Universidade Federal do Rio Grande do Sul

Faculdade de Medicina

Programa de Pós-graduação em Ciências Médicas: Endocrinologia

Relação entre o conteúdo de gorduras da dieta e desfechos clínicos nos pacientes com Diabetes Melito tipo 2: Doença Renal do Diabetes e Mortalidade

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Orientadora: Profa. Dra. Themis Zelmanovitz

Porto Alegre, dezembro de 2014.

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Doutorado

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Tese apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia como requisito parcial para obtenção do título de Doutor.

Porto Alegre, dezembro de 2014.

CIP - Catalogação na Publicação

Teixeira dos Santos, Ana Luiza
Relação entre o conteúdo de gorduras da dieta e desfechos clínicos nos pacientes com Diabetes Melito tipo 2: Doença Renal do Diabetes e Mortalidade / Ana Luiza Teixeira dos Santos. -- 2014.
92 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 Melito. 2. Gorduras da dieta. 3. Doença Renal. 4. Doença Cardiovascular. 5. Mortalidade. 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).

"Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota.".

(Madre Tereza de Calcuta)

AGRADECIMENTOS

A minha orientadora, professora Themis Zelmanovitz, por todo o conhecimento transmitido, pela sempre disponibilidade, pela competência e brilhantismo como profissional e como pesquisadora. Sua afetuosa presença, em todas as etapas da minha formação, foi fundamental na minha trajetória acadêmica. Agradeço profundamente pela dedicação, entusiasmo e sobretudo pela excelência e rigor científico.

A minha família, em especial aos meus pais, por seu amor incondicional, por seu apoio diário incessante, por suas palavras de conforto e de confiança nos momentos de dificuldade. Aos meus irmãos e as minhas sobrinhas por todo o carinho e apoio fornecido. Ao meu namorado, por todo amor e companheirismo, fundamentais para a conclusão desta etapa.

Aos professores, pelos ensinamentos; em especial a Prof.ª Mirela Jobim de Azevedo que me acolheu, permitindo integrar no grupo de Nutrição do Serviço de Endocrinologia do HCPA.

Aos funcionários do Programa de Pós-Graduação e do Serviço de Endocrinologia pela disponibilidade e pelo auxílio prestado em todos os momentos necessários nesses quase 12 anos de convívio.

Às colegas nutricionistas mestrandas e doutorandas, pela convivência, pelas trocas de conhecimento, de angústias, tristezas, alegrias e conquistas. Em especial o meu agradecimento as Nutricionistas Camila Kümmel Duarte e Flávia de Moraes, as quais a amizade será para sempre.

A minha querida aluna de iniciação científica, Maira Zoldan, pela amizade construída e pelo comprometimento e competência em todas as tarefas desempenhadas. E a todos os alunos de iniciação que de alguma forma me ajudaram nessa trajetória, tendo como destaque as alunas do curso de nutrição Bárbara de Deus e Laura Ferraz, e do curso de medicina à aluna Manoella Santos.

Aos pacientes que participaram do estudo, pela disponibilidade, pela colaboração e por contribuírem com a construção do conhecimento.

Ao Programa de Pós-Graduação em Endocrinologia e a Universidade Federal do Rio Grande do Sul, pelo ensino de qualidade e por proporcionar uma formação diferenciada.

FORMATO DA TESE DE DOUTORADO

A presente tese de doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, sendo apresentada através de dois manuscritos originais acerca do tema estudado:

1. Dietary Fat Composition and Chronic Kidney Disease in Patients with Type 2 Diabetes

2. Dietary n-3 Polyunsaturated Fatty Acids and Risk of Mortality in Patients with Type 2 Diabetes

LISTA DE ABREVIATURAS

ACE = angiotensin-converting enzyme

ADA = American Diabetes Association

AG = ácido graxo

AGS = ácido graxo saturado

AGPI = ácido graxo poliinsaturado

ARB = angiotensin-2 receptor blockers

BMI = body mass index

CI = confidence interval

CHD = coronary heart disease

CVD = cardiovascular disease

DCV = doença cardiovascular

DHA = docosahexaenoic acid

DKD = Diabetic Kidney Disease

DM = Diabetes Melito/ Diabetes Mellitus

DRC = doença renal crônica

DRCD = doença renal crônica do diabetes

ECR = ensaio clinico randomizado

EPA = eicosapentaenoic acid

EPIC = European Prospective Investigation of Cancer

FA = fatty acids

eGFR= estimated glomerular filtration rate

KDIGO = Kidney Disease Improving Global Outcomes

NHANES = National Health and Nutrition Education Survey

HUNT = Nord- Trondelag Health

MESA = Multi-Ethnic Study of Atherosclerosis

OR = odds ratio

PI-N = 24-h urinary nitrogen output

PI-WDR = protein intake estimated from WDR

PREDIMED = Prevención con Dieta Mediterránea

PUFA = polyunsaturated fatty acids

REGARDS = Reasons for Geographic and Racial Differences in Stroke

SD = standard deviation

SFA = saturated fatty acids

TFG = taxa de filtração glomerular

UAE = urinary albumin excretion

USRDS = United States Renal Data System

VCT = valor calórico total

VIGITEL = Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico

WDR = weighed diet record

WHO = World Health Organization

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CONTEÚDO

FUNDAMENTAÇÃO TEÓRICA

Importância do problema

O Diabete Melito (DM) é considerado um problema de saúde pública, em razão da acentuada morbimortalidade e dos custos envolvidos no seu tratamento (1). Segundo dados da Organização Mundial da Saúde, a prevalência do DM demonstra tendência de crescimento em todo mundo e estima-se que no ano de 2030 cerca de 300 milhões de indivíduos serão diabéticos (2). No Brasil, dados epidemiológicos do Ministério da Saúde, em seu estudo denominado VIGITEL (Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico), apontou prevalência autorreferida de DM igual a 5,6% em 2011 (2). Estima-se um aumento de 30% na prevalência de DM entre 2010 (prevalência = 6,0%) e 2030 (prevalência = 7,8%) na população brasileira (1). Com relação aos dados regionais apresentados, Porto Alegre foi a terceira capital com o maior número de indivíduos com DM (3). Além disso, tem sido bem estabelecido que indivíduos com DM tipo 2 tenham uma maior taxa de mortalidade do que a população em geral (4,5,6), o que é atribuído principalmente a um risco duas vezes maior de doença coronariana nesse grupo de indivíduos (7). Ainda, pacientes diabéticos têm um prognóstico pior do que a população em geral após desfechos como angina (8), infarto do miocárdio (9,10) e acidente vascular cerebral (11). A mortalidade prematura associada às complicações vasculares representa o maior dano da doença.

As complicações crônicas do DM podem ser divididas em microvasculares ou macrovasculares. Uma das principais complicações microvasculares do DM é a doença renal crônica (DRC), podendo acometer cerca de um terço destes pacientes (12). Segundo

o relatório do *National Health and Nutrition Education Survey (NHANES)*, a prevalência da DRC associada ao DM aumentou de forma constante de 1988 a 2008. Ainda, o relatório mais recente *United States Renal Data System (USRDS)* constatou um aumento de 30% na incidência de doença renal terminal em indivíduos com diabetes nos EUA entre 1992 e 2008 (13,14). Além disso, a DRC do diabetes (DRCD) é a principal causa de início da terapia de substituição renal nos Estados Unidos, bem como no Brasil e está associada com aumento da mortalidade geral e cardiovascular (15). A DRCD é definida pelo aumento da albumina urinária (excreção urinária de albumina [EUA] acima de 30 mg/24 horas ou albumina em amostra acima de 30mg/g de creatinina) e/ou redução da taxa de filtração glomerular (TFG). A *National Kidney Foundation* sugere que tanto a albuminúria como a TFG sejam consideradas na classificação da DRCD, pela evidência de que uma proporção dos pacientes pode apresentar redução da TFG sem aumento da EUA (16,17,18). Ambos os parâmetros são considerados prognósticos de desfechos renais e de mortalidade (19).

Portanto, na nova versão das Diretrizes da *Kidney Disease Improving Global Outcomes* (KDIGO) (20), recomenda-se classificar a DRCD baseando-se na causa, na albuminúria e na categoria da TFG. A albuminúria é considerada normal ou ligeiramente aumentada se <30mg/g de creatinina, moderadamente aumentada se entre 30-300mg/g, e severamente aumentada se >300mg/g; terminologia que substitui as nomenclaturas normoalbuminúria, microalbuminúria e macroalbuminúria anteriormente adotadas (16). De acordo com os valores da TFG, divide-se em seis categorias: normal (≥ 90 ml/min/ 1,73m²), levemente diminuída (60-89 ml/min/ 1,73m²), leve a moderadamente diminuída (45-59 ml/min/ 1,73m²), para severamente diminuída (30-44 ml/min/ 1,73m²), gravemente diminuída (15-29 ml/min/ 1,73m²) e insuficiência renal (<15 ml/min/ 1,73m²) (16).

A Doença Cardiovascular (DCV) é a principal causa de complicação macrovascular, e também a principal causa de morbidade e mortalidade em pacientes com DM, onde entre indivíduos idosos, aproximadamente 22% tem doença do coração (21). Além disso, a doença arterial coronariana é mais freqüente e mais severa em pacientes diabéticos do que nos sem a doença (1). DM em si, em especial devido à hiperglicemia sustentada, contribui grandemente para a gravidade da doença aterosclerótica (2), apesar de alguns grandes estudos com pacientes com DM tipo 2 mostrarem que para desfechos duros o controle glicêmico rigoroso não teve benefício (22, 23). Além disso, a maior gravidade da DCV no DM pode, em parte, ser explicada pela elevada freqüência de fatores de risco cardiovasculares associados, como a obesidade, dislipidemia e hipertensão arterial.

Mesmo com o avanço do tratamento farmacológico para o manejo da doença, a prevalência de complicações associadas ao DM ainda é bastante elevada. A identificação dos fatores de risco não genéticos permite prevenir ou desacelerar a progressão para o surgimento das complicações associadas ao DM. Nesse sentido, a busca de evidências direcionadas à descoberta de medidas não farmacológicas são de grande relevância onde a terapia nutricional assume um papel fundamental. A identificação dos fatores de risco não genéticos permite prevenir ou desacelerar a progressão.

No que diz respeito a terapia nutricional, as diretrizes atuais se focam basicamente em orientações direcionadas ao controle glicêmico, assim como ao manejo das complicações crônicas. Não há uma porcentagem ideal de calorias provenientes de hidratos de carbono, proteína ou gordura ideal para todas as pessoas com DM. Em relação especificamente, à recomendação do consumo de gordura total para pacientes diabéticos, a Associação Americana de Diabetes ADA (24) identifica que existem limitadas evidências

sobre a quantidade ideal deste nutriente para indivíduos com DM. Em sua última recomendação, a ADA alerta que o tipo de gordura consumida parece ser mais importante do que a quantidade total ingerida, principalmente quando avaliado o efeito sobre o controle metabólico e risco para DCV. Para o consumo dos ácidos graxos saturados (AGS), colesterol e ácidos graxos (AG) trans, a ADA recomendada que a quantidade a ser ingerida seja a mesma orientada para a população em geral. No que diz respeito somente à ingestão do AGS, a ADA, em sua última atualização, torna mais flexível o limite de consumo para até 10% do valor calórico total, que anteriormente era restrito a 7%, por falta de evidência científica mostrando benefício para tal restrição. A ingestão de colesterol deve ficar abaixo de 300mg/dia e o consumo de AG trans deve ser o mínimo possível. Recomenda-se o aumento no consumo dos AG polinsaturados (AGPI) da família n-3, seja de origem animal (EPA e DHA) ou de origem vegetal (ácido linolênico), tanto para indivíduos com DM como para a população em geral, devido ao seu benefício sobre as lipoproteínas, prevenção de doenças do coração e associações com resultados positivos de saúde em estudos observacionais. A recomendação para a população geral de ingerir peixe (particularmente peixes gordos) 2x/semana, também se apropria para os pacientes com DM. Embora seja consensual que o consumo regular de peixes ricos em AG Ômega-3 faça parte de uma dieta saudável, a recomendação de suplementação com n-3 (EPA e DHA) a fim de prevenir ou tratar os eventos cardiovasculares cerca-se por controvérsias, fomentadas por resultados conflitantes de estudos clínicos.

