UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

ASSOCIAÇÃO DOS ÁCIDOS GRAXOS SÉRICOS COM DISFUNÇÃO ENDOTELIAL E LIPÍDEOS SÉRICOS EM PACIENTES COM DIABETE MELITO TIPO 2

TESE DE DOUTORADO

MAGDA SUSANA PERASSOLO

PORTO ALEGRE, JUNHO DE 2007.

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MAGDA SUSANA PERASSOLO

PROFESSOR ORIENTADOR: DR. JORGE LUIZ GROSS

Tese apresentada ao PPG em ciências Médicas: Endocrinologia para a obtenção do título de doutor

PORTO ALEGRE, JUNHO DE 2007.

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Sumário

Lista de abreviaturas

AG: ácidos graxos

AGM: ácidos graxos monoinsaturados

AGP: ácidos graxos poliinsaturados

AGS: ácidos graxos saturados

AHA: American Heart Association

CV: cardiovascular

DCV: doença cardiovascular

DHA: ácido docosahexaenóico

EPA: ácido eicosapentaenóico

TG: triglicerídeos

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RESUMO

Os ácidos graxos (AG) são ácidos carboxílicos que compõe a estrutura fundamental dos lipídeos e possuem uma cadeia hidrocarbonada (4 a 26 C) e um grupamento carboxila terminal (ácidos carboxílicos). Eles são capazes de gerar mediadores ativos e modular a resposta a hormônios, como também influenciam a resposta imunológica, inflamatória e secreção de insulina e de outros hormônios. Além do efeito sobre o perfil das lipoproteínas plasmáticas, os AG atuam nos processos inflamatórios, têm papel importante na função endotelial, sensibilidade à insulina e mecanismos trombogênicos. Neste sentido, avaliou-se a associação de disfunção endotelial com a proporção de AG séricos em pacientes com diabete melito tipo 2. Os níveis séricos de endotelina-1 apresentaram correlação positiva com os AG saturados e negativa com os AG poliinsaturados (AGP), demonstrando que a composição de AG séricos está independentemente relacionada com disfunção endotelial, avaliada pela dosagem de endotelina-1. Além de estarem relacionados com disfunção endotelial, os AG medidos nos lipídeos totais, apresentam melhor associação com os AG medidos na fração triglicerídeos e também melhor correlação com os lipídeos séricos (colesterol e triglicerídeos) e se correlacionam com os AG de dieta (AGP). Isto demonstra que os AG dosados nos lipídeos totais representam melhor as funções metabólicas dos AG no soro.

CAPÍTUULO I: **Ácidos Graxos e Doença Cardiovascular***

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Ácidos Graxos e Doença Cardiovascular

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Resumo

A doença cardiovascular (DCV) é a principal causa de mortalidade na população mundial. Isto se deve principalmente à exposição a fatores ambientais, como alimentação não-saudável, sedentarismo, tabagismo e abuso de álcool. Também, co-morbidades como a obesidade, a hipertensão arterial, o diabetes melito e a dislipidemia estão diretamente relacionadas ao aumento na incidência da DCV. A adequada abordagem destes fatores está associada a uma diminuição na incidência e progressão dos eventos cardiovasculares (CV). Neste sentido, várias evidências vêm se acumulando sugerindo que os ácidos graxos (AG) têm um papel importante no desenvolvimento e prevenção da DCV. O objetivo deste manuscrito é revisar aspectos básicos relacionados ao metabolismo e papel fisiológico dos AG e analisar as evidências do impacto dos AG plasmáticos e dietéticos sobre a DCV. Além do efeito sobre o perfil das lipoproteínas plasmáticas, os AG atuam nos processos inflamatórios, têm papel importante na função endotelial, sensibilidade à insulina e mecanismos trombogênicos. Em relação aos AG da dieta, os AG saturados e os AG *trans* apresentam efeito deletério sobre o desenvolvimento da DCV. Já os AG poliinsaturados (AGP) parecem exercer papel protetor, mas existem ainda controvérsias acerca de seus efeitos na prevenção da DCV, em especial dos AGP n-3 e n-6. Em conclusão, as recomendações atuais de modificações dietéticas em relação ao consumo de AG para a prevenção da DCV são baseadas em evidências clínicas razoáveis. Entretanto, serão melhor fundamentadas a partir de novos ensaios clínicos randomizados.

1. Introdução

A doença cardiovascular (DCV) é a principal causa de mortalidade na população em geral, tendo aumentado sua prevalência em 60% nos últimos 30 anos. Estima-se que o aumento será de três vezes nas próximas duas décadas (1). A DCV está relacionada à progressão do processo aterosclerótico e à exposição a fatores de risco, como obesidade, sedentarismo e tabagismo. Também, co-morbidades como hipertensão arterial, diabetes melito, dislipidemia e síndrome metabólica contribuem de forma independente para o aumento na incidência da DCV. A adequada prevenção e a exclusão destes fatores de risco está associada a uma diminuição dos eventos cardiovasculares (CV) (2).

Desde o início dos anos 70, o consumo excessivo de gorduras na dieta tem sido associado ao desenvolvimento da DCV e a redução na ingestão de gorduras totais vem sendo o foco principal nas recomendações dietéticas para indivíduos saudáveis (3). Entretanto, o importante papel dos ácidos graxos (AG) insaturados tem sido ressaltado em estudos observacionais e ensaios clínicos controlados onde a substituição dos AG saturados da dieta por AG insaturados foi mais efetiva na redução do risco CV do que apenas a redução do consumo de gordura total (4,5).

Os AG séricos participam de vários processos no organismo, como resposta inflamatória e fluidez das membranas celulares. Muitos destes processos estão relacionados ao maior ou menor risco de desenvolvimento de patologias CV. Vários estudos (6-10) sugerem que os AG podem apresentar efeito protetor ou deletério sobre a função CV por mecanismos diversos.

O objetivo deste manuscrito é revisar aspectos básicos relacionados ao metabolismo e papel fisiológico dos AG e analisar as evidências do impacto dos AG plasmáticos e dietéticos sobre a DCV.

2. Ácidos Graxos: aspectos bioquímicos e fisiológicos

Os AG são ácidos carboxílicos que compõe a estrutura fundamental dos lipídeos e possuem uma cadeia hidrocarbonada (4 a 26 C) e um grupamento carboxila terminal (ácidos carboxílicos). Os AG são capazes de gerar mediadores ativos e modular a resposta a hormônios, como também influenciam a resposta imunológica, inflamatória e secreção de insulina e de outros hormônios (11).

A presença ou não de duplas ligações (insaturações) nas cadeias hidrocarbonadas dos AG classifica-os como AG saturados (AGS; sem insaturações) ou insaturados [presença de uma – monoinsaturados (AGM) ou mais – AG polinsaturados (AGP) – insaturações; 11,12]. Dependendo da localização da dupla ligação em relação ao carbono mais distante do grupamento carboxila, os AG insaturados são classificados, em n-3, n-6, n-9 e n-7 (12). Nesta classificação utilizam-se os prefixo "n" ou "ômega": ω-3 ou n-3, por exemplo.

No plasma, os AG encontram-se presentes sob a forma não esterificada também conhecidos como AG livres e ligados à albumina sérica, e como AG esterificados a partículas como colesterol, triglicerídios (TG) e fosfolipídios. Ainda, os AG plasmáticos podem ser provenientes da alimentação ou da síntese endógena (13,14).

Os AG insaturados ocorrem nas configurações *cis* ou *trans*. Os isômeros *cis* (os átomos de hidrogênio estão do mesmo lado da liga dupla) apresentam uma dobra da molécula na altura da liga dupla e ocorrem na maioria dos AG naturais. Por outro lado, os AG *trans* são formados principalmente durante a hidrogenação parcial de óleos vegetais utilizados pela indústria de alimentos na produção de margarinas, produtos de padaria, bolachas e alimentos rápidos armazenados (13). O simples aquecimento de um óleo também pode levar à formação de um isômero *trans* (13). Cerca de 0,5% dos ácidos graxos *tran*s ingeridos são provenientes da carne ou produtos lácteos de ruminantes como gado bovino ou ovino.

Uma alternativa tecnológica ao processo de hidrogenação parcial é a interesterificação (14). Este processo não promove a isomerização das duplas ligações dos ácidos graxos e não

afeta o grau de saturação dos mesmos (15). O resultado deste processo forma os chamados lipídeos estruturados (14-16). O processo de interesterificação pode ser químico ou enzimático (14-16). No processo enzimático, biocatalisadores, tais como lípases microbianas, são utilizados para promover a migração acila nas moléculas acilglicerídicas. Na interesterificação química, largamente utilizada, o catalisador empregado com maior freqüência é o metóxido de sódio (MeONa), embora outras bases, ácidos e metais estejam disponíveis. Alquilatos de sódio são reconhecidamente os catalisadores mais ativos, inclusive a temperaturas relativamente baixas, entre 50 e 90 ºC (15).

Síntese de ácidos graxos

O ser humano sintetiza a maioria dos AG que necessita, com exceção dos ácidos linoléico (18:2n-6) e α-linolênico (18:3n-3) que são considerados AG essenciais (13,17). Os AG essenciais pertencem à classe ômega 6 e ômega 3, não são sintetizados endogenamente e devem ser obtidos necessariamente através da dieta.

A síntese dos AG (síntese "de novo") ocorre no citosol das células, principalmente no fígado, glândulas mamárias e tecido adiposo. Os AG são formados a partir do acetil-CoA proveniente quase totalmente da glicose em excesso da dieta. Uma seqüência de reações enzimáticas sucessivas resulta na síntese de ácido palmítico (16:0). A partir deste AG são obtidos os demais AG por processos de alongamento (adição de dois carbonos provenientes do acetil-CoA) e dessaturação (introdução de ligações duplas à molécula de AG). Os AGs da dieta também podem sofrer estes processos de alongamento e dessaturação (13,17,18).

