UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

ASSOCIAÇÃO ENTRE PARÂMETROS DE FUNÇAO VASCULAR E FATORES DE RISCO CARDIOVASCULAR EM PACIENTES COM DIABETES MELITO TIPO 2

DISSERTAÇÃO DE DOUTORADO

Alice Hoefel Nunes

Porto Alegre, 30 de Janeiro de 2014.

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS: ENDOCRINOLOGIA

ASSOCIAÇÃO ENTRE PARÂMETROS DE FUNÇAO VASCULAR E FATORES DE RISCO CARDIOVASCULAR EM PACIENTES COM DIABETES MELITO TIPO 2

Alice Hoefel Nunes

Orientadora: Prof. Dra. Themis Zelmanovitz

Dissertação de Doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul (UFRGS) como requisito parcial para obtenção do título de Doutor em Endocrinologia.

Porto Alegre, 30 de Janeiro de 2014.

CIP - Catalogação na Publicação

Nunes, Alice Hoefel
ASSOCIAÇÃO ENTRE PARÂMETROS DE FUNÇAO VASCULAR E FATORES DE RISCO CARDIOVASCULAR EM PACIENTES COM DIABETES MELITO TIPO 2 / Alice Hoefel Nunes. -- 2014.
56 f.

Orientadora: Themis Zelmanovitz.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Porto Alegre, BR-RS, 2014.

1. Diabetes. 2. Disfunção Endotelial. I. Zelmanovitz, Themis, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

“A verdadeira educação o tornará divino. Educação não é o mero conhecimento de palavras; ela deveria ampliar a mente. A mera aquisição de títulos é sem valor. O caráter é mais importante. De que serve uma educação que não promove boas qualidades? Juntamente com a formação acadêmica você deve adquirir sabedoria e um senso de certo e errado. Conhecimento sem sabedoria, erudição sem determinação, música sem melodia, aprendizado sem humildade, uma sociedade sem disciplina, amizade sem gratidão, e fala sem verdade - todos estes são totalmente inúteis. Por isso, todos devem procurar seguir o caminho correto. Não é a grandeza que importa, mas a bondade. Faça uso adequado de sua educação para o bem da sociedade.”

Sathya Sai Baba

Sumário

Introdução:

Diabetes Mellitus (DM) é uma doença crônica que requer tratamento contínuo e vigilância constante pelo próprio paciente a fim de evitar complicações agudas e reduzir o risco de complicações crônicas (1).

A OMS estima que o número total de pessoas com DM no mundo aumentará, de 171 milhões em 2000 para 366 milhões em 2030; e apenas no Brasil, de 4,5 milhões para 11,3 milhões, no mesmo período, tornando-se o oitavo país no mundo com o maior número de pessoas com diabetes. Entre as complicações do DM, as doenças cardiovasculares e renais apresentam custo elevado, tanto em termos de morbidade quanto de gastos para os sistemas de saúde (2).

A doença arterial coronariana é causa de mais da metade dos óbitos em pacientes com DM tipo 2, sendo a principal complicação crônica dos pacientes diabéticos (3). Quando comparados com indivíduos sem DM, os pacientes com DM têm uma maior prevalência de doença coronariana, maior extensão da isquemia coronariana, maior chance de infarto do miocárdio além de maior frequência de isquemia silenciosa (3, 4). O próprio DM, particularmente o estado de hiperglicemia sustentada, contribui em grande parte para a severidade do processo aterosclerótico(5). Alem disso, a alta prevalência dos fatores de risco tradicionais que são característicos da síndrome metabólica (obesidade, dislipidemia, hipertensão, hiperinsulinemia, hiperglicemia, alterações dos fatores hemostáticos), assim como dos fatores de risco não-tradicionais (inflamação crônica, estresse oxidativo, microalbuminúria, produtos finais da glicação avançada, homocisteína), pode justificar a maior severidade da DCV nos pacientes com DM. O maior risco de doenças cardiovasculares nos

A disfunção do endotélio é um marcador precoce do desenvolvimento das complicações micro e macrovasculares do DM, podendo aparecer antes mesmo do diagnóstico do DM tipo 2 (6).

Em condições basais, o endotélio tem a função de manter o vaso sanguíneo relativamente dilatado, processo mediado principalmente pelo óxido nítrico (NO). Este é sintetizado a partir do aminoácido L-Arginina pela óxido nítrico sintetase endotelial. Além da função vasodilatadora, o NO inibe a agregação plaquetária e a adesão e migração de leucócitos, que é o primeiro indicador morfológico de aterosclerose, inibe a proliferação de células do músculo liso da parede do vaso, reduz a permeabilidade endotelial para macromoléculas e lipoproteínas, assim diminuindo o acúmulo subendotelial de LDL colesterol, inibe a proliferação e migração das células musculares lisas vasculares (7) . Além disso, o endotélio age na modulação da fibrinólise, dissolução de trombos intravasculares e modulação da inflamação através da regulação de quimiocinas. Dentre as substâncias vasoconstritoras destacam-se a Endotelina -1, Angiotensina II e o Tromboxano(8).

O termo disfunção endotelial refere-se à alteração da vasodilatação dependente do endotélio e à má regulação da interação endotélio - células sanguíneas, causando uma inflamação localizada e, posteriormente, lesões vasculares graves e trombose. A disfunção endotelial ocorre quando os efeitos vasoconstritores se superpõem aos efeitos vasodilatadores, geralmente como resultado da diminuição da biodisponibilidade do NO, com perda de sua ação vasoprotetora (9). Essas alterações propiciam um estado pró-constritor, pró-inflamatório e pró-agregante ao vaso sanguíneo (9, 10).

Existem diversas maneiras de se analisar a função endotelial. Marcadores bioquímicos da atividade endotelial, que são secretados pelo endotélio ou liberados da sua superfície podem ser utilizados para medir a atividade endotelial, tais como a fator von Willebrand,

molécula de adesão intracelular (ICAM), molécula de adesão à célula vascular (VCAM) (7), além de fatores inflamatórios como o fibrinogênio e a proteína C-reativa.

Métodos invasivos como angiografia ou Doppler intra-coronário ou pletismografia da artéria braquial antes e após o uso de acetilcolina ou em resposta a diferentes vasodilatadores também são algumas possibilidades (11).

O método não invasivo mais usado na atualidade é a dilatação mediada pelo fluxo [*flow mediated dilation* (FMD)]. Através de eco-Doppler com ultrassom de alta resolução, são determinadas as respostas vasodilatadoras da artéria braquial durante a hiperemia reativa (vasodilatação endotélio dependente) e após a administração de nitroglicerina sublingual (vasodilatador endotélio-independente). Assim, uma resposta alterada ao estímulo do NO endógeno (hiperemia) refletirá uma biodisponibilidade comprometida de NO, ao passo que uma resposta diminuída ao NO exógeno (nitroglicerina) refletirá alterações estruturais do vaso ou sensibilidade diminuída da célula muscular lisa. As respostas alteradas às manobras indicadas têm sido correlacionadas com o comprometimento da vasodilatação endotélio-dependente em artérias coronárias em diversos estudos (7).

Esta técnica é vista como padrão-ouro das técnicas não invasivas atualmente (12). Sua principal vantagem é a possibilidade de aplicação em um número grande de pacientes ou várias vezes no mesmo paciente (8). Principalmente por necessitar apenas de sala, maca e aparelho de ultrassonografia com transdutor linear, e fácil realização para o paciente, pois não necessita de esforço ou causa qualquer lesão a este. O desconforto da técnica consiste na necessidade de jejum de 8 a 12 h, interrupção do fumo por 12 h, a pressão no antebraço por 5 minutos a 200-250 mmHg e o uso de nitrato sublingual na dose de 300mcg, que pode causar tontura ou cefaleia momentânea.

Alguns fatores podem influenciar a avaliação da dilatação mediada pelo fluxo e devem ser aferidos ou controlados como: dislipidemia grave, especialmente hipertrigliceridemia, uso

de estatinas ou colestiramina, hipertensão com controle inadequado e drogas para seu tratamento como inibidores do sistema renina-angiotensina-aldosterona (13, 14), estados de estresse oxidativo elevado (7), uso de antioxidantes (11), alterações do estado de homeostase glicêmica (15), dieta (16), idade (17) e exercício vigoroso (18). A viscosidade sanguínea também pode alterar a velocidade com que o sangue flui pelo vaso e assim o estímulo a liberação de NO após hiperemia reativa. Assim, em 2011, Raffaele Maio e colaboradores também encontraram associação inversa da vasodilatação endotélio dependente com o nível de hemoglobina de pacientes hipertensos ainda não tratados (19).

A desvantagem da técnica é sua necessidade de uso em mais larga escala em outros estudos para ser reconhecida como ferramenta padronizada e a sua grande variabilidade. Além disso, a maneira de ser aplicada também necessita melhor padronização (20).

A avaliação da artéria braquial através da técnica de Doppler permite determinar a dilatação mediada pelo fluxo (endotélio dependente) e a dilatação endotélio independente (14, 21). Recentemente, alguns estudos têm sugerido que a medida do diâmetro da artéria braquial possa também ser uma ferramenta diagnóstica, mais simples, para avaliar o risco cardiovascular (22). Estes estudos têm se baseado em achado de associação entre o diâmetro basal da artéria braquial e risco cardiovascular. A hipótese seria de um remodelamento vascular em pacientes em processo aterosclerótico mais avançado (26).

Alguns estudos avaliaram a reprodutibilidade e a variabilidade da FMD com resultados que variam entre 2 e 84 %. A maioria em pacientes não diabéticos. O estudo que avaliou a variabilidade em diabéticos também encontrou variabilidade elevada (29,7%). De fato, essa população apresenta características peculiares que podem aumentar sua variabilidade como flutuações no perfil lipídico e glicêmico, hipertensão e uso de medicações anti-hipertensivas e estatinas.