No entanto, a maioria dessas recomendações científicas são baseadas em estudos realizados com indivíduos não-diabéticos. E, por serem uma população com maior grau de complicações associadas pela própria doença, se torna de extrema importância um

aumento do número de estudos com esse grupo de pacientes a fim de tentar evidenciar a melhor opção dietética no manejo de cada desfecho clínico associado à doença.

O objetivo da presente revisão é abordar as evidências da relação entre os componentes da gordura da dieta e principais desfechos clínicos associados ao DM, entre eles a doença renal e a doença cardiovascular.

Gorduras da dieta e Doença Renal Crônica

A quantidade e a qualidade dos lipídeos da dieta têm sido debatidas em pesquisas recentes a fim de esclarecer seu papel na DRC. Entretanto, ainda são escassos estudos bem delineados que mostrem essa relação tanto em indivíduos sem DM, como naqueles com DM.

Em relação às gorduras poli-insaturadas, análise transversal de 5.316 adultos jovens sem DM mostrou que o risco de DRC é reduzido no maior quartil de ingestão de ácidos graxos n-6 comparado com o primeiro quartil de ingestão (25). Analisando os AG plasmáticos como biomarcardores da ingestão destas gorduras, estudo transversal prospectivo demonstrou que os AGPI totais, os PUFA n-3, os PUFA n-6, e os AG individuais: linoleico, alfa-linolênico e araquidônico, foram capazes de atenuar a redução da função renal associada à idade, medida pelo *clearence* de creatinina em adultos idosos sadios (26).

Lin et al (27), em estudo transversal avaliando 19.256 participantes do estudo REGARDS (Reasons for Geographic and Racial Differences in Stroke) observou que a ingestão de gordura saturada foi associada significamente com a presença de albuminúria elevada. No entanto, os autores nao observaram associação entre a presença de TFG <60 ml/min/ 1,73m² com nenhuma fração de gordura.

Analisando os padrões alimentares de gordura, especialmente a dieta mediterrânea, que é rica em gordura monoinsaturada, ensaio clínico randomizado mostrou que esta dieta foi tão segura quanto dieta com baixo carboidrato e dieta pobre em gorduras para a preservação da função renal de indivíduos obesos com e sem DM tipo 2 . Entretanto, este benefício pode ter sido mediado pela perda de peso ocorrida ao longo do acompanhamento e que resulta em melhora na sensibilidade a insulina e pressão arterial (28). O mesmo foi observado em ensaio clínico randomizado vinculado ao estudo PREDIMED (Prevención con Dieta Mediterránea) com indivíduos de alto risco para doença cardiovascular. Foram avaliadas diferentes tipos de dietas (mediterrânea + azeite de oliva, mediterrânea + oleaginosas e dieta controle de baixa gordura), sendo as três capazes de melhorar a TFG após 1 ano de seguimento (29).

No que diz respeito à pacientes com DM, foi observada uma associação entre o maior consumo de gordura saturada e a presença de microalbuminúria em pacientes com DM tipo 1 (30). Em estudo realizado com pacientes com DM tipo 1 e 2, seguido por 6 anos, também foi demonstrado que aqueles que evoluíram com regressão da DRCD apresentaram maior ingestão de AGPI e uma menor ingestão de AGS (31). Mais recentemente, em estudo longitudinal com pacientes com DM tipo 1 acompanhados no Estudo DCCT foi observada associação inversa entre a ingestão de AGPI n-3 de cadeia longa (eicosapentaenóico ou EPA e docosaexaenóico ou DHA) e os valores de albuminúria. No entanto, neste estudo não se observou relação entre a ingestão destes AG e a incidência de albuminúria em pacientes normoalbuminúricos (32).

Em pacientes com DM tipo 2, em nosso meio, também foi observado que a alta ingestão de proteína e baixa ingestão de AGPI, principalmente provenientes de óleos vegetais, foram associados com a presença de microalbuminúria (33). A composição de

AG nos lipídios séricos, como biomarcadores da ingestão de gorduras, também tem sido associada à presença de DRCD em pacientes com DM tipo 2. Em estudo caso-controle anteriormente realizado no nosso meio com pacientes DM tipo 2 normo- e microalbuminúricos, foram observados menores níveis de AGPI, em especial ômega 6, nas frações de triglicerídeos dos pacientes com microalbuminúria, sugerindo que esta associação pode contribuir para a progressão da doença renal nesse grupo de pacientes (34). Neste estudo os pacientes haviam recebido orientação dietética prévia e não incluiu pacientes com DRC em estágios mais avançados. Mais recentemente, em um estudo que avaliou os AG eritrocitários de pacientes com DM tipo 2, foi demonstrada uma correlação negativa entre o conteúdo de AGPI, AGPI n-3 e razão n-3/n-6 com o risco de declínio da função renal (35).

Em estudo de coorte que acompanhou pacientes com DM, três padrões alimentares foram identificados; os tercis dos padrões com maior consumo de vegetais e de peixes correlacionaram-se positivamente a redução na creatinina e ligeiramente com aumento na TFG. Entretanto o padrão alimentar com maior gordura não se correlacionou com indicadores de função renal (36).

Em ensaio clínico randomizado (ECR) com indivíduos com DM tipo 1 e elevada EUA, o aumento na razão de ácidos graxos polinsaturados/saturados da dieta de 0.60 para 0.96 aumentou a EUA ao longo do acompanhamento e manteve a TFG (37). Já em ECR realizado em pacientes com DM tipo 2 micro e macroalbuminúricos (38,39), a substituição da carne vermelha pela carne de galinha da dieta a curto-prazo mostrou-se capaz de reduzir a EUA, assim como os níveis séricos de colesterol total, LDL e apolipoproteína B. Estas modificações foram concomitantes com uma maior proporção de AGPI em lipídios séricos após a dieta de galinha e hipoproteica (39). Mais recentemente, outro ECR controlado com adultos com DM e proteinuria, a suplementação diária de AGPI n-3 (4g/dia) durante

quatro semanas foi capaz de reduzir a EUA, mas apenas naqueles pacientes em uso regular de drogas inibidoras do sistema renina-angiotensina (40). No entanto, em meta-análise previa realizada com 17 estudos, observou-se uma redução em torno de 20% da EUA após a suplementação de AGPI n-3, entretanto não significativa no subgrupo de sete estudos realizados em pacientes com DM (41). Sobretudo, neste estudo também nao se observou relação da suplementação com o declínio da TFG.

Assim, os ácidos graxos polinsaturados da dieta parecem ter benefício sobre a função renal, especialmente sobre a redução da albuminúria. O efeito sobre a evolução da TFG ainda é controverso. A gordura saturada parece ter efeito redutor da função renal, porém com menos evidências.

Gorduras da dieta e DCV

As recomendações atuais da Associação Americana de Diabetes para a prevenção e tratamento de Doenças Cardiovasculares preconizam também que a dieta seja rica em frutas, legumes, cereais integrais, nozes e produtos lácteos com baixo teor de gordura, além da restrição de sal, tanto para os pacientes hipertensos como para os normotensos (24).

Em relação ao consumo de gordura saturada, alguns estudos evidenciam seu maior consumo ao risco para o desenvolvimento de DCV (42) apesar de outros não demostrarem o mesmo. Uma revisão sistemática feita por Wheeler et al., observou em apenas um estudo de 3 semanas que comparou uma dieta de baixa gordura saturada (8% do VCT) versus uma dieta rica em gordura saturada (17% do VCT) e não encontrou diferenças nas medidas de risco cardiovascular (43). Estes achados, juntamente com os de outros estudos (44), vão de encontro com as novas recomendações um pouco mais flexíveis para o consumo deste

ácido graxo. A relação entre ácidos graxos saturados e doença isquêmica do coração se mantém controversa e mais estudos são necessários para elucidar esta questão.

O benefício da ingestão de ácido graxo n-3 para prevenção primária ou secundária de DCV em indivíduos com e sem DM foi demonstrados em estudos observacionais e ensaios clínicos, ambos resultantes de suplementação dietética (45) e aumento do consumo de peixes (46). Persiste em discussão a real influência dos ácidos graxos Ômega-3 de origem vegetal sobre a doença cardiovascular. Em relação ao AGPI individuais, muitos estudos observacionais prospectivos sugerem que o consumo de ácido linolênico, o AGPI n-3 essencial e principal componente deste grupo, pode proteger contra eventos cardiovasculares (47). Recentemente, uma meta-análise de estudos prospectivos e retrospectivos reforçou a evidência da relação entre o ácido linolênico e risco de DCV (46). Por outro lado, metanálises e revisões sistemáticas têm mostrado resultados contraditórios (48) e, no estudo randomizado e controlado Alpha Omega, margarina suplementada com ácido linolênico por 40 meses não reduziu a taxa de eventos cardiovasculares maiores em pacientes que já haviam sofrido infarto do miocárdio (49).

Ensaios clínicos randomizados, duplo-cegos, placebo-controlados demonstraram a eficácia da suplementação de AGPI n-3 na prevenção secundária de DCV (50). Entretanto, uma meta-análise recente mostrou que as evidências existentes são insuficientes para a suplementação preventiva de EPA e DHA contra eventos cardiovasculares em pacientes com histórico de DCV (51). O mesmo foi demonstrado no estudo ORIGIN conduzido em pacientes pré-diabéticos e diabéticos, onde a suplementação com AGPI n-3 não reduziu a incidência de eventos cardiovasculares na presença da DCV (52).

Em pacientes com DM, os estudos que avaliaram o efeito dos AG n-3 derivados de fontes alimentares marinhas (EPA e DHA) ou de fontes de origem vegetal (ácido linolênico) são ainda limitados. O efeito benéfico da ingestão de AGPI na progressão da

DCV, assim como em relação ao perfil de risco cardiovascular foi demonstrado em alguns estudos observacionais (33), e ensaios clínicos (50). Em estudo prospectivo envolvendo pacientes com DM tipo 2 e sem doença cardiovascular prévia, o maior consumo de AGPI, principalmente o ácido linolênico, foi associado a proteção para a ocorrência de eventos cardíacos (53). Em relação à prevenção primária, o mesmo foi encontrado em grande estudo de uma coorte feminina de pacientes diabéticas tipo 2 sem doença cardíaca coronariana, que mostrou uma associação inversa do consumo de AGPI n-3 (consumo de peixe) com a incidência de doença cardíaca coronariana (54). Poucos ECR avaliaram o efeito do consumo de AG n-3 na DCV em pacientes com DM, e apenas naqueles já com doença macrovascular (49,50) ou com alto risco de DCV (52). Além disso, estes estudos avaliaram o efeito de suplementação de n-3 e não de fontes alimentares e um efeito benéfico na progressão da DCV foi demonstrado apenas em alguns deles. Assim, ensaios clínicos randomizados ainda não suportam a recomendação da suplementação de n-3 para prevenção primária ou secundária da DCV.

Gorduras da dieta e Mortalidade

Tanto em pacientes com como sem DM, poucos são os estudos que avaliaram a associação do total de gorduras ou de suas frações e mortalidade total, mortalidade cardiovascular e mortalidade não-cardiovascular.

