UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:
ENDOCRINOLOGIA

DISSERTAÇÃO DE MESTRADO

ANÁLISE DA CONDUÇÃO NERVOSA PERIFÉRICA, FUNÇÃO AUTONÔMICA CARDÍACA E DA MORFOLOGIA RETINIANA EM PACIENTES COM DIFERENTES GRAUS DE TOLERÂNCIA À GLICOSE: ESTUDO TRANSVERSAL.

MAYARA ABICHEQUER BEER

Porto Alegre
2017

MAYARA ABICHEQUER BEER

ANÁLISE DA CONDUÇÃO NERVOSA PERIFÉRICA, FUNÇÃO AUTONÔMICA CARDÍACA E DA MORFOLOGIA RETINIANA EM PACIENTES COM DIFERENTES GRAUS DE TOLERÂNCIA À GLICOSE: ESTUDO TRANSVERSAL.

Dissertação de mestrado 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 Mestre em Endocrinologia.

Orientador: Prof. Dr. Fernando Gerchman

Porto Alegre

2017

CIP - Catalogação na Publicação

Beer, Mayara Abichequer
ANÁLISE DA CONDUÇÃO NERVOSA PERIFÉRICA, FUNÇÃO AUTONÔMICA CARDÍACA E DA MORFOLOGIA RETINIANA EM PACIENTES COM DIFERENTES GRAUS DE TOLERÂNCIA À GLICOSE: ESTUDO TRANSVERSAL / Mayara Abichequer Beer. -- 2017.
48 f.

Orientador: Fernando Gerchman.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Porto Alegre, BR-RS, 2017.

1. Complicações do Diabetes. 2. Retinopatia Diabética . 3. Coróide. 4. Estado Pré-Diabético . I. Gerchman, Fernando , orient. II. Título.

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

AGRADECIMENTOS

Aos meus pais João Luiz e Soraya por sempre me incentivarem a buscar e acreditar nos meus sonhos e pelas sábias lições de amor, honestidade e perseverança.

Ao meu orientador Fernando Gerchman, agradeço pelo imenso aprendizado e incentivo à superação e as conquistas. Agradeço principalmente por ter proporcionado o meu desenvolvimento acadêmico, fornecendo subsídios para minha trajetória e levando ao meu aprimoramento como pesquisadora.

Aos colegas e amigos do laboratório do Serviço de Endocrinologia pelo incentivo, troca de conhecimentos e valiosas conversas propiciando um ótimo ambiente de trabalho.

A todos os meus colegas de grupo, que participaram direta e indiretamente na realização desse trabalho, especialmente à Giovana, por apoiar de forma ativa e dedicada nos momentos mais decisivos durante a execução deste projeto. Por fim, agradeço a todos que de alguma forma também contribuíram para a realização deste trabalho.

LISTA DE FIGURAS E TABELAS

ARTIGO ORIGINAL

LISTA DE ABREVIATURAS

ADA	American Diabetes Association
BMI	Body Mass Index
CHEPS	Contact Heat Evoked Potentials
CKD-epi	Filtração glomerular estimada pela equação
DM	Diabetes Melito
DPP	Diabetes Prevention Program
DRC	Doença Renal Crônica
EDI	Enhanced Depth Image
eGRF	Estimated Glomerular Filtration
FPG	Fasting Plasma Glucose
GJA	Glicemia de Jejum Alterada
HbA1c	Glycosylated Hemoglobin
HF	High Frequency Component
HRV	Heart Rate Variability
IFG	Impaired Fasting Glucose
IGM	Impaired Glucose Metabolism
IGT	Impaired Glucose Tolerance
LF	Low Frequency Component
ME	Macular Edema
NGT	Normal Glucose Tolerance
NN	normal to normal intervals
NPDR	Non – proliferative Diabetic Retinopathy
OCT	Tomografia de coerência óptica / Optical Coherence Tomography
OGTT	Oral Glucose Tolerance Test
PDR	Proliferative Diabetic Retinopathy
PNN50	Percentage of difference between R-R intervals > 50 ms.
PSA	Power spectral analysis
QST	Teste Quantitativo Sensitivo / Quantitative Sensory Test
RD	Retinopatia Diabética
RMSSD	Root Mean Square of R – R intervals
SDNN	Standard deviation of normal R – R intervals
SM	Síndrome Metabólica

T2DM	Type 2 Diabetes Mellitus
TDG	Tolerância diminuída à glicose
TP	Total Power
TTOG	Teste de Tolerância Oral à Glicose
US-CRP	C –reactive protein
VFC	Variabilidade da Frequência Cardíaca
VLF	Very Low Frequency Component

SUMÁRIO

RESUMO

O diabetes inclui um grupo de distúrbios metabólicos caracterizados por hiperglicemia, resultante de defeitos na secreção de insulina e / ou na sua ação, ou ambos. Levando em consideração a alta prevalência de diabetes em adultos e a elevada prevalência de complicações micro e macrovasculares nesta população, se faz necessário o estudo de novas estratégias para a identificação precoce dessas complicações. Em um estudo transversal realizado no ambulatório de Pré-diabetes e Síndrome Metabólica do Serviço de Endocrinologia do Hospital de Clínicas de Porto Alegre foram analisados marcadores precoces de retinopatia, nefropatia e neuropatia, com o objetivo de comparar achados iniciais de complicações microvasculares em indivíduos com diferentes graus de tolerância à glicose. Sessenta e quatro sujeitos foram submetidos a um protocolo padrão (vide Figura 1) incluindo avaliação antropométrica, bioquímica contemplando dosagem de glicemia em jejum, 2-h após sobrecarga, hemoglobina glicada, vitamina B12, colesterol HDL, triglicerídeos, albumina urinária em amostra randômica e filtração glomerular estimada pela equação CKD-epi. A avaliação oftalmoscópica de ambos os parâmetros dos olhos por fundoscopia e tomografia de coerência óptica (OCT) de domínio espectral. A neuropatia sensitiva periférica foi avaliada através do questionário de Michigan, limiares vibratórios por diapasão, sensibilidade pelo monofilamento de Semmes-Weinstein 10g, a neuropatia de fibras finas pelo teste sensitivo quantitativo (TSQ) e a neuropatia autonômica cardíaca pelos testes autonômicos de Ewing e análise espectral da variabilidade da frequência cardíaca (VFC). A idade, pressão arterial sistólica diurna, noturna e triglicerídeos foram diferentes entre os grupos. A espessura da coróide no olho direito diminuiu com a piora do grau de tolerância à glicose dos indivíduos. (NGT 331.9 ± 98.8 μm vs. PDM 273.0 ± 83.1 μm vs. DM 217.9 ± 76.7 μm; $p<0.001$) o mesmo comportamento foi visto no olho esquerdo (NGT 356.3 ± 87.1 μm vs. PDM 258.5 ± 86.1 μm vs. DM 229.6 ± 79.8 μm; $p=0.001$). A microalbuminúria de amostra aumentou com a piora do grau de tolerância à glicose. [NGT 5.0 (3.0 – 13.6) vs. PDM 4.0 (3.0 – 13.6) vs. DM 17.0 (6.4 – 57.0); $p=0.033$]. A sensibilidade dos pés ao monofilamento 10g Semmes-Weinstein diminuiu com a piora do grau de tolerância à glicose. [NGT 6.9 ± 0.4 vs. PDM 6.3 ± 0.9 vs. DM 6.1 ± 0.8; $p=0.008$]. Não houve diferença entre os grupos quando avaliados os parâmetros de escore de Michigan, sensibilidade vibratória, e limiares de calor e dor, além da

presença de neuropatia autonômica cardíaca de acordo com os critérios de Ewing. Quando realizada a análise espectral pelo domínio do tempo, a raíz quadrada dos intervalos R-R (RMSSD) apresentou-se reduzida com a piora do grau de tolerância à glicose dos indivíduos. A frequência cardíaca e pressão de pulso, avaliadas através da monitorização da pressão arterial de 24 horas pela MAPA, apresentaram uma tendência de aumento com a piora da tolerância à glicose dos indivíduos.

ABSTRACT

Diabetes includes a group of metabolic disorders characterized by hyperglycemia, resulting from defects in insulin secretion and / or its action or both. Taking into account the high prevalence of diabetes in adults and the association of micro and macrovascular complications in this population, in addition to the appearance of new techniques allowing greater sensitivity in the diagnosis of complications, allowing the development of strategies that enable the prevention of the development of diabetes and its chronic complications. In a cross sectional design study, aiming to compare earlier findings of microvascular complications in subjects with differ degrees of glucose tolerance, 64 subjects underwent a standard protocol comprising anthropometric, biochemical assessment of fasting glycaemia, 2h- glycaemia, glycated hemoglobin, B12 vitamin, HDL-cholesterol, triglycerides, sample albumin and glomerular filtration by CKD-epi. Assessment of fundoscopy and optical coherence tomography (OCT) on both eyes, were made. Autonomic, peripheral, and small fiber neuropathy were assessed by Ewing tests and spectral analysis of the heart rate variability (HRV). Michigan questionnaire, quantitative sensitive test (QST). Vibratory thresholds and foot sensitivity assessed by tuning fork, and Semmes-Weinstein 10g monofilament respectively. Age, daytime, nocturnal systolic blood pressure, and triglycerides were different between groups. Choroidal thickness decreased with decreasing glucose tolerance in the right eye (NGT 331.9 ± 98.8 μm vs. PDM 273.0 ± 83.1 μm vs. DM 217.9 ± 76.7 μm; p <0.001) and left eye (NGT 356, 3 ± 87.1 μm vs. PDM 258.5 ± 86.1 μm vs. DM 229.6 ± 79.8 μm, p = 0.001). Sample microalbuminuria increased with decreased glucose tolerance. [NGT 5.0 (3.0 - 13.6) vs. PDM 4.0 (3.0 - 13.6) vs. DM 17.0 (6.4 - 57.0); P = 0.033]. The foot sensitivity to the Semmes-Weinstein 10g monofilament test decreased with decreasing glucose tolerance (NGT 6.9 ± 0.4 vs. PDM 6.3 ± 0.9 Vs. DM 6.1 ± 0.8; P = 0.008). No differences were found in the Michigan detection parameter, vibration sensitivity, heat and pain thresholds, and autonomic cardiopathy parameters. Although, the mean square root between the R-R intervals (RMSSD), decreased with decreasing glucose tolerance (NGT 911.8 ± 167.0 vs. PDM 875.5 ± 137.9 vs. DM 783.9 ± 113.0; P = 0.005). Heart rate and herat frequency, both assessed by 24-h ambulatory blood pressure monitoring were higher with decreasing glucose tolerance.

