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Mestrado e Doutorado

Efeito da retirada da carne vermelha da dieta sobre a função renal e perfil lipídico sérico de pacientes com Diabetes Melito Tipo 2 e nefropatia diabética

Vanessa Derenji Ferreira de Mello

Orientador: Prof. Dr. Jorge Luiz Gross

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"With a missionary zeal, one must convert not only the patient's mind and soul, but also his doctor to the realisation that it is worth the effort to control the disease as shown by sugar-free urine, normal blood sugar and cholesterol"

Elliot Joslin, 1959

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Conteúdo

Lista de Abreviaturas

AGMI:	ácidos graxos monoinsaturados
AGPI:	ácidos graxos poliinsaturados
AGS:	ácidos graxos saturados
DM:	Diabetes Melito
EUA:	excreção urinária de albumina
FABP2:	gene que codifica a “Intestinal Fatty Acid Binding Protein”
FPR:	fluxo plasmático renal
HAS:	hipertensão arterial sistêmica
ND:	nefropatia diabética
P/S:	relação ácidos graxos poliinsaturados / ácidos graxos saturados
TFG:	taxa de filtração glomerular

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Capítulo I - Introdução

Papel da dieta como fator de risco e progressão da nefropatia diabética

"Abbreviated title": Dieta e nefropatia diabética

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Resumo

A nefropatia diabética (ND) acomete até 40% dos pacientes com diabetes melito (DM) tipo 1 e tipo 2, sendo a principal causa de insuficiênca renal crônica terminal naqueles pacientes que ingressam em programa de tratamento de substituição renal. A dieta parece ter um papel importante no desenvolvimento da doença. Existem evidências reforçando o conceito de que não apenas a quantidade, mas o tipo de proteína ingerida também está associado à ND. Poucos estudos analisaram o papel dos lipídeos na dieta em relação à ND. Dietas hipoprotéicas têm sido úteis em modificar de forma favorável a evolução da ND sendo capazes de desacelerar a progressão da doença para insuficiência renal terminal em pacientes com DM tipo 1 e micro- e macroalbuminúria. Em pacientes com DM tipo 2 existem poucos estudos, porém estudos a curto prazo sugerem que esta conduta dietoterápica reduz a excreção urinária de albumina. Entretanto, a longo prazo, foi observado dificuldade de aderência à restrição protéica e a sua segurança nutricional ainda não é estabelecida. Resultados promissores são observados quando é feita a comparação entre diferentes fontes de ingestão de proteína animal sobre a função renal e perfil lipídico sérico de pacientes com ND, podendo estas intervenções representar uma alternativa à dieta hipoprotéica no manejo dietoterápico nestes pacientes, ao atuar sobre os fatores de risco cardiovasculares e na função endotelial.

Abstract

Diabetic nephropathy (DN) is the leading cause of kidney disease in patients starting renal replacement therapy, and affects up to 40% of type 1 and type 2 diabetic patients. Diet seems to play an important role in the development of disease. There are evidence supporting the concept that not only the amount but also the origin of dietary protein are associated with DN. Few studies analyzed the role of dietary lipids. A low-protein diet slows down the decline of renal function and ameliorates the DN prognosis and death in patients with type 1 diabetes with micro- and macroalbuminuria. Studies in type 2 diabetic patients are scanty but short-term studies suggest that this approach decreases albuminuria. However, the use of low-protein diet for long periods is compromised by poor compliance and its long-term safety is not firmly established. Enthusiastic results come up when comparing the effect of different sources of animal protein on renal function and lipid profile in patients with DN, which may represent an alternatively strategy for low-protein diet on medical nutritional therapy in patients with DN and in cardiovascular risk factors and endothelial function.

I. Introdução

A nefropatia diabética (ND) acomete cerca de 40% dos pacientes com diabetes melito (DM) tipo 1 e tipo 2 (1-4), e é a principal causa de insuficiênca renal crônica naqueles pacientes que ingressam em programa de tratamento de substituição renal (5). Em estudo realizado em 18 centros de diálise de Porto Alegre e da grande Porto Alegre, 26% dos pacientes que ingressam em programa de tratamento de substituição renal apresentam DM (6). A ND está também associada ao aumento da mortalidade cardiovascular (7). A ND é definida tradicionalmente pela presença de proteinúria >0,5 g/24 horas, mas atualmente a medida da excreção urinária de albumina (EUA) é o parâmetro de referência para definir a presença e os estágios da ND. De acordo com a EUA a ND é didaticamente dividida em dois estágios: microalbuminúria ou nefropatia incipiente (EUA $\geq$20 μg/min e $\leq$199 μg/min) e macroalbuminúria ou nefropatia clínica ou proteinúria (EUA $\geq$200 μg/min). A hiperglicemia, o aumento dos níveis de pressão arterial e a predisposição genética são os principais fatores de risco para o desenvolvimento da ND. A dislipidemia e o tabagismo também parecem ter um papel no desenvolvimento da doença. A obtenção do melhor controle glicêmico possível, o tratamento da hipertensão arterial, o uso de drogas com efeito bloqueador do sistema renina-angiotensina-aldosterona, e possivelmente o tratamento da dislipidemia têm sido estratégias eficazes na prevenção da doença renal e na desaceleração da progressão para estágios mais avançados da nefropatia (8). No entanto, apesar das medidas adotadas, um número considerável de pacientes ainda desenvolve ND e esta quando presente continua apresentando uma evolução inexorável, embora mais lenta. Portanto, é necessária a identificação de outros fatores de risco e de progressão da ND para se obter uma prevenção e tratamento mais eficientes.

Vêm se acumulando evidências de que fatores dietéticos podem estar também associados ao maior risco de desenvolvimento da ND (9-11). Recentemente, descrevemos que pacientes com DM tipo 2 e microalbuminúria apresentavam níveis séricos mais elevados de ácidos graxos saturados (AGS) e diminuídos de ácidos graxos poliinsaturados (AGPI) (12). Também demonstramos que a substituição da carne vermelha da dieta por carne de galinha diminuiu os níveis de EUA e melhorou o perfil lipídico, assim como reduziu os AGS e aumentou os AGPI (13,14). Estudos epidemiológicos observacionais, demonstraram que uma maior proporção de AGS séricos foi um fator de risco para o desenvolvimento da doença arterial coronariana em adultos de meia idade (15), assim como foi descrita uma associação de AGS com níveis aumentados de marcadores inflamatórios (16) e com a disfunção endotelial (17,18). Por outro lado, os AGPI da dieta, principalmente os ácidos graxos da família n-3, possuem um efeito benéfico sobre a função endotelial (19). Portanto, pode-se concluir que a composição dos ácidos graxos da dieta está relacionada à disfunção endotelial, microalbuminúria e um padrão alimentar rico em AGS se constitui em fator de risco para morte cardiovascular.

Além disto, fatores genéticos estão associados à ND (20-22). Especula-se que possa haver uma interação entre fatores ambientais dietéticos e genéticos criando condições favoráveis para o desenvolvimento da ND. Assim, por exemplo, um aumento da afinidade da proteína intestinal ligadora de ácidos graxos por ácidos graxos cadeia longa, possivelmente por AGS, poderia induzir uma maior elevação sérica destes nutrientes após as refeições.

O papel específico das modificações da dieta no tratamento da ND ainda não está completamente esclarecido, porém dados recentes demonstraram efeitos benéficos de

algumas intervenções dietéticas, como a restrição protéica da dieta na progressão da ND (23) e a retirada da carne vermelha da dieta nos níveis de EUA (12,13).

Nesta revisão será abordado o papel da ingestão de alguns nutrientes como possíveis fatores de risco para ND em pacientes com DM e também serão analisadas as diferentes estratégias de manejo dietoterápico nas fases de micro- e macroalbuminúria no tratamento da ND levando em conta seus possíveis benefícios, riscos e limitações a longo prazo. O tratamento dietoterápico na fase de insuficiência renal terminal do paciente quando em tratamento de substituição da função renal não será aqui abordado.

II. Efeito dos componentes da dieta na função renal e seu papel como fator de risco para a ND

1. Proteínas da dieta

Há cerca de 25 anos, Brenner e cols (24) postularam a hipótese de que a ingestão de proteínas determinava uma vasodilatação em glomérulos com conseqüente aumento da pressão hidrostática capilar e aumento da taxa de filtração glomerular (TFG). Estas alterações hemodinâmicas renais ocorreriam após a cada refeição que contivesse proteínas e manteriam um estado de vasodilatação renal crônica que favoreceria o desenvolvimento de lesões glomerulares em pacientes que já apresentavam algum grau de lesão renal. De acordo com estes autores, alterações da hemodinâmica renal seriam importantes para o início e a progressão da glomeruloesclerose. O aumento da pressão hidrostática intra-capilar e o aumento da TFG alterariam a seletividade da membrana glomerular favorecendo o maior fluxo de proteínas plasmáticas como, por exemplo, a albumina através da parede capilar do glomérulo. Assim, moléculas como as proteínas se acumulariam à nível de mesângio servindo como um estímulo para a produção de matriz mesangial, contribuindo desta maneira, para o processo de glomeruloesclerose (24). De fato, há um conjunto de evidências que sugere que alterações hemodinâmicas renais em pacientes com DM possam constituir fator de risco para o desenvolvimento futuro de ND (25-27).

Confirmando a hipótese de Brenner, acumulou-se um conjunto de dados que indicam que fatores dietéticos podem determinar alterações importantes na hemodinâmica renal. Indivíduos sadios com hábitos alimentares vegetarianos apresentam valores de TFG e EUA menores do que indivíduos que seguem uma dieta usual (omnívoros), embora a

quantidade total de proteína ingerida tenha sido semelhante (28,29). Por outro lado, a ingestão protéica de forma aguda, principalmente proveniente da carne vermelha, causa um aumento da EUA, do fluxo plasmático renal (FPR) e da TFG em humanos independente da restrição protéica na dieta (30). Em pacientes com DM tipo 1 sem ND, uma restrição protéica de sete dias induziu uma redução significativa da TFG (31).

Não apenas a quantidade, mas o tipo de proteína ingerida poderia influenciar a hemodinâmica renal em indivíduos normais e pacientes com DM. Proteínas de diferentes tipos de carne contêm quantidades diferentes de aminoácidos. A carne de gado, por exemplo, possui níveis de glicina elevados quando comparada às outras carnes. Em pacientes com DM tipo 1 sem ND, Pecis e cols (32) observaram que uma dieta normoprotéica a base de carnes de galinha e peixe reduziu a TFG na mesma magnitude do que uma dieta hipoprotéica, ambas comparadas à uma dieta com ingestão protéica usual. Por outro lado, a ingestão aguda de atum determinou um aumento da TFG quando comparada à ingestão de clara de ovo (33), sugerindo que alguns aminoácidos possam ter um papel mais preponderante na hemodinâmica glomerular. De fato, um aumento da TFG e do FPR foi observado após a infusão do aminoácido arginina em indivíduos normais (34).

Estudos epidemiológicos utilizando inquéritos alimentares em pacientes com DM permitiram estabelecer a associação de determinados nutrientes, em especial a quantidade e o tipo de proteína, com o desenvolvimento de ND. Em um estudo clínico transversal, onde 2696 pacientes com DM tipo 1 foram avaliados por meio de de recordatório alimentar, foi observado que aqueles pacientes que relataram um consumo de proteínas inferior a 20% da ingestão energética total da dieta apresentaram valores de EUA inferiores ao daqueles

pacientes com consumo protéico mais elevado (9). Em outro estudo com 300 pacientes com DM tipo 1 normoalbuminúricos, os autores observaram que uma maior ingestão de proteína oriunda de peixe foi associada ao menor risco para a presença de microalbuminúria (10). Não ficou, entretanto, descartado o papel dos AGPI, os quais representam a classe de lipídeos que predomina na carne de peixe.

Portanto, a quantidade de proteína ingerida e o tipo – principalmente a carne vermelha - interferem na hemodinâmica renal, e podem ter um papel no desenvolvimento da ND, provavelmente devido a diferente composição de aminoácidos. Algumas hipóteses levantadas seriam as de que estes aminoácidos estariam agindo tanto na reabsorção de sódio à nível tubular quanto por meio de estimulação da secreção de hormônios como o glucagon e o de crescimento e de substâncias como as prostaglandinas e o fator endotelial de relaxamento (35) e que estas substâncias promoveriam então, a vasodilatação renal e os distúrbios hemodinâmicos associados envolvidos na lesão do órgão.

2. Lipídeos da dieta

A ingestão de lipídeos pode também interferir na hemodinâmica renal e atuar como fator de risco para o desenvolvimento da ND, mas o seu papel tem sido muito menos estudado do que o das proteínas. Em pacientes com DM tipo 1 normotensos e não-proteinúricos a maior ingestão de proteínas e de gordura, especialmente AGS, foi associada à níveis mais elevados da TFG (36).

Watts e cols (37) foram os primeiros a sugerir que o tipo de gordura poderia estar associada à microalbuminúria. Em um estudo caso-controle de pacientes com DM tipo 1, os autores observaram que pacientes com microalbuminúria apresentavam uma ingestão maior de gorduras em geral do que os normoalbuminúricos. Posteriormente, um estudo

transversal com cerca de 178 pacientes com DM tipo 1 constatou que o consumo de AGS estava fortemente associado ao aparecimento da microalbuminúria. Estes autores não encontraram associação de microalbuminúria com a ingestão de proteínas (38). Outros autores também observaram uma associação significativa da ingestão de AGS, em especial do ácido mirístico, com a microalbuminúria (39).

Mais recentemente, um estudo observacional de sete anos de duração em 192 pacientes com DM tipo 1 e 2, normoalbuminúricos e com diferentes graus de comprometimento renal, a ingestão aumentada de AGPI e diminuída de AGS juntamente com uma maior relação AGPI : AGS e ácidos graxos monoinsaturados (AGMI) : AGS na dieta foi associada a algum grau de regressão da ND (11). Por outro lado, os pacientes que apresentaram uma piora da ND se caracterizaram por um padrão dietoterápico inverso, isto é, ingeriam maior quantidade de AGS. Outra evidência da associação de AGS e microalbuminúria é uma observação recente de nosso grupo de que pacientes com DM tipo 2 e microalbuminúria apresentam uma maior proporção de AGS e menor proporção de AGPI na fração sérica de TG do que os indivíduos normoalbuminúricos, independente da dieta (12).

Há algumas evidências também de que alterações do perfil lipídico, especialmente hipercolesterolemia, possam atuar como fator de risco para o desenvolvimento de ND (40-42). Considerando que o tipo de gordura ingerida pode influir no perfil de lipídeos séricos, pode-se supor que o papel deletério dos AGS na função renal poderia ser mediado em parte pelas alterações induzidas nos níveis de colesterol sérico. É bem conhecido que a ingestão aumentada de AGS e diminuída de AGPI é capaz de aumentar os níveis de colesterol sérico (43). Além disto, AGS podem também induzir diretamente disfunção endotelial

(17,18) e conseqüentemente microalbuminúria. Recentemente, foi descrito que a vasodilatação dependente de endotélio apresentava uma relação inversa com a proporção de AGS, especialmente de ácido mirístico em adultos jovens saudáveis (16). Embora o papel dos lipídeos e em especial dos AGS no desenvolvimento da ND seja ainda controverso as evidências apresentadas sugerem haver uma associação de AGS e doença renal no DM. Os mecanismos patogênicos devem ser mais bem esclarecidos.

