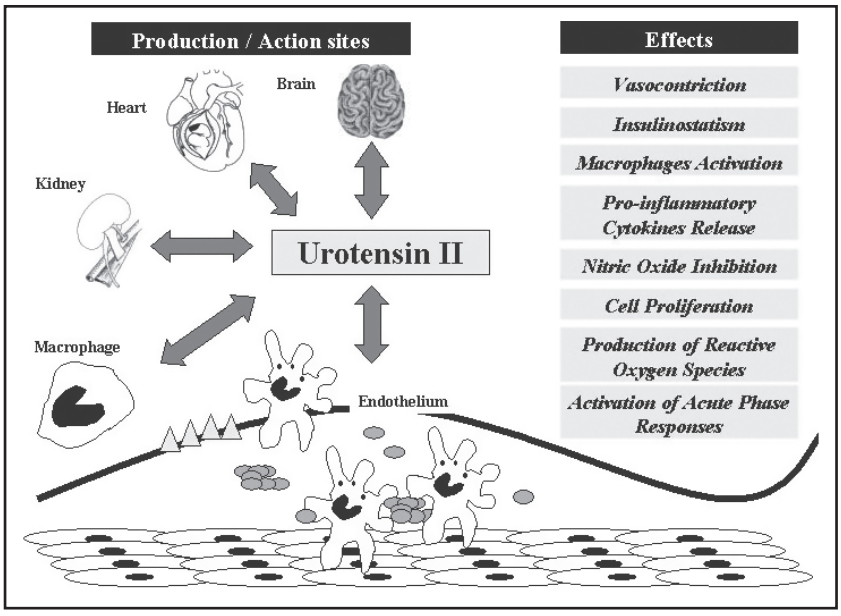

Figure. Production and actions sites of urotensin II and its potential adverse cardiovascular effects.

CI, 0.75–0.88] pg/mL, n=66 in MetS (−); $P<.001$) (Figure). No gradient for UII levels was observed according to stepwise upward scoring (3–5/5) for discrete AHA/NHLBI components defining the MetS (Table II).

Neurohormones Concentrations

No significant differences were observed in circulating NT-proANP or BNP levels between patients with or without MetS, but a significant increase in NT-proANP level was observed in the 3/5 MetS (+) subgroup. On the other hand, Big ET-1 levels were markedly and significantly higher, by a median of 30%, in MetS (+) patients (Table II) ($P<.0001$). There was a nonsignificant uphill and progressive gradient of Big ET-1 crosswide MetS severity scores, in a parallel manner to a lessening in insulin sensitivity as measured with Homeostatic Model Assessment (Table I and Table II).

Spearman correlation coefficients are shown in Table III. In the cohort of T2DM patients, UII plasma concentrations were significantly correlated with age ($r=0.191$, $P<.001$), estimated glomerular filtration rate (GFR) ($r=-0.230$, $P<.001$), diastolic BP ($r=-0.161$, $P<.05$), HbA_{1c} ($r=-0.117$, $P<.05$), NT-proANP ($r=0.194$, $P<.001$), BNP ($r=0.220$, $P<.001$), and Big ET-1 ($r=0.293$, $P<.001$).

Association of UII With MetS

A logistic regression analysis was performed that included age, estimated GFR, BMI, waist circumference, systolic BP, diastolic BP, low-density lipoprotein cholesterol (LDL-C), HDL-C, triglycerides, HbA_{1c}, UII, NT-proANP, BNP, and BigET-1 as independent variables. This analysis showed that UII yielded significantly higher adjusted ORs for having MetS (+) than other variables (odds ratio [OR], 6.41; 95% CI, 1.21–16.04; $P=.02$). The other parameters significantly associated with MetS (+) were BMI (OR, 1.28; 95% CI, 1.06–1.53; $P<.01$), waist circumference (OR, 1.08; 95% CI, 1.00–1.16; $P=.03$), systolic BP (OR, 1.04; 95% CI, 1.01–1.07; $P=.02$), and LDL-C (OR, 0.96; 95% CI, 0.92–0.99; $P=.03$). Stepwise analysis disclosed UII, BMI, waist circumference, systolic BP, and triglycerides as parameters to be retained in the model.

DISCUSSION

Our results show for the first time a significant increase of circulating UII levels associated with the presence of MetS in T2DM patients, but do not demonstrate any significant correlation between UII concentrations and insulinemia or with weighted components of the MetS.