Em relação ao consumo de gordura saturada, uma meta-análise recente de ECR avaliou o efeito da manipulação da gordura da dieta na mortalidade e morbidade cardiovascular sugerindo que a redução da gordura saturada pode proteger para eventos cardiovasculares, reduzindo-os em 14%. (55). Entretanto, é importante enfatizar que o efeito cardioprotetor foi observado em estudos com homens (não mulheres) e em indivíduos com moderado a alto risco cardiovascular prévio. Além disso, este efeito

protetor foi claramente observado quando a gordura saturada foi substituída por insaturada e não por carboidratos.

Em coorte prospectiva, entre indivíduos adultos saudáveis que não estavam recebendo suplementos, valores de AGPI n-3 circulantes foram associadas à menor mortalidade total, especialmente morte por doença arterial coronariana (56). Em outra coorte de população chinesa, observou-se que nos indivíduos do maior quartil de consumo de EPA/DHA e ácido linolênico, o risco foi 33% menor para mortalidade CV, comparados aos indivíduos do menor quartil de ambos os tipos (57). Outra coorte encontrou que a ingestão de ácido linolênico foi associada com menor risco de mortalidade total e mortalidade não CV, que parece ser relacionada, principalmente, com o menor risco de morte de causas como câncer e demência, os quais os mecanismos não são tão bem estabelecidos (58). Entretanto, meta-análise não encontrou associação significativa com ácido linolênico circulante com o risco de DCV fatal e não fatal ou derrame (48).

Em relação a outros subtipos de AGPI, como os AGPI n-6 PUFA, e sua associação com mortalidade, em estudo de coorte com pacientes com idade maior que 65 anos participantes do The Cardiovascular Health Study, o acido linoleico sérico foi inversamente associado com mortalidade total, com uma redução de risco de 13% entre os participantes do menor quartil (referencia: primeiro quintil) (59).

Em pacientes com DM tipo 2 recém diagnosticado que participaram do estudo Nord-Trondelag Health (HUNT), a concentração de EPA em fosfolipídeos plasmáticos foi inversamente associada com mortalidade total, enquanto a concentração de DHA foi positivamente associada (60) . Este estudo sugere um efeito diferente entre EPA e DHA nos pacientes. Em uma coorte retrospectiva, envolvendo pacientes pós infarto agudo do miocárdio, o tratamento precoce com ácido graxo n-3 foi associado com melhora em todas

as causas de mortalidade em pacientes com e sem DM tipo 2, contrapondo um padrão contemporâneo de tratamentos que modificam o risco cardiovascular (61).

Justificativa e objetivo geral da tese

Desta maneira, sendo o DM tipo 2 a quarta causa de morte em países desenvolvidos, com duas vezes mais mortalidade e duas a quatro vezes mais risco para doença coronariana e derrame, cabe o aumento do número de estudos que avaliem o efeito das medidas não farmacológicas, principalmente da terapia nutricional, sobre as principais conseqüências relacionadas ao DM. Considerando que o papel das gorduras da dieta exerce algum tipo de influência sobre os desfechos associados à doença e que ainda muito pouco se sabe a respeito do seu efeito, se torna de extrema importância um maior número de estudos em DM tipo 2 que avaliem essa relação para melhorar os prognósticos nesse grupo de pacientes.

Com base no exposto, a presente Tese de Doutorado foi desenvolvida com o objetivo geral de avaliar, em pacientes com DM tipo 2, o efeito da composição de gordura dietética sobre desfechos clínicos, no caso a Doença Renal do Diabete e o risco de Mortalidade Total e Cardiovascular .

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Capítulo I

Dietary Fat Composition and Chronic Kidney Disease in Patients with Type 2 Diabetes

Dietary Fat Composition and Chronic Kidney Disease in Patients with Type 2 Diabetes

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ABSTRACT

Objective: To evaluate the association of albuminuria and glomerular filtration rate with dietary fat composition of Type 2 diabetes patients, with and without Diabetic Kidney Disease (DKD). **Methods:** In this cross-sectional study, the patients were submitted to anthropometric and dietary assessment, and clinical and laboratory evaluation, emphasizing the chronic complications of diabetes. The diet was evaluated by completing a 3-day weighed diet record (WDR) (analysis using Nutribase 2007® software). Compliance with the WDR technique was assessed by comparing protein intake estimated from 3-day WDR and 24-h urinary nitrogen output. Albuminuria was measured twice and Glomerular Filtration Rate was estimated by using the CKD-EPI equation. **Results:** A total of 368 patients were evaluated (177 [48.1%] male, mean age 60.6 ± 9.7 years, duration of diabetes 12.4 ± 8.1 years, body mass index [BMI] 28.5 ± 4.3 kg/m²). Of these, 256 were normoalbuminurics, 82 presented moderately elevated and 28 severely elevated albuminuria. Patients with DKD presented lower linolenic acid [0.79 % energy (0.00 – 3.2) vs. 0.91 % energy (0.18 – 2.42); P= 0.04] and linoleic acid intake (8 ± 4 % energy vs. 9 ± 3% energy; P=0.01), when compared with patients without DKD. Multivariate analysis showed that the intake of linolenic acid was negatively associated with the presence of DKD (OR 0.62; 95% CI 0.41 – 0.94; P = 0.02), adjusted for age, gender, duration of diabetes, smoking, compliance with WDR, using hypolipidemic agents, BMI and systolic blood pressure. In another model, similar results were observed to linoleic acid, adjusting to the same co-variables (OR 0.92; 95% CI 0.86 – 0.99; p = 0.04). **Conclusion:** In patients with Type 2 diabetes, the lower intake of PUFA, especially linolenic and linoleic acid, are associated with the presence of diabetic kidney disease.

Keywords: Fatty Acids; Diabetic Kidney Disease; Type 2 Diabetes

Introduction

Chronic kidney disease is a major microvascular complication of Diabetes Mellitus (DM) and may affect about one third of the patients (1). According to the report of the National Health and Nutrition Education Survey NHANES, the prevalence of chronic kidney disease associated with DM increased progressively from 1988 to 2008, especially because of the worldwide increased prevalence of DM (2). In addition, diabetic kidney disease (DKD) is the leading cause of entry into renal replacement therapy in the United States and one of the main causes in Brazil, and is significantly associated with increased overall and cardiovascular mortality compared with patients without DM (3). Although aggressive control of important risk factors (hypertension, hyperglycemia, smoking, etc) may attenuate the progression of this complication (4), and consequently has been associated with a reduction of the incidence of new cases of end-stage renal disease, it is still an important global public health problem (5). Hence, this reinforces the importance of identifying other factors that might be associated with the development of DKD.

Among these factors, dietary components also may play a role in the development of DKD. Until a few years ago, the protein content of the diet was the main focus of dietary recommendations and studies that related nutritional factors to chronic kidney disease (6). But, for a long time, there have been many reports about the role of the dietary fat content in chronic kidney disease (7), both in diabetic and non-diabetic people, and with either observational or interventional design.

The studies mainly emphasize the quality and not the quantity of dietary fat. Some cross-sectional and prospective studies have reported a positive association between increasing values of albuminuria and saturated fatty acids (SFA) (8,9), as well as an inverse association with polyunsaturated FA (PUFA) (8), both in type 1 and type 2 diabetic

patients. Previous interventional trials in patients with type 2 diabetes and DKD also demonstrated a reduction of albuminuria after experimental diets with higher PUFA content (e.g., chicken-based or soy enriched diets), when compared to control groups (10,11). On the other hand, a beneficial effect of daily supplementation of n-3 PUFA on the reduction of albuminuria was observed in one meta-analysis of clinical trials (12), but not in others, especially when patients with DM were separately analyzed (13). Therefore, the evidence of the role of dietary fat content modification on the development and progression of DKD is still insufficient to establish specific dietetic recommendations.

The definition of DKD is based on the presence of increased albuminuria and/or decreased kidney function (i.e., glomerular filtration rate [GFR] <60 ml/min per 1.73 m²) for 3 months or more (14). Based on the evidence that a proportion of patients may have reduced GFR without increased albuminuria, it is recommended to evaluate both parameters (14,15,16). Furthermore, both of them are considered prognostic factors of renal and cardiovascular mortality (17,18). Hence, the evaluation of the possible association between dietary fat content and renal dysfunction assessed by GFR is also relevant. In observational studies with non-diabetic patients, a higher consumption of n-6 PUFA (19), as well as long-chain n-3 PUFA (20), was associated with lower risk for CKD, defined as a GFR <60 ml/min/1.73 m. In contrast, the energy-adjusted-linolenic acid intake (the essential n-3 PUFA) was associated with an 18% Increased likelihood of having CKD (20). In a longitudinal study conducted with type 2 diabetic patients, it was observed that high erythrocyte PUFAs, especially an n-3 or n-3/n-6 PUFA ratio, were independently associated with a lower risk of renal function decline (21). On the other hand, some authors did not observe a significant association between subtypes of dietary fat and GFR decline (22) or GFR <60 ml/min/1.73 m² (23). Therefore, the relation

between dietary fat intake and GFR decline is controversial and further studies are necessary to clarify this subject, including patients with DM. This information is essential to design new clinical trials of dietary interventions for these patients. Hence, the aim of the present study is to evaluate the association of albuminuria and GFR with dietary fat composition of type 2 diabetes patients, with and without DKD.

Patients and Methods

Patients

This cross-sectional study was conducted with type 2 diabetes patients from the Diabetes research outpatient clinic at Hospital de Clínicas de Porto Alegre (Rio Grande do Sul, Brazil). The patients were consecutively recruited based on the following exclusion criteria: body mass index (BMI) >40 kg/m^2, heart failure, symptomatic autonomic neuropathy (gastroparesis or diabetic diarrhea), dietary counseling by a registered dietitian during the previous 12 months, and inability to perform the weighed diet records (WDR). The recruitment process occurred during the period of 2001 to 2009. All patients underwent a standardized clinical, nutritional and laboratory examination. The study was approved by the Hospital Ethics Committee and all patients signed a written informed consent form.

Methods

Dietary Assessment

Diet was assessed using a 3-day WDR technique (two nonconsecutive weekdays and one day of the weekend). Patients were issued commercial scales (1-125 g) and measuring cups (25-250 mL; Pirex) and given a detailed explanation and demonstration of the procedures by a trained, registered dietitian. Next, patients performed a training session

for a one-day WDR. The 3-day WDR was performed over two to three weeks and before nutritional recommendations for all patients **(Figure 1).**

Compliance with the WDR technique was assessed by comparison of protein intake estimated from the 3-day WDR (PI-WDR) and from the 24-h urinary nitrogen output (PI-N) performed on the third day of the WDR period (24). According to Vaz et al, patient compliance with the WDR protocol is established if the PI-WDR/ PI-N ratio remain between 0.79 and 1.26.

The analysis of dietary nutrients from the 3-day WDRs was performed using the Nutribase Clinical Nutritional Manager software (version 7.14, 2007). The mean values of each nutrient consumed during the 3-day WDR were calculated. Nutritional data for frequently consumed foods were updated if necessary, and/or complemented with data obtained from local manufacturers of industrialized foods.

Anthropometric Measurements

The body weight and height of patients (without shoes or coats) were collected with an anthropometric scale; measurements were recorded to the nearest 100 g for weight, and to the nearest 0.1 cm for height. Waist circumference was measured midway between the lowest rib and the iliac crest, near the umbilicus.

Clinical evaluation

The clinical evaluation emphasized metabolic and blood pressure assessment as well as the detection of diabetic chronic complications, especially DKD and cardiovascular disease.

Sitting blood pressure was measured twice to the nearest 2 mmHg, after a 10-min rest, using a digital sphygmomanometer (OMRON® Automatic Blood Pressure Monitor, Model HEM-705CP, Vernon Hills, Illinois 60061). Hypertension was defined as blood

pressure ≥140/90 mmHg or use of antihypertensive drugs on at least two separate occasions.