1 REFERENCIAL TEÓRICO

1.1 CARACTERIZAÇÃO DO DIABETES

O diabetes inclui um grupo de doenças metabólicas caracterizadas por hiperglicemia, resultante de defeitos na secreção de insulina e/ou em sua ação ou ambos (1). Estima-se que mundialmente, 415 milhões de adultos tenham diabetes, e que em 2040 este número aumente para 642 milhões de pessoas. O gasto estimado com o controle e prevenção do diabetes é 673 bilhões de dólares, isto é, 12% dos gastos mundiais (2). No Brasil, os custos associados com exames confirmatórios para o diagnóstico de diabetes estão ao redor de 26,19 milhões de dólares, incluindo neste valor, o custo de materiais para o diagnóstico, mídia envolvida em campanhas de conscientização, treinamento de agentes de saúde, e custos de manutenção (3).

Aproximadamente 14,3 milhões de brasileiros têm diabetes, entretanto, acredita-se que ao redor de 1 a cada 2 indivíduos com diabetes não sabem que tem a doença, podendo os dados epidemiológicos brasileiros estarem subestimando a real prevalência da doença no Brasil (2). Quando avaliados os indivíduos do sul e sudeste do Brasil, a prevalência de diabetes autorreferida foi de 6,2% e 11,5% na população que nunca havia realizado medida de glicemia (3). Quanto ao pré-diabetes, um estado intermediário de anormalidade do metabolismo da glicose caracterizado pela presença de glicemia em jejum anormal e/ou tolerância diminuída à glicose, é associado a um risco maior de progressão para o diabetes melito. Pacientes com tolerância diminuída à glicose ou diabetes melito recém-diagnosticado apresentam um risco maior de doença cardiovascular do que indivíduos normoglicêmicos (4).

Levando-se em conta a alta prevalência de pré-diabetes em adultos e a associação de complicações micro e macrovasculares nessa população, torna-se importante estudar como e quando ocorre o desenvolvimento dessas complicações nesse estágio intermediário de anormalidades do metabolismo da glicose, assim, permitindo se desenvolver estratégias que possibilitem a prevenção do desenvolvimento do diabetes e de suas complicações crônicas.

1.2 COMPLICAÇÕES NO PRÉ – DIABETES ANTES ATRIBUÍDAS AO DIABETES

1.2.1 Retinopatia diabética

Em 1997, um estudo realizado em uma coorte de uma população de Pima Indians, avaliando indivíduos com diferentes graus de tolerância à glicose através de fotografias de fundo de olho, identificou retinopatia diabética não proliferativa em 12% de Pima Indians com TDG, de 8,3% naqueles com DM recém-diagnosticado, porém tendo TTOG normal quatro anos antes desse diagnóstico, de 11,2% naqueles com DM recém-diagnosticado. Concluindo que a prevalência de retinopatia nessa população estaria diretamente relacionada à glicemia de jejum e à glicemia de 2h sem uma exposição mais prolongada à hiperglicemia do diabetes para o desenvolvimento da retinopatia (5).

Uma análise realizada pelo *Diabetes Prevention Program* (DPP), estudo de prevenção do diabetes em indivíduos com pré-diabetes randomizados à mudança de estilo de vida, uso de metformina ou grupo controle com intervenção comportamental padrão acessou o fundo de olho anualmente ao longo de 3 anos em 594 de 878 indivíduos que desenvolveram DM ao longo de 3,1 anos de estudos e foram comparados com um grupo com um grupo de 302 participantes que não desenvolveram o DM através de fotografia estereoscópica do fundo do olho. A retinopatia diabética foi identificada em 12,6% dos indivíduos que desenvolveram DM e em 7,9% daqueles sem progressão para o DM (6). Em outro estudo, a prevalência de déficit visual significativo foi avaliada em 1992 idosos brancos com diferentes graus de tolerância à glicose e/ou SM de uma comunidade do sul da Califórnia, Estados Unidos.

Embora a prevalência de déficit visual significativo tenha sido baixa, a frequência encontrada foi duas vezes maior em mulheres com pré-diabetes do que aquelas sem pré-diabetes. Em homens pré-diabéticos encontrou-se uma prevalência também duas vezes maior de perda visual significativa ao comparar-se com homens com diabetes, achado esse considerado inusitado, mas que sugere que anormalidades precoces do metabolismo da glicose estejam relacionadas não só a formas precoces de retinopatia diabética, como encontrado no *Diabetes Prevention Program*, mas também a déficit visual nesses pacientes (7). A tomografia de coerência óptica (OCT) é usada para obter imagens tomográficas da retina, porém a captura de imagens da coróide é dificultada na maioria dos indivíduos (8).

Em alguns estudos buscou-se avaliar alterações na morfologia da retina e nas suas camadas em indivíduos com diferentes graus de retinopatia diabética e edema macular,

identificando-se que a espessura da coróide torna-se mais fina, irregular e lateralizada em relação à mácula quanto pior for o estágio da retinopatia. No entanto, não se realizou este tipo de estudo em indivíduos com pré-diabetes ou síndrome metabólica (9-12).

1.2.2 Neuropatia Diabética

A dor neuropática sem causa definida foi identificada em 35% de pacientes com pré-diabetes, especialmente naqueles com tolerância diminuída à glicose (13). Em outro estudo demonstrou-se haver, em uma pequena quantidade de indivíduos com pré-diabetes, apresentando biópsia de pele anormal e compatível com neuropatia de fibras finas encontradas tipicamente em indivíduos com diabetes e neuropatia (14). Entretanto, não há estudos de condução nervosa que avaliaram a prevalência de neuropatia de fibras finas em pacientes com pré-diabetes e/ou síndrome metabólica, devido à dificuldade de se realizar um estudo utilizando-se de metodologias tradicionais de avaliação de neuropatia sensório motora distal, como a avaliação sensitiva com diapazão, monofilamento e eletroneuromiografia, pois estes recursos não avaliam este tipo de fibra de uma maneira adequada.

A neuropatia autonômica foi avaliada em pacientes com diabetes e neuropatia (n=8), DM recém diagnosticados (n=41) e sua frequência comparada a um grupo controle sem diabetes (n=49) no estudo (14). Outro estudo, avaliando resposta vasoconstritiva a respiração profunda e o resfriamento corporal foram similarmente anormais em pacientes com diabetes e neuropatia e aqueles com diabetes de início recente e normais naqueles sem diabetes, sugeriu que a neuropatia pode já estar presente nas fases iniciais do diabetes e desenvolver-se no pré-diabetes (15).

Adicionalmente, não existem estudos com possíveis marcadores sorológicos de atividade simpática e parassimpática e sua relação com os mecanismos de desenvolvimento da neuropatia nos estágios iniciais de desenvolvimento da hiperglicemia, o que pode ser avaliado respectivamente com a dosagem das metanefrinas urinárias, um metabólito urinário das catecolaminas e, no plasma, do hormônio polipetídeo pancreático, hormônio produzido pela ilhota pancreática e relacionado também a mecanismos de fome e saciedade.

1.2.3 Nefropatia Diabética

Uma série de estudos tem sugerido que o pré-diabetes e a síndrome metabólica associam-se ao desenvolvimento de formas precoces de nefropatia, doença renal crônica (DRC), neuropatia, retinopatia diabética e doença macrovascular (16-21).

A relação do pré-diabetes e a síndrome metabólica com a nefropatia foi mais estudado no AustralianDiabetes, Obesity, and Lifestyle study, que demonstrou uma prevalência de microalbuminúria menor em indivíduos normais (4,3%), intermediária naqueles com glicemia de jejum alterada (8,3%) e tolerância diminuída à glicose (9,9%) e maior naqueles com diabetes de início recente (15,4%) e mais elevada naqueles com diabetes estabelecido (26,5%) (22). Os mecanismos por trás dessa relação não foram identificados de maneira clara, mas possivelmente apresentam relação com anormalidades da homeostase pressórica que ocorrem com o pré-diabetes e a síndrome metabólica, naqueles sem diabetes. Sendo assim, o uso de novas técnicas de avaliação para identificar anormalidades precoces que sugiram o desenvolvimento das complicações microvasculares do diabetes, já no pré-diabetes, pode permitir a melhor compreensão de quando estas complicações começam a se desenvolver e quais fatores que são relacionadas a elas.

2 REFERÊNCIAS BIBLIOGRÁFICAS

1. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2011;34 Suppl 1:S62-9.

2. Federation ID. IDF Diabetes Atlas Brussels, Belgium2015. 7th: Available from: http://www.diabetesatlas.org.

3. Malta DC IB, Chueiri PS, Stopa SR, Szwarcwald CL, Schmidt MI, Duncan BB. Health care among adults with self-reported diabetes mellitus in Brazil, National Health Survey, 2013. Revista Brasileira de Epidemiologia 2015;18:17-32.