Absorção dos ácidos graxos da dieta e transporte no plasma

A absorção de AG da dieta pela mucosa intestinal varia de acordo com o tamanho da cadeia carbônica. Os AG de cadeia curta (2 a 4 átomos de carbono) resultam de fermentação bacteriana de fibras e podem ser absorvidos no cólon por difusão ou por troca iônica. Já os AG de cadeia média (6 a 12 átomos de carbono) são hidrolisados principalmente pela lipase

pancreática e absorvidos no duodeno. Não são incorporados às lipoproteínas, sendo absorvidos diretamente na corrente sanguínea e transportados ao fígado ligados à albumina. Os AG de cadeia longa são absorvidos para dentro do enterócito e utilizados na ressíntese dos TG que serão então incorporados às lipoproteínas sintetizadas no enterócito (quilomícrons). O transporte e distribuição intracelular dos AG de cadeia longa ocorre através da proteína denominada "Fatty Acid Binding Protein 2" (FABP2) (19). Esta proteína intracelular só é expressa no intestino e liga-se aos AG em uma reação não covalente e saturável. A troca de uma guanina (G) por uma alanina (A) no códon 54 do gene de FABP2 (A54T) resulta na substituição de uma alanina (A) por uma treonina (T). A presença do alelo T-54 no polimorfismo do FABP2 foi associada com afinidade aumentada do FABP2 por AG de cadeia longa (20) e com níveis elevados de TG em pacientes com DM tipo 2 (21,22), além de estar relacionado com nefropatia diabética nestes pacientes (22). Polimorfismos no gene da FABP2 poderiam também estar relacionados à resistência à ação da insulina através do aumento da absorção de AG com conseqüente aumento na oxidação destes (23). O polimorfismo A54T do FABP2 foi associado com resistência à insulina em índios Pima sem DM (20) e em aborígines canadenses (24). Entretanto, estudos em japoneses e em finlandeses não observaram uma associação do polimorfismo A54T com sensibilidade à insulina ou obesidade (25,26). É possível que alterações na absorção de AG pela mucosa intestinal, como as descritas em pacientes portadores do alelo T, possam influenciar o perfil sérico de AG.

Os AG oriundos da dieta (cadeia longa) são transportados do intestino para o restante do organismo e atingem a circulação via sistema linfático sob a forma de quilomícrons que são lipoproteínas que apresentam densidade muito baixa. Os TG presentes nos quilomícrons, assim como os dos VLDL, são hidrolisados pela enzima lipase lipoprotéica dos tecidos que liberam os AG para serem utilizados como combustível pelos tecidos periféricos e/ou esterificados em lipídeos complexos (13).

Em algumas situações, os AG séricos refletem os AG ingeridos. Em especial, os AGP dietéticos apresentam uma forte associação com os seus níveis séricos (27,28). Esta relação é mais fraca para os AGS, enquanto que para os AGM ela é ausente (6,28). Ma et al. (27) demostraram que os AGM séricos não refletem a sua ingestão, mas sim a ingestão de AGS, provavelmente devido à síntese endógena de AGM a partir dos AGS.

Medida dos ácidos graxos no plasma

A cromatografia é o método de escolha para a identificação e a quantificação de AG séricos (13,29). Inicialmente, é realizada uma extração dos lipídeos do soro utilizando uma solução de água e metanol (fase aquosa) e clorofórmio (fase orgânica) (30). Quando a dosagem dos AG for realizada nas frações lipídicas (colesterol, ésteres de colesterol, TG, fosfolipídeos, AG livres, lipídeos polares) utiliza-se a cromatografia em camada delgada para a separação das mesmas. Esta etapa não é necessária para a determinação dos AG nos lipídeos totais, portanto, um método menos laborioso (13). A etapa seguinte é a determinação dos AG individuais ou por cromatografia gasosa (CG) – método mais comumente utilizado - ou cromatografia líquida de alta precisão ("High Performance Liquid Chormatography" - HPLC). Para a identificação dos AG, comparam-se os tempos de retenção de cada AG da amostra que está sendo medida com os de uma solução padrão (13,29) (Figura 1). É importante salientar que quando os AG séricos são medidos nos lipídeos totais, sua correlação com os lipídeos plasmáticos (colesterol, TG e HDL) é maior do que quando a medida é realizada nas frações lipídicas (31). Os AG do sangue, após separação por técnica específica (ultracentrifugação, por exemplo), podem também ser medidos por CG ou HPLC em determinadas lipoproteínas, como por exemplo, o quilomicron.

Papel fisiológico dos ácidos graxos

Os AG essenciais (AG ômega 6 e AG ômega 3) são precursores da biossíntese de vários metabólitos importantes, especialmente os eicosanóides (prostaglandinas, leucotrienos,

tromboxanas, lipoxinas, entre outros) que são sintetizados a partir do ácido araquidônico (13). As prostaglandinas têm um importante papel fisiológico, pois participam de diversos processos como a modulação dos processos de contração, dilatação e permeabilidade vascular, adesão leucocitária, promoção da agregação plaquetária, mobilização de cálcio intracelular, entre outros (13,18,32). Os AG essenciais, como o linoléico (18:2n-6) e α-linolênico (18:3n-3), são imprescindíveis ao organismo e têm importante papel no metabolismo celular, incluindo a fluidez das membranas. Desta forma, alterações na sua síntese e metabolismo podem estar vinculadas às alterações endoteliais e hemodinâmicas que contribuem para o aumento da morbi-mortalidade cardiovascular.

3. Ácidos Graxos plasmáticos e doenças cardiovasculares: efeitos relacionados à inflamação e função endotelial

A aterosclerose é um processo inflamatório *per se* (33,34), que se caracteriza por um aumento na produção e liberação de fatores inflamatórios na corrente circulatória. Como os AG estão diretamente relacionados com os processos inflamatórios, pode-se sugerir que alguns dos efeitos destes sobre o processo aterosclerótico ocorram por indução e/ou inibição de processos inflamatórios.

O ácido araquidônico (20:4n-6) é o AGP precursor de alguns eicosanóides, substâncias que têm papel importante no processo inflamatório. Já os AGP n-3 apresentam uma importante ação antiinflamatória (35) que pode contribuir para o efeito benéfico destes AG na função CV. Os principais representantes deste grupo de AG n-3 são os ácidos eicosapentaenóico (EPA; 20:5n-3) e docosahexaenóico (DHA; 22:6n-3) (12,32).

Os AG séricos apresentam uma relação com marcadores inflamatórios e com disfunção endotelial. Fernández-Real et al (36) demonstraram que os AGS e os AGP n-6 séricos apresentam, respectivamente, associação positiva e negativa com interleucina-6 e proteína C reativa em pacientes obesos com resistência insulínica. Ainda, alguns autores

observaram que os AGS apresentam um efeito negativo sobre a função endotelial e que os ácidos linolêico (18:2n-6) e α-linolênico (18:3n-3) apresentam um efeito protetor sobre a função endotelial (37,38).

Os níveis plasmáticos de AGP n-3 de cadeia longa (EPA e DHA) estão inversamente relacionados ao risco de morte súbita (9,39) e podem reduzir o risco de doença cardíaca isquêmica fatal (10). De fato, estes compostos possuem atividade antiinflamatória e seu uso tem sido indicado em doenças inflamatórias como artrite reumatóide e doença de Crohn (40). Os AGP n-3 reduzem o conteúdo de ácido araquidônico nas membranas celulares, resultando na síntese de eicosanóides que têm menor propriedade inflamatória do que aqueles derivados dos AGP n-6 (40). Ainda, os AGP n-3 inibem a síntese de citocinas proinflamatórias, como TNF-alfa, interleucina-1 e interleucina-2 (41) e reduzem a expressão de moléculas de adesão no endotélio (42).

Em relação aos AG livres, seu papel na aterosclerose foi demonstrado pela observação de um risco de DCV aumentado na presença de níveis plasmáticos elevados de AG livres (43). Esta associação foi recentemente confirmada também em pacientes com cardiopatia isquêmica estabelecida. Em uma coorte com cerca de 5 anos de duração um aumento de AG livres séricos foi capaz de prever a mortalidade por todas as causas e por doença cardiovascular em pacientes portadores de doença arterial coronariana (44).

Deve ser salientado que, apesar de os AG de uma maneira geral refletirem a ingestão dietética (20,21) , seus efeitos sobre a inflamação podem ocorrer independente do consumo alimentar, isto é por características peculiares a determinadas condições. Como exemplo, temos a microalbuminuria que é o estágio mais precoce da nefropatia diabética e pode ser considerada uma manifestação renal de um estado inflamatório generalizado (45). Demonstramos que pacientes com diabetes tipo 2 com microalbuminúira apresentam níveis

séricos menores de AGP na fração triglicerídeos do que pacientes normoalbuminúricos quando os dois grupos adotaram a mesma dieta padronizada para DM. (46).

4. Ácidos Graxos da dieta e doenças cardiovasculares

Os efeitos dos AG da dieta ocorrem em geral através da conseqüente modificação dos AG plasmáticos e/ou lipoproteínas a eles relacionadas (12). Os AGP podem reduzir os níveis de colesterol e triglicerídeos por diminuírem a taxa do LDL plasmático, em função de aumentarem o número de receptores de LDL, a síntese endógens de colesterol e a atividade da lipoproteína lípase (12,32) Além disto, os efeitos dos AGP no sistema CV são também decorrentes de sua influência na sensibilidade à insulina (36), nos mecanismos de trombose (39), na função endotelial (47) e nos processos inflamatórios, como referido anteriormente. Os principais efeitos dos AG da dieta sobre os fatores associados a eventos CV estão resumidos na Tabela 1.

Ácidos graxos dietéticos poliinsaturados

A maior ingestão de AGP tem sido associada ao menor risco de morbidade e mortalidade cardiovascular (48). Além disso, questiona-se se o efeito protetor dos AGP n-3 sobre a prevenção de mortalidade cardiovascular é pelo seu efeito sobre fatores de risco cardiovasculares e estabilização de placa aterosclerótica, ou se apenas por seu efeito benéfico sobre as arritmias cardíacas fatais (49).

Estudos que avaliaram a suplementação da dieta com AGP n-3 demonstraram que eles melhoram a função dos barorreceptores em pacientes com insuficiência cardíaca estável (50) e reduzem fatores inflamatórios (47,51,52) e marcadores de dano endotelial (47). De fato, o efeito dos AGP das classes n-3 parece ir além de seu efeito hipolipemiante. Recentemente, observou-se em pacientes com hipercolesterolemia que, associadas à redução de gordura saturada e de colesterol, uma dieta rica em ácido α-linolênico (18:3n-3) promoveu uma maior redução nos níveis de proteína C reativa, molécula de adesão à célula vascular 1 e seletina E,

quando comparada a uma dieta rica em ácido linolêico (18:2n-6) (51). Este efeito benéfico do ácido α-linolênico (18:3n-3) sobre a redução dos níveis de proteína C reativa em pacientes dislipidêmicos foi também confirmado em relação à interleucina-6, independente do perfil lipídico (47). Entretanto, este efeito benéfico deste AG sobre outros marcadores de doença aterosclerótica como o LDL oxidado, interleucinas, espessura da camada íntima da carótida e da femoral não foi observado por outros autores (52).