III. Dietoterapia na Nefropatia Diabética

1. Restrição protéica da dieta

1.1. DM tipo 1

Dietas hipoprotéicas têm sido úteis em retardar a perda da TFG e reduzir a proteinúria em pacientes com ND. Vários ensaios clínicos compararam o efeito de uma restrição protéica sobre a evolução da função renal de pacientes proteinúricos com DM. De uma maneira geral houve uma diminuição da perda da TFG da ordem de 76 a 95% e também da proteinúria de cerca 35% (44-48). Em uma meta-análise dos cinco principais ensaios clínicos realizados entre 1987 e 1993, que incluíram 108 pacientes com DM e ND a restrição protéica determinou uma redução significativa de 44% para o risco de declínio da TFG ou para o aumento da EUA, independente da variação da pressão arterial (49). No entanto, é importante salientar que estes estudos foram realizados em uma época em que o uso de agentes que bloqueiam o sistema-renina-angiotensina (inibidores da enzima conversora da angiotensina ou antagonistas dos receptores da angiotensina II) não era disponível e nem considerado como tratamento de referência para os pacientes com ND. Portanto, é difícil avaliar se o efeito da dieta hipoprotéica ainda persistiria na vigência destes medicamentos. No entanto, mais recentemente, este efeito renoprotetor da dieta hipoprotéica foi confirmado em um estudo prospectivo de intervenção com 82 pacientes com ND conferindo uma redução de 77% no risco de desenvolvimento de insuficiência renal terminal ou morte (risco relativo = 0,23) quando comparada a uma dieta com ingestão protéica usual. Como este estudo foi realizado entre 1996 e 2000 é muito provável que estes pacientes tenham recebido também medicamentos que bloqueiam o sistema-renina-angiotensina (23). As dietas hipoprotéicas empregada nestes estudos, além de conter

menor conteúdo protéico (0,6 g/kg peso), geralmente continham maior quantidade de glicídios e menor quantidade de gorduras em relação à dieta usual dos grupos controle ou da dieta basal do paciente antes de iniciar o estudo. Na maioria dos estudos, a quantidade total de fibras na dieta não variou, pois não havia um aumento do consumo da proteína de origem vegetal, mas sim, apenas diminuição da proteína de origem animal. Entre os minerais, o que geralmente ocorreu, foi uma menor excreção urinária de 24 horas de fósforo e de cálcio. Deve ser lembrado, porém, que os efeitos da restrição protéica sobre o rim não persistem após a suspensão da dieta hipoprotéica, ocorrendo rapidamente um aumento dos níveis de EUA e aumento do declínio da TFG (50).

No entanto, o emprego de uma dieta hipoprotéica a longo prazo apresenta problemas de aderência (51) e de segurança em termos dos seus possíveis efeitos deletérios sobre a nutrição protéica (52,53). Em um estudo de dois anos de duração em apenas 11 pacientes com DM tipo 1 e insuficiência renal a restrição protéica foi mais eficiente em retardar o declínio da TFG no primeiro de ano de tratamento, quando a aderência à dieta hipoprotéica foi maior (51). Em geral, a restrição protéica não determina alterações nutricionais importantes, mas cabe salientar que naqueles estudos onde existe uma perda de peso significativa pode haver diminuição de pré-albumina e albumina séricas (52).

1.2. DM tipo 2

A curto prazo, a restrição protéica da dieta em pacientes com DM tipo 2 (0,8-1,0 g/kg peso) é capaz de reduzir a EUA tanto em pacientes com microalbuminúria (54) como naqueles com proteinúria e nefropatia clínica (55,14). Porém, ainda não existem evidências suficientes de benefício a longo prazo desta conduta dietoterápica. Pijls e cols (56) observaram após seis meses de dieta hipoprotéica uma redução de 14% na EUA em

pacientes com microalbuminúria. Durante a dieta hipoprotéica, houve redução do consumo de proteína animal, porém o de proteína de origem vegetal se manteve o mesmo. Houve também uma tendência à menor ingestão da quantidade de gordura, carboidratos e energia total neste grupo. Frente a estas observações, o presente estudo sugeriu que a restrição protéica responsável pela diminuição da EUA foi a de origem animal e não a de origem vegetal. Entretanto, o prolongamento deste mesmo estudo (57) por um período médio de 16 meses, não demonstrou um efeito benéfico continuado sobre a redução da EUA, provavelmente relacionada a uma diminuição de aderência já observada após os 12 meses de tratamento.

Assim como para os pacientes com DM tipo 1, também não se conseguiu estabelecer com exatidão a quantidade da restrição protéica necessária para se obter um efeito benéfico que retarde a evolução da ND em pacientes com DM tipo 2. Uma abordagem sugerida seria atingir uma ingestão protéica diária da dieta para no máximo entre 1,0 a 0,8 g/kg peso (58). Atualmente, a Associação Americana de Diabetes (ADA) (59) considera adequada a indicação de dieta com restrição moderada de proteínas diária de 0,8 g/kg peso para pacientes com ND sem perda de função renal e de 0,8-1,0 g/kg peso para pacientes ainda em estágio de microalbuminúria. Restrições maiores são indicadas apenas para pacientes que já tenham comprometimento da TFG, para os quais a recomendação é de 0,6 g/kg peso (60). Em casos de síndrome nefrótica é recomendado que o conteúdo protéico da dieta seja restringido a 0,7 g/kg peso com um adicional de 1,0 g/kg peso de proteína de alto valor biológico (AVB) para cada grama de proteína/24 horas perdida na urina considerando valores de proteinúria acima de 5,0 g/24 horas (61). Esta recomendação também é seguida em casos de proteinúria >1 g/24 horas para pacientes

com perda de função renal mais grave (TFG <25 ml/min/ 1,73 m^2) para evitar o balanço nitrogenado negativo. Dietas com restrição protéica de até 0,6 g/kg peso que fornecem ao menos 0,35 g/kg peso de proteínas de AVB asseguram ingestão suficiente de aminoácidos essenciais (61).

2. Dietas com diferentes fontes protéicas

Poucos estudos avaliaram o efeito de diferentes fontes protéicas sobre a função renal em pacientes com DM. Kontessis e cols (62) observaram uma redução da EUA após quatro semanas de uma dieta apenas com proteínas vegetais em pacientes com DM tipo 1 não proteinúricos. Porém, um estudo mais recente que comparou o efeito sobre a função renal de uma dieta com proteína animal e de uma dieta com proteína vegetal da soja em oito pacientes com DM tipo 2 micro- e macroalbuminúricos, não foi capaz de confirmar a hipótese inicial dos autores de que o uso da soja determinaria uma redução da proteinúria (63). Outro estudo recente conduzido em pacientes com DM tipo 2 microalbuminúricos também não encontrou melhora em nenhum dos parâmetros renais avaliados (FPR, TFG e EUA) após comparar o efeito de uma dieta normoprotéica com fontes de proteína vegetal com o efeito de dieta com semelhante conteúdo protéico, mas com predominância de fontes de proteína animal, por um período de seis semanas. Ambas as dietas eram isocalóricas e continham a mesma quantidade de lipídeos totais e de AGS, AGMI e AGPI, de carboidratos, de fibras e de colesterol (64).

Quando a comparação entre os diferentes tipos de proteína foi entre diferentes fontes de proteína animal, os resultados foram mais entusiasmantes. Em pacientes com DM tipo 2 e microalbuminúria, foi demonstrado (13), que uma dieta normoprotéica (1,2 a 1,5 g/kg de peso) - tendo exclusivamente a galinha (partes da coxa e sobrecoxa) como opção

de carne – foi capaz de reduzir a EUA e melhorar o perfil lipídico sérico destes pacientes após 4 semanas de intervenção. Além disto, a dieta à base de carne de galinha reduziu os níveis séricos de colesterol e apo B e foi melhor do que uma dieta hipoprotéica lactovegetariana na redução da EUA. Os efeitos observados com a dieta de galinha podem estar relacionados ao efeito benéfico sobre o perfil lipídico, uma vez que nesta dieta há uma menor ingestão dos AGS, que são um dos determinantes primários dos níveis de colesterol sérico (65,66). A dieta à base de carne de galinha pode representar uma alternativa à dieta hipoprotéica no manejo da progressão da doença renal nestes pacientes com DM e ND.

Conclui-se que o tipo e quantidade de proteína da dieta parecem interferir no surgimento e no curso da doença renal estabelecida. Entretanto, não existem ensaios clínicos a longo prazo, comparando esta conduta dietoterápica com as estratégias atuais de tratamento da ND.

3. Suplementação de ácidos graxos na dieta

Considerando que os AGPI apresentam um efeito benéfico sobre a função endotelial e o perfil lipídico, é possível que a modificação da ingestão dos ácidos graxos da dieta de pacientes com DM e ND poderia ter efeito benéfico também sobre a função renal.

O aumento do conteúdo de ácido linoléico da dieta foi avaliado em um estudo prospectivo com dois anos de seguimento em 36 pacientes com DM tipo 1 com EUA elevada (10 a 200 µg/min) (67). Apesar de ter havido um benefício sobre o perfil lipídico, houve um aumento de 58% na EUA. A administração de óleo de fígado de bacalhau à pacientes com DM tipo 1 e ND não pareceu estar relacionada à alterações na EUA e na TFG (68,69) embora possa haver uma melhora do perfil lipídico e do controle pressórico

em alguns casos (68) e não em outros (69). Apenas dois estudos (70,71) demonstraram haver um efeito benéfico da suplementação de ácidos graxos na dieta - óleo à base de ácidos graxos n-3 - sobre a EUA. Porém, a diminuição da EUA não foi acompanhada de melhora nos perfis lipídico, glicêmico e pressórico após a suplementação. Estes resultados sugerem que a adição de AGPI não é suficiente para reduzir a EUA, mas que também deve haver uma redução da quantidade de AGS da dieta, pois são estes os principais ácidos graxos relacionados à disfunção endotelial e risco de morte cardiovascular (15,17,18).

Portanto, ainda não existem evidências que justifiquem a recomendação do uso de suplementos de ácidos graxos específicos no tratamento nas diversas etapas da ND.

4. Restrição de sódio da dieta

A diminuição dos níveis de pressão arterial usualmente elevados em pacientes com ND é altamente desejável, visto que a hipertensão arterial é um dos principais fatores de risco do desenvolvimento e progressão das complicações micro- e macrovasculares. A restrição moderada de sal determina uma redução da pressão arterial sistólica de 5 mmHg e de 2 mmHg na pressão arterial diatólica (59). Pacientes com DM parecem ser particularmente sensíveis ao efeito do sal nos níveis de pressão arterial, provavelmente pelo fato de terem menor capacidade renal em excretar o sódio (72). Por isso, a ADA recomenda uma ingestão de sódio de 100 mmol/dia (2,4 g de sódio ou 6 g de cloreto de sódio) para indivíduos hipertensos independente da presença de qualquer grau de ND (60). Esta restrição é provavelmente importante já nos estágios iniciais da ND (microalbuminúria), mesmo em indivíduos normotensos. Em pacientes com DM tipo 2, normotensos e microalbuminúricos, a restrição na ingestão de sódio (1,9 vs. 4,8 g de cloreto de sódio) por um período de uma semana reduziu a EUA e a pressão arterial para

valores <130/80 mmHg (73). Além disto, a diminuição do consumo de sódio potencializa o efeito anti-hipertensivo e anti-proteinúrico das drogas antagonistas do receptor de angiotensina em pacientes com DM tipo 2 (74). A magnitude do emprego da restrição de sódio na dieta (1,2-1,7 g de cloreto de sódio) nos níveis de pressão arterial foi similar ao efeito da adição de uma segunda droga anti-hipertensiva, sem os riscos de possíveis efeitos adversos dos medicamentos e de forma mais econômica.

5. Outros nutrientes

O papel de vitaminas antioxidantes como as vitaminas C e E tem sido avaliado na redução da EUA em pacientes com DM com diferentes graus de ND (75,76). A adição destes nutrientes faria parte de um efeito benéfico marcante do tratamento intensificado da ND, onde a redução da progressão da ND é obtida com a associação de antihipertensivos, anti-hiperglicemiantes e hipolipemiantes (77). No entanto, a segurança a longo prazo da suplementação destas vitaminas é questionável e não há quantidade especial indicada na conduta dietoterápica da ND.

6. Outras intervenções dietoterápicas

Dieta restrita em carboidratos e ferro e rica em polifenóis pode estar associada à melhora da sobrevida e atraso na progressão da doença renal em pacientes com ND já estabelecida (78). Esta dieta se caracteriza por uma redução de 50% na quantidade de carboidratos, substituição de fontes protéicas ricas em ferro por fontes protéicas com baixo teor deste mineral e uso de óleo de oliva virgem enriquecido de polifenóis para o preparo e tempero dos alimentos. O efeito desta dieta foi comparado ao de uma dieta com restrição de proteínas conforme recomendação da ADA (0,8 g/kg peso) por um período médio de quatro anos em um estudo randomizado em 191 pacientes com DM tipo 2. Porém, não

houve acompanhamento nutricional em relação à aderência às condutas prescritas. São necessários ainda mais estudos para analisar o possível efeito deste tipo de intervenção alimentar na ND.

IV. Interação genética e dieta

A absorção de ácidos graxos da dieta pela mucosa intestinal ocorre por meio de uma proteína ligadora de ácidos graxos [“Intestinal Fatty Acid Binding Protein” (IFABP)] que é codificada por um gene (FABP2) localizado no braço longo do cromossoma 4. Esta proteína intracelular só é expressa no intestino, e liga-se aos ácidos graxos em uma reação não covalente e saturável. A presença de um polimorfismo no códon 54 deste gene resulta em uma substituição de alanina (A) por treonina (T) na proteína aumentando a sua afinidade para ácidos graxos de cadeia longa e a sua associação com a ND foi recentemente descrita (79). A presença do alelo T-54 no gene FABP2 foi associada também com a presença de níveis elevados de triglicerídeos em pacientes com DM tipo 2 (80) e resistência insulínica (81). Considerando que a ND está associada mais freqüentemente aos componentes da síndrome metabólica (82) e que pacientes com DM tipo 2 e ND apresentam níveis mais elevados de AGS no soro, é lógico supor que a presença do genótipo de risco (TT) possa estar associado a estes fatores de risco para ND e torne estes pacientes mais suscetíveis ao desenvolvimento da mesma. Além disto, os ácidos graxos da dieta podem atuar como moduladores da expressão dos genes que codificam as proteínas reguladoras envolvidas no seu próprio metabolismo (83). Portanto, ácidos graxos específicos poderiam estar de fato envolvidos no processo patogênico da ND. O fator ambiental (dieta rica em AGS) poderia estar interagindo em indivíduos com predisposição genética (genótipo TT) para o desenvolvimento da ND. Esta interação teria conseqüências à nível metabólico ou biomolecular, que estariam fenotipicamente representadas por alterações clínicas e laboratoriais associadas ao aumento do risco cardiovascular: hipertensão arterial, síndrome metabólica, microalbuminúria *per se*, disfunção endotelial e

alterações de marcadores inflamatórios. Estas mesmas alterações clínico-laboratoriais poderiam ser prevenidas ou tratadas de uma maneira mais eficaz a partir da identificação de um genótipo de risco. De fato, observações realizadas em nosso laboratório demonstraram que os pacientes com DM tipo 2 e com o genótipo TT têm um risco 2 vezes maior de desenvolverem ND (79).

V. Conclusões

As evidências disponíveis sugerem que fatores dietéticos podem ter um papel importante no desenvolvimento e na progressão da ND. Atualmente, o efeito benéfico da diminuição da ingestão protéica na evolução da ND apresenta a melhor fundamentação em estudos clínicos bem delineados. No entanto, vêm se acumulando evidências de que a origem da proteína é também muito importante, de igual ou melhor efeito do que o da restrição protéica, o qual pode ser obtido com a retirada da carne vermelha. É necessário também definir com clareza o papel dos ácidos graxos e dos mecanismos pelos quais, os AGS em especial, podem ter um efeito deletério na função endotelial. Finalmente, a interação de fatores alimentares com fatores genéticos poderá auxiliar na identificação de indivíduos de risco e nas estratégias nutricionais que poderiam ser empregadas nestes casos. Deve-se ter em mente a estreita relação entre a presença de ND e o aumento da mortalidade cardiovascular, e idealmente as intervenções dietéticas na ND deveriam também ter um efeito benéfico sobre o perfil lipídico sérico e a função endotelial.