4. Barzilay JI SC, Wahl PW, Kuller LH, Cushman M, Furberg CD, Dobs A, Polak JF, Savage PJ. Cardiovascular disease in older adults with glucose disorders: comparisons of American Diabetes Association of diabetes mellitus with WHO criteria. Lancet. 1999;354:622-5.

5. D.K. Nagi DJP, P.H. Bennett, R. Klein, W.C. Knowler. Diabetic retinopathy assessed by fundus photography in Pima Indians with impaired glucose tolerance and NIDDM. Diabetic Medicine. 1997;14(6):449–56.

6. Group DPPR. The prevalence of retinopathy in impaired glucose tolerance and recent-onset diabetes in the Diabetes Prevention Program. . Diabetic Medicine. 2007;24(2):137 - 44.

7. Klein R B-CE, Blunt BA, Wingard DL, . Visual impairment and retinopathy in people with normal glucose tolerance, impaired glucose tolerance, and newly diagnosed NIDDM. Diabetes Care. 1991;14(10):914-8.

8. Spaide RF KH, Pozonni MC. Enhanced Depth Imaging Spectral-Domain Optical Coherence Tomography. American Journal of Ophthalmology. 2008;146(4):496–500.

9. Regatieri CV BL, Carmody J, Fujimoto JG, Duker JS. Choroidal thickness in patients with diabetic retinopathy analyzed by spectral-domain optical coherence tomography. Retina 2012;32(3):563-8.

10. Adhi M BE, Waheed NK, Duker JS Analysis of morphological features and vascular layers of choroid in diabetic retinopathy using spectral-domain optical coherence tomography. JAMA Ophthalmology. 2013;131(10):1267-74.

11. Vujosevic S MF, Cavarzeran F, Pilotto E, Midena E. Macular and peripapillary choroidal thickness in diabetic patients. Retina. 2012;32(9):1781–90.

12. Kim JT LD, Joe SG, Kim JG, Yoon YH. Changes in choroidal thickness in relation to the severity of retinopathy and macular edema in type 2 diabetic patients. Investigative Ophthalmology & Visual Science. 2013;54(5):3378-84.

13. Singleton JR SA, Bromberg MB. Painful sensory polyneuropathy associated with impaired glucose tolerance. Muscle Nerve. 2001;24(9):1225-8.

14. Smith AG RP, Tripp S, Singleton JR Epidermal nerve innervation in impaired glucose tolerance and diabetes-associated neuropathy. Neurology 2001;57(9):1701-4.

15. McDaid EA MB, Parker AI, Hayes JR, Allen JA. Peripheral Autonomic Impairment in Patients Newly Diagnosed With Type II Diabetes. Diabetes Care. 1994;17(12):1422-7.

16. Tabák AG HC, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. Lancet 2012;379(9833):2279–90.

17. Singleton JR SA. Neuropathy associated with prediabetes: what is new in 2007? Current Diabetes Reports. 2007;7(6):420–4.

18. Shaw JE ZP, de Courten M, Dowse GK, Chitson P, Gareeboo H, Hemraj F, Fareed D, Tuomilehto J, Alberti KG. Impaired fasting glucose or impaired glucose tolerance. What best predicts future diabetes in Mauritius? Diabetes Care. 1999;22(3):399-402.

19. Sega R TG, Lanzarotti A, Carugo S, Cesana G, Schiavina R, Valagussa F, Bombelli M, Giannattasio C, Zanchetti A, Mancia G. Alterations of cardiac structure in patients with isolated office, ambulatory, or home hypertension: Data from the general population (Pressione Arteriose Monitorate E Loro Associazioni [PAMELA] Study). Circulation. 2001;104(12):1385-92.

20. Ninomiya T KY, Kubo M, Yonemoto K, Tanizaki Y, Doi Y, Hirakata H, Iida M. Metabolic syndrome and CKD in a general Japanese population: the Hisayama Study. American Journal of Kidney Diseases. 2006;48(3):383–91.

21. Gerchman F, Tong J, Utzschneider KM, Zraika S, Udayasankar J, McNeely MJ, et al. Body mass index is associated with increased creatinine clearance by a mechanism independent of body fat distribution. The Journal of Clinical Endocrinology and Metabolism. 2009;94(10):3781-8.

22. Tapp RJ, Shaw JE, Zimmet PZ, Balkau B, Chadban SJ, Tonkin AM, et al. Albuminuria is evident in the early stages of diabetes onset: results from the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). American Journal of Kidney Diseases : the official journal of the National Kidney Foundation. 2004;44(5):792-8.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Estudar fatores relacionados ao desenvolvimento de retinopatia e neuropatia de fibras finas e/ou autonômica em pacientes com PDM.

3.2 OBJETIVO SECUNDÁRIO

Estudar a prevalência das outras complicações microvasculares no PDM e DM de início recente, dentre elas, marcadores de nefropatia diabética, como a excreção urinária de albumina e a filtração glomerular estimada.

4 ARTIGO ORIGINAL

Título

Relationship between choroidal thickness on optical cohrence tomography, microalbuminuria, neuropathy and metabolic abnormalities in subjects with different degrees of glucose tolerance.

Autores

Beer, M.A, Piccoli, G.F, Araújo, C., Fitz, R., Schestatsky, P., Lavinsky, D., Gerchman, F.

Relationship between choroidal thickness on optical cohrence tomography, microalbuminuria, neuropathy and metabolic abnormalities in subjects with different degrees of glucose tolerance.

Short title: Earlier microvascular complications and glucose tolerance.

Mayara Abichequer Beer [1], Giovana Piccoli[2], Carina Araújo[1], Raquel Fitz[1], Pedro Schestatsky[3], Daniel Lavinsky[4], Fernando Gerchman[5*]

[1] Post Graduate Medical Science Program: Endocrinology. Faculdade de Medicina, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil.
[2] Medicine course, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil.
[3] Division of Internal Medicine, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
[4] Division of Ophthalmology, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
[5] Division of Endocrinology, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil

***Corresponding author**

Email: fgerchman@gmail.com

Abstract

Introduction: Diabetic microvascular complications are the leading cause of blindness, end-stage renal disease and amputation in subjects with type 2 diabetes (T2DM). Several studies have suggested that these abnormalities developed in early stages of impaired glucose tolerance metabolism, previously defined by consensus as pre-diabetes. **Objective:** To assess earlier findings of microvascular complications in subjects with different degrees of glucose tolerance. **Methods:** In a cross-sectional study, subjects (n=64, 44 females, age 54.6 ± 12.6; mean±SD) from a Metabolism Clinic of a university hospital were submitted to a 2-h 75g oral glucose tolerance test (OGTT): 14 with normal glucose tolerance [NGT], 20 with impaired glucose metabolism [IGM], 30 with type 2 diabetes [T2DM]; according to ADA criteria. Central choroidal thickness was measured by enhanced depth image (EDI) spectral domain optical coherence tomography (Spectralis OCT) in 115 eyes while retinal examination was measured using non-mydriatic retinography. Glycated hemoglobin (A1c) and albuminuria (spot urine) were measured. C-fiber neuropathy was assessed by Quantitative Sensory Testing (QST-CHEPS), and cardiac autonomic neuropathy was measured by the Ewing tests and assessed by spectral domain analysis. A two-sided P value <0.05 was considered significant. **Results:** Moderate and severe diabetic retinopathy were found with digital retinography examination in 3 subjects with T2DM and 1 with NGT. Choroidal thickness decreased with decreasing glucose tolerance in the right eye (NGT 331.9 ± 98.8 μm vs. IGM 273.0 ± 83.1 μm vs. DM 217.9 ± 76.7 μm; p <0.001) and left eye (NGT 356 , 3 ± 87.1 μm vs. IGM 258.5 ± 86.1 μm vs. DM 229.6 ± 79.8 μm, p = 0.001). Random spot microalbuminuria increased with decreasing glucose tolerance. [NGT 5.0 (3.0 - 13.6) vs. PDM 4.0 (3.0 - 13.6) vs. DM 17.0 (6.4 - 57.0); P = 0.033]. While foot sensitivity tested with the Semmes-Weinstein 10g monofilament test decreased with glucose tolerance (NGT 6.9 ± 0.4 vs. PDM 6.3 ± 0.9 Vs. DM 6.1 ± 0.8; P = 0.008). No differences were found in the Michigan score for neuropathy screening, vibration perception, heat and pain thresholds, and autonomic cardiac neuropathy tests. The mean square root between the R-R intervals (RMSSD) decreased with decreasing glucose tolerance (NGT 911.8 ± 167.0 vs. PDM 875.5 ± 137.9 vs. DM 783.9 ± 113.0; P = 0.005). Pulse pressure increased with decreasing glucose tolerance, suggesting a loss of parasympathetic function in earlier abnormalities of glucose metabolism. The choroidal thickness decreased with age, 24-h systolic blood pressure and

increasing urinary albumin excretion rate. By multiple linear regression analysis, progressive age was independently associated with decreased choroidal thickness. **Conclusions:** Subjects with IGM presented increased albumin excretion rates and a discrete disruption of the autonomic parasympathetic nervous system. To our knowledge, this is the first study suggesting that pathological changes in choroidal thickness may be an earlier marker of diabetic retinopathy in subjects with abnormal glucose metabolism and DM.

