Universidade Federal do Rio Grande do Sul

Programa de Pós-Graduação em Ciências Médicas: Endocrinologia

Anize Delfino von Frankenberg

ANORMALIDADES METABÓLICAS RELACIONADAS AOS NÍVEIS DE ADIPONECTINA E À GORDURA DA DIETA

Porto Alegre

2015

Anize Delfino von Frankenberg

ANORMALIDADES METABÓLICAS RELACIONADAS AOS NÍVEIS DE ADIPONECTINA E À GORDURA DA DIETA

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de Doutor em endocrinologia.

Orientador: Prof. Dr. Fernando Gerchman

Porto Alegre

2015

CIP - Catalogação na Publicação

Delfino von Frankenberg, Anize
ANORMALIDADES METABÓLICAS RELACIONADAS AOS NÍVEIS DE ADIPONECTINA E À GORDURA DA DIETA / Anize Delfino von Frankenberg. -- 2015.
177 f.

Orientador: Fernando Gerchman.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Porto Alegre, BR-RS, 2015.

1. Adiponectina. 2. Síndrome metabólica. 3. Lipídios dietéticos. I. Gerchman, Fernando , orient. II. Título.

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

AGRADECIMENTOS

Ao meu orientador Fernando Gerchman por me incentivar desde o início a permanecer no meio acadêmico e dedicar grande parte da minha vida profissional à pesquisa. Agradeço imensamente pelo apoio para a realização de parte do doutorado na Universidade de Washington em Seattle. Agradeço à credibilidade para o desenvolvimento do projeto de pós-doutorado junto a este PPG. Obrigada pela amizade ao longo desses anos de trabalho, acredito que tenha sido o grande diferencial nos momentos mais difíceis dessa caminhada.

À pesquisadora Kristina Utzschneider e ao pesquisador Steven E. Kahn pela acolhida em suas equipes de pesquisa e colaboração com meu crescimento profissional e pessoal. Além de todo o conhecimento obtido, a permanência em Seattle me possibilitou a convivência com *fellows* de diversas partes do mundo. Agradeço de coração a todo o auxílio e amizade de vocês: Meghan, Daniel e família, Rene, Leon, Tanya e Trish. Vocês me proporcionaram viver outras culturas, fazer *hikings*, churrascos na simpática West Seattle e pedaladas nos belos parques em tardes ensolaradas de Seattle.

Ao professor André F. Reis pela colaboração nos artigos e disponibilização do banco de dados para análise e escrita do artigo 1 desta tese.

A todos os meus colegas pós-graduandos do grupo Pré-DM em especial, Vanessa Piccoli e Filipe do Nascimento, que estão nessa caminhada

desde o início, que me apóiam e escutam. Aos acadêmicos, sem o apoio de vocês não seria possível desenvolver esse belo trabalho em equipe.

Às colegas nutricionistas, grandes amigas, que sabem o trabalho que temos ao entrar no universo da pesquisa. Thaís Rasia, Fernanda Bernaud e Oellen Franzosi, obrigada pelo apoio, pelo ombro amigo e pelas risadas.

À minha família, que me deu a base para que pudesse alcançar os meus sonhos. Pai, mãe, manão, cunhada e amado sobrinho, obrigada pelo carinho e apoio em todas as horas. Mãe, sem a tua visita, Seatte não teria a mesma graça, fico muito feliz de teres feito parte desse lindo capítulo da minha vida.

Ao meu amor, Márcio, minha mais nova família. São 14 anos de crescimento e admiração mútua. Agradeço por todo incentivo, cuidado e apoio nas minhas decisões profissionais e pessoais. Sabes que mesmo nossas diferenças são complementares. Sou grata ao apoio que deste sem pestanejar quando falei da ideia de passar seis meses realizando o doutorado nos Estados Unidos. Sou mais grata ainda por teres encurtado a distância física com as visitas. Também agradeço a ti por me dar mais uma família amada, família Mello, com a qual posso contar sempre.

Esta tese de doutorado segue o formato proposto pelo Programa de Pós Graduação em Ciências Médicas: Endocrinologia: Faculdade de Medicina, Universidade Federal do Rio Grande do Sul. Será constituída de uma introdução em português e três artigos em inglês. O artigo do capítulo 2 está publicado na revista Diabetology & Metabolic Syndrome. O artigo do capítulo 3 está publicado na revista British Journal of Nutrition. O artigo do capítulo 4 foi submetido para a revista European Journal of Nutrition.

SUMÁRIO

LISTA DE TABELAS E FIGURAS

CAPÍTULO 1

CAPÍTULO 2

CAPÍTULO 3

CAPÍTULO 4

LISTA DE ABREVIATURAS

ABPM: Ambulatory 24-h blood pressure monitoring [Monitoramento ambulatorial de pressão arterial de 24h]

ADA: American Diabetes Association [Associação Americana de Diabetes]

AHA: American Heart Association

AIRg: Acute Insulin Response to Glucose [Resposta Aguda de Insulina à Glicose]

ALT: Alanine Aminotransferase [Alanina Aminotransferase]

AMPK: AMP-activated Protein Kinase [Proteína Quinase Ativada por AMP]

APM1: aminopeptidase M1

AUC: Area Under the Curve [Área Sob a Curva]

BMI: Body Mass Index [Índice de Massa Corporal]

CDC: Centers for Disease Control and Prevention [Centros de Controle e Prevenção de Doenças norte-americano]

CLA: Conjugated linoleic acid [ácido linoléico conjugado]

CV: Coefficient of Variation [Coeficiente de Variação]

CVD: Cardiovascular Disease [Doença Cardiovascular]

DBP: Diastolic Blood Pressure [Pressão Arterial Diastólica]

DM: Diabetes Mellitus [Diabete Mellito]

DM2: Diabete Mellito tipo 2

DNL: *de novo* lipogenesis

EGP: Endogenous Glucose Production [Produção Endógena de Glicose]

FPG: Fasting Plasma Glucose [Glicose Plasmática de Jejum]

GEE: Generalized Estimating Equation [Modelo de Equações de Estimações Generalizadas]

HbA1c: Glycated Hemoglobin [Hemoglobina Glicada]

HbA1c: Glycated Hemoglobin [Hemoglobina Glicada]

HDL-cholesterol: High Density Lipoprotein-cholesterol [Lipoproteína de Alta Densidade]

HFD: High Fat Diet [Dieta com Alto Teor de Gorduras]

HIR index: Hepatic Insulin Resistance index [Índice Hepático de Resistência à Insulina]

HMW: High molecular weight [Alto Peso Molecular]

HOMA IR: homeostatic model assessment of insulin resistance

IAF: Intra-abdominal Fat [Gordura Intra-abdominal]

IDF: International Diabetes Federation [Federação Internacional de Diabetes]

IFG: Impaired Fasting Glucose [Glicose de Jejum Alterada]

IGT: Impaired Glucose Tolerance [Tolerância Diminuída à Glicose]

IVGTT: Intravenous-glucose Tolerance Test [Teste Endovenoso de Tolerância à Glicose]

Kg: Glucose Disappearance Constant [Constante de Desaparecimento de Glicose]

LDL-cholesterol: Low Density Lipoprotein-cholesterol [Lipoproteína de Baixa Densidade]

LFD: Low Fat Diet [Dieta com Baixo Teor de Gorduras]

Matsuda ISI: Matsuda insulin sensitivity index [ìndice de Sensibilidade à Insulina de Matsuda]

MetS: Metabolic Syndrome [Síndrome Metabólica]

MRI: Magnetic Resonance Imaging [Ressonância Magnética]

MS: Metabolic Syndrome [Síndrome Metabólica]

MUFA: Monounsaturated Fat [Gordura Monoinsaturada]

n-3 PUFA: Omega-3 Polyunsaturated Fatty Acid [Ácido Graxo Poliinsaturado Ômega-3]

n-6 PUFA: Omega-6 Polyunsaturated Fatty Acid [Ácido Graxo Poliinsaturado Ômega-6]

NEFAs: Non-esterified Fatty Acids [Ácidos Graxos Não Esterificados]

NGT: Normal Glucose Tolerance [Tolerância Normal à Glicose]

NHANHES study: National Health and Nutrition Examination Survey study

OGTT: Oral Glucose Tolerance Test [Teste Oral de Tolerância à Glicose]

PICP: procollagen type I carboxyterminal propeptide [propeptídeo carboxiterminal do pró-colágeno tipo I]

PPAR-γ: peroxisome proliferator-activated receptor gamma

PPAR-α: peroxisome proliferator-activated receptor alfa

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PUFA: Polyunsaturated Fatty Acid [Ácido Graxo Poliinsaturado]

Ra: Rates of Glucose Appearance [Taxa de Aparecimento de Glicose]

RCT: Randomised Controlled Trials [Ensaio Clínico Randomizado Controlado]

Rd: Rates of Glucose Disappearance [Taxa de Desaparecimento de Glicose]

Rd-high: Rate of Glucose Disposal During High-level Insulin Infusion [Taxa de Eliminação de Glicose Durante a Alta Infusão de Insulina]

Rd-low: Rate of Glucose Disposal During Low-level Insulin Infusion [Taxa de Eliminação de Glicose Durante a Baixa Infusão de Insulina]

SBP: Systolic Blood Pressure [Pressão Arterial Sistólica]

SD: Standard Deviation [Desvio Padrão]

SE: Standard Error [Erro Padrão]

SQF: Subcutaneous Fat [Gordura Subcutânea]

US-CRP: Ultra-sensitive C-reactive Protein [Proteína C Reativa Ultra Sensível]

VLDL: Very Low Density Lipoprotein [Lipoproteína de Muito Baixa Densidade]

WMD: Weighted Mean Differences [Diferenças de Médias Ponderadas]

RESUMO

O diabetes tipo 2 está atingindo proporções epidêmicas em todo o mundo. A Organização Mundial de Saúde estima que 9% dos adultos com mais de 18 anos têm diabetes, sendo a maioria classificada como tendo diabetes tipo 2. A elevada produção de glicose hepática, a redução da secreção de insulina, a resistência à insulina e as alterações do metabolismo da glicose normalmente encontrados em indivíduos com obesidade, são os principais fatores relacionados à patogênese do diabetes tipo 2 e da síndrome metabólica.

A adiponectina é um hormônio regulador da homeostase da glicose e dos lipídios ao sensibilizar a ação da insulina. Uma vez que a redução da sensibilidade à insulina está relacionada ao diabetes tipo 2 e à síndrome metabólica, a diminuição dos níveis de adiponectina pode estar relacionada ao desenvolvimento dessas anormalidades metabólicas. Menores concentrações de adiponectina estão relacionadas com maior massa de gordura intra-abdominal. Portanto, a adiponectina pode estabelecer um elo entre gordura intra-abdominal, resistência à insulina e desenvolvimento da síndrome metabólica. Dessa forma, o objetivo do primeiro estudo foi investigar a relação entre os níveis de adiponectina e a síndrome metabólica em uma análise transversal com duas populações distintas. Primeiramente, foram analisados indivíduos encaminhados para triagem e avaliação de anormalidades do metabolismo da glicose e síndrome metabólica, submetidos a um teste de tolerância oral à glicose, na unidade de diabetes do Serviço de Endocrinologia do Hospital de Clínicas de Porto Alegre e analisados pela presença da

síndrome metabólica. Uma replicação da análise foi realizada em indivíduos submetidos à angiografia cardíaca no Hospital São Paulo da Universidade Federal de São Paulo. Neste estudo, também foi de nosso interesse determinar quais os componentes da síndrome metabólica estavam relacionadas aos níveis de adiponectina. Além disso, analisamos como a resistência e a sensibilidade à insulina e a inflamação subclínica estavam relacionadas com os níveis de adiponectina. Os níveis de adiponectina total e de alto peso molecular estão mais baixos na presença da síndrome metabólica e reduzem com o aumento do número de critérios para síndrome metabólica. Os níveis de adiponectina são, em parte, determinados por sua relação com o colesterol HDL, os triglicerídeos e a adiposidade abdominal. Além disso, a inflamação subclínica e a resistência à insulina podem explicar parcialmente por que os níveis de adiponectina são menores em indivíduos com síndrome metabólica em comparação com indivíduos sem síndrome metabólica.

Estratégias terapêuticas de modificação de estilo de vida, que envolvem atividade física de intensidade moderada ou alta e perda de peso, com enfoque na redução da síndrome metabólica e seus componentes, mostraram-se efetivas para aumentar as concentrações de adiponectina. Diferentes intervenções dietéticas também foram identificadas como potenciais modificadores das concentrações de adiponectina e da sensibilidade à insulina, que podem ser moduladas pela ingestão de lipídios. O efeito das dietas que contém baixa ou elevada quantidade de gordura ou de tipos diferentes de gorduras nos níveis de adiponectina foi analisado em diversos estudos que mostraram resultados heterogêneos. De fato, maior adesão à dieta

mediterrânea tem sido associada com concentrações mais elevadas de adiponectina, que pode ser explicado pela composição da dieta que é rica em nozes, óleo de oliva e peixes, que são fontes alimentares de ácidos graxos insaturados. Outros lipídios dietéticos, tais como, o ácido linoléico conjugado, o colesterol e o ácido graxo poli-insaturado ômega-3 têm sido associados com diferentes respostas nas concentrações de adiponectina. A fim de esclarecer o efeito dos lipídios da dieta sobre os níveis de adiponectina, o segundo estudo teve como objetivo revisar e analisar sistematicamente ensaios clínicos randomizados que investigaram os efeitos de lipídios da dieta nas concentrações circulantes de adiponectina em adultos. A revisão sistemática com meta-análise mostrou que nos estudos de intervenção que compararam as dietas com baixo e elevado teor de gordura não houve associação entre a quantidade total de gordura com diferenças nos níveis circulantes de adiponectina. Observou-se que a suplementação de ômega-3 aumentou modestamente as concentrações circulantes de adiponectina. Por outro lado, a suplementação de ácido linoléico conjugado reduziu as concentrações de adiponectina quando comparada com a suplementação de ácido graxo insaturado utilizado como placebo ativo.

As dietas contendo baixas ou elevadas quantidades de gordura podem também afetar os fatores determinantes da tolerância à glicose, especialmente a sensibilidade à insulina. Entretanto, este achado aparentemente não pode ser explicado pelo efeito da gordura dietética total sobre os níveis de adiponectina. Estudos de intervenção de médio e longo prazo têm avaliado se as dietas isocalóricas hiperlipídicas podem modificar a sensibilidade à insulina. No

entanto, os dados apresentados na literatura atual são contrários à crença de que dietas ricas em gordura levam à resistência à insulina. Em contraste, dieta com baixo teor de gordura, com menos de 10% de energia proveniente de gordura saturada, parece melhorar a sensibilidade à insulina. O terceiro estudo buscou determinar, em indivíduos com excesso de peso/obesidade e peso estável, o efeito de dietas com baixo ou elevado teor de gordura total e gordura saturada na sensibilidade à insulina, através da avaliação da supressão da produção de glicose endógena e da captação de glicose promovidas pela insulina. Quatro semanas em uma dieta rica em gordura total e gordura saturada diminuiu significativamente a sensibilidade à insulina em indivíduos com sobrepeso/obesidade, apesar da ausência de ganho de peso. No entanto, a dieta com baixo teor de gorduras não melhorou a sensibilidade à insulina. A diminuição na sensibilidade à insulina na dieta rica em gordura não pôde ser explicada por alterações na gordura intra-abdominal e hepática ou pela alteração das adipocinas. No entanto, foram observadas correlações positivas entre alterações de sensibilidade à insulina e gordura subcutânea.

ABSTRACT

Type 2 diabetes is reaching worldwide epidemic proportions with the World Health Organization estimating that 9% of adults older than age 18 have diabetes, with the vast majority having type 2 diabetes. Elevated hepatic glucose production, impaired insulin secretion, and insulin resistance, abnormalities of glucose metabolism typically found in subjects with obesity, are major factors underlying the pathogenesis of type 2 diabetes and metabolic syndrome.

Adiponectin is a major regulator of glucose and lipid homeostasis by its insulin sensitizer properties. Since decreased insulin sensitivity is linked to type 2 diabetes and metabolic syndrome, decreased adiponectin levels may be related to its development. Lower adiponectin concentrations are related with higher intra-abdominal fat mass. Therefore, adiponectin could link intra-abdominal fat with insulin resistance and the development of metabolic syndrome. Therefore, the purpose of the first study was to investigate the relationship between adiponectin levels with metabolic syndrome in two different cohorts. Firstly, screened and evaluated for abnormalities of glucose metabolism and metabolic syndrome were submitted to an oral glucose tolerance test at the diabetes unit of the Endocrine Department of Hospital de Clínicas de Porto Alegre and were cross-sectionally examined according to the presence of metabolic syndrome. A replication analysis was performed in subjects undergoing cardiac angiography at Hospital São Paulo, from Federal University of São Paulo. We were also interested in finding out which metabolic syndrome components are mostly related to adiponectin levels. Additionally, we

analyzed how insulin resistance, sensitivity and subchronic inflammation were related to adiponectin levels. Our results have shown that total and high-molecular weight adiponectin levels not only are lower in the presence of metabolic syndrome, but it also decreases by increasing number of metabolic syndrome criteria. These levels are partly determined by their relationship with HDL cholesterol, triglycerides and abdominal adiposity. Furthermore, chronic inflammation and insulin resistance may partially explain why adiponectin levels are lower in subjects with metabolic syndrome compared to subjects without metabolic syndrome.