Bibliografia

1. Hovind P, Tarnow L, Rossing P, Jensen BR, Graae M, Torp I et al: Predictors of the development of microalbuminuria and macroalbuminuria in patients with type 1 diabetes: inception cohort study. **Br Med J 2004**;328:1105-8.
2. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR: Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). **Kidney Int 2003**;63:225-32.
3. Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in type 1 (insulin-dependent) diabetes: an epidemiological study. **Diabetologia 1983**;25:496-501.
4. Wong TY, Shankar A, Klein R, Klein BE: Retinal vessel diameters and the incidence of gross proteinuria and renal insufficiency in people with type 1 diabetes. **Diabetes 2004**;53:179-84.
5. U.S. Renal Data System, USRDS 2003 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, **2003**.
6. Bruno RM, Gross JL: Prognostic factors in Brazilian diabetic patients starting dialysis. A 3.6-year follow-up study. **J Diabetes Complications 2000**;14:266-71.
7. Valmadrid CT, Klein R, Moss SE, Klein BE: The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. **Arch Intern Med 2000**;160:1093-100.

8. Gross JL, Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T: Diabetic nephropathy: diagnosis, prevention and treatment. **Diabetes Care 2005**;28:168-76.
9. Toeller M, Buyken A, Heitkamp G, Brämswing S, Mann J, Milne R et al: Protein intake and urinary albumin excretion rates in the EURODIAB IDDM complications study. **Diabetologia 1997**;40:1219-26.
10. Möllsten AV, Dahlquist GG, Stattin E, Rudberg S: Higher intakes of fish protein are related to a lower risk of microalbuminuria in young swedish type 1 diabetic patients. **Diabetes Care 2001**;24:805-10.
11. Cardenas C, Bordiu E, Bagazgoitia J, Calle-Pascual AL; Diabetes and Nutrition Study Group, Spanish Diabetes Association: Polyunsaturated fatty acid consumption may play a role in the onset and regression of microalbuminuria in well-controlled type 1 and type 2 diabetic people: a 7-year, prospective, population-based, observational multicenter study. **Diabetes Care 2004**;27:1454-7.
12. Perassolo MS, Almeida JC, Prá RL, Mello VD, Maia AL, Moulin CC et al: Fatty acid composition of serum lipid fractions in type 2 diabetic patients with microalbuminuria. **Diabetes Care 2003**;26:613–8.
13. Gross JL, Zelmanovitz T, Moulin CC, De Mello V, Perassolo M, Leitão C et al: Effect of a chicken-based diet on renal function and lipid profile in patients with type 2 diabetes. **Diabetes Care 2002**;25:645–51.
14. Azevedo MJ, Mello VD, Zelmanovitz T, Perassolo MS, Gross JL: Reduction of urinary albumin excretion and improvement of lipid profile after a chicken-diet in

macroalbuminuric type 2 diabetic patients. **12th International Congress of Endocrinology, 2004** (Abstract).

15. Ohrvall M, Berglund L, Salminen I, Litchell H, Aro A, Vessby B: The serum cholesterol ester fatty acid composition but not the serum concentration of alpha tocopherol predicts the development of myocardial infarction in 50-year-old men. **Atherosclerosis 1996**;127:65-71.
16. Fernandez-Real JM, Broch M, Vendrell J, Ricart W: Insulin resistanse, inflammation, and serum fatty acid composition. **Diabetes Care 2003**;26:1362-8.
17. Steer P, Vessby B, Lind L: Endothelial vasodilatory function is related to the proportions of saturated fatty acids and alpha-linolenic acid in young men, but not in women. **Eur J Clin Invest 2003**;33:390-6.
18. Sarabi M, Vessby B, Millgard J, Lind L: Endothelium-dependent vasodilation is related to the fatty acid composition of serum lipids in healthy subjects. **Atherosclerosis 2001**;156:349-55.
19. De Caterina R, Liao JK, Libby P: Fatty acid modulation of endothelial activation. **Am J Clin Nutr 2000**;71:213S–23S.
20. Quinn M, Angelico MC, Warram JH, Krolewski AS. Familial factors determine the development of diabetic nephropathy in patients with IDDM. **Diabetologia 1996**;39:940-5.
21. Canani LH, Gerchman F, Gross JL: Familial clustering of diabetic nephropathy in Brazilian type 2 diabetic patients. **Diabetes 1999**;48:909-13.

22. Freedman BI, Tuttle AB, Spray BJ: Familial predisposition to nephropathy in African-Americans with non-insulin-dependent diabetes mellitus. **Am J Kidney Dis 1995**;25:710-3.
23. Hansen HP, Tauber-Lassen E, Jensen BR, Parving HH: Effect of dietary protein restriction on prognosis in patients with diabetic nephropathy. **Kidney Int 2002**;62:220-8.
24. Brenner BM, Meyer TW, Hostetter TH: Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. **N Engl J Med 1982**;307:652-9.
25. Mogensen CE, Christensen CK: Predicting diabetic nephropathy in insulin-dependent patients. **N Engl J Med 1984**;311:89–93.
26. Rudberg S, Persson B, Dahlquist G: Increased glomerular filtration rate as a predictor of diabetic nephropathy: an 8-year prospective study. **Kidney Int 1992**;41:822–8.
27. Silveiro SP, da Costa LA, Beck MO, Gross JL: Urinary albumin excretion rate and glomerular filtration rate in single-kidney type 2 diabetic patients. **Diabetes Care 1998**;21:1521-4.
28. Wiseman NJ, Hunt R, Goodwin A, Gross JL, Keen H, Viberti G: Dietary composition and renal function in healthy subjects. **Nephron 1987**;46:37-42.
29. Kontessis PS, Jones S, Dodds R, Trevisan R, Nosadini R, Fioretto P et al: Renal, metabolic, and hormonal responses to ingestion of animal and vegetable proteins. **Kiney Int 1989**;38:136-44.

30. Viberti G, Bognetti E, Wiseman MJ, Dodds R, Gross JL, Keen H: Effect of protein-restricted diet on renal response to a meat meal in humans. **Am J Physiol 1987**;253:F388-93.
31. Azevedo MJ, Padilha LM, Gross JL. A short-term low-protein diet reduces glomerular filtration rate in insulin-dependent diabetes mellitus patients. **Brazilian J Med Biol Res 1990**;23:647-54.
32. Pecis M, Azevedo MJ, Gross JL: Chicken and fish diet reduces glomerular hyperfiltration in IDDM patients. **Diabetes Care 1994**;17:665-72.
33. Nakamura H, Ito S, Ebe N, Shibata A: Renal effects of different types of protein in healthy volunteer subjects and diabetic patients. **Diabetes Care 1993**;169:1071-5.
34. Hirschberg RR, Zipser RD, Slomowitz LA, Kopple JD: Glucagon and prostaglandins are mediators of amino acid-induced rise in renal hemodynamics. **Kidney Int 1988**;33:1147-55.
35. Woods LL: Mechanisms of renal hemodynamic regulation in response to protein feeding. **Kidney Int 1993**;44:659-75.
36. Bouhanick B, Suraniti S, Berrut G, Blet F, Simard G, Lejeune JJ et al: Relationship between fat intake and glomerular filtration rate in normotensive insulin-dependent diabetic patients. **Diabete Metab 1995**;21:168–72.
37. Watts GF, Gregory I, Naoumova R, Kubal C, Sham KM: Nutrient intake in insulin-dependent diabetic patients with incipient nephropathy. **Eur J Clin Nutr 1988**;42:697-702.

38. Riley MD, Dwyer T: Microalbuminuria is positively associated with usual dietary saturated fat acid intake and negatively associated with usual dietary protein intake in people with insulin-dependent diabetes mellitus. **Am J Clin Nutr 1998**; 67:50-7.
39. Holler C, Abrahamian H, Auinger M: Effect of nutrition on microalbuminuria in patients with type 1 diabetes: prospective data evaluation over 5 years. **Acta Med Austriaca 1999**;26:168-72.
40. Krolewski AS, Warram JH, Christlieb R: Hypercholesterolemia – a determinant of renal function loss and deaths in IDDM patients with nephropathy. **Kidney Int 1994**;45(suppl.45):S125-S31.
41. Appel GB, Radhakrishnan J, Avram MM, DeFronzo RA, Escobar-Jimenez F, Campos MM et al: Analysis of metabolic parameters as predictors of risk in the RENAAL study. **Diabetes Care 2003**;26:1402-7.
42. Jenkins AJ, Lyons TJ, Zheng D, Otvos JD, Lackland DT, McGee D et al: DCCT/EDIC Research Group. Lipoproteins in the DCCT/EDIC cohort: associations with diabetic nephropathy. **Kidney Int 2003**;64:817-28.
43. Mensink RP, Katan MB: Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. **Arterioscler Thromb 1992**;338:911–9.
44. Zeller K, Whittaker E, Sullivan L, Raskin P, Jacobson HR: Effect of restricting dietary protein on the progression of renal failure in patients with insulin-dependent diabetes mellitus. **N Engl J Med 1991**;324:78-84.
45. Walker JD, Bending JJ, Dodds RA, Mattock MB, Murrells TJ, Keen H: Restriction of dietary protein and progression of renal failure in diabetic nephropathy. **Lancet 1989**;16:1411-5.

46. Dullaart RPF, Beusekamp BJ; Meijer S; Van Doormaal JJ; Slutter WJ: Long-term effects of protein-restricted diet on albuminuria and renal function in IDDM patients without clinical nephropathy and hypertension. **Diabetes Care 1993**;16:483-92.

47. Ciavarella A, DiMizio G, Stefoni S, Borgnino L, Vannini P: Reduced albuminuria after dietary protein restriction in insulin-dependent diabetic patients with diabetic nephropathy. **Diabetes Care 1987**;10:407-13.

48. Barsotti G, Ciardella F, Morelli E, Cupisti A, Mantovanelli A, Giovannetti S: Nutritional treatment of renal failure in type 1 diabetic nephropathy. **Clin Nephrol 1988**;29:280-7.

49. Pedrini MT, Levey AS, Lau J, Chalmers TC, Wang PH: The effect of dietary protein restriction on the progression of diabetic and nondiabetic renal diseases: a meta-analysis. **Ann Intern Med 1996**;124:627-32.

50. Hansen HP, Christensen PK, Tauber-Lassen E, Klausen A, Jensen BR, Parving HH: Low protein diet and kidney function in insulin-dependent diabetic patients with diabetic nephropathy. **Kidney Int 1999**;55:621-8.

51. Evanoff G, Thompson C, Brown J, Weinman E: Prolonged dietary protein restriction in diabetic nephropathy. **Arch Intern Med 1989;**149:1129-33.

52. Meloni C, Morosseti M, Suraci C, Pennafina MG, Tozzo C, Gallucci T et al: Severe dietary protein restriction in overt diabetic nephropathy: benefits or risks? **J Renal Nutr 2002**;12:96-101.

53. Brodsky IG, Robbins DC, Hiser E, Fuller SP, Fillyam M, Devlin JT: Effect of low-protein diets on protein metabolism in insulin-dependent diabetic patients with early nephropathy. **J Clin Endocrinol Metab 1992**;75:351-7.

54. Pomerleau J, Verdy M, Garrel DR, Nadeau MH: Effect of protein intake on glycaemic control and renal function in type 2 (non-insulin-dependent) diabetes mellitus. **Diabetologia 1993**;36:829:34.

55. Sugimoto T, Kikkawa R, Haneda M, Shigeta Y: Effect of dietary protein restriction on proteinuria in non-insulin-dependent diabetic patients with nephropathy. **J Nutr Sci Vitaminol 1991**;37:S87-S92.

56. Pijls LTJ, de Vries H, Donker AJM, Van Eijk JThM: The effect of protein restriction on albuminuria in patients with type 2 diabetes mellitus: a randomized trial. **Nephrol Dial Transplant 1999**;14:1445-53.

57. Pijls LTJ, de Vries H, Van Eijk JThM, Donker AJM: Protein restriction, glomerular filtration rate and albuminuria in patients with type 2 diabetes mellitus: a randomized trial. **Eur J Clin Nutr 2002**;56:1200-7.

58. Waugh NR, Robertson AM: Protein restriction for diabetic renal disease. Cochrane Review. In: The Cochrane Library, Issue 1. **2004**. Oxford: Update software.

59. American Diabetes Association. Nutrition principles and recomendations for people with diabetes mellitus. **Diabetes Care 2004**;27(Suppl 1):S36-S46.

60. American Diabetes Association. Nephropathy in diabetes. **Diabetes Care 2004**;27(Suppl 1): S79-S83.

61. Kopple JD. Renal disorders and nutrition. In: Shils ME, Olson JA, Shike M, Ross AC, editors. **Modern Nutrition in Health and Disease**. 9th edition. Baltimore:Williams&Wilkins, 1999:1439-72.

62. Kontessis PS, Bossinakou I, Sarika L, Iliopoulou E, Papantoniou A, Trevisan R et al: Renal, metabolic, and hormonal responses to proteins of different origin in normotensive, nonproteinuric type I diabetic patients. **Diabetes Care 1995**;18: 1233-40.

63. Anderson JW, Blake JE, Turner J, Smith BM: Effect of soy protein on renal function and proteinuria in patients with type 2 diabetes. **Am J Clin Nutr 1998**;68 (Suppl 6):1347S-53S.

64. Wheeler ML, Fineberg SE, Fineberg NS, Gibson RG, Hackward LL: Animal versus plant protein meals in individuals with type 2 diabetes and microalbuminuria: effects on renal, glycemic, and lipid parameters. **Diabetes Care 2002**;25:1277-82.

65. Mello VDF: O papel dos componentes nutricionais de dietas com diferentes fontes protéicas relacionado ao perfil lipídico sérico de pacientes com diabete melito tipo2 com e sem nefropatia diabética. Dissertação de Mestrado apresentada no programa de Pós-Graduação em Ciências Médicas: **Endocrinologia, 2000**

66. Caggiula AW, Mustad VA: Effects of dietary fat and fatty acids on coronary artery disease risk and total and lipoprotein cholesterol concentrations: epidemiologic studies. **Am J Clin Nutr 1997**;65(Suppl 5):1597S-610S.

67. Dullaart RP, Beusekamp BJ, Meijer S, Hoogenberg K, van Doormaal JJ, Sluiter WJ: Long-term effects of linoleic-acid-enriched diet on albuminuria and lipid levels

in type 1 (insulin-dependent) diabetic patients with elevated urinary albumin excretion. **Diabetologia 1992**;35:165-72.

68. Jensen T, Stender S, Goldstein K, HØlmer G, Deckert T: Partial normalization by dietary cod-liver oil of increased microvascular albumin leakage in patients with insulin-dependent diabetes and albuminuria. **N Engl J Med 1989**; 321:1572-77.
69. Rossing P, Hansen BV, Nielsen F, Myrup B, HØlmer G, Parving HH: Fish oil in diabetic nephropathy. **Diabetes Care 1996**;11:1214-19.
70. Hamazaki T, Takazakura E, Osawa K, Urakaze M, Yano S: Reduction in microalbuminuria in diabetics by eicosapentaenoic acid ethyl ester. **Lipids 1990**; 25:541-5.
71. Shimizu H, Ohtani K, Tanaka Y, Sato N, Mori M, Shimomura Y: Long-term effect of eicosapentaenoic acid ethyl (EPA-E) on albuminuria of non-insulin dependent diabetic patients. **Diabetes Res Clin Pract 1995**;28:35-40.
72. DeFronzo RA: The effect of insulin on renal sodium metabolism. A review with clinical implications. **Diabetologia 1981**;21:165-71.
73. Imanishi M, Yoshioka K, Okumura M, Konishi Y, Okada N, Morikawa T et al: Sodium sensitivity related to albuminuria appearing before hypertension in type 2 diabetic patients. **Diabetes Care 2001**;24:111-6.
74. Houlihan CA, Allen TJ, Baxter AL, Panangiotopoulos S, Casley DJ, Cooper ME, et al: A low-sodium diet potentiates the effects of losartan in type 2 diabetes. **Diabetes Care 2002**;25:663-71.