Introduction

Diabetic retinopathy is the leading cause of blindness in adults between 20 and 74 years in developed countries (1). Diabetes control and duration are major risk factors for its development (2). Other risk factors are ageing, hypertension, diabetic neuropathy and nephropathy (3-7). Although it was previously defined that the development of microvascular complications may happen after 7 years of the development of diabetes (2), data from the Diabetes Prevention Program have shown that subjects with prediabetes have already earlier signs of diabetic retinopathy (8). Current studies have also demonstrated the occurrence of milder sensory neuropathy, dysfunction of small and autonomic fibers in metabolic syndrome, impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) and newly onset diabetes (< 3 years) (9-12). The ocular choroid has a crucial role in supplying blood to the outer retina.

According to experimental findings, a dysfunction of its homeostasis seems to play a role in the development of diabetic retinopathy (13-16). Choroid irregularities were demonstrated in 89% of T2DM subjects with non-proliferative diabetic retinopathy (NPDR), in 90% of those with proliferative diabetic retinopathy (PDR) and in 93% of those with macular edema (ME), compared to none of those considered as having healthy eyes (13).

While some studies have shown that the choroidal thickness is reduced in subjects with DME and PDR (13, 14), others did not confirm these results (15, 16) . New data has suggested that the choroid may be affected in subjects with prediabetes (17). In this study, subjects with prediabetes had a greater choroidal thickness compared to normal subjects, suggesting that earlier abnormalities of the choroidal circulation may be detected with earlier abnormalities of glucose metabolism (17).

In order to better understand how the retina and the peripheric nervous system is affected by early abnormalities of glucose metabolism we compared in a cross-sectional study different retinal, sensitive and autonomic nervous parameter in subjects with different degrees of glucose tolerance.

Subjects and methods

Subjects

This is a cross-sectional study using data from 223 consecutive patients who did not have a previous diagnosis of metabolic syndrome and were referred for outpatient care in the Metabolism Unit of Hospital de Clínicas de Porto Alegre. The glucose tolerance status of the majority of these patients was not known. Forty patients were not included based on exclusion criteria, which comprised insulin, glucocorticoid or anti-retroviral treatment, menopause hormone replacement therapy, clinically significant autoimmune diseases, uncompensated hypo or hyperthyroidism, malignant disease that could affect 5-year survival, stage IV or V chronic kidney disease, AIDS, pregnancy/lactation, dementia, cirrhosis, hepatitis, and malnutrition. From the remaining 183 subjects who were included at the baseline assessment, 55 subjects had the same assessment repeated in a follow up visit. Additionally, 13 new subjects were included in the protocol for the baseline assessment and were submitted to a first protocol visit and were also submitted to the eye and neurologic examination (Figure 1). The protocol was approved by the institutional review board of Hospital de Clínicas de Porto Alegre and the subjects provided written informed consent.

Study procedures

Ethnicity classification was based on self-reported skin color and recorded as white or non-white, which included black, mixed, yellow, Indigenous and undeclared, according to the national definition of race and ethnicity used in Brazil (18). Physical activity was classified in four categories adapted from the classification proposed by Hu et al (19): sedentary, light exercise, moderate exercise and heavy exercise. Body weight was recorded in light clothing without shoes. Height was measured on a stadiometer. Body mass index (BMI) was calculated by weight (kg)/height (m^2). Waist circumference was taken at the midpoint between the lower costal margin and the iliac crest measured to the nearest 0.5 cm.

Blood pressure measurements

Office BP was measured in the right arm with oscillometric monitor device OMRON® (H-003D), with cuff adjusted for arm circumference, while the participant was seated. The mean of the last two measurements was used to estimate systolic and diastolic BP.

Ambulatory blood pressure measurements (ABPM) was performed by oscillometric method (Spacelabs 90207, with validated certificate), with recordings taken every 15 min in daytime and every 20 min during night. ABPM was performed on an ordinary working day and patients were advised to maintain their usual daily activities. Sleep time was recorded as the period between the time when the patient went to bed and the time when the patient woke up the next morning. The parameters evaluated were mean systolic (SBP) and diastolic (DBP) 24-h, daytime and nighttime blood pressure. Pulse pressure (difference between SBP and DBP) was recorded by 24-ABPM.

Blood Assays

Serum total and HDL-cholesterol, triglycerides, glucose, insulin and HbA1c were measured in the Clinical Pathology Unit. Cholesterol and triglycerides were determined by means of an enzymatic method (Ádvia 1800); HbA1c by high performance liquid chromatography (Tosoh Plus); US-CRP by turbidimetry (Ádvia 1800); glucose by an enzymatic method (Cobas c501 by Roche); and insulin by electrochemiluminescence (inter-assay coefficient of variability (CV) of 2.2% for high-controlled tests, general CV inferior to 7%; Centaur XP).

Classification of glucose tolerance

Based on HbA1c, fasting and 2 h-plasma glucose concentrations, subjects were categorized according to ADA criteria as having normal glucose tolerance (NGT: fasting plasma glucose [FPG] <6.1 mmol/L, 2 h-plasma glucose level <7.8 mmol/L), and HbA1c <5.7% [39 mmol/mol]); impaired fasting glucose (IFG; FPG 6.1–6.9 mmol/L and 2 h-plasma glucose level <7.8 mmol/L), or impaired glucose tolerance (IGT; FPG <6.1 mmol/L and 2 h-plasma glucose level 7.8–11.0 mmol/L); and diabetes (FPG ≥7.0

mmol/L and/or 2-h PG ≥11.1 mmol/L or HbA1c ≥ 6.5% [48 mmol/mol] or use of medication for diabetes control) (20). Subjects with IFG and/or IGT were considered to have prediabetes (impaired glucose metabolism; IGM). HbA1c levels between 5.7% (39mmol/mol) and 6.4% (48mmol/mol) were also used for definition of prediabetes.

Retinopathy Classification

Colored fundus photographs were taken from all subjects by nonmydriatic retinal camera (CR2-45 NM Nonmydriatic Fundus Camera, Canon Inc, Japan). Two photos of patient's both eyes in different position were taken. The images were accessed by an ophthalmologist skilled in diagnosing diabetic retinopathy (DL) to determinate the presence of diabetic retinopathy and its classification according to the severity of the findings. The severity of diabetic retinopathy was classified based on the American Diabetes Association's recommendations (20).

In order to standardize the process, the ophthalmologist, who was blinded for the glucose tolerance status, classified diabetic retinopathy as mild, moderate or severe (21). Additionally, the identification of the following signs was performed: retinal venous and arterial occlusion, retinal emboli, retinal macroembolysm, anterior ischemic optic neuropathy, glaucoma and age macular degeneration (21).

Retinal and macular measurements

For additional data about central macular attachments and development of retinal atrophy, choroidal thickness was performed using Spectralis SD-OCT (Heidelberg Engenieering Co, Heidelberg, Germany) with EDI mode after pupil dilatation centering on the fovea, which operates at a wavelength of 870 µm. Ten sections comprising 100 averaged scans were obtained at an angle of 5° to 30° in a rectangle centered on the fovea, covering 750 µm temporal and nasal to the fovea. Choroid thickness was defined as the vertical distance from the outer edge of the hyperreflective retinal epithelium pigmented to the inner sclera using a measuring tool built-in linear measuring tool. At least one eye of each study participant was assessed. OCT images were obtained by the same physician, and the images were assessed by a masked Ophthalmologist.

Assessment of diabetic neuropathy

Subjects underwent sensitive examination of extremities through tactile and nociceptive sensitivity to determine the cutaneous pressure perception threshold using monofilament (Semmes-Weinstein (10g)), adjusted adding the following points: plantar surface of the first, third and fifth toes, plantar surface at the level of the metatarsal phalangeal joints, central point of plantar face, the heel. With eyes closed, the patients were required to provide a "yes/no" response to monofilament pressure and in addition identify correctly the site of contact. Each filament was placed against a plantar surface of the four sites in a perpendicular fashion so that it bent with a constant force (22).

Vibratory thresholds were assessed using the 128 Hz tuning fork. The participants were asked to close their eyes and report when they felt a vibrating sensation (for the tuning fork) (23). The skin point where the tuning fork was placed was the medial malleolus (24).

Autonomic nervous system evaluation by spectral analysis of HRV

For the assessment of autonomic neuropathy we used the Ewing protocol described elsewhere (25).

In order to diagnose subjects with autonomic neuropathy, at least 2 out of the 5 need to be abnormal. This cut point was used according to previous studies (26, 27).

Heart rate variability (HRV)

The regulation of HRV originates from both sympathetic and parasympathetic nervous systems, and thus HRV can be used as a quantitative marker of autonomic function (25). Spectral analysis of HRV provides the basic information of how power of the R-R variation distributes as a function of frequency. It uses the timing variation between consecutive heartbeats measured using the R-R interval on an ECG. For this, HRV was evaluated using ECG recording by time and frequency domain methods, called the power spectral analysis (PSA) assessed by a computerized system called VNS-MICRO(25, 27).

Subjects were assessed with a 5 min ECG recording while the patient was resting in a supine position after a period of adaptation. Subjects were advised to refrain from any caffeine or alcohol for 12 h prior to the examination, and any subjects with hypoglycemia in the preceding 24 h had their examination postponed. All examinations took place in the morning. The software and digital signal processing algorithms followed established guidelines for HRV analysis (27). Three main components are distinguished in a spectrum calculated from an R-R time series derived from short-term ECG recordings:

In addition, various time-domain measures can be calculated based on the intervals between successive normal complexes with the so-called normal-to-normal (NN) intervals. The following indices were calculated from the ECG recording along the test: mean of all the R-R intervals, standard deviation (SD) of the R-R intervals (SDNN), root mean square of successive differences of adjacent R-R intervals (RMSSD).