Estudos observacionais demonstraram que, além de reduzirem a mortalidade total e CV (53), as dietas ricas em AGP n-3 de cadeia longa, EPA e DHA, também reduziram o risco de desenvolvimento de DCV (54) e diminuiram o risco de desenvolvimento de doença isquêmica cardíaca fatal (10). Pacientes com alto consumo de EPA e DHA têm menor risco de morte súbita do que aqueles com baixo consumo destes AG (54). O AG α-linolênico (18:3n-3) também apresenta efeito protetor sobre a função CV (10,53).

No entanto, recentemente, uma meta-análise que avaliou estudos de coorte e ensaios clínicos randomizados e controlados demonstrou que os AGP n-3 não apresentam um definitivo efeito protetor sobre a mortalidade total ou eventos CV (55). Contudo, esta meta-análise incluiu o estudo DART-2 (56) no qual a análise dos AG séricos para avaliação da aderência ao tratamento foi realizada em apenas 2% dos participantes e apenas nos 6 primeiros meses do estudo. Quando este estudo foi excluído da análise os AGP n-3 passaram a apresentar um efeito protetor sobre os eventos CV [RR = 0,83 (0,75 – 0,91)] (56). Também em relação ao AGP n-6 existem controvérsias. O consumo aumentado do ácido linoléico, um AG n-6, foi considerado um fator de risco para DCV (10), observação não confirmada por outros autores (57). Ainda, a possível interferência no consumo dos AGP n-6 sobre o efeito benéfico do AGP n-3 foi avaliada. A ingestão de AGP n-3 foi capaz de reduzir o risco de DCV, com mínima influência do consumo dos AGP n-6 (54). Já Leskinen et al. (6) em um estudo de caso-controle observaram que pacientes após infarto do miocárdio tiveram valores

mais elevados de AG palmítico (16:0) e oléico (18:1), e menor de linoléico (18:2 n–6) comparados ao grupo controle. Além disto, os pacientes pós-infarto apresentaram índices maiores da enzima delta-6 dessaturase, responsável pela síntese de alguns AGP n-6. A enzima delta-6 dessaturase converte o ácido linoléico (18:2n-6) em γ-linolênico (18:3-n6) e homogamalinolêncio (20:3n-6) e encontra-se com maior atividade na síndrome metabólica (28), sabidamente uma situação de elevado risco CV (58). Além de estar relacionada ao infarto do miocárdio, uma menor ingestão de ácido linoléico, associada à maior ingestão de AGS e AGM foi associada a um aumento da prevalência de hipertrofia de ventrículo esquerdo (7). Ainda, o ácido linoléico (18:3-n6) da alimentação e os seus níveis séricos têm sido associados a uma menor mortalidade global em homens de meia idade (8). E, finalmente, na análise mais recente da coorte do "Nurses Health Study", o consumo do ácido linoléico (18:2n-6) apresentou uma associação inversa e significativa com o risco de eventos coronarianos, sugerindo um efeito também protetor sobre a DCV (48).

De fato, o papel do AGP n-3 na prevenção de eventos CV é baseado em dados contundentes, conforme demonstrado em estudos epidemiológicos e de intervenção clínica (56,59). Revisões atuais (56,59) sugerem a suplementação dietética de AGP n-3, em especial EPA e DHA, para a prevenção de eventos CV.

Embora a maioria das evidências sugira que os AGP tenham efeitos benéficos sobre a DCV, seus efeitos ainda não estão completamente esclarecidos, sendo necessário a realização de ensaios clínicos randomizados com desfechos bem determinados para avaliar o efeito dos AGP n-3 e dos AGP n-6, individualmente e associados.

Ácidos graxos dietéticos saturados e monoinsaturados

Os AGS da alimentação aumentam os níveis de colesterol total, principalmente os níveis de LDL colesterol, por diminuir a expressão dos receptores hepáticos de LDL,

reduzindo a captação do colesterol e sua utilização intracelular. Também os AGM, quando substituem os AGS, apresentam um efeito hipocolesterolêmico.

Um estudo realizado com uma coorte de 505 homens com um seguimento de 20 anos demonstrou uma associação entre a maior ingestão de AGS e a perda de função ventricular esquerda, podendo estes AGs estarem associados a uma possível disfunção diastólica (60).

O efeito do consumo dos AGM tem sido demonstrado através de estudos sobre a dieta mediterrânea que caracteriza-se por ser rica em fibras, grãos integrais, vegetais, nozes e azeite de oliva e apresentar uma grande quantidade de AGM. Numerosas evidências sugerem que a aderência à dieta mediterrânea está associada a menos eventos CV, especialmente em pacientes pós-infarto do miocárdio (61,62). O efeito protetor da dieta mediterrânea é provavelmente superior ao da dieta pobre em gordura. Neste sentido, recentemente, um ensaio clínico randomizado comparou o efeito a curto-prazo da dieta mediterrânea sobre marcadores intermediários de risco CV, com uma dieta apenas restrita em gordura de acordo com as recomendações da American Heart Association. Foram avaliados 772 pacientes assintomáticos, mas de alto risco CV. A dieta mediterrânea (com adição de azeite de oliva ou suplementação com nozes) resultou em uma maior redução dos níveis de glicose, níveis pressóricos, proteína C reativa e melhora do perfil lipídico quando compara da a uma dieta pobre em gordura (63). Além do efeito benéfico sobre o perfil lipídico, a dieta mediterrânea também tem se mostrado eficaz em melhorar a função endotelial (64). Entretanto, acredita-se que seu efeito benéfico seja não somente devido ao seu elevado conteúdo de AGM, mas também devido a outros componentes desta dieta como o conteúdo de polifenóis, que também apresentam efeito favorável sobre os lipídeos séricos (aumento de HDL e redução de LDL) (65).

Ácidos graxos dietéticos *trans*

A ingestão de AG *trans,* assim como de AGS*,* foi associada a um maior risco de DCV (66) e o impacto desta associação se torna significativo especialmente em populações com grande ingestão destes AG. A ingestão média de AG *trans* tem sido estimada em torno de 2,7% do valor energético total diário (67), valores estes bem acima das recomendações atuais.

A maior ingestão de AG *trans* foi positivamente associada ao maior risco de doença coronariana, mesmo quando analisada em diferentes faixas etárias e em diferentes valores de índice de massa corporal (48). Esta associação foi confirmada em uma meta-análise recente realizada com quatro estudos prospectivos com aproximadamente 140 mil pacientes (68). Foi demonstrado que o aumento de dois pontos percentuais no consumo de AG *trans* resultou em um acréscimo de 23% na incidência de DCV (68). Ainda, os AG *trans* podem aumentar o risco de morte súbita por problemas CV (69).

Os ácidos graxos *trans* possuem um efeito deletério sobre as lipoproteínas plasmáticas, pois além de aumentarem os níveis de LDL colesterol, triglicerídeos e lipoproteína (a), também reduzem os níveis de HDL colesterol (12,13,32). Deve ser salientado que a associação da ingestão de AG *trans* com a incidência de DCV em estudos prospectivos é maior do que aquela decorrente somente da melhora no perfil lipídico, sugerindo que os AG trans influenciem também outros fatores de risco cardiovasculares (70). Os AG *trans* parecem aumentar o risco de DCV mais do que qualquer outro nutriente, conferindo um aumento importante de risco mesmo com baixos valores de consumo (68). De fato, os AG *trans* também apresentam efeito adverso sobre a função endotelial, sensibilidade à insulina e trombose (4,57,71).

Recomendações dietéticas para prevenção de DCV

Com base nas evidências existentes sobre a influência dos AG sobre a DCV, a *American Heart Association* (72) recomenda que, associado a outras estratégias para modificações de estilo de vida, a ingestão de gorduras saturadas da dieta seja menor do que

7% e de gordura *trans* menor que 1% do valor energético total, sendo que o colesterol não deve ultrapassar 300 mg/dia. Estes valores podem ser alcançados através de modificações na qualidade da dieta como a escolha de carnes magras e de alternativas de vegetais e legumes; ingestão de produtos lácteos desnatados com até 1% de gordura na sua composição e redução na ingestão de gorduras parcialmente hidrogenadas. O papel protetor dos AGP n-3 na prevenção de eventos CV é aceito pela AHA que recomenda a suplementação dietética de 1g/dia dos ácidos EPA e DHA, preferencialmente provenientes de óleo de peixe para os pacientes com DCV e de 2 a 4g/dia para aqueles pacientes que apresentam também hipertrigliceridemia (72).

5. Conclusão

Os ácidos graxos apresentam um papel importante tanto na prevenção quanto no desenvolvimento da DCV. Além do seu efeito sobre as lipoproteínas plasmáticas, os AG atuam nos processos inflamatórios, tem papel importante sobre a função endotelial, sensibilidade à insulina e sobre os mecanismos trombogênicos. A ingestão de AG trans e de AGS apresentam efeito deletérios sobre o processo aterosclerótico e podem ter um efeito arritmogênico. Efeito deletério sobre a DCV foi também demonstrado na presença de concentrações elevadas de AG livres. Embora a maioria dos dados existentes até o presente momento demonstrem que os AGP têm um papel protetor em relação à DCV, estas evidências serão melhor fundamentadas a partir de novos ensaios clínicos randomizados.

