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Mestrado e Doutorado

Lipídeos dietéticos em pacientes com Diabetes Melito tipo 2: aspectos relacionados à nefropatia diabética e efeitos do polimorfismo Ala54Thr do gene *FABP2*

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Orientadora: Profa Dra Mirela Jobim de Azevedo

Porto Alegre, 16 de Junho de 2008.

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Doutorado

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Esta Tese de Doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da UFRGS, sendo apresentada na forma de três manuscritos sobre o tema da Tese:

1. Artigo de revisão geral sobre o tema, que deverá ser submetido para publicação em periódico científico nacional, conforme as normas do periódico;
2. Artigo original referente ao trabalho de pesquisa propriamente dito que deverá ser submetido para publicação em periódico científico de circulação internacional, conforme as normas do periódico;
3. Artigo original referente ao trabalho de pesquisa propriamente dito que deverá ser submetido para publicação em periódico científico de circulação internacional, conforme as normas do periódico.

Conteúdo

Lista de Abreviaturas

AA:	Ala54 homozygote to *FABP2* gene
ACE:	*Angiotensin-converting enzyme*
ADA:	*American Diabetes Association*
AGMIs:	Ácidos graxos monoinsaturados
AGPIs:	Ácidos graxos poliinsaturados
AGs:	Ácidos graxos
AGSs:	Ácidos graxos saturados
Apo E:	Apolipoproteína E *(apolipoprotein E)*
BMI:	*Body mass index*
DAGs:	Diacilgliceróis
DHA:	Docosahexaenóico
DM:	Diabetes melito *(Diabetes mellitus)*
DN:	*Diabetic nephropathy*
ECR:	Ensaio clínico randomizado
EPA:	Eicosapentaenóico
EUA:	Excreção urinária de albumina
FAs:	*Fatty acids*
HDL:	*High-density lipoprotein*
HSPG:	*Heparin sulfate proteglycane*
IDL:	*Intermediary-density lipoprotein*
I-FABP:	*Intestinal fatty acid binding protein*
LDL:	*Low-density lipoprotein*
LLP:	Lipase lipoprotéica
MACRO:	Macroalbuminúricos
MICRO:	Microalbuminúricos *(microalbuminuric)*
MUFAs:	*Monounsaturated fatty acids*
n3-PUFAs:	*Omega 3 polyunsaturated fatty acids*
n6-PUFAs:	*Omega 6 polyunsaturated fatty acids*
ND:	Nefropatia diabética

NORMO:	Normoalbuminúricos *(normoalbuminuric)*
OR:	*Odds ratio*
PPARγ2:	*Peroxisome proliferators-activated receptor- γ2*
PUFAs:	*Polyunsaturated fatty acids*
RC:	Razão de chances
SFAs:	*Saturated fatty acids*
TAGs:	Triacilgliceróis
TFG:	Taxa de filtração glomerular
TG:	Triglicerídeos
TT:	Thr54 homozygote to *FABP2* gene
UAE:	*Urinary albumin excretion*
VLDL:	*Very low-density lipoprotein*

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Capítulo I

PAPEL DOS LIPÍDEOS DA DIETA NA NEFROPATIA DIABÉTICA*

*Artigo submetido ao periódico *Arquivos Brasileiros de Endocrinologia e Metabologia*

PAPEL DOS LIPÍDEOS DA DIETA NA NEFROPATIA DIABÉTICA

“Role of dietary lipids in diabetic nephropathy”

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Título resumido: Papel dos lipídeos na nefropatia diabética

Resumo:

O objetivo do presente manuscrito foi revisar o possível papel dos lipídios dietéticos na nefropatia diabética (ND), considerando as alterações do perfil lipídico associadas e a interação entre aspectos dietéticos e genéticos. Os lipídios dietéticos podem ter um papel importante no desenvolvimento e na progressão da ND. A composição das gorduras da dieta tem sido associada com a ND, particularmente à microalbuminúria, e às anormalidades lipídicas e de função endotelial. Entretanto, ainda não está comprovado o benefício da modificação da ingestão de gorduras em pacientes com ND, em especial sobre desfechos definitivos como incidência e progressão da ND, insuficiência renal e morte. Além disso, a resposta do perfil lipídico à ingestão de gorduras pode ser influenciada por fatores genéticos. A identificação de polimorfismos genéticos específicos associados a esta interação poderá permitir a individualização de estratégias nutricionais na ND.

DESCRITORES: nefropatia diabética; microalbuminúria; lipídeos da dieta; ácidos graxos; lipídeos séricos; dislipidemia.

Abstract:

The aim of the present manuscript was to review the possible role of dietary lipids in diabetic nephropathy (DN), taking into account associated abnormalities of serum lipids and interaction of dietary and genetic aspects. Dietary lipids may have an important role in the development and progression of DN. The fat diet composition has been associated with DN, particularly with microalbuminuria, serum lipids abnormalities, and endothelial function. However, the beneficial effect of fat intake modification for these patients is not fully established, especially regarding hard outcomes such as DN incidence and progression, kidney failure, and death. Moreover, genetic factors may influence the response of serum lipids to fat intake. The identification of specific genetic polymorphisms associated with this interaction could allow adoption of individual nutritional strategies in DN.

KEYWORDS: diabetic nephropathy; microalbuminuria; dietary lipids; fatty acids; serum lipids; dyslipidemia.

INTRODUÇÃO

A nefropatia diabética (ND) acomete até 40% dos pacientes com diabetes melito (DM) (1-4) e, além de estar associada a uma mortalidade cardiovascular aumentada (5), é considerada a principal causa de insuficiência renal crônica naqueles pacientes que ingressam em programa de tratamento para a substituição renal (6, 7).

A ND é definida pelo aumento da excreção urinária de albumina (EUA) na ausência de outras doenças renais e tem sido classificada em estágios. O estágio denominado de microalbuminúria, ou de nefropatia incipiente, caracteriza-se por valores de EUA de 30 a 299 mg/ 24 h. No estágio de macroalbuminúria, ou nefropatia clínica, os valores correspondentes de EUA são ≥300 mg/ 24 h (4, 7). Já no estágio de microalbuminúria ocorre um aumento de mortalidade, em especial cardiovascular, e um risco aumentado de progressão para insuficiência renal (4).

A "American Diabetes Association" recomenda que para o diagnóstico da ND seja também estimada a taxa de filtração glomerular (TFG), independente dos valores de EUA (8). Alguns pacientes com DM tipo 1 (9) ou tipo 2 (10) podem apresentar diminuição da TFG na presença de valores normais de EUA. Estes pacientes normoalbuminúricos e com TFG <60 ml/ min apresentam mais freqüentemente hipertrigliceridemia e síndrome metabólica quando comparados com aqueles com valores superiores de TFG (11). Deve ser também lembrado que valores elevados de EUA em pacientes com DM tipo 2 podem também estar relacionados à presença de hipertensão arterial, freqüentemente observada nestes pacientes (3). De fato, a microalbuminúria pode estar presente em até 40% de pacientes não diabéticos portadores de hipertensão essencial (12).

A hiperglicemia e o aumento dos níveis de pressão arterial, associados à predisposição genética, são os principais fatores de risco para o desenvolvimento da

ND. A obtenção de um controle glicêmico próximo à normalidade, o tratamento rigoroso da hipertensão arterial, o uso de drogas bloqueadoras do sistema renina-angiotensina-aldosterona, e possivelmente o tratamento da dislipidemia têm sido medidas utilizadas para a prevenção e desaceleração da progressão da ND (4). No entanto, ainda há um número considerável de pacientes que desenvolve ND e sua progressão não é completamente evitada com os tratamentos disponíveis atualmente. Portanto, é necessário que outros fatores associados à ND, como o tipo de dieta, sejam mais bem estudados para otimizar a prevenção e o tratamento desta complicação crônica.

No final da década de 90, estudos observacionais em pacientes com DM sugeriram que a quantidade (13) ou o tipo (14) de proteína estavam associados à microalbuminúria. Também, o conteúdo de gordura (15-19) consumido na dieta usual parece ser um fator de risco para o desenvolvimento e progressão da microalbuminúria. De fato, em pacientes com DM tipo 2 já foi demonstrado que quando é utilizada carne branca em substituição à carne vermelha, a conseqüente modificação do conteúdo de ácidos graxos (AGs) poliinsaturados (AGPIs) e saturados (AGSs) da dieta reduz a EUA e melhora o perfil lipídico (20-22). Postula-se que estes efeitos ocorram devido à alteração dos ácidos graxos séricos ou ainda, pela absorção intestinal modificada de AGs dietéticos. Deve ser lembrado, entretanto, que as alterações nos lipídeos séricos que influenciam o curso clínico da ND, podem ocorrer independente da dieta (23, 24).

A dislipidemia está presente em pacientes com ND desde a fase de microalbuminúria. É possível que estas alterações nos AGs séricos estejam associadas às alterações inflamatórias e de função endotelial, como já descrito em pacientes sem DM (25, 26). Recentemente, em pacientes com DM tipo 2, os AGSs séricos se correlacionaram com os valores de endotelina-1 (27). Reforça esta observação a

associação positiva entre EUA e endotelina-1 em pacientes com DM tipo 2 dislipidêmicos (28) ou não (29). As anormalidades dos lipídeos séricos podem contribuir para a elevada mortalidade cardiovascular observada em pacientes com DM microalbuminúricos. Deve ser ainda considerada a possibilidade de interação entre fatores ambientais dietéticos e genéticos (30, 31) na promoção de condições favoráveis para o desenvolvimento da ND.

O objetivo do presente manuscrito foi revisar o possível papel dos lipídeos dietéticos na ND, considerando as alterações do perfil lipídico e a interação entre aspectos dietéticos e genéticos.

DISLIPIDEMIA E NEFROPATIA DIABÉTICA

A associação entre um perfil lipídico aterogênico e a EUA tem sido observada em pacientes com DM tipo 1 (32, 33) e tipo 2 (23, 24, 34, 35)**.** As associações positivas dos lipídeos séricos com a ND foram descritas em relação aos valores de colesterol total, colesterol LDL ("low-density lipoprotein"), triglicerídeos, colesterol VLDL ("very low-density lipoprotein") e apolipoproteína B, além de alterações no tamanho das partículas de colesterol LDL (24, 32-35). AGs séricos foram também associados à microalbuminúria no DM. Pacientes com DM tipo 2 e microalbuminúria apresentaram um percentual sérico menor de AGPIs e maior de AGSs na fração triglicerídeo, quando comparados aos pacientes normoalbuminúricos (23). É possível que o tipo de anormalidade lipídica seja distinto em diferentes fases da ND. Em pacientes com DM tipo 1, a progressão da ND foi associada com o colesterol não esterificado (forma livre) do colesterol LDL nos pacientes normoalbuminúricos, com os triglicerídeos das VLDL e IDL ("intermediary-density lipoprotein") nos microalbuminúricos e com o menor tamanho das partículas de colesterol LDL nos pacientes macroalbuminúricos (33).

É provável que as alterações lipídicas precedam o desenvolvimento da micro- ou macroalbuminúria, e não o contrário, embora este tópico não esteja completamente esclarecido. Em um estudo onde 133 pacientes com DM tipo 2 foram acompanhados por um período de 5 anos, a presença de microalbuminúria foi um fator de risco para desenvolvimento de hipertrigliceridemia e valores diminuídos de colesterol HDL ("high-density lipoprotein") (35). Entretanto, em fases mais avançadas da ND, os valores elevados de colesterol sérico parecem ser determinantes da perda de função renal em pacientes com DM tipo 1 (36) e DM tipo 2 (37).

Um dos prováveis mecanismos da dislipidemia que contribui para o desenvolvimento e progressão da ND pode estar relacionado ao efeito nefrotóxico dos lipídeos séricos. O aumento da permeabilidade de macromoléculas no mesângio, incluindo lipídeos, levaria à progressão da lesão glomerular inicial para glomerulosclerose (36). Outro aspecto interessante em relação ao mecanismo de ação dos lipídeos – em especial o colesterol – na ND é a semelhança histológica e imunohistoquímica entre a lesão glomerular progressiva e o desenvolvimento de estrias gordurosas no processo de aterosclerose. É possível que a patogênese de ambos os processos seja compartilhada (38).

A resistência à ação da insulina, de uma maneira geral presente nos pacientes com DM tipo 2, está também entre os prováveis mecanismos relacionados às alterações lipídicas séricas em pacientes com ND (35). A redução do efeito da insulina em situações de hiperglicemia favorece a mobilização dos AGs livres do tecido adiposo que por sua vez, estimulam a síntese hepática de apolipoproteína B, principal componente protéico das VLDL. Com um conseqüente aumento da produção destas lipoproteínas que são ricas em triglicerídeos (39). Além disso, a resistência à ação da insulina pode inibir a atividade da enzima lipase lipoprotéica (LLP), responsável pela conversão de

lipoproteínas ricas em triglicerídeos em AGs livres (40). Como conseqüência, os valores de colesterol HDL (39) e a depuração de colesterol VLDL ficam reduzidos (40). A proteína transportadora de ésteres de colesterol (CETP) também é responsável pela elevação dos valores de triglicerídeos séricos e redução dos valotres de colesterol HDL (40). O papel da LLP é reforçado pela observação de que pacientes com DM tipo 2 e microalbuminúria possuem uma redução na atividade desta enzima associada à hipertrigliceridemia e a baixos valores de colesterol HDL (41, 42).

Estes achados sugerem que as anormalidades lipídicas observadas em pacientes com ND precedam o desenvolvimento da própria ND, em especial em sua fase inicial. Os possíveis mecanismos relacionados à dislipidemia na ND incluem a resistência à ação da insulina e a redução da atividade da enzima LLP. As anormalidades lipídicas resultantes teriam efeito nefrotóxico contribuindo para a patogênese da ND.

EFEITOS DOS LIPÍDEOS DA DIETA SOBRE O PERFIL LIPÍDICO SÉRICO

Os efeitos dos lipídeos da dieta sobre o perfil lipídico sérico são atribuídos em especial aos AGs, de acordo com seus graus de saturação. Estes efeitos são distintos e independem da presença do DM. Na Tabela 1 estão resumidos as principais gorduras da dieta, suas respectivas fontes alimentares e seu impacto sobre os lipídeos séricos, componentes inflamatórios e de coagulação. O colesterol, os AGSs e os AGs insaturados *trans-isômeros* provenientes da dieta desempenham um papel importante nas anormalidades lipídicas, principalmente na elevação de colesterol total e colesterol LDL séricos. Os AGs monoinsaturados (AGMIs) por sua vez têm um efeito hipocolesterolêmico ao substituírem os AGSs da dieta. A ingestão de AGPIs, em especial os AGs ômega 3, além de reduzir os valores de colesterol e triglicerídeos séricos, possui efeitos benéficos nos processos inflamatórios, (44), assim como um efeito antitrombogênico (46).

Entre os mecanismos benéficos da modificação do perfil lipídico causada pela quantidade e qualidade de gordura da dieta está a redução da resistência à ação da insulina e da atividade inflamatória. Os AGPIs de cadeia muito longa, principalmente o AG docosahexaenóico (DHA), diminuem a expressão das moléculas de adesão endotelial assim como reduzem as interações entre as células do sistema imunológico e as células do endotélio (48). Também já foi demonstrado que a suplementação oral de eicosapentaenóico (EPA) e DHA diminuiu a produção de citoquinas pró-inflamatórias pelas células mononucleares periféricas (49).

Uma maior proporção de AGs insaturados de cadeia longa e uma menor proporção de AGSs na dieta estão associadas à melhora da ação da insulina (50). Em pacientes obesos com resistência à ação da insulina, os AGSs apresentaram associação positiva com marcadores inflamatórios séricos (interleucina-6, proteína C reativa), enquanto que para os AGPIs, a associação foi negativa (25). Além disto, evidências a partir de revisões sistemáticas mostram que a suplementação dos AGPIs de cadeia muito longa, EPA e DHA, reduziu a mortalidade geral e cardiovascular (51). Em pacientes com DM tipo 2 a suplementação de EPA e DHA (~4,3 g/dia) por cerca de 9 semanas reduziu em cerca de 25% os triglicerídeos séricos e em 38% o colesterol VLDL e aumentou em 25% o fator VII de coagulação quando comparado com placebo (52-54).