Finalmente, pode-se avaliar o espessamento da camada íntima-média de grandes artérias (carótida), que embora não sendo um marcador direto da função endotelial, este espessamento é uma consequência física de uma disfunção endotelial antecedente, sendo um bom indicador prognóstico de aterosclerose inicial na circulação cerebral e coronariana. Estudos analisaram a correlação entre disfunção endotelial estimada pela dilatação mediada pelo fluxo e o espessamento da íntima média (23), além da correlação destas com diversos aspectos clínicos. Neste estudo, a correlação da FMD e da espessura da íntima média foi significativa apenas nos pacientes que tinham esta última medida nos menores valores, ou seja, com processo aterosclerótico mais leve ou inicial.

Esta tese está composta de dois artigos que abordam o estudo da artéria braquial através da técnica de Doppler em pacientes com DM tipo 2 acompanhados no ambulatório de Endocrinologia do Hospital de Clínicas de Porto Alegre. No primeiro artigo, avaliamos a variabilidade intra-individual da técnica em 29 pacientes com DM tipo 2 e procuramos determinar os fatores associados a esta variabilidade. No segundo artigo, avaliamos a associação de parâmetros de medida da função vascular através desta técnica com fatores de risco cardiovascular em um estudo transversal de 240 pacientes com DM tipo 2.

Referências Bibliográficas

1. Association AD. Standards of medical care in diabetes--2014. Diabetes Care. 2014;37 Suppl 1:S14-80.

2. Sowers JR, Epstein M, Frohlich ED. Diabetes, hypertension, and cardiovascular disease: an update. Hypertension. 2001;37(4):1053-9.

3. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR, et al. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int. 2003;63(1):225-32.

4. Cooper ME. Pathogenesis, prevention, and treatment of diabetic nephropathy. Lancet. 1998;352(9123):213-9.

5. Dronavalli S, Duka I, Bakris GL. The pathogenesis of diabetic nephropathy. Nat Clin Pract Endocrinol Metab. 2008;4(8):444-52.

6. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. 2005;28(1):164-76.

7. Giunti S, Cooper M. Management strategies for patients with hypertension and diabetes: why combination therapy is critical. J Clin Hypertens (Greenwich). 2006;8(2):108-13.

8. Andrade LJ, Cruz TR, Daltro C, Soares FJ, França CS, Sampaio AP, et al. [Myocardial perfusion scintigraphy in type 2 diabetic patients with atypical chest pain]. Arq Bras Endocrinol Metabol. 2004;48(3):362-73.

9. Deedwania PC, Fonseca VA. Diabetes, prediabetes, and cardiovascular risk: shifting the paradigm. Am J Med. 2005;118(9):939-47.

10. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. Diabetes. 2002;51(4):1157-65.

11. B.L. w. Endothelial Disfunction in typer 2 diabetes. Arq Bras Endocrinol Metab2002.

12. Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. J Am Coll Cardiol. 1999;34(3):631-8.

13. Aguiar LG, Villela NR, Bouskela E. [Microcirculation in diabetes: implications for chronic complications and treatment of the disease]. Arq Bras Endocrinol Metabol. 2007;51(2):204-11.

14. Keogh JB, Grieger JA, Noakes M, Clifton PM. Flow-mediated dilatation is impaired by a high-saturated fat diet but not by a high-carbohydrate diet. Arterioscler Thromb Vasc Biol. 2005;25(6):1274-9.

15. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation. 2004;109(23 Suppl 1):III27-32.

16. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115(10):1285-95.

17. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol. 1994;24(6):1468-74.

18. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65.

19. West SG, Wagner P, Schoemer SL, Hecker KD, Hurston KL, Likos Krick A, et al. Biological correlates of day-to-day variation in flow-mediated dilation in individuals with Type 2 diabetes: a study of test-retest reliability. Diabetologia. 2004;47(9):1625-31.

20. Ma Y, Njike VY, Millet J, Dutta S, Doughty K, Treu JA, et al. Effects of walnut consumption on endothelial function in type 2 diabetic subjects: a randomized controlled crossover trial. Diabetes Care. 2010;33(2):227-32.

21. Jensen-Urstad K, Johansson J. Gender difference in age-related changes in vascular function. J Intern Med. 2001;250(1):29-36.

22. Kitzman DW, Brubaker PH, Herrington DM, Morgan TM, Stewart KP, Hundley WG, et al. Effect of endurance exercise training on endothelial function and arterial stiffness in older patients with heart failure and preserved ejection fraction: a randomized, controlled, single-blind trial. J Am Coll Cardiol. 2013;62(7):584-92.

23. Maio R, Sciacqua A, Bruni R, Pascale A, Carullo G, Scarpino PE, et al. Association between hemoglobin level and endothelial function in uncomplicated, untreated hypertensive patients. Clin J Am Soc Nephrol. 2011;6(3):648-55.

24. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol. 1994;24(2):471-6.

25. Montalcini T, Gorgone G, Gazzaruso C, Romeo S, Bosco D, Pujia A. Brachial artery diameter measurement: a tool to simplify non-invasive vascular assessment. Nutr Metab Cardiovasc Dis. 2012;22(1):8-13.

26. Yeboah J, McClelland RL, Polonsky TS, Burke GL, Sibley CT, O'Leary D, et al. Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals. JAMA. 2012;308(8):788-95.

27. Yokoyama H, Sone H, Saito K, Yamada D, Honjo J, Haneda M. Flow-mediated dilation is associated with microalbuminuria independent of cardiovascular risk factors in type 2 diabetes - interrelations with arterial thickness and stiffness. J Atheroscler Thromb. 2011;18(9):744-52.

Variability of flow mediated dilation of brachial artery and association with clinical parameters in type 2 diabetic patients

Running head: Variability of flow mediated dilation in diabetes

[1] Endocrine Division, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

[2] Cardiology Division, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Address for correspondence and reprint requests: Themis Zelmanovitz, Serviço de Endocrinologia do Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350/ Prédio 12 - 4º andar CEP 90035-003 - Porto Alegre - RS, Brazil.

Phone/Fax: (55-51) 3359.8127 / 3331.3312

E-mail: themis.voy@terra.com.br

Sources of support: FIPE- Hospital de Clínicas de Porto Alegre; CAPES; CNPq; Propesq-Universidade Federal do Rio Grande do Sul

Abstract

Objective – To determine the intra-individual coefficient of variation of flow mediated dilation (FMD) in type 2 diabetic patients, and to evaluate the factors associated to this variability.

Patients and Methods – Patients with type 2 diabetes underwent endothelial function assessment by ultrasound of the brachial artery to determine FMD, and repeated three times within a period of 30 days. Clinical evaluation and laboratory tests were performed at baseline, and blood pressure and glycemic control was assessed during the visits.

Results – Twenty-nine patients (14 men, 59.8 years, 14 years from diabetes diagnosis) completed the evaluation. The average CV of basaline and post hyperemia diameter of brachial artery, and FMD were 3.4% 3.6% and 37.3%, respectively. There was an inverse correlation between intra-individual CV of FMD with albuminuria and triglycerides levels, as well as, with FMD values. Blood pressure and glycemic control maintained stable among the visits in all patients.

Conclusion– In type 2 diabetic patients with other comorbidities and under diverse medications, the variability of FMD is large, superior to healthy individuals. Low FMD value was the single factor associated to this variability in the studied sample.

Introduction

Cardiovascular disease (CVD) is the mainly cause of mortality among diabetic patients(24). For several forms of CVD, diabetes characterizes itself as an independent risk factor, and higher severity and poorer prognosis of this complication is common to be observed(25). Furthermore, type 2 diabetes is associated with endothelial dysfunction, considered a precursor of the atherosclerosis process. The endothelium is the major modulator of vascular homeostasis and is responsible for a number of vasoprotective effects (e.g. vasodilation, suppression of smooth muscle cell growth, and inhibition of inflammatory responses)(11).

There are several ways to analyze endothelial function. The noninvasive method most used nowadays is the flow mediated dilation (FMD), which measures the vasodilator response of the brachial artery to reactive hyperemia caused by transient ischemia of the arm. This technique is considered as the gold standard of noninvasive techniques(12). Besides its noninvasive nature, some of its main advantages are the capacity to perform multiple tests in the same patient and the study of a large number of patients(8). It causes little discomfort and almost no risk for individuals. Nevertheless, there are many variables that potentially affect FMD measurement, which could interfere with its reliability. These include medications as statins, cholestyramine, angiotensin-converting enzyme inhibitors and antioxidants(11); metabolic factors as glycemic fluctuations and triglycerides values(26); dietary factors (27); age(17) and vigorous exercise(18).

Many studies had evaluated FMD variability, reporting a large range of values, from 2 to 84%. Most of them were with non-diabetic patients(28-30) . In a previous study of our group, the local intra-individual CV of FMD of young healthy individuals was 4%. As far as we know, the single study that evaluated type 2 diabetic patients, from West el al(15), also reported a high coefficient of variation (29.7%). In fact, these patients present peculiar

characteristics that could maximize this variability, such as fluctuations of glycemic control and lipid profile, specifically triglycerides levels, blood pressure control and the use of some drugs that influence endothelial functions, like anti-hypertensive drugs and statins. West el al. observed that the subjects with a higher coefficient of variation of FMD also showed large fluctuations in glucose, insulin and heart rate.

It is well known that the variability of a diagnostic test interferes with its accuracy and, more importantly, influences its association with diseases. So, FMD is a valid and widespread instrument for assessing endothelial function and, as a diagnostic test, it is necessary to quantify the variability of this parameter in the various subpopulations. This study aims to determine the inter-individual coefficient of variability of FMD in type 2 diabetic patients, and to evaluate the possible factors associated to this variability.

Patients and Methods

Patients with type 2 diabetes (World Health Organization criteria) (31) were recruited from Endocrine Division's outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil. Exclusion criteria were the presence of heart failure (class III or IV), acute cardiovascular event in the preceding 6 months, uncontrolled blood pressure, use of phosphodiesterase type 5 inhibitors for erectile dysfunction, presence of other renal disease except diabetic nephropathy, severe autonomic neuropathy, and smoke habit. The treatment with antihypertensive, hypolipidemic and oral antidiabetic agents was maintained during the study. Patients were oriented to stop medications in the night before the exam.