The renal function was evaluated by serum creatinine and 24-h urinary albumin excretion (UAE) (25). Because of the high intra-individual variability of UAE, we calculated the mean value of two measurements (within a period of 3 to 6 months) to establish persistent albuminuria (≥ 30 mg/24-h) (ADA). Glomerular Filtration Rate (eGFR) was estimated by using the CKD-EPI equation:

eGFR = 141 x min (Scr/k,1)^{a} x max (Scr/k,1)$^{-1.209}$ x 0.993^{Age} x 1.018 (if female) x 1.159 (if black)

Where Scr is serum creatinine, k is 0.7 for females and 0.9 for males, ^{a} is -0.329 for females and -0.411 for males, min indicates minimum of Scr/k or 1, and max indicates maximum of Scr/k or 1 (26). The DKD was defined when UAE ≥ 30 mg/24 h or when eGFR <60ml .min^{-1}. 1.73 m^{-2}.

Ischemic heart disease was defined as: presence of angina and/or possible infarction, using a World Health Organization (WHO) cardiovascular questionnaire as previously standardized in patients with type 2 diabetes by our group (27), or abnormalities on resting electrocardiogram (Minnesota Codes: Q and QS patterns [1-1 to 1-3]; S-T junction and segment depression [4-1 to 4-4]; T-wave items [5-1 to 5-3], or complete left bundle branch block [7-1]). In those cases, the ischemic heart disease was always confirmed by exercise electrocardiogram or radionuclide myocardial perfusion imaging with stress (exercise or pharmacological) compatible with the presence of myocardial ischemia (28). Peripheral vascular disease was determined based on the presence of intermittent claudication and/or absence of posterior tibial pulse at clinical examination. The presence of cerebrovascular disease was established if there was a

history of cerebrovascular accident (ischemic stroke) and /or compatible findings (sequelae).

Patients were classified as “nonsmoker” or “smoker” (smoker in the past or currently a smoker), regardless how many cigarettes per day. Physical activity was classified into four levels based on a standardized questionnaire, which was adapted to local habits (29).

Laboratory analyses

Glycemic control was evaluated by serum glucose (glucose-peroxidase colorimetric enzymatic method) and glycated hemoglobin (high precision chromatography, Merck-Hitachi 9100 apparatus). The lipid profile consisted of the measurement of total cholesterol and triglycerides using colorimetric assay. HDL cholesterol was measured using the direct enzymatic method. LDL cholesterol was calculated using the Friedewald formula. Serum creatinine was measured by the Jaffe method and urea by kinetic UV assay. Urinary albumin excretion was evaluated by the immunoturbidimetry technique (Kit MICROALB, AMES) (31).

Statistical analysis

The Student t test, Mann-Whitney test for independent samples was applied as shown in the comparison of clinical, laboratory and diet characteristics among patients with and without DKD. For categorical variables we used Fisher's exact test or chi-square. The correlation coefficients of Pearson or Spearman were also analyzed, as indicated. Univariate and multivariable logistic regression models were constructed to assess adjusted associations of dietary fats with albuminuria or eGFR <60 $ml.min^{-1}$. 1.73 m^{-2}. DKD is the dependent variable and nutrients are independent variables. Other independent

variables were selected as potential confounders (age, gender, presence of hypertension, and fasting plasma glucose) according to univariate analyses or biological relevance.

Data were expressed as mean ± standard deviation or as median (minimum-maximum) unless otherwise stated. The level of significance adopted was 5%. Software SPSS 18.0 (SPSS®, Chicago, IL) was used for the analysis.

Results

A total of 368 patients (177 male) underwent clinical, laboratory, and nutritional evaluation. The mean age of patients was 61 ± 10 years, duration of diabetes 12 ± 8 years, and a mean body mass index (BMI) of 28.5 ± 4.3 Kg/m². Also, 79% had hypertension and 20% had ischemic heart disease. Thirty percent of the patients presented DKD, 22% with moderately elevated UAE (30-300mg/24-h) and 8% with markedly elevated UAE (>300mg/24-h). The great majority of patients (78%) was in Stage 1 and 2 of chronic kidney disease classification of the National Kidney Foundation, with a mean eGFR = 86 ± 18 $ml.min^{-1}.1.73\ m^{-2}$ body surface area (**Table 1).**

According to albuminuria values, the patients were divided into two groups: with and without DKD (UAE<30mg/24-h). The main clinical and laboratory characteristics of patients are shown in **Table 2.** The patients with DKD were younger and there were a higher proportion of males. As expected, the presence of hypertension was more frequent in patients with DKD, and the values of diastolic blood pressure were higher, compared with the patients without DKD. Regarding the medications in use, patients with DKD more frequently used the angiotensin-converting enzyme (ACE) inhibitors or angiotensin-2 receptor blockers (ARBs) (70% vs. 56%; P=0.01), and less frequently the lipid-lowering drugs (23% vs. 36%; P=0.01), compared with the patients without DKD. The patients with DKD presented worse glycemic control and lower levels of HDL cholesterol, compared

with the patients without DKD.

Regarding the characteristics of the diet, participants with DKD had a higher protein intake and a lower total PUFA when compared to those without DKD **(Table 3)**. Analyzing the individual dietary PUFA intake, subjects with DKD presented lower linolenic acid (C18: 3n-3) [0.79 % energy (0.00 – 3.2) vs. 0.91 % energy (0.18 – 2.42); P= 0.04] and linoleic acid (C18: 2n-6) intake (8 ± 4 % energy vs. 9 ± 3% energy; P=0.01), when compared with patients without DKD. No statistical difference was observed for other evaluated PUFA: eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid. Multivariate analysis showed that the intake of linolenic acid was negatively associated with the presence of DKD (≥ 30 mg/24-h) (OR 0.62; 95% CI 0.41 – 0.94; P = 0.02), adjusted for age, gender, duration of diabetes, smoking, compliance with WDR, using hypolipidemic agents, BMI and systolic blood pressure **(Table 4)**. In another model, similar results were observed to linoleic acid, the principal component of n-6 PUFA intake, adjusting to the same co-variables (OR 0.92; 95% CI 0.86 – 0.99; p = 0.04) **(Table 4)**.

When the patients were divided according to the degrees of albuminuria, it was observed that the consumption of total PUFA, as well as linoleic acid, was significantly higher in the normoalbuminuric group, compared to the group with markedly elevated UAE. When the linolenic acid intake was analyzed, both the normoalbuminuric group and those with moderately elevated UAE presented a higher intake of this FA, compared to the group with markedly elevated UAE **(Table 5).**

The patients were then divided according to eGFR values: above or below 60 ml/min/1.73 m^{-2}. The participants with lower eGFR were older (68 ± 7 vs 60 ± 10 years; P=<0.001) and had higher serum levels of triglycerides (181 mg/dl (71-414) vs. 134 mg/dl

(25-573); P=0.03) when compared to participants with higher eGFR. Regarding the characteristics of the diet, no statistical difference was observed for analyzed nutrients between the two groups **(Table 6).**

Given that a proportion of patients may have reduced GFR without increased albuminuria, we also performed an analysis from the point of view of the two outcomes together (presence of high albuminuria or eGFR <60 $ml.min^{-1}$. 1.73 m^{-2}) **(Table 7).** Patients with DKD presented a higher proportion of hypertension and of smoking habit, when compared to those without CKD. Worse glycemic control and lower HDL cholesterol were also observed in patients with DKD. The characteristics of the diet of the patients with and without DKD are demonstrated in **Table 8.** Individuals with DKD had a higher protein intake and a lower total PUFA, linolenic acid and linoleic acid intake, when compared to those without DKD. Multivariate analysis showed that the total PUFA consumption was negatively associated with the presence of DKD (UAE ≥ 30 mg/24-h or eGFR <60 $ml.min^{-1}$. 1.73 m^{-2}) (OR 0.93; 95% CI 0.87 – 0.99; P = 0.03), adjusted for systolic blood pressure, gender, smoking and glycated hemoglobin. When individual FA were separately analyzed in different models, similar results were observed both to linolenic acid (OR 0.60; 95% CI 0.40 – 0.90; P = 0.01) and to linoleic acid (OR 0.92; 95% CI 0.86 – 0.99; p = 0.03), adjusting to the same co-variables. Instead, in another model, it was observed that the dietary protein intake was positively associated with the presence of DKD (OR 1.10; 95% CI 1.00 – 1.14; P = 0.04), also adjusted to the same variables **(Table 9).**

Discussion

The current study demonstrated a negative association of PUFA intake with the presence of DKD in type 2 diabetes patients, with DKD defined by increased albuminuria or decreased eGFR. However, no statistical association was found with the isolated

presence of eGFR < 60 ml/min/1.73 m^{-2}. Moreover, our findings suggest that this association is stronger in more advanced stages of renal involvement, i.e., the patients with severely increased UAE presented lower intake of these FA. We had previously reported that low intake of total PUFA, mainly from vegetable oils, was associated with the presence of microalbuminuria in patients with type 2 DM, but we did not include patients with severely increased albuminuria nor evaluated GFR (31). In a previous longitudinal study that included patients with type 1 and 2 diabetes, and with micro and macroalbuminuria, it had been shown that those who presented regression of albuminuria had a higher intake of PUFA and a lower intake of saturated fatty acids (SFA), independent of treatment with ACE inhibitors and/or angiotensin receptor blockers. However, the investigators did not analyze specific FA, not identifying which dietary components contributed to the renoprotective effect (8). Also, in randomized and crossover clinical trials performed in type 2 diabetes patients with micro and macroalbuminuria (9,10), replacement of dietary red meat by the chicken meat in the short term proved capable of reducing the UAE, concomitant with a higher proportion of PUFA on serum lipids after a chicken diet (9).

Regarding the individual PUFA, the present study demonstrated an inverse association between linoleic (18:2n-6) and linolenic acids (18:3n-3) consumption and the presence of DKD. Concerning linoleic acid, the data about its relation with CKD is scarce. In our previous study, we also observed the lower intake of n-6 PUFA in microalbuminuric patients. Likewise, this inverse association was reinforced when fatty acid composition of serum lipids, used as a biological marker of fat intake (32), was evaluated in type 2 diabetic patients. In this study, a lower proportion of n-6 PUFA was observed in the triglyceride fraction in microalbuminuric patients as compared with normoalbuminuric patients. On the other hand, contrary to our findings, in a prospective randomized study

with type 1 diabetic patients with increased albuminuria, the UAE increased by 58% after a higher intake of linoleic acid, albeit an improvement in serum lipid profile (33). This study did not include patients with macroalbuminuria. In fact, more recently, Gopinath et al, in 2,600 healthy adults, observed a non-significant increase in the prevalence of moderate CKD with increasing consumption of n-6 PUFA. In this way, the effect of n-6 PUFA on renal disease is controversial and not well established (20)

The relation between CKD and dietary linolenic acid is also poorly explored and opposing results were observed (20). In the same cohort of 2,600 healthy adults of the Blue Mountains Eye Study, ∝-linolenic acid intake was positively associated with CKD (20). Significant positive associations were observed in participants with an eGFR of 60 to 75 ml/min per 1·73m2 and of 45 to 60 ml/min per 1·73m2. The authors themselves argued that this finding was not expected, and emphasize that one of the possibilities for these results is the fact that this FA, a plant n-3 PUFA, is poorly converted (less than 5%) to EPA and DHA, which are supposed to have beneficial effects on renal function. But the linolenic acid itself could have some beneficial effects, like the anti-inflammatory properties that may be the link between this fatty acid and the presence of CKD (34). In fact, the role of linolenic acid in cardiometabolic risk is still to be discussed (35).