Previous studies have reported that UII plasma levels are raised in diabetic patients.[10,23] The physiopathological role of the UII/UT system in conditions associated with insulin resistance and/or hyperinsulinemia is poorly documented. Recent reports implicated the UII system in the pathophysiology of diabetes mellitus.[10,24,25] In a perfused rat pancreas model, Silvestre and colleagues[26] reported that UII is present in pancreatic extracts and may

Table III. Correlation Between UII Plasma Concentration and Baseline Characteristics for Diabetic Patients

Variable	Spearman Correlation Coefficient	*P* Value
Age, y	0.191	<.001
GFR, mL/min	−0.230	<.001
BMI, kg/m^2	0.018	.73
Waist circumference, cm	0.112	.03
Systolic blood pressure, mm Hg	0.037	.49
Diastolic blood pressure, mm Hg	−0.161	<.05
LDL-C, mg/dL	0.002	.78
HDL-C, mg/dL	−0.014	.79
Triglycerides, mg/dL	0.045	.39
Insulin, pmol/mL	0.083	.33
HbA_{1c}, %	−0.117	<.05
NT-proANP, pg/mL	0.194	<.001
BNP. pg/mL	0.220	<.001
Big ET-1, pg/mL	0.293	<.001

Abbreviations: Big ET-1, big endothelin-1; BMI, body mass index; BNP: brain natriuretic peptide; NT-proANP: N-terminal pro-atrial natriuretic peptide; GFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UII, urotensin II.

play through its cognate UT receptor, a regulatory role in insulin secretion. More recently, Marco and colleagues[27] demonstrated in the same experimental model that UII signaling inhibits ß-cell secretion and confirmed the putative insulinostatic role of this peptide. Other studies in humans found that the UII gene S89N polymorphism was associated with the development of T2DM in Japanese persons through a potential effect on insulin sensitivity.[24,25] Haplotypes and SNPs in the UII and UT receptor genes in Chinese persons were also associated with pancreatic ß-cell function, insulin resistance, and 2-hour plasma glucose.[28] These observations support that UII and its receptor are associated with insulin resistance, hyperinsulinemia, and/or hyperglycemia.

Recently, activation of the UII system was proposed to be associated with the development of MetS and its discrete components such as hypertension, insulin resistance, hyperglycemia, inflammation, dyslipidemia, and obesity.[29] The observations that UII is described as the most potent naturally occurring vasoconstrictor identified so far[3,30] and also modulates plasma free fatty acids, enhances lipogenesis, and stimulates depot lipase activity in fish, suggesting a putative involvement in dyslipidemia, reinforces this hypothesis.[29,31] Our data confirm for the first time that T2DM MetS (+) patients display increases of UII circulating concentrations and we therefore hypothesize that an increase of UII circulating levels may represent another putative mechanism relating insulin resistance, hyperinsulinemia, and the development of hypertension and atherosclerotic vascular disease. The rise in UII levels observed in T2DM, MetS, and states of insulin resistance is likely to be multifactorial. Thus, Totsune and colleagues reported that the diabetic state itself may elevate plasma UII levels, and that decreased renal function is another independent factor that raises plasma UII in T2DM patients.[10,23] Our data also hint toward a significant correlation between UII and estimated GFR, which may partly account for the increased UII levels in T2DM.

Stimulation of the UII system is also related to adverse vascular effects (Figure) and increased cardiac risk.[6,9,30] UII is involved in vascular remodelling through its growth-promoting effects and acts synergistically with oxidised LDL in inducing vascular smooth muscle cell proliferation via the cSrc/PKC/MAPK pathway.[32] UII may also act as an effector inducing a proatherothrombotic phenotype in coronary vascular cells through increases in tissue factor mRNA, VCAM1, and ICAM1. [33] UII and UT receptor RNA and protein expression are significantly enhanced in streptozotocin-induced diabetic rat cardiomyocytes.[34] Recent studies also underlined the putative contributive role of UII in human atherosclerosis progression. Thus, plasma UII levels are correlated with carotid atherosclerosis in hypertensive[33] and coronary artery disease patients.[36] These results suggest a possible role for the UII/UT system in the pathophysiology of diabetic cardiomyopathy and vascular dysfunction through autocrine/paracrine pathways.

The evidence of raised UII plasma immunoreactivity in insulin resistance states may also provide new therapeutic insight. Recent reports demonstrated the benefits of pharmacologic treatment with UII antagonists in various conditions.[37–39] By virtue of their insulinotropic effect, UII receptor antagonists may be considered as potential agents to target the impaired insulin secretion in certain hyperglycemic states, including T2DM. Long-term treatment of streptozotocin-induced diabetic rats with palosuran improved survival, increased insulin secretion, and slowed the rise in glycemia in HbA_{1c} and serum lipids associated with insulin deficiency.[40] UII antagonists may represent new approaches in addressing endothelial dysfunction and diabetes-associated vascular disease.

LIMITATIONS

The sample population was selected and is neither representative of people with the MetS nor representative of the general population. In our selected sample population, there is a significant heterogeneity that may be related to the heterogeneity of the MetS itself.

CONCLUSIONS

This study demonstrates for the first time that increased UII levels, a peptide with insulinostatic effects associated with cardiovascular diseases, are related to the MetS phenotype. These observations bring further evidence of a potential reciprocal feedback loop between the UII system and metabolic states characterized by insulin resistance and/or hyperinsulinemia. Prospective studies are required to validate the potential contribution of UII as a cardiometabolic risk marker in patients with T2DM and/or MetS and the therapeutic potential of UII antagonists in insulin resistance and in preventing cardiovascular complications.

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