A adoção de uma dieta rica em AGMIs, semelhante à adotada em países mediterrâneos, é uma estratégia primária para a redução de mortalidade por todas as causas (55), de risco para doença coronariana (56) e de eventos cardiovasculares (57). Na dieta Mediterrânea há um consumo abundante de vegetais, óleo de oliva (principal fonte de gordura desta dieta) e pequena quantidade de lácteos e carne vermelha. Frango e peixes são as fontes protéicas mais consumidas. Ovos estão presentes no máximo

quatro unidades por semana e o vinho tinto é consumido em quantidade moderada (58). O seguimento de dietas do tipo mediterrânea reduz o colesterol total, colesterol LDL e apolipoproteína B e está associado à melhora da função endotelial (59). Este último efeito pode estar relacionado à oferta aumentada de AGPIs ômega 3 decorrente do consumo de peixes e óleo de canola (com redução da razão AGPIs ômegas 6:3 e menor atividade inflamatória) (60) e à presença de polifenóis antioxidantes oriundos do vinho e do óleo de oliva (61). A presença do AG oléico na dieta Mediterrânea, um AGMI, já foi relacionada à melhora da função endotelial por promover o deslocamento seletivo de AGSs das membranas celulares, efeito de menor magnitude do que o dos AGPIs (62). Uma ação anti-trombogênica da dieta Mediterrânea foi demonstrada em um ensaio clínico de 2 meses de duração em pacientes com DM tipo 2. Quando o AG linoléico (ômega 6) foi substituído pelo AG oléico (AGMI) ocorreu uma redução de resistência à ação da insulina e restauração da vasodilatação endotélio-dependente (63).

Com base nestas observações, a "American Diabetes Association" elaborou recomendações em relação à ingestão de gorduras na dieta para pacientes com DM (64). Resumidamente: limitar o consumo de AGSs em até 7% das calorias ingeridas e de colesterol da dieta em até 200 mg ao dia; consumir o mínimo de AGs insaturados *trans-isômeros* e AGSs e consumir 2 ou mais porções de peixe por semana para fornecer AGPIs ômega 3. Estas recomendações são semelhantes às preconizadas para tratamento de dislipidemia na população em geral (65, 66).

EFEITOS DOS LIPÍDEOS DA DIETA SOBRE A FUNÇÃO RENAL

A ingestão de gorduras, através da modificação dos lipídeos séricos e dos possíveis efeitos destes sobre a função renal (ver tópico anterior neste manuscrito), pode atuar como um fator associado ao desenvolvimento e progressão da ND. Dietas habituais com diferentes composições de gorduras podem representar proteção ou risco

para a ND. Modificações do conteúdo de gordura da dieta, através de substituição de alimentos ou de suas fontes, de nutrientes específicos ou ainda de suplementos já demonstraram efeitos benéficos sobre a albuminúria em pacientes com DM.

O efeito deletério dos AGSs da dieta na função renal pode ser mediado em parte pela conseqüente elevação do colesterol sérico após o aumento de sua ingestão com concomitante redução da ingestão de AGPIs (67). A hipercolesterolemia atuaria como fator de risco para o desenvolvimento da ND (32, 37, 68). Além disto, os AGSs podem também induzir diretamente à disfunção endotelial (25-27) que, por sua vez, está associada à microalbuminúria (28). De fato, a função endotelial é modulada diretamente por fatores dietéticos, especialmente pelos AGPIs ômega 3 (48) que têm potencial efeito benéfico sobre a doença cardiovascular**.** Além disto, a gordura da dieta, tanto o tipo quanto a quantidade, podem influenciar processos inflamatórios (69) e estados de doença a eles relacionados (70).

Na revisão sobre os efeitos da gordura da dieta sobre a função renal em pacientes com ND foram encontrados ensaios clínicos e estudos observacionais selecionados no *Medline* e *Lilacs* (língua portuguesa, inglesa e espanhola), além de publicações específicas da área médica e de nutrição até fevereiro de 2008 (descritores utilizados: *lipid, fat, fatty acid, diet OR dietary AND diabetic nephropathy, microalbuminuria OR renal disease AND diabetes).* Entre os 15 estudos selecionados, cinco estudos foram de associação de lipídeos dietéticos com albuminúria, um estudo foi associação com hiperfiltração glomerular e oito foram ensaios clínicos (Tabelas 2 e 3).

Estudos de associação

Na Tabela 2, em ordem cronológica de publicação, estão descritas as principais características dos estudos observacionais onde foi demonstrada a associação de

lipídeos dietéticos com o desenvolvimento e/ou progressão da ND. A maioria foi realizada em pacientes com DM tipo 1 com normo- e microalbuminúria (15- 17, 71), sendo três estudos transversais, um estudo de casos e controles e dois estudos prospectivos. Apenas dois estudos incluíram pacientes com DM tipo 2 (18, 19), sendo que o estudo prospectivo incluiu pacientes macroalbuminúricos (18). Os instrumentos de avaliação de consumo alimentar utilizados foram: históricos alimentares, recordatórios com ou sem pesagem de alimentos, diários alimentares ou questionários de 13ódon13aca13 alimentar. Somente três estudos avaliaram o impacto da dieta no perfil lipídico sérico dos pacientes (16, 18, 19).

No final da década de 80 foi descrita uma associação positiva de ingestão de gorduras totais com a microalbuminúria (15). Posteriormente, foi também demonstrada uma associação do maior consumo de AGSs com a presença de microalbuminúria (16), em especial com o ácido mirístico (17). Recentemente, a ingestão diminuída de AGPIs foi associada com microalbuminúria (19). Também a ingestão aumentada de AGPIs e diminuída de AGSs foi associada à regressão para estágios menos avançados da ND em um estudo prospectivo (18). A maior ingestão de gorduras totais e de AGSs foi associada à hiperfiltração glomerular (>137 ml. Min^{-1}) (71), sendo o aumento da TFG considerado como um possível fator de risco para a ND (6).

Ensaios clínicos

Na Tabela 3, em ordem cronológica de publicação, estão resumidos oito ensaios clínicos randomizados que avaliaram os efeitos da modificação da ingestão de lipídeos sobre a ND. Além da modificação do tipo de gordura consumida (tipo de óleo para o preparo dos alimentos e coberturas para passar no pão) (72-74), foram incluídos estudos nos quais a manipulação da gordura ocorreu pela substituição do tipo de carne (carne vermelha por carne de galinha) (21-23) ou ainda pela suplementação na dieta usual com

AGPIs ômega 3 (75, 76). O tempo médio de intervenção dietética dos estudos variou de três semanas a 24 meses, sendo que quando o delineamento foi um ensaio clínico randomizado cruzado (20-22) o período de "washout" entre as dietas foi de três a quatro semanas.

Efeitos benéficos sobre a ND com redução da EUA foram demonstrados com a adoção de dieta vegetariana pobre em gordura (73), com substituição de óleos ricos em triacilgliceróis por diacilgliceróis (74) e com substituição da carne vermelha da dieta por carne de galinha (21, 22) e suplementação com AGPIs ômega 3 (75, 76). Com a dieta a base de carne de galinha foi também observado um efeito benéfico sobre a hiperfiltração glomerular (20). Alguns autores demonstraram também uma melhora concomitante no perfil lipídico, tanto em pacientes com microalbuminúria (21, 73) quanto naqueles com macroalbuminúria (22, 74). Em um único estudo, após o acréscimo da ingestão de ácido linoléico (ômega 6), a EUA aumentou em 58%, embora tenha ocorrido uma melhora do perfil lipídio sérico (72). Uma explicação para este resultado não esperado sobre a EUA seria os efeitos distintos dos AGPIs ômega 6 e ômega 3 no organismo, já que os AGs ômega 3 reduzem a EUA (75, 76).

Conclui-se que, de uma maneira geral, a manipulação de gorduras específicas da dieta é capaz de trazer benefícios sobre a EUA, idealmente em associação com uma melhora do perfil lipídico. Entretanto, deve ser lembrado que os estudos que mostram estes efeitos são de curta duração, avaliam um número de paciente relativamente limitado e que não há evidências contundentes de benefícios sobre desfechos clínicos definitivos como a falência renal e/ou morte. Não existem ainda recomendações definitivas para manipulação das gorduras da dieta com vistas à proteção da função renal na ND.

INTERAÇÃO GENÉTICA: GORDURAS DA DIETA E PERFIL LIPÍDICO

A influência de fatores genéticos na presença da ND tem sido demonstrada por vários autores (77), assim como em pacientes brasileiros (78). É provável que a susceptibilidade genética para a presença de valores anormais de albuminúria e de TFG (doença renal terminal) seja distinta (79). Aspectos genéticos relacionados à patogênese da ND podem também estar associados com fatores dietéticos que levem às alterações nos lipídeos séricos. Polimorfismos genéticos explicariam a variabilidade inter-individual da resposta de lipoproteínas aos componentes dietéticos (80, 81), como ocorre com determinados AGs (82).

Para a revisão de artigos relacionados a polimorfismos possivelmente associados à dislipidemia ou lipídeos da dieta na ND foram utilizados o *Medline* e *Lilacs* (língua portuguesa, inglesa e espanhola), além de publicações específicas da área até fevereiro de 2008. Os descritores utilizados foram: *polymorphism AND diabetic nephropathy, microalbuminuria OR renal disease AND diabetes*. Quinze estudos foram selecionados: um sobre o gene que modula a proteína intestinal carreadora de ácidos graxos (I-FABP – "intestinal fatty acid binding protein"), nove estudos sobre as variações do gene da apolipoproteína E (apoE) e quatro sobre o gene que modula o receptor ativado por proliferadores do peroxissoma (PPARγ2 – "peroxisome proliferator-activated receptor-γ2"). Dois estudos foram transversais, 11 de casos e controles e um prospectivo (4,5 anos de acompanhamento). Os estudos foram realizados em diferentes populações, com pacientes com DM tipo 1 (83, 84) e com DM tipo 2 (85-96). A maioria deles observou uma associação positiva com a ND (83, 85, 86, 88, 89, 91, 92). As descrições das características gerais dos polimorfismos genéticos associados aos lipídeos em estudos com pacientes com ND estão resumidas na Tabela 4.

Os seguintes polimorfismos foram associados com a presença e/ou progressão da ND: o Ala54Thr do gene do *FABP2* (91) e o polimorfismo da *apoE* (alelo $\in 2$) (83,

82, 83, 85, 86). Já uma associação negativa, portanto representando uma provável proteção, foi demonstrada para o polimorfismo Ala12Pro do gene do *PPARγ2*, tanto para microalbuminúria (87) quanto para estágios mais avançados de ND (90, 96). No caso do polimorfismo da *apoE*, a associação com a ND ainda é controversa quando o alelo presente for o $\in 4$ (84, 92-94).

Em conclusão, as alterações genéticas parecem modificar os efeitos das gorduras da alimentação sobre o perfil lipídico e, desta forma, influenciar o desenvolvimento e progressão da ND. Entretanto, quase todos os estudos de associação são transversais. São necessários mais estudos de coorte para analisar a evolução da doença renal e dieta. Idealmente deveriam ser realizados ensaios clínicos para avaliar a resposta à ingestão de gordura em pacientes com diferentes genótipos de risco para ND.

COMENTÁRIOS FINAIS

As evidências disponíveis sugerem que os lipídios dietéticos e séricos podem ter um papel importante no desenvolvimento e na progressão da ND. Um perfil lipídico sérico desfavorável (valores aumentados de colesterol total, de triglicerídeos, de apolipoproteína B e de colesterol LDL – além de partículas mais densas – com valores diminuídos de colesterol HDL), incluindo alterações de AGs, provavelmente precede a instalação da ND. Estas alterações lipídicas estão relacionadas à ingestão de gorduras. De fato, a composição dos AGs séricos associa-se à microalbuminúria, disfunção endotelial e a um padrão alimentar rico em AGSs e pobre em AGPIs. Este tipo de dieta provavelmente representa um fator de risco para a ND, além do já estabelecido aumento de risco cardiovascular. Embora as evidências atuais sugiram fortemente que a manipulação de gorduras da dieta é capaz de reduzir a EUA com concomitante melhora do perfil lipídico, não existe até o presente momento uma recomendação específica neste sentido para prevenção ou tratamento de ND.

Finalmente, cabe salientar a existência da interação entre fatores alimentares e genéticos na resposta intra-individual do perfil lipídico à ingestão de gorduras. A identificação de pacientes com risco aumentado para desenvolvimento ou progressão da ND pela presença de polimorfismos genéticos específicos e suas inter-relações permitirá a adoção de estratégias nutricionais individualizadas.

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Tabela 1. Gorduras da dieta, fontes alimentares e seu impacto no organismo.

Gordura da dieta	Fonte alimentar (43)	Impacto no organismo (44)
Colesterol dietético	Produtos de origem animal: vísceras (fígado, coração, cérebro), manteiga, ovos (gema), tortas com creme ou queijo.	↑ Colesterol total, ↑ colesterol LDL, ↑ colesterol HDL séricos.
AG Saturados (AGSs) - *efeito aterogênico em ordem decrescente*		
AG palmítico (C16) AG mirístico (C14) AG láurico (C12)	**Produtos de origem animal**: lácteos integrais, carnes de gado gordas (costelas, cupim, fraldinha, charque, picanha), toucinho e rabo de porco, ovelha. **Produtos de origem vegetal:** óleo de coco, babaçu, dendê, amendoim.	↑ Colesterol total (↓ da atividade do receptor hepático de colesterol LDL leva a ↓ da depuração do colesterol LDL e ↑ do colesterol sérico). Efeito maior do que o efeito resultante da ingestão do próprio colesterol.
AG com ≤ 10 carbonos	Óleo de babaçu e côco (mas possuem AG palmítico também).	Neutro em relação aos lipídeos séricos.
AG Esteárico (C18)	Chocolates[#], carne de cabra.	↑ Colesterol total de pouca magnitude, possivelmente pela rápida conversão a ácido oléico (C18:1ω9) no organismo. [#] *efeito final dependente do conteúdo de compostos fenólicos do cacau (45)*
AG Monoinsaturados		
AG Oléico (C18:1ω9): *- representante mais abundante na dieta*	Óleos de oliva e canola, nozes, amendoim e castanhas.	↓ Colesterol total, ↓ colesterol LDL quando substituem os AGSs na dieta; não altera colesterol HDL.
AG Poliinsaturados (AGPIs)		
Família ω 6:		
AG linoléico (C18:2 ω 6): *- representante mais abundante na dieta; AG essencial.*	Óleos vegetais (girassol, milho, arroz, soja), nozes e sementes.	↓ Colesterol total, ↓ colesterol LDL e HDL quando substituem os AGSs na dieta. Precursores de componentes inflamatórios *.
Família ω -3		
AG linolênico (C18:3 ω 3) *- AG essencial.*	Óleos de soja e canola, linhaça (semente e óleo) e em pequenas quantidades na carne de frango.	Precursor dos demais AGPIs da família ω 3.
AG EPA (C20:5 ω 3) AG DHA (C22:6 ω 3)	Peixes gordurosos (sardinha), óleos de peixe, em algumas carnes e ovos (em pequenas quantidades).	↓ colestero lLDL colesterol total, ↓ triglicerídeos. Precursores de componentes antiinflamatórios* Efeitos benéficos na atividade anti-trombótica**.
AG insaturados *trans* isômeros		
AG C18:1*t* AG C18:2*t*	Margarinas de consistência firme e gorduras vegetais parcialmente hidrogenadas. Em pequenas quantidades em carnes, produtos cárneos e lácteos.	↑ Colesterol total, ↑ relação colesterol LDL/HDL, ↑ triglicerídeos; ↑ Lipoproteína (a).