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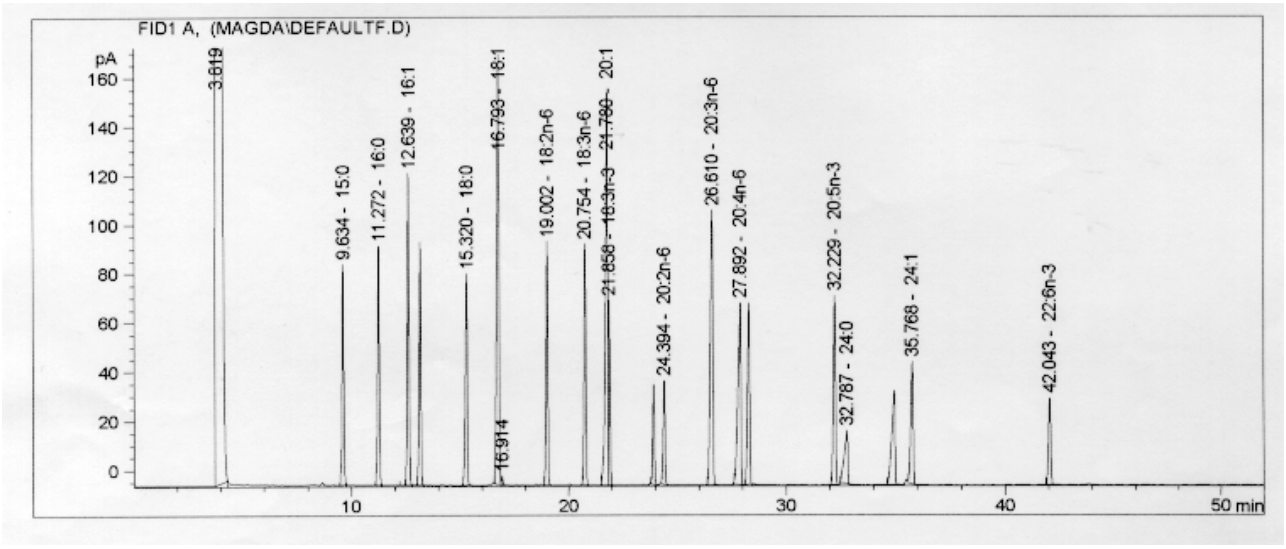

Figura 1: Exemplo de cromatograma (cromatografia gasosa) obtido a partir de uma amostra padrão contendo 20 diferentes ácidos graxos

Tabela 1: Efeitos dos ácidos graxos da dieta sobre os fatores que influenciam os eventos cardiovasculares

Ácido graxo	**Efeito**
AGS	- Elevação dos níveis de LDL (redução de receptores LDL); - Associação positiva com níveis de IL-6 e PCR; - Associação com perda de função ventricular esquerda.
AG *trans*	- Elevação dos níveis de LDL, triglicerídeos e lipoproteína (a); - Redução dos níveis de HDL.
AGM	- Redução dos níveis de LDL.
AGP n-6	- Redução dos níveis de LDL; - Precursores de substâncias inflamatórias.
AGP n-3	- Redução dos níveis de LDL e triglicerídeos; - Efeito antiinflamatório; - Melhora na função de barorreceptores; - Redução nos níveis de PCR e seletina 1.

AG: ácidos graxos; AGS: ácidos graxos saturados; AGM: ácidos graxos monoinsaturados; AGP: ácidos graxos poliinsaturados; IL-6: interleucina 6; PCR: proteína C reativa.

CAPÍTULO II: Endothelial dysfunction and serum fatty acid composition in patients with type 2 diabetes*

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Endothelial dysfunction and serum fatty acid composition in patients with type 2 diabetes

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Abstract

The aim of this study was to evaluate the possible association between serum fatty acids composition and endothelial dysfunction in patients with type 2 diabetes. A cross-sectional study was conducted with 125 type 2 diabetic patients normo- or microalbuminuric with serum creatinine <1.5 mg/dL. Serum fatty acids composition (gas chromatography), serum levels of endothelin-1 [(ET-1), ELISA], fibrinogen, serum C-reactive protein, lipids, HOMA resistance index (HOMA-R), and 24-h urinary albumin excretion rate (UAER) were measured. Serum levels of ET-1 were positively correlated with saturated fatty acids [(SFAs), r = 0.257; P = 0.025) and negatively correlated with polyunsaturated fatty acids [(PUFAs), r = -0.319; P = 0.005]. Serum ET-1 levels were also positively correlated with systolic blood pressure, waist circunference, total cholesterol levels, triglycerides and HOMA-R. In multiple linear regression models only SFAs (R^2 = 0.317, P = 0.002) or PUFAs (R^2 = 0.314, P = 0.001) remained associated with ET-1 levels. Models were adjusted for systolic blood pressure, HOMA-R, waist circumference, triglycerides, BMI and smoking habit. The serum total PUFA levels showed an inverse correlation with UAER (r = -0.248; P = 0.012). In conclusion, in type 2 diabetic patients the serum fatty acids composition was independently related to endothelial function evaluated by serum ET-1. SFAs were associated with endothelial dysfunction (high levels of ET-1), while PUFAs had a protective role in endothelial function.

1. Introduction

In patients with type 2 diabetes mellitus, endothelial dysfunction, increased urinary albumin excretion and chronic inflammation are interrelated processes that develop in parallel, and are strongly and independently associated with risk of death (1). Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced by endothelial and vascular smooth muscle cells and it has been used as a marker of endothelial function (2). Experimental studies in patients with and without diabetes have consistently shown that ET-1 had a significant correlation with flow-mediated vasodilation of brachial artery (3). Endothelial dysfunction increases ET-1 production leading to vascular hypertrophy, atherogenesis and to glomerulosclerosis in the kidney (2).

We have previously reported that normoalbuminuric dyslipidemic type 2 diabetic patients had increased levels of ET-1 (4) and this was associated with urinary albumin levels and insulin resistance. It has been shown that patients with diabetic nephropathy (DN) had higher levels of ET-1 compared to patients without DN (5).

Serum fatty acids composition has been associated with cardiovascular mortality (6) and sudden death (7). Patients with serum polyunsaturated fatty acids (PUFAs) in the upper tertile had a lower cardiovascular mortality rate (6). We have demonstrated that type 2 diabetic patients with microalbuminuria had lower proportion of serum PUFAs (8). Moreover, the replacement of red meat [high content of saturated fatty acids (SFAs)] in the usual diet by chicken meat (high PUFAs content) reduced albumin excretion rate in micro- and macroalbuminuric type 2 diabetic patients (9,10) and increased the serum levels of PUFAs (10). PUFAs may have a beneficial effect on endothelial function, since microalbuminuria appears to represent the glomerular involvement in a state of generalized vascular dysfunction (11,12). Consequently, PUFAs may have a beneficial effect on endothelial function, but this possible association has not yet been analyzed in patients with type 2 diabetes. Therefore, this

study was conducted to evaluate the possible association between serum fatty acids composition and endothelial dysfunction in patients with type 2 diabetes.

2. Methods

2.1. Patients

One hundred and twenty-five patients with type 2 diabetes mellitus (WHO criteria) attending the Endocrine Division's outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil were selected on the basis of the following criteria: body mass index (BMI) <40 kg/m^2; A_{1c} test <9.0%; triglyceride levels <400 mg/dL; urinary albumin excretion rate (UAER) <200 μg/min; serum creatinine ≤1.5 mg/dL, normal liver and thyroid function; absence of urinary tract infection (negative urine culture), presence of other renal disease, heart failure (class III or IV) or acute cardiovascular event in the preceding 6 months. Treatment with antihypertensive and oral antidiabetic agents was maintained during the study. None of the patients were using hypolipidemic agents. The local Ethics Committee approved the protocol and patients gave their written informed consent.

Eligible patients entered a run-in period of approximately one month, during which they were instructed to perform a 3-day weighed diet records, as previously reported (13). At the end of the run-in period, patients underwent a clinical and laboratoty evaluation. The body weight and height of patients (without shoes or coats) were obtained with an anthropometric scale. BMI [weight (kg)/height^2 (m)] was then calculated. Waist circumference was measured midway between the lowest rib margin and the iliac crest, near the umbilicus. Flexible, nonstretch fiberglass tape was used for these measurements. 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. The presence of metabolic

syndrome was established according to the National Cholesterol Education Program (NCEP) criteria (14).

2.2. Laboratory measurements

Blood samples were collected after a 12-hour overnight fast. For the measurement of plasma ET-1, venous blood (5 ml) was drawn and put into a refrigerated tube containing EDTA. Serum and plasma were separated after centrifugation at 1,500 g and 4°C for 15 min, and stored at –80°C for later measurements. ET-1 was measured by ELISA using a commercial kit (R&D Systems, Minneapolis, USA). Plasma glucose was measured by a glucose oxidase method, serum creatinine by the Jaffé reaction, A1c test by ion-exchange HPLC (Merck-Hitachi L-9100 glycated hemoglobin analyzer, reference range: 4.7 – 6.0%; Merck, Darmstadt, Germany), and insulin by a chemoluminescent method (Elecsys 2010, Basel, Switzerland). Insulin resistance was estimated by HOMA resistance index (HOMA-R = fasting serum insulin (μU/ml) X fasting plasma glucose (mmol/l)/22.5; (15)). Fibrinogen was measured by a coagulometric method (Sta Compact: Cedex, France), serum C reactive protein (CRP) by nephelometry (reference range: 1–4 mg/L). Urinary albumin was measured in 24-h timed sterile urine samples by immunoturbidimetry (Sera-Pak immuno microalbuminuria; Bayer, Tarrytown, NY). Microalbuminuria was considered to be present when UAER was 20–200 μg/min at least twice in a 6-month period. Serum total cholesterol and triglycerides were measured by enzymatic-colorimetric methods and HDL cholesterol by a direct selective inhibition method. LDL cholesterol was calculated by using Friedewald's formula. Fatty acids were determined in total lipids. Lipids were extracted from serum with chloroform-methanol (2:1; by volume) and converted into fatty acid methyl esters by boron trifluoride catalysis as described previously (8). In brief, the methyl esters were then separated and measured by gas chromatography on a 60 m fused silica capillary column with an internal diameter of 0.20 μm (CP – Sil 88). Analysis was performed on a Hewlett-Packard 6890 gas

chromatograph equipped with a flame ionization detector. Helium was used as carrier gas and nitrogen as make-up gas. The split-ratio was 5:1. The injection port temperature was 200°C and the detector temperature was 250°C. The column temperature was held at 160°C for 5 min and increased to 190°C at a rate of 2°C/min; it was then held at this temperature for 2 min, and increased again to 220°C at a rate of 1°C/min. The identity of each fatty acid peak was ascertained by comparing the peak retention time with a previously characterized mixture of 24 fatty acids. The relative amount of each fatty acid (proportion of total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids.

2.3. Statistical analysis

Partial correlation coefficients were used for testing the relationships between the ET-1 levels and serum fatty acids, using LDL, HDL and triglycerides as covariates. Multiple linear regression models were carried out to test the association of ET-1 (dependent variable) and factors with possible biological relevance or significant at univariate analysis. The serum ET-1 levels and fatty acids composition for patients grouped according to the presence of the metabolic syndrome components were analyzed using the one-way ANOVA, followed by a Tukey post-hoc test for multiple comparisons. Variables with non-Gaussian distribution were log transformed before analysis. Data are presented as medians and 95% CI or means ± SD unless otherwise stated. P values <0.05 were considered statistically significant. SPSS software version 12.0 for Windows (SPSS Inc., Chicago, IL) was used.