In our study, no association was observed between long-chain n-3 PUFA (eicosapentaenoic or EPA and docosahexaenoic or DHA) and DKD. A renoprotective effect of these FA has been suggested by some observational studies conducted with diabetic patients, but not all. In the European Prospective Investigation of Cancer (EPIC), consumption of two or more portions of fish per week, compared with less than one portion per week, was associated with a lower risk of macroalbuminuria in individuals with diabetes (36). More recently, in a longitudinal study of type 1 diabetes patients from

the DCCT study, an inverse association was observed between intake of EPA and DHA and the values of albuminuria. However, in this study in normoalbuminuric patients no relationship between the intake of these FA and the incidence of albuminuria was observed after a mean follow-up of 6.5 years (37). Similarly, but evaluating a large number (5,042) of participants without cardiovascular disease and diabetes, in the Multi-Ethnic Study of Atherosclerosis (MESA), it was observed that total fish consumption, including fatty fish, lean fish, fried fish, and shellfish, was not associated with albuminuria. The benefit of the intake of n-3 PUFA on renal disease in individuals with diabetes also has been demonstrated in clinical trials, but as a result of dietary supplementation (38), and it was not confirmed in a previous meta-analysis (13). The possible explanations for these controversial findings might be the differences among the selected populations (ex.: different degrees of increased albuminuria), and the fact that some studies focused on n-3 PUFA intake, while others on dietary sources of these FA (marine sources). Another possible reason that could be hypothesized is that the varied amount of consumed dietary n-3 PUFA among the populations could have influenced the different results. In fact, In the present study, the mean EPA and DHA intake was low. As previously analyzed, less than 20% of our population consume marine foods, such as fish, which are known to be rich sources of EPA and DHA (39).

The possible mechanisms of the beneficial effect of PUFA on kidney function may be through both blood pressure levels (40) and improvement in lipid profile. In a cohort of 4508 American adults aged 18-30, without hypertension at baseline, the participants in the highest quartile of long chain n-3 PUFA intake had a significantly lower incidence of hypertension during the follow-up, compared to those in the lowest quartile (40). In relation to the improvement of the lipid profile, in a meta-analysis of randomized placebo-controlled trials of monotherapy with EPA (n=10), DHA (n=17), or EPA versus DHA

(n=6), both EPA and DHA significantly reduced triglycerides compared with placebo in the pooled analyses (41). In the present study, no difference of systolic and diastolic blood pressure was observed between patients with higher and lower intake of PUFA, when they were divided according to the mean intake of this FA (data not shown). Regarding the lipid profile, the group of patients with PUFA intake above average had lower levels of triglycerides when compared to the group with the lowest intake of the FA (data not shown). It is likely that lipid abnormalities may precede the development of micro- or macroalbuminuria - and not vice versa - though this topic is not completely understood.

Regarding SFA, the present study did not observed an association between their consumption and the presence of DKD. This finding is consistent with previous studies (31,42), but not all. A previous cross-sectional study observed a positive association between SFA intake and microalbuminuria in type 1 diabetic patients (8). Another study with middle-aged and older adults, with and without diabetes, demonstrated that a higher SFA intake was independently associated with the presence of high albuminuria, and that this association did not vary with the presence or not of diabetes (43).

Most of the studies that evaluated the impact of dietary fat intake on renal disease, predominantly reported the effect on albuminuria and only a few have examined the association between these nutrients and the eGFR decline. In the present study, we did not find an association between any FA and the loss of renal function (eGFR < 60 ml/min/1.73 m^{-2}). Our findings are consistent with some studies conducted with non-diabetic populations, which demonstrated no correlation between GFR < 60 ml/min/1.73 m^{-2} and total or subtypes of dietary fat (43,44). But it is not unanimous. In a study with participants of The Blue Mountain Eye Study, with 2,600 healthy adults aged 50 years or older, the dietary intake of long-chain n-3 PUFAs and fish was inversely associated with the risk of prevalent CKD, defined as an eGFR less than 60 ml/min/1.73 m^{-2} (20). Another study with

an older population demonstrated that a high plasma PUFA concentration, both n-3 FA and n-6 FA, may attenuate the age-associated decline in renal function (42) On the other hand, a meta-analysis of 12 clinical trials in individuals with CKD of any cause, in which seven studies were with diabetic patients, demonstrated that oral supplementation of long-chain n-3 PUFAs would reduce albuminuria, but not the decline in GFR (median follow-up was 9 months)(13). So, this subject needs further studies in order to assert that the dietary fat composition can influence the progression of renal outcomes.

Some limitations of this study must be acknowledged. The study design is cross-sectional and does not allow for inference of causality. Furthermore, the small number of patients with more advanced renal involvement would have impaired the power of the study to detect an association between dietary FA and GFR decline. However, the proportion of patients with severely increased UAE in the present study reflects the prevalence of this complication in the diabetic population. And, finally, linked to dietary data limitation, the measurement of a biological marker of fat intake, like serum FA, would have reinforced our results. Serum FA has been used to evaluate fat intake in patients with type 2 diabetes (45). However, our dietary data were accurate. We used a standardized 3-day weighed-diet record technique, which includes a 24-h urinary urea measurement to confirm dietary intake estimated from records (46). In fact, this dietary tool has been largely used to confirm dietary compliance in studies with diabetic patients (31, 47, 48).

In conclusion, higher dietary intake of PUFA, especially linoleic and linolenic acids, was associated with a reduced prevalence of DKD in type 2 diabetes patients. In addition to this, an increase in protein intake in the diet appears to be associated with increased risk for worsening kidney disease. These associations appeared to be stronger when DKD was defined by increased albuminuria or decreased eGFR. The availability of

fish and other marine products is limited in many countries (49) and linoleic and linolenic acids from plant oils and nuts is the predominant source of PUFA in the typical Western diet. Perhaps, these dietary FA may be a renoprotective alternative and could be very important for public health. However, further large-scale longitudinal studies and clinical trials are needed to confirm these findings in patients with type 2 diabetes, in order to try to understand the mechanism by which this benefit occurs and thus to provide a non-drug strategy for these patients.

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Table 1. Renal status of patients

Moderately elevated UAE (%)	**22**
Elevated UAE (%)	**8**
eGFR (mL/min/1,73m2)	**86 ± 18**
Stage 1 (%)	**37,2**
Stage 2 (%)	**41,1**
Stage 3 (%)	**5,2**
Stage 4 (%)	**0,3**
UAE (mg/24h)	**11 (3 – 5750)**

UAE: urinary albumin excretion; eGFR= estimated glomerular filtration rate

Table 2. Clinical and laboratory characteristics of type 2 diabtes patients with and without Diabetic Kidney Disease based on the presence of urinary albumin excretion ≥ 30 mg/24-h.

	Without DKD (n = 247)	**With DKD (n = 111)**	**P**
Age (years)	61 ± 10	58 ± 10	**0.01**
Male (%)	44	59	**0.01**
Duration of diabetes (years)	13 ± 8	12 ± 8	0.69
Ethnicity: White (%)	85	86	0.94
Hypertension (%)	75	90	**<0.001**
Ischemic Heart Disease (%)	18	23	0.26
Smoking (%)	49	61	0.05
BMI (kg/m^2)	28.5 ± 4.4	28.7 ± 4.2	0.64
Waist Circumference (cm)			
Men	100 ± 10	103 ± 12	0.12
Women	99 ± 11	99 ± 10	0.67
Systolic Blood Pressure (mmHg)	137 ± 21	141 ± 21	0.06
Diastolic Blood Pressure (mmHg)	79 ± 11	82 ± 12	**0.03**
Fasting plasma glucose (mg/dL)	146 ± 51	155 ± 59	**0.02**
Glycated hemoglobin (%)	7.4 ± 1.5	7.8 ± 1.6	**0.02**
Total cholesterol (mg/dL)	200 ± 41	204 ± 46	0.36
HDL cholesterol (mg/dL)	51 ± 13	48 ± 11	**0.02**
LDL cholesterol (mg/dL)	121 ± 34	123 ± 40	0.55
Triglycerides (mg/dL)	134 (25 – 421)	143 (49 - 573)	0.28
eGFR ($ml .min^{-1} . 1.73 m^{-2}$)	85 ± 17	88 ± 21	0.22

BMI: body mass index; eGFR: estimated glomerular filtration rate.

Table 3. Daily dietary intake of type 2 diabetes patients with and without Diabetic Kidney Disease based on the presence of urinary albumin excretion ≥30 mg/24-h

	Without DKD (n = 257)	**With DKD (n = 111)**	**P**
Energy (Kcal)	1829 ± 506	1808 ± 486	0.71
Carbohydrates (% of energy)	47 ± 6	47 ± 8	0.83
Proteins (% of energy)	19 ± 3	20 ± 4	**0.03**
Lipids (% of energy)	34 ± 7	33 ± 8	0.31
Saturated FA (% of energy)	9.5 ± 2.5	9.4 ± 3.0	0.69
Monounsaturated FA (% of energy)	11 ± 3	12 ± 3	0.39
Polyunsaturated FA (% of energy)	10 ± 4	9 ± 4	**0.02**
P/S Ratio	1.1 ± 0.4	1.0 ± 0.5	0.12
Cholesterol (mg)	200 ± 106	222 ± 100	0.17
***Trans* FA (% of energy)**	1.2 ± 0.7	1.3 ± 0.7	0.28

FA: fatty acid; P/S ratio: polyunsaturated/saturated ratio

Table 4. Logistic regression analysis (dependent variable: the presence of renal disease in diabetes based on the presence of urinary albumin excretion ≥30 mg/24-h)

Variáveis Independentes	RC	IC – 95%	P
Proteins (% of energy) *	**1,06**	**0,99 – 1,13**	**0,08**
Polyunsaturated FA (% of energy) *	**0,93**	**0,87 – 1,00**	**0,05**
Linoleic FA (18:2n-6) (% of energy) *	**0,92**	**0,86 – 0,99**	**0,04**
Linolenic FA (18:3n-3) (% of energy) *	**0,62**	**0,41 – 0,94**	**0,02**

FA: fatty acid.

***** Models adjusted for gender, age, duration of diabetes, smoking, use of hypolipidemic agents, body mass index, systolic blood pressure and compliance with weighed diet record.

Table 5. Dietary polyunsaturated fatty acids content of type 2 diabetic patients according to the degree of albuminuria.

Albuminuria	Normo (n=256)	Moderately increased (Micro) (n=82)	Severely increased (Macro) (n=29)	P
Polyunsaturated FA	**9.9 ± 3.4** [#]	**9.2 ± 4.1**	**8.1 ± 3.2**	**0.02**
Linoleic FA (18:2n-6)	**8.7 ± 3.0** [#]	**8.1 ± 3.8**	**7.1 ± 3.3**	**0.02**
Linolenic FA (18:3n-3)	**0.91 (0.18 – 2.42)**	**0.83 (0.00 – 3.02)**	**0.69 (0.01 – 2.12)***	**0.03**

FA: fatty acid

[#] Normo *vs* Severely increased

* Normo and Moderately increased *vs* Severely increased

Table 6. Daily dietary intake of patients with type 2 diabetes based on the presence or absence of chronic kidney disease as the values of GFR

	Without CKD (n = 342)	With CKD (n = 25)	P
Energy (Kcal)	1829 ± 496	1692 ± 503	0.19
Carbohydrates (% of energy)	47 ± 7	48 ± 7	0.32
Proteins (% of energy)	19 ± 4	20 ± 4	0.50
Lipids (% of energy)	34 ± 7	32 ± 9	0.23
Saturated FA (% of energy)	9.6 ± 2.7	8.9 ± 2.7	0.26
Monounsaturated FA (% of energy)	11 ± 3	11 ± 3	0.65
Polyunsaturated FA (% of energy)	10 ± 4	9 ± 4	0.61
Linoleic FA (18:2n-6) (% of energy)	9 ± 3	8 ± 4	0.43
Linolenic FA (18:3n-3) (% of energy)	0.90 (0.00 – 3.02)	0.75 (0.01 – 2.42)	0.17
Arachidonic FA (20:4n-6) (% of energy)	0.05 (0.00 – 0.27)	0.05 (0.00 – 0.16)	0.51
Eicosapentaenoic FA (20:5n-3) (% of energy)	0.00 (0.00 – 0.17)	0.00 (0.00 – 0.12)	0.35
Docosahexaenoic FA (22:6n-3) (% of energy)	0.01 (0.00 – 0.50)	0.02 (0.00 – 0.36)	0.46
P/S Ratio	1.1 ± 0.5	1.1 ± 0.4	0.98
Cholesterol (mg)	213 ± 106	183 ± 73	0.16
***Trans* FA (% of energy)**	1.04 (0.00 – 5.67)	0.91 (0.07 – 3.75)	0.15