Therapeutic strategies that target the metabolic syndrome and its components have been shown to increase adiponectin concentrations, such as lifestyle modification involving moderate- or high intensity physical activities and weight loss. Different dietary interventions have also been identified as potential modifiers of adiponectin concentrations and insulin sensitivity, and they may be influenced by lipid intake. The relationship of the effect of diets containing either low or high amounts of fat or different type of fat have been analyzed in different studies and has shown heterogeneous results. Indeed, close adherence to a Mediterranean diet has been associated with higher adiponectin concentrations which can be also explained by its composition that is rich in nuts, olive oil and fish, all of which are dietary sources of unsaturated fatty acids. Other dietary lipids such as conjugated linoleic acid, dietary cholesterol and long-chain n-3 polyunsaturated fatty acids have been associated with a variable response to adiponectin concentrations. In order to clarify the effect of dietary lipids on adiponectin levels, the second study aimed to systematically review and

analyze randomised controlled trials investigating the effects of dietary lipids on circulating adiponectin concentrations in adults. The meta-analysis has shown that intervention studies that compared diets with low and high fat content were not associated with differences in adiponectin concentrations. However, it was observed that n-3 polyunsaturated fatty acids supplementation modestly increased the circulating concentrations of adiponectin, whereas conjugated linoleic acid supplementation reduced the concentrations when compared with unsaturated fatty acid supplementation used as an active placebo.

Diets containing either low or high amounts of fat can also affect the determinants of glucose tolerance, specifically insulin sensitivity. These findings apparently cannot be explained by the effect of total dietary fat on adiponectin levels. Medium to long-term diet intervention studies have examined whether isocaloric high-fat diets modify insulin sensitivity. However, the data presented in the current literature are at odds with the preconception that high fat diets lead to insulin resistance. In contrast, a low-fat diet with less than 10% of energy from saturated fat seems to improve insulin sensitivity. The third study sought to determine the effect of diets containing either low or high amounts of fat and saturated fat on insulin sensitivity, both in insulin's ability to suppress endogenous glucose production and to promote glucose uptake, and insulin secretion in weight-stable overweight/obese subjects. Four weeks on a diet high in fat and saturated fat significantly decreased insulin sensitivity in overweight/obese subjects despite the absence of weight gain. However, a diet low in fat and saturated fat did not improve insulin sensitivity. The decrease in insulin sensitivity on the high-fat diet could not be explained by changes in intra-

abdominal fat, liver fat, or adipokines. However, positive correlations between changes in subcutaneous fat and insulin sensitivity were observed.

CAPÍTULO 1

INTRODUÇÃO

A prevalência de síndrome metabólica (SM) e de diabetes melito tipo 2 (DM2) na população norte-americana é de 22,9% e 9,3% respectivamente conforme os dados do NHANHES e CDC (Beltran-Sanchez et al., 2013 e National Diabetes Statistics Report, 2014). No Brasil, a prevalência da SM na população adulta é elevada e similar à descrita na população norte-americana. Em uma recente revisão sistemática de estudos transversais, a média ponderada da prevalência de SM foi de 29,8%, 20,1% e 41,5% na população adulta brasileira das regiões urbana, rural e indígena respectivamente (de Carvalho Vidigal *et al.*, 2013). De acordo com resultados de um estudo conduzido com trabalhadores de turnos fixos de uma indústria do Rio Grande do Sul, ser do sexo feminino, ter idade mais avançada e ter privação de sono mostraram-se potenciais fatores de risco para SM, enquanto ter maior escolaridade e realizar maior número de refeições ao dia foram fatores de proteção para SM (Canuto *et al.*, 2015). Em relação ao DM2, o Estudo Multicêntrico sobre a Prevalência do Diabetes no Brasil estimou uma prevalência de DM na população adulta em 7,6% no final da década de 1980 (Malerbi *et al.*, 1992). Dados de 2012 indicam que no conjunto da população adulta das 26 capitais e do Distrito Federal, 7,4% dos entrevistados referiu diagnóstico médico de DM2 através de inquérito telefônico (Vigitel, 2012). Outros estudos epidemiológicos indicam taxas mais elevadas, como 13,5% em São Carlos-SP e de 15% em Ribeirão Preto-SP (Bosi *et al.*, 2009 e Moraes *et al.*, 2010). Já na cidade de Pelotas-RS, a prevalência de DM2 diagnosticado

pelo médico referida em entrevista era de 7,1% no ano de 2010 (da Costa *et al.*, 2010). As complicações decorrentes do DM2 resultam em uma elevada morbimortalidade e redução de seis anos em média na expectativa de vida quando a doença é diagnosticada aos 50 anos (Emerging Risk Factors Collaboration, 2011). Os gastos diretos e indiretos com o DM2 são de 245 bilhões de dólares por ano nos Estados Unidos. O DM2 dobra os gastos em saúde quando comparados aos gastos realizados em indivíduos sem a doença (CDC, 2014). No Brasil, a doença também é considerada um importante problema de saúde pública. O custo per capita estimado da doença é de 1.527,6 de dólares por ano. Tendo em vista que a população com DM2 no Brasil é estimada em 11,6 milhões de casos em adultos entre 20 e 79 anos, são gastos em torno de 17 bilhões de gastos diretos ao ano (IDF, 2010).

A obesidade está associada ao desenvolvimento da SM, que é caracterizada pelo conjunto de fatores de risco para doença cardiovascular e DM2, tais como, hiperglicemia, pressão arterial elevada, triglicerídeos elevados, colesterol HDL baixo e obesidade central (Malik *et al.,* 2004). Além disso, a obesidade e o acúmulo de gordura abdominal causam uma série de anormalidades metabólicas que resultam no aumento da produção hepática de glicose e na redução da sensibilidade à insulina no músculo esquelético, fígado e tecido adiposo, processos intimamente relacionados à patogênese do DM2 (Kahn *et al.,* 2006).

Adiponectina

A adiponectina, hormônio expresso principalmente no tecido adiposo, é codificada pelo gene *APM1* (cromossomo 3q27). Em humanos, os níveis plasmáticos de adiponectina variam de 3 a 30 µg/mL, sendo uma das proteínas com maior concentração plasmática. A molécula possui 247 aminoácidos e é secretada na circulação em três isoformas oligoméricas: trímeros (baixo peso molecular), hexâmeros (moderado peso molecular) e complexas, de alto peso molecular (Pajvani *et al.*, 2003). Alguns estudos sugerem que a isoforma de alto peso molecular é biologicamente mais ativa e seus menores níveis relacionados ao DM e à doença arterial coronariana (Aso *et al.*, 2006, Kobayashi *et al.*, 2004 e Pajvani *et al.*, 2004). A adiponectina atua através de dois receptores chamados AdipoR1 e AdipoR2, sendo o primeiro mais expresso no tecido muscular e o segundo no tecido hepático. Estudos subsequentes demonstraram que o receptor AdipoR1 também está presente nas células endoteliais (Motoshima *et al.*, 2004), cardiomiócitos (Pineiro *et al.*, 2005) e células beta-pancreáticas (Kharroubi *et al.*, 2003), já o receptor AdipoR2 está expresso nas células endoteliais (Tan *et al.*, 2004) e ambos receptores estão expressos no hipotálamo (Coope *et al.*, 2008). A resistência à ação da insulina resultante da obesidade causa redução na expressão dos receptores da adiponectina nos tecidos muscular e hepático (Tsuchida *et al.*, 2004). Além disso, a expressão da adiponectina reduz com o aumento da insulina, do TNF-α, da endotelina-1 e dos glicocorticóides, fatores implicados na fisiopatogênse da resistência à insulina, inflamação subclínica, disfunção endotelial e regulação do metabolismo energético, aspectos intimamente

relacionados ao desenvolvimento da SM, DM2 e doença cardiovascular (Gil-Campos *et al.*, 2004).

Nesse sentido, uma série de estudos demonstraram que os níveis de adiponectina encontram-se reduzidos na obesidade (Heid *et al.*, 2006 e Kern *et al.*, 2003), no DM2 (Hara *et al.*, 2002 e Heid *et al.*, 2006) e na doença arterial coronariana (Menzaghi *et al.*, 2007 e Schulze *et al.*, 2005).

A fim de testar o efeito *in vivo* da adiponectina na sensibilidade à insulina, um modelo de camundongo lipoatrofiado e que apresenta deficiência de adiponectina foi desenvolvido. Nesses animais, a reposição em doses fisiológicas de adiponectina melhorou a resistência à insulina (Yamauchi *et al.*, 2001). A adiponectina estimulou a oxidação dos ácidos graxos no músculo através do aumento da expressão de moléculas envolvidas no transporte de ácidos graxos (CD36), na sua combustão (acetil coenzima A oxidase) e na dissipação da energia via aumento de expressão da proteína desacopladora tipo 2 (UCP-2) (Yamauchi *et al.*, 2001). A redução do conteúdo de triglicerídeos no tecido muscular esquelético associou-se ao aumento da translocação do GLUT-4, que levou à melhora da sensibilidade à insulina (Yamauchi *et al.*, 2001). A reposição de adiponectina nesses animais aumentou a expressão de PPAR-alfa, aumento da oxidação de ácidos graxos e maior gasto energético, ocasionando uma redução dos triglicerídeos no músculo e no fígado (Yamauchi *et al.*, 2001). Em outro estudo conduzido pelo mesmo grupo, o tratamento agudo (de até seis horas) de mioblastos (células C2C12) com adiponectina, além de aumentar a oxidação de ácidos graxos, estimulou a captação de glicose via ativação da AMPK (Yamauchi *et al.*, 2002) levando à redução das

enzimas que sinalizam a gliconeogênese hepática. Em outro estudo, camundongos selvagens, que receberam uma dieta rica em lipídios totais tiveram uma redução dos níveis de adiponectina comparada a uma dieta rica em carboidratos. A reposição de adiponectina nesses animais também melhorou a resistência à insulina e a hipertrigliceridemia induzidas especificamente pela dieta hiperlipídica (Yamauchi *et al.*, 2001). Adicionalmente, foi demonstrado em camundongos selvagens e em modelos de camundongos *ob/ob ou* com DM2 induzido por estreptozotocina que um aumento agudo dos níveis circulantes de adiponectina leva a uma redução transitória do nível basal de glicose através da inibição das enzimas relacionadas à gliconeogênese hepática e à taxa de produção hepática de glicose (Berg *et al.*, 2001 e Combs *et al.*, 2001). Com base nesses estudos, foi possível demonstrar que a estimulação da oxidação de ácidos graxos no músculo e no fígado, o aumento da captação de glicose no músculo esquelético e a supressão da gliconeogênese hepática são potenciais vias através das quais a adiponectina regula a sensibilidade à insulina (Yamauchi *et al.*, 2001, Berg *et al.*, 2001 e Combs *et al.*, 2001).Os dados sugerem que a resistência à insulina associada à dieta com alto teor de gorduras e à obesidade relacionam-se em parte com a redução dos níveis circulantes de adiponectina e que o aumento dos seus níveis protejam contra o desenvolvimento de diferentes aspectos da SM, especialmente aqueles relacionados a modulação da sensibilidade à insulina, distribuição de gordura corporal e metabolismo das lipoproteínas (Yamauchi *et al.*, 2001). Entretanto, não está claro como diferentes intervenções dietéticas especialmente

relacionadas com mudanças na ingestão de gorduras resultam em mudanças nos níveis circulantes de adiponectina.

Associação entre polimorfismos do gene da adiponectina, níveis de adiponectina circulante e DM2

Estudos epidemiológicos demonstraram que o DM2 agrega-se em famílias, sugerindo haver uma contribuição genética para o seu desenvolvimento. O risco cumulativo de DM2 aos 65 anos foi de 14,8% para indivíduos sem história familiar de DM2, 22% para indivíduos com apenas um dos pais com DM2 e 41% para indivíduos em que ambos os pais são acometidos pela doença (Weijnen *et al.*, 2002).

Estudos mais recentes têm demonstrado que uma série de polimorfismos genéticos associa-se ao desenvolvimento da obesidade e do DM2 (Keramati *et al.*, 2014 e Smemo *et al.*, 2014). Genes que modulam o metabolismo do tecido adiposo e, por consequência, estão implicados nos processos de síntese e metabolismo dos ácidos graxos, são importantes determinantes da distribuição de gordura corporal e da sensibilidade à insulina, aspectos também relacionados às anormalidades do metabolismo da glicose e do desenvolvimento do DM2. Variantes genéticas do gene da adiponectina também foram associados com resistência à ação da insulina e ao DM2 (Florez, 2008).

Estima-se que de 39 a 46% da variabilidade dos níveis de adiponectina circulante devem-se a fatores genéticos (Comuzzie *et al.*, 2001 e Lindsay *et al.*, 2003). Neste sentido, uma recente revisão sistemática com metanálise compilou os dados de sete estudos que exploraram a associação entre três polimorfismos genéticos (do inglês *single nucleotide polymorphisms*; SNPs -11391G→A, +45T→G e +276G→T) e os níveis de adiponectina plasmática (Menzaghi *et al.*, 2007). O SNP -11391G→A foi associado com maiores níveis de adiponectina circulante nos sujeitos que carregavam o alelo A comparados aos sujeitos que carregavam apenas o alelo G. Não foi observada associação para o SNP +45T→G e os níveis de adiponectina. Em relação ao SNP +276G→T, os níveis de adiponectina mostraram uma tendência de aumento progressivo dos homozigotos para o alelo G quando comparados aos heterozigotos e os homozigotos para o alelo T (Menzaghi *et al.*, 2007).

Associações entre polimorfismos do gene da adiponectina e risco para DM2 também tem sido amplamente explorado na literatura. Dentre as nove regiões cromossômicas relacionadas ao DM2, três (3q, 15q e 20q) são encontrados em diversos grupos étnicos, tais como, japoneses, alemães e franceses (Okamoto Y *et al*., 2006). Curiosamente, a região 3q27 contém o gene da adiponectina sugerindo mais uma vez o papel da adiponectina como um determinante para a susceptibilidade ao DM2. O SNP 276 no íntron 2 (G vs. T) relacionou-se com fenótipos distintos em relação aos níveis de adiponectina, resistência à insulina e suscetibilidade para DM2. Indivíduos com o genótipo G/G na posição 267 tinham menores níveis de adiponectina e risco aumentado para DM2 comparados aos com genótipo T/T (Hara K *et al*., 2002).

Associações similares entre o gene da adiponectina e susceptibilidade para desenvolver DM2 também foi demonstrado em alemães e franceses (Vasseur F, *et al*., 2002 e Stumvoll M *et al*., 2002).

Tendo em vista a grande quantidade de estudos que buscaram associações entre diferentes polimorfismos do gene da adiponectina e o DM2, uma recente revisão sistemática com metanálise agrupou o resultado de mais de 2.000 indivíduos com DM2 vs. controles para quatro SNPs -11391G→A, -11377C→G, +45T→G, e +276G>T (Menzaghi *et al.*, 2007). Não foi demonstrada associação entre os SNPs avaliados e risco para DM2. Posteriormente, outra revisão sistemática com metanálise de 45 estudos (9.986 indivíduos com DM2 vs. 16.222 controles) avaliou apenas o polimorfismo +45T→G e, através de uma análise de subgrupo, demonstrou que nos estudos que incluíram asiáticos houve uma associação entre +45T→G e risco para DM2. Entretanto, nos estudos que incluíram caucasianos não foi observada associação (Fan Y *et al.*, 2015). Em relação à resistência à ação da insulina, foi observada uma associação entre o SNP +276G→T e a resistência à insulina estimada pelo HOMA-IR. A resistência à ação da insulina era maior nos indivíduos homozigóticos para o alelo G comparados aos heterozigóticos e os homozigóticos para o alelo T, indicando uma maior sensibilidade à insulina nos sujeitos que carregavam o alelo T, o mesmo alelo que mostrou uma tendência à associação com maiores níveis de adiponectina (Menzaghi *et al.*, 2007).

Adiponectina e Síndrome Metabólica

Estudos têm sugerido que a expressão do gene *APM1* em tecido adiposo abdominal visceral é menor do que em tecido adiposo abdominal subcutâneo (Lihn *et al.*, 2004 e Fisher *et al.*, 2002). Essa expressão gênica em tecido adiposo se correlaciona de forma significativa com seus níveis plasmáticos, sendo maior em indivíduos magros e naqueles com maior sensibilidade à ação da insulina (Kern *et al.*, 2003). Ainda, a menor concentração de adiponectina relaciona-se mais fortemente a quantificação de gordura abdominal visceral comparado a gordura abdominal subcutânea sugerindo uma possível relação com a SM (Cnop *et al.*, 2003).