LDL = "low-density lipoprotein cholesterol"; HDL = "high-density lipoprotein cholesterol"; AG = ácido graxo; EPA = ácido graxo eicosapentaenóico; DHA = ácido graxo docosahexaenóico.(46) ** (44)

Tabela 2. Estudos observacionais: associação de gorduras da dieta com nefropatia diabética

Estudo	Origem	n	Pacientes	Delineamento	Avaliação de dieta	Tipo de gordura (expresso como)	Resultados	
							Efeito na nefropatia diabética	Efeito nos lipídeos séricos
Watts e cols. 1988 (15)	UK	30	DM tipo 1 NORMO/ MICRO (1:1)	Casos e controles	Históricos alimentares 7 dias	Gordura total (g e % de energia)	**MICRO *vs.* NORMO:** ↑ Consumo de gorduras total > nos MICRO: 138,4 ± 11,5 *vs.* 94,4 ± 5,7 g/ dia 44,4 ± 1,3 *vs.* 39,5 ± 1,2 % de energia.	Não avaliado.
Bouhanick e cols. 1995 (71)	França	110	DMtipo 1 Não- proteinúricos	Transversal	Recordatório alimentar 1 dia	Gordura total (g/ kg de peso corporal)	**Associação com hiperfiltração glomerular:** Regressão linear multivariada: r = 0,25 (idade e gordura)	Não avaliado.
RILEY MD e cols. 1998 (16)	Austrália	178	DM tipo 1 NORMO e MICRO	Transversal de base populacional	Questionário de freqüência alimentar 152 itens	AGSs categorizado em quintis (% de energia)	**Associação com Microalbuminúria:** Maior quintil (19,2%) *vs.* Menor quintil (13,1%) Análise multivariada (ajustada colesterol HDL): RC = 4,9 (IC 95% = 1,2 – 20,0).	Colesterol HDL dos MICRO > NORMO: 61,5±16,6 *vs.* 53,4±15,9 mg/dL.
HOLLER H e cols. 1999 (17)	Alemanha	37	DM tipo 1 MICRO	Coorte prospectivo Seguimento=5 anos	Recordatórios alimentares 4-dias bimestrais	Gordura total (g) AGs mirístico, araquidônico e linoléico (mg)	**Associação com Albuminúria:** Positiva: AGS, AG mirístico, AG arquidônico. Negativa: AG linoléico. Regressão múltipla: R^2 = 0,589.	Não avaliado.
Cardenas e cols. GSEDNu 2004 (18)	Espanha	192	99 DM tipo 2 93 DM tipo 1 NORMO, MICRO, MACRO	Coorte de base populacional Seguimento=7 anos	Diários alimentares 7 dias início e final do estudo	AGSs, AGPIs e AGMIs (% de energia)	**Algum grau de regressão de estágio de ND** quando: Ingestão ↑ AGPIs e ↓ AGSs. **Progressão da ND** quando: Ingestão ↓ AGPIs e ↑ AGSs.	↓ Colesterol total e colesterol LDL nos pacientes que regrediram. ↓ TG séricos mais acentuada nos pacientes que regrediram: -12,2 *vs*. -4,6%.
Almeida e cols. 2008 (19)	Brasil	181	DM tipo 2 NORMO e MICRO	Transversal	Recordatórios alimentares com pesagem de alimentos 3 dias	AGPIs, AGPIs ômega 6 Fontes alimentares de gorduras: óleos vegetais e manteiga (Nutrientes: % de energia) (Alimentos: mg/ kg ou g)	Análise multivariada: **Associação positiva com Microalbuminúria** : Manteiga: RC = 1,39 (IC 95% = 1,03-1,86). **Associação negativa com Microalbuminúria**: AGPIs: RC = 0,86 (IC 95% = 0,76-0,96); AGPIs de origem vegetal: RC = 0,87 (IC 95% = 0,79-0,97); AGPIs omega 6: RC = 0,82 (IC 95% = 0,72-0,93); Óleos vegetais: RC = 0,04 (0,01-0,52).	Sem diferença entre os pacientes NORMO e MICRO.

DM = diabetes melito; NORMO = normoalbuminúricos; MICRO = microalbuminúricos; AGSs= ácidos graxos saturados; RC=razão de chances; HDL = "high-density lipoprotein cholesterol"; AGs =ácidos graxos; MACRO = macroalbuminúricos; AGPIs= ácidos graxos poliinsaturados; AGMIs= ácidos graxos monoinsaturados; ND = nefropatia diabética; TG = triglicerídeos.

Tabela 3. Ensaios clínicos: manipulação das gorduras na dieta e efeitos na nefropatia diabética.

Estudo	n	Pacientes	Delineamento e tempo de intervenção	Intervenção	Controle	Resultados	
						Efeito na nefropatia diabética	Efeito no perfil lipídico
Hamazaki e cols. 1990 (75)	26	DM tipo 1 e tipo 2: MICRO	ECR Tempo = 6 meses (cada dieta)	Dieta usual + suplemento de AGPIs ω 3 (1800 mg/dia)	Placebo	↓ 45% na EUA; Sem alteração de glicose, teste A1C e pressão arterial.	Ausente.
Dullaart e cols. 1992 (72)	36	DM tipo 1: EUA de 10 a 200 µg/min	ECR Tempo = 2 anos (cada dieta)	Dieta usual com: AG linoléico (12% energia) e razão AGPIs:AGSs = 1,0	Dieta usual com: AG linoléico (7% energia) e razão AGPIs:AGSs = 0,6	↑ 58% na EUA.	↓ colesterol LDL e apolipoproteína B após intervenção; ↓ colesterol HDL na intervenção e controle.
Pecis e cols. 1994 (20)	15	DM tipo 1 NORMO: 9 normofiltrantes 6 hiperfiltrantes	ECR cruzado Tempo = 3 semanas (cada dieta)	1. Dieta normoprotéica a base de carne branca (galinha e peixe) 2. Dieta hipoprotéica	Dieta usual (carne vermelha)	↓ TFG após dieta normoprotéica a base de carne branca e da dieta hipoprotéica.	Benefício somente nos normofiltrantes: ↓ Colesterol total e HDL na dieta hipoprotéica.
Shimizu e cols. 1995 (76)	45	DM tipo 2: MICRO e MACRO (índice albumina/creatinina em amostra urinária)	ECR Tempo = 12 meses (cada dieta)	Suplemento de AGPIs ω 3 (900 mg/dia de EPA)	Placebo	↓ 58% da albuminúria aos 3 meses e mantém até 12 meses. Sem alteração de teste A1C e pressão arterial.	Ausente.
Gross e cols. 2002 (21)	28	DM tipo 2: 15 NORMO 13 MICRO	ECR cruzado Tempo = 4 semanas (cada dieta)	1. Dieta normoprotéica a base de carne de galinha 2. Dieta hipoprotéica	Dieta usual (carne vermelha)	NORMO: ↓ TFG após a dieta a base de galinha e dieta hipoprotéica. MICRO: Maior ↓ EUA após dieta a base de galinha (46%) quando comparada com dieta hipoprotéica (18%) e dieta usual.	Benefício somente nos MICRO: ↓ apolipoproteína B e colesterol total após dieta a base de galinha e da hipoprotéica.
Barnard e cols. 2006 (73)	99	DM tipo 2: 79 NORMO 20 MICRO	ECR Tempo = 22 semanas (cada dieta)	Dieta vegetariana restrita: 15% proteínas 10% de gordura total: <5% de AGSs e <50 mg de colesterol	Dieta preconizada pela ADA: 15–20% proteínas 7% de AGSs 60–70% de carboidratos e AGMIs <200 mg de colesterol	Maior ↓ EUA após dieta vegetariana restrita (56%) quando comparada com dieta ADA (21%).	↓ colesterol LDL após dieta vegetariana restrita e dieta preconizada pela ADA.
Mello e cols. 2006 (22)	17	DM tipo 2: MACRO	ECR cruzado Tempo = 4 semanas (cada dieta)	1. Dieta hipoprotéica 2. Dieta normoprotéica a base de carne de galinha	Dieta usual (carne vermelha)	↓ 20,6% da EUA após dieta a base de galinha e ↓ 31,4% da EUA após dieta hipoprotéica.	↓ Colesterol não-HDL e ↑ AGPIs séricos após dietas a base de galinha e hipoprotéica. ↓ TG e AG palmítico sérico após dieta a base de galinha.

Yamamoto e cols. 2006 (74)	15	DM tipo 2: MACRO (cretinina sérica 0,48-4,84 mg/dl)	ECR Tempo = 6 meses (cada dieta)	Dieta usual com 10g de óleo rico em DAGs	Dieta usual com 10g de óleo habitual (rico em TAGs)	Menor perda de função renal (manutenção da creatinina sérica) na dieta com DAGs.	↓ TG na dieta com DAGs.

DM = diabete melito; MICRO = microalbuminúricos; ECR=ensaio clínico randomizado; AGPIs= ácidos graxos poliinsaturados; EUA = excreção urinária de albumina; AG = ácido graxo; AGSs = ácidos graxos saturados; LDL = "low-density lipoprotein cholesterol"; HDL = "high-density lipoprotein cholesterol"; NORMO = normoalbuminúricos; TFG = taxa de filtração glomerular; MACRO = macroalbuminúricos; EPA = ácido graxo eicosapentaenóico; ADA = "American Diabetes Association"; TG = triglicerídeos; DAGs = diacilgliceróis; TAGs = triacilgliceróis;

Tabela 4. Características gerais dos polimorfismos genéticos associados a lipídeos em estudos relacionados à nefropatia diabética.

Polimorfismo		Lipoproteína ou receptor relacionado		Efeito do polimorfismo no metabolismo lipídico	Associação na ND	n	Estudo Pacientes
Gene	**Descrição**	**Lipoproteína ou receptor**	**Ação**				
FABP2	Substituição de uma alanina por uma treonina (Ala54Thr) no 35ódon 54 (cromossomo 4): Genótipos: AlaAla, ThrThr, AlaThr Alelo de risco = Thr	Proteína intracelular expressa no intestino (I-FABP)	Carreadora de Ags longos da dieta.	**Pacientes com alelo Thr:** ↑ TG séricos em pacientes DM tipo 2 (97).	Positiva	1042	DM tipo 2, brasileiros Transversal, multicêntrico (91)
ApoE	Variações do gene produzem três alelos: ∈2, ∈3 e ∈4 (cromossomo 19): Genótipos homozigotos: *E2/2, E3/3 e E4/4* Genótipos heterozigotos: *E2/3, E2/4 e E3/4*	Glicoproteína polimórfica, presente nas lipoproteínas	Mediadora do receptor de LDL, atuando no catabolismo das lipoproteínas ricas em TG e no transporte reverso do colesterol.	**Pacientes com alelo ∈2:** ↓ Resposta do colesterol total, colesterol LDL e HDL após manipulação das gorduras da dieta (80).	Possível associação positiva com macroalbuminúria.	167	DM tipo 2, coreanos Casos e controles (85)
					Positiva para macroalbuminúria e diálise.	464	DM tipo 2, tailandeses Casos e controles (86)
					Positiva para progressão dos estágios de ND.	429	DM tipo 2, japoneses Prospectivo de 4,5 anos seguimento (88)
					Positiva para falência renal (hemodiálise).	419	DM tipo 1, americanos Casos e controles (83)
					Risco aumentado de albuminúria: co-herança com polimorfismo do gene HSPG.	298	DM tipo 2, chineses Casos e controles (89)
				Todos os alelos.	Sem associação.	166	DM tipo 1, russos Casos e controles (84)
				Pacientes com alelo ∈4: ↑Resposta do colesterol total, HDL e LDL após manipulação das gorduras da dieta (80).	Positiva para ND.	108	DM tipo 2, turcos Casos e controles (92)
					Negativa para microalbuminúria.	94	DM tipo 2, coreanos Casos e controles (93)
					Negativa para falência renal (hemodiálise).	85	DM tipo 2, chilenos Casos e controles (94)
PPAR γ2	Substituição de uma alanina por prolina na posição 12 (Ala12Pro) no ponto de mutação no exon B da parte NH2 terminal do *PPAR γ2*. Genótipo: *AlaAla; AlaPro; ProPro* Alelo de proteção = *Ala*	Receptor ativado por proliferadores do peroxissoma (PPAR) γ2	Atua na diferenciação de adipócitos; ↓ Desensibilização da ação à insulina pelos Ags séricos livres, fator-α de necrose tumoral e resistina e ↑ação da adiponectina;	**Pacientes com alelo Ala:** ↑ Sensibilidade à ação da insulina (98).	Negativa para macroalbuminúria e falência renal (hemodiálise).	445	DM tipo 2, alemães Casos e controles (87)
					Sem associação. (exon 2 e exon 6)	141	DM tipo 2, turcos Casos e controles (95)
					Negativa para proteínúria e falência renal.	316	DM tipo 2, brasileiros Casos e controles (90)
					Negativa para microalbuminúria.	159	DM tipo 2, aborígenes canadenses Transversal (96)

ND = nefropatia diabética; I-FABP = "intestinal fatty acid binding protein"; AG = ácidos graxos; TG = triglicerídeos; DM = diabete melito; ApoE = apolipoproteína E; LDL = "low-density lipoprotein cholesterol"; HDL = "high-density lipoprotein cholesterol"; HSPG = "heparan sulfate proteoglycane"; PPAR = "peroxisome proliferator-activated receptor";

Capítulo II

Sources of protein and polyunsaturated fatty acids of the diet and microalbuminuria in type 2 diabetes mellitus*

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Sources of protein and polyunsaturated fatty acids of the diet and microalbuminuria in type 2 diabetes mellitus

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ABSTRACT

Background: Albuminuria excretion rate above the reference range and below albustix positive proteinuria (20 – 199 μg/min) is known as microalbuminuria and has been associated with an increased risk of death and progression to renal failure. Besides hyperglicemia and high blood pressure levels, dietary factors can also influence albuminuria.

Objective: To evaluate possible associations of dietary components (macronutrients and selected foods) with microalbuminuria in type 2 diabetic patients.

Methods: In this cross-sectional study, 119 normoalbuminuric [NORMO; 24-h urinary albumin excretion (UAE) <20 μg/min; immunoturbidimetry] and 62 microalbuminuric (MICRO; UAE 20–199 μg/min) type 2 diabetic patients, attending the Endocrine Division, Hospital de Clínicas de Porto Alegre (Brazil), without previous dietary counseling, underwent 3-day weighed-diet records, and clinical and laboratory evaluation.

Results: MICRO patients consumed more protein (20.5 ± 4.4 *vs.* 19.0 ± 3.5 % of total energy; $p = 0.01$) with a higher intake from animal sources (14.5 ± 4.7 *vs.* 12.9 ± 3.8 % of total energy; $p = 0.015$) than NORMO patients. The intakes of polyunsaturated fatty acids (PUFAs; 8.6 ± 2.9 *vs.* 9.7 ± 3.3 % of total energy; $p < 0.03$), PUFAs from vegetable sources (7.3 ± 3.4 *vs.* 8.6 ± 3.7 % of total energy; $p = 0.029$), plant oils [0.2 (0.1-0.6) *vs.* 0.3 (0.1-0.9) mg/kg weight; $p = 0.02$] and margarines [3.3 (0-75.7) *vs.* 7.0 (0-51.7) g/day; $p = 0.01$] were lower in MICRO than in NORMO. In multivariate logistic regression models, adjusted for age, gender, presence of hypertension and fasting plasma glucose, intake of total protein (% of total energy; OR 1.104; 95% CI 1.008-1.208; $p = 0.032$) was positively associated with microalbuminuria. The intakes of total PUFAs (% of total energy; OR 0.855; 95% CI 0.762-0.961; $p = 0.008$), PUFAs

from vegetable sources (% of total energy; OR 0.874; 95%CI 0.787-0.971; p = 0.012) and plant oils (mg/kg weight; OR 0.036; 95%CI 0.003-0.522; p = 0.015) were negatively associated with microalbuminuria.

Conclusions: In type 2 diabetic patients, the high intake of protein and the low intake of PUFAs, particularly from plant oils, were associated with the presence of microalbuminuria. Reducing protein intake from animal sources and increasing the intake of lipids from vegetable origin might-reduce the risk of microalbuminuria.

KEY WORDS: dietary intake; type 2 diabetes mellitus; microalbuminuria; protein intake; polyunsaturated fatty acids; plant oils

INTRODUCTION

Diabetic nephropathy (DN) is the leading cause of kidney disease in patients starting renal replacement therapy and affects up to 40% of diabetic patients [1]. DN is defined by increased urinary albumin excretion (UAE) in the absence of other renal diseases, and has been categorized as either microalbuminuria or incipient nephropathy (UAE 20 to 199 μg/min) and macroalbuminuria or clinical nephropathy (UAE ≥200 μg/min). Microalbuminuria has been associated with increased risk of death, mainly from cardiovascular causes, and progression to renal failure [1]. Hyperglycemia [2], increased blood pressure levels [3], and genetic predisposition [4, 5] are the main known risk factors for the development of DN. Dietary intakes also appear to play a role as a risk factor for DN.