3. Results

3.1. Patient characteristics

The main clinical and laboratory characteristics of the patients are shown in Table 1. The patients presented a reasonable glycemic control and none were using hypolipemic agents (fibrates or statins). Ninety-three patients (74.4%) met the NCEP criteria for the metabolic

syndrome. Two women were using hormone replacement therapy and one was using an oral contraceptive; nine patients were currently smokers. The majority of the patients were not engaged in any physical activities (51%) nor taking light physical exercise (42%). The type of antihypertensive treatment was diuretics (59 patients), direct vasodilators (one patient), ACE inhibitors (65 patients), angiotensin II receptor antagonist (12 patients) and beta-blockers (41 patients). Only one patient did not use antihypertensive drugs.

3.2. Dietary intake and serum fatty acid composition

The mean dietary daily intake of the patients assessed by 3-day weighed diet records was: total energy = 1883 ± 475 Kcal; carbohydrates = 46.29 ± 6.57%; proteins = 18.84 ± 3.21%; lipids = 34.88 ± 6.78%; cholesterol = 210 ± 102 mg; SFAs = 9.58 ± 2.52%; monounsaturated fatty acids (MUFAs) = 11.90 ± 2.92%; PUFAs = 10.37 ± 3.48%. The intake of *trans* fatty acids was 0.97% (0.36 to 4.39%) of the total energy intake.

The levels of serum fatty acids (% of total fatty acids) were: SFAs = 39.19 ± 5.38%; MUFAs = 23.00 ± 3.93%; PUFAs = 37.79 ± 5.70%; total n-6 PUFAs = 36.83 ± 5.78 and total n-3 PUFAs = 0.71% (0 to 6.21%).

The proportion of serum PUFAs was positively correlated with the intake of the PUFAs ($r = 0.320$; $P = 0.001$). This correlation was particularly evident between the serum n–6 PUFAs and the diet content of n-6 PUFAs ($r = 0.305$; $P = 0.001$). No significant association was observed between serum levels and intake of MUFAs ($r = 0.069$; $P = 0.467$) and SFAs ($r = -0.101$; $P = 0.288$).

3.3. Variables associated with serum ET-1 levels

Serum levels of ET-1 had a positive correlation with systolic blood pressure, waist circumference, serum total cholesterol and triglycerides levels, HOMA-R, and serum CRP concentration. Serum ET-1 levels were also positively correlated with serum SFAs and had a

negative correlation with serum PUFAs, especially with the n-6 fatty acids group and with the individual fatty acids: linoleic and arachidonic acid (Table 2).

Considering that blood pressure, waist circumference and serum triglycerides are part of the metabolic syndrome we also analyzed the association of ET-1 levels with the number of components of the metabolic syndrome (Table 3). A progressive increase in serum ET-1 levels was observed according to the increase in the number of metabolic syndrome components ($P < 0.001$). However, significance was only observed between patients with five components as compared with the patients without metabolic syndrome ($P = 0.035$). Interestingly, a decreased proportion of serum PUFAs ($P = 0.002$) and an increase in the proportions of serum MUFAs ($P = 0.001$) according to the number of metabolic syndrome components was also observed. Patients with five components had a higher proportion of MUFAs and a lower proportion of PUFAs as compared to patients with only three components ($P = 0.001$; $P = 0.020$, respectively) or to patients without the metabolic syndrome ($P = 0.001$; $P = 0.002$). We did not observe statistical significance on patients with four components of metabolic syndrome when compared with patients with three components or patients without metabolic syndrome. The serum proportion results of total n-6 PUFAs were similar to those of PUFA. On the other hand, these associations were not observed in n-3 PUFAs and SFAs ($P = 0.479$ and $P = 0.079$, respectively).

Multiple linear regression analyses were performed with serum ET-levels as the dependent variable and serum PUFAs proportion, systolic blood pressure, HOMA-R, waist circumference, triglycerides, BMI and smoking as independent variables. Only the proportion of serum PUFAs remained significantly associated with serum ET-1 ($R^2 = 0.329$; $P = 0.002$), especially n-6 PUFAs ($R^2 = 0.324$; $P = 0.002$). The results did not change when systolic blood pressure, HOMA-R, waist circumference, triglycerides and BMI were replaced by the presence or absence of metabolic syndrome ($R^2 = 0.264$; $P < 0.001$). In another model, the

proportion of serum SFAs was included instead of PUFAs and systolic blood pressure, HOMA-R, waist circumference, triglycerides, BMI and smoking were considered as independent variables. Again, only SFAs remained significantly associated with serum ET-1 levels (R^2 = 0.317; P = 0.002) (Figure 1). Once more, when the components of metabolic syndrome were replaced by the presence or absence of the metabolic syndrome only SFAs were significantly associated with serum ET-1 levels (R^2 = 0.184; P = 0.001).

3.4. Other associations

The serum proportion of PUFAs and total n-6 PUFAs showed an inverse correlation with UAER (respectively, r = -0.248; P = 0.012, r = -0.217; P = 0.027). The proportion of serum linoleic acid was inversely correlated with HOMA-R (r = -0.254; P = 0.034). Serum arachidonic acid also correlated with HOMA-R (r = -0.228) but did not reach statistical significance (P = 0.057).

4. Discussion

In this sample of patients with type 2 diabetes, it was observed that the serum SFAs had a positive correlation with serum levels of ET-1. The results showed that one third of the variability of ET-1 was independently predicted by levels of serum fatty acids. Additionally, an increase in the number of components of the metabolic syndrome was associated with an increase in ET-1 levels, serum MUFAs and SFAs levels, and with a decrease in PUFA levels.

Very few studies have analyzed the association of fatty acids and endothelial function in individuals without diabetes and, as far as we know, there are no data in patients with diabetes. Other authors have observed that the proportion of SFAs had a negative effect on endothelial function assessed by endothelium-dependent vasodilation and venous occlusion plethysmography (16,17). Interestingly, they also reported that PUFA had a protective effect on the endothelial function. In a study with healthy subjects, palmitoleic acid was inversely associated with the index of endothelial function, while stearic and linoleic acids were

positively correlated with this index (16). In young men, elevated serum SFAs and decreased of alpha-linolenic acid were related to a reduced endothelial vasodilatory function evaluated by venous occlusion plethysmography (17). Moreover, improved endothelial function mediated by PUFAs could also lead to a beneficial effect on glomerular membrane properties. In fact, in the present study it was observed a negative correlation between serum PUFAs and UAER.

The possible mechanism of the deleterious effect of SFAs on endothelial function is still largely unidentified. It is well known that SFAs may increase serum cholesterol levels (18) and in fact we observed a positive correlation between serum cholesterol and ET-1. Moreover, we observed that the aggregation of cardiovascular risk factors was associated with increased serum ET-1 levels and a concomitant increase in serum SFAs. As the association of serum fatty acids and ET-1 remained significant after controlling for the other factors we may speculate that serum SFAs may have a direct effect on endothelial function. Previous studies have shown that a single meal containing high levels of saturated fat impairs endothelial function for 2 to 6 hours (19).

The serum fatty acids composition depends on the fatty acid content of the diet and on the metabolism of the fatty acid beginning by its synthesis and also taking into consideration the elongation, desaturation and oxidation steps (20). In this sample of type 2 diabetic patients, dietary factors may have had a significant contribution to the serum fatty acid composition, since there was a positive correlation between the intake and the serum levels of PUFA. Moreover, the replacement of beef meat by chicken meat reduced the UAER in micro- (9) and macroalbuminuric type 2 diabetic patients (10) and increased the serum PUFA levels. Chicken meat presents a higher content of PUFA than the beef (21). Although dietary factors may have a significant impact on serum PUFA levels, other factors may also be involved. We observed previously, that microalbuminuric type 2 diabetic patients following a standardized

diet had lower levels of PUFA as compared with normoalbuminuric patients (8). This observation suggests that other factors related to renal involvement associated with diabetes may also have an influence on serum fatty acids. Furthermore, one should take into account that specific genetic polymorphism of the fatty acids binding protein 2 are more frequent in type 2 diabetic patients with renal disease (22) and may influence the intestinal absorption of dietary fatty acids (23).

The observation that serum SFAs levels increased in parallel with the increase in the number of the components of the metabolic syndrome and that both influence the ET-1 serum levels suggests that the role of SFAs in endothelial function is as deleterious as the presence of metabolic syndrome. The concomitant decrease in serum PUFA under these circumstances may suggest that patients with metabolic syndrome had a low intake of foods rich in PUFA. Alternatively, PUFA, especially linoleic and arachidonic acids may have a beneficial effect on insulin sensitivity (24).

The serum proportion of MUFAs did not correlate with serum ET-1 levels,-probably by the conversion of dietary oleic acid (a MUFA) in a SFA in the blood (20). It was demonstrated that the benefic effect of dietary MUFAs probably was to the polyphenols constituents of the foods riches in MUFAs (25). Therefore is most unlikely that *trans* fats may have had an influence on the results presented. Another limitation was the cross sectional design of the study which allows only for the description of the association between the proportion of serum fatty acids and endothelial function and not for establishing a cause and effect relationship between these parameters.

In conclusion, in type 2 diabetic patients the serum fatty acids composition was independently related to endothelial function evaluated by serum ET-1. SFAs were associated with endothelial dysfunction (high levels of ET-1), while PUFAs had a protective role in

endothelial function. In addition, PUFAs were inversely correlated with UAER, a surrogate marker of renal and cardiovascular disease.

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

Characteristic	
Age (years)	59. 6 ± 10.7
Gender (male)	62 (49.6%)
Diabetes duration (years)	10.8 ± 7.0
Hypertension	100 (80%)
Microalbuminuria	42 (33.6%)
Diabetes treatment (D/OA/I/I+OA)	6/77/16/26
Body mass index (kg/m^2)	28.8 ± 4.1
Fasting plasma glucose (mg/dl)	145 ± 52
A_{1C} test (%)	7.1 ± 1.4
Serum creatinine (mg/dl)	0.89 ± 0.20
Total cholesterol (mg/dl)	202 ± 43
HDL cholesterol (mg/dl)	49 ± 10
LDL cholesterol (mg/dl)	124 ± 35
Triglycerides (mg/dl)	134 (40 – 485)
HOMA resistance index	3.6 (0.17 – 74)
Fibrinogen (mg/dl)	388 ± 85
Endothelin-1 (pg/ml)	0.67 ± 0.39
C reactive protein (mg/l)	2.9 (0.18 – 10)

Data are expressed as mean ± SD, median (95% CI) or number of patients (%) with the characteristic. D/OA/I/I+OA: diet only/oral antidiabetic agents/insulin/insulin associated with oral antidiabetic agents.