A associação inversa entre os níveis de adiponectina e os critérios que compõe a SM tem sido descrita na literatura (Arita *et al.*, 1999, Daimon *et al.*, 2003, Iwashima *et al.*, 2004, Okamoto *et al.*, 2006, Matsubara *et al.*, 2002). Está bem demonstrado que indivíduos com excesso de peso apresentam menores níveis de adiponectina comparados a indivíduos sem sobrepeso e que os níveis deste hormônio diminuem com o aumento do IMC em homens e mulheres (Arita *et al.*, 1999). Além disso, maiores níveis de adiponectina associaram-se a uma menor incidência de DM2 em uma coorte japonesa acompanhada por 5 anos com o objetivo de se melhor entender os fatores relacionados ao desenvolvimento do DM. Os indivíduos que pertenciam ao menor tercil de adiponectina desenvolveram aproximadamente nove vezes mais DM2 do que aqueles indivíduos que pertenciam ao maior tercil (Daimon *et al.*, 2003). Adicionalmente, indivíduos com menores níveis de adiponectina plasmática apresentam moléculas de colesterol LDL de menor tamanho, menor

atividade da lipoproteína lipase, menores níveis de colesterol HDL e maiores níveis de triglicerídeos (Okamoto *et al.*, 2006, Matsubara *et al.*, 2002). Em relação à pressão arterial, menores níveis de adiponectina circulante foram observados em indivíduos hipertensos comparados aos não hipertensos, mesmo após ajustes para obesidade, resistência à insulina e DM2 (Iwashima *et al.*, 2004). Estudos sugerem um efeito da adiponectina sobre a homeostase pressórica. Um aumento da deposição de colágeno promovido pelo aumento do nível sérico do propeptídeo carboxiterminal do pró-colágeno tipo I (PICP) está associado a uma aceleração do processo de rigidez arterial, fenômeno intimamente relacionado com o processo de desenvolvimento da hipertensão (Liao *et al*., 1999) e da SM (Schillaci G *et al*., 2005). Foi demonstrado em um estudo transversal com 188 indivíduos hipertensos e sem DM2, que maiores níveis de adiponectina relacionam-se com menores níveis circulantes de PICP (Tsai *et al*., 2008). Reforçando essa hipótese, menores níveis de adiponectina associaram-se a maior rigidez da parede arterial em uma coorte de idosos (Snijder *et al*., 2009). Também já foi demonstrado um efeito da adiponectina na função endotelial. A adiponectina aumenta a expressão gênica e ativa a óxido nítrico sintase endotelial através da ativação da AMPK (Vaiopoulos *et al*., 2012), estimulando a síntese de óxido nítrico, importante fator endotelial e potente vasodilatador (Xu e Vanhoutte *et al*., 2012). Adicionalmente, sabe-se que o sistema renina-angiotensina desempenha um papel importante na regulação da pressão arterial e que quando está ativado perpetua o processo de inflamação na parede arterial, aumentando o estresse oxidativo e desenvolvimento de aterosclerose (Brasier *et al*. , 2002 e De Kloet *et al*., 2010). Através de seus efeitos antioxidantes e anti-inflamatórios, o aumento dos níveis

da adiponectina inibe o efeito vascular deletério da ativação do sistema renina-angiotensina e que está intimamente relacionado a desregulação da homeostase pressórica na SM (Van Stijn *et al*., 2014). A figura 1 mostra os diferentes mecanismos envolvidos na gênese da SM relacionado à hipoadiponectinemia.

Apenas um estudo avaliou a relação dos diferentes critérios para SM e os níveis de adiponectina (Koh *et al.*, 2011). Esse estudo incluiu apenas indivíduos asiáticos com mais de 40 anos e os resultados demonstraram que menores níveis de adiponectina relacionam-se com maior circunferência da cintura, maiores níveis de triglicerídeos, PCR, glicose de jejum e insulina. Ainda, os indivíduos com maior adiponectina circulante apresentavam maiores níveis de HDL-colesterol. Embora a relação entre adiponectina circulante e os diferentes componentes da SM tenham sido estabelecidas em diferentes estudos, não existem estudos que avaliem a relação dos níveis de adiponectina com a SM em populações não asiáticas sem e com ateroesclerose.

Efeito dos lipídios dietéticos nos níveis circulantes de adiponectina

Estudos de intervenção demonstraram que os níveis de adiponectina podem ser determinados, em parte, por diferentes tipos de dieta. Visto o importante papel que a adiponectina desempenha no metabolismo dos carboidratos e lipídios, incluindo melhora da sensibilidade à insulina e aumento da oxidação de ácidos graxos, as dietas que modificam a quantidade e a qualidade dos lipídios podem ter um impacto no metabolismo e nos níveis

plasmáticos da adiponectina (Mantzoros *et al.*, 2006). Diversos estudos buscaram mostrar o efeito de dietas com elevado ou baixo teor de lipídios totais na regulação dos níveis de adiponectina (Arvidsson *et al.*, 2004, Marina *et al.*, 2015 e Wycherley *et al.*, 2010). Em um ensaio clínico randomizado, que comparou dietas hipocalóricas restrita em gordura (27% de lipídios, 52% de carboidratos e 21% de proteínas) *versus* dieta elevada em gorduras (41% de lipídios, 39% de carboidratos e 20% de proteínas), não foram observadas mudanças nos níveis de adiponectina ao final de 10 semanas de intervenção (Arvidsson *et al.*, 2004). Entretanto, outro estudo que comparou uma dieta hipocalórica normolipídica (30% de lipídios) *versus* hiperlipídica (61% de lipídios) demonstrou aumento de 30% e 18% nos níveis de adiponectina, após 52 semanas de intervenção (Wycherley *et al.*, 2010). Por outro lado, ao fornecer dietas isocalóricas para manutenção de peso com alto teor de gorduras (55% de lipídios, 27% de carboidratos, 18% de proteínas) ou baixo teor de gorduras (20% de lipídios, 62% de carboidratos, 18% de proteínas) não foi demonstrada diferença nos níveis de adiponectina após quatro semanas de intervenção (Marina *et al.*, 2015). Estes resultados sugerem que a quantidade dos lipídios na dieta não parece ter um papel significativo na modulação dos níveis de adiponectina em seres humanos, diferentemente dos resultados encontrados no modelo de roedores citado anteriormente (Yamauchi *et al.*, 2001).

A maior aderência à dieta de estilo mediterrânea, que apresenta elevado teor de gorduras insaturadas, tem sido associada a níveis mais elevados de adiponectina (Trichopoulou *et al.*, 2003). Essa relação se deve, possivelmente,

não apenas à baixa carga glicêmica e ao moderado consumo de álcool dessa dieta, mas também ao elevado teor de oleaginosas, óleo de oliva e peixes, que são alimentos fonte de ácidos graxos poliinsaturados (Mantzoros *et al.*, 2006). Os mecanismos através dos quais o padrão de dieta mediterrânea impacta nos níveis circulantes de adiponectina ainda é desconhecido, mas algumas hipóteses foram levantadas. Os ácidos graxos poli-insaturados do tipo ômega-3 (AGPI ômega-3) encontrados nessa dieta podem modular os níveis de adiponectina através da interação com o *peroxisome proliferator-activated receptor alfa (PPAR-α) e gamma (PPAR-γ)* (Neschen *et al.*, 2006). A ativação do receptor PPAR-α, estimulada pelo consumo de AGPI ômega-3, aumenta a expressão dos receptores de adiponectina (AdipoRs 1 e 2) no músculo e fígado melhorando a sensibilidade desse hormônio nesses tecidos (Yamauchi e Kadowaki, 2008). Dessa forma, a adiponectina atua reduzindo a inflamação e o stress oxidativo, o que leva, por fim, a uma melhora da sensibilidade à insulina (Tsuchida *et al.*, 2005). Ainda, o AGPI ômega-3 ativa o *PPAR-γ*, atuando no aumento dos níveis de adiponectina através de uma segunda via. Em estudo experimental, o consumo de AGPI ômega-3 aumentou duas vezes a expressão do gene da adiponectina que ocorreu paralelamente ao aumento de 2-3 vezes na expressão do gene que codifica o *PPAR-γ* (Neschen *et al.*, 2006). Dessa forma, a ativação de ambos fatores de transcrição *PPAR-α* e *PPAR-γ*, promovida pelo AGPI ômega-3, aumenta os níveis bem como a ação da adiponectina, o que resulta na melhora da inflamação e da resistência à insulina induzida pela obesidade (Yamauchi e Kadowaki, 2008).

Além dos AGPI ômega-3, outros tipos de lipídios tem demonstrado efeito na regulação da adiponectina dentre os quais, destaca-se o ácido linoléico conjugado (CLA). O CLA pode causar resistência à ação da insulina por reduzir os níveis de adiponectina plasmática (Kennedy *et al.*, 2010). Os níveis de RNA mensageiro da adiponectina foram reduzidos após a suplementação de CLA em ratos (Poirier *et al.*, 2006) e em cultura de células de adipócitos humanos (Brown *et al.*, 2004). Visto que o gene da adiponectina é modulado pela ativação do receptor PPAR-γ, a supressão do gene da adiponectina pode ser atribuída em parte pelo efeito antagonista do trans-10,cis-12 CLA ao PPAR-γ (Iwaki *et al.*, 2003).

Apesar da maior parte dos estudos de intervenção com modificação da quantidade ou qualidade dos lipídios realizados em humanos terem como objetivo primário o efeito dessas intervenções em diferentes parâmetros metabólicos e não, necessariamente, nos níveis de adiponectina, sua dosagem foi avaliada antes e depois da intervenção em uma série de estudos. A análise conjunta dos resultados desses ensaios clínicos em populações de diferentes etnias, sexo, perfil metabólico e de doenças permitiria esclarecer o efeito da ingestão de lipídios nos níveis circulantes de adiponectina.

Efeito dos lipídios dietéticos na resistência insulínica

Uma série de estudos demonstrou que o ganho de peso e o acúmulo de gordura intra-abdominal estão implicados no desenvolvimento da resistência à insulina, SM e DM2 (Cnop *et al*., 2003, Cameron *et al*., 2008 e Tong J *et al*.,

2007). A piora da resistência à insulina deve-se, em parte, a um desbalanço na síntese e secreção de adipocitocinas produzidas pelo tecido adiposo visceral que estão implicadas no desenvolvimento dos principais aspectos relacionados à SM e DM2: inflamação subclínica, menor captação de glicose pelo tecido muscular e adiposo, inibição da oxidação de ácidos graxos e estimulação das citocinas que ativam a gliconeogênese hepática (TNF-α e interleucina-6). Adicionalmente, o acúmulo de gordura intra-abdominal associa-se a uma diminuição dos níveis de adiponectina (Gil-Campos *et al*., 2004 e Kadowaki *et al*., 2006). Após um mês de intervenção com dieta isocalórica hiperlipídica (55% de lipídios totais e 25% de gordura saturada) em indivíduos obesos com peso estável, não houve aumento na quantidade de gordura hepática nem diminuição dos níveis de adiponectina, mecanismos relacionados ao desenvolvimento de resistência insulínica e DM2, sugerindo mais uma vez que a quantidade de gordura da dieta não foi capaz de se relacionar com mudanças nos níveis de adiponectina (Marina A *et al.*, 2014). Estes resultados sugerem que a dieta hiperlipídica rica em ácidos graxos saturados induz a resistência à insulina e relaciona-se ao desenvolvimento do DM2 através de mecanismos independentes dos relacionados a variações nos níveis de adiponectina. Ainda, a melhora do controle glicêmico é observada em intervenções com dietas hipocalóricas com alto ou baixo teor de lipídios (Shai *et al.*, 2008 e Guldbrand *et al.*, 2012). Sendo assim, esse efeito parece ser atribuído pela redução de peso em si e não pela modificação da composição da dieta. Para que seja possível determinar o efeito da composição dos macronutrientes da dieta, especialmente dos lipídios, na sensibilidade à insulina é importante que o peso se mantenha constante ao longo do estudo.

Poucos estudos com intervenções de médio e longo prazo avaliaram o efeito de dieta isocalórica com diferentes teores de gordura na sensibilidade à insulina (Tabela 1). Dois estudos que compararam dietas com alto teor de gorduras (50-55% do VCT) *versus* baixo teor de gorduras (20-25% do VCT) não encontraram diferença na medida da sensibilidade à insulina pelo clamp em indivíduos saudáveis (van Herpen *et al.*, 2011 e Eckel *et al.*, 2006). Após quatro semanas de dieta com alto teor de gorduras, também não foi observada mudança na sensibilidade à insulina estimada pelo índice Matsuda, em idosos que mantiveram o peso constante (Utzschneider *et al.*, 2013). Esses resultados contradizem a crença de que elevado teor de gordura na dieta resultam em redução na sensibilidade à insulina.

Em relação à qualidade do lipídio, alguns estudos buscaram comparações entre diferentes tipos de gorduras de dietas com elevado teor de gorduras totais na sensibilidade à insulina. Em um ensaio clínico que testou uma dieta com alto teor de gorduras saturadas (17% de gordura saturada) houve uma redução de 12,5% na sensibilidade à insulina em indivíduos saudáveis ao final de três meses (Vessby *et al.*, 2001). Alguns mecanismos através dos quais a gordura saturada reduz a sensibilidade à insulina tem sido avaliados. Primeiramente, a gordura saturada reduz a afinidade da membrana à insulina gerando uma redução da ação da insulina nas células alvo (Manco *et al.*, 2004). Ainda, o aumento na síntese de ceramidas através do consumo excessivo de gorduras saturadas também induz a resistência à insulina (Eissing *et al.*, 2013). Adicionalmente, o aumento de citocinas inflamatórias, como o TNF-α e a interleucina-6, através do consumo elevado de gorduras

saturadas, reduz a sensibilidade à insulina (Kennedy *et al.*, 2009). Por fim, as gorduras saturadas e insaturadas podem exercer efeitos distintos no acúmulo de gordura (visceral ou subcutâneo), impactando de forma distinta o metabolismo da glicose (Gouk *et al.*, 2013 e Gouk *et al.*, 2014). Em um estudo de hiper-alimentação com indivíduos saudáveis, o aumento da ingestão de gordura saturada foi associado a um aumento duas vezes maior de gordura visceral abdominal quando comparado ao aumento da ingestão de gorduras poliinsaturadas, mesmo com o ganho de peso similar nos grupos após 7 semanas de intervenção (Rosqvist *et al.*, 2014). Entretanto, ainda não foi demonstrado na literatura o efeito de uma dieta rica em gorduras e com alto teor de gorduras saturadas na sensibilidade à insulina de indivíduos com o peso estável.

Diante do exposto acima, os objetivos desta tese consistem em:

- Avaliar a associação entre os níveis de adiponectina total e de alto peso molecular com a presença de SM.

- Revisar sistematicamente ensaios clínicos randomizados e controlados que investigaram o efeito dos lipídios dietéticos nos níveis de adiponectina em adultos.

- Determinar o efeito de dietas com baixo ou elevado teor de gorduras e gordura saturada na sensibilidade à insulina em indivíduos com sobrepeso e peso estável.

Tabela 1: características dos estudos que avaliaram o efeito de intervenções dietéticas com modificação no conteúdo de lipídios na sensibilidade à insulina

Autor Ano	Desenho do estudo Tempo de seguimento	Características dos participantes	Composição da dieta em % kcal (gordura: carboidrato: proteína, VCT)	Método de avaliação da sensibilidade à insulina	Mudança da sensibilidade à insulina
Bisschop 2001	ECR cruzado 11 dias	6 homens Idade: 29–55 anos IMC: 21–26 kg/m^2	LFD: 0:85:15 HFD: 83:0:17 Dietas isocalóricas. Fórmula líquida. VCT NR.	CLAMP	Dados gráficos. Melhora da SI em ambos. Na dieta LFD maior SI comparada à dieta HFD.
Ebbeling 2012	ECR cruzado 4 semanas	21 indivíduos 8 mulheres Idade: 30,3±5,7 anos IMC: 34,4±4,9 kg/m^2	LFD: 20:60:20, alta carga glicêmica HFD1: 40:40:20, moderada carga glicêmica HFD2: 60:10:30, baixa carga glicêmica Dietas isocalóricas, após perda de 10-15% do peso inicial. VCT NR.	OGTT	LFD: ↑120% HFD1: ↑263% HFD2: ↑288% Final da fase de manutenção comparada ao período basal.
Eckel 2006	ECR paralelo 15 dias	39 indivíduos 17 mulheres Idade: 25–36 anos IMC: 18,7–50,2 kg/m^2	LFD: 25:55:20 HFD: 50:30:20 Dietas isocalóricas. VCT NR.	CLAMP	LFD: ↑11,3% HFD: ↑1,1%
Gadgil 2013	ECR cruzado 6 semanas	164 indivíduos com HAS 73 mulheres Idade: 53,6±10,9 anos IMC: 30,2±6,1 kg/m^2	LFD (↑carb): 27:58:15 LFD (↑prot): 27:48:25 HFD (↑AGMI): 37:48:15 AGMI CARB e PROT = 13% e UNSAT = 21%. VCT NR.	QUICKI	LFD (↑carb): ↑0,6% ¥ LFD (↑prot): 1,1↑% HFD (↑AGMI): ↑2%* ¥
Jebb 2010	ECR paralelo 2x2 24 semanas	548 indivíduos 318 mulheres Idade: 30–70 anos IMC: 28,6±5,3 kg/m^2	LFD1 (↑IG): 28:55:17 LFD2 (↓IG): 28:55:17 HFD1 (↑AGMI /↑IG): 38:45:17 HFD2 (↑AGMI /↓IG): 38:45:17 HFD = 20% AGMI e LFD = 10% AGMI. VCT NR.	IVGTT	LFD1: ↓8,6 (-15,4, -1,1)* LFD2: ↑9,9 (2,4, 18,0)* HFD1: ↑2,1 (-5,5, 9,8) HFD2: ↓3,5 (-10,6, 4,3)
Kirk 2009	ECR 11 semanas	22 obesos 18 mulheres	LFD: 20:65:15 HFD: 75:10:15	CLAMP	↑3,7%* (análise com ambos os grupos após 11

		Idade: 43,6±2,5 anos IMC: 36,5±0,8kg/m2	Dietas com restrição calórica (1000 kcal/dia)		semanas). Sem diferença entre as intervenções.
Utzschneider 2013	ECR paralelo 4 semanas	20 idosos 13 mulheres Idade: 69,3±1,6 anos IMC: 26,9± 0,8 kg/m^2	LFD: 23% gordura/7% saturada e IG < 55 HFD: 43% gordura/24% saturada e IG > 70 Dietas isocalóricas. VCT NR.	OGTT (Matsuda índex)	LFD: ↑22,9%* HFD: ↑21,8%
van Herpen 2011	ECR 3 semanas	20 homens com sobrepeso Idade: 54±2,3 anos IMC: 29,3±0,6 kg/m^2	LFD: 20:65:15 HFD: 55:30:15 Dietas isocalóricas. VCT NR.	CLAMP	LFD: ↑13,5% HFD: ↑9,6%
Vessby 2001	ECR paralelo 12 semanas	162 indivíduos 76 mulheres Idade: 30-65 anos IMC: 22-32 kg/m^2	HFD1 (↑sat): 37% gordura/17% sat/14%MUFA/6%PUFA HFD2 (↑AGMI): 37% gordura/8% sat/23%AGMI/6%PUFA Dietas isocalóricas. VCT NR.	IVGTT	HFD1: ↓12,5%*¥ HFD2: ↑2%

Abreviaturas: ECR = ensaio clínico randomizado, VCT = valor calórico total, LFD = *low-fat diet* (dieta com baixo teor de gorduras), HFD = *high-fat diet* (dieta com elevado teor de gorduras), IMC = índice de massa corporal, TOTG = teste oral de tolerância à glicose, TTG-IV= teste intravenoso de tolerância à glicose, CLAMP = clamp euglicêmico hiperinsulinêmico.