75. Gaede P, Poulsen HE, Parving HH, Pedersen O: Double-blind, randomised study of the effect of combined treatment with vitamin C and E on albuminuria in Type 2 diabetic patients. **Diabetic Medicine 2001**;18:756-60.

76. McAuliffe AV, Brooks BA, Fisher EJ, Molyneaux LM, Yue DK: Administration of ascorbic acid and an aldose reductase inhibitor (tolrestat) in diabetes: effect on urinary albumin excretion. **Nephron 1998**;80:277-84.

77. Gæde P, Vedel P, Parving HH, Pedersen O: Intensified multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: the Steno type 2 randomised study. **Lancet 1999**;353:617-22.

78. Facchini FS, Saylor KL: A low-iron-available, polyphenol-enriched, carbohydrate-restricted diet to slow progression of diabetic nephropathy. **Diabetes 2003**;52:1204-9.

79. Canani LH, Costa LA, Maia AL, Gross JL. Associação do polimorfismo A54T do gene FABP2 e nefropatia diabética em pacientes com diabete melito tipo 2. **12º Congresso Latino Americano de Diabetes, 2004** (Abstract).

80. Georgopoulos A, Aras O, Tsai M: Codon-54 polymorphism of the fatty acid-binding protein 2 gene is associated with elevation of fasting and postprandial triglycerides in type 2 diabetes. **J Clin Endocrinol Metab 2000**;85:3155–60.

81. Baier LJ, Sacchettini JC, Knowler WC, Eads J, Paolisso G, Tataranni PA, et al: An amino acid substitution in the human intestinal fatty acid binding, increased fat oxidation, and insulin resistance. **J Clin Invest 1995**; 95:1281-7.

82. Costa LA, Canani LH, Lisboa HRK, Tres GS, Gross JL. Aggregation of features of the metabolic syndrome is associated with increased prevalence of chronic complications in type 2 diabetes. **Diabetic Med 2004**;21:252-4.

83. Pégorier JP, LeMay C, Girard J: Control of gene expression by fatty acids. **J Nutr 2004**;134:2444S-9S.

Capítulo II:

Withdrawal of red meat from the usual diet reduces albuminuria and improves the lipid profile in type 2 diabetic patients with macroalbuminuria

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Withdrawal of red meat from the usual diet reduces albuminuria and improves the lipid profile in type 2 diabetic patients with macroalbuminuria

Running title: Chicken and low-protein diet reduces macroalbuminuria

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Abstract

Objective - To assess the effect of replacing red meat of the usual diet with chicken (CD) and of a low-protein diet (LPD) on the renal function and lipid profile in macroalbuminuric type 2 diabetic patients.

Research design and methods - A crossover controlled trial was conducted in 17 patients with type 2 diabetes and macroalbuminuria [24-hour urinary albumin excretion rate (UAER) ≥200 μg/min] (14 male; age: 59 ± 11 years). Each patient followed for a 4-week period: their usual diet (UD), a CD and a lacto-vegetarian LPD, with a 4-week washout period. At the end of each diet glomerular filtration rate, UAER, lipids and fatty acids profile, glycemic control indices, body mass index and blood pressure were measured.

Results – Compared to UD, CD and LPD induced a reduction in UAER [CD: 269.4 (111-1128) μg/min, LPD: 229.3 (76.6-999.3) μg/min, UD: 312.8 (223.7-1223.7) μg/min; P <0.01], and in non-HDL cholesterol [CD (3.92 ± 0.99 mmol/l); LPD (3.92 ± 0.93 mmol/l; UD: 4.23 ± 1.06 mmol/l; P<0.05)], and an increase in serum total polyunsaturated fatty acids (CD: 39.8 ± 2.6%; LPD: 39.7 ± 4.4%; UD: 37.3±3.1%; P <0.05). Triglycerides were lower [1.22 (0.5-3.88) mmol/l; P <0,05] after CD than after UD [1.46 (0.6-4.73) mmol/l] and LPD [1.51 (0.62-7.35) mmol/l].

Conclusion – Withdrawing red meat from the diet, either by replacing it with chicken or by following a LPD, reduced UAER and had beneficial effect on lipid and fatty acid profile in macroalbuminuric patients with type 2 diabetes and may represent an additional strategy for treatment of patients with diabetic nephropathy.

Introduction

Diabetic Nephropathy (DN) develops in approximately 40% of patients with diabetes and is still the leading cause of chronic kidney disease in patients starting renal replacement therapy (1). Furthermore it is associated with increased cardiovascular mortality (2). The available therapeutic strategies consist of achieving the best metabolic control, treating hypertension, using drugs with blockade of the renin-angiotensin-aldosterone system, and treating dyslipidemia. They are effective in delaying the progression to more advanced stages of nephropathy, and also in reducing cardiovascular mortality in diabetic patients (3). Although some patients may regress to early stages of DN, progressive decline of glomerular filtration rate (GFR) is still observed and therefore additional strategies are necessary to arrest the progression of macroalbuminuria. A low-protein diet slows down the decline of renal function in proteinuric patients with type 1 diabetes (4) and ameliorates the DN prognosis and death in these patients (5). However, the long-term safety of this diet is not firmly established nor its effect in type 2 diabetic patients with macroalbuminuria.

Higher intake of fish protein has a protective effect for the development of microalbuminuria in young patients with type 1 diabetes (6). We have previously described that replacing red meat with chicken in the usual diet reduced UAER by 46%, and also improved serum lipid profile in patients with type 2 diabetes and microalbuminuria (7). Therefore, this study was conducted to assess the effect of replacing the red meat from the diet with chicken and the effect of a lacto-vegetarian low-protein diet on the renal function and lipid profile in patients with type 2 diabetes and macroalbuminuria.

Research design and methods

Patients

This study was conducted in patients with type 2 diabetes defined according to World Health Organization criteria and attending the Endocrine Division's outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil. Patients were selected according to the following criteria: age <75 years, body mass index (BMI) <30 kg/m^2, urinary albumin excretion (UAER) ≥200 μg/min confirmed at least twice in a 6-month period, serum triglycerides <4.52 mmol/l, normal liver and thyroid function, compliance with diabetes treatment (A_{1c} test <10%), serum creatinine levels ≤132.6 mmol/l, proteinuria <3.0g/24-hour, absence of urinary tract infection or other renal diseases, symptomatic autonomic neuropathy and heart failure. To avoid potential confounding factors on the effect of diets on renal function and lipid profile, none of the patients were using ACE inhibitors, ARBs or hypolipidemic agents during the study. Eligible patients entered a run-in period of approximately 2 months, during which they were oriented to achieve the best possible metabolic control through dietary and oral antidiabetic agents or insulin adjustments. The antihypertensive and antidiabetic drugs were maintained during the study. Each patient received standardized nutritional guidelines from a nutritionist (V.D.F.M.) following American Diabetes Association (ADA) recommendations (8). At the end of the run-in period, patients underwent a clinical and laboratory evaluation. BMI [weight (kg)/height^2 (m)] was calculated. 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 or use of antihypertensive drugs.

Study design

This study followed a crossover, controlled clinical trial design. The Ethics Committee at Hospital de Clínicas approved the protocol and all patients gave their written informed consent. After the run-in period, as shown in Fig.1, patients were assigned to follow three types of diets for a period of 4 weeks each: the patient's usual diet (UD), a low-protein diet (LPD) and a chicken diet (CD), with a 4-week washout period between them. During the washout period the patients maintained their UD. All participants were instructed to maintain their usual physical activities and not to make any changes in their lifestyles or medications throughout the study period. Consecutive eligible patients were randomly assigned to one of the sequence of diets as follows: 1) UD, LPD, CD; 2) UD, CD, LPD; 3) LPD, UD, CD; 4) LPD, CD, UD; 5) CD, LPD, UD and 6) CD, UD, LPD. The primary outcome measures were UAER, serum lipids and fatty acids profile. Since there were no studies on the effect of the chicken diet on UAER in macroalbuminuric patients with diabetes, the sample size was estimated based on the UAER reduction after a LPD in macroalbuminuric patients (9). To obtain a UAER reduction of 34.6%, it was estimated that 15 macroalbuminuric patients had to be included considering an $\propto = 0.05$ and $\beta = 0.80$.

Diet composition and prescription

The dietary adjustments on patients' UD during the run-in period were designed to meet the nutritional ADA´s recommendations, but the usual amount and source of protein from each individual patient were not modified (1.2 to 1.5g/kg body wt). All prescribed diets were isoenergetic, with the same proportion of lipids [30% (26-35%)], and the UD and CD were isoproteic [20% (17-25%)]. The CD was created by replacing the red meat of the UD with dark chicken meat (skinless leg quarter). The protein content of the LPD was

0.5 to 0.8 g/kg per day (vegetable and dairy protein only). No food was supplied to patients. Compliance was assessed by means of 2-day weighed diet records and 24-hour urinary nitrogen output at the end of the second and the fourth weeks, as previously reported (10). Dietary macronutrients and micronutrients from diet records were analyzed using the Nutribase 98 Clinical Nutritional Manager software (Cybersoft Phoenix, AZ) (11). The composition of the diets followed by the patients and assessed by the weighed record method were expressed as a percentage of total daily energy for macronutrients and as an absolute amount for the other nutrients.

Laboratory measurements

GFR was measured using the ^{51}Cr-EDTA single-injection technique (coefficient of variation 12%. GFR reference range: 72-137.5 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}). Urinary albumin was measured in 24-hour timed sterile urine samples by immunoturbidimetry (Sera-Pak immuno microalbuminuria; Bayer, Tarrytown, NY; mean intra-assay and interassay coefficients of variation 4.6 and 7.5 %, respectively).

Plasma glucose level was determined by a glucose oxidase method, serum creatinine level by the Jaffé reaction, A_{1c} test by ion-exchange high-performance liquid chromatography (Merck-Hitachi L-9100 glycated hemoglobin analyzer, reference range 4.7 – 6.0%; Merck, Darmstadt, Germany) and fructosamine by a colorimetric method (reference range 1.87 – 2.87 mmol/l). Urinary urea was measured by an enzymatic ultraviolet method (mean intra-assay coefficient of variation 3.8%). The nitrogen intake was estimated assuming patients presented a nitrogen balance (12) and protein intake was calculated by the following formula: protein intake (g/day) = nitrogen intake x 6.25.

Serum total cholesterol and triglycerides were determined by enzymatic-colorimetric methods (Merck Diagnostica, Darmstadt, Germany; Boeringher Mannheim, Buenos Aires, Argentina) and HDL cholesterol by a direct selective inhibition method. LDL cholesterol was calculated using Friedewald's formula (LDL = total cholesterol – HDL – triglycerides/5). Non-HDL cholesterol was determined according to the difference between total and HDL cholesterol.

The fatty acid (FA) composition on serum total lipids was determined by extraction with chloroform-methanol (2:1; by volume) according to the method of Folch et al (13) as previously described (13) but without separating the lipid fractions. The relative amount of each FA (% of total FAs) was quantified by integrating the area under the peak and dividing the result by the total area for all FAs.

Statistical analysis

Differences among the three types of diets were tested by repeated-measures ANOVA, followed by pairwise comparisons between treatments using Bonferonni's correction. Non-parametric variables were log transformed before analysis. Pearson or Spearman correlation coefficients were used to test the associations between variables after diets or between changes in these variables after CD and LPD compared to UD. P values < 0.05 were considered statistically significant. Results were expressed as means ± SD, as median (range) or as mean (95% CI). SPSS software 10.0 version (SPSS, Chicago, IL) was used for the analyses.

Results

Patient characteristics

Sixty-nine eligible patients were invited to enter the study protocol. Forty-seven patients were not included for several reasons (dislike of chicken and/or red meat, difficulties with: dietary records skills, appointments schedule and following dietary prescription) and 19 of the 22 patients that followed the run-in period were randomized to one of the six diet sequences described in Fig. 1. All the randomized patients completed the study protocol, but two patients were not included in the final analysis: one patient developed a proteinuria of >3g/24-hour and another spontaneously regressed to microalbuminuria, during the study. Seventeen patients were then included in the final analysis. Patients were 59 ± 11 years old, had a BMI of 26.2 ± 2.6 kg/m^2, a mean blood pressure of 93.7 ± 8.5 mmHg, and a reasonable metabolic control [fasting glucose level: 8.04 ± 3.27 mmol/l and $A1_c$ test: 7.6 ± 2.6 %; total cholesterol: 5.34 ± 0.93 mmol/l, HDL cholesterol: 1.17 ± 0.18 mmol/l, LDL cholesterol: 3.42 ± 0.91 mmol/l, non-HDL cholesterol: 4.23 ± 1.01 mmol/l and triglycerides: 1.57 (0.88-3.27)]. Fourteen of the 17 patients analysed were male, 8 were hypertensive, 10 had some evidence of diabetic retinopathy, which was proliferative in 4 patients, four patients had coronary artery disease diagnosis and only two patients were smokers. Two of the three women were postmenopausal but none were using hormone replacement therapy. Most of the patients were treated with antidiabetic agents and/or insulin, and the oral antidiabetic agents mostly used were sulfonylurea and metformin. Anti-hypertensive treatment used was calcium channel blockers (n = 4), diuretics (n = 4), beta-blockers (n = 4) and direct vasodilators (n

= 1) or combination of medications: diuretic and calcium channel blockers (n = 1); diuretic and beta-blockers (n = 3); beta-blockers and direct vasodilators (n = 1).

Composition of the diets

The intake of polyunsaturated fatty acids (PUFA) and PUFA to saturated fatty acids (SFA) ratio were higher in the CD (PUFA: 9.3 ± 2.5%; PUFA to SFA ratio: 1.24 ± 0.32) and in the LPD (PUFA: 9.5 ± 2.6%; PUFA to SFA ratio: 1.13 ± 0.43) when compared to the UD (PUFA: 8.1 ± 2.6%; PUFA to SFA ratio: 0.96 ± 0.44; P = 0.022). SFA intake was lower in the CD (7.8 ± 2.0%) and in the LPD (8.7 ± 2.1%) compared to the UD (9.2 ± 2.5%), but did not reach conventional statistical significance (P = 0.103). Total energy intake was lower only during the LPD (1634 ± 451 kcal; P < 0.001) when compared to the UD (1901 ± 480 kcal) and the CD (1870 ± 452 kcal). As expected, protein content was lower during the LPD (11.6 ± 1.5%; P < 0.0001) compared to the CD (21.2 ± 3.9%) and the UD (21.9 ± 3.4%), without difference between the UD and the CD. During LPD, the patients' intake of carbohydrate (58.7 ± 6.8%) and total fiber (27.0 ± 8.1%) were higher compared to both UD and CD [carbohydrate: UD 46.9 ± 6.7%; CD 50.0 ± 7.0% (P < 0.0001), total fiber: UD 20.0 ± 7.5%; CD 22.8 ± 9.3% (P = 0.0001)]. Cholesterol, zinc, iron and phosphorus intake, however, were lower in the LPD compared to the other two diets and total fat, monounsaturated fatty acids (MUFA), potassium, sodium and calcium intake were not different between diets (data not shown).

When all diets were evaluated, total protein intake (g/kg weight per day) as assessed by nitrogen output, was high correlated to that estimated by weighed-diet records (r = 0.749; P < 0.001).

Effect of the diets on renal function

Renal parameters after the diets are described in Table 1. UAER levels were significantly lower after the CD and the LPD as compared to the UD. The relative difference of UAER levels after the CD (20.6%; 95% CI: 4.8 – 36.4) and the LPD (31.4%; 95% CI: 12.7 – 50) from UAER levels after the UD was significant (P = 0.014 and P = 0.003, respectively). The reduction after the CD and the LPD was similar (P = 0.249). Three patients (17.6%) after the CD and 8 patients (47.1%) after the LPD had a regression of UAER levels to the microalbuminuria range (P = 0.143). GFR values did not change after the three diets.