The effect of specific dietary changes on renal function has been evaluated. A low protein diet (about 0.6 g/kg of body weight per day) can slow the increase of UAE and also the decline of the glomerular filtration rate in clinical nephropathy in patients with type 1 diabetes [6, 7]. However, this effect was not confirmed in a long–term study in microalbuminuric type 2 diabetic patients [8]. In normoalbuminuric type 1 diabetic patients, the reduction of protein intake [9], or the replacement of red meat from the usual diet by fish or chicken without protein restriction [10], could also reduce glomerular hyperfiltration, a putative risk factor for DN. The mechanisms behind the beneficial effect related to a low protein diet on renal function have not been fully elucidated, but there is evidence that improvements in glomerular hemodynamics and/or lipid profile are probably related [1]. Recently, decreases of low-grade inflammation indexes have been postulated as a potential mechanism of albuminuria reduction after a low protein diet [11]. Also, the replacement of red meat with chicken in the usual diet reduced UAE and improved the lipid profile in micro- and macroalbuminuric type 2

diabetic patients [12, 13]. The nutrients specifically related to renal effects of these dietary interventions are not clearly defined.

Data from observational studies suggested that in patients with diabetes, not only the amount [14] or source [15] of protein, but also the fat content [16, 17, 18] in the usual diet could be risk factors for the development and progression of DN. Total fat intake seems to be associated with glomerular hyperfiltration in nonproteinuric patients with type 1 diabetes [19]. There are no data about the role of specific foods or groups of foods on the development of DN in type 2 diabetic patients. Furthermore, the potential associations of DN with the intake of different sources of protein or fat were not studied in these patients. Therefore, the aim of this study was to evaluate the possible association of dietary components, macronutrients and selected foods from the usual diet, with microalbuminuria in type 2 diabetic patients.

SUBJECTS AND METHODS

Patients

This cross-sectional study was conducted in patients with type 2 diabetes mellitus defined as patients over 30 years of age at onset of diabetes mellitus, no previous episode of ketoacidosis or documented ketonuria, and treatment with insulin only after 5 years of diagnosis.

Patients consecutively attending the outpatient clinic of the Endocrine Division at Hospital de Clínicas de Porto Alegre (Porto Alegre, Brazil) were prospectively selected based on the following criteria: no dietary counseling by a registered dietitian during the previous 12 months, age <80 years, BMI <35 kg/m^2, UAE <200 μg/min, normal liver and thyroid function tests, and absence of urinary tract infection or other renal disease and cardiac failure. The recruitment process occurred during the period of March 2006 to April 2007.

Among 201 eligible patients, 20 patients were unable to complete diet records and/or had time constraints. Therefore, 181 patients were included and underwent a clinical and laboratory evaluation. Sitting blood pressure was measured twice to the nearest 2 mmHg, after a 10-min rest, using a standard mercury sphygmomanometer (phases I and V of Korotkoff). Hypertension was defined as blood pressure >140/90 mmHg measured on two occasions or use of antihypertensive drugs [20]. The frequency of exercise, according to activities during a typical day, was graded into four levels based on the following sentences*:* 1. "I read, watch television, and work in the household at tasks that don't strain me physically"; 2. "I walk, cycle, or exercise lightly in other ways at least four hours per week"; 3. "I exercise to maintain my physical condition by running, jogging, doing gymnastics, swimming, playing ball games, etc, for at least 3 hours per week"; 4. "I exercise competitively several times a week by running, orienteering, playing ball games, or engaging in others sports involving heavy exertion" [21]. Patients were self-identified as white or non-white (mixed or black). According to a random spot urine sample or 24-h timed urine collection, patients were classified as normoalbuminuric (NORMO: UAE <17 mg/L or <20 μg/min), or microalbuminuric (MICRO: UAE 17-174 mg/L or 20-199 μg/min). The diagnosis of microalbuminuria was always confirmed by a 24-h timed urine sample collected over a 3- to 6- month period [1] prior to the recruitment process.

The Ethics Committee at Hospital de Clínicas approved the protocol and participants gave their written informed consent before entering the study.

Dietary Assessment

The patient's usual diet was assessed by means of 3-day weighed-diet record technique (two non-consecutive weekdays and one-weekend day). Patients were issued commercial scales (1-125 g; H.R. Deutschendorf & Cia. Ltda, Brazil) and measuring

cups (25-250 mL; Marinex, Brazil) and a detailed explanation and demonstration was given to each subject by the dietitian. Patients carried out a one-day training period on the weighed-diet record technique before starting the protocol.

Compliance with the weight-record technique, besides an interview with the dietitian was assessed by comparison of protein intake estimated from the mean value of the 3-day weighed-diet records and from the 24-h urinary nitrogen output, performed on the third day of the weighed-diet record period [22]. In a subset of patients (62 NORMO and 26 MICRO) who did not use hypolipidemic agents, compliance was also evaluated by correlations between the intake of polyunsaturated fatty acids (PUFAs) with total serum PUFAs and the intake of linoleic fatty acid with serum linoleic fatty acid [23]. Serum fatty acids were measured at the end of the 3-day weighed-diet record period.

Dietary nutrients from diet records were analyzed using the Nutribase 98 Clinical Nutritional Manager software v.1.0 (Cybersoft Phoenix, AZ). Nutrient data on frequently consumed foods were updated if necessary [24] and/or complemented with data obtained from local manufacturers of specific industrialized foods. Data from dietary components in the diet were expressed as crude intake (g/day; mg/day), percentage of total energy intake, and for animal and vegetable sources of protein or lipids, as percentage of total intake of protein and lipids, respectively. The intakes of protein and food groups were expressed in g per kg of body weight, in order to allow a simple comparison with routine dietary counseling. The *trans* fatty acids food composition was derived from “Tabela de Composição dos Alimentos – TACO” [25], U.S. Department of Agriculture [26], Slover et al. [27], and the TRANSFAIR Study [28]. The total, soluble and insoluble dietary fiber content was estimated according to the data provided in the CRC Handbook of Dietary Fiber in Human Nutrition [29].

In the description of selected foods, dairy products included milk, yoghurt and cheese. The term “all types of meats” indicated red and white meat, sausages and their products. Red meat included beef and pork with their processed products, and lamb. White meat included fish, shellfish and its products, and poultry (chicken and turkey) and their processed products. The type and amount of plant oil daily used for cooking and salad dressings were recorded as ml per day and mg per kg of body weight. The oil intake took into account the total amount of oil consumed by daily table participants at each shared meal time. Total margarine intake was analyzed separately, as was butter intake. Foods were also classified according to their carbohydrate content (%) of crude weight: vegetables from group A (5%), vegetables from group B (10%), and vegetables and foods from group C (20%).

Anthropometric Assessment

The body weight and height of patients (without shoes or coats) were obtained with measurements recorded to the nearest 100 g for weight and to the nearest 0.1 cm for height. BMI (kg/m^2) was then calculated. Waist circumference was measured midway between the lowest rib margin and the iliac crest, near the umbilicus measured once to the nearest 1 mm. Flexible, non-stretch fiberglass tape was used for these measurements.

Laboratory Measurements

UAE was measured in sterile urine samples by immunoturbidimetry [MicroAlb Sera-Pak® immuno microalbuminuria; Bayer, Tarrytown, NY on Cobas Mira Plus (Roche®); mean intra-assay and interassay CVs of 4.5 and 7.6%, respectively]. Urinary urea was measured by an enzymatic ultraviolet method (mean intra-assay coefficient of variation 3.8%).

Blood samples were obtained after a 12-h overnight fast. Plasma glucose was determined by a glucose oxidase method, serum and urinary creatinine level by Jaffé´s reaction and the A1C test by ion-exchange high-performance liquid chromatography (Merck-Hitachi L-9100 glycated hemoglobin analyzer; reference range 4.7-6.0%; Merck, Darmstadt, Germany). Serum total cholesterol and triglycerides were measured by enzymatic-colorimetric methods (Merck Diagnostica, Darmstadt, Germany; Boeringher Mannheim, Buenos Aires, Argentina), HDL cholesterol by homogeneous direct method (autoanalyzer, ADVIA 1650). LDL cholesterol was calculated using the Friedewald equation only for patients with triglycerides ≤400 mg/dl. One MICRO patient had triglycerides of 409 mg/dl and one NORMO patient had triglycerides of 421 mg/dl.

Serum samples for lipid analysis were separated after centrifugation and stored at -70ºC for later laboratory measurements. The total PUFA and linoleic fatty acid were determined in serum total lipids by gas chromatography (60 m fused silica capillary column with an internal diameter of 0.20 μm; CP-Sil 88; Hawlett-Packard 6890), as previously described [13, 30]. Fatty acid results were expressed as the percent of total fatty acids.

Statistical Analysis

Variables were compared by Student's *t* test, Mann-Whitney *U* test, Pearson Chi Square, as appropriate. The mean daily intake of nutrients obtained from 3-d weighed record was used in all statistical analyses. Correlations were analyzed by the Spearman or Pearson coefficients as appropriate and agreement between estimated protein intakes was established by the Bland & Altman graphical method [31]. Multivariate logistic regression analyses were performed with microalbuminuria as the dependent variable and nutrients or selected foods as 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. Results were expressed as medians (range) or mean ± SD, unless otherwise stated. *P* values <0.05 were considered to be statistically significant. SPSS 14.0 (SPSS®, Chicago, IL) was used for the analyses.

RESULTS

Patients

The main clinical and laboratory characteristics of NORMO and MICRO patients are shown in Table 1. MICRO patients were younger (p = 0.049) and had hypertension more frequently (p = 0.007), although blood pressure levels were not different, and they used Angiotensin-Converting Enzyme (ACE) inhibitors more commonly as compared to NORMO patients. Regarding other medicines, the use of oral agents to treat diabetes was less frequent in MICRO than NORMO patients. Proportion of patients using hypolipidemic agents was not different between the groups. Statins (90% of patients) and fibrates were the used drugs. Other evaluated variables were not different between the two groups.

Compliance with the 3-day Weighed Diet Record Technique

The total protein intake (g/kg weight/day) estimated by nitrogen output and by the 3-day weighed diet records was not different (n = 181; 1.2 ± 0.3 *vs.* 1.2 ± 0.4; p = 0.688). The coefficient of correlation ® between these two protein intakes estimates was 0.546 (p <0.0001), but the value was 0.640 (p <0.0001) if this correlation was calculated on the same day as the 24-h urine collection. The agreement between estimated protein intakes using the nitrogen output method and 3-day weighed diet records evaluated by Bland & Altman graphics [31] was -0.01 (-0.64; 0.62) g/kg of body weight. Respective

values for protein intake using the nitrogen output method and one-day weighed diet record method were -0.05 (-0.63; 0.52) g/kg of body weight.

In a subset of 88 patients (62 NORMO and 26 MICRO) coefficient of correlation between the intake of total PUFAs evaluated as % of total or g per day and serum total PUFAs were: 0.271 (% of total energy *vs.* % of total serum fatty acids; p = 0.011) and 0.386 (g per day *vs.* % of total serum fatty acids; p <0.001). Corresponding values of correlation coefficients between the intake and serum linoleic fatty acid were 0.301 (% of total energy *vs*. % of total serum fatty acids; p = 0.004) and 0.379 (g per day *vs.* % of total serum fatty acids; p <0.001).

Diet Characteristics

Mean daily intake of nutrients of type 2 diabetic patients based on 3-day weighed diet records is described in Table 2. MICRO patients consumed more proteins (% of total energy; p = 0.010) than NORMO patients, especially from animal sources (p = 0.015). The total energy and the intakes of carbohydrate, total lipids, cholesterol and fibers were not different between the two groups. MICRO patients tended to have a lower intake from vegetable lipids as compared to NORMO patients, but conventional statistical significance was not reached.

Table 3 shows data from the intake of different sources of animal protein. The intake of protein from red meat was higher in MICRO as compared to NORMO patients (p = 0.045), without a difference in other sources of proteins.

The intake of fatty acids is shown in Figure 1. PUFAs intake (8.6 ± 2.9 *vs.* 9.7 ± 3.3 % of total energy; p = 0.028) and n6-PUFA intake (7.0 ± 2.9 *vs.* 8.6 ± 3.2 % of total energy; p = 0.002) were lower in MICRO than in NORMO patients. The low PUFA-to-saturated fatty acids ratio observed in MICRO patients had a borderline significance (0.95 ± 0.39 vs. 1.08 ± 0.45; p = 0.068). The mean daily intake of saturated (9.7 ± 2.7

vs. 9.7 ± 2.7 % of total energy), *trans* (1.2 ± 0.6 *vs.* 1.3 ± 0.8 % of total energy), n3-PUFAs (0.1 ± 0.3 *vs.* 0.1 ± 0.3 % of total energy), and monounsaturated fatty acids (11.9 ± 2.9 *vs.* 11.3 ± 2.7 % of total energy) was not different between MICRO and NORMO patients (p >0.10). Vegetables represent the main source of PUFAs intake in MICRO (86.1%) and in NORMO (87.1%) patients, without difference between the two groups of patients (p = 0.968). However, MICRO patients consumed less PUFAs (% of total energy) from vegetable sources (7.3 ± 3.4) than NORMO patients (8.6 ± 3.7; p = 0.029), without difference regarding PUFAs from animal sources (1.3 ± 0.6 *vs.* 1.2 ± 0.8; p = 0.237).

The daily intake of selected groups of foods from animal and from vegetable sources is shown in Table 4. The intake of all types of meat (g/day) was higher in MICRO than in NORMO patients (p = 0.007) without any difference between red and white meat intakes. Data from poultry and fish meat were grouped as white meat since only 16.6% of patients consume fish. Also, no difference was observed in the fish intake between NORMO and MICRO patients [6.8 ± 18.3; 0(0-103.3) *vs.* 9.9 ± 24.5; 0(0-108.3) g per day; p = 0.467] as well as intake of poultry [51.0 ± 55.8; 35(0-293.3) *vs.* 58.7 ± 58; 40(0-293.3); p = 0.310]. Eggs were consumed by 22.1%_of patients, without a difference between MICRO [4.5 ± 11.4; 0(0-50) g/day] and NORMO patients [3.3 ± 7.9; 0(0-40) g/day; p = 0.883]. Daily plant oils intake (mg/kg weight; p = 0.020) and margarines (g/day; p = 0.014) were lower in MICRO as compared to NORMO patients. The intake of butter was higher in MICRO [0.8 ± 2.8; 0(0-16.7) g/day] as compared to NORMO patients [0.1 ± 0.9; 0(0-6.7) g/day; p = 0.024].

The intakes of dairy products, legumes and beans, vegetables from groups A and B, and foods from group C, and fruits were not different between MICRO and NORMO patients (Table 4). Regarding dairy products, when the intake of milk, yoghurt, and

cheese was evaluated separately, no differences were observed between NORMO and MICRO patients (data not shown). The most frequent types of plant oil consumed were soy (53%) and sunflower (12.2%). The frequencies of intake of other plant oils were: 7.2%, corn; 2.8%, rice; 1.1%, canola, and 0.6% olive. Forty-two patients (23.2%) had a mixed consume of oils. There was no difference in the frequency of types of oils between NORMO and MICRO patients (p = 0.555). The frequencies of intake of different types of margarines were: 42.0%, soy; 22.7%, halvarines (soft margarines with 40% lipids); 3.3%, corn, and 1.7%, mixed types. Fifty-four patients (29.8%) did not consume margarines. There was no difference in the frequency of margarine types between NORMO and MICRO patients (p = 0.477).

The association of daily intake of different nutrients and selected group of foods with the presence of microalbuminuria in multivariate logistic regression models is shown in Table 5. In this table each line corresponds to one multivariate logistic regression model, and in each model, nutrients or foods were included one at a time, as continuous variables (without categorization). Constructed logistic regression models were adjusted for age, gender, presence of hypertension, and fasting plasma glucose. The total protein consumed (% total energy intake; OR 1.104; 95%CI 1.008-1.208; p = 0.032) and butter intake (g per day; OR 1.386; 95%CI 1.033-1.860; p = 0.030) was positively associated with the presence of microalbuminuria. The intakes of total PUFAs (% total energy intake; OR 0.855; 95%CI 0.762-0.961; p = 0.008), PUFAs from vegetable sources (% total energy intake; OR 0.874; 95%CI 0.787-0.971; p = 0.012), n6-PUFA (% total energy intake; OR 0.818; 95%CI 0.723-0.926; p = 0.002), and plant oils (mg/kg weight; OR 0.036; 95%CI 0.003-0.522; p = 0.015) were negatively associated with microalbuminuria. The inclusion of frequency of exercise in these regression models did not change the results. We also constructed multiple linear

regression models with UAE-log transformed and the same independent variables used in the logistic regression models. The association of protein, PUFA, n6-PUFA, butter, and plant oils with UAE-log was maintained (data not shown).