Figura 1. Relação entre hipoadiponectinemia e desenvolvimento de SM.

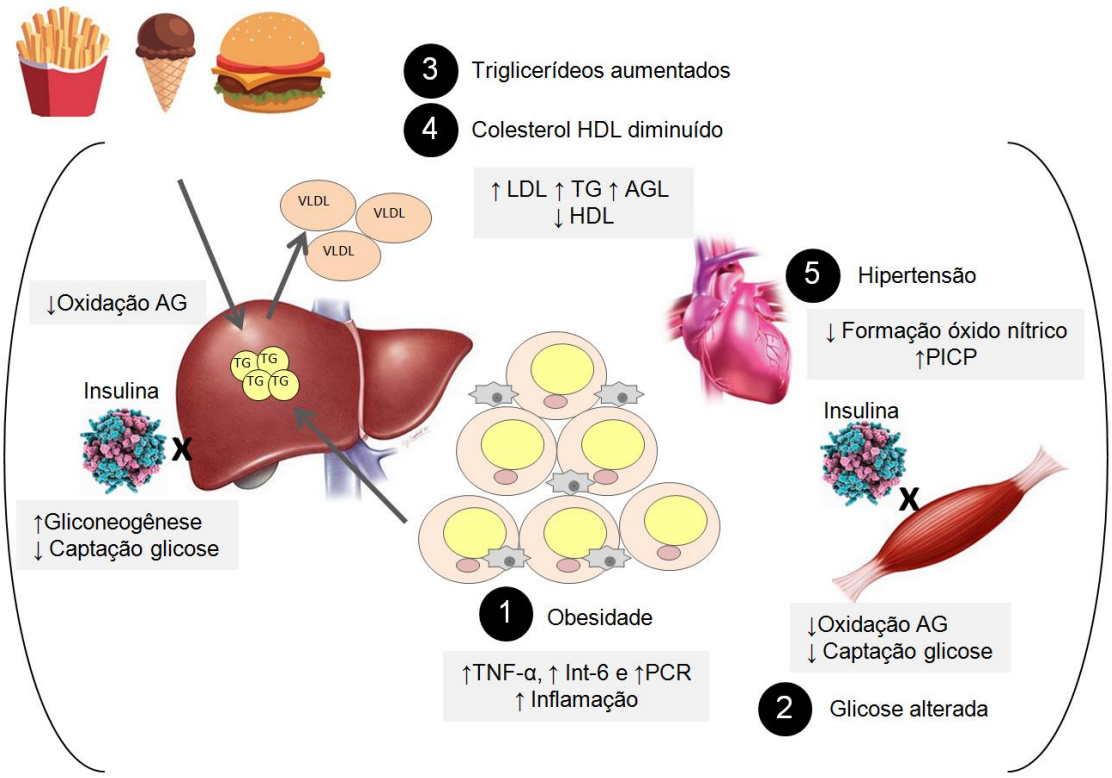


Figura 1. A figura mostra os mecanismos através dos quais a redução dos níveis de adiponectina associa-se ao desenvolvimento da SM. **Número 1:** o acúmulo de gordura visceral reduz a produção de adiponectina. A inflamação do tecido aumenta os níveis de proteína-C reativa (PCR) e de citocinas inflamatórias (TNF-α e interleucina-6) que ativam a gliconeogênese hepática. **Número 2:** a gliconeogênese hepática está ativada e adicionalmente a sensibilidade à insulina no músculo e no fígado está reduzida gerando aumento dos níveis de glicose circulantes. **Número 3 e 4:** a redução da oxidação dos triglicerídeos provenientes do tecido adiposo e dos lipídios dietéticos pelo fígado aumenta os níveis de ácidos graxos livres (AGL) e a produção de VLDL gerando desbalanço do perfil lipídico (aumento do colesterol LDL, triglicerídeos (TG) e redução do colesterol HDL). **Número 5:** o aumento do nível sérico do propeptídeo carboxiterminal do pró-colágeno tipo I (PICP) intensifica a rigidez arterial e a redução da produção de óxido nitrico contribui para redução da vasodilatação. Esses mecanismos somados ao ambiente pró-inflamatorio promovem alterações da homeostase pressórica que contribuem com o desenvolvimento da hipertensão arterial sistêmica.

REFERÊNCIAS

1. Arita Y *et al*. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. **Biochem Biophys Res Commun**, v. 257, n. 1, p. 79–83, Apr 2 1999.
2. Arvidsson E *et al*. Effects of different hypocaloric diets on protein secretion from adipose tissue of obese women. **Diabetes**, v. 53, n.8, p. 1966–1971, Aug 2004.
3. Aso Y *et al*. Comparison of serum high-molecular weight (HMW) adiponectin with total adiponectin concentrations in Type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. **Diabetes**, v. 55, p. 1954–1960, 2006.
4. Beltran-Sanchez H, Harhay MO, Harhay MM, McElligott S: Prevalence and trends of metabolic syndrome in the adult US population, 1999-2010. **J Am Coll Cardiol**, v. 62, n. 8, p. 697-703, Aug 20 2013.
5. Berg AH *et al*. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. **Nat Med**, v. 7, p. 947–953, 2001.
6. Bisschop PH *et al*. Dietary fat content alters insulin-mediated glucose metabolism in healthy men. **Am J Clin Nutr**, v. 73, n. 3, p. 554-559, 2001.
7. Bosi PL *et al*. Prevalência de diabetes mellitus e tolerância à glicose diminuída na população urbana de 30 a 79 anos da cidade de São Carlos, São Paulo. **Arq Bras Endocrinol Metab.**, v. 53, n. 6, p.726-32, 2009.
8. Brasier AR e Recinos A. Vascular inflammation and the renin-angiotensin system. **Arterioscler Thromb Vasc Biol.**, v. 22, p. 1257–1266, 2002.
9. Brown J *et al*. Conjugated linoleic acid (CLA) induces human adipocyte delipidation: autocrine/ paracrine regulation of MEK/ERK signaling by adipocytokines. **J Biol Chem**., v. 279, p. 26735–26747, 2004.
10. Cameron AJ *et al*. Central obesity as a precursor to the metabolic syndrome in the AusDiab study and Mauritius. **Obesity**, v. 16 n. 12, p. 2707-16. Dec 2008.

11. Canuto R *et al.* Metabolic syndrome in fixed-shift workers. **Rev Saude Publica,** v. 49, n.30, Jun 2015.
12. Centers for Disease Control and Prevention (CDC). National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. US Department of Health and Human Services, Atlanta 2014.
13. Cnop M *et al*. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. **Diabetologia**, v. 46, n. 4, p. 459–469, Apr 2003.
14. Combs TP *et al*. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. **J Clin Invest**, v. 108, p.1875–1881, 2001.
15. Comuzzie AG *et al.* The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. **J Clin Endocrinol Metab**, v. 86, n. 9, p.4321-5. Sep, 2001.
16. Coope A *et al*. AdipoR1 mediates the anorexigenic and insulin/leptin-**l**ike actions of adiponectin in the hypothalamus. **FEBS Lett**, v. 582, n. 10, p. 1471-6, Apr 2008.
17. Da Costa JS *et al*. Prevalence of Diabetes Mellitus in Southern Brazil: a population-based study. **Rev Saúde Pública**, v. 40 n. 3, p. 542-5, Jun 2006.
18. Daimon M *et al*. Decreased serum levels of adiponectin are a risk factor for the progression to Type 2 diabetes in the Japanese population: the Funagata study. **Diabetes Care**, v. 26, n.7, p. 2015–20, Jul 2003.
19. De Carvalho Vidigal F *et al.* Prevalence of metabolic syndrome in Brazilian adults: a systematic review. **BMC Public Health**, v. 13, n. 1, p. 1198, Dec 18 2013.
20. De Kloet AD, *et al*. The renin angiotensin system and the metabolic syndrome. **Physiol Behav.**, v.100, p. 525–534, 2010.
21. Ebbeling, CB. Effects of Dietary Composition on Energy Expenditure During Weight-Loss Maintenance. **JAMA**, v. 307, n. 24, p. 2627-2634, 2012.

22. Eckel RH *et al*. Carbohydrate balance predicts weight and fat gain in adults. **Am J Clin Nutr**, v. 83, n. 4, p. 803-808, 2006.
23. Eissing L *et al*. De novo lipogenesis in human fat and liver is linked to ChREBP-beta and metabolic health. **Nat Commun**, v. 4, p. 1528 2013.
24. Emerging Risk Factors Collaboration *et al*. Diabetes Mellitus, Fasting Glucose, and Risk of Cause-Specific Death. **N Engl J Med**, v. 364 n. 9, p. 829–841, March 3 2011.
25. Fan Y *et al*. Association between ADIPOQ +45T>G Polymorphism and Type 2 Diabetes: A Systematic Review and Meta-Analysis. **Int. J. Mol. Sci.**, v. 16, p. 704-723, 2015.
26. Fisher FM *et al*. Differences in adiponectin protein expression: effect of fat depots and type 2 diabetic status. **Horm. Metab. Res.**, v. 34, n. 11-12, p. 650–654, Nov-Dec 2002.
27. Florez JC: Clinical review: the genetics of type 2 diabetes: a realistic appraisal in 2008. **J Clin Endocrinol Metab**, v. 93, n. 12, p. 4633-4642, Dec 2008.
28. Gadgil MD *et al*. The effects of carbohydrate, unsaturated fat, and protein intake on measures of insulin sensitivity: results from the OmniHeart trial. **Diabetes care**, v. 36, n. 5, p. 1132-1137, 2013.
29. Gil-Campos M *et al*. Adiponectin, the missing link in insulin resistance and obesity. **Clin Nutr**, v. 23: p. 963–974, 2004.
30. Gouk SW *et al*. Long-chain SFA at the sn-1, 3 positions of TAG reduce body fat deposition in C57BL/6 mice. **Br J Nutr**, v. 110, n. 11, p. 1987-1995, Dec 14 2013.
31. Gouk SW *et al*. Stearic acids at sn-1, 3 positions of TAG are more efficient at limiting fat deposition than palmitic and oleic acids in C57BL/6 mice. **Br J Nutr**, v. 111, n. 7, p. 1174-1180, Apr 14 2014.
32. Guldbrand H *et al*. In type 2 diabetes, randomisation to advice to follow a low-carbohydrate diet transiently improves glycaemic control compared with advice to follow a low-fat diet producing a similar weight loss. **Diabetologia**, v. 55, n. 8, p. 2118-2127, Aug 2012.

33. Hara K *et al.* Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. **Diabetes**, v. 51, n. 2, p. 536-540, Feb 2002.
34. Heid IM *et al.* Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. **Diabetes,** v. 55, n. 2, p. 375-384, Feb 2006.
35. International Diabetes Federation (IDF), 2010. Disponível em: <http://www.idf.org/node/23640> Acesso em: 22 jun. 2015.
36. Iwaki M *et al*. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. **Diabetes**, v. 52, p. 1655–1663, 2003.
37. Iwashima Y *et al*. Hypoadiponectinemia is an independent risk factor for hypertension. **Hypertension**, v. 43, n.6, p. 1318–1323, Jun 2004.
38. Jebb SA *et al*. Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity and cardiovascular risk: the RISCK (Reading, Imperial, Surrey, Cambridge, and Kings) trial. **Am J Clin Nutr**, v. 92, n. 4, p.748-758, 2010.
39. Kadowaki T *et al.* Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. **J Clin Invest**, v. 116, n. 7, p. 1784-1792, Jul 2006.
40. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. **Nature**, v. 444, n. 7121, p. 840-6, Dec 14 2006.
41. Kennedy A *et al*. Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. **J Nutr**, v. 139, n. 1, p. 1-4, Jan 2009.
42. Keramati AR *et al*. A form of the metabolic syndrome associated with mutations in DYRK1B. **N Engl J Med**, v. 370, n. 20, p. 1909-19, May 15 2014
43. Kern PA *et al.* Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. **Diabetes**, v. 52, n. 7, p. 1779-1785, Jul 2003.

44. Kharroubi I *et al*. Expression of adiponectin receptors in pancreatic beta cells. **Biochem Biophys Res Commun**, v. 312, p. 1118–1122, 2003.
45. Kirk E *et al*. Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. **Gastroenterology**, v. 136, n. 5, p. 1552–1560, May 2009.
46. Kobayashi H *et al*. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. **Circ Res**, v. 94, p. e27–e31, 2004.
47. Koh SB *et al*. Relationships between Serum Adiponectin with Metabolic Syndrome and Components of Metabolic Syndrome in Non-Diabetic Koreans: ARIRANG Study. **Yonsei Med J**, v. 52, n. 2, p. 234-24, March 2011.
48. Liao D, *et al*. Arterial stiffness and the development of hypertension the ARIC study. **Hypertension**, v. 34 p.201–206, 1999.
49. Lihn AS *et al.* Lower expression of adiponectin mRNA in visceral adipose tissue in lean and obese subjects. **Mol Cell Endocrinol**, v. 219, n. 1-2, p. 9-15, Apr 30 2004.
50. Lindsay RS, *et al.* Genome-wide linkage analysis of serum adiponectin in the Pima Indian population. **Diabetes**, v. 52, n. 9, p. 2419-25, Sep 2003.
51. Malerbi D *et al*. The Brazilian Cooperative Group on the Study of Diabetes Prevalence. Multicenter study of the prevalence of diabetes mellitus and impaired glucose tolerance in the urban Brazilian population aged 30 a 69 years. **Diabetes Care**, v. 15, n. 11, p.1509-16, 1992.
52. Malik S *et al*. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. **Circulation**, v. 110, n. 10, p. 1245-50, Sep 7 2004.
53. Manco M, Calvani M, Mingrone G. Effects of dietary fatty acids on insulin sensitivity and secretion. **Diabetes Obes Metab**, v. 6, n. 6, p. 402-413, Nov 2004.
54. Mantzoros CS *et al*. Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. **Am J Clin Nutr**, v. 84, n. 2, p. 328–335, Aug 2006.

55. Marina A *et al*. Effects of dietary fat and saturated fat content on liver fat and markers of oxidative stress in overweight/obese men and women under weight-stable conditions. **Nutrients**, v. 6, n. 11, p. 4678-90, Oct 28 2014.
56. Matsubara, M., Maruoka, S., Katayose, S. Decreased plasma adiponectin concentrations in women with dyslipidemia. **J. Clin. Endocrinol. Metab.**, v. 87, n.6, p. 2764–2769, Jun 2002.
57. Menzaghi C, Trischitta V, Doria A: Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. **Diabetes**, v. 56, n. 5, p. 1198-1209, May 2007.
58. Moraes AS *et al*. Prevalência de diabetes mellitus e identificação de fatores associados em adultos residents em área urbana de Ribeirão Preto, São Paulo, Brasil 2006: Projeto OBEDIARP. **Cad Saúde Pública.**, v. 26, n. 5, p. 929-41, 2010.
59. Motoshima H *et al*. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. **Biochem Biophys Res Commun**, v. 315, p. 264–271, 2004.
60. Neschen S *et al*. Fish oil regulates adiponectin secretion by a peroxisome proliferator-activated receptor-gamma-dependent mechanism in mice. **Diabetes**, v. 55, n., p. 924-8, Apr 2006.
61. Okamoto Y *et al*. Adiponectin: a key adipocytokine in metabolic syndrome. **Clin Sci (Lond)**, v 110, n. 3, p. 267–278, Mar 2006.
62. Pajvani UB *et al*. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. **J Biol Chem**, v. 279, p. 12152–12162, 2004.
63. Pajvani UB *et al*. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. **J Biol Chem**, v. 278, p. 9073–9085, 2003.
64. Pineiro R *et al*. Adiponectin is synthesized and secreted by human and murine cardiomyocytes. **FEBS Lett**, v. 579, p. 5163–5169, 2005.