Table 2: Partial correlations of serum endothelin-1 (pg/ml) levels with clinical and laboratory variables in type 2 diabetic patients.

Variables	**R**	**P**
Systolic blood pressure (mmHg)	0.216	0.041
Diastolic blood pressure (mmHg)	0.129	0.225
Body mass index (kg/m^2)	0.182	0.086
Waist circumference (cm)	0.255	0.015
Total cholesterol (mg/dl)	0.262	0.013
LDL cholesterol (mg/dl)	0.117	0.275
HDL cholesterol (mg/dl)	-0.051	0.632
Triglycerides (mg/dl)	0.377	<0.001
Fasting plasma glucose (mg/dl)	0.127	0.237
A1c test (%)	0.156	0.145
Creatinine (mg/dl)	-0.118	0.269
HOMA resistance index	0.232	0.048
C reactive protein (mg/l)	0.241	0.028
Fibrinogen (mg/dl)	0.201	0.082
Saturated fatty acids (%)	0.257	0.025
Monounsaturated fatty acids (%)	0.075	0.521
Polyunsaturated fatty acids (%)	-0.319	0.005
Total n-6 fatty acids (%)	-0.302	0.005
Total n-3 fatty acids (%)	0.017	0.881
Linoleic acid (18:2n-6)	-0.261	0.023
Arachidonic acid (20:4n-6)	-0.227	0.049

Table 3: Serum endothelin-1 levels (pg/ml) and serum fatty acids (%) in type 2 diabetic patients according to the presence of metabolic syndrome and its components.

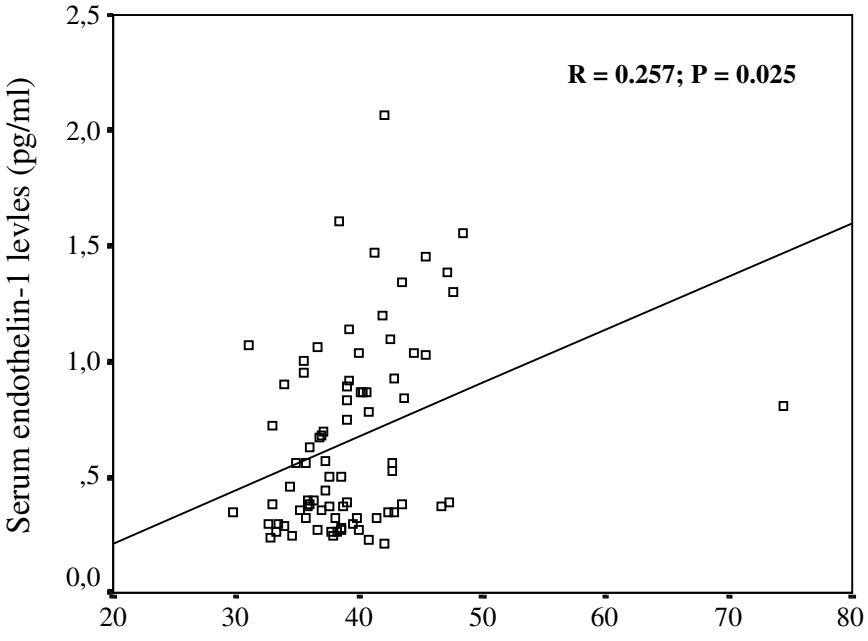
	Without MS (n = 32)	**3 components** (n = 42)	**4 components** (n = 30)	**5 components** (n = 21)	**P**
ET-1	0.52 ± 0.25	0.64 ± 0.35	0.80 ± 0.52	0.86 ± 0.42	0.023*
SFAs	38.0 ± 4.1	39.3 ± 4.6	40.2 ± 8.2	39.8 ± 4.2	0.079
MUFAs	21.9 ± 2.7	22.2 ± 3.8	23.4 ± 4.4	26.1 ± 3.7	0.002#
PUFAs	40.0 ± 4.7	38.6 ± 5.1	36.4 ± 7.1	34.0 ± 4.6	0.001#
Total n-6 fatty acids	39.3 ± 4.8	37.4 ± 5.4	35.7 ± 6.9	32.9 ± 4.9	0.002#
Total n-3 fatty acids	0.79 ± 0.90	1.21 ± 1,.29	0.74 ± 0.49	1.06 ± 0.84	0.479

Data are expressed as mean ± SD. ET-1: endothelin-1; MS: metabolic syndrome; SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids.

* Five components vs. without MS

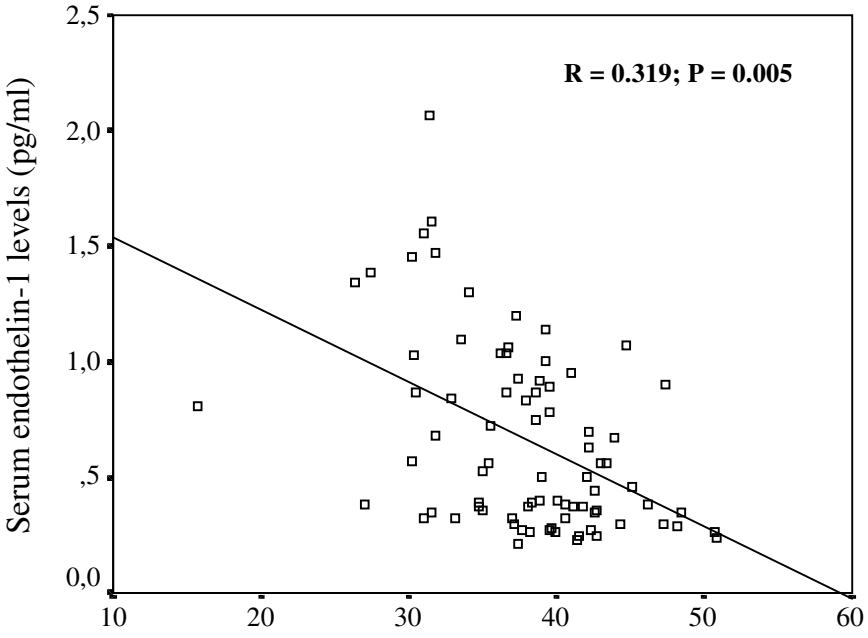
Five components vs. without MS and three components

A



Proportion of serum saturated fatty acids (%)

B



Proportion of serum polyunsaturated fatty acids (%)

Figure 1: Partial correlations between serum ET-1 levels and serum fatty acids: A – Saturated Fatty Acids, B – Polyunsaturated Fatty Acids.

CAPÍTULO III: Measurement of serum fatty acids in total lipids and in lipid fractions in type 2 diabetic patients.

Measurement of serum fatty acids in total lipids and in lipid fractions in type 2 diabetic patients.

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Abstract

The aim of this study was to analyze the possible associations between serum fatty acids (FAs) measured in total lipids and the FAs measured in lipid fractions and with serum lipid levels in type 2 diabetic patients. The FA composition of total lipids and of the lipid fractions (phospholipid, triglyceride and cholesterol ester) were determined by gas chromatography in the fasting state in 70 type 2 diabetic patients (age = 57.4 ± 9.9 years, 37 males, BMI= 27.1 ± 3.2 kg/m^2) after a four-week standardized diet. Lipid fractions were separated by thin layer chromatography. The proportion of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) in total lipids had a correlation only with the proportion of FAs in the triglyceride fraction ($r = 0.205$, $P = 0.089$; $r = 0.485$, $P<0.001$; $r = 0.238$, $P = 0.031$, respectively) but not with FAs in the phosphoplipid and cholesterol ester fractions. Dietary and serum PUFA in total lipids were also correlated ($r = 0.396$; $P = 0.001$) but this was not observed with the others classes of FAs, either in total lipids or in lipid fractions. Regarding associations with serum lipids, serum MUFA in total lipids had a positive correlation with total cholesterol ($r = 0.288$; $P = 0.016$) and triglycerides ($r = 0.754$; $P<0.001$), and a negative correlation with HDL cholesterol ($r = -0.308$; $P = 0.011$). Serum PUFA in total lipids was negatively correlated with cholesterol ($r = -0.291$; $P = 0.014$) and triglycerides ($r = -0.633$; $P<0.001$) and serum SFAs did not correlate with any lipid fraction. In conclusion, measurement of FAs in total lipids express only the content of FAs in the triglyceride fraction but not in phospholipid and cholesterol fractions. These measurements may represent a better functional assessment of FAs, especially PUFA and MUFA, since they were associated with lipid fractions.

Introduction

Fatty acids (FAs) are the principal component of most serum lipids. The role of serum FAs in atherosclerosis, endothelial function and cardiovascular events has becoming increasingly relevant (1,2). Patients with diabetes presented an accelerated atherosclerosis process and an increased risk for microvascular disease due to hyperglycemia and the concomitant aggregation of cardiovascular risk factors. Increased proportion of levels of serum saturated fatty acids (SFAs) and/or decreased proportion of polyunsaturated fatty acids (PUFA) influence endothelial function (3,4) and consequently may be a risk factor for the development of microvascular complications in patients with diabetes. We have previously reported that type 2 diabetic patients with microalbuminuria presented lower levels of PUFA, especially of the n-6 family, in triglyceride fraction (5). Decreased levels of total PUFA, mainly of the n-6 family, has also been observed in type 2 diabetic patients with dyslipidemia (6).

FAs in serum are distributed as free forms, or non estherified bound to albumin, and as estherified to phospholipids, triglycerides and cholesterol (7). Serum FAs can be measured in total lipids or in the lipid fractions. The measurement of FAs in the lipid fraction is more laborious and requires a previous extraction process.

In order to have a better understanding of the characteristics of the serum measurement of FAs in total lipids or in lipid fractions in type 2 diabetes we analyzed the possible associations of serum FAs measured in total lipids with FAs measured in lipid fractions (triglycerides, cholesterol esters and phospholipids) and with serum lipoproteins in type 2 diabetic patients. Moreover we also assessed the influence of the diet on the proportion of FAs in total lipids and lipid fractions.