DISCUSSION

In this sample of type 2 diabetic patients the protein intake (% of total energy intake) was higher in MICRO than in NORMO patients. This higher protein intake was probably related to the intake of protein from animal origin. A positive association of protein intake with UAE has already been described for type 1 diabetic patients [14]. In addition, the source of the protein might have different effects in renal function. The intake of fish protein seems to have a renoprotective effect reducing the increased glomerular filtration rate [10], and on the presence of microalbuminuria [15]. The reduction of glomerular filtration rate was probably not related to different protein content of meats, since this effect was not associated with changes in plasma amino acids [10]. In type 2 diabetic patients there are very few data about association of intake of protein and UAE. Restriction of protein reduced the UAE in patients with microalbuminuria. [8, 12, 32, 33]. More interestingly, the change of the protein source with no alteration of total protein intake was equally effective in improving renal function. We have already described that replacing red meat with chicken in the usual diet reduced UAE in micro- and macroalbuminuric patients with type 2 diabetes [12, 13]. It is well known that chicken meat has a higher PUFAs content than red meat. The beneficial effect of chicken meat in UAE probably occurs due to concomitant rise in serum PUFAs [13]. These observations suggest that the fatty acid content of the protein food source rather than its amino acid composition may mediate the renal effect of the consumed protein. In fact, the results of the present study reinforce our previous hypothesis, since it was demonstrated a protective role of PUFAs for the presence of

microalbuminuira. We probably did not find a protective role of chicken meat because in the present study only 5% of patients (n = 9) consumed only chicken meat.

In the present study the observation that the intake of PUFAs, mainly from vegetable sources (n6-PUFA), was lower in MICRO as compared to NORMO patients is consistent with the hypothesis that a high consumption of PUFAs may have a protective role for microalbuminuria. This effect was possibly related to beneficial effect of PUFAs on endothelial function improving glomerular selectivity [34]. In this study, the role of the origin of PUFAs was demonstrated in a multivariate logistic model where PUFAs from vegetable sources and n6-PUFA, but not from animal sources, was associated with microalbuminuria. In addition, in an observational study [18], diabetic patients who had a regression of any diabetic nephropathy stage consumed more PUFAs and less saturated fatty acids than those who had progression. Actually, microalbuminuric type 2 diabetic patients had a low proportion of serum PUFAs, especially n6-PUFA, in triglyceride fraction [30].

Although only seven percent of type 2 diabetic patients in the present sample mentioned consuming butter, whose lipid content is mostly SFA, a positive association of butter intake with microalbuminuria was demonstrated. The role of SFA intake as a risk factor for DN was previously described by other authors [17, 18]. This possibility was reinforced by recent observation of a positive correlation between serum SFA and serum endothelin-1 [35]. In addition, endothelin-1 (a marker of endothelial dysfunction) was also positively associated with urinary albumin levels [36].

The intake of patients included in the present study is representative of their usual consumption since they did not receive expert dietary counseling in the previous 12 months. In fact, even NORMO patients did not follow all current dietary recommendations for diabetes mellitus [37], especially regarding SFA intake. The

intake of PUFA was adequate in NORMO and MICRO patients. However, a low intake of this type of fatty acids, especially n6-PUFA, was associated with the presence of microalbuminuria. This observation emphasizes the probable role of low dietary intake of PUFA in the development of microalbuminuria.

A possible limitation of this study could be related to dietary data records, since most observational studies are limited due to the lack of accuracy for quantitative data. In the present study dietary daily intake was assessed by a 3-day weighed-diet record technique that was previously standardized in patients with type 2 diabetes [22] and has been used to ultimately confirm general dietary compliance [12, 13, 22, 30]. The accuracy of the recorded dietary intake was confirmed by significant correlations between protein intake as evaluated by weighed diet records and 24-h urinary nitrogen output, and between PUFAs intake and its serum measurements. Another limitation of this study, as expected, could be attributed to its cross-sectional design that doesn't permit a causal relationship between the intake of fatty acids and microalbuminuria.

There is no strong evidence on dietary advice to reduce the risk of developing DN. The American Diabetes Association [37] recommends moderate reduction of protein intake, in the early stages or after established DN. Based on the present study, we could consider that reducing animal protein intake, and consuming rich or PUFAs-enriched foods might be recommended to type 2 diabetic patients to prevent microalbuminuria. However, prospective clinical trials should be performed to confirm this suggestion.

In conclusion, the high intake of protein and the low intake of PUFAs, particularly from plant oils, were associated with the presence of microalbuminuria.

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ABBREVIATIONS: DN = Diabetic Nephropathy; UAE = Urinary Albumin Excretion; NORMO = Normoalbuminuric; MICRO = Microalbuminuric; PUFAs = Polyunsaturated Fatty Acids.

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Table 1 – Clinical and laboratory characteristics of type 2 diabetic patients

	NORMO	MICRO	*p*
N	119	62	-
Age (years)	60.9 ± 9.7	57.7 ± 10.7	0.049[1]
Gender (female)	69 (58.0 %)	27 (43.5 %)	0.065[2]
Ethnicity: white	107 (89.9 %)	54 (87.1 %)	0.566[2]
Diabetes duration (years)	12.7 ± 7.8	12.1 ± 8.6	0.668[2]
Hypertension	87 (73.1 %)	56 (90.3 %)	0.007[2]
Systolic blood pressure (mmHg)	140 ± 23	145 ± 18	0.155[1]
Diastolic blood pressure (mmHg)	83 ± 10	83 ± 11	0.769[1]
Current smoking (self-reported)	18 (15.1 %)	9 (14.5 %)	0.913[2]
Frequency of exercise: level 1	58 (50.0 %)	21 (36.8 %)	0.108[2]
Body weight (kg)	73.9 ± 12.7	76.7 ± 12.9	0.166[1]
BMI (kg/m²)	28.3 ± 4.2	28.6 ± 3.6	0.602[1]
Waist circumference (cm)			
Female	99.9 ± 10.2	102.5 ± 9.7	0.246[1]
Male	98.7 ± 11.6	100.3 ± 8.9	0.520[1]
Diabetes treatment			
Diet only	3.4 %	9.7 %	0.078[2]
Oral agents	66.4 %	48.4 %	0.019[2]
Insulin or insulin plus oral agents	30.3 %	41.9 %	0.116[2]
Angiotensin-Converting Enzyme inhibitors	56 (47.1 %)	39 (62.9 %)	0.043[2]
Hypolipidemic agents	21 (17.6 %)	9 (13.5 %)	0.715[2]
Fasting plasma glucose (mg/dL)	146.5 ± 47.9	159. 9 ± 62.7	0.112[1]
A_{1C} test (%)	7.5 ± 1.5	7.6 ± 1.7	0.832[1]
Total cholesterol (mg/dL)	211 ± 40	209 ± 39	0.675[1]
HDL cholesterol (mg/dL)	50 ± 11	48 ± 12	0.144[1]
LDL cholesterol (mg/dL) *	131 ± 35	132 ± 37	0.964[1]
Triglycerides (mg/dL)	134 (40-421)	133 (40-409)	0.726[3]
Serum creatinine (mg/dL)	0.86 ± 0.16	0.86 ± 0.20	0.831[1]
24-h UAE (μg/min)	3.5 (2.6-18.9)	60.4 (20-180.6)	-

Data are means ± SD, median (range) or number of patients (%) with analyzed characteristics.
NORMO = normoalbuminuric patients; MICRO = microalbuminuric patients.
UAE = urinary albumin excretion.
[1]Student's *t* test; [2] Pearson Chi Square; [3] Mann-Whitney U test.

0. LDL was not calculated in 2 patients who had triglycerides >400 mg/dL.

Table 2. Mean daily intake of nutrients of type 2 diabetic patients

Nutrient	NORMO	MICRO	*p*
N	119	62	
Total energy (kcal)	1797 ± 503	1801 ± 505	0.957[1]
Carbohydrates			
Crude intake (g)	212.6 ± 65.2	206.9 ± 65.8	0.583[1]
Total energy intake (%)	47.5 ± 6.6	46.3 ± 7.4	0.261[1]
Protein			
Crude intake (g)	85.3 ± 28.1	90.7 ± 27.7	0.218[1]
Total energy intake (%)	19.0 ± 3.5	20.5 ± 4.4	0.010[1]
from animal sources (% of total energy)	12.9 ± 3.8	14.5 ± 4.7	0.015[1]
from vegetable sources (% of total energy)	6.4 ± 1.8	5.9 ± 1.4	0.083[1]
Intake (g/kg weight)	1.17 ± 0.36	1.20 ± 0.33	0.592[1]
Lipids			
Crude intake (g)	67.5 ± 24.1	66.6 ± 24.8	0.803[1]
Total energy intake (%)	33.5 ± 6.7	33.2 ± 6.8	0.775[1]
from animal sources (% of total energy)	13.8 ± 5.5	15.1 ± 5.8	0.140[1]
from vegetable sources (% of total energy)	19.6 ± 6.2	17.9 ± 6.8	0.092[1]
Cholesterol (mg)	207.9 ± 105.3	230.7 ± 92.4	0.151[1]
Fiber			
Crude intake (g)	17.4 ± 6.8	16. 5 ± 7.4	0.379[1]
Soluble fiber (g)	5. 6 ± 2.2	5.2 ± 2.4	0.333[1]
Insoluble fiber (g)	12.0 ± 4.9	11.3 ± 5.3	0.360[1]

Data are expressed as mean ± SD.
NORMO = normoalbuminuric patients; MICRO = microalbuminuric patients.
[1] Student's *t* test.

Table 3. Mean daily intake of proteins from animal sources of type 2 diabetic patients

Protein (% of total energy)	**NORMO**	**MICRO**	***p***
N	119	62	
From red meat	5.6 (0-17.3) [6.1 ± 3.9]	6.9 (0-29.9) [7.5 ± 4.9]	0.045[1]
From chicken meat	2.4 (0-20.3) [3.0 ± 3.2]	3.0 (0-12.6) [3.5 ± 3.2]	0.206[1]
From fish meat	0 (0-5.6) [0.4 ± 1.0]	0 (0-6.0) [0.5 ± 1.1]	0.359[1]
From dairy products	3.1 (0-15.5) [3.5 ± 2.5]	2.5 (0-8.3) [3.0 ± 2.0]	0.226[1]
From milk	1.7 (0-6.7) [1.8 ± 1.4]	1.6 (0-4.9) [1.7 ± 1.5]	0.226[1]
From animal sources excluding fish	12.2 (2.8-27.8) [12.5 ± 3.9]	13.1 (7.2-36.1) [14.0 ± 4.9]	0.028[1]

Data are expressed as median (range) and [mean ± SD].
NORMO = normoalbuminuric patients; MICRO = microalbuminuric patients.
Red meat = beef and pork with their processed products, and lamb; chicken meat = poultry (chicken and turkey) and their processed products; fish meat = fish, shellfish and its products; dairy products = milk, yoghurt and cheese.
[1] Mann-Whitney U test.

○ NORMO patients
• MICRO patients

Table 4. Daily intake of selected groups of foods by type 2 diabetic patients

Selected foods	NORMO	MICRO	*p*
N	119	62	
	Foods from animal sources		
All types of meats			
Intake (g)	139.7 (19-436.7) [153.9 ± 77.5]	172.8 (46.5-528.3) [187.1 ± 88.5]	0.007[1]
Intake (g/kg weight)	2.0 (0.3-8.0) [2.2 ± 1.1]	2.2 (0.7-5.0) [2.4 ± 1.0]	0.073[1]
Red meat			
Intake (g)	81.7 (0-358.8) [98.7 ± 72.3]	105.5 (0-465.0) [118.6 ± 79.9]	0.067[1]
Intake (g/kg weight)	1.2 (0-5.0) [1.4 ± 1.0]	1.4 (0-4.4) [1.5 ± 0.9]	0.163[1]
White meat (chicken and fish)			
Intake (g)	48.3 (0-293.3) [57.8 ± 55.5]	40 (0-293.3) [68.5 ± 64.4]	0.488[1]
Intake (g/kg weight)	0.7 (0-3.5) [0.8 ± 0.7]	0.6 (0-3.6) [0.9 ± 0.9]	0.718[1]
Dairy products			
Intake (g)	282.3 (0-1114.7) [298.6 ± 207.3]	227.5 (0-810.7) [267.9 ± 196.9]	0.271[1]
Intake (g/kg weight)	3.8 (0-22.8) [4.2 ± 3.2]	2.9 (0-11.0) [3.6 ± 2.8]	0.224[1]
	Foods from vegetable sources		
Plant oils			
Intake (ml)	20.0 (3.8-60.0) [21.0 ± 11.2]	16.6 (3.3-42.9) [17.6 ± 9.5]	0.065[1]
Intake (mg/kg weight)	0.3 (0.1-0.9) [0.3 ± 0.1]	0.2 (0.1-0.6) [0.2 ± 0.1]	0.020[1]
Margarines			
Intake (g)	7.0 (0-51.7) [9.7 ± 11.2]	3.3 (0-75.7) [7.2 ± 12.8]	0.014[1]
Intake (g/kg weight)	0.1 (0-0.6) [0.1 ± 0.1]	0.0 (0-1.1) [0.1 ± 0.2]	0.014[1]

Legumes and beans			
Intake (g)	66.7 (0-306.7) [81.2 ± 72.7]	55.9 (0-422.0) [70.6 ± 69.7]	0.412[1]
Intake (g/kg weight)	0.9 (0-4.8) [1.1 ± 1.0]	0.7 (0-5.6) [0.9 ± 0.9]	0.266[1]
Vegetables of groups A+B			
Intake (g)	126.1 (3.3-437.7) [138.1 ± 88.4]	122.8 (0-626.9) [154.4 ± 128.4]	0.920[1]
Intake (g/kg weight)	1.6 (0.1-6.7) [1.9 ± 1.3]	1.5 (0-8.5) [2.0 ± 1.7]	0.693[1]
Foods of group C			
Intake (g)	295.8 (91.7-825.0) [327.3 ± 143.1]	326.9 (110.2-724.7) [352.2 ± 133.3]	0.092[1]
Intake (g/kg weight)	4.0 (1.4-10.6) [4.5 ± 1.9]	4.4 (1.1-10.4) [4.7 ± 2.0]	0.194[1]
Group C, whole grains			
Intake (g)	0 (0-328.4) [30.5 ± 52.4]	0 (0-211.7) [23.8 ± 43.9]	0.532[1]
Intake (g/kg weight)	0 (0-4.9) [0.4 ± 0.8]	0 (0-3.2) [0.3 ± 0.7]	0.483[1]
Fruits			
Intake (g)	218.6 (0-881.7) [241.4 ± 169.2]	185.4 (0-786.7) [208.4 ± 164.1]	0.148[1]
Intake (g/kg weight)	2.8 (0-16.3) [3.4 ± 2.5]	2.3 (0-9.4) [2.7 ± 2.1]	0.079[1]

Data are expressed as median (range) and [mean ± SD].
NORMO = normoalbuminuric patients; MICRO = microalbuminuric patients.
All types of meats = red and white meat, sausages and their products; Red meat = beef and pork with their processed products, and lamb; Vegetables of groups A+B = vegetables with 5-10 % of carbohydrate content; group C = vegetables and foods with 20% of carbohydrate content.
[1] Mann-Whitney U test.

Table 5. Multivariate logistic regression analysis models: Daily intake of different nutrients and selected groups of foods and their odds ratios for microalbuminuria* (dependent variable)

	Odds Ratio	95% CI	p
Nutrients			
Protein (% total energy intake)	1.104	1.008 - 1.208	0.032
from animal sources (% of total energy intake)	1.081	0.996 - 1.173	0.062
from red meat (% of total energy intake)	1.039	0.958 - 1.127	0.355
from vegetable sources (% of total energy intake)	0.883	0.711 - 1.097	0.262
Lipids			
from animal sources (% of total energy intake)	1.038	0.979 - 1.101	0.213
from vegetable sources (% of total energy intake)	0.949	0.900 - 1.000	0.048
Polyunsaturated fatty acids (% of total energy intake)	0.855	0.762 - 0.961	0.008
from animal sources (% of total energy intake)	1.265	0.816 - 1.961	0.294
from vegetable sources (% of total energy intake)	0.874	0.787 - 0.971	0.012
n6-polyunsaturated fatty acids (% of total energy intake)	0.818	0.723 - 0.926	0.002
Polyunsaturated-to-saturated fatty acids ratio	0.394	0.170 – 0.915	0.030
Foods from animal sources			
All types of meats (g)	1.003	0.998-1.007	0.238
All types of meats (g/kg weight)	1.150	0.855 - 1.547	0.354
Red meat (g)	0.999	0.994-1.004	*0.789*
Butter (g)	1.386	1.033-1.860	*0.030*
Foods from vegetable sources			
Plant oils (ml)	0.947	0.914-0.981	0.003
Plant oils (mg/kg weight)	0.036	0.003 - 0.522	0.015
Margarines (g)	0.977	0.946 - 1.009	0.152
Group C (g)	1.000	0.998-1.003	0.765
Fruits (g)	0.999	0.997-1.001	0.334

* All logistic regression models were adjusted for age, gender, presence of hypertension, and fasting plasma glucose. Nutrients or selected foods were included in each model, one at a time.