65. Poirier H *et al*. Nutritional supplementation with trans-10,cis-12 conjugated linoleic acid induces inflammation of white adipose tissue. **Diabetes**, v. 55, p. 1634-40, 2006.
66. Rabe K *et al.* Adipokines and insulin resistance. **Mol Med**, v. 14, n. 11-12, p. 741-751, Nov-Dec 2008.
67. Rosqvist F *et al*. Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. **Diabetes**, v. 63, n. 7, p. 2356-2368, Jul 2014.
68. Schillaci G, *et al*. Metabolic syndrome is associated with aortic stiffness in untreated essential. **Hypertension**, v 45, p. 1078–1082, 2005.
69. Schulze MB *et al.* Adiponectin and future coronary heart disease events among men with type 2 diabetes. **Diabetes**, v. 54, n. 2, p. 534-539, Feb 2005.
70. Shai I *et al*. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. **New Engl J Med**, v. 359, n. 3, p. 229-24, Jul 17 2008.
71. Smemo S *et al*. Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature, v. 507, n. 7492, p. 371–375, March 20 2014.
72. Snijder MB, *et al*. Relationship of adiposity with arterial stiffness as mediated by adiponectin in older men and women: the Hoorn Study. **Eur J Endocrinol**,v. 160, p. 387–395, 2009.
73. Stumvoll M *et al*. Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. **Diabetes**, v. 51, p.37–41, 2002.
74. Tan KC *et al*. Hypoadiponectinemia is associated with impaired endothelium-dependent vasodilation. **J Clin Endocrinol Metab**, v. 89, p. 765–769, 2004.
75. Tong J *et al*. Intra-abdominal fat accumulation predicts the development of the metabolic syndrome in non-diabetic Japanese-Americans. **Diabetologia,** v. 50, n. 6, p. 1156-60, Jun 2007.
76. Trichopoulou A *et al*. Adherence to a Mediterranean diet and survival in a Greek population. **N Engl J Med**, v. 348, n. 26, p. 2599–2608, Jun 26 2003.

77. Tsai WC, *et al*. Association of adiponectin with procollagen type I carboxyterminal propeptide in non-diabetic essential hypertension. **Blood Press**, v. 17, p. 233–238, 2008.
78. Tsuchida A *et al*. Peroxisome proliferator-activated receptor (PPAR)alpha activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPARalpha, PPARgamma, and their combination. **Diabetes**, v. 54, n. 12, p. 3358–3370, Dec 2005.
79. Tsuchida A *et al*. Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. **J Biol Chem**, v. 279, p. 30817–30822, 2004.
80. Utzschneider KM *et al*. Beneficial effect of a weight-stable, low-fat/low-saturated fat/low-glycaemic index diet to reduce liver fat in older subjects. **Br J Nutr**, v. 109, n. 6, p. 1096-1104, Mar 2013.
81. Vaiopoulos AG, *et al*. The role of adiponectin in human vascular physiology**. Int J Cardiol.**, v. 155, p. 188–193, 2012.
82. van Herpen NA *et al*. Three weeks on a high-fat diet increases intrahepatic lipid accumulation and decreases metabolic flexibility in healthy overweight men. **J Clin Endocrinol and Metab**, v. 96, n. 4, p. E691-695, Apr 2011.
83. Van Stijn CMW, *et al*. Adiponectin expression protects against angiotensin II-mediated inflammation and accelerated atherosclerosis. **PLoS One**, v. 9, p. e86404, 2014.
84. Vasseur F, *et al*. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. **Hum Mol Genet**, v. 11, p. 2607–2614, 2002.
85. Vessby B *et al*. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. **Diabetologia**, v. 44 n. 3, p. 312-319, 2001.
86. Vigitel Brasil 2012. Vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico. Ministério da Saúde, 2012.

87. Weijnen CF *et al*. Risk of diabetes in siblings of index cases with Type 2 diabetes: implications for genetic studies. **Diabet Med,** v. 19, n. 1, p. 41-50 Jan 2002.
88. Wycherley TP *et al*. Long term effects of weight loss with a very low carbohydrate and low fat diet on vascular function in overweight and obese patients. **J Intern Med**, v. 267, n. 5, p. 452–461, May 2010.
89. Xu A e Vanhoutte PM. Adiponectin and adipocyte fatty acid binding protein in the pathogenesis of cardiovascular disease. **Am J Physiol Heart Circ Physiol**, v.302, p. H1231–H1240, 2012.
90. Yamauchi T *et al*. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. **Nat Med,** v. 8, p. 1288–1295, 2002.
91. Yamauchi T *et al*. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. **Nat Med**, v. 7, p. 941–946, 2001.
92. Yamauchi T, Kadowaki T. Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. **Int J Obes (Lond)**, v. 32, Suppl 7:S13-8 Dec 2008.

CAPÍTULO 2

Major components of metabolic syndrome and adiponectin levels:

a cross-sectional study

Anize D von Frankenberg[1,4,*], Filipe V do Nascimento[1], Lucas Eduardo Gatelli[1], Bárbara L Nedel[1], Sheila P Garcia[1], Carolina SV de Oliveira[3], Pedro Saddi-Rosa[3], André F Reis[3], Luis H Canani[1,2], Fernando Gerchman[1,2]

[1] Post-Graduate Endocrinology Program, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil. [2] Metabolism Unit, Endocrinology Division, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil. [3] Endocrinology Unit, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil. [4] Hospital de Clínicas de Porto Alegre. Rua Ramiro Barcelos, 2350 – Prédio 12. 4° andar, 90035-003 Porto Alegre, RS, Brazil

[*] Corresponding author. Hospital de Clínicas de Porto Alegre. Rua Ramiro Barcelos, 2350 – Prédio 12. 4° andar, 90035-003 Porto Alegre, RS, Brazil

Keywords: Adiponectin, Metabolic syndrome, Obesity

Artigo publicado na revista Diabetology & Metabolic Syndrome 2014 Feb 26;6(1):26. doi: 10.1186/1758-5996-6-26.

Abstract

Background: Adiponectin is a major regulator of glucose and lipid homeostasis by its insulin sensitizer properties. Since decreased insulin sensitivity is linked to metabolic syndrome (MS), decreased adiponectin levels may be related to its development. The purpose of the study was to investigate the relationship between adiponectin levels and MS.

Methods: Firstly, we cross-sectionally examined subjects with or without MS submitted to an oral glucose tolerance test at Hospital de Clínicas de Porto Alegre (n = 172). A replication analysis was performed in subjects (n = 422) undergoing cardiac angiography at Hospital São Paulo. Subchronic inflammation (US-CRP), coagulation marker (fibrinogen), insulin sensitivity and resistance (Matsuda ISI and HOMA-IR) were estimated. Plasma total and high molecular weight (HMW) adiponectin were measured.

Results: Total and HMW adiponectin levels were lower in MS subjects (P< 0.05). Total adiponectin levels were lower in the presence of high waist circumference, low HDL-cholesterol and elevated triglyceride criteria in both samples and by elevated blood pressure and glucose criteria in Porto Alegre. HMW adiponectin levels were lower in the presence of low HDL-cholesterol, elevated triglycerides, and glucose criteria. Total adiponectin levels were positively related with HDL-cholesterol and ISI Matsuda, negatively related with waist circumference, glucose, triglycerides, HOMA-IR, and US-CRP and not related with blood pressure. While adjusting for sex and age, increased adiponectin levels remained associated with a reduced prevalence ratio for MS in both cohorts (P = 0.001).

Conclusions: Adiponectin levels decreased with increasing number of MS criteria, and it is in part determined by its relationship with HDL, triglycerides and abdominal adiposity.

Background

Obesity is a major public-health problem worldwide. It has been associated with the development of metabolic syndrome (MS) [1] which is an interrelated cluster of risk factors for cardiovascular disease (CVD) and type 2 diabetes [2,3] such as hyperglycemia, raised blood pressure, elevated triglyceride levels, low high-density lipoprotein cholesterol levels, and central obesity [4]. Data from the NHANHES study has shown that 22.9% of United States adult population had MS in 2009/10 [5].

The growing rates of this obesity-related syndrome have spurred the search for greater insight about mechanisms contributing to the development of MS, especially those reflecting a dysfunction of adipose tissue, which probably plays a major role in its development [6].

Adiponectin, a hormone expressed in adipose tissue and encoded by the ADIPOQ gene (chromosome 3q27), plays an important role in regulating insulin sensitivity, glucose and lipid metabolism besides anti-inflammatory and anti atherogenic properties [7]. Its high molecular weight (HMW) isoform is the major responsible for these functions [8]. In the presence of obesity, adiponectin release is down regulated resulting in reduced circulating levels [9].

In order to investigate the relationship between total and HMW adiponectin levels and the presence of MS we measured its levels in two different cohorts. We were also interested in finding out which MS components are mostly related to adiponectin levels. Additionally, we analyzed how insulin resistance, sensitivity and subchronic inflammation were related to adiponectin levels.

Methods

Subjects

In order to determine the relationship between MS and adiponectin, we undertook a two-stage study using data from two cohorts. In the first stage, the relationship between adiponectin and MS was assessed in consecutive subjects who were referred for ambulatory care in the Metabolism Unit of Hospital de Clínicas de Porto Alegre, Federal University of Rio Grande do Sul (n = 172), whose glycemic status was not previously defined. Exclusion criteria included clinically significant autoimmune diseases, uncompensated hypo or hyperthyroidism, malignant disease that may affect 5-year survival, stage IV or V chronic kidney disease, AIDS, pregnancy/lactation, dementia, cirrhosis, hepatitis, alcohol or illicit drug abuse, glucocorticoid or anti-retroviral treatment, and malnutrition. The protocol was approved by the institutional review board of Hospital de Clínicas de Porto Alegre and the subjects provided written informed consent.

In order to confirm the relationship between adiponectin levels and MS we performed a second stage study (replication analysis) with data from the Endocrinology and Cardiovascular Units of the hospital of the Federal University of São Paulo (São Paulo cohort, UNIFESP) of 422 consecutive subjects who had complete assessment of MS criteria and underwent cardiac angiography for investigation of coronary heart disease, according to an indication of their clinician as previously described [10]. Exclusion criteria included creatinine clearance <50 mL/min, abnormal thyroid function, presence of active inflammatory disease and neoplasia. The protocol was approved by the

institutional committee of ethical practice of UNIFESP and subjects provided written informed consent.

Study procedures and assays

Subjects from the Porto Alegre and the São Paulo cohorts underwent a standard evaluation which included medical history, physical examination and anthropometric measurements. Ethnicity was classified based on self-reported skin color and recorded as white and non white. Current smoking was defined by active consumption in the last three months. Habitual alcohol consumption was accessed by a yes or no question. Subjects were classified as physically active if they practiced moderate-intensity activity for at least 150 minutes per week. Body weight was recorded in light clothing without shoes. Height was measured on a stadiometer. BMI was calculated by weight (kg)/height (m^2) [11]. Waist circumference was taken at the midpoint between the lower costal margin and the iliac crest measured to the nearest 0.5 cm.

In the Porto Alegre cohort, blood pressure measurements were performed one week after withdrawal of all antihypertensive medications whereas in the São Paulo cohort subjects were not withdrawal of medications in use. Office blood pressure were measured by the arm blood pressure oscillometric monitor device OMRON® (H-003D) with cuff adjusted for arm circumference while the participant was seated, in the right arm three times by auscultation, and the mean of the last two measurements was used to estimate systolic and diastolic arterial blood pressure in both cohorts. For a better understanding of the relationship between adiponectin and arterial blood pressure, ambulatory 24-h blood pressure monitoring (ABPM) was performed

by oscillometry (Spacelabs 90207), with a 15-min interval during the daytime and a 20-min interval during the nighttime in 129 subjects from the Porto Alegre cohort. Subjects were advised to maintain their usual daily activities. Sleep time was recorded as the period between the time when the patient went to bed and the time when the patient woke up the next morning. The means of 24-h, daytime, and nighttime systolic and diastolic blood pressure were recorded.

Blood samples were drawn after an overnight fast for analysis of lipids (total, HDL cholesterol, and triglycerides), glycated hemoglobin (HbA1c), insulin, and total adiponectin in subjects from both cohorts. HMW adiponectin was measured in blood samples of subjects from the São Paulo cohort. Ultra-sensitive C-reactive protein (us-CRP) and fibrinogen were measured in blood samples of subjects from the Porto Alegre cohort. Subjects from the Porto Alegre cohort were also submitted between 8:00 and 9:00 AM to a 75-g oral glucose tolerance test (OGTT) in which both glucose and insulin were measured at 0, 30, 60, 90 and 120 minutes. All subjects were classified according to glucose tolerance status [12] and presence of MS [4].

Assays

Serum total and HDL-cholesterol, triglycerides, insulin, HbA1c, US-CRP and fibrinogen were measured in the Clinical Pathology Unit. Cholesterol and triglycerides were determined by means of an enzymatic method (Ádvia 1800); insulin by chemiluminescence (Centaur XP); HbA1c by high performance liquid chromatography (Tosoh Plus); US-CRP by turbidimetry (Ádvia 1800) and fibrinogen by coagulometric method (BCS).

In the Porto Alegre cohort, plasma samples for adiponectin were analyzed in duplicate by using ELISA kits (Invitrogen®; intra-assay coefficient of variation [CV] < 3.84% and inter-assay CV < 5.50%). Plasma adiponectin study samples respectively presented an intra and inter-assay CV of 2.99% and 4.26%. In the São Paulo cohort, total and HMW adiponectin levels were measured in plasma samples using commercial ELISA kits (EZHADP-61 K and EZHMWA-64 K, respectively, Millipore, Saint Charles, MO). Intra- and interassay CV were respectively 7.4% and 10.6% for total adiponectin and 3.41% and 9.0% for HMW adiponectin.

Classification of metabolic syndrome

MS was defined as the presence of three out of five criteria described below: high waist circumference (≥ 80 cm for women and ≥ 94 cm for men); elevated triglyceride levels (≥ 150 mg/dL [1.7 mmol/L]) or drug treatment for elevated triglycerides levels; reduced HDL-cholesterol (<40 mg/dL [1.0 mmol/L] for men and <50 mg/dL [1.3 mmol/L] for women) or drug treatment for reduced HDL-cholesterol levels; elevated blood pressure (systolic ≥ 130 mm Hg or diastolic ≥ 85 mm Hg) or antihypertensive drug treatment; and elevated glucose (fasting plasma glucose ≥100 mg/dL [6.1 mmol/L]) or drug treatment for hyperglycemia according to Consensus Societies/Joint Interim Statement [4]. Since subjects from the Porto Alegre cohort were submitted to the OGTT, we have also used the 2 h-plasma glucose criteria for impaired glucose tolerance (≥ 140 mg/dL[7.8 mmol/L]) in order to define the glucose criteria [4].

Classification of glucose tolerance

Based on fasting and 2 h-plasma glucose concentrations, subjects from the Porto Alegre cohort were categorized according to the American Diabetes Association criteria as having normal glucose tolerance (NGT: fasting plasma glucose [FPG] <100 mg/dL [6.1 mmol/L] and 2 h-plasma glucose level <140 mg/dL [7.8 mmol/L]); impaired fasting glucose (IFG; FPG 100-125 mg/dL [6.1–6.9 mmol/L] and 2 h-plasma glucose level <140 mg/dL [7.8 mmol/L], or impaired glucose tolerance (IGT; FPG <100 mg/dL [6.1 mmol/L] and 2 h-plasma glucose level 140-199 mg/dL [7.8–11.0 mmol/L] and diabetes (FPG ≥126 mg/dL [7.0 mmol/L] and/or 2-h PG ≥ 200 mg/dL [11.1 mmol/L) [12]. Subjects with IFG and/or IGT were considered to have prediabetes and this was also used to define the glucose criteria of the MS. Subjects from the São Paulo cohort were classified based on fasting glucose as described above and/or using HbA1c levels according to the American Diabetes Association criteria as NGT (FPG <100 mg/dL [6.1 mmol/L] and HbA1c <5.7%); prediabetes (FPG 100-125 mg/dL [6.1–6.9 mmol/L] and/or HbA1c between 5.7 and 6.4%), and diabetes (FPG ≥126 mg/dL [7.0 mmol/L] and/or HbA1c ≥ 6.5%).

Estimation of insulin sensitivity, insulin resistance, inflammation and endothelium dysfunction

Indices of insulin sensitivity or insulin resistance were determined, including: the homeostatic model assessment of insulin resistance (HOMA IR = [fasting glucose (mmol/L) * fasting insulin (μU/mL)] / 22.5) [13] and the Matsuda insulin sensitivity index (Matsuda ISI) that was calculated as 10,000/√ [fasting glucose (mmol/L) × fasting insulin (mU/L)] × [mean glucose × mean insulin

during OGTT] [14]. Subchronic inflammation was estimated by US-CRP and coagulation marker by fibrinogen [15,16].