Effect of the diets on serum lipids

In general, the lipid profile improved after the CD and the LPD (Table 1). Total and LDL cholesterol had a borderline reduction after the CD (P = 0.067) and the LPD (P = 0.123) and only non-HDL cholesterol levels had a significant and similar reduction after the CD and the LPD compared to the UD. Triglycerides levels were lower only after the CD. HDL cholesterol levels were not different after the diets.

Fatty acid composition after diets

Fatty acid composition of serum total lipids is depicted in Table 2. The total PUFA proportion was higher after the CD and the LPD, than after the UD. Total SFA were lower after the CD and the LPD, although without reaching conventional statistical significance (P = 0.068). The proportion of palmitic acid was lower after the CD compared to the UD and the LPD, and stearic acid only after the LPD compared to the UD. The ratio of SFA to total n-6 FAs was lower after the CD and the LPD compared to the UD, but did not reach conventional statistical significance (P = 0.059). After the UD, there was a negative correlation between UAER levels and the ratio of PUFA to SFA in serum total lipids (r = -

0.628; P = 0.022), as well as a strong positive correlation of UAER levels and the ratio of SFA to total n-3 (r = 0.713; P = 0.006). Considering the fatty acid profile after the CD and the LPD together, significant correlations were observed between UAER reduction after diets and increase of both eicosapentaenoic acid (r_s = 0.498, P = 0.010) and total n-3 FAs (r_s = 0.416; P = 0.035). A positive correlation was also found between reduction in UAER and reduction in the ratio of SFA to total n-3 FAs after the CD and the LPD (r_s = 0.432; P = 0.032).

Effect of diets on glycemic control, blood pressure levels and nutritional indices

No difference was observed in glycemic control indices at the end of each diet as evaluated by fasting plasma glucose (UD = 6.33 ± 2.31 mmol/l, CD = 6.66 ± 1.93 mmol/l and LPD = 5.89 ± 2. 51 mmol/l; P = 0.467) and fructosamine (UD = 3.7 ± 0.7 mmol/l, CD = 3.4 ± 0.6 and LPD = 3.6 ± 0.7 mmol/l; P = 0.119). Mean blood pressure levels were also similar after the diets (UD = 86.9 ± 10.1 mmHg, CD = 87.3 ± 11.2 mmHg and LPD = 88.3 ± 9.5 mmHg; P = 0.726). BMI did not differ after the UD (26.1 ± 2.5 kg/m^2) and the CD (26.0 ± 2.6 kg/m^2), but it was lower after the LPD (25.7 ± 2.7 kg/m^2; P < 0.05).

Conclusions

In this sample of macroalbuminuric type 2 diabetic patients the CD and the LPD induced a significant reduction in UAER levels, an increase in the serum proportion of PUFA and a decrease in serum SFA. Moreover, there was an improvement in the lipid profile.

The UAER reduction observed in this study in macroalbuminuric type 2 diabetic patients after the LPD are in accordance with previous observations in macroalbuminuric patients with type 1 diabetes (4), which reported an average reduction of 30% in UAER after a low-protein diet intervention. Although less remarkable, the decrease in UAER and serum cholesterol levels after the CD observed in these macroalbuminuric type 2 diabetic patients extends our previous results in patients with microalbuminuria (7).

The reduction of UAER after the LPD and the CD might be related to the decrease of proportion of serum SFA and an increase in the proportion of serum PUFA, due to the withdraw of red meat from the diet. Red meat consumed by our patients had approximately 15% of fat and the composition of this fat was: SFA: 40%; MUFA: 45% and PUFA: 5% (11). A high proportion of serum SFA has been considered a risk factor for coronary artery disease in middle aged healthy men (15), and it was also associated with an increase of inflammatory markers levels (16) and endothelial dysfunction (17,18). In type 2 diabetes, increased UAER, endothelial dysfunction and chronic inflammation are interrelated processes and are strongly associated with the risk of death (19). In fact, in this study we observed a negative correlation between the ratio of PUFA to SFA in serum lipids and UAER after the UD, suggesting that the relative proportion of these FAs influenced UAER. Furthermore, the observed positive correlation of the increased proportion of serum total n-3 FAs and especially eicosapentaenoic acid with UAER reduction after the

CD and the LPD reinforces the suggestion that an increased proportion of PUFA might have a favorable effect on the endothelial function and is in accordance with previous observations (20) that purified eicosapentaenoic acid administration decreased albumin excretion in patients with type 2 diabetes and DN. Interestingly, a higher intake of PUFA and lower intake of SFA were also recently reported to be associated with DN regression in patients with type 1 and type 2 diabetes (21).

The improvement of cholesterol levels may also have contributed to the reduction of UAER after the LPD and the CD. Lipid reduction by hypolipidemic agents might preserve GFR and decrease proteinuria in diabetic patients (22). The decrease in non-HDL cholesterol was probably related to the lower intake of SFA and the higher intake of PUFA which is known to reduce cholesterol levels (23)

The possible limitations of this study were the crossover design, with the possibility of a carry-over effect. This probably did not interfere in the observed results because the diets had a 4-week washout period between them, and this seems to be enough to wean patients from the previous diet. Moreover, plasma lipids and lipoproteins reach stable values after a period of 3-4 weeks of dietary modification (24) and when comparing data from baseline and after the UD intervention, there were no differences regarding BMI, glycemic and lipids control indices (data not shown). Compliance with the diets was probably adequate since its assessment by weighed-diet records method and urea measurements performed during each diet in our study showed a good correlation between these two tools. Another aspect was that multiple correlations were performed in our study, especially between FAs and UAER, and this might lead to statistically significant results

only by chance. However, the results observed on serum FAs profile correlations with UAER, are consistent with the intervention performed.

In conclusion, these results indicate that the withdrawal of red meat from the diet, either replacing it with chicken or following a lacto-vegetarian low-protein diet, promotes a beneficial effect on reno- and cardiovascular risk factors associated with DN in patients with type 2 diabetes and macroalbuminuria, and may represent an additional therapeutic strategy.

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References

1. U.S. Renal Data System, USRDS 2003 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2003
2. Valmadrid CT, Klein R, Moss SE, Klein BE: The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. *Arch Intern Med* 160:1093-1100, 2000
3. Gross JL, Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T: Diabetic nephropathy: diagnosis, prevention and treatment. *Diabetes Care* 28:168-176, 2005
4. Pedrini MT, Levey AS, Lau J, Chalmers TC, Wang PH: The effect of dietary protein restriction on the progression of diabetic and nondiabetic renal diseases: a meta-analysis. *Ann Intern Med* 124:627-632, 1996
5. Hansen HP, Tauber-Lassen E, Jensen BR, Parving HH: Effect of dietary protein restriction on prognosis in patients with diabetic nephropathy. *Kidney Int* 62:220-228, 2002
6. Möllstein AV, Dahlquist GG, Stattin EL, Rudberg S: Higher intakes of fish protein are related to a lower risk of microalbuminuria in young Swedish type 1 diabetic patients. *Diabetes Care* 24:805-810, 2001
7. Gross JL, Zelmanovitz T. Moulin CC, De Mello V, Perassolo M, Leitão C, Hoefel A, Paggi A, Azevedo ML: Effect of a chicken-based diet on renal function and lipid profile in patients with type 2 diabetes: a randomized crossover trial. *Diabetes Care* 25:645-651, 2002

8. Franz MJ, Horton ES, Bantle JP, Beebe CA, Brunzell JD, Coulston AM, Henry RR, Hoogwerf BJ, Stacpoole PW: Nutrition principles for the management of diabetes and related complications (Technical Review). *Diabetes Care* 17: 490-518, 1994
9. Bending JJ, Dodds RA, Keen H, Viberti GC: Renal response to restricted protein intake in diabetic nephropathy. *Diabetes* 37: 1641-1646, 1988.
10. Moulin CC, Tiskievicz F, Zelmanovitz, Oliveira J, Azevedo MJ, Gross JL: Use of weighed diet records in the evaluation of diets with different protein contents in patients with type 2 diabetes. *Am J Clin Nutr* 67: 853-857, 1998
11. United States Department of Agriculture, Agriculture Research Service: US Department of Agriculture Nutrient Data Base for Standard Reference, Release 14. Washington, DC: US Department of Agriculture, 2001 (USDA Handbook No 8)
12. Maroni BJ, Steinman TI, Mitch WE: A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 27:58-65, 1985
13. Folch J, Less M, Stanley S: A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497-509, 1957
14. Perassolo MS, Almeida JC, Prá RL, Mello VD, Maia AL, Moulin CC, Camargo JL, Zelmanovitz T, Azevedo MJ, Gross JL: Fatty acid composition of serum lipid fractions in type 2 diabetic patients with microalbuminuria. *Diabetes Care* 26:613–618, 2002
15. Ohrvall M, Berglund L, Salminen I, Litchell H, Aro A, Vessby B: The serum cholesterol ester fatty acid composition but not the serum concentration of alpha tocopherol predicts the development of myocardial infarction in 50-year-old men. *Atherosclerosis* 127:65-71, 1996

16. Fernandez-Real JM, Broch M, Vendrell J, Ricart W: Insulin resistanse, inflammation, and serum fatty acid composition. *Diabetes Care* 26:1362-1368, 2003

17. Steer P, Vessby B, Lind L: Endothelial vasodilatory function is related to the proportions of saturated fatty acids and alpha-linolenic acid in young men, but not in women. *Eur J Clin Invest* 33:390-396, 2003

18. Sarabi M, Vessby B, Millgard J, Lind L: Endothelium-dependent vasodilation is related to the fatty acid composition of serum lipids in healthy subjects. *Atherosclerosis* 156:349-355, 2001

19. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH: Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. *Diabetes* 51:1157-1165, 2002

20. Shimizu H, Ohtani K, Tanaka Y, Sato N, Mori M, Shimomura Y: Long-term effect of eicosapentaenoic acid ethyl (EPA-E) on albuminuria of non-insulin dependent diabetic patients. *Diabetes Res Clin Pract* 28:35-40, 1995

21. Cardenas C, Bordiu E, Bagazgoitia J, Calle-Pascual AL; Diabetes and Nutrition Study Group, Spanish Diabetes Association: Polyunsaturated fatty acid consumption may play a role in the onset and regression of microalbuminuria in well-controlled type 1 and type 2 diabetic people: a 7-year, prospective, population-based, observational multicenter study. *Diabetes Care* 27:1454-1457, 2004

22. Fried LF, Orchard TJ, Kasiske BL: Effect of lipid reduction on the progression of renal disease: a meta-analysis. *Kidney Int* 59:260-269, 2001

23. Mensink RP, Katan MB: Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler Thromb* 338: 911–919, 1992.
24. Kris-Etherton P, Dietschy J: Design criteria for studies examining individual fatty acids effects on cardiovascular disease risk factors: human and animal studies. *Am J Clin Nutr* 65 (Suppl.):1590S-1596S, 1997

Table 1 - ***Renal function and serum lipid profile after diets***

	Usual diet	Chicken diet	Low-protein diet	*P*
UAER (μg/min)	312.8 (223.7-1223.7)	269.4 (111-1128)	229.3 (76.6-999.3)	0.0003*
GFR ($ml \cdot min^{-1} \cdot 1.73\ m^2$)	81.8 ± 22.2	83.3 ± 26.1	81.9 ± 25.3	0.860
Total cholesterol (mmol/l)	5.37 ± 1.18	5.08 ± 0.96	5.06 ± 0.91	0.069
HDL cholesterol (mmol/l)	1.14 ± 0.26	1.14 ± 0.23	1.14 ± 0.21	0.989
LDL cholesterol (mmol/l)	3.48 ± 0.88	3.27 ± 0.78	3.16 ± 0.75	0.123
Non-HDL cholesterol (mmol/l)	4.23 ± 1.06	3.92 ± 0.99	3.92 ± 0.93	0.042*
Triglycerides (mmol/l)	1.46 (0.6-4.73)	1.22 (0.5-3.88)	1.51 (0.62-7.35)	0.0121†

Data are means ± SD or median (range). *P* referes to repeated measures ANOVA. * Bonferonni´s correction: usual diet versus chicken and low-protein diets ($P < 0.01$ and $P < 0.001$, respectively); † chicken diet versus usual and low-protein diets ($P < 0.01$).

Table 2 - ***Serum fatty acid composition of total lipids after diets***

	Usual diet	Chicken diet	Low-protein diet	*P*
Total SFA	40.6 ± 3.8	37.8 ± 2.5	38.8 ± 3.4	0.0680
16.0 Palmitic	30.8 ± 9.3	28.1 ± 2.2	30.5 ± 3.5	0.0490[*]
18.0 Stearic	9.2 ± 1.4	8.8 ± 1.5	7.9 ± 1.3	0.0250[†]
Total MUFA	22.2 ± 2.6	22.5 ± 2.2	21.6 ± 3.06	0.5910
16.1 Palmitoleic	3.5 (0-4.24)	2.9 (0-4.84)	2.17 (0-3.58)	0.0940
18.1 Oleic	18.4 ± 1.5	18.6 ± 1.6	19.0 ± 2.6	0.6300
Total PUFA	37.3 ± 3.1	39.8 ± 2.6	39.7 ± 4.4	0.0290[‡]
18.2 n-6 Linoleic	27.6 ± 2.6	28.2 ± 2.4	29.2 ± 4.0	0.2480
18.3 n-6 Linolenic	0 (0-0.86)	0 (0-0.78)	0 (0-0.58)	0.549
18.3 n-3 Linolenic	0.04 (0-0.54)	0 (0-1.68)	0.44 (0-1.38)	0.0690
20.4 n-6 Arachidonic	5.96 ± 1.48	6.43 ± 1.06	5.19 ± 1.24	<0.0001[§]
20.5 n-3 Eicosapentaenoic	0.40 (0.18-0.82)	0.43 (0.16–0.62)	0.36 (0.16–0.86)	0.5380
22.6 n-3 Docosahexaenoic	1.64 (0.13-3.8)	2.22 (0.85-4.84)	1.83 (0.39-7.83)	0.4150
Total n-6 fatty acids	34.8 ± 2.7	37.0 ± 1.8	36.3 ± 4.8	0.1290
Total n-3 fatty acids	2.68 (0.19-4.9)	2.08 (1.43-5.79)	3.1 (1.3-8.24)	0.6610
PUFA/SFA ratio	0.96 ± 0.16	1.05 ± 0.11	1.04 ± 0.19	0.1840
SFA/total n-6 fatty acids ratio	1.17 ± 0.17	1.03 ± 0.10	1.09 ± 0.21	0.0590
SFA:total n-3 fatty acids ratio	15.5 (7.5-36.1)	13.7 (6.3-28.6)	13.0 (4.4-29.0)	0.6740
MUFA/SFA	0.56 ± 0.10	0.60 ± 0.09	0.56 ± 0.10	0.4110

Data are means ± SD or median (range). *P* referes to repeated measures ANOVA. [*] Bonferonni's correction: chicken diet versus usual and low-protein diets (P <0.05). [†] low-protein versus usual diet (P <0.01); [‡] usual diet versus chicken and low-protein diets (P < 0.05); [§] low-protein diet versus usual and chicken diets (P <0.05).