All types of meats = red and white meat, sausages and their products; Red meat = beef and pork with their processed products, and lamb; group C = vegetables and foods with 20% of carbohydrate content.

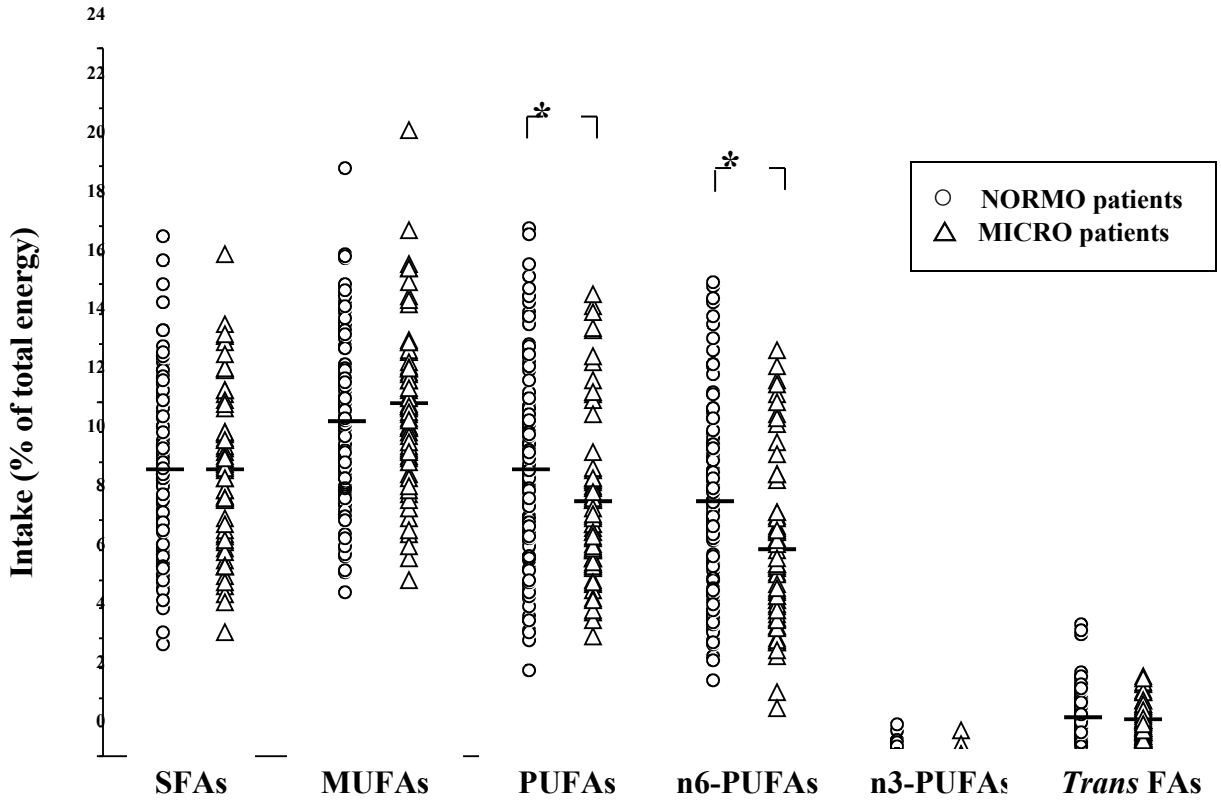

Figure 1. Mean daily intake of fatty acids of type 2 diabetic patients.

NORMO = normoalbuminuric patients (n = 119); MICRO = microalbuminuric patients (n=62).

SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; n6-PUFAS = omega 6 polyunsaturated fatty acids; n3-PUFAs = omega 3 polyunsaturated fatty acids; *trans* FAs = *trans* unsaturated fatty acids.

* = $p < 0.05$.

Capítulo III

Polymorphism Ala54Thr of the *FABP2* Gene Increases Postprandial Fatty Acids in Patients with Type 2 Diabetes

*** Artigo será submetido para publicação**

Polymorphism Ala54Thr of the *FABP2* Gene Increases Postprandial Fatty Acids in Patients with Type 2 Diabetes

Short running title: Postprandial fatty acids and *FABP2* in diabetes

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ABSTRACT

Objective: To evaluate whether the Ala54Thr polymorphism of *FABP2* gene influences the fatty acid (FA) composition in chylomicrons after a standard meal in patients with type 2 diabetes.

Research Design and Methods: Patients homozygous for Ala54Thr polymorphism of *FABP2* gene (TT; AA) were selected from a type 2 diabetic Brazilian multicentric cohort. FAs in chylomicrons (gas-chromatography), plasma glucose, and serum triglycerides were measured at baseline (12-h fasting) and two-hour interval during 8-h after a sandwich (7.1 kcal/kg of total energy, 40.8% fat; 19.8% protein; 38.4% carbohydrate).

Results: Only patients with TT genotype (n = 11) had an increase of FAs after the standard meal which peaked at 6-h. Only long-chain FAs increased [0.95 (0.10-4.13) to 3.10 (0.73-20.67) g/L; P = 0.021]. Saturated FAs increased from 0.46 (0.06-1.60) to 1.37 (0.22-7.15) g/L, monounsaturated FAs from 0.39 (0.03-1.52) to 0.93 (0.35-5.55) g/L, polyunsaturated FAs from 0.26 (0-1.05) to 0.71 (0.14-7.99) g/L, and *trans*-FAs from 0.02 (0-0.07) to 0.07 (0.01-0.45) g/L ($P < 0.05$ for all comparisons). Diabetes treatment, previous diet, and baseline serum triglycerides [1.6(1.0-3.4) *vs.* 1.6(0.6-3.7) mmol/L], LDL (3.0 ± 1.0 *vs.* 3.2 ± 0.6 mmol/L), HDL (1.3 ± 0.2 *vs.* 1.3 ± 0.4 mmol/L), and all FAs did not differ between patients with TT and AA (n = 15) genotypes. The increase of plasma glucose and serum triglycerides was not different in patients with TT and AA genotypes.

Conclusion: TT genotype in Ala54Thr polymorphism of *FABP2* gene in patients with type 2 diabetes increased dietary long-chain FAs absorption, including *trans*-FAs, and this might favor increased susceptibility to the effects of dietary lipids.

KEY WORDS: Serum Fatty Acids; *FABP2* Gene; Ala54Thr polymorphism, type 2 diabetes, postprandial lipemia

INTRODUCTION

Dietary and serum polyunsaturated fatty acids (PUFAs) have been negatively associated with cardiovascular mortality (1) and sudden death (2). Moreover, high values of serum saturated fatty acids (SFAs) might induce endothelial dysfunction (3). Patients with type 2 diabetes, in addition to frequently increased serum fasting triglycerides and decreased HDL cholesterol (4), also have increased postprandial serum triglycerides (5). Furthermore, serum fatty acid (FA) abnormalities have been described in type 2 diabetes (6, 7), especially in the presence of microalbuminuria (8) or hyperlipidemia (9). These lipid alterations, besides a shift in fat metabolism associated with insulin resistance (6, 7, 10), could be related to changes in the absorption of dietary lipids.

Absorption of FAs, especially long-chain FAs, across the intestinal mucosa is carried out by the intestinal FA binding protein (IFABP) encoded by the *FABP2* gene (11). A polymorphism at codon 54 of the *FABP2* gene, changing alanine to threonine (Ala54Thr), increases the affinity of IFABP for long-chain FAs (12). In non diabetic subjects the presence of Ala54Thr polymorphism had been associated with increases in serum postprandial lipids (13-16) and insulin resistance (13-18), although this postprandial response is controversial (19, 20). In patients with type 2 diabetes the Ala54Thr polymorphism has been associated, besides renal disease, with increased fasting triglycerides (21). Only one study evaluated the postprandial lipid response related to this polymorphism in patients with type 2 diabetes (22).

The aim of this study was to evaluate whether the Ala54Thr polymorphism of *FABP2* gene influences the FA composition in chylomicrons after a standard meal in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Patients

Patients were identified from a Brazilian multicentric cohort of 1,042 patients with type 2 diabetes (21). Patients were selected from the outpatient clinic of the Endocrine Division of the Hospital de Clínicas de Porto Alegre, if they had the Ala54Thr polymorphism genotyped, <75 years age, A1C test <8%, serum fasting triacylglycerols <4.52 mmol/L, estimated glomerular filtration rate >30ml min^{-1}, 24-h total urinary protein <1.0 g, and no cardiovascular event in the previous year. Twenty-one patients homozygous for Thr54 *FABP2* polymorphism (TT) were identified, but six patients refused to participate, and four could not be found. In addition, 15 Ala54 homozygous patients (AA) were selected based on similar gender, age, BMI, and diabetes treatment as the patients with TT genotype. The Ethics Committee at Hospital de Clínicas approved the protocol and patients gave their written informed consent.

Twenty-six patients entered a run-in period of approximately 11 (6-28) weeks, in which they were advised to achieve the best possible metabolic and blood pressure controls. Dietary adjustments were performed (23), and the use of antidiabetic agents was standardized to metformin and/or NPH insulin to achieve the goal of A1C < 8.0%. Hypolipidemic agents were temporarily discontinued 6 weeks before the day of the meal test. During the run-in period, patients underwent a “sham” test to evaluate the insulin adjustments needed the night before and during the day of the test. In the sham test each patient ate the same standard meal, a sandwich, and underwent capillary glucose measurements (Accu Chek Advantage®, Roche, Germany) performed at 0, 2, 4 and 6-h.

Clinical and nutritional evaluation

Sitting blood pressure was measured twice to the nearest 2 mmHg after a 10-minute rest, using a standard mercury sphygmomanometer (phases I and V of Korotkoff). Hypertension was defined as blood pressure > 140/90 mmHg measured on two occasions or use of antihypertensive drugs. According to 24-h urinary albumin excretion (UAE) patients were classified as normoalbuminuric (UAE < 20 μg/min), microalbuminuric (20 - 199 μg/min) or macroalbuminuric (UAE ≥ 200 μg/min). Micro- or macroalbuminuria was confirmed in a second urine sample (24). Fundus examination was performed (MJA) through dilated pupils, and diabetic retinopathy was graded (25). Frequency of exercise, according to activities during a typical day, was graded into four levels: 1-none, 2-low, 3-moderate and 4-high, based on a questionnaire (26) adapted to local habits. Patients were self-identified as white or non-white.

The usual diet was assessed by a 24-h recall on the day of the meal test by the research dietitian (JCA), and the diet composition was calculated (27). The body weight and height of patients (without shoes or coats) were obtained with an anthropometric scale, with measurements recorded to the nearest 100 g for weight and to the nearest 0.1 cm for height. BMI (kg/m^2) was then calculated. Waist circumference was measured midway between the lowest rib margin and the iliac crest, near the umbilicus, measured once to the nearest 1 mm. A flexible, no-stretch fiberglass tape was used for these measurements.

Standard meal test procedures

A sliced white bread (1.29 g/kg) sandwich with butter (0.07 g/kg), sliced cheddar cheese (0.43 g /kg), and 80% fat ham (0.86 g /kg), providing 7.13 kcal/kg of body weight as total energy was the standard meal. The sandwich total amount of fat

was 25.7 g / 80 kg body weight or 13.7 ± 1.0g of fat/m^2 of body surface and its detailed estimated nutrient content (27) is shown in Table 1.

The test started at 08:00 A.M. after a 12-h fast. Patients were advised not to drink alcohol and to avoid strenuous exercise in the previous three days. Patients who used insulin reduced their nocturnal dose about 20% the day before the test and about 30-40 % on the morning of the test, according to the "sham" test performed in the run in period. At baseline, plasma glucose, serum creatinine, insulin, A1C test, lipid profile, endothelin-1, fibrinogen, C-reactive protein and FAs in chylomicrons were measured. Rapid-acting insulin analog (lispro) was administered before the standard meal, depending on the calculated carbohydrate content of the sandwich (1U per 15 g of carbohydrate). Patients took their usual medicines after eating the sandwich. Blood samples were collected 2, 4, 6 and 8-h after the standard meal to measure serum triglycerides, plasma glucose, and FAs. In addition, at the 8th hour, endothelin-1, fibrinogen and C-reactive protein measurements were performed.

Laboratory Measurements

Plasma glucose was measured by a glucose oxidase method, serum creatinine by Jaffé's reaction, and A1C test by ion-exchange HPLC (Merck-Hitachi L-9100 glycated hemoglobin analyzer; reference range 4.7-6.0 %; Merck, Darmstadt, Germany). Fasting insulin was measured, only in patients not using insulin, by a chemoluminescent method (Elecsys 2010, Basel, Switzerland). Insulin resistance was estimated by HOMA resistance index [HOMA1-IR = fasting serum insulin (μU/mL) x fasting plasma glucose (mmol/L) / 22.5] and β-cell function, by HOMA1-%β [= 20 x fasting serum insulin (μU/mL) / fasting plasma glucose (mmol/L) x 3.5] (28) in patients not using insulin. Endothelin-1 was measured by ELISA (R&D Systems, Minneapolis, USA), fibrinogen

by a coagulometric method (Sta Compact: Cedex, France), and serum C-reactive protein by nephelometry (Behring Nephelometer II; reference range: 1-3 mg/L).

Serum total cholesterol and triglycerides were measured by enzymatic-colorimetric methods (Merck Diagnostica, Darmstadt, Germany; Boeringher Mannheim, Buenos Aires, Argentina), HDL cholesterol by homogeneous direct method (autoanalyzer, ADVIA 1650). LDL cholesterol was calculated using Friedewald's formula (29). FAs were measured by gas chromatography in plasma chylomicrons. Blood samples for plasma chylomicrons analysis were separated after centrifugation and stored at -70ºC for later laboratory measurements. To separate chylomicrons, 5 mL of plasma adjusted by adding solid KBr were overlaid with saline gradients (30) and ultracentrifuged (Beckman®; TFT 38000 g at 20° C for 33 min). The top 1 mL was carefully aspirated to remove the chylomicrons fraction. FAs were extracted from chylomicrons with chloroform-methanol (2:1, by vol), and converted into FA methyl esters by boron trifluoride catalysis (8). In brief, the methyl esters were then separated and measured by gas chromatography on a 100 m fused silica capillary columm (CPSil 88, Varian®, Palo Alto, California, USA) with an internal diameter of 0.20 μm (Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector, Agilent Technologies®, Santa Clara, California, USA) as previously described (8, 31). Methyl heneicosanoate (C21:0) was used as an internal standard (Sigma-Aldrich®, St Louis, USA) and the identity of each FA peak was ascertained by comparing the peak retention time with a previously characterized mixture of 37 FAs (Sigma-Aldrich®, St Louis, USA). In our laboratory, the coefficient of variation of different FAs in this mixture range from 0.03 to 3.5% (n = 4). FAs were classified according to the length of chains (32). Delta desaturase activities were estimated by the following formulas: delta

5 desaturase = 20:4n-6 / 20:3n-6; delta 6 desaturase = 18:3n-6 / 18:2n-6, and delta 9 desaturase = 16:1n-7 / 16:0 (36).

Molecular analysis was performed by isolating DNA from lymphocytes using standard procedures (33). The genotyping of the A54T polymorphism was performed by PCR amplification as previously described (8, 21) and all amplification reactions were performed twice.