FA: fatty acid; P/S ratio: polyunsaturated/saturated ratio

Table 7. Clinical and laboratory characteristics of patients with type 2 diabetes divided according to the presence or not of Diabetic Kidney Disease, defined based on the values of albuminuria (> 30 mg/day) or eGFR <60 ml.min^{-1} . 1.73 m^{-2})

	Without CKD (n = 241)	**With CKD (n = 127)**	**P**
Age (years)	61 ± 9	60 ± 11	0.34
Male (%)	44	57	**0.02**
Duration of diabetes (years)	12 ± 8	13 ± 8	0.90
Ethnicity: White (%)	85	87	0.88
Hypertension (%)	73	90	**<0.001**
Ischemic Heart Disease (%)	17	25	0.07
Smoking (%)	48	62	**0.01**
BMI (kg/m^2)	28.4 ± 4.4	28.7 ± 4.2	0.52
Waist Circumference (cm)			
Men	100 ± 10	102 ± 12	0.13
Women	98 + 11	100 + 10	0.42
eGFR (ml .min^{-1} . 1.73 m^{-2})	87 + 15	82 + 23	0.11
Sistolic Blood Pressure (mm Hg)	136 ± 20	142 ± 22	**0.01**
Diastolic Blood Pressure (mm Hg)	79 ± 11	82 ± 12	**0.07**
Fasting plasma glucose (mg/dL)	146 ± 51	163 ± 67	**0.01**
Glycated hemoglobin (%)	7.4 ± 1.5	7.6 ± 1.6	**0.03**
Total cholesterol (mg/dL)	201± 40	204 ± 47	0.50
HDL cholesterol (mg/dL)	51 ± 13	48 ± 12	**0.04**
LDL cholesterol (mg/dL)	121 ± 34	123 ± 40	0.55
Triglycerides (mg/dL)	134 (25 – 421)	147 (49 - 573)	0.11

BMI: body mass index; eGFR: estimated glomerular filtration rate.

Table 8. Daily dietary intake of patients with type 2 diabetes divided according to the presence or not of Diabetic Kidney Disease, defined based on the values of albuminuria (> 30 mg/day) or eGFR <60 ml.min^{-1} . 1.73 m^{-2})

	Without CKD (n = 241)	**With CKD (n = 127)**	**P**
Energy (Kcal)	1833 ± 505	1793 ± 482	0.47
Carbohydrates (% of energy)	47 ± 6	47 ± 8	0.98
Proteins (% of energy)	19 ± 3	20 ± 4	**0.01**
Lipids (% of energy)	34 ± 7	33 ± 8	0.21
Saturated FA (% of energy)	9.5 ± 2.5	9.4 ± 3.0	0.51
Monounsaturated FA (% of energy)	11 ± 3	12 ± 3	0.41
Polyunsaturated FA (% of energy)	10 ± 3	9 ± 4	**0.01**
Linoleic FA (18:2n-6) (% of energy)	9 ± 3	8 ± 4	**0.01**
Linolenic FA (18:3n-3) (% of energy)	0.93 (0.18 – 2.29)	0.79 (0.00 – 3.02)	**0.02**
Arachidonic FA (20:4n-6) (% of energy)	0.05 (0.01 – 0.27)	0.05 (0.00 – 0.18)	0.19
Eicosapentaenoic FA (20:5n-3) (% of energy)	0.00 (0.00 – 0.16)	0.00 (0.00 – 0.12)	0.34
Docosahexaenoic FA (22:6n-3) (% of energy)	0.01 (0.00 – 0.50)	0.01 (0.00 – 0.36)	0.39
P/S Ratio	1.1 ± 0.5	1.0 ± 0.5	0.12
Cholesterol (mg)	183 (39 – 696)	212 (51 – 551)	0.09
***Trans* FA (% of energy)**	0.01 (0.00 – 0.50)	0.02 (0.00 – 0.36)	0.54

FA: fatty acid; P/S ratio: polyunsaturated/saturated ratio

Table 9. Logistic regression analysis (dependent variable: the presence of renal disease in diabetes based on the values of albuminuria (> 30 mg/day) or eGFR <60 $ml.min^{-1}$. 1.73 m^{-2})

Independent Variables	OR	IC – 95%	P
Proteins (% of energy) *	**1,10**	**1,00 – 1,14**	**0,04**
Polyunsaturated FA (% of energy) *	**0,93**	**0,87 – 0,99**	**0,03**
Linoleic FA (18:2n-6) (% of energy) *	**0,92**	**0,86 – 0,99**	**0,03**
Linolenic FA (18:3n-3) (% of energy) *	**0,60**	**0,40 – 0,90**	**0,01**

FA: fatty acid

* Models adjusted for gender, smoking, glycated hemoglobin and systolic blood pressure.

Capítulo II

Dietary n-3 Polyunsaturated Fatty Acids and Risk of Mortality in Patients with Type 2 Diabetes

Dietary n-3 Polyunsaturated Fatty Acids and Risk of Mortality in Patients with Type 2 Diabetes

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ABSTRACT

Introduction: Diabetes has an important impact on length and quality of life and, despite the more intensive management of glycemic control and of the concomitant morbidities, the frequency of diabetic chronic complications is still high resulting in higher mortality. There are many reports relating the composition of dietary fat, especially n-3 polyunsaturated fatty acids to the incidence of mortality, but mostly in non-diabetic people. **Objective:** This cohort study aims at assessing the association between the dietary fat composition and total and cardiovascular mortality in patients with type 2 diabetes. **Methods:** At baseline, the patients were submitted to nutritional assessment (anthropometry and assessment of usual diet) and clinical and laboratory evaluation. After a minimum follow up of one year, a new clinical assessment was performed. The diet was assessed by completing three-day weighed diet records (Nutribase 2007®software). The adequacy of the diet records was confirmed by the estimate of protein ingestion by 24-hour urinary urea. **Results:** Three hundred and sixty-eight patients were assessed (177 [48.1%] male, mean age 61 ± 8 years, duration of DM 12 ± 8 years, body mass index [BMI] 28.6 ± 4.3 kg/m²). In the women, multivariate cox analysis showed that the intake of total n-3 PUFA was negatively associated with the risk of total mortality (HR: 0.08; 95% 0.01, 0.85; p= 0.036), adjusted for age, presence of ischemic heart disease, systolic blood pressure, values of GFR, glycated hemoglobin and compliance with the WDR. When the individual PUFA were separately analyzed, it was observed that linolenic acid (18: 3n-3) was negatively associated with the risk of total mortality (HR: 0:35; 95% 0.12 – 1.00; P= 0.050), but marginally significant, adjusted for the same co-variables. In men, no association was observed between dietary fat composition and mortality risk. Also, no relation was observed between dietary fat and cardiovascular mortality both in women and men. Conclusion: In women with type 2 diabetes, the higher total n-3 polyunsaturated fatty

acid consumption, especially linolenic acid, is associated with lower total mortality. This association was not found in the men.

Introduction

The prevalence of diagnosed diabetes has increased worldwide, and in the same proportion, the prevalence of its chronic complications (1). Furthermore, diabetes has an important impact on length and quality of life, independently of age at diagnosis and whether it is type 1 or type 2 diabetes (2). Despite the more intensive management of glycemic control and the concomitant morbidities (hypertension, dyslipidemia), the frequency of diabetic chronic complications remains high, also resulting in higher mortality. It is estimated that if an individual is diagnosed at 40 years of age, men will lose 11.6 life-years and women will lose 14.3 life-years (3).

For a long time, there has been an interest in comparing the composition of dietary fat to the incidence of mortality, but mostly in non-diabetic people (4). Among the few studies involving patients with diabetes, the emphasis is on cardiovascular outcomes, and less on total and cause-specific mortality (5), and yet the findings remain controversial (6,7).

Regarding the subtypes of dietary fat, the family of n-3 PUFA is of greatest interest in most studies, involving patients with diabetes (8) and without the disease (9). However, some of them show a benefit with higher consumption of this FA (10) while others do not (8,11). Among the individual n-3 PUFA, the major components are linolenic acid, and the long-chain n-3 (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]). Linolenic acid is the essential n-3 and the FA that comprise most of the dietary n-3 PUFA. Its main dietary sources are vegetable oils. EPA and DHA are long-chain n-3 and about 5% of circulating values come from the conversion of ingested linolenic acid. Dietary EPA and DHA come mainly from marine sources. Most of the previous studies have suggested that long-chain n-3 FA derived from seafood are associated with a lower risk of mortality,

CHD and stroke (12), but not all (13). Lesser studies focus on the relationship between linolenic acid and cardiovascular and total mortality, some demonstrating positive results (14,15). Also, the early studies on this subject demonstrated a stronger beneficial effect of PUFA intake than the more recent ones, maybe due to concomitant aggressive pharmacological management of cardiovascular risk factors, which could influence the incidence of cardiovascular outcomes.

Recently, we published that in patients with type 2 diabetes without ischemic heart disease, a high intake of polyunsaturated fatty acids, especially alpha linolenic acid, was protective for the development of cardiac events (16). As type 2 diabetic patients have peculiar characteristics, such as concomitant important cardiovascular risk factors, and higher risk of some non-cardiovascular diseases (e.g. cancer), it is relevant to evaluate to which extent the composition of dietary FA could be associated with total and cardiovascular mortality, but in a population sample with and without cardiovascular disease, as well as with different degrees of diabetic complications. Hence, the aim of the present study was to examine the association of dietary fat composition with the risk of total and cardiovascular mortality among patients with type 2 diabetes.

Patients and methods

Patients

We carried out a retrospective data analysis of patients with type 2 diabetes belonging to a prospective cohort study. The patients included in the study were from the Diabetes research outpatient clinic at Hospital de Clínicas de Porto Alegre (Rio Grande do Sul, Brazil) and were followed for at least 12 months. At baseline, the patient exclusion criteria were: body mass index (BMI) > 40 kg/m2, symptomatic autonomic neuropathy (gastroparesis or diabetic diarrhea), dietary counseling by a registered dietitian during the

previous 12 months, and inability to perform the weighed diet records (WDR). The study was approved by the Hospital Ethics Committee and all patients signed a written informed consent form.

All patients underwent a standardized clinical, nutritional and laboratory examination at baseline. A three-day WDR was carried out, 24-h urine was collected, and on the 3rd day (after patients fasted overnight), blood samples were also collected. During the reevaluation period, patients were submitted to the same clinical and laboratory examinations.

The follow-up was determined as the period between the first assessment and the last medical evaluation or the date of death. Patients were divided into two groups: if they were alive or not at the reevaluation period.

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Methods

Dietary assessment

Diet was assessed using a 3-day WDR technique (two nonconsecutive weekdays and one day of the weekend). Patients were issued commercial scales (1-125 g) and measuring cups (25-250 mL;Pirex) and given a detailed explanation and demonstration of the procedures by a trained, registered dietitian. Next, patients performed a training session for a one-day WDR. The 3-day WDR was performed over two to three weeks and before nutritional recommendations for all patients (Figure 1). Later, dietary advice was individualized, based on the 3-day WDR and according to recommendations from the American Diabetes Association (ADA) guidelines (17).

Compliance with the WDR technique was assessed by comparison of protein intake estimated from the 3-day WDR (PI-WDR) and from the 24-h urinary nitrogen output (PI-N) performed on the third day of the WDR period (18). According to Vaz et al (18),

patient compliance with the WDR protocol is established if the PI-WDR/PIN ratio is between 0.79 and 1.26.