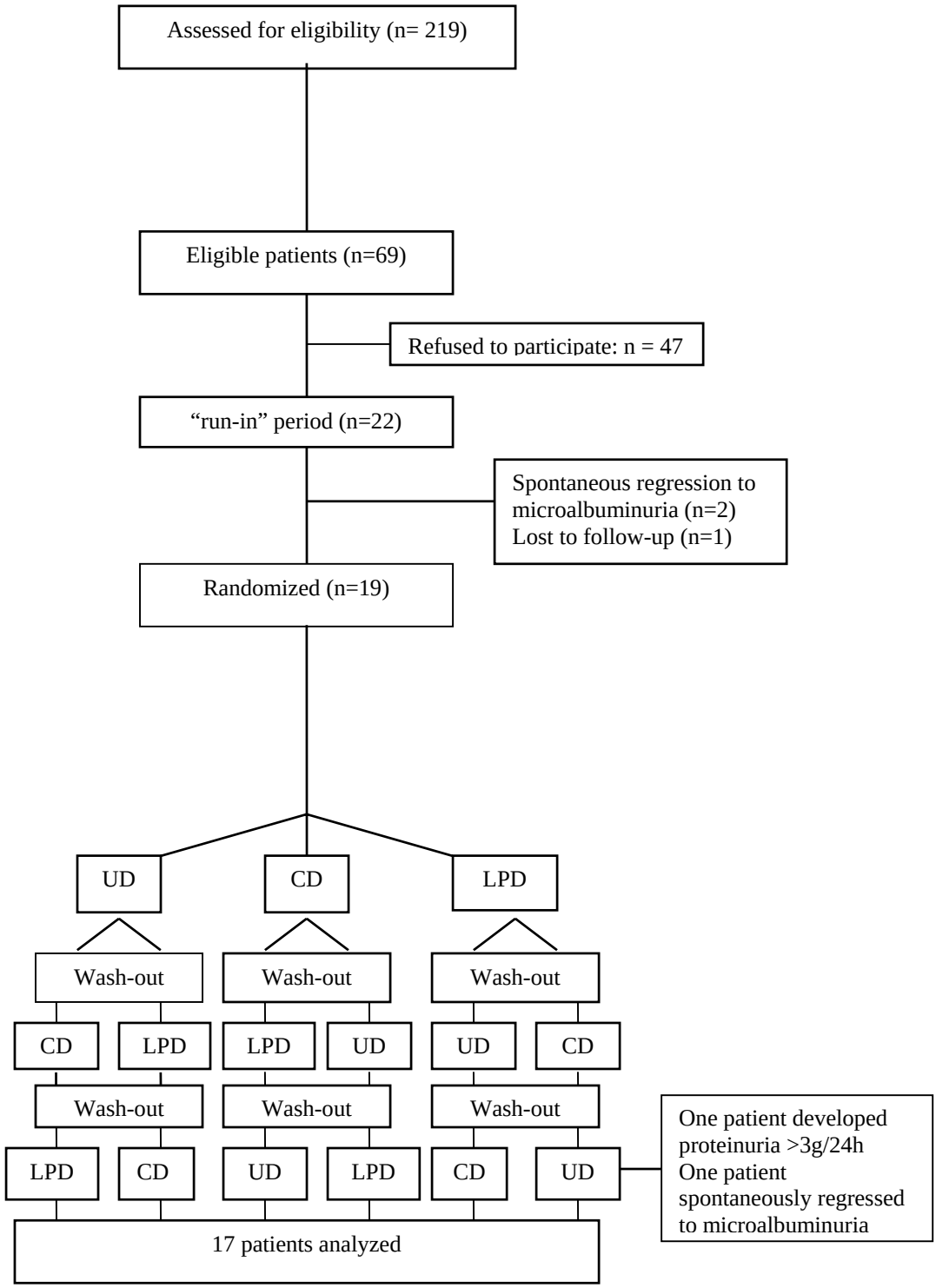

Figure 1. ***Flow of patients***

Capítulo III:

Effect of chicken-based diet versus enalapril on albuminuria in patients with type 2 diabetes and microalbuminuria: a one-year randomized controlled study

Artigo a ser submetido para publicação no periódico *Diabetes Care*, com algumas modificações

Effect of chicken-based diet versus enalapril on albuminuria in patients with type 2 diabetes and microalbuminuria: a one-year randomized controlled study

Running title: Long-term use of chicken-diet and enalapril reduces microalbuminuria

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Abstract

Objective – To compare the long-term effect of a chicken-based diet (CD) or enalapril on renal function and lipid profile in type 2 diabetic patients with microalbuminuria.

Research design and methods - In this controlled clinical trial, 22 patients were randomized to one of the following treatments: experimental diet [CD plus active placebo (verapamil or hydralazine)] or enalapril (patient's usual diet plus enalapril 10 mg/day) for 12 months. Glomerular filtration rate [GFR (^{51}Cr-EDTA)], 24-hour urinary albumin excretion rate [UAER (immunoturbidimetry)], lipid profile, metabolic control and nutritional indices were measured at baseline, and quarterly for one year. Blood pressure (BP), anthropometric indices, UAER and compliance with the diet (24-hour urinary urea and weighed-diet records) were evaluated monthly.

Results – Eleven patients concluded the CD and 11 the enalapril treatment. UAER was reduced after CD treatment [100.6 (38.4-125.1) vs. 49.8 (6.2-146.5) μg/min; $P<0.05$] and after enalapril treatment [63.9 (22.6-194.3) vs. 23.1 (4.0-104.9) μg/min; $P<0.01$]. There was no difference between the UAER reduction after CD [31% (95% CI: 0.39-61.7) or after enalapril treatment [46.6% (95% CI: 27.9-67.9); $P=0.533$]. UAER reduction in the enalapril group, but not in the CD group, was positively correlated to decrease in mean BP levels.

Conclusion - CD and ACE inhibitors promote similar UAER reduction in patients with type 2 diabetes and microalbuminuria, and the CD might represent an additional therapeutic approach to manage diabetic nephropathy.

Introduction

Diabetic nephropathy (DN) is the leading cause of chronic kidney disease in patients starting renal replacement therapy (1) and is associated with increased cardiovascular mortality (2). Microalbuminuria or incipient nephropathy is a risk factor for the development of more advanced stages of DN and it is also a risk factor for cardiovascular mortality (3). Microalbuminuria is present in about 7% of people with type 2 diabetes at the time of diagnosis (3) and in 24% of the patients with 25 years duration of the disease. Blockade of the renin-angiotensin system has been considered the first line of treatment in patients with microalbuminuria and type 2 diabetes (4,5). Large scale randomized controlled trials have shown that angiotensin converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor blockers (ARBs) reduce albuminuria and slow down the decline of renal function in these patients (6,7). Despite this, a progression to more advanced stages of DN is still seen in approximately 15% of the patients (6,7,8). Moreover, ACE inhibitors might be associated with some adverse effects, such as cough, hypersensitivity reaction, decreased renal function, and severe hipoglycemia episodes requiring hospitalization (9). Therefore additional therapeutic strategies are still needed.

We have previously reported that withdrawing red meat from the usual diet and replacing it by chicken or employing a low-protein diet not only reduced the urinary albumin excretion rate (UAER) but also improved the lipid profile in micro- and macroalbuminuric patients with type 2 diabetes in short-term proof of concept studies (10,11).

Therefore, the aim of this study was to compare the long-term effect of a chicken-based diet versus the use of the ACE inhibitor enalapril on renal function [UAER and

glomerular filtration rate (GFR)] and lipid profile in type 2 diabetic patients with microalbuminuria.

Research design and methods

Patients

This study was conducted in type 2 diabetes mellitus patients (according to World Health Organization criteria) attending the Endocrine Division's outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil. Patients were selected according the following criteria: age <75 years, compliance with diabetes treatment (A_{1c} test <10%), 24-hour UAER ≥20 μg/min and ≤199 μg/min confirmed at least twice in a 6-month period, serum triglycerides <4.52 mmol/l and normal liver and thyroid function tests. Patients were excluded from the study if they had: body mass index (BMI) >34 kg/m^2, serum creatinine levels >132.6 μmol/l, repeated episodes of urinary tract infection, other renal diseases, symptomatic autonomic neuropathy, cardiac failure, acute myocardial infarction or stroke within the last 6 months or if they had been submitted to coronary artery revascularization procedures within the last 6 months. To avoid potential confounding factors in the effect of experimental diet on the lipid profile, none of the patients were using hypolipidemic agents during the study. Eligible patients entered a run-in period of approximately 2 months, in which they were oriented to achieve the best possible metabolic control through dietary adjustments and use of oral antidiabetic agents or insulin adjustments. The dietary adjustments were designed to meet the American Diabetes Association (ADA) recommendations as closely as possible (12). Sitting blood pressure (BP) was measured twice to the nearest 2 mmHg, after a 5-min rest using a standard mercury sphygmomanometer (phases I and V of Korotkoff sounds). The average BP was recorded and mean BP was calculated as the diastolic BP plus one third of the pulse pressure. Hypertension was defined as BP ≥140/90 mmHg or when the patient was taking antihypertensive drugs. Patients using ACE inhibitors or ARBs had these drugs suspended

at least 6 weeks before entering the study period and other antihypertensive drugs were added as needed following this order: diuretics, beta-blockers and calcium channel blockers.

Study design

This study followed a randomized, controlled clinical trial design. The Ethics Committee of Hospital de Clínicas de Porto Alegre approved the protocol and all patients gave written informed consent. After the run-in period, patients were randomly assigned to follow either the experimental diet treatment or the enalapril treatment for a 12-month period. Randomization was performed in blocks of four with two choices for enalapril treatment and two choices for the experimental diet treatment. For every four patients who had entered the study, one block was used no matter whether the patient had continued or not throughout the study period. Experimental diet treatment consisted on a chicken-based diet (CD) plus an active placebo defined as verapamil (240 mg/day) or hydralazine (80 mg/day), and the enalapril treatment consisted on a usual diet (UD) according to the ADA's diet recommendations and enalapril (10 mg/day).

After randomization, patients underwent a baseline clinical and laboratory evaluation, including BP, renal function, glycemic control indices (fasting plasma glucose and A_{1c} test), serum lipid profile, compliance with the diet and nutritional indices (anthropometric: body weight and height, waist and hip circumferences, triceps skinfold thickness and mid-upper arm muscle area; laboratory: hematocrit, hemoglobin, total serum proteins and serum zinc). The glomerular filtration rate (GFR) and 24-hour UAER measured in sterile samples, were assessed twice during the next two weeks and the mean of the two measurements was used as the baseline value. The completeness of the urine collection was evaluated by measuring urinary creatinine excretion in each 24-hour urine

sample. All participants were instructed to maintain their usual physical activities and not to make any marked change in their lifestyle throughout the study period. Changes in medication prescriptions were not made unless it were for the purpose of stabilizing glycemic or BP controls. Patients had monthly visits with the dietitian (V.D.F.M.) and physician (T.Z.) to evaluate compliance to the diet prescription and medication therapy. At these visits anthropometric indices and BP values were recorded, and a 24-hour urine collection was obtained for UAER measurement. At the 4^{th}, 8^{th} and 12^{th} months of the study period the evaluation consisted of monthly measurements plus lipid profile, glycemic control indices, nutritional laboratorial indices, and GFR determination. For data analysis the variables measured monthly (24-hour UAER, BP control, compliance with diet prescription and anthropometric measurements) in the previous four months were grouped and the mean was recorded representing a time period, named: 4^{th} month, 8^{th} month and 12^{th} month.

The authors supplied the antihypertensive drugs used as active placebo during CD treatment and enalapril and compliance with their prescriptions were checked by tablet counts at each visit. Authors also supplied the oil (corn oil) consumed by the patients during the study, and its use was checked monthly by the difference in amount received and amount returned.

The primary outcome measure was UAER. Sample size was calculated based on the reduction of 24-hour UAER after a CD in microalbuminuric patients (10). To obtain a mean reduction of 36% on 24-hour UAER, it was estimated that 15 microalbuminuric patients had to be included for a significance level of $\propto = 0.05$ and a power of $1-\beta = 0.80$ in each treatment group.

Diet composition and prescription

During the run-in period, dietary adjustments were made in the patients' UD but the usual amount and source of protein of each individual patient was not modified (1.0 to 1.5g/kg body wt). In the CD group, diet consisted on a normoproteic chicken-based diet described elsewhere (10). Briefly, the CD was created by replacing the red meat of the UD with dark chicken meat (skinless leg quarter) and the protein content of both diets was not different. Patients in the enalapril group followed the UD adjusted during the run-in period. The patients consumed only corn oil throughout the study period and they were instructed not to reuse it. Compliance with the diet was assessed monthly by 3-day weighed-diet records (2-week-day and one weekend-day) and measurement of urea in 24-hour urine samples. The standard criterion for the estimation of protein intake was 24-hour urinary nitrogen output. The protein intake estimated by 24-hour urinary urea was calculated by the following formula: protein intake (g/day) = nitrogen intake x 6.25. The nitrogen intake was estimated by urinary urea nitrogen + non-urea nitrogen, where urinary urea nitrogen = urinary urea / 2 and non-urea nitrogen = 0.031 g N /kg body wt /day, assuming that patients presented a positive nitrogen balance (13).

Dietary macronutrients and micronutrients were analyzed using the Nutribase 98 Clinical Nutritional Manager software (Cybersoft Phoenix, AZ) (14). Data on macronutrients in the diet were expressed as a percentage of total energy intake.

Laboratory measurements

GFR was measured using the ^{51}Cr-EDTA single-injection technique [coefficient of variation (CV) 12%. GFR reference range: 72-137.5 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}]. Urinary albumin was measured in 24-hour timed sterile urine samples by immunoturbidimetry [MicroAlb Sera-Pak® immuno microalbuminuria; Bayer, Tarrytown, NY on Cobas Mira Plus

(Roche®)]; mean intra-assay and interassay CVs were 4.5 and 7.6 %, respectively). Urinary urea was measured by an enzymatic ultraviolet method (mean intra-assay CV 3.8%).

Blood samples were obtained after a 12-hour fast. Plasma glucose level was determined by a glucose oxidase method, serum and urinary creatinine level by the Jaffé reaction and the A_{1c} test by an ion-exchange high-performance liquid chromatography procedure (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) and HDL cholesterol by a direct selective inhibition method. LDL cholesterol was calculated using the Friedewald formula (LDL = total cholesterol – HDL – triglycerides/5). Non-HDL cholesterol was determined by the difference between total and HDL cholesterol.

Nutritional assessment

Nutritional evaluation was performed as described previously (15). Briefly, the body weight and height of patients (without shoes or coats) were obtained using an anthropometric scale, with measurements recorded to the nearest 100 g for weight and to the nearest 0.1 cm for height. BMI was then calculated. Triceps skinfold thickness, mid-upper arm circumference, waist circumference and hip circumference were measured and mid-upper arm muscle area was obtained by appropriate calculations. Flexible, nonstretch fiberglass tape was used for these measurements.

Total serum proteins were determined by the biuret colorimetric method and serum zinc by atomic absorption spectrophotometry (Toxilab® Análises Clínicas e Toxicológicas

laboratory, Porto Alegre, Brazil). Hematocrit and hemoglobin were measured with a Coulter counter (Pentra Retic 120®) by light impedance and absorbance.

Statistical analysis

The characteristics of the patients randomized to CD treatment and to the enalapril treatment were compared using the unpaired t test, χ^2 test and Fisher's exact test, as appropriate. The variables with a non-gaussian distribution were log-transformed before analysis, except for comparisons of the relative changes on UAER between the CD group and enalapril group, where the Mann-Whitney U test was used. Differences among the periods during each treatment were tested by repeated-measures ANOVA and Student-Newman-Keuls (SNK) post-hoc test. The Spearman correlation coefficient was used for testing the associations between UAER reduction and BP level reductions in each treatment group. A simple linear regression analysis was performed to evaluate the influence of BP changes on UAER reduction (dependent variable).

Only data from patients who completed the one-year study were included in these analyses. Results were expressed as means ± SD, median (range) or as mean (95% CI). P values of <0.05 were considered statistically significant. SPSS software 10.0 version (SPSS, Chicago, IL) was used for the analyses.

Results

Patients

Forty-seven eligible patients were invited to enter the study protocol. Eleven patients refused to participate. Thirty-six patients started the run-in period, but 12 patients were not randomized due to several reasons. Therefore, 24 patients were randomized to one of the two treatments arms (Fig. 1): 12 patients to CD and 12, to enalapril. Two patients did not complete the trial: one male patient in the enalapril arm was discontinued due to the progression to macroalbuminuria during the third month of the study, which was confirmed in the next subsequent two months and one female patient in the CD arm due to intolerance of chicken meat. Therefore, 11 patients in each arm were included in the final analysis. The baseline main clinical and laboratory characteristics of the two groups of treatments are shown in Table 1. The patients were well matched regarding age, gender, ethnicity, BMI, diabetes duration, proportion of hypertensive and smokers, diabetes treatment, glycemic control, lipid profile and renal function. Regarding treatment of diabetes, no patient was using thiazolidinediones compounds. The active placebo used by the patients in the CD were verapamil (n=7) and hydralazine (n=4). Antihypertensive agents besides active placebo or enalapril, were: diuretics (n=10; six in the CD), beta-blockers (n=7; four in the CD), calcium channel blockers (n=6; two in the CD), direct vasodilators (n=1; in the enalapril) and alpha-blockers (n=1; in the enalapril). One of the six women was postmenopausal in the CD and two of the five women in the enalapril treatment, but none were using hormone replacement therapy.