Statistical Analyses

Sample size was estimated based on the difference (140.7 mg h/L - area under the curve) between palmitic acid in chylomicrons after an oral-fat-loading in non diabetic subjects with AA and TT genotypes (14). It was estimated that 11 patients with TT and 11 with AA genotypes had to be included to have an 80% power and 0.05 alpha. Student's *T* test, Mann-Whitney *U* tests, and Chi Square test were used to compare characteristics of the two groups, as appropriate. Differences between baseline and postprandial variables values (maximum value or value at 8th hour) were evaluated by Wilcoxon *U* test. Individual FAs that had a median serum value at peak equal to zero were not analyzed. During the meal test plasma glucose values were analyzed by repeated-measures ANOVA (post hoc test: LSD), and triglycerides by Friedman's ANOVA (post hoc test: NP MC). Spearman correlation was used to evaluate the relationship of postprandial maximum FAs values with the increase in FA desaturases. Results were expressed as medians (range) or mean ± SD, unless otherwise stated. *P* values < 0.05 (two-tail) were considered to be statistically significant. SPSS 14.0 (SPSS®, Chicago, IL) was used for analyses.

RESULTS

Patients' characteristics

The baseline clinical and laboratorial characteristics of patients homozygous for the Ala54Thr polymorphism of *FABP2* gene are shown in Table 2. There were no differences between patients with TT (n = 11) and AA (n = 15) genotypes regarding age, gender, ethnicity, diabetes duration, smoking habit, physical activity, anthropometric measurements, blood pressure levels, proportion of patients with microvascular complications, and medicines in use. Serum creatinine, glycemic and lipid control parameters, HOMA indexes, serum endothelin-1, fibrinogen, and C-reactive protein did not differ either.

The patient's usual daily total energy intake as evaluated by 24-h diet recall did not differ in patients with TT (1626.5 ± 380.3 kcal) and AA (1570.3 ± 456.8 kcal; P = 0.749) genotypes. Also macronutrients intakes (% of energy) were not different between patients with TT and AA genotypes: carbohydrate = 42.6 ± 8.3 *vs.* 45.5 ± 6.6 %; protein = 19.6 ± 3.1 *vs.* 19.9 ± 4.0 %; fat = 38.0 ± 9.2 *vs.* 35.7 ± 7.3 %; SFAs = 10.7 ± 3.2 *vs.* 10.3 ± 2.0 %; monounsaturated FAs (MUFAs) = 14.1 ± 4.2 *vs.* 12.5 ± 3.0 %; PUFAs = 10.6 ± 4.4 *vs.* 9.9 ± 3.9 %, and *trans*- FAs = 0.6 ± 0.7 *vs.* 0.5 ± 0.4 % (P > 0.05 for all). Daily intakes of fiber (18.1 ± 6.6 *vs.* 21.5 ± 11.39 g; P = 0.403) and cholesterol (189 ± 121 *vs.* 184 ± 119 mg; P = 0.922) did not differ either. There was no difference in the proportion of alcohol consumers in patients with TT (50 %) and AA (50%; P = 1.000) genotypes. The mean alcohol intake (1 drink = ~14ml of ethanol) also did not differ between the two groups (TT = 1.50 ± 0.6 *vs.* AA = 2.0 ± 1.15 drinks per month; P = 0.446).

Standard meal test

The test conditions were compared between patients with TT and AA genotypes. The amount of carbohydrate intake (52.7 ± 8.2 *vs.* 53.1 ± 8.0 g; P = 0.907), the dose of rapid-acting insulin used before the standard meal (3.8 ± 1.6 *vs.* 4.9 ± 2.0 U; P = 0.159),

carbohydrate to rapid-acting insulin ratio (15.6 ± 5.4 *vs.* 12.3 ± 4.0 g/U; P = 0.089), and the time-span of standard meal intake during the test (17.45 ± 5.22 *vs.* 17.85 ± 5.64 min; P = 0.856) were not different between the two groups.

Fatty acids in chylomicrons during the meal test

Chylomicrons FAs at baseline were not different in patients with TT or AA genotypes (P > 0.10). Only patients with TT genotype had an increase of FAs after the standard meal which peaked at 6-h (Fig. 1, Fig. 2). Table 3 shows baseline and 6-h postprandial FAs values in chylomicrons of patients homozygous for the Ala54Thr polymorphism of *FABP2* gene. The long-chain FAs in chylomicrons increased, but short-chain and medium-chain FAs did not change after the meal, as expected. Total FAs, SFAs, MUFAs, PUFAs, and *trans*- FAs increased 6-h postprandially in patients with TT genotype. An increase of palmitic acid, oleic acid, 18:2 *trans*- fatty acid, and alpha-linolenic acid was also observed only in patients with TT genotype.

In order to evaluate whether the increase in postprandial FAs was associated with changes in plasma FA desaturases (delta 5, delta 6, and delta 9), the activities of desaturases were estimated. In all patients an increase of delta 6 desaturase from 0.08 (0.00 - 0.52) to 0.62 (0.00 - 2.08); P < 0.0001 and delta 9 desaturase from 4.54 (0.64 - 9.79) to 11.15 (1.30 - 68.97; P < 0.0001 occurred, but not in delta 5 desaturase from 0.07 (0.00 - 0.35) to 0.61 (0.00 - 7.08; P = 0.317. The increases in delta 6 and delta 9 desaturase activities were not different between patients with TT and AA genotypes (data not shown). The correlations of the maximum postprandial values of plasma FAs (at 6^{th} hour) with postprandial increases of plasma desaturase activities were analyzed in patients with TT and AA genotypes. Only in patients with TT genotype the maximum SFA values were positively associated with the increase in delta 9 desaturase activity (*r*

= 0.645; P = 0.032) and negatively associated with the increase in delta 6 desaturase activity (r = -0.700; P = 0.024). On the other hand, the maximum postprandial MUFA values were negatively associated with the increase in delta 6 desaturase activity (r = -0.750; P = 0.012) in the TT group.

Endothelin-1, fibrinogen, and C-reactive protein after the standard meal

The increase of plasma endothelin-1 did not reach the adopted statistical significance in patients with TT [0.94 (0.18-1.76) to 1.36 (0.54-2.42) pg/mL; P = 0.119] and AA [0.91 (0.13 – 1.58) to 1.10 (0.70 – 3.78) pg/mL; P = 0.054] genotypes. However, when all studied patients (n = 26) were analyzed, endothelin-1 was higher at 8-h [1.20 (0.54-3.78) pg/mL] as compared to baseline [1.03 (0.13-1.76) pg/mL; P = 0.013]. In all patients serum fibrinogen [359 (124-457) to 350 (297-510) mg/dL; P = 0.135] and C-reactive protein [1.93 (0.39-23) to 1.59 (0.44-23.0) mg/L; P = 0.360] did not change after the standard meal, as well as in patients with TT and AA genotypes (data not shown).

Plasma glucose and serum triglycerides during the meal test

Figure 3 shows the values of plasma glucose and serum triglycerides after the standard meal. Plasma glucose increased in both the TT (P = 0.013) and the AA groups (P < 0.001) after the standard meal. The maximum increase of glucose occurred 2-h postprandially and was not different between patients with TT [0.3 (-1.8; 4.4) mmol/L] and AA genotypes [1 (-2.0; 5.9) mmol/L; P = 0.281]. Serum triglycerides also increased during the standard meal in TT (P < 0.001) and AA groups (P < 0.001). The maximum increase of serum triglycerides was reached 4-h postprandially and was not different in patients with TT [0.5 (0.2-1.8) mmol/L] and AA [0.9 (0.02-2.5) mmol/L; P = 0.886] genotypes.

DISCUSSION

In patients with type 2 diabetes, after a standard meal, FAs in chylomicrons increased more in patients homozygous for the Thr54 than in patients homozygous for Ala54 polymorphism in the *FABP2* gene. This increase occurred in SFAs, MUFAs and PUFAs, including *trans*-FAs. It is possible that patients with TT genotype could be more susceptible to dietary effects of fat. This hypothesis is reinforced by our previous observation that patients with type 2 diabetes and TT genotype had high fasting serum triglycerides and a high prevalence of diabetic nephropathy (21).

This postprandial increase observed was mainly related to long-chain FAs. The chylomicron FA composition has been a high concordance with meal FA composition, although the postprandial absorption of short or medium dietary FAs occurs mostly by the portal route as an alternative of chylomicrons secretion (35). In addition, the preferential postprandial increase of long-chain FAs in patients with TT genotype, instead of short and medium chain FAs, was also demonstrated in non diabetic subjects (14). These observations are explained by a high binding affinity for both saturated and unsaturated long-chain FAs in the single ligand binding site in IFABP (11).

The analyses of desaturase activities were in accordance with the higher intestinal absorption of FAs by patients with TT genotype. The delta 6 and delta 9 desaturase activities were higher after the standard meal as compared to baseline, without changes in delta 5 desaturase activity. In addition, the activity of delta 6 desaturase was inversely correlated with delta 9 desaturase activity and with the increases of FAs after the meal only in TT patients. This pattern of desaturase activities was associated with postprandial increase of FAs and is probably due to FA content of the standard meal. Changes in the proportions of SFAs and unsaturated FAs in the diet can affect the desaturase activities by competing FAs in the tissues and in the activity of the enzymes (36). The fat content of the sandwich, rich in SFAs (mainly palmitic acid)

could have induced an increase in the rate of desaturation of palmitic and stearic acids to their MUFAs counterparts (36), and reduced rate of conversion of linoleic acid and alpha-linolenic acid to their metabolites.

The increase in plasma endothelin-1 could reflect the absorption of dietary fat from the standard meal. In healthy subjects a reduction in the endothelium-dependent vasodilatation was observed after a meal rich in fat as compared to a low fat meal (37). In fact, plasma endothelin-1 increased in all studied patients, although FAs increased only in patients with TT genotype. Although the degree of metabolic control or the presence of macrovascular complications could have influenced the responses of endothelin-1 (38), patients with TT and AA genotypes did not differ regarding these features. It is possible that an unidentified factor besides fat intake influenced the endothelin-1 increases observed in all patients.

In the present study patients were well paired on the basis of gender, age, BMI and diabetes treatment, and as expected, no differences at baseline were observed in lipid profile, including all FAs. Probably these results were from the run-in period management such as standardized diabetes treatment and dietary counseling. In fact, the effect of genetic variation may be more evident in the postprandial state than in the less-common fasting state (39). As far as we know there is no data on postprandial responses of individual FAs in chylomicrons in patients with type 2 diabetes and Ala54Thr polymorphism of *FABP2* gene. The use of a standard meal that corresponds to an ordinary meal, such as a sandwich, supports the results of the present study.

The influence of the Ala54Thr polymorphism in fat intestinal absorption was evaluated in patients with type 2 diabetes and TT genotype in only one study (22). An increase in postprandial serum triglycerides in patients with TT genotype after non-habitual fat ingestion overload was demonstrated. The FAs were not measured. The

observed increase in triglycerides was probably due to the intake of a meal with higher fat content (55 g *vs.* 14 g of body surface) as compared to our sandwich. Actually, a meal with high amount of fat (50-80 g) promotes an exaggerated postprandial triglycerides increase (35). Furthermore, patients had poorer glucose control (A1C test: 10.8 *vs.* 6.6 %) than patients from the present study.

One possible limitation of the present study could be related to the methodology used to isolate the chylomicrons fraction in plasma. A fixed rotor was used after layering the plasma with saline and density gradient centrifugation. This technique does not completely separate the VLDL from chylomicrons, and therefore, the isolated chylomicrons sub fraction could include some residual VLDL particles (40). However if this possibility occurred it took place in measurements from both patients with TT and AA genotypes. Moreover, a concomitant increase of FAs in VLDL is also expected in the postprandial period. Finally, not performing the apolipoprotein E (apo E) genotyping could be a confounding factor in the present study. This possibility is unlikely, since we studied the postprandial acute effect of a meal and the most important effect of the presence of apo E4 allelle was in the LDL response to long-term dietary intervention (41).

In conclusion, TT genotype in Ala54Thr polymorphism of *FABP2* gene in patients with type 2 diabetes increased dietary long-chain FAs absorption, including *trans*-FAs, and this might favor increased susceptibility to the effects of dietary lipids. It can be speculated whether the polymorphism of *FABP2* gene could identify a subset of patients with diabetes who might receive specific dietary counseling regarding FAs intake.

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Table 1. Estimative of nutrient content of the standard meal *

Nutrients	Content
Energy (kcal)	566.9 ± 82.1
(kcal/kg of body weight)	7.13 ± 0.28
Carbohydrate (% of energy)	38.40 ± 1.10
Protein (% of energy)	19.80 ± 0.30
Lipid (% of energy)	40.80 ± 0.90
Saturated fatty acid (% of energy)	20.90 ± 0.60
Butyric acid (C4:0; % of energy)	0.60 ± 0.08
Hexanoic acid (C6:0; % of energy)	0.30 ± 0.04
Caprylic acid (C8:0; % of energy)	0.20 ± 0.02
Capric acid (C10:0; % of energy)	0.60 ± 0.02
Lauric acid (C12:0; % of energy)	0.60 ± 0.02
Miristic acid (C14:0; % of energy)	2.70 ± 0.10
Palmitic acid (C16:0; % of energy)	10.10 ± 0.30
Margaric acid (C17:0; % of energy)	0.10 ± 0.01
Stearic acid (C18:0; % of energy)	4.80 ± 0.10
Arachidic acid (C20:0; % of energy)	0.02 ± 0.002
Monounsaturated fatty acid (% of energy)	13.10 ± 0.30
Oleic acid (C18:1; % of energy)	11.80 ± 0.30
Eicosenoic acid (C20:1; % of energy)	0.10 ± 0.01
Polyunsaturated fatty acid (% of energy)	3.90 ± 0.03
Linoleic acid (C18:2; % of energy)	3.30 ± 0.03
Alpha-linolenic acid (C18:3; % of energy)	0.50 ± 0.01
Arachidonic acid (C20:4; % of energy)	0.04 ± 0.01
Eicosapentaenoic acid (C20:5; % of energy)	0.00
Docosahexaenoic acid (C22:6; % of energy)	0.00
Trans-fatty acid (% of energy)	1.90 ± 0.60
Cholesterol (mg)	89.0 ± 12.4
Total fiber (g)	3.3 ± 0.5

Data are means ± SD. * sandwich: sliced bread with butter, cheddar slice and fat ham. Data from USDA SR 17 Research Quality nutrient data (27).

Table 2. Baseline clinical and laboratory characteristics of patients homozygous for the Ala54Thr polymorphism of *FABP2* gene

	***FABP2* Genotype**		
	AA	**TT**	**P**
N	15	11	-
Age (years)	62.0 ± 7.7	61.6 ± 6.7	0.902
Gender (M)	13 (86.7 %)	8 (72.7 %)	0.346
Ethnicity: white	13 (86.7 %)	11 (100 %)	0.452
Diabetes duration (years)	13.1 ± 5.6	12.7 ± 6.9	0.891
Current smoking	2 (13.3 %)	2 (18.2 %)	0.574
Frequency of exercise: none	4 (26.7 %)	4 (36.4 %)	0.457
Body Mass Index (kg/m²)	28.8 ± 4.1	29.2 ± 3.7	0.824
Waist circumference (cm)			
Female (n = 5)	92.5 ± 11.3	102.5 ± 6.4	0.390
Male (n = 21)	100.5 ± 10.2	98.4 ± 11.1	0.659
Abnormal waist circumference*	12 (80%)	8 (72.7%)	0.509
Hypertension	13 (86.7 %)	9 (81.7 %)	0.735
Systolic blood pressure (mmHg)	133 ± 22	136 ± 10	0.731
Diastolic blood pressure (mmHg)	72 ± 11	71 ± 10	0.932
Diabetic nephropathy			
Microalbuminuria	7 (46.7 %)	3 (27.3 %)	0.187
Macroalbuminuria	0	2 (18.2 %)	
Diabetic retinopathy			
Proliferative	2 (13.3 %)	3 (30.0 %)	0.326
Non-proliferative	5 (33.3 %)	1 (10.0 %)	
Diabetes treatment			
Metformin	6 (40.0 %)	5 (45.5 %)	0.918
Metformin + NPH insulin	8 (53.3 %)	5 (45.5 %)	
NPH insulin only	1 (6.7 %)	1 (9.1 %)	
ACE inhibitors	11 (73.3 %)	9 (81.8 %)	0.491
Fasting plasma glucose (mmol/L)	6.9 ± 1.3	6.3 ± 1.7	0.362
A1C test (%)	6.7 ± 0.4	6.5 ± 0.6	0.305
HOMA1-IR **	2.73 (1.33-3.78)	2.83 (0.05-8.06)	1.000

HOMA1-% β **	5.29 (2.78-7.50)	8.20 (0.19-19.91)	0.548
Total cholesterol (mmol/L)	5.00 ± 1.22	5.32 ± 0.91	0.475
HDL cholesterol (mmol/L)	1.26 ± 0.20	1.33 ± 0.43	0.630
LDL cholesterol (mmol/L)	2.96 ± 1.01	3.20 ± 0.64	0.493
Triglycerides (mmol/L)	1.57 (1.01-3.41)	1.60 (0.59-3.72)	0.493
Serum creatinine (μmol/L)	95 ± 13	99 ± 35	0.330
Endothelin-1 (pg/mL)	1.08 (0.13-1.58)	0.97 (0.18-1.76)	0.683
Fibrinogen (mg/dL)	345 (302-417)	390 (297-510)	0.097
C-reactive protein (mg/L)	1.30 (0.47-23.00)	4.38 (0.44-11.20)	0.413

Data are means ± SD, median (range) or number of patients with analyzed characteristics (%). *Abnormal waist circumference according to the International Diabetes Federation (33) = men ≥94cm, women ≥80cm; ACE = Angiotensin-Converting Enzyme; **Analysis performed only in patients not using insulin: 5 patients with AA genotype and 5 patients with TT genotype.