Statistical analysis

Data were expressed as absolute number (%), mean ± standard deviation (SD) or median (P25-P75). To compare demographic, clinical, and laboratory data utilizing the presence or the different components of MS, the chi-square test, independent-samples t test and one-way ANOVA were used as appropriate. Prevalence of MS by adiponectin tertiles was compared by chi-square test. Variables with a non-normal distribution were log transformed before analyses. The significance of the correlations was examined by using the parametric Pearson's correlation coefficient for normally distributed or log transformed variables. To test the independent association of adiponectin and MS, multivariate regression analyses were performed using three different Poisson regression models. Prevalence ratio and 95% CI for continuous variables are shown for a 1-SD-magnitude increase (equal to 7.063 μg/mL). The first model contained MS as the dependent variable and age, gender and adiponectin as the independent variables. In the second model, US-CRP and HOMA-IR were added. In the third model, HOMA-IR was replaced by ISI-Matsuda into the model. We did not include as independent variables those that define a criterion for MS in order to avoid double correction. A P value or a P for trend < 0.05 was chosen as the level of significance. Calculations were made by using SPSS (version 19.0; SPSS Inc, Chicago).

Results

Subjects' characteristics

Porto Alegre cohort

The Porto Alegre cohort comprised 172 subjects, of whom 124 (72%) were females. The clinical and laboratory characteristics are summarized in Table 1. Subjects were subdivided by absence (21%) or presence (79%) of MS. Although these two groups did not differ by gender distribution, ethnicity, smoking status, alcohol consumption, physical activity, total cholesterol, and fibrinogen subjects with MS were older and presented higher HbA1c levels, HOMA-IR, and US-CRP than those without MS. Prediabetes and type 2 diabetes prevalence were higher in the presence of MS. BMI, waist circumference, fasting plasma glucose, triglycerides, and office blood pressure were also greater in subjects with MS. By ambulatory blood pressure monitoring, 24-h, day and night systolic blood pressures were also greater in subjects with than without MS (Table 1).

Plasma adiponectin was lower in the presence of MS (11.0 [7.9 – 13.7 μg/mL] vs 16.4 [10.2 – 22.7 μg/mL]; median [P25-P75], $p < 0.001$) (Figure 1A) and decreased significantly with an increasing number of MS criteria (Figure 1B). Furthermore, when participants were stratified into three groups by adiponectin tertiles, the prevalence of MS decreased from the lowest to the highest adiponectin tertile (tertile 1 = 89.5%, tertile 2 = 87.9%, and tertile 3 = 59.6%; P for trend < 0.001).

While comparing by each MS criterion, adiponectin levels were significantly lower in the presence of following criteria: high waist circumference

(12.4 [8.1 – 14.5 µg/mL] vs 18.5 [11.9 – 26.3 µg/mL]; P = 0.002), low HDL-cholesterol (10.9 [7.3 – 13.2 µg/mL] vs 15.6 [10.0 – 20.4 µg/mL]; P <0.001), elevated triglycerides (10.4 [7.1 – 12.6 µg/mL] vs 14.3 [8.7 – 18.7 µg/mL]; P = 0.001), elevated glucose (11.8 [8.0 – 13.7 µg/mL] vs 15.7 [9.9 – 20.2 µg/mL]; P = 0.032), and elevated blood pressure (12.1 [8.0 – 14.0 µg/mL] vs 15.8 [9.7 – 20.6 µg/mL]; P = 0.030).

To examine the relationship between adiponectin levels and the different MS components, we determined their correlations. Adiponectin levels were positively correlated with HDL-cholesterol (r = 0.452, P < 0.001) and negatively correlated with waist circumference (r = -0.269, p < 0.001), fasting glucose (r = -0.289; P = 0.001), and triglycerides (r = -0.252, p < 0.001). There was no significant correlation between adiponectin concentrations and systolic (r = -0.135; P = 0.081) or diastolic (r = -0.143; P = 0.066) office blood pressures (Figure 2). There was also no significant correlation between adiponectin concentrations and systolic 24-h (r = -0.087; P = 0.414), systolic daytime (r = -0.071; P = 0.504), systolic nighttime (r = -0.093; P = 0.383), diastolic 24-h (r = -0.042; P = 0.695), diastolic daytime (r = -0.030; P = 0.779), and diastolic nighttime (r = -0.042; P = 0.692) blood pressures.

Furthermore, adiponectin was negatively related to US-CRP (r = -0.154; P = 0.047) whereas this relationship was not found with fibrinogen (r = -0.048; P = 0.552). Regarding resistance and insulin sensitivity indexes (ISI), adiponectin was negatively associated with HOMA-IR (r = -0.218; P = 0.005) and positively associated with Matsuda ISI (r = 0.191; P = 0.014).

São Paulo cohort

In order to confirm the relationship between adiponectin levels and MS we performed a second stage study in 422 subjects who were submitted to cardiac angiography for evaluation of coronary heart disease as indicated by their clinician, of whom 190 (45%) were females (Table 1). Subjects were subdivided by absence (9%) or presence (91%) of MS. Age, ethnicity, smoking status, physical activity, and total cholesterol were similar between groups. However, alcohol consumption was lower in the presence of MS. As we have already observed in the Porto Alegre cohort, glucose tolerance decreased whereas HbA1C and HOMA-IR increased in the presence of MS. BMI, waist circumference, fasting plasma glucose, triglycerides and blood pressure were greater in subjects with MS as expected.

Plasma adiponectin had a similar pattern as observed in subjects from the Porto Alegre cohort. It was lower in the presence of MS (8.2 [5.5 – 13.8 µg/mL] vs 12.8 [9.0 – 20.5 µg/mL]; median [P25-P75], p = 0.005) (Figure 1A) and it significantly decreased with an increasing number of MS criteria (Figure 1B). Furthermore, when participants were stratified into three groups by adiponectin tertiles, the prevalence of MS decreased from the lowest to the highest adiponectin tertile (tertile 1 = 96.4%, tertile 2 = 91.5%, and tertile 3 = 90.8%; P for trend = 0.001).

Comparing according to the presence or absence of each MS criterion, adiponectin levels were significantly lower in the presence of following criteria: high waist circumference (10.4 [7.1 – 12.6 µg/mL] vs 18.5 [11.9 – 26.3 µg/mL]; P = 0.001), low HDL-cholesterol (10.6 [5.5 – 13.7 µg/mL] vs 14.4 [7.8 – 17.2 µg/mL]; P = 0.010), and elevated triglycerides (6.7 [7.6 – 9.6 µg/mL] vs 10.3

[11.7 – 14.0 µg/mL]; P < 0.001). However, this relationship was not found with elevated glucose (11 [5.6 – 14.0 µg/mL] vs 12.3 [5.8 – 15.7 µg/mL]; P = 0.110) and elevated blood pressure (11.0 [5.7 – 14.1 µg/mL] vs 10.9 [5.5 – 14.5 µg/mL]; P = 0.570).

While analyzing the relationship between adiponectin and the different MS components, adiponectin concentrations were positively correlated with HDL-cholesterol (r = 0.371; P < 0.001) and negatively correlated with waist circumference (r = -0.317; P < 0.001), fasting glucose (r = -0.118; P = 0.015), and triglycerides (r = -0.359; P < 0.001). Similar to the Porto Alegre cohort, there was no significant correlation between adiponectin concentrations with diastolic and systolic blood pressures (Figure 2).

HMW adiponectin had similar results as observed with total adiponectin. It was lower in the presence of MS (4.9 [6.2 – 7.3 µg/mL] vs 7.8 [7.9 – 13.5 µg/mL]; median [P25-P75], p < 0.001) and it significantly decreased with an increasing number of MS criteria (p < 0.001). Furthermore, when participants were stratified into three groups by adiponectin tertiles, the prevalence of MS decreased from the lowest to the highest adiponectin tertile (tertile 1 = 96.2%, tertile 2 = 91.5%, and tertile 3 = 83.8%; P for trend = 0.007).

Comparing according to the presence or absence of each MS criterion, HMW adiponectin levels were significantly lower in the presence of following criteria: low HDL-cholesterol (4.9 [6.3 – 7.4 µg/mL] vs 7.7 [7.3 – 11.5 µg/mL]; P = 0.005), elevated triglycerides (4.0 [4.6 – 5.9 µg/mL] vs 6.8 [7.7 – 9.4 µg/mL]; P < 0.001), and elevated glucose (5.0 [6.4 – 7.5 µg/mL] vs 7.0 [6.7 – 10.6 µg/mL]; P = 0.035). However, this relationship was not found with high waist circumference (5.0 [6.2 – 7.3 µg/mL] vs 5.9 [7.0 – 10.6 µg/mL]; P = 0.065), and

elevated blood pressure (5.0 [6.5 – 7.7 µg/mL] vs 6.0 [5.8 – 9.3 µg/mL]; P = 0.170).

While analyzing the relationship between adiponectin and the different MS components, HMW adiponectin concentrations were positively correlated with HDL-cholesterol (r = 0.378; P < 0.001) and negatively correlated with waist circumference (r = -0.246; P < 0.001), fasting glucose (r = -0.128; P = 0.008), and triglycerides (r = -0.320; P < 0.001).

Association between MS and adiponectin levels while adjusting for possible confounders

Porto Alegre cohort

To confirm the relationship between adiponectin levels and the presence of MS, we performed three different multiple Poisson regression models with MS as the dependent variable. In the first model an increment of 1-SD of adiponectin levels was significantly associated with a lower prevalence ratio of MS (0.84 [95% CI 0.75 – 0.93; P = 0.001]) while adjusting for sex and age. In the second model, when US-CRP and HOMA-IR were added to the model, 1-SD of adiponectin remained associated with a lower prevalence ratio of MS (0.88 [95% CI 0.79 – 0.97; P = 0.012]). The same is true while replacing HOMA-IR by ISI Matsuda in the model (0.88 [95% CI 0.80 – 0.97; P = 0.011]). Adding separately smoking status, alcohol consumption, physical activity, and waist circumference to this model did not change these results.

São Paulo cohort

We replicated similar models with data from the São Paulo cohort and observed similar results. In the first model, 1-SD of adiponectin levels was significantly associated with a lower prevalence ratio of MS (0.94 [95% CI 0.90 – 0.97; P = 0.001]) while adjusting for sex and age. In the second model, 1-SD of adiponectin remained associated with decreased prevalence ratio of MS (0.93 [95% CI 0.88 – 0.98; P = 0.001]) when HOMA-IR were added to the model. Adding separately smoking status, alcohol consumption, physical activity, and waist circumference to this model did not change these results.

Discussion

The present study assessed the relationship between adiponectin levels and MS in a two-stage study using data from two cohorts. We demonstrated that lower circulating total and HMW adiponectin levels were associated with the presence of MS. Decreasing total and HMW adiponectin plasmatic levels were related with an increasing number of MS criteria in both cohorts. This association was independent of age and sex, smoking status, alcohol consumption, physical activity, waist circumference, insulin sensitivity, and subchronic inflammation as shown while adjusting for confounders in different multivariate models with data from the Porto Alegre cohort. In order to confirm these findings, we applied the same approach in an independent cohort of subjects with different clinical characteristics who were being investigated for coronary artery disease in São Paulo. The confirmation of the same associations in these two different cohorts would underscore that this was not a

spurious finding. Additionally, total adiponectin levels were lower in the presence of each MS criterion in the Porto Alegre cohort whereas similar findings were observed in the São Paulo cohort, except for the elevated blood pressure and elevated glucose criteria. We found similar findings with HMW adiponectin levels which were lower in the presence of low HDL-cholesterol and elevated triglycerides criteria. Different from total adiponectin, HMW adiponectin levels were lower in the presence of elevated glucose criterion whereas their levels were not different in the presence of high waist circumference criterion. Since HMW adiponectin levels were inversely related with waist circumference, we believe that HMW adiponectin levels were not statistical lower in the presence of MS as a matter of sample size ($P = 0.065$).

Our results corroborate the findings of other studies which have analyzed the relationship between adiponectin levels and the MS [6,17]. Vega and Grundy showed that overweight and obese men with a high adiponectin/leptin ratio have a lower triglyceride, triglyceride/HDL levels and higher insulin sensitivity than those with a lower adiponectin/leptin ratio (6). Besides, Mente et al. suggested a possible causal relationship between low serum adiponectin levels and insulin resistance as measured by HOMA-IR (15).

As an extension to these studies, our findings suggest that adiponectin is inversely related to MS ratios while adjusting for possible confounders. We also tried to explore the possible factors related to lower adiponectin levels by the presence of MS. Lower adiponectin levels were observed with increasing US-CRP and HOMA-IR whereas higher adiponectin levels were related to increasing insulin sensitivity. Several studies have demonstrated that adiponectin is related to insulin sensitivity, since it sensitizes hepatocytes to the

effects of insulin, suppressing hepatic glucose output [18]. Adiponectin also promotes fatty acid oxidation in the liver and adipocytes, decreasing triglycerides levels [19-22] and increasing glucose uptake by skeletal muscles [23,24].

Central obesity has been related to adipose cell enlargement and the development of a proinflammatory state [25]. We have found a significant and inverse correlation in both cohorts between waist circumference and adiponectin plasma levels. Additionally, low adiponectin levels were related to high US-CRP, a marker of subchronic inflammation. In fact, the hypoadiponectinemia, per se, may partly determine the proinflammatory state found in subjects with MS in our study, especially those with central obesity.

The strongest relationship between adiponectin levels and MS criteria was observed with HDL cholesterol. Some studies suggest that the association between adiponectin and HDL cholesterol may result from the effect of adiponectin in reducing triglycerides, apo A-I fractional catabolic rate [26,27], and hepatic lipase activity [28,29].

Although we found that decreasing adiponectin levels were related to increasing blood pressure in the Porto Alegre cohort, we were not able to find the same results in the São Paulo cohort with both, the total and HMW adiponectin. Additionally, we believe that in São Paulo, where subjects were investigated for coronary artery disease by coronary arteriography, a high rate of anti-hypertensive medications used by this population affected this relationship by their direct action on adiponectin levels and by their action on blood pressure.

There are potential limitations to our study. Firstly, the cross-sectional study design makes it difficult to infer causal relationship between low total and HMW adiponectin levels and MS. Secondly, the subjects are not representative of the general population, since they were referred for assessment and evaluation of MS in Porto Alegre and to coronary artery disease confirmation by coronary angiography in São Paulo. However, by using two samples with completely different profiles, we were able to replicate our results which strength the direction of our findings. Additionally, our study goes in the same direction of others that have shown an effect of adiponectin in regulating lipid and glucose metabolism, which corroborates our findings [6,17].

Conclusions

In conclusion, total and HMW adiponectin levels not only are lower in the presence of MS, but it also decreases by increasing number of MS criteria. These levels are partly determined by their relationship with HDL cholesterol, triglycerides and abdominal adiposity. Furthermore, chronic inflammation and insulin resistance may contribute to the decrease in adiponectin levels. Longitudinal data of prospective population based studies might be used to understand the role of adiponectin in the development of MS.

Competing interests

None of the authors had a conflict of interest.

Authors' contributions

1) Designed research: ADvonF, FG; 2) conducted research: ADvonF, AFR, FG; 3) analyzed data or performed statistical analysis: ADvon F, FG; 4) wrote paper: ADvonF, LEG, AFR, LHC, FG; 5) had primary responsibility for final content: ADvonF, FG; 6) critical review of the manuscript: all authors. All authors read and approved the final manuscript.

Acknowledgments

This study received financial support from Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS), Hospital de Clínicas de Porto Alegre Research Fund (FIPE 11-226), Brazilian National Research Council (CNPq); F.G. was supported by a scholarship from the International Scholarship Program of the Endocrine Society.

References

1. WHO: **Obesity: preventing and managing the global epidemic of obesity**. In *Book Obesity: preventing and managing the global epidemic of obesity.* ; 2005.

2. Ford ES: **Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence.***Diabetes Care* 2005, **28:**1769–1778.

3. Malik S, Wong ND, Franklin SS, Kamath TV, L'Italien GJ, Pio JR, Williams GR: **Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults.***Circulation* 2004, **110:**1245–1250.

4. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr: **Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; american heart association; world heart federation; international atherosclerosis society; and international association for the study of obesity.***Circulation* 2009, **120:**1640–1645.

5. Beltran-Sanchez H, Harhay MO, Harhay MM, McElligott S: **Prevalence and trends of Metabolic Syndrome in the adult US population, 1999-2010.***J Am CollCardiol* 2013, **62:**697-703.

6. Vega GL, Grundy SM: **Metabolic risk susceptibility in men is partially related to adiponectin/leptin ratio.***J Obes* 2013, **2013:**409679.

7. Ahima RS: **Metabolic actions of adipocyte hormones: focus on adiponectin.***Obesity (Silver Spring)* 2006, **14**(Suppl 1)**:**9S–15S.

8. Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT: **Adiponectin multimeric complexes and the metabolic syndrome trait cluster.***Diabetes* 2006, **55:**249–259.

9. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y:**Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity.***BiochemBiophys Res Commun* 1999, **257:**79–83.

10. Oliveira CS, Saddi-Rosa P, Crispim F, Canani LH, Gerchman F, Giuffrida FM, Vieira JG, Velho G, Reis AF: **Association of ADIPOQ variants, total and high molecular weight adiponectin levels with coronary artery disease in diabetic and non-diabetic Brazilian subjects.***J Diabetes Complications* 2012, **26:**94–98.