Research design and methods

Patients

This cross-sectional study was conducted in 70 patients with type 2 DM defined as age over 30 years of age at onset of DM, no previous episode of ketoacidosis or documented ketonuria, and treatment with insulin only after 5 years of diagnosis.

Patients attending the Endocrine Division's outpatient clinic at Hospital de Clínicas de Porto Alegre, Brazil were selected on the basis of the following criteria: age <75 years; body mass index (BMI) <35 kg/m^2; good compliance with diabetes treatment; triglyceride levels <400 mg/dl; urinary albumin excretion rate (UAER) <200 μg/min; A1c test < 7,0%; normal liver and thyroid function; and absence of urinary tract infection, other renal disease, and cardiac failure. Treatment with antihypertensive and oral antidiabetic agents was maintained during the study. None of the patients were using hypolipidemic agents. The Ethics Committee at Hospital de Clínicas approved the protocol and patients gave their written informed consent before entering 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 .

At the end of the run-in period, patients underwent a clinical evaluation. The body weight and height of patients (without shoes or coats) were obtained with an anthropometric scale, with measurements recorded to the nearest 100g for weight and to the nearest 0.1cm for height. BMI [weight (kg)/height^2 (m)] was then calculated. Waist circumference was measured midway between the lowest rib margin and the iliac crest, near the umbilicus. Flexible, nonstretch fiberglass tape was used for these measurements. 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.

Diet

During the run-in period, each patient received standardized nutritional guidelines developed by a nutritionist following American Diabetes Association recommendations (8) as close as possible. The amount and source of protein from the patient's usual diet was not modified. Patients were also given corn oil [fatty acid composition as indicated by the manufacturer: palmitic acid (10%); stearic acid (2%); oleic acid (31%); linoleic acid (56%); other acids (1%)] to prepare their food during this period. Compliance with the prescribed diet, was assessed by an interview with the nutritionist, and confirmed by comparison of the daily protein intake estimated from the 2-day weighed-diet records with 24-h urinary nitrogen output at the end of the second and the fourth weeks, as previously reported (9). Dietary nutrients from diet records were analyzed using the Nutribase 98 Clinical Nutritional Manager software v.1.0 (Cybersoft Phoenix, AZ). Data intake from nutrients were expressed as a percentage of total energy (%). Patients were assessed after a minimum of 4 weeks following the end of the run-in period.

Laboratory measurements

Blood samples were obtained after a 12-hour fast. Serum was separated after centrifugation at 1,500g for 15-min, and stored at –80°C for later laboratory measurements.

Fatty acids measurements

FAs were determined in phospholipid, triglyceride and cholesterol ester fractions and in the total lipids.

FA measurements in lipid fractions

Lipids were extracted from serum with chloroform-methanol (2:1; by volume) according to the method of Folch et al. (10). FAs fractions were separated by thin-layer chromatography using a silica gel plate (Silica Gel F240, Merck) and mobile-phase

development, using a mixture of hexane, diethyl ether, and acetic acid glacial (80:20:1, respectively; by volume) (11). Fractions were visualized by iodine vapor. Phospholipid, triglyceride and cholesterol ester bands were scraped into separate tubes; lipids were extracted from silica with chloroform-methanol and converted into fatty acid methyl esters by boron trifluoride catalysis (12). The methyl esters were then separated and measured by gas chromatography on a 60 m fused silica capillary column with an internal diameter of 0.20 μm (CP – Sil 88). Analysis was performed on a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector. Helium was used as carrier gas and nitrogen as make-up gas. The split-ratio was 5:1. The injection port temperature was 200°C and the detector temperature was 250°C. The column temperature was held at 160°C for 5 min and increased to 190°C at a rate of 2°C/min; it was then held at this temperature for 2 min, and increased again to 220°C at a rate of 1°C/min. The identity of each FA peak was ascertained by comparison of peak retention time with a previously characterized mixture of 20 FAs. The relative amount of each FA (% of total FA) was quantified by integrating the area under the peak and dividing the result by the total area for all FAs.

FA measurements in total lipids

Lipids extracted from serum (10) were converted into fatty acid methyl esters by boron trifluoride catalysis (12). Then, the methyl esters were evaluated by gas chromatography.

Other measurements

HDL cholesterol was separated by precipitation with heparin and $MnCl_2$. Then, total cholesterol, HDL and triglycerides were measured by enzymatic-colorimetric methods (Merck Diagnostica, Darmstadt, Germany; Boeringher Mannheim, Buenos Aires, Argentina). LDL cholesterol was calculated using Friedewald's formula (LDL = TC – HDL – TG/5). Apolipoprotein B was determined by immunoturbidimetric assay (Kit Unimate 3, Roche Diagnostic System, Basel, Switzerland; intra-assay coefficient of variation 4.2%).

Urinary albumin was measured in 24-h timed sterile urine samples by immunoturbidimetry (Sera-Pak immuno microalbuminuria; Bayer, Tarrytown, NY; mean intra-assay and interassay coefficients of variation 4.5 and 11.0 %, respectively). According to a random spot urine sample or 24-h timed urine collection, patients were defined as normoalbuminuric (UAE <17 mg/l or UAE <20 μg/min) or microalbuminuric (UAE 17-174 mg/l or UAE 20-199 μg/min). The diagnosis of microalbuminuria was always confirmed in a 24-h timed urine sample (13). Plasma glucose level was determined by a glucose oxidase method, serum creatinine level by the Jaffé reaction and A1c test by an ion exchange high-performance liquid chromatography procedure (Merck-Hitachi L-9100 glycohemoglobin analyzer; Merck, Darmstadt, Germany). Urinary urea was measured by an enzymatic ultraviolet method (mean intra-assay coefficient of variation 3.8%). The protein intake was calculated using 24-h urine 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 weight /day, assuming patients presented nitrogen balance (14).

Statistical analysis

The Pearson correlation coefficient was used for testing the correlations among the FAs measurements in total lipids with FA in different FA fractions, and serum lipoproteins. Variables with non-Gaussian distribution were log transformed before analysis. Results were expressed as medians and 95% CI or means ± SD unless otherwise stated. SPSS 14.0 (SPSS Inc., Chicago, IL) was used for the analyses.

Results

Patient characteristics

The main clinical and laboratory characteristics of the patients are shown in Table 1. All women were postmenopausal and only two were using hormone replacement (conjugated

estrogens). Anti-hypertensive treatment was calcium channel blockers, diuretics, direct vasodilators, ACE inhibitors, and beta-blockers.

Characteristics of the diet

During standardized diet, the estimated intake by weighed diet records was: total energy intake = 1680 ± 435 Kcal; protein = 22.37 ± 3.68%; carbohydrates = 49.38 ± 5.98%; lipids = 28.46 ± 5.38%; SFA = 8.32 ± 2.18%; monounsaturated fatty acid (MUFA) = 9.71 ± 2.41%; PUFA = 7.64 ± 2.2%, and cholesterol = 198 ± 79mg.

The compliance with diet prescription was confirmed. The total protein intake (g/kg body weight) assessed by nitrogen output (1.33 ± 0.30) and by weighed diet records (1.28 ± 0.31; P = 0.162) were not different

Also, the dietary PUFA correlated with serum PUFA measured in total lipids (r = 0.396; P = 0.001). Others dietary and serum FAs were not correlated, either in total lipids or in lipid fractions.

Fatty acid distribution

The distribution of serum FAs in the total lipids and in lipid fractions (triglyceride, cholesterol ester, and phospholipid) is described in Table 2 and Figure 1. These lipids presented different distribution of FAs. In total lipids and cholesterol esters the most frequent FA was PUFA, while SFA was the most frequent FA observed in the triglycerides and phospholipids.

Correlation of individual serum fatty acids in total lipids with fatty acids in lipids fraction

The correlations of each FAs of total lipids with the corresponding FA of different lipid fractions are described in Table 3. All FAs from total lipids presented a positive correlation with their corresponding FA from triglyceride fraction. Only the correlation with

SFA did not reach conventional statistical significance. A positive correlation between the PUFA n-3 of total lipids and the PUFA n-3 of phospholipid fraction was also observed. Other correlations between FAs in total lipids and lipid fractions were not significant.

Correlation of serum fatty acids in total lipids and serum lipid levels

The proportion of serum FAs in total lipids was related to serum lipoproteins, in special with cholesterol and triglycerides (Table 4). The serum MUFA presented a positive correlation with cholesterol and with triglycerides and a negative association with serum HDL. The serum PUFA had a negative correlation with cholesterol and with triglycerides. The same pattern of correlations was observed regarding PUFA n-6 and n-3. SFA did not correlated with any of the evaluated serum liproproteins. In these patients, serum FAs in total lipids were not associated with LDL contents. The individual FAs (SFA, MUFA, PUFA) measured in all lipid fractions (triglyceride fraction, cholesterol ester fraction, phospholipid fraction) did not correlate with total cholesterol, HDL and triglycerides (data not shown).

Discussion

In this sample of type 2 diabetic patients, a significant positive correlation was observed between the proportion of FAs measured in total lipids and triglycerides fraction regarding MUFA, PUFA, PUFA n-6 and PUFA n-3. In addition, it was observed that the serum FAs in total lipids had significant correlations with serum lipid levels. Serum PUFA had a negative correlation with total cholesterol and triglycerides and serum MUFA was positively correlated with both cholesterol and triglycerides levels and inversely correlated with HDL levels. Finally there was a correlation between serum PUFA measured in total lipids and dietary PUFA.

The FAs measured in total lipids represent the FA composition of all serum lipid esters (triglycerides, phospholipids and cholesterol esters) and the free FAs or nonesterified FAs taken together (6). In the fasting sate, the source of FAs in the plasma are the lipolysis of

triglycerides stored in adipose tissue and the hepatic secretion of triglycerides particles (15,16). The source of FAs used in the synthesis of the triglycerides produced by liver is mainly those mobilized from adipose tissue (17). Esters of cholesterol and phospholipids do not undergo a major metabolic transformation in the fasting state. The esterification process leading to the synthesis of triglycerides utilize random FAs and do not have a specific preference for one or other family of FAs (17). On the contrary, most of the cholesterol esters to HDL in the plasma are synthesized by the enzyme lecithin-cholesterol acyltransferase (LCAT) which has a preference for PUFAs (18) mainly linoleic acid (19); on the other hand, the cholesterol esters to VLDL and LDL are synthesized by the enzyme acyl CoA-cholesterol acyltrasnferase (ACAT), which has a preference for oleic acid (18). Also, the esterification process of the phospholipids fraction utilizes preferentially SFA in position 1 and PUFA in position 2 (18).