Clinical and Laboratory Evaluation

A clinical history of the patients was taken, and all subjects underwent to physical and laboratory examination. Endothelial function assessment was repeated three times within a period of 30 days after an overnight fasting. In all visits, blood pressure and capillary glycemia were measured.

Clinical evaluation consisted of blood pressure assessment, renal and cardiovascular evaluation. Sitting blood pressure was measured twice to the nearest 2 mm Hg after a 10-min rest by using a digital sphygmomanometer (OMRON ® Automatic Blood Pressure Monitor, Model HEM-705CP, Vernon Hills, Illinois 60061). Hypertension was defined as blood pressure ≥ 140/90mmHg on at least 2 separate occasions or use of antihypertensive drugs(32). Renal function was evaluated by serum creatinine and 24-h urinary albumin excretion (UAE). Microalbuminuria and macroalbuminuria were considered to be present when UAE was between 17 - 174 mg/L and ≥ 174 mg/L, respectively, at least twice in a 6-month period (33, 34) . Cardiovascular evaluation consisted of applying the World Health Organization cardiovascular questionnaire and resting electrocardiogram (Rose). Exercise electrocardiogram testing or radionuclide myocardial perfusion imaging (exercise or pharmacological – dipyridamol) were done, when indicated, to evaluate the presence of myocardial ischemia (35).

Blood samples were collected after a 12-hour overnight fast. Plasma glucose and serum lipids were determined by enzymatic methods (Advia® 1800, Siemens Healthcare, Munich, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula (36) (LDL = TC – HDL – TG/5). Hemogram was performed by flow citometry (ABX Pentra DX 120, HORIBA, Kyoto, Japan). Glycated haemoglobin was determined by HPLC method (Variant II Turbo HbA1c, BioRad Laboratories, Hercules, CA, USA). Urinary albumin was measured in sterile urine samples by immunoturbidimetry (Advia® 1800, Siemens Healthcare, Munich, Germany).

Endothelial Function Assessment

Patients underwent assessment of endothelium–dependent FMD, according to *Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilatation of the Brachial Artery (37).* Patients were oriented not to exercise or ingest

substances that might affect FMD such as caffeine and vitamin C, and after overnight fasting, subjects were kept quiet for five minutes before FMD measurement. The recording of the brachial artery was obtained two to four cm above the antecubital fossa. The artery segment was imaged using an ultrasound system equipped with vascular software for two-dimensional imaging, color and spectral Doppler and an internal electrocardiogram monitor. The brachial artery was imaged above the antecubital fossa in the longitudinal plane 7MHz linear array ultrasound transducer (En Visor). The perpendicular distance between the M-lines (border between media and adventitia) was measured at end-diastole as detected by the R wave on the electrocardiogram. The average of three measures of vessel diameter was calculated from three resting cycles taken before the cuff inflation. (a total of nine measures). To create a flow stimulus in the brachial artery, a sphygmomanometric (blood pressure) cuff was first placed on the forearm. A baseline rest image was acquired, thereafter arterial occlusion was created by cuff inflation to supra-systolic pressure. Typically, the cuff is inflated to at least 200 mm Hg for five minutes to occlude arterial inflow for a standardized length of time. This causes ischemia and consequent dilation of downstream resistance vessels via auto-regulatory mechanisms. Subsequent cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress causes the brachial artery to dilate. The longitudinal image of the artery was recorded 45 seconds to two min after cuff deflation. The average of three measures of vessel diameter was calculated from three cycles taken after cuff deflation. (a total of nine measures). FMD was expressed as the percentage of post-ischemic increase of the diameter at rest.

Statistical analysis

Spearman correlation coefficients were used for testing the relationships between the FMD and clinical and laboratory characteristics. The CV was calculated for each subject from

the mean and SD for the three measurements as follows: CV = (100 × SD) / mean. The Student t or Mann-Whitney test for independent continuous variables and the Exact Fisher or Chi-Square test for categorical variables were applied to test clinical differences between men and women. General linear model repeated measure was used to compare variables among the visits.

P values < 0.05 were considered significant. Results were expressed as mean ± SD or median (range). SPSS software (version 18.0; SPSS, Chicago, IL) was used for the analyses.

Results

Thirty-one patients were recruited to the study, but two patients did not complete the protocol. Therefore, twenty-nine patients were included in the study (14 men and 15 women). Table 1 shows clinical and laboratory characteristics of patients. No significant difference was observed between men and women. Seventy one percent of patients were in use of statins, 57% in use of renin-angiotensin system inhibitors and 59% were using insulin.

The mean FMD in the whole group was 6.29 ± 3.72 %, 5.62 % ± 3.15 in men and 6.93 ± 4.19 % in women. Table 2 presents the average values of resting and post-hyperemia artery diameter and FMD of all patients, as well as, the intra-individual CV of each variable. Post-hyperemia artery diameter in men and FMD in all group were significantly lower in the third day. The mean CV of resting and post-hyperemia artery diameter and of FMD were not different between men and women.

Table 3 describes the correlations between clinical and laboratory variables and vascular parameters assessed by Doppler in the brachial artery. It was observed an inverse correlation of FMD with patients' age. Values of total cholesterol, LDL and triglycerides were positively correlated with FMD. No associations between FMD and other variables were found, as well as, with other vascular parameters. In women, FMD is correlated with age and

BMI, and in men there was a positive correlation of post-hyperemia diameter with total cholesterol, LDL and UAE.

Table 4 describes the correlation between clinical and laboratory variables and CV of vascular parameters. There was an inverse correlation between intra-individual CV of FMD with UAE and triglycerides levels, as well as with the values of FMD. This was not observed when patients were divided by sex. No correlation between the CVs of resting and post-hyperemia diameters and clinical and laboratory variables was observed.

In linear regression analysis, CV of FMD was inversely associated with and FMD values (r^2=0.54; P=0.10; standardized β=-0.54; p=0.012) after adjustment to age, gender, glycated hemoglobin and triglycerides values.

Discussion

The present study reported a mean CV of resting and post-hyperemia diameters and of FMD of 3.4±2.6%, 3.6±2.5% and 37.3±14.5% in type 2 diabetic patients. Furthermore, these CV values were not different between men and women.

FMD has been suggested as a non-invasive cardiovascular risk measure with possible large-scale application. Several studies have shown the association of endothelial dysfunction determined by FMD measures with cardiovascular disease(38), either in relation to incidence of early events (20) and to new events in individuals at high risk(39). The variability of FMD has been well studied in non-diabetic subjects but not in diabetic individuals. It is well known that diabetic patients are at risk for CVD(24) independently of other factors. However, our population usually has several associated comorbidities and is on use of a wide range of medications, which may influence vasomotor measures. Thus, already published studies with CV of FMD may not reflect our population.

In diabetic patients, West et al (15) already determined CV of FMD, but in a group of "healthy" diabetic individuals, i.e., with no other comorbidities. In fact, the reported CV in the West study was slightly smaller (29% vs. 37%), that might be influenced by some peculiar characteristics of that sample of patients. They presented lower glycemic, lipid and blood pressure levels than our patients, what could reflect more controlled and less advanced disease. Furthermore, they studied younger population, without other comorbidities besides diabetes. As in our study, they measure resting and post-hyperemia diameters found lower variability than FMD (2,5 and 2,7 % respectively). They didn't analyze men and women separately.

West et al. has also asserted that the variability of FMD can result from alterations (subtle) in the technique's application among different days (over repeated testing sessions), measurement errors and biological variability. Some adjustments to reduce application's issues and attempts to minimize technique's variability, such as fasting, monitoring temperature of the room, resting period before the exams, dietary and exercise measures that may alter the results, have already been proposed. But, despite this variability, FMD has been well correlated with major comorbidities, even in subclinical level (40). As this test might present possible large-scale application, new rules and calibrations can be established over time, which commonly occurred with many other tests.

The present study observed that CV of FMD was positively associated to FMD values. No other studied variables were associated to CV of FMD or with resting and post-hyperemia diameters. As in the study of West et al., our results did not correlate with glucose and blood pressure measurements. They found positively correlation with age, higher systolic BP and triglycerides.

This study presents the limitation of assessment being performed by a single operator and haven't been applied with the device that holds the probe in a static position, which was

held by the operator. Measurements were performed by operators and not by specific software as in some studies. However, the results don't differ from those found in literature, both in CV and FMD values. Patients were fasting, under controlled temperature, but only stopped their antihypertensive medications the day before assessment and not in many half lives, which would be scientifically ideal, but inadequate for the population in question.

In conclusion, in diabetic patients with other comorbidities and under different medications, the variability of FMD is large, but similar (slightly higher) to those found in literature in healthy diabetic and healthy individuals. Large-scale studies with diabetic patients and FMD may elucidate the validity of vasomotor measure as a diagnostic and prognostic tool in this population.

References

1. Association AD. Standards of medical care in diabetes--2014. Diabetes Care. 2014;37 Suppl 1:S14-80.

2. Sowers JR, Epstein M, Frohlich ED. Diabetes, hypertension, and cardiovascular disease: an update. Hypertension. 2001;37(4):1053-9.

3. Andrade LJ, Cruz TR, Daltro C, Soares FJ, França CS, Sampaio AP, et al. [Myocardial perfusion scintigraphy in type 2 diabetic patients with atypical chest pain]. Arq Bras Endocrinol Metabol. 2004;48(3):362-73.

4. Deedwania PC, Fonseca VA. Diabetes, prediabetes, and cardiovascular risk: shifting the paradigm. Am J Med. 2005;118(9):939-47.

5. Wei M, Gaskill SP, Haffner SM, Stern MP. Effects of diabetes and level of glycemia on all-cause and cardiovascular mortality. The San Antonio Heart Study. Diabetes Care. 1998;21(7):1167-72.

6. Wajchenberg BL. Disfunção Endotelial no Diabetes do Tipo 2. Arquivos Brasileiros de Endocrinologia & Metabologia. 2002;46:514-9.