11. *Global database on body mass index. BMI classification.* [http://www.who.int/bmi/index.jsp?introPage=intro_3.html].

12. **Executive summary: Standards of medical care in diabetes--2010.***Diabetes Care* 2010, **33**(Suppl 1)**:**S4–10.

13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: **Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.***Diabetologia* 1985, **28:**412–419.

14. Matsuda M, DeFronzo RA: **Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp.***Diabetes Care* 1999, **22:**1462–1470.

15. Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, Wilson AC, Folsom AR, Wu K, Benderly M,Goldbourt U, Willeit J, Kiechl S, Yarnell JW, Sweetnam PM, Elwood PC, Cushman M, Psaty BM, Tracy RP, Tybjaerg-Hansen A, Haverkate F, de Maat MP, Fowkes FG, Lee AJ, Smith FB, Salomaa V, Harald K, Rasi R, Vahtera E, Jousilahti P,*et al*: **Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis.***JAMA* 2005, **294:**1799–1809.

16. Kostapanos M, Florentin M, Elisaf MS, Mikhailidis DP: **Hemostatic factors and the metabolic syndrome.***CurrVascPharmacol* 2013, **11:**880-905.

17. Mente A, Meyre D, Lanktree MB, Heydarpour M, Davis AD, Miller R, Gerstein H, Hegele RA, Yusuf S, Anand SS: **Causal relationship between adiponectin and metabolic traits: a mendelian randomization study in a multiethnic population.***PLoS One* 2013, **8:**e66808.

18. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: **The adipocyte-secreted protein Acrp30 enhances hepatic insulin action.***Nat Med* 2001, **7:**947–953.

19. Asterholm IW, Scherer PE: **Enhanced metabolic flexibility associated with elevated adiponectin levels.***Am J Pathol* 2010, **176:**1364–1376.

20. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T, Durand JL, Li H, Li G, Jelicks LA, Mehler MF, Hui DY, Deshaies Y, Shulman GI, Schwartz GJ, Scherer PE: **Obesity-associated improvements in metabolic profile through expansion of adipose tissue.***J Clin Invest* 2007, **117:**2621–2637.

21. Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J: **Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis.***Metabolism* 2011, **60:**313–326.

22. Shetty S, Ramos-Roman MA, Cho YR, Brown J, Plutzky J, Muise ES, Horton JD, Scherer PE, Parks EJ: **Enhanced fatty acid flux triggered by adiponectin overexpression.***Endocrinology* 2012, **153:**113–122.

23. Chen MB, McAinch AJ, Macaulay SL, Castelli LA, O'Brien PE, Dixon JB, Cameron-Smith D, Kemp BE, Steinberg GR: **Impaired activation of AMP-kinase and fatty acid oxidation by globular adiponectin in cultured human skeletal muscle of obese type 2 diabetics.***J ClinEndocrinolMetab* 2005, **90:**3665–3672.

24. Debard C, Laville M, Berbe V, Loizon E, Guillet C, Morio-Liondore B, Boirie Y, Vidal H: **Expression of key genes of fatty acid oxidation, including adiponectin receptors, in skeletal muscle of Type 2 diabetic patients.***Diabetologia* 2004, **47:**917–925.

25. Fuentes E, Fuentes F, Vilahur G, Badimon L, Palomo I: **Mechanisms of chronic state of inflammation as mediators that link obese adipose tissue and metabolic syndrome.***Mediators Inflamm* 2013, **2013:**136584.

26. Chan DC, Barrett PH, Ooi EM, Ji J, Chan DT, Watts GF: **Very low density lipoprotein metabolism and plasma adiponectin as predictors of high-density lipoprotein apolipoprotein A-I kinetics in obese and nonobese men.***J ClinEndocrinolMetab* 2009, **94:**989–997.

27. Verges B, Petit JM, Duvillard L, Dautin G, Florentin E, Galland F, Gambert P: **Adiponectin is an important determinant of apoA-I catabolism.***ArterioscIerThrombVascBiol* 2006, **26:**1364–1369.

28. Clarenbach JJ, Vega GL, Adams-Huet B, Considine RV, Ricks M, Sumner AE: **Variability in postheparin hepatic lipase activity is associated with plasma adiponectin levels in African Americans.***J Investig Med* 2007, **55:**187–194.

29. Schneider JG, von Eynatten M, Schiekofer S, Nawroth PP, Dugi KA: **Low plasma adiponectin levels are associated with increased hepatic lipase activity in vivo.***Diabetes Care* 2005, **28:**2181–2186.

Table 1

Subjects' demographic, clinical and laboratory characteristics according to the presence of metabolic syndrome (MS)

	Porto Alegre cohort MS			São Paulo cohort MS		
	Absence	**Presence**	**P value[a]**	**Absence**	**Presence**	**P value[a]**
N	36	136	-	38	382	-
Female sex	28 (78%)	96 (71%)	0.531	10 (26%)	181 (47%)	0.010
Age (years)	48 ± 12	54 ± 11	0.010	59 ± 12	60 ± 10	0.647
Etnicity (% of white)	28 (78%)	114 (84%)	0.676	20 (51%)	233 (61%)	0.286
Tabagism	5 (14%)	18 (13%)	0.740	26 (67%)	302 (79%)	0.334
Habitual alcohol consumption	3 (8%)	11 (8%)	0.937	5 (13%)	10 (3%)	0.016
Physical activity			0.082			0.061
Sedentary	17 (46%)	79 (58%)		18 (46%)	111 (29%)	
Active (≥150 min per week)	19 (54%)	57 (42%)		21 (54%)	271 (71%)	
BMI (Kg/m^2)	28 ± 6	33 ± 6	-	23 ± 3	29 ± 5	-
Overweight	12 (33%)	48 (36%)	-	9 (24%)	130 (34%)	-
Obesity	12 (33%)	79 (59%)	-	1 (3%)	149 (39%)	-
Waist circumference (cm)						
Females	91.4 ± 14.2	105.5 ± 12.5	-	80.7 ± 8.0	97.8 ± 12.4	-
Males	101.1 ± 16.8	106.7 ± 11.8	-	84.9 ± 6.2	100.6 ± 1.1	-
Glucose Tolerance Status			<0.001			<0.001
Normal Glucose Tolerance	32 (89%)	16 (12%)		20 (53%)	50 (13%)	
Prediabetes	1(3%)	76 (56%)		12 (32%)	123 (31%)	
Type 2 diabetes	3 (8%)	44 (32%)		6 (16%)	209 (56%)	
Fasting plasma glucose (mg/dL)	91 ± 11	114 ± 41	-	100 ± 24	126 ± 47	-
2 h-plasma glucose (mg/dL)	111 ± 43	191 ± 81	-	-	-	-
HbA1c (%)	5.5 ± 0.6	6.4 ± 1.2	0.001	5.7 ± 0.8	6.9 ± 1.6	<0.001
HOMA-IR	1.6 (1.1 – 2.4)	3.3 (1.9 – 4.7)	0.002	0.5 (0.3-0.6)	1 (0.6-1.6)	<0.001
Total cholesterol (mg/dL)	201 ± 41	205 ± 42	0.749	272 ± 48	270 ± 54	0.861
HDL- cholesterol (mg/dL)	55 ± 13	47 ± 12	-	46 ± 12	38 ± 10	-

Triglycerides (mg/dL)	100 ± 40	162 ± 91	-	96 ± 26	163 ± 92	-
US-CRP (mg/L)	1.8 (1.5 – 4.6)	4.0 (5.4 – 8.3)	0.003	-	-	-
Fibrinogen (mg/dL)	349 (300 – 385)	384 (372 – 412)	0.107	-	-	-
Systolic Blood Pressure (mm Hg)	125 ± 19	144 ± 22	-	138 ± 24	141 ± 23	-
Diastolic Blood Pressure (mm Hg)	79 ± 11	87 ± 13	-	79 ± 15	80 ± 13	-
Systolic 24-h Blood Pressure (mm Hg)	118.4 ± 12.6	134.3 ± 15.2	0.010	-	-	-
Diastolic 24-h Blood Pressure (mm Hg)	71.5 [65 – 79.3]	79.0 [70.5 – 87.0]	0.087	-	-	-
Systolic daytime Blood Pressure (mm Hg)	121.3 ± 12.8	137.9 ± 15.1	0.010	-	-	-
Diastolic daytime Blood Pressure (mm Hg)	75.0 [68.3 – 83.0]	83.0 [75.0 – 90.0]	0.107	-	-	-
Systolic nighttime Blood Pressure (mm Hg)	112.3 ± 13.3	126.6 ± 17.0	0.023	-	-	-
Diastolic nighttime Blood Pressure (mm Hg)	65.6 ± 10.0	71.5 ± 11.9	0.114	-	-	-
Medicines:						
Antihypertensive	8 (22)	68 (52)	0.014	20 (54)	316 (86)	<0.001
Statin	3 (9)	23 (18)	0.671	11 (28)	235 (61)	<0.001
Hypoglycemic	0 (0)	0 (0)	0.998	9 (23)	85 (20)	0.854

Data expressed as absolute number (%), mean ± SD or median (P25-75). SI conversion factors: triglycerides, mg/dL x 0.01129 = mmol/L; cholesterol, HDL-cholesterol, and LDL-cholesterol, mg/dL x 0.02586 = mmol/L; glucose, mg/dL x 0.0556 = mmol/L. [a]P value for comparisons between two groups was tested by χ^2 test for categorical variables or Student's t test for continuous variables and were adjusted for age and sex by multiple logistic regression analysis. Since the sample was grouped by the presence of MS, P values were not expressed for comparison of its components (all P values were < 0.05).

Figure 1

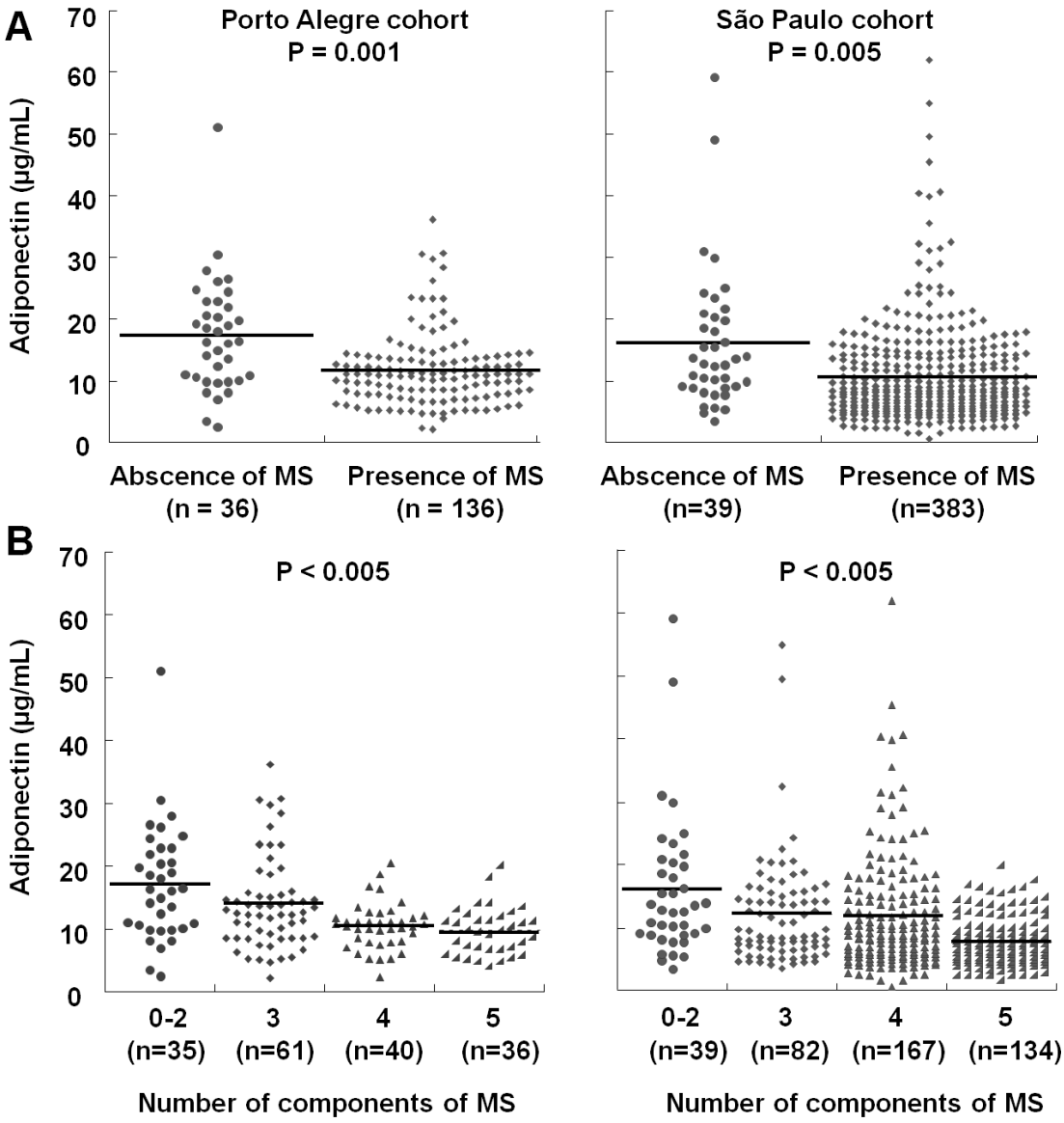


Figure 1. Adiponectin levels according to the presence of metabolic syndrome (MS). Comparison by independent T test **(A).** Adiponectin levels according to the number of components of MS. Comparison by *ANOVA* **(B)**.

Figure2

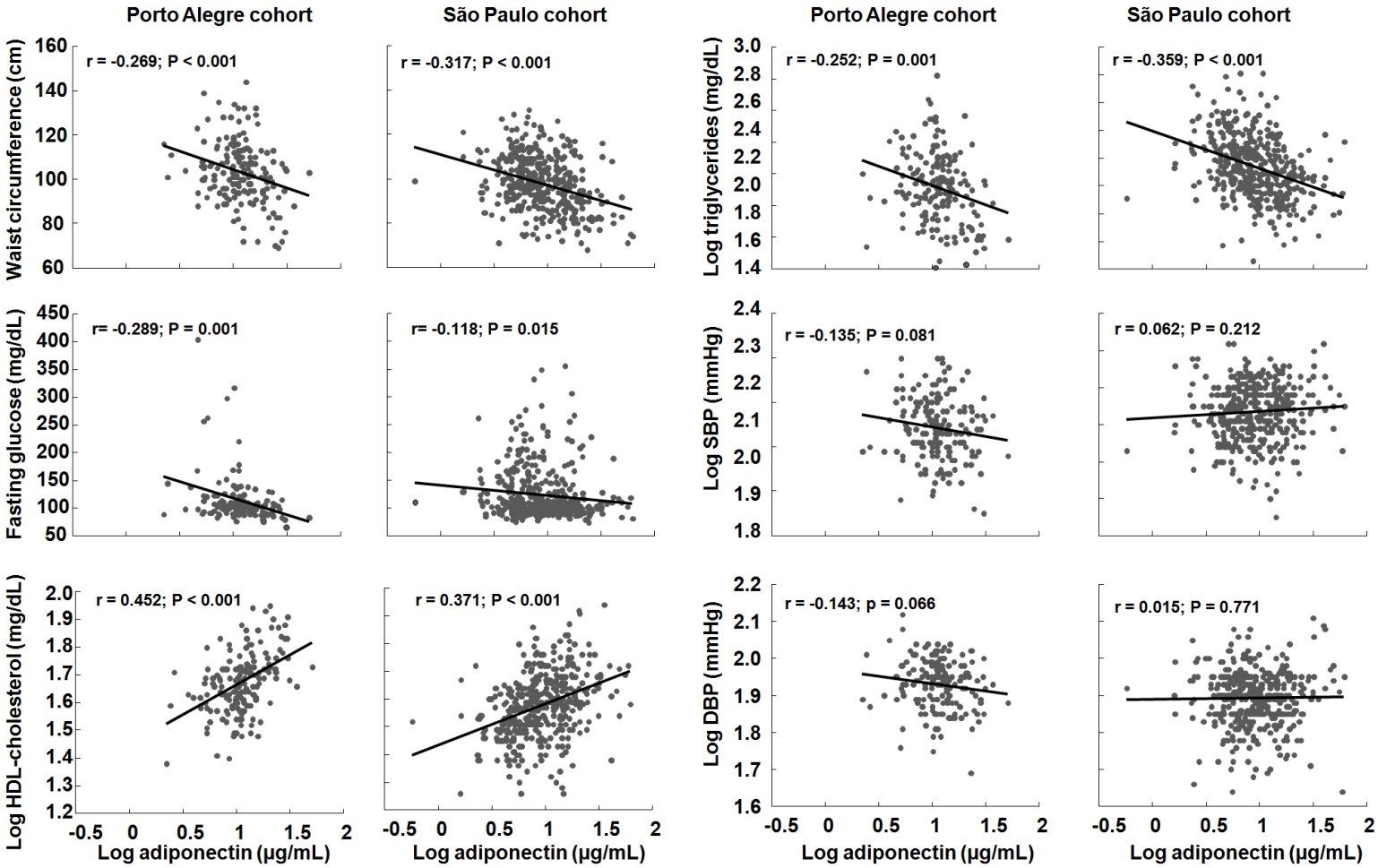


Figure 2. Relationship between adiponectin levels and metabolic syndrome criteria. DBP = diastolic blood pressure; SBP = systolic blood pressure.