The results observed in this study are in accordance with the metabolic pathways described above. The observed correlation of FAs measured in total lipids with only the triglyceride fraction is probably the reflection of the random utilization of FAs to form the molecule of triglyceride.

In the present study, a positive correlation of dietary PUFA with PUFA measured in total lipids was used to confirm the patients' adherence to the standardized prescribed diet. This correlation was not observed in the lipid fractions or with other FAs. Other studies have also demonstrated an association between dietary and serum PUFAs measured in total lipids (20,21). This significant correlation of only PUFA of the diet with PUFA in total lipids is probably due to a significant proportion of linoleic acid, (about 30%), an essential FA, in the composition of total lipids (22). Moreover, linoleic acid is also the predominant PUFA ingested (18). Finally, serum PUFA did not undergo a metabolic transformation to other classes of FAs (16).

PUFAs measured in total lipids, but not in fractions, were inversely correlated with cholesterol and triglycerides levels. It was already discussed that PUFAs in total lipids are influenced by dietary PUFA and it well known that PUFAs reduce cholesterol levels due an increase in the expression of LDL receptors and, consequently enhancing the cellular uptake of cholesterol (23,24). The role of PUFA in the reduction of triglycerides is probably related to a decrease in the hepatic secretion of VLDL and also to an increase in the fraction catabolic rate of this lipoprotein (24). Regarding the role of serum MUFA, in this study we observed that serum MUFA was positively correlated with cholesterol and triglycerides and inversely correlated with HDL cholesterol. This may be explained once oleic acid (the principal dietary MUFA) is converted to SFA in the organism (16), which in turn may influence the metabolism of cholesterol and triglycerides in an opposite direction as described for PUFA.

In conclusion, the measurement of FAs composition in total lipids is a reliable assessment of the metabolic functions of the FAs in the serum, especially regarding its relationship with the lipoproteins and PUFAs from the diet. Moreover, it is a cheaper and less laborious alternative than the determination of FAs in the lipid fractions.

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

Age (years)	57.4 ± 9.9
Male gender	37 (52.9%)
Diabetes duration (years)	10.8 ± 7.6
Hypertension	42 (60%)
Microalbuminuria	33 (47.1%)
Diabetes treatment (D/OA/I/I+OA)	7/44/6/13
Body mass index (kg/m^2)	27.1 ± 3.2
Fasting plasma glucose (mg/dl)	124 ± 32
A1c test (%)	5.2 ± 1.0
Serum creatinine (mg/dl)	0.85 ± 0.18
Apolipoprotein B (mg/dl)	119 ± 31
Cholesterol (mg/dl)	200 ± 38
Triglycerides (mg/dl)	122 (93 – 185)
HDL (mg/dl)	50 ± 18
LDL (mg/dl)	119 ± 31

Data are expressed as mean ± SD, median (95% CI) or number of patients (%) with the characteristic. D/OA/I/I+OA: diet only / oral antidiabetic agents / insulin / insulin associated with oral antidiabetic agent.

Table 2: Distribution of serum fatty acid in the total lipids and in lipids fractions of type 2 diabetic patients

	TL	TG fraction	CE fraction	PL fraction
SFA (%)	37.37 ± 2.82	38.76 ± 16.38	23.82 ± 10.90	55.67 ± 15.25
MUFA (%)	21.76 ± 3.17	31.40 ± 13.36	17.51 ± 11.23	7.67 (1.75 – 51.15)
PUFA (%)	40.87 ± 4.23	29.84 ± 12.10	58.51 ± 14.38	32.61 ± 12.41
PUFA n-6 (%)	38.58 ± 4.20	26.95 ± 12.02	53.14 ± 14.87	30.29 ± 11.32
PUFA n-3 (%)	2.13 ± 0.77	1.27 (0 – 6.83)	3.25 (0 – 6.13)	1.94 (0 – 7.41)

Data are expressed as mean ± SD or median (variation). SFA: Saturated Fatty Acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, TL: total lipids, TG: triglycerides, CE: cholesterol esters, PL: phospholipids.

Table 3: Correlations of each total lipid fatty acids with the corresponding fatty acids in lipid fractions

	r	P
Triglyceride fraction		
SFA	0.205	0.089
MUFA	0.485	< 0.001
PUFA	0.258	0.031
PUFA n-6	0.317	0.007
PUFA n-3	0.387	0.001
Cholesterol ester fraction		
SFA	0.104	0.391
MUFA	0.023	0.852
PUFA	-0.028	0.816
PUFA n-6	0.084	0.488
PUFA n-3	-0.126	0.297
Phospholipid fraction		
SFA	0.149	0.217
MUFA	0.151	0.213
PUFA	0.086	0.481
PUFA n-6	0.171	0.158
PUFA n-3	0.277	0.020

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Table 4: Correlations of serum fatty acids in total lipids with lipid levels

	Cholesterol	HDL	LDL	Triglycerides
SFA	0.113 (0.353)	0.105 (0.396)	-0.093 (0.453)	0.107 (0.160)
MUFA	0.288 (0.016)	-0.366 (0.002)	0.141 (0.259)	0.676 (<0.001)
PUFA	-0.291 (0.014)	0.183 (0.135)	-0.035 (0.781)	-0.619 (<0.001)
PUFA n-6	-0.2536 (0.034)	0.202 (0.099)	-0.017 (0.894)	-0.587 (<0.001)
PUFA n-3	-0.207 (0.086)	-0.086 (0.488)	-0.100 (0.422)	-0.171 (0.157)

Data are expressed as correlation coefficient (statistic significance - P). SFA: saturated fatty acids. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids.

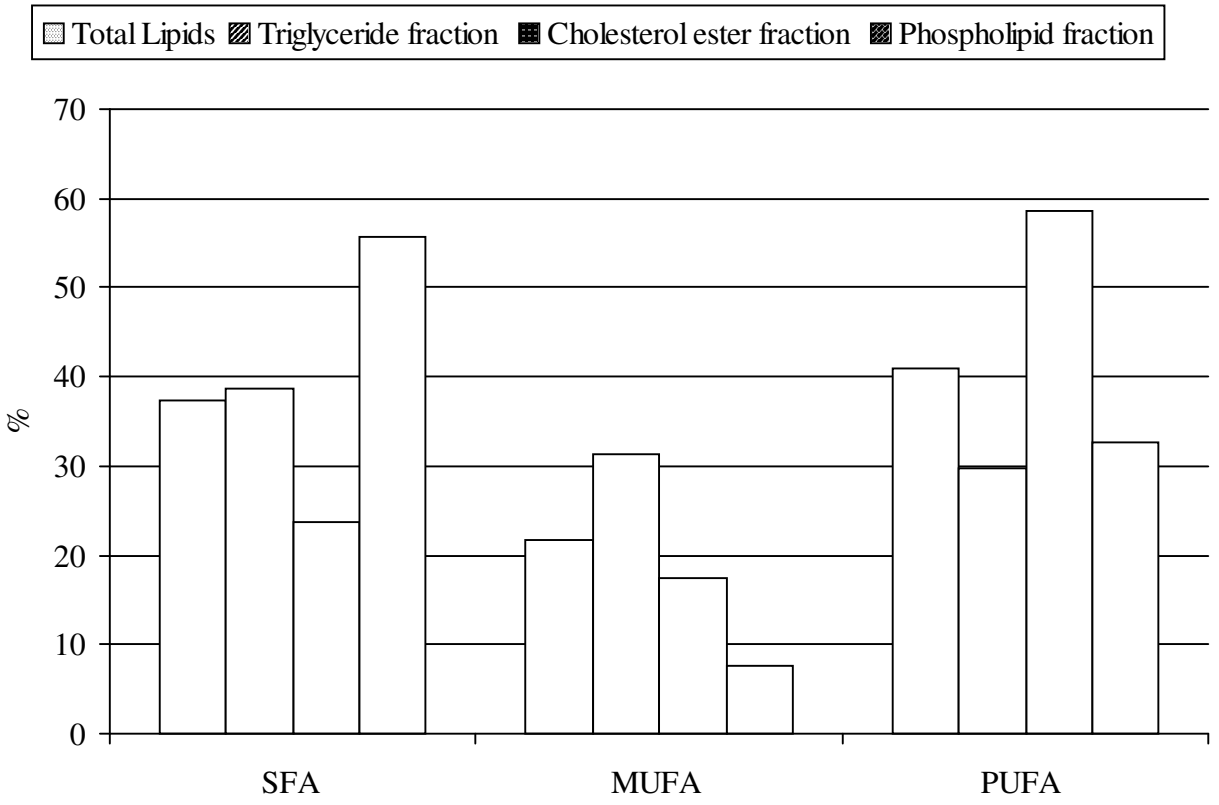

Figure 1: Serum fatty acid composition in total lipids and lipid fractions (% of the total amount of fatty acids) in type 2 diabetic patients (SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid).

CONSIDERAÇÕES FINAIS

A função endotelial dos pacientes foi avaliada por apenas um parâmetro laboratorial (endotelina-1). No entanto, para uma avaliação mais completa destes pacientes seria importante a utilização de outros parâmetros de disfunção endotelial, como moléculas de adesão vascular e pleitismografia, por exemplo. Estes parâmetros, juntamente com a endotelina-1 podem fornecer uma explicação fisiopatológica mais completa da associação entre dano endotelial, risco cardiovascular e ácidos graxos em pacientes com diabete melito tipo 2.

Pelo exposto nesta tese, fica também evidente que as diferentes frações de ácidos graxos no soro ou ácidos graxos em lipídeos totais têm funções fisiológicas e refletem situações diferentes no organismo; e que as mesmas apresentam diferentes relações com as lipoproteínas plasmáticas. Portanto, a cada situação onde serão utilizados ácidos graxos séricos, deve-se optar pela melhor alternativa, se em lipídeos toais ou em frações.

Apesar dos ácidos graxos séricos apresentarem relação com disfunção endotelial, esta associação precisa ser mais bem elucidada com estudos observacionais a longo prazo e de intervenção, com estratégias dietéticas que contemplem diferentes ácidos graxos na sua composição. Neste sentido, torna-se necessário a realização de ensaios clínicos randomizados que contemplem a suplementação da dieta com ácidos graxos poliinsaturados n-6 para a avaliação da função endotelial e outros fatores de risco para doença cardiovascular em pacientes com diabete melito tipo 2.