The analysis of dietary nutrients intake from the 3-day WDR was performed using the Nutribase Clinical Nutritional Manager software (version 7.14, 2007). The mean values of each nutrient consumed during the 3-day WDR were calculated. Nutritional data for frequently consumed foods were updated if necessary, and/or complemented with data obtained from local manufacturers of industrialized foods.

Anthropometric measurements

The body weight and height of patients (without shoes or coats) were collected with an anthropometric scale; measurements were recorded to the nearest 100 g for weight, and to the nearest 0.1 cm for height. Waist circumference was measured midway between the lowest rib and the iliac crest, near the umbilicus.

Clinical evaluation at baseline and end-of-stu*dy*

The clinical assessment emphasized cardiovascular evaluation (blood pressure, WHO cardiovascular questionnaire, resting electrocardiogram, and evaluation of peripheral arterial pulses) and renal function.

Patients were classified as “nonsmoker” or “smoker” (smoker in the past or currently a smoker). Physical activity was classified into four levels based on a standardized questionnaire, which was adapted to local habits (19).

Mean blood pressure was calculated based on two separate measures using a digital sphygmomanometer (OMRON® Automatic Blood Pressure Monitor, Model HEM-705CP, Vernon Hills, Illinois 60061). Hypertension was defined as blood pressure ≥ 140/90 mmHg or use of antihypertensive drugs on at least two separate occasions.

Peripheral vascular disease was determined based on the presence of intermittent claudication and/or absence of posterior tibial pulse at clinical examination. The presence

of cerebrovascular disease was established if there was a history of cerebrovascular accident (ischemic stroke) and/or compatible findings (sequelae).

The renal function was evaluated by serum creatinine and 24-h urinary albumin excretion (UAE) (20). Because of the high intra-individual variability of UAE, we calculated the mean value of two measurements (within a period of 3 to 6 months) to establish persistent albuminuria (≥ 30 mg/24-h) (ADA). Glomerular Filtration Rate (eGFR) was estimated by using the CKD-EPI equation (21).

Information on coexisting conditions and causes of death were retrieved from the patient's hospital records, or if it was incomplete, further information was provided by family members. We counted the time of follow-up for each participant from the date of filling out the baseline 3-day WDR to the date of death from any cause or to the date of the nutritional and clinical re-evaluation. Non-CVD mortality included deaths due to cancer, pulmonary diseases, infection, dementia, fractures/trauma, and other causes. Cardiovascular disease mortality was defined as deaths attributable to CHD, stroke, other atherosclerotic disease, and other CVD.

Laboratory analyses

Glycemic control was evaluated by serum glucose (glucoseperoxidase colorimetric enzymatic method) and glycated hemoglobin (high precision chromatography, Merck-Hitachi 9100 apparatus). The lipid profile consisted of the measurement of total cholesterol and triglycerides using colorimetric assay. HDL cholesterol was measured using the direct enzymatic method. LDL cholesterol was calculated using the Friedewald formula. Serum creatinine was measured by the Jaffe method and urea by kinetic UV assay. Urinary albumin excretion was evaluated by the immunoturbidimetry technique (Kit MICROALB, AMES) (22).

Statistical analysis

Student t test, Mann-Whitney U test, and the Exact Fisher or Chi-Square tests were applied. Multivariate Cox's proportional hazard model was used to estimate the risk factors for the risk of total and cardiovascular mortality (dependent variables). Different models were generated to evaluate the association of each nutrient (independent variables) with mortality. Independent variables were selected based on their significance ($P < 0.10$) at univariate analysis or according to their biological relevance.

Data were expressed as mean ± standard deviation, or as median (P^{25} - P^{75}) unless otherwise stated. The level of significance adopted was 5%. The software SPSS 18.0 (SPSS®, Chicago, IL) was used for the analysis.

Results

Characteristics of patients according to progression to death

A total of 368 patients underwent clinical, laboratory, and nutritional evaluation at baseline. The mean age of patients was 61 ± 8 years, duration of diabetes 12 ± 8 years, and BMI 28.6 ± 4.3 kg/m^2. During the mean follow-up of 5.8 years (from one to 13.3 years) forty-one deaths occurred: sixteen patients died due to cardiovascular disease, twenty-three patients died from non-cardiovascular causes (complications due to systemic lupus erythematosus, sepsis, postoperative complications, neoplasms, and respiratory insufficiency), and two died from a non-identified cause.

Baseline clinical and laboratory characteristics of the patients are shown in **Table 1.** As expected, patients who progressed to death were older compared with those who survived. Furthermore, the proportion of men was significantly higher in the group that died compared to the live patients. The presence of ischemic heart disease and diabetic kidney disease at baseline were also predictors of higher mortality during the follow-up period. In the group that progressed to death, higher values of systolic and diastolic blood

pressure and lower values of glomerular filtration rate were also observed. Other clinical and laboratory characteristics were not different between the groups. Regarding the medications frequently used by diabetic patients and that could have an impact on mortality rates (ACE inhibitors, beta-blockers and hypolipidemic agents), as well as drugs used for glycemic control (oral anti-diabetics or insulin), no difference was observed between the groups.

Diet characteristics and total and cardiovascular mortality

Compliance with the WDR technique was observed in 85% and 83% of patients of the group that progressed to death or not, respectively (P = 0.83). The daily dietary intake evaluated by 3-day WDR of patients with type 2 diabetes, categorized according to being alive or not, is shown in **Table 2.** The dietary PUFA intake was lower in the group that progressed to death, but did not achieve statistical significance. The other macronutrient consumption was not different between the groups. When the fractions of PUFA were separately evaluated, it was observed that the dietary intake of total n-6 PUFA were significantly lower in the group that progressed to death, as well as the consumption of linoleic acid (18: 2n-6), the most abundant n-6 PUFA in the diet, when compared with the group of live patients.

When the patients were divided according to cardiovascular mortality, two patients, in whom the cause of death was not identified, were excluded from the analysis. No difference was observed between the patients with and without cardiovascular deaths regarding the dietary nutrients intake.

Association between dietary PUFA content and the risk of total mortality

As men presented significantly higher mortality compared with women (15.8% *vs*. 6.8, p= 0.01), the following analysis of data was made with the patients divided according to gender.

In the women, multivariate analysis showed that the intake of total n-3 PUFA was negatively associated with the risk of total mortality (HR: 0.08; 95% 0.01, 0.85; p= 0.036), adjusted for age, presence of ischemic heart disease, systolic blood pressure, values of GFR, glycated hemoglobin and compliance with WDR. When the individual PUFA were separately analyzed, it was observed that linolenic acid (18: 3n-3) was negatively associated with the risk of total mortality (HR: 0:35; 95% 0.12 – 1.00; P= 0.050), but marginally significant, adjusted for the same co-variables. On the other hand, the inverse association between total PUFA, n-6 PUFA, and especially linoleic acid was not confirmed. In men, no association between dietary fat intake and the risk of total mortality was observed.

As the consumption of marine sources in our population was extremely low (about 14%), to test the association between EPA and DHA intake and total mortality, we categorized the patients according to having any intake of these FA or not. Among men, to eat any amount of EPA was associated with a 58% reduction in the risk of total mortality after adjustment for age, presence of ischemic heart disease, systolic blood pressure, values of GFR, glycated hemoglobin and compliance with WDR (HR: 0.42; 95% 0.19, 0.92; p= 0.031). No association was observed in relation to DHA intake in both men and women.

Analyses according to quartiles of n-3 PUFA intake and total mortality

Fractions of PUFA were analyzed according to the quartiles of intake in women and men separately. n-3 PUFA intake were linearly associated with a decrease in total mortality, as follows: 1st quartile (≤0.65% of energy): 12.5% of deaths; 2nd quartile (0.66 to 0.91% of energy): 4.9% of deaths; 3rd quartile (0.92 to 1.31% of energy): 6.5% of deaths; and 4th quartile (>1.32% of energy): 4.1% of deaths (*P* for trend = 0.04). In multivariable regression models, women in the highest quartile of n-3 PUFA intake presented a

significantly lower risk of any-cause mortality, compared with those in the lowest quartile of intake **(Table 3 and Figure 1)**, after adjustment to age, presence of ischemic heart disease, systolic blood pressure, values of GFR, glycated hemoglobin and compliance with WDR. For linolenic acid intake, the same inverse association was observed. The any-cause mortality rates for each quartile were as follow: 1st quartile (≤0.61% of energy): 10.4% of deaths; 2nd quartile (0.62 to 0.87% of energy): 6.8% of deaths; 3rd quartile (0.88 to 1.28% of energy): 6.5% of deaths; and 4th quartile (>1.29% of energy): 4.3% of deaths (*P* for trend = 0.05). Women in the highest quartile of linolenic acid intake, compared with those in the lowest quartile of intake, seems to have a 90% reduced risk of total mortality, but it was not statistically significant (P = 0.09).

In men, no association between dietary n-3 PUFA divided into quartiles and total mortality was observed.

Discussion

In this cohort of patients with type 2 diabetes, dietary n-3 PUFA, mainly linolenic acid, were independently associated with a lower risk of total mortality in female group. Women in the higher quartile compared with those in the lower quartile of n-3 intake and linolenic acid, had 93% and 90% reduced risk of dying from any cause, respectively, after adjustment to risk factors. In general, the larger proportion of total n-3 PUFA consists of linolenic acid, a plant-derived essential n-3 FA, found in selected seed and vegetable oils, such as soybean and rapeseed oils. In fact, in the population of the present study, soybean oil was the most widely consumed vegetable oil (67%), whereas only 14% of patients consumed marine foods. As the availability of fish and other marine products is limited in many countries (23) and linolenic acid from plant oils and nuts is the predominant source of n-3 PUFA in the typical Western diet, these findings are so relevant as a alternative dietary intervention.

The finding that the protective effect of n-3 PUFA on all-cause mortality was only in women is in consonance with some other studies. In a report based on the Blue Mountains Eye Study (participants aged ≥ 49y), dietary intake of total n-3 PUFA (from the nuts and not the fish) demonstrated a protective effect against inflammatory diseases mortality only in older women (10), and not in men. Some evidence suggests that older women are more sensitive to the ability of n-3 PUFA to reduce the production of inflammatory cytokines (24). Furthermore, nuts are rich sources of alpha linolenic and linoleic acid, the two essential PUFA. As in our study, the benefit with the higher consumption of the total n-3 PUFA was derived from the plant sources (linolenic acid) and not from animal sources (EPA and DHA).

Regarding the observed inverse association between dietary linolenic acid intake (the principal n-3 PUFA) and total mortality, some studies reported similar results, but in non-diabetic populations. In the Iowa Women's Health Study cohort of healthy postmenopausal women, it was demonstrated that dietary linolenic acid intake was associated with a lower risk of all-cause mortality, after adjustment to many risk factors (14). Also, in a 15-year follow-up of a population-based cohort of 1551 middle-aged men who were free of cardiovascular disease, cancer, and diabetes at baseline, the individuals with a lower dietary intake of linoleic and linolenic acid had a higher total and cardiovascular mortality (15). Moreover, in a recent large prospective cohort study in older adults, higher dietary linolenic acid was associated with a lower risk of total mortality and non-cardiovascular mortality, in particular, deaths due cancer and dementia (25). Interestingly, even with a much lower number of participants and of outcomes, but with high-risk patients (presence of diabetes), our study reported a marginally significant association like these large-scale prospective cohort studies.

Further research is needed to better understand the mechanisms by which linolenic acid may be associated with a lower risk of non-cardiovascular mortality. Clinical anti-inflammatory effects of n-3 FA have been documented in several chronic inflammatory conditions including rheumatoid arthritis and inflammatory bowel diseases (26). Linolenic acid has been shown to exert favorable effects on cytokine production. This FA has also shown to significantly decrease cell adhesion molecules, including E-selectin (27). Another potential biochemical pathway by which total n-3 FA attenuates inflammation and endothelial activation is by reducing the baseline production of hydrogen peroxide (28).