For spectral domain evaluation, total power (TP; 0.003-1Hz) was assessed and its different components analyzed: very low frequency (VLF) (<0.04 Hz) component is closely related to humoral modulation while low frequency (LF) (0.04 – 015 Hz), reflects manly sympathetic tones, and high frequency (HF) (0.15 – 0,5Hz) component is more closely related to respiratory frequency, which reflects vagal modulation. LF/HF ratio reflecting the sympathetic/vagal balance.

Small fiber neuropathy

CHEPS is able to measure the heat perception thresholds and heat pain (Quantitative Sensory Testing – QST) according to different protocols (28, 29).

Thermoalgesic stimuli were applied at the skin of the anterior aspect of both forearms, approximately 5 – 10 cm up hand, with a Peltier contact thermode of 9 cm2 (CHEPS, Medoc Ltd., Ramat Yishai, Israel) at a ramp rate of 1 °C/s. We used the method of limits to determine warm and heat pain thresholds in all patients. Thresholds were defined as the mean value of temperature in each forearm in 3 stimuli separated by inter-stimuli intervals of at least 30 s.

Data Analysis and Statistical Methods

Data was described as mean ± standard deviation (SD) or median (P25 - P75). Categorical data, such as presence or absence of retinopathy were compared by $\chi 2$ test. Comparisons among glucose tolerance groups were performed by one-way ANOVA or *Kruskal-Wallis* tests, if variables were normal or non-normal distributed, respectively. Correlations were performed by *Pearson's* correlation test for normally distributed variables and by *Spearman's* test when at least one of the variables in the analysis had a skewed distribution.

A two-sided P value < 0.05 was considered significant. Statistical analysis was performed using the Statistical Package for the Social Science program (SPSS, version 22.0 for Windows, Chicago, IL).

Results

Subjects' characteristics

The protocol including eye and neuropathy evaluation comprised a total of 64 subjects, of whom 68.8% were female. Subjects were subdivided according to glycemic status as NGT (20.6%), IGM (31.7 %.) and T2DM (47.6%). NGT subjects were younger than the other 2 groups. They also presented a lower systolic blood pressure (SBP) by 24-h arterial blood pressure monitoring during the day and the night and lower levels of triglycerides. The groups did not differ by BMI, RMR, waist, HDL and vitamin B12 as shown in Table 1.

Retinal status

A comparison of how retinal morphology differs by glucose tolerance status was performed. Fundoscopic examination was possible to be made in 58 subjects (NGT =13, IGM= 18, T2DM= 27).

In 6 subjects it was not possible to perform fundoscopic examination due to blindness (n=2), eyelid edema (n=1) diagnosed as the Melkersson-Rosenthal syndrome, epiretinal membrane (n=1) and difficulty in fixing the pupil during the digital retinal examination (n=2). Findings compatible with glaucoma and macular degeneration of age

were not found, arterial occlusion was found in one subject and multiple findings compatible with hypertensive retinopathy in two subjects.

One subject with T2DM had bilateral mild cataract and cotton wool spots, characterizing him as having moderate nonproliferative diabetic retinopathy. One subject with long term T2DM had proliferative diabetic retinopathy and was submitted to laser therapy.

We did not find patterns compatible with diabetic retinopathy in other subjects. Thus, the prevalence of findings compatible with hypertensive and diabetic retinopathy were higher in subjects with T2DM, but the rates were low and did not show statistical differences among groups.

While assessing the choroidal layers by OCT, its thickness decreased with decreasing glucose tolerance in the right eye (NGT 331.9 ± 98.8 μm vs. IGM 273.0 ± 83.1 μm vs. DM 217.9 ± 76.7 μm; p <0.001) and left eye (NGT 356 , 3 ± 87.1 μm vs. IGM 258.5 ± 86.1 μm vs. DM 229.6 ± 79.8 μm, p = 0.001). (Figure 2).

Glomerular and renal function

Random sample albuminuria increased with decreasing glucose tolerance [NGT 5.0 (3.0 - 13.6) vs. IGM 4.0 (3.0 - 13.6) vs. DM 17.0 (6.4 - 57.0); P = 0.033]. This statistical difference was significant between subjects with T2DM compared to subjects with NGT and IGM. Estimated GFR by CKD-EPI did not differ among groups (Table 1).

Neuropathic signs and symptoms

In order to determine the presence of painful and non-painful diabetic neuropathy, subjects underwent clinical and laboratory assessment. When asked about the presence of symptoms of peripheral neuropathy by Michigan Questionnaire, the number of positive responses increased with decreasing glucose tolerance, although this difference was not statistical different. While assessing the sensibility in 7 different areas in both foot with the 10-g *Semmes-Weinstein* monofilament, sensibility decreased with decreasing glucose tolerance (NGT 6.9 ± 0.4 vs. IGM 6.3 ± 0.9 vs. DM 6.1 ± 0.8; P = 0.008). Reduced sensation of vibration detection with the use of a 128-Hz tuning fork was not different among NGT, IGM and T2DM subjects (Table 2).

Small fiber neuropathy was assessed by comparing the thresholds of heat and pain obtained by QST test in both hands. Temperature thresholds for heat and pain sensation were similar among subjects with NGT, IGM and T2DM in both hands.

Autonomic function

We assessed pulse pressure parameters by 24h ABPM. These parameters shown to be increased with decreasing glucose tolerance (NGT 40.5 ± 4.5 vs. IGM 54.3 ± 9,9 vs. DM 45.3 ± 11.6; P = 0.001). In order to study how the cardiac autonomic nervous system is affected by earlier abnormalities of glucose homeostasis, subjects were submitted to the standard battery of Ewing tests, including the Valsalva maneuver (Valsalva ratio), the deep breathing test of expiration-to-inspiration ratio (E/I), the lying to standing test (30:15 test) and the orthostatic hypotension test. The patterns of heart rate, heart ventricular rate and blood pressure responses to these tests did not differ.

Additionally, we performed a categorization of subject's scores reached in Ewing parameters as normal, borderline and pathological values based on the results of the VNS-MICRO software. Considering the criteria of two positive tests as compatible with the presence of cardiac autonomic neuropathy, we did not detect differences in such rates among groups (Table 2). The percentage of subjects with borderline or presence of autonomic neuropathy was not statistically different among groups.

Table 2 also summarizes the parameters of time and frequency spectral domain analysis comparing subjects with different degrees of glucose tolerance. A 5-min record during rest showed a non-significant decrease in the frequency (VLF, LF, HF and total power) domain spectral analysis in subjects with IGM and T2DM compared to those with NGT. The LF/HF ratio had a non-significant increase with decreasing glucose tolerance. While SDNN had also a non-significant decrease, the RMSSD time-domain analysis had a significant decrease (Table 2), suggesting a disruption of cardiac autonomy with abnormal glucose tolerance.

Relationship of anthropometric, metabolic, renal and subclinical inflammation with choroidal thickness

In order to understand the relationship between glucose tolerance and choroidal thickness we analyzed its possible determinants (Table 3). While BMI, waist circumference, total body fat, day, night and 24-h diastolic blood pressures, fasting plasma glucose, HbA1c, HOMA-IR, eGFR, US-CRP were not related with choroidal thickness, age, 2-h plasma glucose and sample microalbuminuria were inversely related to choroidal thickness in right, left and both eyes. While day, night and 24-h systolic blood pressures were inversely related with left eye it was not statistically related with choroidal thickness in the right eye.

Relationship of peripheral, small fiber and autonomic neuropathy with choroidal thickness

The parameters of peripheric sensitivity, small fiber and cardiac autonomic neuropathy examines by QST, Ewing tests and HRV frequent and time domain spectral analysis did not correlate significantly with choroidal thickness.

Multivariate analysis: adjustments for factors significantly associated with choroidal thickness

We performed multiple linear regression analysis to adjust for the potential confounders. We investigated whether age, gender, sample microalbuminuria and glucose tolerance, variables that were independently associated with subfoveal thickness in univariate models, were independent determinants of early subfoveal thickness alterations. While age was inversely related with subfoveal thickness of the right and left eyes, gender, microalbuminuria, and glucose tolerance were not associated with them. (Table 4).

Discussion

In the present study, we analyzed how glucose tolerance is associated with microvascular complications related to diabetes. Our data indicate that subfoveal thickness decreases in subjects with impaired fasting glucose and/or impaired glucose tolerance, namely IGM , and in subjects with recent diagnosis of type 2 diabetes, also IGM subjects have already an increased urinary albumin excretion. In order to assess the role of earlier abnormalities of glucose metabolism in the development of diabetic neuropathy we developed a protocol where we were able to study different peripheric and autonomic nerve fibers while using different approaches. We were not able to show differences in the rates of symptoms or signs of diabetic sensory neuropathy in subjects with different degrees of glucose tolerance.

Additionally, we studied if the small peripheral fibers are affected precociously in subjects with early abnormalities of glucose metabolism and T2DM by QST, since these fibers have a role in thermal regulation and skin thermal sensation (30). QST is able to measure the heat perception thresholds and heat pain. Again, we were not able to find differences in the perception thresholds for heat pain in subjects with different degrees of glucose tolerance, suggesting that these fibers are not sensitive for earlier exposure of IGM. One of the reasons for such finding is that we actually have 5 types of fiber C and probably the C-nociceptors were more resistant to the effects of abnormal glucose regulation (31).