Characteristics of the diets

The characteristics of the diets followed during each treatment as assessed by the weighed-diet record method are described in Table 2. Energy, carbohydrate, protein, total lipids, saturated fatty acids (SFA), cholesterol and total fiber intake remained stable during the study period in the CD and in the enalapril treatments. Monounsaturated fatty acids (MUFA) intake at the 12th month was lower compared to baseline, but only in the CD. However, polyunsaturated fatty acids (PUFA) and PUFA to SFA (P/S) ratio were significantly different among periods during each treatment. In the CD group, patients reported a higher intake of PUFA at the 8th month period compared to baseline and a higher P/S ratio at the 4th, 8th and 12th months compared to baseline, without a difference among them. In the enalapril group, a rise in the PUFA intake at the 4th, 8th and 12th months compared to baseline was recorded by the patients. Consequently, the P/S ratio was also higher at these times, but this difference only reached statistical significance compared to baseline at the 8th month. The total protein intake (grams per kilogram body weight), as assessed by nitrogen output also did not change during both treatment study periods at baseline, 4th, 8th and 12th month: CD: 1.32 ± 0.32 vs. 1.28 ± 0.29 vs. 1.27 ± 0.30 vs. 1.34 ± 0.29, respectively (P = 0.686) and enalapril: 1.32 ± 0.36 vs. 1.30 ± 0.32 vs. 1.22 ± 0.22 vs. 1.30 ± 0.23, respectively (P = 0.488).

Renal function

UAER levels during the study are depicted in Fig. 2. The UAER levels decreased during the study period after CD (ANOVA, P=0.01) and enalapril treatment (ANOVA, P = 0.001). As compared to baseline levels [median and range; CD: 100.6 (38.4-125.1) μg/min; enalapril [63.9 (22.6-194.3) μg/min], UAER levels were already lower at the 4th month [CD: 46.8 (21.2-109.7) μg/min; enalapril: 41.1 (6.3-78) μg/min] and remained stable

during the 8th [CD: 49.4 (11.3-136) μg/min; enalapril: 37 (6.5-87.1) μg/min] and 12th [CD: 49.8 (6.2-146.5) μg/min; enalapril: 23.1 (4-104.9) μg/min] months (SNK $P < 0.01$ baseline vs. 4th, 8th and 12th months for CD and SNK $P < 0.05$ baseline vs. 4th, 8th and 12th months for enalapril), even in the enalapril group where the small difference observed between 8th and 12th months periods which was not statiscally different.

The relative reduction in UAER after the one-year period was similar in both groups: CD: 31% (95% CI: 0.39 - 61.7) vs. enalapril: 47.8% (95% CI: 27.9 - 67.9), $P > 0.05$. A total of three patients (27.7%) regressed to normoalbuminuria range at the end of the study period in each group of treatment.

The GFR was measured at baseline, 4th, 8th and 12th months, but unfortunately GFR was not available in three patients due to technical problems at the 4th month. Therefore GFR was analyzed at baseline, 8th and 12th months. GFR did not change during the study period in both groups (CD: 103.8 ± 19.0 vs. 104.9 ± 17.6 vs. 105.5 ± 22.1 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^2; $P > 0.05$ and enalapril: 103.4 ± 22.9 vs. 96.3 ± 19.2 vs. 102.3 ± 23.1 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^2; $P > 0.05$).

Serum lipids

Lipid levels during CD and enalapril treatments are shown in Table 3. In the CD group, total, LDL and non-HDL cholesterol were higher at baseline especially compared to the 4th and 8th months, without a difference between the 4th and 8th months. In the enalapril group, a significant change was observed in total and non-HDL cholesterol levels at the 12th month compared to baseline. There were no differences regarding other lipid fractions during the study period in both treatments, just a trend to lower levels of HDL cholesterol in the enalapril group.

Glycemic control and BP levels

Glycemic control, assessed by fasting plasma glucose (FPG) and A_{1c} test were stable during the study period, from baseline to the 12th month, in both treatments arms FPG: CD 7.59 ± 1.89 vs. 6.54 ± 1.94 vs. 6.10 ± 1.78 vs. 7.21 ± 2.94, P = 0.348 and enalapril 7.93 ± 1.66 vs. 7.32 ± 1.28 vs. 6.60 ± 1.05 vs. 6.88 ± 1.83, P = 0.071; A_{1c} test: CD 7.6 ± 2.1 vs. 7.4 ± 2.8 vs. 7.4 ± 2.5 vs. 7.4 ± 2.6, P = 0.830 and enalapril 6.8 ± 2.0 vs. 6.6 ± 1.9 vs. 6.6 ± 2.2 vs. 6.9 ± 2.2, P = 0.229. Mean BP levels were higher at baseline than during the study in both treatments but without attaining conventional statistical significance (CD: 92 ± 8 vs. 90 ± 7 vs. 89 ± 8 vs. 87 ± 6, P = 0.122 and enalapril: 90 ± 6 vs. 88 ± 7 vs. 87 ± 6 vs. 85 ± 7, P = 0.212). Analyzing data considering the reduction of mean BP from baseline to 12th month, [CD: 5.1 mmHg (95% CI: -0.8 – 10.9) and enalapril: 5.0 mmHg (95% CI: 0.3 – 9.7)] it was not found any difference between treatments (P > 0.05). However, reductions in mean BP levels were strongly correlated to UAER reduction in the enalapril group (r_s = 0.700, P < 0.05), but not in the CD. In a simple linear regression model analysis, a reduction of 5 mmHg in mean BP explained 36% of the observed UAER reduction.

Nutritional indices

The anthropometric indices were stable during all the study period in both treatment groups, as shown in Table 4. Biochemical parameters evaluated to monitor nutritional status were also stable during each treatment period and its values were not different between groups at baseline and at the end of the 12th month period.

Conclusions

In this sample of patients with type 2 diabetes and microalbuminuria it was observed that a chicken-based diet or enalapril induced a prompt and a similar UAER reduction of about 30 to 50% that remained stable throughout the one-year study period.

This long-term study confirms our previous observations in short-term studies that a chicken-based diet induced a rapid reduction in UAER in type 2 diabetic patients with micro- and macroalbuminuria (10,11). This reduction was sustained during the study period and was probably not related to glycemic and BP control or hemodynamic factors. Glycemic control remained stable during the study period and there was a non-significant decrease of about 5 mmHg in mean BP. It is unlikely that this influenced the UAER reduction during the CD since there was no correlation between mean BP and UAER changes in this arm of the study. Hemodynamic factors probably did not contribute to UAER reduction because GFR did not change during the one-year period of the study. The UAER reduction observed might be related to the beneficial effect of decrease in SFA and the increase in PUFA intake during CD on endothelial function, probably improving the glomerular capillary membrane barrier size selectivity. In type 2 diabetes, endothelial dysfunction and increased UAER are interrelated processes, which are strongly associated with risk of death (16). PUFA can improve endothelial dysfunction by acting as a modulator of cell responsiveness to cytokines decreasing the expression of cell adhesion molecules involved in thrombosis and inflammation processes including ICAM-1, VCAM-1 and E-selectin. Furthermore, PUFA might also increase nitric oxide production, a potent vasodilator, which counteracts the endothelial vasoconstrictors thromboxane A2, and endothelin 1 (17). Reduction in the cholesterol levels especially at the 4^{th} and 8^{th} months, probably related to the lower intake of SFA and the higher intake of PUFA during the CD,

could also have contributed to the UAER reduction. A meta-analysis of 13 prospective controlled trials showed that hypolipidemic agents might preserve GFR and decrease proteinuria (18). Therefore, the UAER reduction after CD could be considered as a specific effect of diet intervention. Finally, CD was considered safe since no deficiency was detected on the nutritional indices evaluated.

The effect of enalapril on UAER reduction, as well as the proportion of patients that regressed to normoalbuminuria, were similar to that observed after the CD. This reduction in UAER levels and the regression rates to normoalbuminuria have also been described in microalbuminuric type 2 diabetic patients by other authors using ACE inhibitors or ARBs (7,8,19). ACE inhibitors are considered to have a specific renoprotective effect, independent of BP reduction, probably related to the reduction in glomerular capillary pressure (20) and/or its effect in delaying the progression of structural glomerular damage (21,22). However, the effect of enalapril on UAER reduction observed in our study might be partially explained by a BP reduction. In fact, the mean BP decline in the enalapril arm of the study was significant and accounted for 36% of the observed UAER reduction. Significant BP changes with the use of ACE inhibitors were also reported in large-scale studies where UAER reduction was observed, especially in hypertensive patients (6,19).

The main limitation of this study was the small sample size but the UAER reduction observed in our patients was consistent and very similar to those reported in other interventional studies (7,8,19). However, long-term studies with a larger number of patients should be performed to confirm these results. Compliance with the CD was probably adequate since it was frequently assessed by the weighed-diet records method and urea measurements along the study period.

In conclusion, this long-term study indicates that a chicken-based diet induced a similar reduction in UAER as that caused by ACE inhibitors, and reinforces the concept that the withdrawal of red meat without concomitant reduction of protein intake might represent an additional approach that can be included in the therapeutic strategies used in the management of patients with diabetic nephropathy.

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References

1. U.S. Renal Data System, USRDS 2003 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2003
2. Valmadrid CT, Klein R, Moss SE, Klein BE: The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. *Arch Intern Med* 160:1093-1100, 2000
3. Adler AI, Stratton IM, Neil HA, Yudkin JS, Matthews DR, Cull CA, Wright AD, Turner RC, Holman RR: Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. *Br Med J* 321:412-419, 2000
4. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jr., Jones DW, Materson BJ, Oparil S, Wright JT, Jr., Roccella EJ: The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 289:2560-2572, 2003
5. American Diabetes Association: Nephropathy in Diabetes. *Diabetes Care* 27:S79-83, 2004
6. Heart Outcomes Prevention Evaluation Study Investigators: Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *Lancet* 355:253-259, 2000
7. Parving HH, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Arner P: The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med* 345:870-878, 2001

8. Chan JC, Ko GT, Leung DH, Cheung RC, Cheung MY, So WY, Swaminathan R, Nicholls MG, Critchley JA, Cockram CS: Long-term effects of angiotensin-converting enzyme inhibition and metabolic control in hypertensive type 2 diabetic patients. *Kidney Int* 57:590-600, 2000
9. Morris AD, Boyle DIR, McMahon AD: ACE inhibitor use is associated with hospitalization for severe hypoglicemia in patients with diabetes. *Diabetes Care* 20:1363-1367, 1997
10. Gross JL, Zelmanovitz T, Moulin CC, De Mello V, Perassolo M, Leitao C, Hoefel A, Paggi A, Azevedo MJ: Effect of a chicken-based diet on renal function and lipid profile in patients with type 2 diabetes: a randomized crossover trial. *Diabetes Care* 25:645-651, 2002
11. Azevedo MJ, Mello VDF, Zelmanovitz T, Perassolo M, Gross JL: Reduction of urinary albumin excretion and improvement of lipid profile after a chicken-diet in macroalbuminuric type 2 diabetic patients. *12th International Congress of Endocrinology (International Society of Endocrinology)*, OR53, Lisbon, 2004. abstract
12. American Diabetes Association: Nutrition recommendations and principles for people with diabetes mellitus. *Diabetes Care* 21 (Suppl. 1):S32-S35, 1998.
13. Maroni BJ, Steinman TI, Mitch WE: A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 27:58-65, 1985
14. United States Department of Agriculture, Agriculture Research Service: US Department of Agriculture Nutrient Data Base for Standard Reference, Release 14. Washington, DC: US Department of Agriculture, 2001 (USDA Handbook No 8)

15. Moulin CC, Tiskievicz F, Zelmanovitz, Oliveira J, Azevedo MJ, Gross JL: Use of weighed diet records in the evaluation of diets with different protein contents in patients with type 2 diabetes. *Am J Clin Nutr* 67: 853-857, 1998
16. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH: Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. *Diabetes* 51:1157-1165, 2002
17. Brown AA, Hu FB: Dietary modulation of endothelial function: implications for cardiovascular disease. *Am J Clin Nutr* 73:673-686, 2001
18. Fried LF, Orchard TJ, Kasiske BL: Effect of lipid reduction on the progression of renal disease: a meta-analysis. *Kidney Int* 59:260-269, 2001
19. Mogensen CE, Viberti G, Halimi S, Ritz E, Ruilope L, Jermendy G, Widimsky J, Sareli P, Taton J, Rull J, Erdogan G, De Leeuw PW, Ribeiro A, Sanchez R, Mechmeche R, Nolan J, Sirotiakova J, Hamani A, Scheen A, Hess B, Luger A, Thomas SM: Effect of low-dose perindopril/indapamide on albuminuria in diabetes: preterax in albuminuria regression: PREMIER. *Hypertension* 41:1063-71, 2003
20. Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM: Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. *J Clin Invest* 77:1925-30, 1986
21. Ahmad J, Shafique S, Abidi SM, Parwez I: Effect of 5-year enalapril therapy on progression of microalbuminuria and glomerular structural changes in type 1 diabetic subjects. *Diabetes Res Clin Pract* 60:131-8, 2003

22. Rudberg S, Osterby R, Bangstad HJ, Dahlquist G, Persson B: Effect of angiotensin converting enzyme inhibitor or beta blocker on glomerular structural changes in young microalbuminuric patients with Type I (insulin-dependent) diabetes mellitus. *Diabetologia* 42:589-95, 1999

Table 1. ***Baseline characteristics of the microalbuminuric type 2 diabetic patients***

	Chicken Diet	Enalapril	*P*
n	11	11	
Age (years)	54.8 ± 11.8	57 ± 9.5	0.637
Gender (male)	6 (50%)	6 (54.5%)	1.000
Ethnicity (black)	3 (27.3%)	2 (18.2%)	1.000
BMI (kg/m^2)	28.6 ± 2.3	27.8 ± 2.3	0.433
DM duration (years)	11.6 ± 7.2	9 ± 6.2	0.369
Mean blood pressure (mmHg)	92 ± 8	90 ± 6	0.515
Hypertension (yes)	8 (72.8%)	7 (63.6%)	1.000
Current smoking (yes)	4 (36.4%)	5 (45.5%)	0.631
Diabetes treatment (D/OA/I/I+OA)	3/4/2/2	2/5/1/3	0.839
Fasting plasma glucose (mmol/l)	7.49 ± 1.89	7.93 ± 1.66	0.570
A_{1c} (%)	7.6 ± 2.2	6.8 ± 2.0	0.065
Total cholesterol (mmol/l)	6.08 ± 0.80	5.95 ± 0.91	0.756
HDL cholesterol (mmol/l)	1.29 ± 0.21	1.17 ± 0.21	0.558
LDL cholesterol (mmol/l)	3.96 ± 0.67	3.80 ± 0.88	0.509
Non-HDL cholesterol (mmol/l)	4.78 ± 0.75	4.63 ± 0.91	0.684
Triglycerides (mmol/l)	1.75 (0.88 – 3.58)	1.72 (0.82 – 3.06)	0.914
Serum creatinine (μmol/l)	66.3 ± 10.6	66.4 ± 10.6	0.857
GFR ($ml \cdot min^{-1} \cdot 1.73\ m^2$)	103.8 ± 19.0	102.7 ± 21.9	0.896
24h – UAER (μg/min)	100.6 (38.4 – 125.1)	63.9 (22.6 – 194.3)	0.448

Data are means ± SD, median (range) or number of patients (%) with analyzed characteristic. D: diet only; OA: oral antidiabetic agents; I: insulin.