7. B.L. w. Endothelial Disfunction in typer 2 diabetes. Arq Bras Endocrinol Metab2002.

8. Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. J Am Coll Cardiol. 1999;34(3):631-8.

9. Aguiar LG, Villela NR, Bouskela E. [Microcirculation in diabetes: implications for chronic complications and treatment of the disease]. Arq Bras Endocrinol Metabol. 2007;51(2):204-11.

10. Keogh JB, Grieger JA, Noakes M, Clifton PM. Flow-mediated dilatation is impaired by a high-saturated fat diet but not by a high-carbohydrate diet. Arterioscler Thromb Vasc Biol. 2005;25(6):1274-9.

11. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation. 2004;109(23 Suppl 1):III27-32.

12. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115(10):1285-95.

13. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol. 1994;24(6):1468-74.

14. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65.

15. West SG, Wagner P, Schoemer SL, Hecker KD, Hurston KL, Likos Krick A, et al. Biological correlates of day-to-day variation in flow-mediated dilation in individuals with Type 2 diabetes: a study of test-retest reliability. Diabetologia. 2004;47(9):1625-31.

16. Ma Y, Njike VY, Millet J, Dutta S, Doughty K, Treu JA, et al. Effects of walnut consumption on endothelial function in type 2 diabetic subjects: a randomized controlled crossover trial. Diabetes Care. 2010;33(2):227-32.

17. Jensen-Urstad K, Johansson J. Gender difference in age-related changes in vascular function. J Intern Med. 2001;250(1):29-36.

18. Kitzman DW, Brubaker PH, Herrington DM, Morgan TM, Stewart KP, Hundley WG, et al. Effect of endurance exercise training on endothelial function and arterial stiffness in older patients with heart failure and preserved ejection fraction: a randomized, controlled, single-blind trial. J Am Coll Cardiol. 2013;62(7):584-92.

19. Maio R, Sciacqua A, Bruni R, Pascale A, Carullo G, Scarpino PE, et al. Association between hemoglobin level and endothelial function in uncomplicated, untreated hypertensive patients. Clin J Am Soc Nephrol. 2011;6(3):648-55.

20. Yeboah J, McClelland RL, Polonsky TS, Burke GL, Sibley CT, O'Leary D, et al. Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals. JAMA. 2012;308(8):788-95.

21. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol. 1994;24(2):471-6.

22. Montalcini T, Gorgone G, Gazzaruso C, Romeo S, Bosco D, Pujia A. Brachial artery diameter measurement: a tool to simplify non-invasive vascular assessment. Nutr Metab Cardiovasc Dis. 2012;22(1):8-13.

23. Yokoyama H, Sone H, Saito K, Yamada D, Honjo J, Haneda M. Flow-mediated dilation is associated with microalbuminuria independent of cardiovascular risk factors in type 2 diabetes - interrelations with arterial thickness and stiffness. J Atheroscler Thromb. 2011;18(9):744-52.

24. Bloomgarden ZT. Diabetes and cardiovascular disease. Diabetes Care. 2011;34(3):e24-30.

25. Grundy SM, Benjamin IJ, Burke GL, Chait A, Eckel RH, Howard BV, et al. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation. 1999;100(10):1134-46.

26. Cortés B, Núñez I, Cofán M, Gilabert R, Pérez-Heras A, Casals E, et al. Acute effects of high-fat meals enriched with walnuts or olive oil on postprandial endothelial function. J Am Coll Cardiol. 2006;48(8):1666-71.

27. Ros E, Núñez I, Pérez-Heras A, Serra M, Gilabert R, Casals E, et al. A walnut diet improves endothelial function in hypercholesterolemic subjects: a randomized crossover trial. Circulation. 2004;109(13):1609-14.

28. Herrington DM, Fan L, Drum M, Riley WA, Pusser BE, Crouse JR, et al. Brachial flow-mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter. J Cardiovasc Risk. 2001;8(5):319-28.

29. De Roos NM, Bots ML, Schouten EG, Katan MB. Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. Ultrasound Med Biol. 2003;29(3):401-6.

30. Malik J, Wichterle D, Haas T, Melenovsky V, Simek J, Stulc T. Repeatability of noninvasive surrogates of endothelial function. Am J Cardiol. 2004;94(5):693-6.

31. Diabetes mellitus. Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1985;727:1-113.

32. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003;42(6):1206-52.

33. Gross JL, Zelmanovitz T, Oliveira J, de Azevedo MJ. Screening for diabetic nephropathy: is measurement of urinary albumin-to-creatinine ratio worthwhile? Diabetes Care. 1999;22(9):1599-600.

34. Zelmanovitz T, Gross JL, Oliveira JR, Paggi A, Tatsch M, Azevedo MJ. The receiver operating characteristics curve in the evaluation of a random urine specimen as a screening test for diabetic nephropathy. Diabetes Care. 1997;20(4):516-9.

35. Standards of medical care in diabetes--2010. Diabetes Care.33 Suppl 1:S11-61.

36. Friedewald W, Levy R, Fredrickson D. Estimation of the concentration of Low-density Lipoprotein Cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18:499-502.

37. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65.

38. Schächinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation. 2000;101(16):1899-906.

39. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. Circulation. 2007;115(18):2390-7.

40. Kobayashi K, Akishita M, Yu W, Hashimoto M, Ohni M, Toba K. Interrelationship between non-invasive measurements of atherosclerosis: flow-mediated dilation of brachial artery, carotid intima-media thickness and pulse wave velocity. Atherosclerosis. 2004;173(1):13-8.

Table 1: Clinical and laboratory characteristics of patients

	All (n=29)	Men (n=14)	Women (n=15)	P
Age (years)	59.8 ± 10.7	60.0 ± 10.0	59.7 ± 11.8	0.948
Time of DM diagnosis (years)	14.1 ± 8.5	12.6 ± 6.3	15.5 ± 10.2	0.370
BMI (kg/m²)	30.1 ± 3.5	30.7 ± 2.1	29.5 ± 4.4	0.355
Hypertension (%)	86	86	87	0.941
Ischemic heart disease (%)	10.3	7.1	20	0.135
Diabetic Nephropathy (%)	58.3	54.5	61.5	0.729
Glycemia (mg/dL)	159.8 ± 56.7	157.7 ± 54.8	161.8 ± 60.7	0.856
A1c (%)	8.4 ± 1.7	7.8 ± 1.5	9.1 ± 1.8	0.057
SBP (mmHg)	139.6 ± 15.2	141.4 ± 13.4	138.1 ± 16.9	0.541
DBP (mmHg)	79.7 ± 8.8	82,3 ± 8.4	77.5 ± 8.8	0.145
Total-cholesterol (mg/dL)	156 ± 38.2	153.6 ± 38,8	158.3 ± 39.1	0.765
HDL- cholesterol (mg/dL)	40.9 ± 10.6	41.7 ± 14.1	40.1 ± 6.5	0.702
Triglycerides (mg/dL)	118 (33-371)	90.5 (33-371)	134.5 (39-264)	0.995

Data are expressed in mean ±SD or median (min-max) if non-parametric variable.
P for difference between men and woman, DM: diabetes mellitus , BMI: body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure;

Table 2. Vascular and metabolic parameters measured under fasting conditions across the three visits.

	Visit Day			P	Average CV (%)
	1 Day	2 Day	3 Day		
Resting Artery diameter (mm)					
All	0,379±0,068	0,379 ± 0,063	0,373± 0,064	0.252	3,4±2,6
Men	0,420±0,054	0,415±0,057	0,412±0,052	0.338	2,9±2,1
Women	0,341±0,058	0,346±0,051	0,337±0,053	0.322	3,9±3,1
Post-hyperemia Artery diameter (mm)					
All	0,403±0,072	0,403±0,066	0,391±0,066	0.004	3,6±2,5
Men	0,444±0,062	0,438±0,060	0,429±0,056	0.021	3,3±1,5
Women	0,364±0,058	0,369±0,052	0,355±0,055	0.080	3,9±3,2
FMD (% change)					
All	6,29± 3,72	6,19± 3,87	4,72± 3,27	0.026	37,3±14,5
Men	5,62±3,15	5,59±3,02	4,01±2,41	0.198	41,3±14,2
Women	6,93±4,19	6,75±4,57	5,38±3,87	0.171	33,7±14,4
SBP (mm Hg)					
All	139,7±17,4	139,0±15,5	139,6±15,3	0.951	-
Men	141,5±15,3	144,0±10,2	144,0±11,5	0.362	-
Women	138,1±19,5	135,6±17,9	136,6±17,4	0.920	-
DBP (mm Hg)					
All	79,9±12,4	80,4±9,4	81,7±9,9	0.799	-
Men	81,9±13,9	86,3±6,1	85,5±7,7	0.931	-
Women	78,3±11,2	76,2±9,1	79,0±10,7	0.482	-
Capillary glycaemia(mg/dl)					
All	156±34	153± 59	161± 55	0.774	-
Men	137±28	125±35	160±43	0.697	-
Women	169±32	171±65	162±57	0.149	-

FMD: Flow-mediated Dilation; SBP: Systolic blood pressure, DBP: Diastolic blood pressure;
Values are means ± SEM unless otherwise indicated. P for differences between visits in general linear model analisys

Table 3. Correlation of basal, post-hyperemia diameters and FMD absolute values with clinical and laboratory variables.

	Baseline BAD (mm)		**Post-hyperemia Diameter (mm)**		**FMD (%)**	
	r^2	P	r^2	P	r^2	P
Age (years)	0,322	0,089	0,258	0,176	-0,540	0,003
Time of DM diagnosis (years)	0,164	0,397	0,165	0,391	0,015	0,937
BMI (Kg/m²)	0,114	0,555	0,159	0,411	0,342	0,070
SBP (mmHg)	0,137	0,487	0,128	0,516	-0,059	0,766
DBP (mmHg)	-0,102	0,606	-0,091	0,643	0,145	0,463
Glycemia (mg/dL)	-0,188	0,357	-0,192	0,347	0,030	0,883
A1c (%)	-0,334	0,095	-0,313	0,120	0,180	0,380
Total-cholesterol (mg/dL)	-0,059	0,778	-0,013	0,951	0,418	0,038
HDL-cholesterol (mg/dL)	0,120	0,568	0,101	0,630	-0,041	0,845
LDL-cholesterol (mg/dL)	-0,059	0,778	-0,007	0,972	0,395	0,051
Triglycerides (mg/dL)	-0,158	0,441	-0,172	0,245	0,431	0,028
UAE (mg/L)	0,265	0,212	0,400	0,249	0,381	0,066

DM: diabetes mellitus; BMI: body mass index; A1c: Glycated Hemoglobin; FMD: Flow-mediated Dilation; SBP: Systolic blood pressure, DBP: Diastolic blood pressure; UAE: Urinary albumin excretion.