Table 3. Baseline and 6-h postprandial fatty acids in chylomicrons of patients homozygous for the Ala54Thr polymorphism of *FABP2* gene

***FABP2* Genotype**	**AA patients (n=15)**			**TT patients (n=11)**		
	baseline	**6-h postprandial**	**P**	**baseline**	**6-h postprandial**	**P**
Total fatty acids (g/L)	1.35 (0.59-3.68)	1.44 (0.74-11.10)	0.256	1.13 (0.24-4.35)	3.28 (0.86-21.12)	0.026
Short chain fatty acids† (g/L)	0.03 (0-0.10)	0.03 (0-0.30)	0.638	0.02 (0-0.12)	0.01 (0-0.20)	0.260
Medium chain fatty acids‡ (g/L)	0.01 (0-0.22)	0.01 (0-0.05)	0.334	0.01 (0-0.05)	0.01 (0.01-0.10)	0.155
Long chain fatty acids§ (g/L)	1.20 (0.43-3.48)	1.29 (0.59-10.55)	0.281	0.95 (0.10-4.13)	3.10 (0.73-20.67)	0.021
Saturated fatty acids (g/L)	0.63 (0.19-1.29)	0.67 (0.21-3.67)	0.078	0.46 (0.06-1.60)	1.37 (0.22-7.15)	0.041
Palmitic acid; C16:0 (g/L)	0.06 (0-0.12)	0.07 (0.02-0.81)	0.053	0.05 (0-0.11)	0.11 (0-0.52)	0.017
Stearic acid; C18:0 (g/L)	0.05 (0-0.24)	0.10 (0-0.75)	0.117	0.05 (0-0.14)	0.12 (0-0.92)	0.878
Arachidic acids; C20:0 (g/L)	0.25 (0.02-0.86)	0.24 (0.04-2.03)	0.733	0.22 (0.02-0.93)	0.51 (0.09-4.33)	0.155
Monounsaturated fatty acids (g/L)	0.39 (0.16-0.94)	0.46 (0.22-3.46)	0.281	0.39 (0.03-1.52)	0.93 (0.35-5.55)	0.026
Oleic acid; C18:1 (g/L)	0.11 (0.03-0.22)	0.11 (0.03-0.73)	0.650	0.10 (0.01-0.27)	0.22 (0.02-1.21)	0.041
Polyunsaturated fatty acids (g/L)	0.27 (0.04-1.33)	0.26 (0.10-3.41)	0.427	0.26 (0-1.05)	0.71 (0.14-7.99)	0.021
Linoleic acid; C18:2n6 (g/L)	0.18 (0.04-0.67)	0.22 (0.08-2.38)	0.650	0.18 (0-0.87)	0.50 (0.07-3.92)	0.155
Alpha-linolenic acid; C18:3n3 (g/L)	0 (0-0.65)	0 (0-0.23)	0.753	0 (0-0.15)	0.08 (0-1.01)	0.036
***Trans*- fatty acids (g/L)**	0.01 (0-0.07)	0.02 (0.01-0.46)	0.394	0.02 (0-0.07)	0.07 (0.01-0.45)	0.023
C18:2 trans (g/L)	0.01 (0-0.07)	0.02 (0.01-0.35)	0.394	0.02 (0-0.07)	0.06 (0-0.32)	0.037

Data are median (range). † fatty acids with < 8 carbons; ‡ fatty acids with 8-12 carbons; § fatty acids with ≥ 14 carbons.

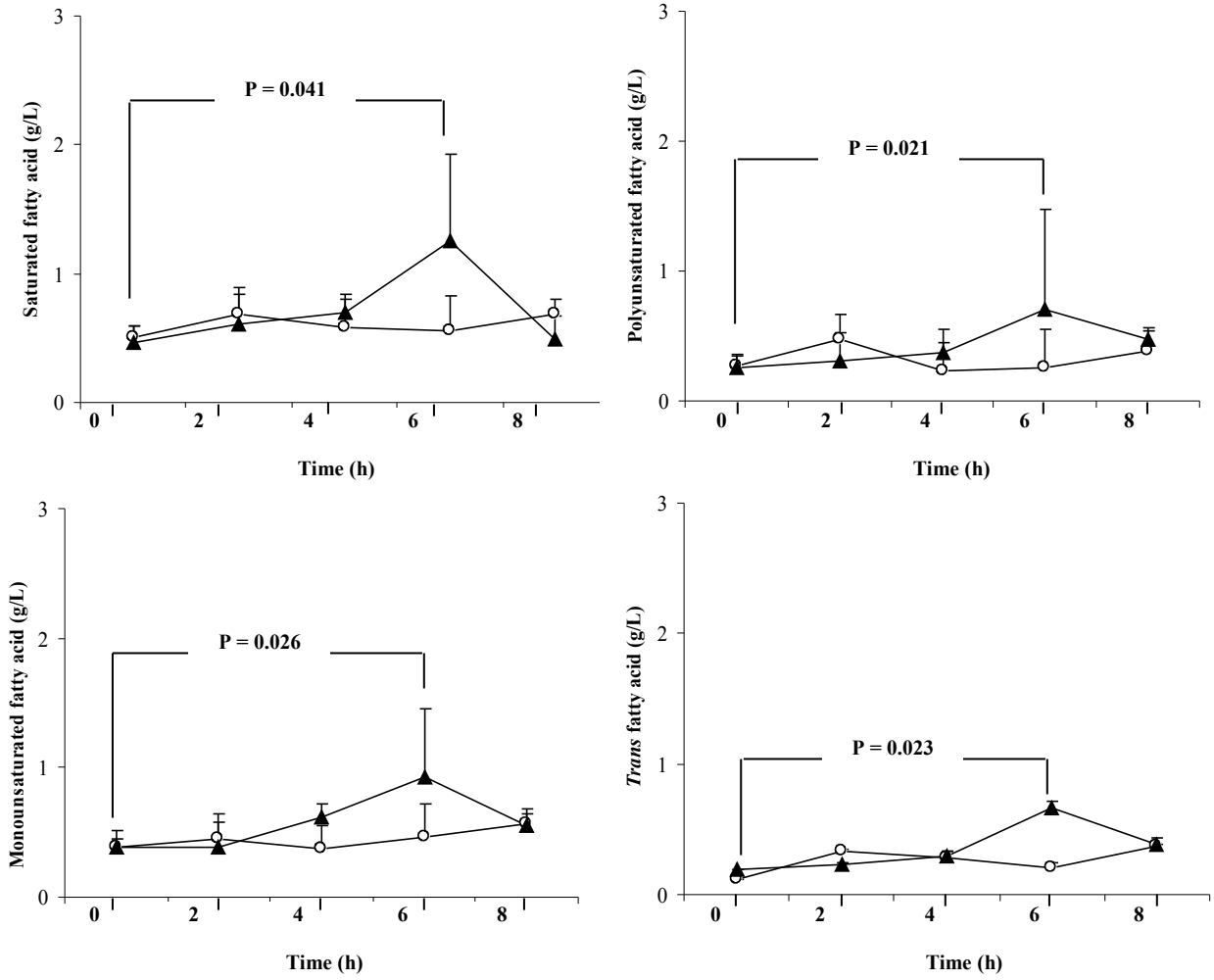

Figure 1. Saturated, monounsaturated, polyunsaturated and *trans*- fatty acid in chylomicrons of patients with type 2 diabetes homozygous for the Ala54Thr polymorphism of *FABP2* gene after standard meal. Patients with TT genotype (Δ), n = 11; patients with AA genotype (●) n = 15.

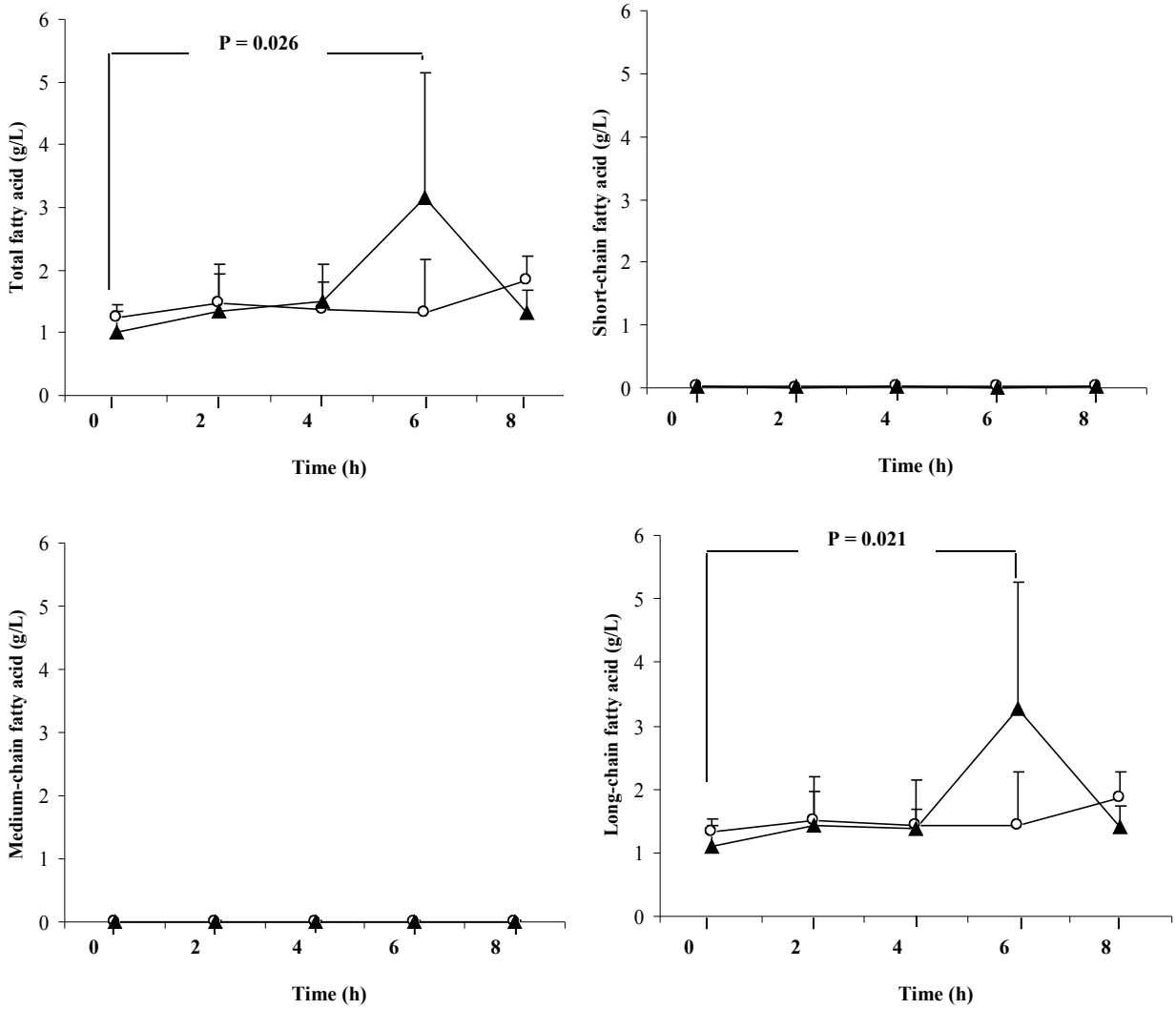

Figure 2. Total, short, medium, and long-chain fatty acids in chylomicrons of patients with type 2 diabetes homozygous for the Ala54Thr polymorphism of *FABP2* gene after standard meal. Patients with TT genotype (Δ), n = 11; patients with AA genotype (●) n = 15.

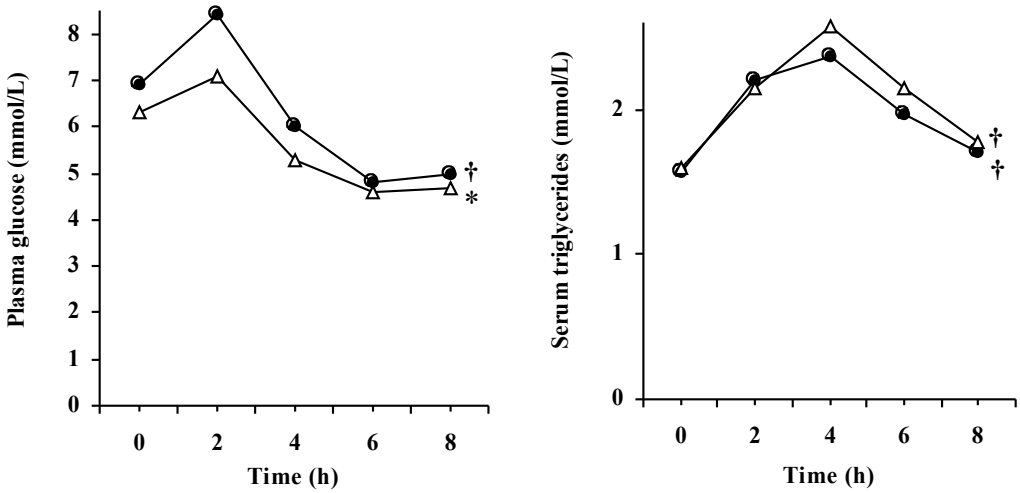

Figure 3. Plasma glucose and serum triglycerides in patients with type 2 diabetes homozygous for the Ala54Thr polymorphism of *FABP2* gene after standard meal. Patients with TT genotype (Δ), n = 11; patients with AA genotype (●) n = 15. * $P < 0.01$. † $P < 0.001$.

CONSIDERAÇÕES FINAIS

As evidências disponíveis sugerem que as gorduras da dieta, através da modificação de lipídeos séricos e da função endotelial, podem ter um papel importante no desenvolvimento e na progressão da ND. Um padrão alimentar rico em AGSs e pobre em AGPIs, além de representar um risco cardiovascular elevado, provavelmente é um fator de risco para a ND.

De fato, demonstramos uma associação negativa entre ingestão de AGPIs, particularmente oriundos de óleos vegetais, com a presença de microalbuminúria em pacientes com DM tipo 2. A ingestão de proteínas também foi associada positivamente à microalbuminúria.

A importância dos AGs alimentares e suas associações com a ND fica também evidente com a observação da resposta de AGs plasmáticos à refeição rica em gordura. Pacientes com DM tipo 2 homozigotos para o alelo Thr54 do polimorfismo do *FABP2* são provavelmente um grupo de risco para complicações crônicas relacionadas ao DM. Quando submetidos a uma refeição rica, especialmente em AGs saturados, estes pacientes apresentam uma absorção aumentada de AGs quando comparados aos pacientes sem este genótipo. É possível que estes pacientes tenham uma suscetibilidade aumentada aos efeitos das gorduras da dieta, que por sua vez, leve-os a uma maior freqüência das complicações relacionadas ao DM.

Como pespectiva futura e necessária, o papel dos componentes da dieta especificamente relacionados à microalbuminúria deve ser confirmado em estudos prospectivos e ensaios clínicos randomizados. Mais ainda, o acompanhamento de pacientes com DM tipo 2 com o perfil genético de risco para complicações crônicas relacionado a

absorção de gorduras da dieta permitirá a adoção de estratégias nutricionais individualizadas, com vistas a prevenção das complicações micro- e macrovasculares do DM nestes pacientes. Para isto, outros polimorfismos envolvidos com o metabolismo lipídico devem também ser identificados nestes pacientes.

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