When only cardiovascular deaths were separately evaluated, no association was observed with any component of dietary fat. This finding is in accordance with some few studies that also did not reported association of cardiovascular mortality with dietary fat content or individual FA (25). However, many others, mostly with non-diabetic patients, observed inversely association with dietary PUFA intake (7,15,29). A recent meta-analysis from Pan et al (29) suggests that linolenic acid consumption may confer cardiovascular benefits, and each 1 g/d increment of linolenic acid intake was associated with a 10% lower risk of CHD death. Additionally, Mozaffarian et al (7) demonstrated that circulating individual and total n3-PUFA are associated with lower total mortality, especially coronary heart disease death, in older adults. Probably, the low number of cardiovascular deaths (N=16) in our sample of patients followed in this period of time could have influenced the absence of relation between dietary fat components and this outcome.

In the present study, we did not find an association between dietary total n-6 PUFA, or the linoleic acid (the major PUFA), and risk of total mortality. In fact, the beneficial effect of n-6 PUFA on health, and especially on cardiovascular disease, is a subject of controversy. Some older and yet recent studies did not demonstrate protective results on coronary heart disease as on non-cardiovascular mortality, respectively (10,30).

However, in a recent cohort study, in which 2792 older adults participating in The Cardiovascular Health Study were evaluated, serum linoleic acid was inversely associated with total mortality, with a risk reduction of 13% (31). The difference among the findings may be related to the studied population samples. The former studies were conducted with patients with established coronary disease or diverse inflammatory non-cardiac disease, i.e., linoleic acid effect was evaluated as a secondary prevention. Whereas, the later study was a primary prevention study. Some concerns about n-6 PUFA are related to their theoretical proinflammatory and prothrombotic effects, which may be associated to harm to cardiovascular or non-cardiovascular health. Furthermore, it is also emphasized its potential competition with n-3 PUFA in metabolism pathways, interfering with the beneficial effects of these FA. In our study, no association was also observed between the n-6/n-3 ratio and dying from total mortality (data not shown).

In the current study, initially it was not observed association between long-chain n-3 PUFA and total or cardiovascular mortality both in men or women. However, when the patients were categorized according to having any intake of these FA or not, an inverse association of EPA with total mortality was observed in men. These findings might be related to the extremely low amount of these FA in the habitual dietary fat intake of our population. Some previous studies conducted with diabetic patients had reported beneficial effects of EPA (6) or fish intake (5) on total mortality. However, it is not unanimous in the literature (14). It is yet unclear whether potential cardiovascular benefits of n-3 PUFA could result into lower total mortality. Furthermore, other non-cardiovascular conditions (e.g., cancer, lung disease), important causes of death, may be unaffected by n-3 PUFA, lessening the effects on total mortality. In fact, n3-PUFA supplementation was not associated with a lower risk of all-cause mortality (32).

Several limitations should be considered in the present study. The number of deaths for the analysis was small, which might explain the weaknesses of the study to observe the association of some fat components with reduction of mortality risk. Also linked to dietary data, the measurement of a biological marker of fat intake would have reinforced our results. However, our dietary data were accurate. We used a standardized 3-day weighed-diet record technique, which includes a 24-h urinary urea measurement to confirm dietary intake estimated from records (33). In fact, this dietary tool has been largely used to confirm dietary compliance in studies with diabetic patients (34,35,36). Finally, the retrospective data analyses of patients who belong to a prospective cohort study might be considered as a study weakness. However, all patients were treated in the same outpatient division by the same experienced team using a standardized clinical and laboratory protocol. This cohort has been prospectively followed by our research group since 2000 (18,34-37).

In summary, the present study suggests an association between total n-3 PUFA intake, especially linolenic acid, with the prevention of total mortality in women with type 2 diabetes. However, these findings require further investigation and replication in additional studies, especially randomized controlled clinical trials, to confirm these findings in diabetic patients. The determination of the dietary fat composition associated to increased or decreased risk of total mortality of this specific population may be valuable information to reinforce dietary recommendations.

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Table 1. Baseline clinical and laboratory characteristics of the patients categorized according to progression to death or not.

	No Death (n = 327)	**Death (n = 41)**	**P**
Age (years)	60 ± 8	67 ± 7	**0.00**
Gender (male) (%)	45.6	68.3	**0.01**
Follow-up time (months)	70 ± 32	63 ± 34	0.20
Ethnicity: White	86	83	0.54
Duration of diabetes (years)	12 ± 8	14 ± 7	0.33
Hypertension (%)	78	90	0.07
Ischemic Heart Disease (%)	18	34	**0.02**
Diabetic Kidney Disease (%)	28	44	0.05
Smoking (self-reported) (%)	52	59	0.51
Alcohol (self-reported) (%)	46	39	0.66
Sedentary lifestyle (%)	57	68	0.18
BMI (kg/m^2)	28 ± 4	29 ± 5	0.40
SBP (mmHg)	137 ± 20	148 ± 24	**0.00**
DBP (mmHg)	80 ± 11	84 ± 14	0.05
GFR ($ml .min^{-1} . 1.73 m^{-2}$)	87 ± 18	81 ± 21	**0.04**
Fasting plasma glucose (mg/dL)	151 ± 57	159 ± 59	0.40
Glycated hemoglobin (%)	7.5 ± 1.5	7.9 ± 1.9	0.10
Total cholesterol (mg/dL)	201 ± 43	207 ± 38	0.45
HDL cholesterol (mg/dL)	48 ± 12	7.8 ± 1.6	0.23
LDL cholesterol (mg/dL)	121 ± 36	129 ± 35	0.20
Triglycerides (mg/dL)	135 (25-575)	133 (61-358)	0.93

BMI: body mass index ; SBP: systolic blood pressure; DBP: diastolic blood pressure; GFR: glomerular filtrate rate

Table 2. Daily dietary intake of the patients categorized according to progression to death or not.

	No death (n = 327)	**Death (n = 41)**	**P**
Energy (Kcal)	1818 ± 503	1860 ± 473	0.61
Carbohydrates (% of energy)	47 ± 7	48 ± 7	0.24
Proteins (% of energy)	19 ± 4	20 ± 4	0.42
Lipids (% of energy)	34 ± 7	32 ± 7	0.12
Saturated FA (% of energy)	9.5 ± 2.7	9.3 ± 2.9	0.64
Monounsaturated FA (% of energy)	11 ± 3	11 ± 3	0.37
Polyunsaturated FA (% of energy)	9.3 (2.8-25.3)	8.6 (4.6-16.0)	0.060
Total n-3 (% of energy)	0.90 (0.00-3.02)	0.73 (0.01-1.86)	0.099
Linolenic Acid (% of energy)	0.93 (0.02-3.02)	0.83 (0.01-1.87)	0.152
Total n-6 (% of energy)	8.6 ± 3.3	7.5 ± 2.9	**0.045**
Linoleic Acid (% of energy)	8.6 ± 3.3	7.6 ± 2.9	**0.044**
Arachidonic Acid (% of energy)	0.05 (0.00 – 0.27)	0.05 (0.00–0.17)	0.526
***Trans* FA (% of energy)**	1.04 (0.00-5.67)	1.00 (0.40-4.10)	0.37
P/S Ratio	1.1 ± 0.5	1.0 ± 0.4	0.38
Cholesterol (mg)	199 (39-696)	181 (84-644)	0.51
Total fiber (g/1000Kcal)	11 ± 4	12 ± 4	0.39

FA: fatty acid; P/S ratio: polyunsaturated/saturated ratio

Table 3. Cox regression analysis in women (dependent variable: death)

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P
N-3 total					
Model I	1.0	0.39 (0.08-2.02)	0.73 (0.1-3.02)	0.31 (0.06-1.62)	0.16
Model II	1.0	0.32 (0.06-1.72)	0.67 (0.15-3.07)	0.07 (0.01-0.78)	**0.03**
Linolenic Acid					
Model I	1.0	0.68 (0.16-2.92)	0.89 (0.21-3.83)	0.50 (0.09-2.61)	0.41
Model II	1.0	0.52 (0.10-2.60)	0.78 (0.17-3.64)	0.10 (0.01-1.11)	0.09

MODEL I: adjusted for age
MODEL II: adjusted for age, ischemic heart disease, glomerular filtration rate, systolic blood pressure, glycated hemoglobin and compliance with WDR.

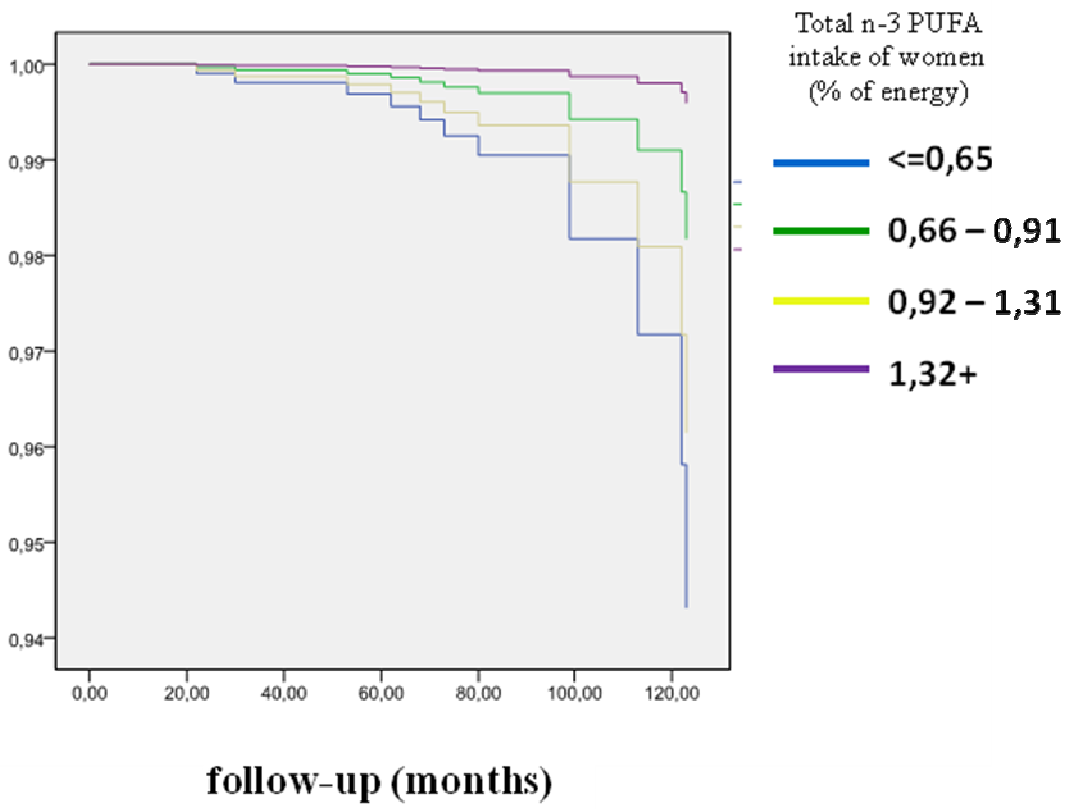

Figure 1. Multivariate Cox regression analysis to evaluate the association between the rate of total mortality (dependent variable) and total n-3 PUFA intake of women with type 2 diabetes. Patients were divided according to the quartiles of intake of this nutrient: ≤ 0.65% of energy = blue line; 0.66-0.91% of energy = green line; 0.92-1.31% of energy = yellow line; and > 1.32% of energy = purple line. The analysis was adjusted for age, ischemic heart disease, glomerular filtration rate, systolic blood pressure, glycated hemoglobin and compliance with WDR.