Additionally, we studied by different approaches how the cardiac autonomic nervous system is earlier affected by IGM (32) Although we found an increased rate of cardiac autonomic neuropathy than expected in our sample, even in subjects with NGT. We were not able to find differences in the rates of this diabetic complication in subjects with IGM and early diabetes. Some data suggest that the parasympathetic nervous system is more affected by hyperglycemia than the sympathetic system (33)

As a result, with the more significant loss of parasympathetic fibers in subjects with DM, one of the classical manifestations of cardiac autonomic neuropathy is tachycardia at rest (33). We were able to measure HR by 24-h arterial blood pressure monitoring. Although heart rate increased with decreasing glucose tolerance, this difference was not statistical significant. However, we were able to find that pulse pressure increased with decreasing glucose tolerance, what suggest a deregulation of the

autonomic nervous system in controlling blood pressure homeostasis more than heart rate, which could explain why we were not able to find differences in autonomic function by time and frequent domain spectral analysis. It is well know that an increased pulse pressure is associated with cardiovascular endpoints.

Although we believe that prospective data needs to be development to confirm these findings, pulse pressure may be considered a possible marker of abnormality of the autonomous nervous system and, thus, a sign that is possible to be identified on physical examination that is able to predict the development of complications in subjects with prediabetes. Although with heart rate variability by time and frequent domain spectral analysis our data suggest that there is a decrease in humoral, sympathic and parasympathic nervous system activity with decreasing glucose tolerance, these differences were not statistically significant, with the exception of RMSSD, which is a measure of HRV and reflects the integrity of vagus nerve-mediated autonomic control of the heart (34). Our results have shown that RMSSD is progressively lower with decreasing glucose tolerance. This data strength our findings suggesting a damage of cardiac parasympathetic fibers in subjects with IGM and early T2DM as was shown by increased pulse pressure in subjects with IGM (34).

Finally, in order to determine if glucose tolerance was a main and independent determinant of choroidal thickness, we performed a multiple linear regression model which has shown that age was significantly related with diminished choroidal thickness while adjusting for other possible determinants.

This study has some limitations and strengths that need to be addressed. First, its cross-sectional design does not allow establishing a cause consequence relationship. Secondly, the small sample size may result in type 2 error and may explain why we were not able to find differences in autonomic neuropathy while analyzing most of the time and frequent-domain spectral analysis parameters in subjects with different degrees of glucose tolerance. The complexity of the protocol does not allow recruiting our entire original sample for the second data collection that composes this study. However, the well characterized sample with complete demographic, clinical and laboratorial characteristics, allow us to define how to study earlier signs of microvascular complications with IGM.

In conclusion, this study provides evidence that subjects with earlier abnormalities of glucose metabolism have already an increased level of renal damage and diminished

choroidal thickness, suggesting that these subjects may have earlier abnormalities of blood flow and supply to the retina. Subjects with IGM presented decreased pulse pressure and sensibility to the 10-g *Semmes-Weinstein* monofilament, but not Michigan Questionnaire, tuning fork and temperature heat thresholds in QST. This finding suggests that peripheral neuropathic alterations could be found even in earlier abnormalities of glucose metabolism.

Also, a decreasing glucose tolerance shown increased pulse pressure by 24h ABPM, while cardiac autonomy function by time and frequency spectral domain significant decrease, suggesting that earlier abnormalities of glucose homeostasis could increase pulse pressure and disrupt cardiac autonomy. In our population, systolic blood pressures were inversely related with choroidal thickness in left eye but not in the right eye. Nevertheless, age was significantly related with diminished choroidal thickness while adjusting for other possible determinants.

Prospective observational and interventional studies needs to be designed to confirm these findings and to understand how these early abnormalities are related to the development of micro and macrovascular complications of T2DM. A more clear picture of how we could intervene by preventing early development of T2DM complications in subjects with earlier abnormalities of glucose metabolism may be a fundamental point of care in order to ameliorate quality of life on these population.

Acknowledgments

This study was partially supported by grants from Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundo de Incentivo à Pesquisa e Eventos (FIPE) at Hospital de Clínicas de Porto Alegre.

Competing interests / financial disclosure: Nothing to declare.

References

1. Fong DS, Aiello LP, Ferris FL, 3rd, Klein R. Diabetic retinopathy. Diabetes Care. 2004;27(10):2540-53.

2. Harris MI, Klein R, Welborn TA, Knuiman MW. Onset of NIDDM occurs at least 4-7 yr before clinical diagnosis. Diabetes Care. 1992;15(7):815-9.

3. Klein R, Klein BE, Moss SE, Cruickshanks KJ. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XV. The long-term incidence of macular edema. Ophthalmology. 1995;102(1):7-16.

4. King P, Peacock I, Donnelly R. The UK prospective diabetes study (UKPDS): clinical and therapeutic implications for type 2 diabetes. British Journal of Clinical Pharmacology. 1999;48(5):643-8.

5. Leibowitz HM, Krueger DE, Maunder LR, Milton RC, Kini MM, Kahn HA, et al. The Framingham Eye Study monograph: An ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973-1975. Survey of Ophthalmology. 1980;24(Suppl):335-610.

6. Wong TY, Klein R, Sharrett AR, Manolio TA, Hubbard LD, Marino EK, et al. The prevalence and risk factors of retinal microvascular abnormalities in older persons: The Cardiovascular Health Study. Ophthalmology. 2003;110(4):658-66.

7. Knowler WC, Bennett PH, Ballintine EJ. Increased incidence of retinopathy in diabetics with elevated blood pressure. A six-year follow-up study in Pima Indians. The New England Journal of Medicine. 1980;302(12):645-50.

8. Diabetes Prevention Program Research G. The prevalence of retinopathy in impaired glucose tolerance and recent-onset diabetes in the Diabetes Prevention Program. Diabetic Medicine : a Journal of the British Diabetic Association. 2007;24(2):137-44.

9. Papanas N, Ziegler D. Prediabetic neuropathy: does it exist? Current Diabetes Reports. 2012;12(4):376-83.

10. Vinik AI, Erbas T, Casellini CM. Diabetic cardiac autonomic neuropathy, inflammation and cardiovascular disease. Journal of Diabetes Investigation. 2013;4(1):4-18.

11. Divisova S, Vlckova E, Hnojcikova M, Skorna M, Nemec M, Dubovy P, et al. Prediabetes/early diabetes-associated neuropathy predominantly involves sensory small fibres. Journal of the Peripheral Nervous System : JPNS. 2012;17(3):341-50.

12. Papanas N, Vinik AI, Ziegler D. Neuropathy in prediabetes: does the clock start ticking early? Nature Reviews Endocrinology. 2011;7(11):682-90.

13. Adhi M, Brewer E, Waheed NK, Duker JS. Analysis of morphological features and vascular layers of choroid in diabetic retinopathy using spectral-domain optical coherence tomography. JAMA Ophthalmology. 2013;131(10):1267-74.

14. Nagaoka T, Kitaya N, Sugawara R, Yokota H, Mori F, Hikichi T, et al. Alteration of choroidal circulation in the foveal region in patients with type 2 diabetes. The British Journal of Ophthalmology. 2004;88(8):1060-3.

15. Regatieri CV, Branchini L, Carmody J, Fujimoto JG, Duker JS. Choroidal thickness in patients with diabetic retinopathy analyzed by spectral-domain optical coherence tomography. Retina. 2012;32(3):563-8.

16. Lee HK, Lim JW, Shin MC. Comparison of choroidal thickness in patients with diabetes by spectral-domain optical coherence tomography. Korean Journal of Ophthalmology : KJO. 2013;27(6):433-9.

17. Yazgan S, Arpaci D, Celik HU, Dogan M, Isik I. Macular Choroidal Thickness May Be the Earliest Determiner to Detect the Onset of Diabetic Retinopathy in Patients with Prediabetes: A Prospective and Comparative Study. Current eye research. 2017;42(7):1039-47.

18. IBGE. Instituto Brasileiro de Geográfia e Estatística Características Étnico-raciais da População. Brasil 2013. Available from: https://www.ibge.gov.br/estatisticas-novoportal/sociais/populacao/9372-caracteristicas-etnico-raciais-da-populacao.html.

19. Hu G, Lindstrom J, Valle TT, Eriksson JG, Jousilahti P, Silventoinen K, et al. Physical activity, body mass index, and risk of type 2 diabetes in patients with normal or impaired glucose regulation. Archives of Internal Medicine. 2004;164(8):892-6.

20. American Diabetes A. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37 Suppl 1:S81-90.

21. Wilkinson CP, Ferris FL, 3rd, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110(9):1677-82.

22. Mayfield JA, Sugarman JR. The use of the Semmes-Weinstein monofilament and other threshold tests for preventing foot ulceration and amputation in persons with diabetes. The Journal of Family Practice. 2000;49(11 Suppl):S17-29.

23. O'Neill J, McCann SM, Lagan KM. Tuning fork (128 Hz) versus neurothesiometer: a comparison of methods of assessing vibration sensation in patients with diabetes mellitus. International Journal of Clinical Practice. 2006;60(2):174-8.

24. Richardson JK. The clinical identification of peripheral neuropathy among older persons. Archives of Physical Medicine and Rehabilitation. 2002;83(11):1553-8.

25. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. British Medical Journal. 1982;285(6346):916-8.

26. Kahn R. Proceedings of a consensus development conference on standardized measures in diabetic neuropathy. Autonomic nervous system testing. Diabetes Care. 1992;15(8):1095-103.

27. Canani LH, Copstein E, Pecis M, Friedman R, Leitao CB, Azevedo MJ, et al. Cardiovascular autonomic neuropathy in type 2 diabetes mellitus patients with peripheral artery disease. Diabetology & Metabolic Syndrome. 2013;5(1):54.

28. Schestatsky P, Llado-Carbo E, Casanova-Molla J, Alvarez-Blanco S, Valls-Sole J. Small fibre function in patients with meralgia paresthetica. Pain. 2008;139(2):342-8.