Table 4. Correlation of basal and post hyperemia diameters and FMD CV values with clinical and laboratory variables

	Basal diameter (CV)		**Post-hyperemia diameter (CV)**		**FMD (CV)**	
	r^2	P	r^2	P	r^2	P
Age (years)	0,111	0,566	0,054	0,782	0,062	0,749
Time of DM diagnosis (years)	0,113	0,560	0,022	0,909	-0,102	0,600
BMI (Kg/m²)	-0,065	0,736	-0,016	0,934	-0,308	0,104
SBP (mmHg)	-0,173	0,379	-0,273	0,160	0,017	0,932
DBP (mmHg)	-0,172	0,380	-0,233	0,233	-0,215	0,271
Glycemia (mg/dL)	-0,009	0,966	-0,032	0,878	0,037	0,856
A1c (%)	0,081	0,695	0,089	0,666	-0,131	0,523
Total-cholesterol (mg/dL)	-0,307	0,135	-0,242	0,243	-0,218	0,296
HDL-cholesterol (mg/dL)	0,196	0,349	0,031	0,883	0,148	0,479
LDL-cholesterol (mg/dL)	-0,337	0,100	-0,214	0,304	-0,128	0,541
Triglycerides (mg/dL)	-0,306	0,128	-0,368	0,064	-0,454	0,020
UAE mg	-0,376	0,070	-0,254	0,231	-0,460	0,024
Baseline BAD (mm)	-0,298	0,116	-	-	-0,177	0,359
Post-hyperemia diameter (mm)	-	-	-0,247	0,197	-0,127	0,513
FMD (%)	-	-	-	-	-0,449	0,015

DM: diabetes mellitus; BMI: body mass index; A1c: Glycated Hemoglobin; SBP: Systolic blood pressure, DBP: Diastolic blood pressure; UAE: Urinary albumin excretion; BAD: Brachial artery diameter FMD: Flow-mediated Dilation;

Endothelial dysfunction and association with cardiovascular risk factors in type 2 diabetic patients

Running head: Endothelial dysfunction in type 2 diabetes

Address for correspondence and reprint requests: Themis Zelmanovitz, Serviço de Endocrinologia do Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350/ Prédio 12 - 4º andar CEP 90035-003 - Porto Alegre - RS, Brazil.

Phone/Fax: (55-51) 3359.8127 / 3331.3312

E-mail: **themis.voy@terra.com.br**

Sources of support: FIPE- Hospital de Clínicas de Porto Alegre; CAPES; CNPq; Propesq- Universidade Federal do Rio Grande do Sul

Abstract

Objective – To determine clinical and laboratorial factors, especially traditional and non-traditional cardiovascular risk factors, associated to vascular function parameters assessed by ultrasound of the brachial artery in type 2 diabetic patients with and without established cardiovascular disease.

Patients and Methods – This cross-sectional study evaluated type 2 diabetic patients that were submitted to clinical evaluation (metabolic and blood pressure control, detection of chronic complications of diabetes mellitus and cardiovascular evaluation). Patients underwent to ultrasound assessment of the brachial artery to determine endothelium-dependent and endothelium-independent vasodilation.

Results – Two hundred and forty patients were evaluated (age: 62.9 ± 9.4 years; 43 % male; mean diabetes duration: 15.6 ± 8.9 years, 21% with ischemic heart disease). Vascular measurements were: baseline brachial artery diameter (BAD) of 0.354 ± 0.6 cm, flow-mediated vasodilation (FMD) of 6.07 ± 4.13% and nitrate-mediated vasodilatation (NMD) of 18.53 ± 7.07%. Adjusting for age, multiple cardiovascular risk factors, independent predictors of lower FMD were higher systolic blood pressure, lower fibrinogen, and glomerular filtration rate (higher proportion of patients in category 2) ($p = 0.001$). Increased hemoglobin (Hb) was the single predictor of increased BAD ($p = 0.017$) which was maintained significant even when evaluating only patients without established cardiovascular disease or with severely increased urinary albumin. There were no predictors of NMD. Among female patients, elevated systolic blood pressure, microalbuminuria, decreased glomerular filtration rate and fibrinogen were independent risk factors for lower FMD.

Conclusion -- The present study reported an association between lower FMD and the presence of early loss of renal function, especially in women. Also, a positive association between baseline BAD and hemoglobin levels was observed, reinforcing the suggestion that higher hemoglobin is related to endothelial dysfunction.

Introduction

Atherosclerosis is the consequence of a chronic inflammatory condition, in which endothelial dysfunction plays a key role(1). Flow-mediated vasodilation (FMD) of the brachial artery is considered a reliable assessment of endothelium-dependent vasodilation and also a surrogate measure of nitric oxide production(2), which has been used in early stages of vascular injury to assess subclinical atherosclerotic process (3) and in patients with more advanced atherosclerotic disease(4, 5) It has been also questioned their capacity to improve diagnostic performance in patients with some degree of cardiovascular risk (6).

Some studies, however, have assessed the value of the basal artery diameter measurement as a simple diagnostic tool to access the cardiovascular risk (7). BAD certainly represents a simple parameter to assess, considering aspects like variability, once the last one is affected by an intra-observer coefficient of variation (CV) of more than 30% while the CV of BAD is less than 5%(8). Furthermore, many factors that limit FMD reproducibility do not affect BAD measurement(7) . Some related concepts for improvement of BAD diagnosis is the fact that the larger arteries tend to have less dilatory response and that, in cases of more advanced atherosclerotic disease, vascular remodeling, as systemic response, would produce a less vigorous response to endothelium-dependent dilation

Regarding nitrate-mediated dilation (NMD), it has been used as a measurement of vascular smooth muscle cell function and was independently associated with older age, the presence of hypertension and higher fasting glucose (9).

Both FMD and NMD have been evaluated in type 2 diabetic patients (9), as well as FMD was evaluated in different UAE groups in DM(10). Most previous reports studying diabetes and endothelial dysfunction, included patients in who did not have diabetic complications or any other apparent risk factor for endothelial damage apart from diabetes and/or dyslipidemia or hypertension.

Since diabetic patients present increased cardiovascular risk, the use of a wide range of medications is often observed and can cause acute and chronic vascular changes. The assessment of vascular function by tools that can measure these two artery stages and correlate them with several risk factors may help elucidate whether these measures can provide additional information for the population's care.

The aim of this study was to determine clinical and laboratorial factors, especially traditional and non-traditional cardiovascular risk factors, associated to vascular parameters assessed by ultrasound of the brachial artery in type 2 diabetic patients with and without established cardiovascular disease.

Population and methods

Patients

We studied 240 patients with type 2 diabetes attending the Endocrine Division's outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil, in a cross-sectional study design. Type 2 diabetes mellitus was defined by the World Health organization(11). Consecutive patients in routine clinical visits were invited to the study.

Exclusion criteria were > 85 years of age, presence of heart failure (class III or IV), an acute cardiovascular event in the preceding 6 months, and severe autonomic neuropathy. Treatment with antihypertensive and oral anti-diabetic agents was maintained during the study. The institutional local Research Ethics Committee approved the study protocol, and patients signed a written informed consent prior to inclusion.

Methods

Clinical evaluation

Clinical evaluation consisted of blood pressure assessment, renal and cardiovascular evaluation. Participants were instructed to maintain their medications and usual physical activities throughout the study period.

Sitting blood pressure was measured twice to the nearest 2 mm Hg after a 10-min rest by using a digital sphygmomanometer (OMRON ® Automatic Blood Pressure Monitor, Model HEM-705CP, Vernon Hills, Illinois 60061), to evaluate blood pressure control. Hypertension was defined as blood pressure ≥ 140/90mmHg on at least 2 separate occasions or use of antihypertensive drugs(12).Renal function was evaluated by serum creatinine and urinary albumin in random sample(13). Glomerular filtration rate (GFR) was estimated by CKD-EPI equation(14) **(***Chronic Kidney Disease Epidemiology Collaboration*). Urinary albumin was considered moderately and severely increased when the values were between 17 and 174 mg and ≥ 174 mg, respectively, at least twice in a 6-month period prior to inclusion (15, 16). Cardiovascular evaluation consisted of applying the World Health Organization cardiovascular questionnaire and resting electrocardiogram(17). Exercise electrocardiogram testing or radionuclide myocardial perfusion imaging (exercise or pharmacological – dipyridamol) were done, if necessary, to evaluate the presence of myocardial ischemia. Prior cardiovascular disease was considered ischemic heart disease, stroke and peripheral vascular disease(18).

Endothelial function assessment

Using vascular ultrasonography, arterial endothelium function was measured by examining brachial artery responses to endothelium-dependent and endothelium-independent stimuli. Patients underwent assessment of endothelium–dependent FMD, according to

*Guidelines for the Ultrasound Assessment of Endothelial-Dependent Flow-Mediated Vasodilation of the Brachial Artery(19) . A*fter an overnight fasting period, subjects were kept quiet for 5 minutes before measurement. Ultrasound systems were equipped with vascular software for two-dimensional imaging, color and spectral Doppler and an internal electrocardiogram monitor, image resolution was enhanced with a broad-band (multiple-frequency: 7 to 12 MHz) linear array transducer. The brachial artery was imaged above the antecubital fossa in a longitudinal section and a sphygmomanometric cuff was placed on the forearm. After a baseline rest image was acquired; arterial occlusion was created by cuff inflation at least 200 mm Hg for 5 minutes. Subsequent cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia). The diameter of brachial artery was scanned and recorded after 45 seconds to 2 min after cuff deflation. Following reactive hyperemia, 10 minute resting period was allowed prior to acquisition of a new baseline image. Subsequently, sublingual glyceryl trinitrate spray (300mg) was administered to evaluate endothelium-independent dilation. A final image was obtained 5 minutes after the administration. Our laboratory inter-individual coefficient of variation of basal artery diameter, FMD and of NMD previously determined in a group of 29 type 2 diabetic patients were 3.4 ± 2.6%, 37.3 ± 14.5% and 3.6 ± 2.5%, respectively.