Table 2. ***Nutrient intake during experimental diet and enalapril, according to weighed-diet records***

	baseline	4th month	8th month	12th month	*P*
Daily energy (kcal)					
Chicken diet	1626 ± 373	1712 ± 440	1718 ± 450	1695 ± 517	0.455
Enalapril	1533 ± 474	1637 ± 296	1578 ± 321	1576 ± 297	0.633
Carbohydrate (% en)					
Chicken diet	45.6 ± 4.9	48.5 ± 5.3	46.4 ± 4.0	48.2 ± 5.1	0.536
Enalapril	48.6 ± 6.2	48.3 ± 6.4	47.3 ± 4.3	46.9 ± 3.9	0.703
Protein (% en)					
Chicken diet	23.5 ± 4.6	25.9 ± 10.2	22.8 ± 4.4	23.2 ± 5.0	0.616
Enalapril	23.9 ± 4.9	23.4 ± 3.2	24.2 ± 4.1	23.0 ± 3.1	0.655
Protein (g/kg body wt)					
Chicken diet	1.32 ± 0.39	1.25 ± 0.37	1.29 ± 0.39	1.30 ± 0.41	0.556
Enalapril	1.29 ± 0.51	1.32 ± 0.29	1.31 ± 0.34	1.26 ± 0.33	0.899
Total lipids (% en)					
Chicken diet	29.5 ± 7.0	29.1 ± 4.7	30.4 ± 5.2	28.6 ± 2.2	0.661
Enalapril	28.4 ± 7.3	29.2 ± 3.8	28.5 ± 2.3	30.4 ± 1.8	0.584
SFA (% en)					
Chicken diet	8.6 ± 2.1	8.0 ± 1.6	8.0 ± 2.3	7.5 ± 1.3	0.103
Enalapril	8.4 ± 2.5	8.4 ± 1.5	8.0 ± 1.1	8.9 ± 1.6	0.302
MUFA (% en)					
Chicken diet	10.2 ± 2.5	9.0 ± 1.5	9.3 ± 1.7	8.6 ± 1.0	0.024 *
Enalapril	9.4 ± 2.2	9.5 ± 1.0	9.5 ± 0.7	10.1 ± 1.2	0.467
PUFA (% en)					
Chicken diet	7.9 ± 3.1	9.3 ± 1.8	10.2 ± 2.0	9.1 ± 1.2	0.016 †
Enalapril	6.8 ± 1.8	7.9 ± 2.0	8.0 ± 1.6	8.0 ± 1.6	0.027 ‡
P/S ratio					

Chicken diet	0.93 ± 0.26	1.22 ± 0.19	1.36 ± 0.33	1.27 ± 0.33	<0.0001 ‡
Enalapril	0.83 ± 0.16	0.97 ± 0.26	1.03 ± 0.23	0.95 ± 0.29	0.017 †
Cholesterol (mg)					
Chicken diet	215 ± 94	237 ± 98	247 ± 78	248 ± 91	0.240
Enalapril	200 ± 96	218 ± 53	212 ± 71	208 ± 62	0.807
Total fiber (g)					
Chicken diet	18.7 ± 5.5	19.5 ± 6.8	19.7 ± 7.1	20.3 ± 7.3	0.773
Enalapril	19.4 ± 9.7	19.2 ± 4.8	18.5 ± 5.2	18.0 ± 5.8	0.876

Data are means ± SD. en: daily energy. SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids. P/S: polyunsaturated to saturated FA.

P referes to repeated measures ANOVA. SNK: * baseline vs. 12th month (P <0.05); † baseline vs. 8th month (P <0.05); ‡ baseline vs. 4th, 8th and 12th months (P <0.01).

Table 3. ***Lipid profile during experimental diet and enalapril***

	baseline	4th month	8th month	12th month	*P*
Total C (mmol/l)					
Chicken diet	6.08 ± 0.80	5.64 ± 0.85	5.43 ± 0.93	5.79 ± 1.01	0.007*
Enalapril	5.95 ± 0.91	5.53 ± 0.93	5.64 ± 0.75	5.30 ± 1.09	0.017†
HDL C (mmol/l)					
Chicken diet	1.29 ± 0.23	1.29 ± 0.26	1.24 ± 0.26	1.32 ± 0.21	0.393
Enalapril	1.35 ± 0.23	1.29 ± 0.18	1.37 ± 0.28	1.27 ± 0.28	0.075
LDL C (mmol/l)					
Chicken diet	3.98 ± 0.67	3.54 ± 0.72	3.36 ± 0.78	3.67 ± 0.70	0.007*
Enalapril	3.72 ± 0.93	3.52 ± 0.75	3.52 ± 0.78	3.34 ± 0.93	0.248
Non-HDL C (mmol/l)					
Chicken diet	4.78 ± 0.75	4.37 ± 0.80	4.22 ± 0.78	4.47 ± 0.96	0.009*
Enalapril	4.60 ± 0.93	4.27 ± 0.93	4.27 ± 0.75	4.03 ± 1.06	0.035†
Triglycerides (mmol/l)					
Chicken diet	1.75 (0.88-3.58)	1.58 (0.88-3.07)	1.65 (0.75-3.79)	1.25 (0.73-3.66)	0.915
Enalapril	1.72 (0.82-3.06)	1.57 (0.99-3.29)	1.42 (0.87-2.51)	1.48 (0.69-2.86)	0.189

Data are means ± SD or median (range). C: cholesterol *P* referes to repeated measures ANOVA.

SNK: * baseline vs. 4th and 8th months ($P < 0.05$); † baseline vs. 12th month ($P < 0.05$).

Table 4. ***Anthropometric and biochemical indices during experimental diet and enalapril***

	baseline	4th month	8th month	12th month	*P*
BMI (kg/m^2)					
Chicken diet	28.6 ± 2.3	28.8 ± 2.4	29.0 ± 2.6	29.0 ± 2.6	0.149
Enalapril	27.9 ± 2.3	27.7 ± 2.4	27.9 ± 2.5	27.8 ± 2.4	0.632
Triceps skinfold thickness (mm)					
Chicken diet	22.6 ± 10.6	21.4 ± 9.6	21.6 ± 9.8	21.0 ± 8.7	0.434
Enalapril	19.5 ± 7.2	18.9 ± 7.7	18.9 ± 8.6	18.8 ± 8.6	0.596
Mid-upper arm muscle area (mm^2)					
Chicken diet	54.7 ± 12.4	57.4 ± 13.9	55.7 ± 12.9	56.3 ± 11.8	0.556
Enalapril	57.8 ± 8.0	58.9 ± 8.0	57.7 ± 7.9	56.3 ± 6.2	0.302
Waist circunference (cm)					
Chicken diet	101.7 ± 5.5	101.3 ± 5.1	101.8 ± 5.5	102.3 ± 5.5	0.523
Enalapril	99.8 ± 4.1	99.6 ± 4.5	99.9 ± 5.1	99.8 ± 4.4	0.959
Waist-to-hip ratio					
Chicken diet	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05	1.01 ± 0.05	0.386
Enalapril	1.02 ± 0.05	1.02 ± 0.04	1.02 ± 0.05	1.01 ± 0.03	0.968
Hematocrit (%)					
Chicken diet	43.1 ± 4.4	43.2 ± 3.6	42.9 ± 3.2	43.5 ± 3.7	0.830
Enalapril	43.2 ± 3.8	41.4 ± 2.8	42.0 ± 4.0	41.7 ± 3.3	0.062
Hemoglobin (mg/dl)					
Chicken diet	14.0 ± 1.4	14.0 ± 1.1	14.0 ± 1.1	14.0 ± 1.2	0.990
Enalapril	14.1 ± 1.2	13.5 ± 1.0	13.6 ± 1.3	13.8 ± 1.0	0.170
Total proteins (g/l)					
Chicken diet	73 ± 5	71 ± 5	73 ± 4	72 ± 5	0.729
Enalapril	72 ± 3	72 ± 6	73 ± 6	73 ± 5	0.987

Serum zinc (μmol/l)					
Chicken diet	14.7 ± 2.0	15.8 ± 4.1	15.5 ± 4.0	17.8 ± 3.1	0.441
Enalapril	14.4 ± 1.4	15.2 ± 2.6	14.7 ± 3.2	14.4 ± 2.3	0.937

Data are means ± SD. *P* referes to repeated measures ANOVA.

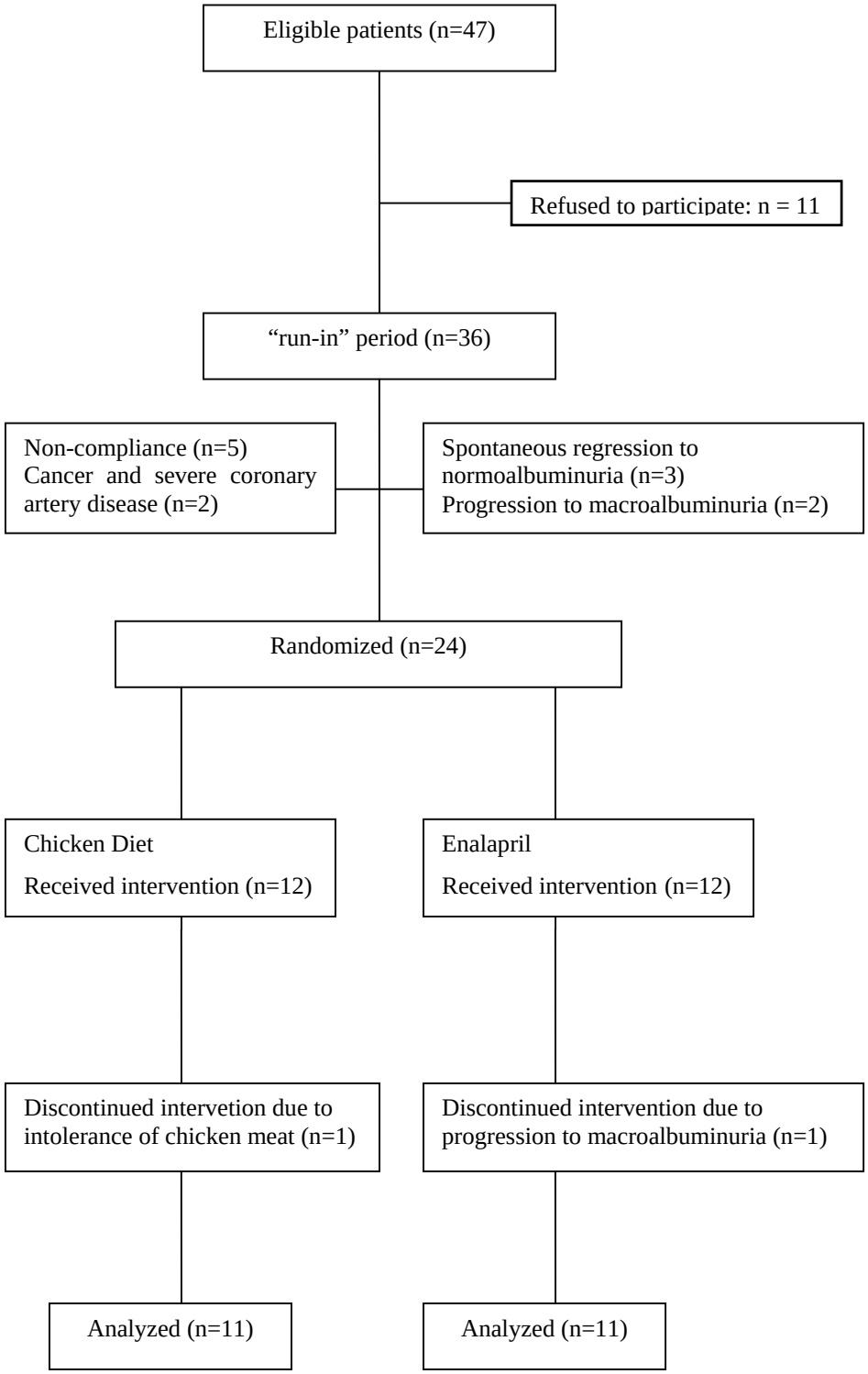

Figure1. ***Flow of patients***

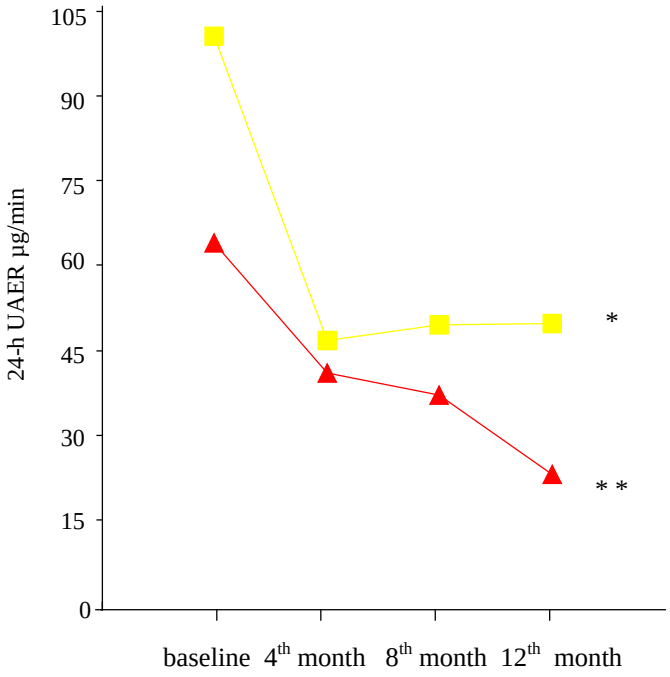

Figure 2. *24h-urinary albumin excretion rate during the study:*

Values are expressed as medians during treatment with chicken diet (■)

and enalapril (▲).

*ANOVA for repeated measurements: * P = 0.010*

** P = 0.001

CONSIDERAÇÕES FINAIS

O papel da dieta como fator de risco e progressão da ND ainda não está entendido na sua plenitude. Poucos estudos têm analisado o efeito de intervenções dietoterápicas como alternativas adicionais às estratégias atualmente empregadas, especialmente se compararmos com os grandes ensaios clínicos que vêm sendo relizados para analisar o efeito de medicamentos. As intervenções dietéticas são baratas, podem atingir um número grande de pacientes, quando adequadamente divulgadas e usualmente não associadas a efeitos colaterias graves.

A análise do conjunto de dados apresentados nesta Tese justifica a continuação desta linha de pesquisa, enfocando especialmente duas perguntas principais:

Por que os ácidos graxos saturados se encontram elevados na ND em pacientes com DM tipo 2?

Qual é o efeito dos ácidos graxos saturados na função endotelial e em particular na EUA?

Para responder a estas questões delineou-se um conjunto de projetos inter-relacionados brevemente descritos a seguir.

Estudo transversal em uma população de pacientes com DM tipo 2 (normo- e microalbuminúricos) para analisar a possível associação entre o perfil de ácidos graxos séricos e a composição dos ácidos graxos na dieta, levando em consideração o papel de polimorfismos da enzima intestinal ligadora de ácidos graxos e presença de resistência insulínica. Esta última poderia influenciar os níveis e os tipos de ácidos graxos séricos atuando através das enzimas saturases e dessaturases.

Análise do papel do polimorfismo do gene FABP2 nos níveis séricos de AGS através de uma refeição padrão usual em um estudo de casos (polimorfismo de risco TT) e controles (polimorfismo AA) em pacientes com DM tipo 2 microalbuminúricos.

Avaliação de possível efeito dos AGS na função endotelial e marcadores inflamatórios através de um estudo transversal em uma amostra de pacientes com DM tipo 2 normo- e microalbuminúricos. A função endotelial seria avaliada através de marcadores como a endotelina-1 e pletismografia do antebraço.

Os resultados destes estudos poderão ajudar a entender o papel da dieta na patogênese da ND e fornecer evidências de suporte para intervenções dietoterápicas.