29. Fruhstorfer H, Lindblom U, Schmidt WC. Method for quantitative estimation of thermal thresholds in patients. Journal of Neurology, Neurosurgery, and Psychiatry. 1976;39(11):1071-5.

30. Guy RJ, Clark CA, Malcolm PN, Watkins PJ. Evaluation of thermal and vibration sensation in diabetic neuropathy. Diabetologia. 1985;28(3):131-7.

31. Yeap BB, Russo A, Fraser RJ, Wittert GA, Horowitz M. Hyperglycemia affects cardiovascular autonomic nerve function in normal subjects. Diabetes Care. 1996;19(8):880-2.

32. Ziegler MG, Lake CR, Kopin IJ. The sympathetic-nervous-system defect in primary orthostatic hypotension. The New England Journal of Medicine. 1977;296(6):293-7.

33. Nonogaki K, Iguchi A. Stress, acute hyperglycemia, and hyperlipidemia role of the autonomic nervous system and cytokines. Trends in Endocrinology and Metabolism: TEM. 1997;8(5):192-7.

34. Strandberg TE, Salomaa VV, Vanhanen HT, Pitkala K, Miettinen TA. Isolated diastolic hypertension, pulse pressure, and mean arterial pressure as predictors of mortality during a follow-up of up to 32 years. Journal of Hypertension. 2002;20(3):399-404.

TABLE 1 – Demographic, clinical and laboratory characteristics according to glucose tolerance status.

	NGT (n=14)	***PDM (n=20)***	***DM (n=30)***	***Valor p***
Age (years)	45.1 ± 12.5	58.4 ± 12.3	55.8 ± 11.1	0.006
Female sex (n-%)	10 (71.4%)	17 (85.0%)	17 (56.7%)	0.103
Years of diagnosis	-	2.0 (2- 3)	3.0 (2-4)	0.317
Height (m)	1.67 ± 0.12	1.59 ± 0.08	1.61 ± 0.09	0.070
BMI (kg/m^2)	30.6 ± 4.7	32.8 ± 7.7	32.1 ± 6.2	0.638
RMR (kcal/day)	1627.0 (1267 – 1789)	1454 (1346 – 1562)	1627 (1397 – 1987.0)	0.342
Waist (cm)	101.0 ± 13.3	101.4 ± 13.3	105.1 ± 12.0	0.484
SBP day (mmHg)	121.4 ± 13.7	131.8 ± 14.4	133.3 ± 14.1	0.035
SBP night (mmHg)	109.4 ± 12.5	124.9 ± 18.3	125.6 ± 15.5	0.009
Hypertension (n-%)	6 (46.2%)	15 (75%)	24 (71.4%)	0.072
Fasting plasma glucose (mg/dl)	83 (80 – 89)	99 (93.75 – 109.25)	117 (107 – 168)	-
2-h plasma glucose (mg/dl)	106.0 (78 – 126)	165.5 (145.75 – 177.0)	276.0 (211 – 341)	-
HbA1c (%)	5.0 ± 0.3	5.4 ± 0.4	7.5 ± 2.3	-
HDL (mg/dl)	54.2 ± 13.9	50.2 ± 12.8	45.3 ± 11.1	0.082
Triglycerides (MG/dl)	103 (71 – 145)	108.5 (71.5 – 143.5)	150 (116 – 202)	0.028
Vitamin B12 (pg/mL)	411.6 (349.9 – 505)	416 (336.5 – 517.75)	352 (281 – 584)	0.898
Alcoholic n (%)	1 (7.7%)	0 (0%)	3 (10%)	0.374
Microalbuminuria (mg/g)	5.0 (3.0-13.6)	4.0 (3.0-24.3)	17.0 (6.4 - 57.0)	0.033
eGFR (CKD-EPI -ml/min/$1.73m^2$)	98.9 ± 22.3	90.2 ± 16.6	91.3 ± 19.4	0.428

Data described as mean ± standard deviation, absolut number (%) or median (P25 - P75). RMR = resting metabolic rate

PDM X DM: valor p = 0.039

TABLE 2- Assessment of clinical neuropathy, C-fiber and autonomic nervous system function in subjects with different degrees of glucose tolerance.

	NGT (n=14)	IGM (n=20)	T2DM (n=30)	P value
Michigan score	4.4±3.2	6.4±2.2	6.6±3.2	0.104
Foot sensitivity	6.9±0.4	6.3±0.9	6.1±0.8	0.008
Vibration sensation thresholds	100%	95%	96,7%	0.709
Small fiber function tests				
Heat potential right hand (°C)	34.3 (33.9-34.7)	34.7 (34.1-37.2)	34.9 (34.0 - 35.8)	**0.173**
Heat potential left hand (°C)	34.3 (33.7-34.7)	34.9 (34.0-35.4)	34.7 (34.0 - 35.4)	**0.621**
Pain potential right hand (°C)	41.3 (39.8-43.7)	43.4 (40.1-47.0)	41.3 (38.8 – 44.6)	**0.211**
Pain potential left hand (°C)	42.2 (38.7-44.8)	43.0 (39.9-45.4)	43.0 (39.2 – 44.5)	**0.166**
Ewing cardiovascular autonomic function tests				
heart rate (24-h)	72.6 ± 6.9	78.0 ± 6.2	78.6 ± 10.4	0.076
Pulse pressure (mmHg)	40.5 ± 4.5	54.3 ± 9.9	54.3 ± 11.6	0.001
R-R variation (deep-breathing)	1.2 (1.1 – 1.3)	1.2 (1.1 – 1.4)	1.2 (1.0 – 1.3)	0.423
R-R variation (standing)	1.3 (1.2 – 1.8)	1.8 (1.1 – 5.0)	1.7 (1.1 – 2.9)	0.113
R-R variation (Valsalva)	1.4 (1.2 – 2.8)	1.8 (1.3 – 2.4)	1.4 (1.2 – 2.4)	0.647
Postural hypotension (systolic BP)	-2 (-9.3; 0)	-2 (-4.0; 2.0)	-2 (-5.3; 1.0)	0.220
Cardiovascular Neuropathy (%)	6 (42.9%)	5 (25.0%)	14 (46.7%)	0.290
Ewing criteria (n)	1.0 (0.0 – 2.0)	0.5 (0.0 – 1.8)	1.0 (0.0 – 2.0)	0.197

HRV frequency domain analysis				
VLF (ms^2/Hz)	922.5 (366.5-1734.7)	768.0 (406.7 – 1325.8)	692.5 (343.8 – 1289.8)	0.837
LF (ms^2/Hz)	604.0 (264 - 1256)	550 (264.3 - 1308)	454.5 (99.8 – 1073.8)	0.530
HF (ms^2/Hz)	892 (309.5-1547.8)	445 (223.3 – 3255.3)	259 (60.3 – 1490.8)	0.420
LF/HF ratio	0.96 (0.48-1.93)	0.94 (0.55-1.83)	1.15 (0.53 – 2.75)	0.494
Total Power (ms^2/Hz)	2860.5 (992.8-5039.8)	2294.0 (1295.3-5220.8)	1642.0 (663.5-3784.0)	0.608
HRV time-domain analysis				
RMSSD	911.8 ± 167.0	875.5 ± 137.9	783.9 ± 113.0	0.005
SDNN	51.5 (29.3-75.5)	47.0 (34.3-81.3)	36.5 (23.0-70.3)	0.567

Data described as mean ± standard deviation or median (P25 - P75)

TABLE 3 – Relationship between demographic, clinical, blood pressure, biochemical, peripherical, small fiber and autonomic neuropathy parameters with subfoveal choroidal thickness.

	Subfoveal Choroidal Thickness Right Eye		**Subfoveal Choroidal Thickness Left Eye**		**Mean Subfoveal Choroidal Thickness**	
	Partial r	P	Partial r	P	Partial r	P
Age	**-0.428**	**0.001**	**-0.491**	**<0.001**	**-0.476**	**<0.001**
BMI	0.066	0.062	0.026	0.842	0.056	0.669
Waist Circumference	-0.062	0.651	-0.084	0.528	-0.060	0.652
Total body fat	-0.071	0.601	-0.163	0.216	-0.138	0.298
24h SBP	-0.233	0.080	**-0.287**	**0.026**	**-0.265**	**0.041**
24h DBP	-0.079	0.558	-0.110	0.403	-0.079	0.547
Day SBP	-0.219	0.102	**-0.287**	**0.026**	**-0.265**	**0.041**
Day DBP	-0.008	0.954	-0.096	0.464	-0.058	0.655
Night SBP	-0.220	0.100	**-0.307**	**0.018**	**-0.299**	**0.210**
Night DBP	-0.145	0.283	-0.210	0.110	-0.195	0.140
Fasting Plasma Glucose	-0.231	0.082	-0.224	0.082	-0.241	0.061
2h post load glycemia	**-0.497**	**<0.001**	**-0.447**	**<0.001**	**-4.80**	**<0.001**
HbA1c	-0.242	0.07	-0.249	0.05	-0.255	0.05
eGFR	0.171	0.266	0.257	0.081	0.226	0.669
Sample microalbuminuria	-0.302	0.022	-0.335	0.009	-0.343	0.008