Laboratory measurements

Blood samples were collected after a 12-hour overnight fast. Plasma glucose, serum lipids, C reactive protein (CPR) and fibrinogen were determined by enzymatic methods and creatinine by the Jaffe reaction traceable to IDMS.9 (Advia® 1800, Siemens Healthcare, Munich, Germany). *Low-density lipoprotein* (LDL) cholesterol was calculated using the Friedewald formula (20). Hemogram was performed by flow citometry (ABX Pentra DX 120, HORIBA, Kyoto, Japan). HbA1c was determined by HPLC method (Variant II Turbo HbA1c, BioRad Laboratories, Hercules, CA, USA). This method is a National Glycohemoglobin

Standardization Program (NGSP) certified method (http://www.ngsp.org/prog/index.html) and it is aligned to International Federation of Clinical Chemistry (IFCC) reference method. The Clinical Pathology department at our institution is a HbA1c External Quality Assurance Program participant with adequate performance.

Urinary albumin was measured in sterile urine samples by immunoturbidimetry (Advia® 1800, Siemens Healthcare, Munich, Germany).

Statistical analysis

Pearson or Spearman correlation coefficients were used for testing the relationships between BAD, FMD e NMD and clinical and laboratory characteristics. The Student t or Mann-Whitney test for independent continuous variables and the Exact Fisher or Chi-Square test for categorical variables were applied, as indicated.

Because of the lack of a well-established cutoff point of normalcy of vascular function parameters, the patients were stratified according to the median of each vascular measure in the present sample to analyze association with categorical variables. Multiple logistic regression models were carried out to test the association of baseline BAD, FMD e NMD (dependent variables) and factors with possible biological relevance or significance at univariate analysis, and results were described using odds ratios (OR) and their 95% confidence interval (CI).

Variables with non-Gaussian distribution (FMD, plasma TG, CRP and UAE) were log transformed before analysis. P values < 0.05 were considered significant. Results were expressed as mean ± SD or median (range). SPSS software (version 18.0; SPSS, Chicago, IL) was used for the analyses.

Results

Two hundred and forty patients were evaluated (age: 62.9 ± 9.4 years; 43 % male; mean diabetes duration: 15.6 ± 8.9 years, 21% with ischemic heart disease).Clinical and laboratorial characteristics of the studied patients are described in Table 1. When patients were divided according to gender, it was observed that women presented longer time of diabetes diagnosis, higher BMI, and higher values of total and LDL cholesterol, glycated hemoglobin and fibrinogen than men. On the other hand, men presented higher proportion of peripheral cardiovascular disease and diabetic nephropathy, as well as higher levels of creatinine, hemoglobin and baseline basal artery diameter.

Association of clinical parameters and vascular function in the overall population

Table 2 illustrates the associations between baseline BAD, FMD and NMD measurements and clinical variables of interest. Baseline BAD correlated negatively with diabetes duration, total cholesterol and HDL-cholesterol levels, and positively with waist circumference, serum creatinine levels, urinary albumin and hemoglobin values. FMD correlated negatively with age, creatinine and urinary albumin, and positively with GFR and fibrinogen. NMD presented negative correlation only with age.

When the patients were stratified according to the median of each measure (baseline BAD = 0.345 cm, FMD = 5.8 % and NMD = 17.5 %), patients with higher baseline BAD were mostly male. The group with higher FMD was younger, had a lower systolic blood pressure, higher proportion of patients in stage 1 of CKD (stage 1 = 54.2%, stage 2 = 35.8% and stage 3 = 10 %, p = 0.012), lower proportion of ischemic heart disease (14 vs 28 %, p = 0.020) and tendency of female predominance (Table 3). Clinical variables did not differ between groups above and below the median NMD (data not shown).

In logistic regression analysis, the dependent variable of the constructed models were to be in the group with values above or below the median for each measure of vascular

function, and the independent variables were the following factors: use of lipid-lowering medication, systolic blood pressure, DM duration, fibrinogen, glycaemia, use of antihypertensive drugs that act on the renin-angiotensin system, hemoglobin, HDL-C, waist circumference, age, stage of CKD.

Therefore, in multivariate analyses, the presence of baseline BAD above 0.345 cm was linked only to Hb values, after adjustment to these covariates. An FMD below 5.08% was positively associated with systolic blood pressure and with the presence of CKD stage 2 (CKD stage 1 as the reference), and inversely associated with higher fibrinogen (Table 5). No factor appeared as an independent predictor of NMD in the assessed models.

Association between clinical parameters and vascular function measures according to gender

Table 4 shows the correlation of clinical parameters with vascular function measures in patients according to gender.

In women, it was observed a positive correlation between baseline BAD with BMI, and an inverse correlation with total cholesterol and HDL-cholesterol levels. FMD was inversely correlated with age and glycaemia, and positively correlated with estimated GFR and serum fibrinogen. The NMD correlated negatively with age and glycaemia.

In women, in multivariate analysis, the presence of lower FMD was positively associated with systolic blood pressure (OR:1.09, 95 % CI:1.02-1.15, p = 0.01), urinary albumin (OR 1.002; 95%CI 1001-1004, p = 0.023), and with CKD stage 3 (CKD stage 1 as reference; OR 88.22; 3.82 - 2037.56; 95 % CI, P = 0.01), and inversely associated with fibrinogen (OR 0.099, 95% CI 0.97-0.99; p = 0.003). No factor appeared as an independent predictor of BAD in the assessed models. The presence of NMD below median was linked to

higher waist circumference, glycemia, UAE, hemoglobin, statins use and to lower fibrinogen and GFR categories.

When men were analyzed, was observed a positive correlation between BAD with triglycerides. FMD was inversely correlated with UAE and NMD correlated negatively with systolic blood pressure.

In multivariate analysis, these associations were not confirmed, after adjustment to the same covariates as described above.

Association between traditional and nontraditional cardiovascular risk factors and vascular function measures in patients without cardiovascular disease

The association between cardiovascular risk factors and measures of vascular function was reanalyzed excluding the patients with established CVD. One hundred and seventy-six patients were analyzed, with average baseline BAD of 0.352 cm, FMD of 6.3% and 18.6% of NMD.

In the correlation analysis, the baseline BAD was inversely correlated with serum cholesterol ($r = -0151$, $p = 0.048$), HDL-c ($r = -0273$, $p < 0.001$) and fibrinogen ($r = -0.162$, $p < 0.039$), and positively correlated with hemoglobin (r - 0351, $P < 0.001$) and urinary albumin ($r = 0.189$, $p < 0.015$). FMD showed a negative correlation with age ($r = -0149$, $P < 0.049$) and urinary albumin ($r = -0.183$, $p < 0.019$), and positive with fibrinogen ($r = 0.155$, $p < 0.049$) and GFR ($r = 0.161$, $p < 0.034$). The NMD showed negative correlation with age ($r = -0173$, $p < 0.024$) and positive with HDL-c ($r = 0.158$, $p < 0.042$).

In multivariate analysis, e presence of lower BAD was positively associated with hemoglobin and FMD with lower fibrinogen (table5).

Association between clinical and laboratorial characteristics and vascular function measures in patients without overt nephropathy

Also, patients with normal to moderately increased urinary albumin, i.e., without overt nephropathy, were separately analyzed. Two hundred and nine patients were analyzed, with average baseline BAD of 0.350 cm, FMD of 6.3 % and 18.68 % of NOMD.

In the correlation analysis, the baseline BAD was negatively correlated to diabetes duration ($r = -0140$, $p = 0.045$), fibrinogen ($r = -0155$, $p < 0.032$), HDL-c ($r = -0221$, $p < 0.001$), and positively with waist circumference ($r = 0.150$, $p = 0.042$), hemoglobin (r - 0301, $P < 0.001$). FMD demonstrated a negative correlation with age ($r = -0.150$, $p < 0.031$), and positive with GFR ($r = 0.174$, $p < 0.012$) and fibrinogen ($r = 0.164$, $p < 0.023$). NMD showed negative correlation with age ($r = -0189$, $p < 0.007$) and diabetes duration ($r = -0189$, $p = 0.007$), and positively with HDL-c ($r = 0.158$, $p < 0.042$).

In multivariate analysis, we observed similar results to those when all the sample of patients was included (Table 5).

Discussion

In this population of type 2 diabetic patients, we found an association between higher FMD and systolic blood pressure and stages of CKD. The progression to stage 2 CKD was associated with higher values of FMD, when compared with patients in stage 1 CKD. These findings were observed both in the whole population and when only patients without overt nephropathy were separately analyzed, showing that endothelial function is intimately linked to better kidney condition, as previously demonstrated by Reffelmann and Yokoyama(10, 21). In population-based study(21), they found correlation of FMD and GFR in entire population, in men and women, but only in the later after multiple adjustments. Our population of diabetic patients found similar results.

On the other hand, in the present study, an inverse association was observed between the presence of lower FMD values and fibrinogen levels, especially in women. This suggests that higher levels of fibrinogen are associated with a lower chance to have lower FMD, i.e., endotelial dysfunction, contrary to what is reported in the literature(22, 23). Increased fibrinogen, as an inflammatory, haemostatic component, is present in diabetic patients and some studies have shown that fibrinogen is associated with diabetes regulation, age, hypertension and components of the metabolic syndrome(23).

The present study also observed an association between baseline BAD and hemoglobin levels, when all patients were analyzed, and also when patients without CVD or overt nephropathy were separately evaluated. Recently, some researchers have suggested baseline BAD as a tool to evaluate vascular function(7) , but few studies have analyzed BAD association with cardiovascular risk factors(24).

It is known that increased hematocrit and hemoglobin are associated with lower FMD in both diabetic and hypertensive patients (25, 26) due to lower bioavailability of nitric oxide, since hemoglobin acts as a nitric oxide buffer solution. Also, a higher hematocrit is a negative predictor for cardiovascular events (27). Increased basal diameter of the brachial artery may reflect atherosclerosis and vascular remodeling. Therefore, our findings are consistent with the results observed by other researchers, and confirmation with further studies that can demonstrate some causality of hemoglobin as an independent factor for the increase of baseline BAD in diabetic patients would be necessary. Furthermore, there is a positive correlation, although weak, between baseline BAD and microalbuminuria, which also is a risk factor for cardiovascular disease.

Recently, Naka (9)and Yokoyama(9, 10) studied vascular function in diabetic patients. The first found time since diagnosis of diabetes as the single independent predictor of an

increased FMD and increased age, fasting glucose and hypertension as independent predictors of decreased NMD.

The later show UAE as determinant of FMD and that the relation of FMD with intima-media thickness is less pronounced in patients more advanced atherosclerotic disease. Our population had a similar age, but longer diabetes duration, when compared to the population of the Naka's study (9). In addition, our patients were with a not adequate glycemic control, which may potentially interfere the variability of the FMD method (8). Furthermore, the type 2 diabetic patients frequently present comorbidities are in use of many medications that alter the outcome of vascular evaluation. All these factors may have influenced the results of the present study, especially the lack of association with some traditional cardiovascular risk factors.

The majority of the female population in the present study was already in the post-menopausal period, and they presented a higher number of risk factors correlated with vascular function. When the patients were divided according to the gender, the female population had showed several significant clinical differences when compared to male. Despite being a population with higher glycated hemoglobin, total cholesterol and longer diabetes duration, women have less peripheral vascular disease, as well as less diabetic nephropathy, suggesting lighter atherosclerotic disease than males.

In conclusion, the present study reported an association between lower FMD and the presence of early loss of renal function, in patients with diabetes, with and without established cardiovascular disease, especially in women. Also, a positive association between baseline BAD and hemoglobin levels was observed, reinforcing the suggestion that higher hemoglobin is related to endothelial dysfunction. These findings are relevant in the research of factors associated to the subclinical atherosclerotic process in type 2 diabetic patients and further studies are necessary to confirm these results.

Table 1. Clinical and laboratory characteristics of studied patients

	All (n=240)	Men (n=103)	Women (n=137)	P
Demographics				
Age (years)	62.9 ± 9.4	62.7 ± 9.4	63.0 ± 9.2	0.78
Time of DM diagnosis (years)	15.6 ± 8.9	13.9 ± 8.2	16.9 ± 9.4	0.01
Hypertension (%)	82	79	84	0.31
Ischemic Heart Disease (%)	21	21	20	0.74
Peripheral Cardiovascular Disease (%)	24	33	16	0.01
Diabetic Nephoopathy (%)	36	47	29	0.00
Category of GFR				
1 (%)	45	47	42	0.68
2 (%)	43	42	44	0.59
3 (%)	12	11	13	0.26
Use of				
RAAS inhibitors (%)	67	71	84	0.19
Statins (%)	57	57	64	0.34
Waist circumference (cm)	100.7 ± 9.8	102.0 ± 8.7	99.8 ± 10.4	0.09
BMI (Kg/m²)	29.3 ± 4.2	28.5 ± 3.3	29.9 ± 4.6	0.01
Laboratory				
Glycemia (mg/dL)	155 ± 61	153 ± 60	156.4 ± 62.6	0.63
A1c (%)	8.2 ± 1.8	7.8 ± 1.5	8.5 ± 1.9	0.00
Total-cholesterol (mg/dL)	179 ± 42	168 ± 41	186 ± 39	0.00
HDL-Cholesterol (mg/dL)	46 ± 12	45 ± 11	48 ± 12	0.09
LDL-cholesterol (mg/dL)	101 ± 35	95 ± 35	105 ± 34	0.02
Triglycerides (mg/dL)	136 (41 -780)	125 (41 – 780)	146 (44-540)	0.11
Creatinine (mmol/L)	0.87 ± 0.29	0.98 ± 0.21	0.79 ± 0.32	<0.001
CKD-EPI (mL/min/1.73m²)	84.1 ± 18.3	84.4 ± 17.3	83.6 ± 19.2	0.80
C-reactive protein (mg/L)	2.6 (0.12 – 9.9)	1.8 (0.12 – 8.8)	2.9 (0.12-9.1)	0.80
Fibrinogen (mg/dL)	392 ± 100	375 ± 94	406 ± 104	0.03
Hemoglobin (g/dL)	13.7 ± 2.9	14.2 ± 1.2	12.9 ± 1.16	<0.001
Vascular Measurements				
Baseline BAD (mm)	0.354 ± 0.061	0.389 ± 0.058	0.326 ± 0.049	<0.001
SBP (mmHg)	138 ± 18	136 ± 18	140 ± 18	0.21
DPB (mmHg)	78 ± 10	77 ± 9	78 ± 10	0.46
FMD (%)	6.07 ± 4.13	5.57 ± 3.79	6.35 ± 4.38	0.22
NMD (%)	18.53 ± 7.07	17.95 ± 6.10	18.97 ± 7.66	0.28

GFR: Glomerular filtration rate; BMI: Body mass index; RAAS: renin- angiotensin-aldosterone system; CKD-EPI: COROnic Kidney Disease epidemiology collaboration; A1c: Glycated Hemoglobin; BAD: Brachial artery diameter; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FMD: Flow-mediated dilation; NMD: Nitrate-mediated dilation;

Data are expressed in MEAN ±SD or median (min-max) if non parametric.

Table 2. Correlation between clinical and laboratorial parameters with measures of vascular function in studied patients.

	Baseline BAD (mm)		FMD (%)		NMD (%)	
	r^2	P	r^2	P	r^2	P
Age (years)	0.075	0.26	-0.147	0.02	-0.170	0.01
Time of DM diagnosis (years)	-0.134	0.04	-0.016	0.81	-0.118	0.08
BMI (Kg/m²)	0.073	0.28	0.011	0.87	0.008	0.91
SBP (mmHg)	-0.060	0.38	-0.102	0.14	-0.110	0.12
DBP (mmHg)	-0.005	0.95	-0.049	0.47	-0.027	0.70
Waist Circunference (cm)	0.205	0.00	-0.048	0.49	-0.007	0.92
Glycemia (mg/dL)	0.004	0.96	-0.106	0.10	-0.059	0.39
A1C (%)	-0.075	0.25	0.048	0.46	-0.030	0.66
Total-Cholesterol (mg/dL)	-0.150	0.02	0.061	0.35	0.001	0.95
HDL-Cholesterol (mg/dL)	-0.233	0.00	0.093	0.15	0.122	0.07
LDL- Cholesterol	-0.124	0.06	0.066	0.32	0.001	0.99
Triglicerídeos (mg/dL)	0.035	0.59	-0.056	0.39	-0.102	0.13
Creatinine (mg/dL)	0.222	0.00	-0.188	0.00	-0.006	0.93
Urinary albumin (mg/L)	0.152	0.02	-0.159	0.02	-0.090	0.19
Estimated GFR (mL/min/1.73m²)	0.027	0.68	0.168	0.01	0.032	0.63
C-reactive protein (mg/L)	0.015	0.84	-0.032	0.67	-0.106	0.17
Fibrinogen (mg/dL)	-0.126	0.06	0.142	0.04	-0.025	0.71
Hemoglobin (mg/L)	0.345	0.000	0.892	0.89	-0.039	0.60

BAD: Brachial artery diameter ;A1c: Glycated Hemoglobin; BMI: Body mass index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; UAE: BMI: Body mass index; UAE: Urinary albumin excretion; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; A1c: Glycated Hemoglobin; Urinary albumin excretion; CKD-EPI: CORonic Kidney Disease epidemiology collaboration;

Table 3. Clinical and laboratorial characteristics of the patients divided according to the median FMD of the studied population.

	Flow-mediated Dilation		
	< 5.08 %	**> 5.08 %**	**P**
Age (years)	64.2 ± 9.0	61.5 ± 9.8	0.028
Time of DM diagnosis (years)	15.8 ± 9.0	15.6 ±9.1	0.89
BMI (Kg/m²)	29.6 ± 4.1	29.2 ±4.2	0.53
Male (%)	37	49	0.07
SBP (mmHg)	140.6 ± 17.5	135.7 ± 17.6	0.04
DBP (mmHg)	77.6 ± 9.3	77.7 ± 10.3	0.96
Hypertension (%)	53	47	0.13
Ischemic Heart Disease (%)	28	14	0.01
Peripheral vascular Disease (%)	28	21	0.34
Waist Circunference (cm)	101.0 ± 9.3	100.6 ± 10.2	0.78
Glycemia (mg/dL)	158 ± 61	152 ± 62	0.46
A1C (%)	8.0 ± 1.7	8.3 ±1.8	0.24
Total-Cholesterol (mg/dL)	177 ± 40	181 ± 43	0.48
HDL-Cholesterol (mg/dL)	46 ± 12	47 ± 12	0.59
LDL- Cholesterol	99 ±33	103 ± 36	0.41
Triglicerídeos (mg/dL)	146 (44- 508)	132 (41-780)	0.42
Creatinine (mg/dL)	0.92 ± 0.35	0.83 ± 0.21	0.01
Urinary albumin (mg/L)	14.6 (0-2488)	8.6(0-2395)	0.01
Estimated GFR (mL/min/1.73m²)	80.8 ± 18.67	86.9 ± 17.8	0.01
Category of GFR			0.012
1 (%)	35	54	
2 (%)	50	36	
3 (%)	14	10	
C-reactive protein (mg/L)	2.29 (0.12-8.87)	1.7 (0.12-9.88)	0.83
Fibrinogen (mg/dL)	382 ± 96	403 ± 104	0.12
Hemoglobin (mg/L)	13.5 ± 1.3	13.3 ± 1.2	0.26
Baseline BAD (mm)	0.371 ± 0.057	0.336 ± 0.061	0.00

BMI: Body mass index; UAE: SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; A1c: Glycated Hemoglobin; Urinary albumin excretion; CKD-EPI: CORonic Kidney Disease epidemiology collaboration; BAD: Brachial artery diameter. Data are expressed in mean ± SD or median (min-max) if non parametric.