US-CRP	-0.056	0.707	-0.178	0.211	-0.154	0.280
			Ewing tests			
24hheart rate (bpm)	-0.169	0.237	-0.180	0.192	-0.185	0.179
Pulse pressure (mmHg)	-0.217	0.125	-0.277	0.043	-0.280	0.040
R-R variation (deep-breathing)	0.166	0.240	-0.008	0.952	0.061	0.677
R-R variation (standing)	-0.069	0.616	-0.080	0.549	-0.084	0.526
R-R variation (Valsalva)	-0.055	0.689	-0.182	0.168	-0.143	0.279
Postural hypotension	-0.210	0.472	0.024	0.936	-0.105	0.721
			HRV frequency domain analysis			
VLF (ms^2/Hz)	0.040	0.767	0.045	0.734	0.035	0.789
LF (ms^2/Hz)	0.108	0.426	0.051	0.696	0.074	0.577
HF (ms^2/Hz)	0.195	0.147	0.067	0.613	0.105	0.425
LF/HF ratio	-0.232	0.082	-0.061	0.644	-0.105	0.425
Total Power (ms^2/Hz)	0.114	0.398	0.042	0.749	0.063	0.635
			HRV time-domain analysis			
RMSSD	0.167	0.211	0.165	0.204	0.148	0.254
SDNN	0.132	0.326	0.043	0.746	0.065	0.620
			Small fiber neuropathy component			
Heat potential right hand (°C)	0.099	0.464	-0.010	0.940	0.033	0.801
Heat potential left hand (°C)	-0.116	0.387	-0.082	0.531	-0.106	0.416

Pain potential right hand (°C)	0.118	0.379	0.036	0.784	0.059	0.650
Pain potential left hand (°C)	0.100	0.454	0.051	0.699	0.064	0.624
24h SBP (mmHg)	-0.233	0.080	-0.290	0.025	-0.272	0.035
SBP day (mmHg)	-0.219	0.102	-0.287	0.026	-0.265	0.041
SBP night (mmHg)	-0.268	0.044	-0.320	0.013	-0.320	0.013

TABLE 4 – Multiple linear regression analyses of the association of subfoveal choroidal thickness with age, gender, microalbuminuria, 24-h systolic blood pressure and glucose tolerance

	Subfoveal choroidal thickness in right eye				Subfoveal choroidal thickness in left eye			
	B	**Partial r**	**SD**	**P**	**B**	**Partial r**	**SD**	**P**
Age	**-0.004**	**-0.287**	**(2.28; 2.75)**	**0.045**	**-0.004**	**-0.308**	**(-0.008; -0.001)**	**0.024**
Gender	0.073	0.188	(-0.40; 0.19)	0.199	0.113	0.276	(-0.001; 0.226)	0.052
Microalbuminuria	-0.001	0.019	(-0.01; 0.01)	0.509	0.001	-0.102	(-0.001; 0.01)	0.441
NGT	0.156	0.361	(0.21; 0.29)	0.024	0.183	0.369	(0.046; 0.321)	0.100
IGM	0.134	0.344	(0.02; 0.25)	0.028	0.117	0.288	(-0,002; 0.236)	0.053

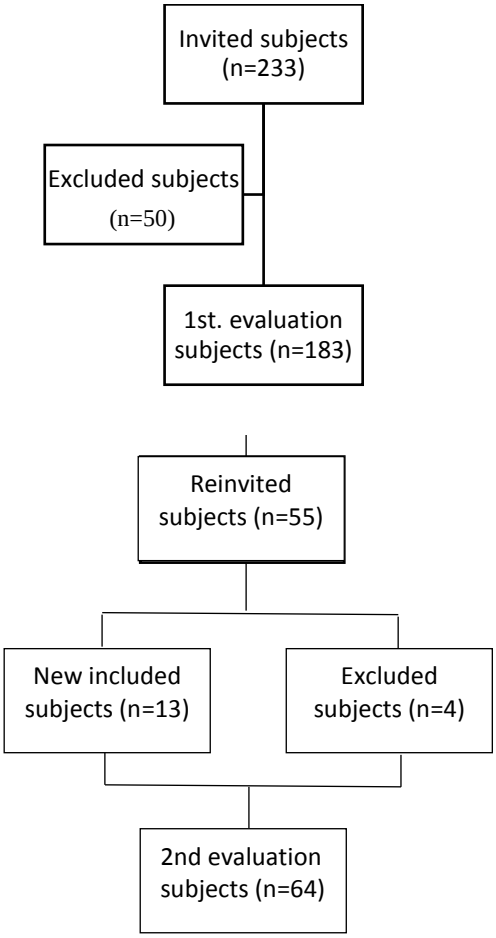

Figure 1 - Subjects evaluation during the study.

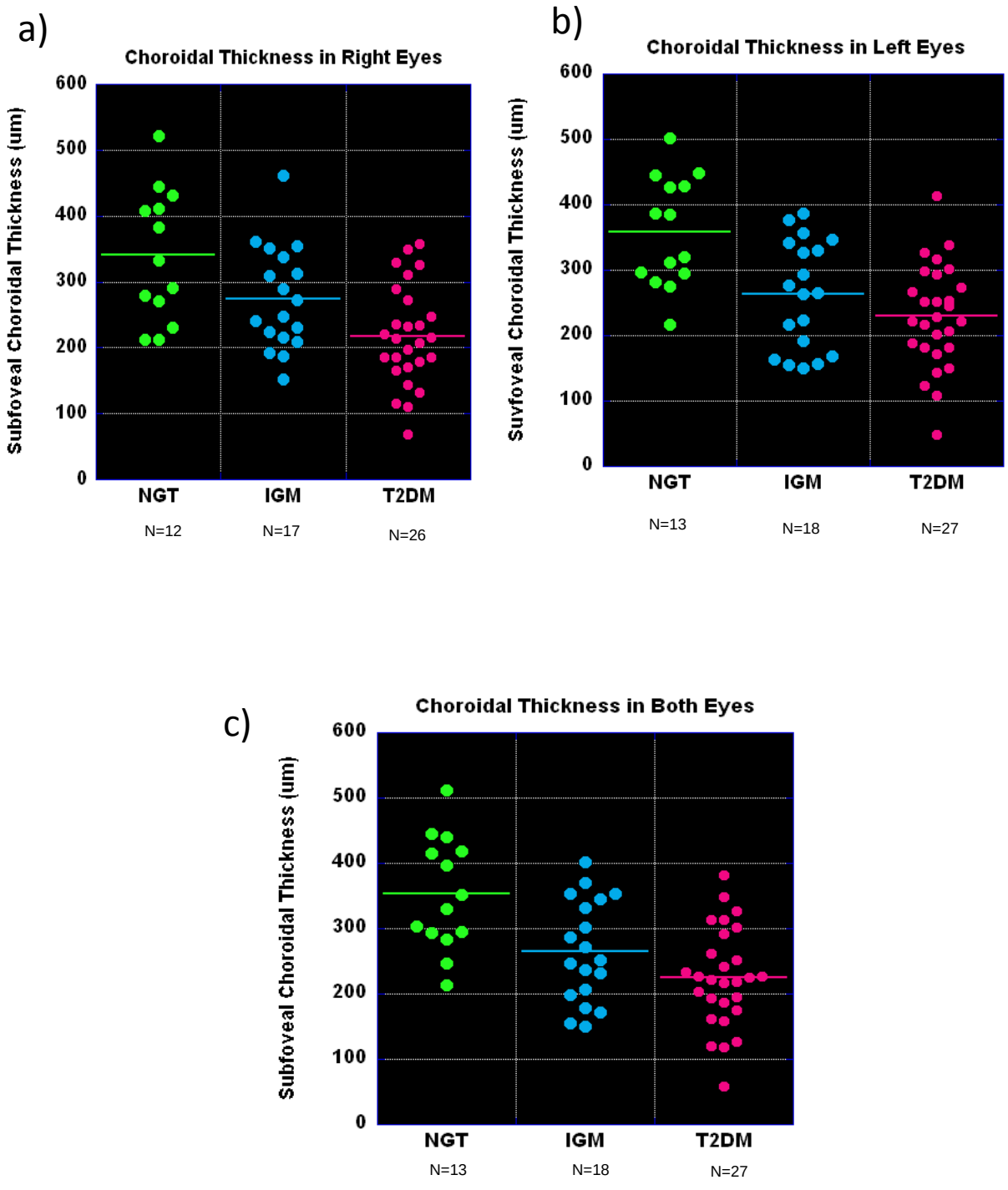

Figure 2 - Subfoveal choroidal thickness in the right (a), left (b) and the mean of both eyes eye of subjects with different degrees of glucose tolerance.

5 CONCLUSÕES

Demonstrou-se que a espessura da coróide diminuiu em ambos os olhos de acordo com a diminuição da tolerância à glicose, aumento da idade, pressão arterial sistólica de 24 horas e a taxa de excreção urinária de albumina.

Quando analisadas por regressão linear múltipla, a diminuição progressiva da tolerância à glicose e idade foram associadas independentemente com a redução da espessura da coróide.

Além disso, verificamos um aumento dos níveis de microalbuminúria com a diminuição da tolerância à glicose, esta diferença é resultante de uma albuminúria maior em indivíduos com DM, pois apresentaram taxas aumentadas de excreção de albumina.

Apesar da sensibilidade ao monofilamento de Semmes-Weinstein ter reduzido com a piora da tolerância a glicose, não foram encontradas diferenças na escala de Michigan, limiar de sensibilidade à vibração, limiar de calor e dor pelo CHEPS e a maioria dos testes autonômicos cardíacos.

A análise do domínio do tempo por RMSSD diminuiu e a pressão de pulso aumentou com diminuição da tolerância à glicose sugerindo uma perda de função parassimpática em anormalidades do metabolismo da glicose. Indivíduos com pré-diabetes apresentaram discreta anormalidade no funcionamento do sistema nervoso parassimpático autônomo.

Este é o primeiro estudo que sugere que mudanças na espessura da coroide possam ser marcadores precoces de retinopatia diabética em indivíduos com pré-diabetes e DM de início recente.