Table 4. Correlation between clinical characteristics and vascular function measures separated by gender.

	Male						Female					
	Baseline BAD		**FMD%**		**NMD**		**Baseline BAD**		**FMD%**		**NMD%**	
	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P	r^2	P
Age (years)	0.099	0.322	-0.009	0.926	-0.145	0.153	0.106	0.219	-0.230	0.007	-0.187	0.032
Time of DM diagnosis (years)	-0.117	0.239	0.012	0.908	-0.115	0.255	0.010	0.906	-0.054	0.533	-0.149	0.094
BMI (kg/m²)	0.003	0.976	0.002	0.981	-0.073	0.497	0.277	0.002	-0.013	0.881	0.013	0.884
SBP (mmHg)	0.086	0.404	-0.138	0.180	-0.255	0.014	-0.024	0.798	-0.107	0.248	-0.025	0.791
DBP (mmHg)	0.064	0.536	-0.043	0.678	-0.191	0.068	0.039	0.676	-0.062	0.504	0.083	0.382
Waist circunference (cm)	0.017	0.870	-0.156	0.134	0.004	0.969	0.266	0.266	0.029	0.752	-0.024	0.798
Glycemia (mg/dL)	-0.107	0.285	-0.023	0.817	0.175	0.085	0.110	0.206	-0.187	0.030	-0.197	0.026
A1c (%)	0.060	0.552	0.108	0.280	0.036	0.727	0.006	0.948	-0.015	0.863	-0.080	0.367
Total-Cholesterol (mg/dL)	0.079	0.432	0.024	0.807	0.011	0.914	-0.180	0.038	0.066	0.450	-0.020	0.823
HDL-Cholesterol (mg/dL)	-0.075	0.455	0.014	0.886	0.067	0.514	-0.322	0.000	0.000	0.093	0.164	0.064
LDL-Cholesterol (mg/dL)	-0.033	0.743	0.007	0.947	-0.005	0.965	-0.116	0.185	0.097	0.270	-0.008	0.932
Tryglicerides (mg/dL)	0.213	0.032	0.034	0.735	-0.065	0.525	0.106	0.223	-0.139	0.110	-0.152	0.087
Creatinine (mg/dL)	-0.116	0.245	-0.041	0.679	-0.085	0.405	-0.008	0.925	-0.296	0.000	0.094	0.285
Urinary albumin (mg/dL)	0.006	0.956	-0.237	0.019	-0.079	0.448	0.101	0.258	-0.081	0.366	-0.093	0.300
Estimated GFR (mL/min/1.73m²)	0.096	0.335	-0.034	0.734	0.064	0.528	-0.024	0.781	0.303	0.001	0.007	0.939
C-reactive protein (mg/L)	0.025	0.830	-0.123	0.288	-0.152	0.190	0.155	0.128	0.008	0.938	-0.077	0.458
Fibrinogen (mg/dL)	-0.088	0.391	0.013	0.903	-0.164	0.117	-0.056	0.537	0.216	0.016	0.055	0.550
Hemoglobin (mg/L)	0.249	0.023	0.002	0.984	0.035	0.755	-0.062	0.521	0.125	0.198	0.024	0.806

BAD: Brachial artery diameter BMI: Body mass index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; A1c: Glycated Hemoglobin; UAE: Urinary albumin excretion; CKD-EPI: CORonic Kidney Disease epidemiology collaboration.

Table 5: Multivariated analysis of risk factors for BAD and FM

	All (n = 240)		Patients without CVD (n = 176)		Patients without macroalbuminuria (n = 209)	
	OR (95% CI)	**P**	**HR (95% CI)**	**P**	**HR (95% CI)**	**P**
Baseline BAD (cm)						
Hemoglobin (g/dL)	1.58 (1.11 – 2.24)	0.011	1.54 (1.02 – 2.32)	0.039	1.54 (1.07 – 2.25)	0.02
Time of DM (years)	-	-	-	-	0.94 (0.89 – 0.99)	0.02
FMD (%)						
Fibrinogen (mg/dL)	0.99 (0.986 – 0.997)	0.004	0.99 (0.988 – 0.998)	0.03	0.99 (0.989 – 0.997)	0.004
SBP (mmHg)	1.03 (1.01 – 1.06)	0.013	-	-	1.03 (1.00 – 1.06)	0.04
GFR category	4.11 (1.39– 12.10)	0.010	-	-	-	-

BAD: Brachial artery diameter; FMD: flow-mediated dilation; NMD: nitrite-mediated dilation; CVD: cardiovascular disease; OR: odds ratio; CI: confidence interval; SBP: systolic blood pressure; GFR: glomerular filtration rate (category 1 *vs.* category 2).

References

1. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115(10):1285-95.

2. Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. J Am Coll Cardiol. 1999;34(3):631-8.

3. Shechter M, Shechter A, Koren-Morag N, Feinberg MS, Hiersch L. Usefulness of brachial artery flow-mediated dilation to predict long-term cardiovascular events in subjects without heart disease. Am J Cardiol. 2014;113(1):162-7.

4. Ciccone MM, Iacoviello M, Puzzovivo A, Scicchitano P, Monitillo F, De Crescenzo F, et al. Clinical correlates of endothelial function in chronic heart failure. Clin Res Cardiol. 2011;100(6):515-21.

5. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. Circulation. 2007;115(18):2390-7.

6. Yeboah J, McClelland RL, Polonsky TS, Burke GL, Sibley CT, O'Leary D, et al. Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals. JAMA. 2012;308(8):788-95.

7. Montalcini T, Gorgone G, Gazzaruso C, Romeo S, Bosco D, Pujia A. Brachial artery diameter measurement: a tool to simplify non-invasive vascular assessment. Nutr Metab Cardiovasc Dis. 2012;22(1):8-13.

8. West SG, Wagner P, Schoemer SL, Hecker KD, Hurston KL, Likos Krick A, et al. Biological correlates of day-to-day variation in flow-mediated dilation in individuals with Type 2 diabetes: a study of test-retest reliability. Diabetologia. 2004;47(9):1625-31.

9. Naka KK, Papathanassiou K, Bechlioulis A, Kazakos N, Pappas K, Tigas S, et al. Determinants of vascular function in patients with type 2 diabetes. Cardiovasc Diabetol. 2012;11:127.

10. Yokoyama H, Sone H, Saito K, Yamada D, Honjo J, Haneda M. Flow-mediated dilation is associated with microalbuminuria independent of cardiovascular risk factors in type 2 diabetes - interrelations with arterial thickness and stiffness. J Atheroscler Thromb. 2011;18(9):744-52.

11. Diabetes mellitus. Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1985;727:1-113.

12. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003;42(6):1206-52.

13. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. 2005;28(1):164-76.

14. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

15. Zelmanovitz T, Gross JL, Oliveira JR, Paggi A, Tatsch M, Azevedo MJ. The receiver operating characteristics curve in the evaluation of a random urine specimen as a screening test for diabetic nephropathy. Diabetes Care. 1997;20(4):516-9.

16. Gross JL, Zelmanovitz T, Oliveira J, de Azevedo MJ. Screening for diabetic nephropathy: is measurement of urinary albumin-to-creatinine ratio worthwhile? Diabetes Care. 1999;22(9):1599-600.

17. GA R. Cardiovascular survery methods. In: B.H. GR, Pirineas RJ, editor. *WHO Monograph Series*1982. p. 123-65.

18. Association AD. Standards of medical care in diabetes--2010. Diabetes Care. 2010;33 Suppl 1:S11-61.

19. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39(2):257-65.

20. Friedewald W, Levy R, Fredrickson D. Estimation of the concentration of Low-density Lipoprotein Cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18:499-502.

21. Reffelmann T, Krebs A, Ittermann T, Empen K, Hummel A, Dörr M, et al. Mild renal dysfunction as a non-traditional cardiovascular risk factor?-Association of cystatin C-based glomerular filtration rate with flow-mediated vasodilation. Atherosclerosis. 2010;211(2):660-6.

22. Bosevski M, Borozanov V, Peovska I, Georgievska-Ismail L. Endothelial dysfunction correlates with plasma fibrinogen and HDL cholesterol in type 2 diabetic patients with coronary artery disease. Bratisl Lek Listy. 2007;108(7):297-300.

23. Bosevski M, Bosevska G, Stojanovska L. Influence of fibrinogen and C-RP on progression of peripheral arterial disease in type 2 diabetes: a preliminary report. Cardiovasc Diabetol. 2013;12:29.

24. Holewijn S, den Heijer M, Swinkels DW, Stalenhoef AF, de Graaf J. Brachial artery diameter is related to cardiovascular risk factors and intima-media thickness. Eur J Clin Invest. 2009;39(7):554-60.

25. Maio R, Sciacqua A, Bruni R, Pascale A, Carullo G, Scarpino PE, et al. Association between hemoglobin level and endothelial function in uncomplicated, untreated hypertensive patients. Clin J Am Soc Nephrol. 2011;6(3):648-55.

26. Natali A, Toschi E, Baldeweg S, Casolaro A, Baldi S, Sironi AM, et al. Haematocrit, type 2 diabetes, and endothelium-dependent vasodilatation of resistance vessels. Eur Heart J. 2005;26(5):464-71.

27. Lowe G, Rumley A, Norrie J, Ford I, Shepherd J, Cobbe S, et al. Blood rheology, cardiovascular risk factors, and cardiovascular disease: the West of Scotland Coronary Prevention Study. Thromb Haemost. 2000;84(4):553-8.