CAPÍTULO 3

Effect of dietary lipids on circulating adiponectin: a systematic review with meta-analysis of randomised controlled trials

Anize D von Frankenberg [1], Flávia M Silva [1], Jussara C de Almeida [1,3], Vanessa Piccoli [1], Filipe V do Nascimento [1], Mônica M Sost [1], Cristiane B Leitão [1,3], Luciana L R Remonti [1,2], Daniel Umpierre [4], André F Reis [5], Luis H Canani [1,2,3], Mirela J de Azevedo [1,2,3], Fernando Gerchman [1,2]

[1] Post-Graduate Endocrinology Program, School of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. [2] Metabolism Unit, Endocrinology Division, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil. [3] Department of Internal Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. [4] Exercise Pathophysiology Research Laboratory, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil. [5] Endocrinology Unit, Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil.

Corresponding author: Anize D von Frankenberg

Hospital de Clínicas de Porto Alegre. Rua Ramiro Barcelos. 2350–Prédio 12. 4° andar. 90035-003. Porto Alegre. RS – Brazil. Phone/FAX+55 33598127 / 8777.

E-mail: anize.frankenberg@gmail.com

Running title: Circulating adiponectin and dietary lipid.

Key words: adiponectin, dietary lipid, n-3 polyunsaturated fatty acid, conjugated linoleic acid.

Artigo publicado na revista British Journal of Nutrition. 2014 Oct 28;112(8):1235-50.

Abstract

Different dietary interventions have been identified as potential modifiers of adiponectin concentrations, and they may be influenced by lipid intake. We identified studies investigating the effect of dietary lipids (type/amount) on adiponectin concentrations in a systematic review with meta-analysis. A literature search was conducted until July 2013 using databases such as Medline, Embase and Scopus (MeSH terms: 'adiponectin', 'dietary lipid', 'randomized controlled trials (RCT)'). Inclusion criteria were RCT in adults analysing adiponectin concentrations with modification of dietary lipids. Among the 4930 studies retrieved, fifty-three fulfilled the inclusion criteria and were grouped as follows: (1) total dietary lipid intake; (2) dietary/supplementary n-3 PUFA; (3) conjugated linoleic acid (CLA) supplementation; (4) other dietary lipid interventions. Diets with a low fat content in comparison to diets with a high-fat content were not associated with positive changes in adiponectin concentrations (twelve studies; pooled estimate of the difference in means: -0.04 (95% CI -0.82, 0.74) µg/ml). A modest increase in adiponectin concentrations with n-3 PUFA supplementation was observed (thirteen studies; 0.27 (95% CI 0.07, 0.47) µg/ml). Publication bias was found by using Egger's test (P=0.01) and funnel plot asymmetry. In contrast, CLA supplementation reduced the circulating concentrations of adiponectin compared with unsaturated fat supplementation (seven studies; -0.74 (95% CI -1.38, -0.10) µg/ml). However, important sources of heterogeneity were found as revealed by the meta-regression analyses of both n-3 PUFA and CLA supplementation. Results of new RCT would be necessary to confirm these findings.

Introduction

Adiponectin, a hormone expressed mostly in adipose tissue and encoded by the APM1 gene (chromosome 3q27), plays an important role in regulating insulin sensitivity, glucose and lipid metabolism besides its anti-inflammatory and anti-atherogenic properties (1). It has been suggested that the synthesis and secretion of adiponectin are influenced by body fat distribution, sex and ethnicity. Low levels of adiponectin are found in patients with obesity, type 2 diabetes mellitus and coronary artery disease (1,2). More recently, we also found that the presence of the metabolic syndrome and the increasing number of its components are associated with decreased adiponectin concentrations (3). Therapeutic strategies that target the metabolic syndrome and its components have been shown to increase adiponectin concentrations, such as lifestyle modification involving moderate- or high-intensity physical activities and weight loss (4,5).

Although different nutrients may affect adiponectin concentrations,it is not clear how changes in the amount and quality of macronutrients affect its concentrations (6). In one study (7) where subjects were randomised to receive hypoenergetic moderate-fat/moderate-carbohydrate v. low-fat/high-carbohydrate diets, no changes in adiponectin concentrations were observed over 10 weeks of dietary intervention. In other intervention studies, the comparison between diets with low and high fat content showed conflicting results. While adiponectin concentrations were not affected in one study (8), intake of a low-fat diet was associated with a 30% increase in the concentrations of adiponectin in another study (9). These differences probably

suggest that the quality rather than the amount of fat may have a significant influence on adiponectin concentrations. This may be exemplified by analysing the effect of a Mediterranean diet on adiponectin concentrations. Close adherence to a Mediterranean diet has been associated with higher adiponectin concentrations (10). This may be explained not only by its low glycaemic load and moderate alcohol consumption, but also by its composition that is rich in nuts, olive oil and fish, all of which are dietary sources of unsaturated fatty acids (6,11,12). As a result, these data pointed out that lipids are outstanding among potential dietary modulators of circulating adiponectin.

Other dietary lipids such as conjugated linoleic acid (CLA), dietary cholesterol and long-chain n-3 PUFA have been associated with a variable response to adiponectin concentrations (12). Regarding n-3 PUFA, a well-conducted systematic review and meta-analysis showed that its intake was associated with a significant increase in adiponectin concentrations (13). However, the results of that meta-analysis need to be interpreted with caution as a significant and unexplained heterogeneity was present between studies included in its results. Therefore, the present meta-analysis aimed to systematically review and analyse randomised controlled trials (RCT) investigating the effects of dietary lipids on circulating adiponectin concentrations in adults.

Methods

A systematic review was conducted using a predetermined protocol established according to the Cochrane Handbook's recommendations (14). Results are reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (15).

Search strategy

A literature review was conducted by searching the electronic databases Medline, Embase and Scopus until July 2013 to identify RCT that reported the effect of dietary lipids on adiponectin concentrations in adults. The initial search included the key search terms 'dietary lipid' and 'adiponectin'. It also included the entry terms associated with a high-sensitivity strategy for the search of RCT (available at http://www.sign.ac.uk/methodology/filters.html#random). The complete 'Medline' search strategy is described in the online supplementary material.

Inclusion and exclusion criteria

We included only those RCT that analysed the effect of dietary lipids on fasting concentrations of circulating total adiponectin. The outcome was changes in adiponectin concentrations from baseline to the end of the study. Studies that met the initial criteria were identified, regardless of language or publication date.

We excluded the studies that did not report the outcome, were not randomised, or included children or pregnant women. Controlled trials that analysed the interaction between dietary interventions and changes in adiponectin concentrations corresponding to different polymorphisms were also excluded if they did not report the overall outcome regardless of polymorphisms. Although studies that did not report means and standard deviations for the outcome (separately for each group at baseline and at the end of the intervention, or changes from baseline for each group) were included in the review, these studies were not included in the meta-analysis. If data necessary for the review were missing, we contacted the authors by e-mail and/or telephone. The study was excluded if the reply was not received within 4 weeks. Of the thirteen authors who were contacted, nine (16 – 24) replied back. Of these nine authors, three (16,17,24) provided the requested data to be included in the present meta-analysis.

Study selection and data extraction

For the present meta-analysis, two reviewers (A. D. v. F. and F. M. S.) independently analysed the titles and abstracts of the articles retrieved from the literature search, reviewed the full text of the published articles, and extracted the data using a standard data extraction protocol. Any disagreements between the reviewers regarding study inclusion were resolved by a third investigator (J. C. d. A. or F. G.).

The extracted data included the number of participants, study design, trial duration, and patients' demographic and anthropometric characteristics (age, sex, height, weight, BMI, presence of obesity, the metabolic syndrome, hypertension, and dyslipidaemia). Data on total energy, macronutrients (type and amount) and dietary compliance were collected from the description of the intervention and control diets. Data extracted for dietary fat composition included the following: total, saturated, monounsaturated and polyunsaturated fats (g or percentage of total energy intake); n-3 PUFA (g); n-6 PUFA (g); cholesterol (mg). However, data for n-3 and n-6 PUFA were not available in most of the included studies. Data on means and statistical dispersion for adiponectin concentrations at baseline and at the end of the study were extracted. Percentage changes in adiponectin concentrations at the end of each study were calculated for all studies that presented baseline adiponectin values.

The included studies were grouped according to the following interventions: (1) total dietary lipid intake; (2) dietary/supplementary n-3 PUFA; (3) CLA supplementation; (4) other dietary lipid interventions.

Assessment of bias and quality of studies

The quality of the studies was assessed independently by two reviewers (A. D. v. F. and F. M. S.), and any disparity was resolved by a third reviewer (J. C. d. A. or F. G.). Biases were classified into six domains: selection; performance; detection; attrition; reporting; other (15,25). The 'other' domain included the assessment of dietary compliance. The risk of bias in each domain

was classified as high, low or unclear. Regarding dietary compliance, the risk of bias was classified as 'low' if the study described the method for the assessment of dietary compliance.

Statistical analyses

Changes in adiponectin concentrations were reported as absolute differences between the values of arithmetic means and standard deviations at baseline and at the end of the study (26). Heterogeneity between studies was assessed by Cochran's Q test, and a P for trend ≤0.10 was considered statistically significant. The I^2 test was also performed to evaluate the magnitude of heterogeneity, which was considered high if $I^2 \geq 50.0$ %. Pooled estimates of the weighted mean differences (WMD) between dietary intervention and control groups were calculated using a random-effects model of DerSimonian & Laird (27) because a significant heterogeneity between the included studies was identified in preliminary models. Furthermore, this approach provides a more conservative assessment of the average effect size.

Potential sources of heterogeneity between trials were assessed by meta-regression analyses. Variables were chosen based on biological relevance before the meta-analysis was conducted. All meta-regression models included the following variables: age (less than the mean value, or equal to or greater than the mean value); sex (male, %); study location (Europe/North America v. others); time of the follow-up (equal to or less than the mean value, or greater than the mean value); BMI (<30 and ≥30 kg/m²); differences in weight

change between groups. Blinding of participants/personnel was included in the n-3 PUFA meta-analysis as a meta-regression variable. This variable was neither included in the total dietary lipid meta-analysis as blinding was not clear in all studies, nor in the CLA supplementation meta-analysis as the risk of bias was low in all the studies. Additionally, specific variables were included in the three different meta-analyses according to relevance and availability. For total dietary lipid intake, a cut-off point for the amount of lipid intake was not defined as exclusion criteria. The difference in total energy intake, total dietary lipid intake between groups (difference in total caloric intake < 1256 kJ/day *v.* > 1256 kJ/day [300 kcal/day]), median percentage point difference in lipid intake between groups (≤10% *v.* > 10% of lipid intake), and mean carbohydrate content in control groups (<30% *v.* >30% of TCI) were included in the meta-regression models. Mean carbohydrate content was analysed only in the control group because it is expected to be dependent on the differences in the amount of lipid intake between groups. For n-3 PUFA and CLA supplementation, the amount of supplementation and the type of oil used as a placebo were also considered in the meta-regression models.

Subsequently, sensitivity (subgroup) analyses were conducted by including the variables with a positive adjusted R^2 value in meta-regression analyses, to determine how much of the between-study difference could be explained by these variables.

Publication bias was assessed by funnel plot asymmetry and Begg's or Egger's tests (28 – 30). The bias was considered significant if P<0.10 (29,30). The non-parametric trim-and-fill method was used to assess the potential

influence of publication bias on sensitivity analyses, and provided a theoretical pooled estimate accounting for estimated missing studies (28).

All statistical analyses were performed using Stata 11.0 software (Stata). Significance was set at $P<0.05$, and 95% CI are quoted throughout.

Results

A total of 4930 studies were identified from the literature search (Fig. 1). On the basis of the titles and abstracts, ninety-one studies were selected for the full-text review, of which fifty-three fulfilled the final inclusion criteria. The included studies were grouped according to the following interventions: (1) total dietary lipid intake (7–9,16,18,19,31 – 39); (2) n-3 PUFA intake (21,22,24,40–55); (3) CLA supplementation (17,56–61); (4) other dietary lipid interventions(20,23,62–71). The main results of the studies included in the meta-analysis are presented in Tables 1–3, whereas those included only in qualitative analyses are presented in online supplementary Table S2.

Total dietary lipid intake

Of the total selected studies, fifteen (7–9,16,18,19,31–39) investigated the effects of a diet with a low-fat content (20–37% of energy from lipids) on the circulating concentrations of adiponectin compared with a control diet with a high fat content (35–61% of energy from lipids), as shown in Table 1. To test how differences in lipid quantity (expressed as the percentage of daily energy)

may affect adiponectin concentrations, the diet with the lowest fat content was classified as an intervention diet in each study.

The median follow-up time was 14 weeks (5 d–144 weeks). These studies included seventeen to 322 participants (mean age 50 years). Most (71·4 %) of the studies included both sexes (8,16,19,31,33–36,38,39). The mean difference in total dietary lipid intake between the intervention and control groups was 12.0% of the total energy intake. Of these studies, seven (16,31,32,35,37–39) did not describe the lipid type and four (7,18,36,38) had no information about energy consumption. Differences in energy intake were not found to be significant in most of the studies, but were statistically significant only in one study (32). Among all the other studies that did not report a statistical difference in energy intake between the intervention and control groups, two studies were found to have an energy intake difference of 2173 and 1382 kJ (519 and 330 kcal). In the first study, there were no changes and differences in adiponectin concentrations between the groups throughout the study (34), while in the other study, there was an increase in adiponectin concentrations within the groups, but not between the groups (35).

The risk of bias in the studies included in the quantitative analysis is summarised in online supplementary Table S1. The risk of selection bias was unclear in the majority of the studies, taking into account the lack of information about random sequence generation and allocation concealment. Performance bias was also unclear in all studies. Information about the blinding of outcome assessors was described in only one study (39). Regarding attrition bias, the rates of dropouts and/or withdrawals were less than 20% in nine studies

(7,9,16,18,32–34,37,38). Reporting bias was low in all studies. Dietary compliance was assessed in most studies.

Among the fifteen selected studies, twelve (7–9,16,18,31,32,34,35,37–39) reported sufficient data and were thus included in the meta-analysis. The remaining three studies (19,33,36) were excluded due to the lack of sufficient data for quantitative analysis. Among these three studies that were excluded from the quantitative analysis, one (36) showed a greater increase in adiponectin concentrations in the control group than in the intervention group, another (19) showed an increase in the concentrations of adiponectin in the control group than in the intervention group, and in the last one it was not possible to describe the differences between intervention and control groups because the results were not described separately by groups (33).

Overall, the intervention diet (28–37% of the total energy intake from fat) did not increase adiponectin concentrations compared with the control diet (39–61% of the total energy intake from fat) (WMD -0.04 (95% CI -0.82, 0.74) µg/ml; I^2 83.7 %, P for heterogeneity <0.001; Fig. 2(a)). Given the significant heterogeneity between the included studies, we performed a meta-regression analysis by including one variable per model age (adjusted R^2 = -9.6 %, *P* = 0.63), sex (adjusted R^2 = -16.5, *P* = 0.90), study location (adjusted R^2 = -9.8%, *P* = 0.61), time of follow-up (adjusted R^2 = -12.7%, *P* = 0.87), BMI (adjusted R^2 = -10.3%, *P* = 0.91), weight loss difference between intervention and control groups (adjusted R^2 = -11.1%, *P* = 0.66), differences in caloric intake between intervention and control group (adjusted R^2 = -5.7%, *P* = 0.41), percentage points difference in total dietary lipid intake between intervention and control

diets (adjusted R^2 = -15.1%, *P* = 0.65), and carbohydrate content in control group (adjusted R^2 = -16.4%, *P* = 0.88). In three studies (32,34,37), a significant change in body weight between the intervention and control groups was observed at the end of each trial. We also performed a sensitivity analysis with body weight used as a variable, which showed no significant change in the results. Publication bias was not observed in the present meta-analysis (Begg's test, P=0.89; Egger's test, P=0.21), and asymmetry was also not detected, as shown in the funnel plot (Fig. 3(a)).

n-3 PUFA intake

Of the total selected studies, nineteen analysed the effect of n-3 PUFA intake on adiponectin concentrations: sixteen (21,22,24,40 – 42,45 – 52,54,55) with n-3 PUFA supplementation and three (43,44,53) with diets composed of n-3 PUFA-rich foods. The details of these studies are summarised in Table 2. The median follow-up time was 10 weeks (3–24 weeks). These studies included twenty-six to 324 participants, and most studies (54 %) included both sexes.

Dietary composition was described in ten studies (21,22,40,41,43,44,50,51,53,54). Comparisons between intervention (diet or supplementation) and fatty acid intake from different sources (placebo) were made in twelve studies (21,22,24,40,42,45–48,51,52,55). Among the dietary intervention studies, one (43) used different amounts of n-3 PUFA from plant and marine sources, while two (44,53) used different types of fish.

The risk of selection bias was unclear in the majority of the studies, taking into account the lack of information about random sequence generation and allocation concealment. In general, performance bias was low in most studies. Information about the blinding of outcome assessors was described in only three studies (24,42,51). Attrition bias was low in ten studies (21,24,41,42,46–50,55). Reporting bias was low in all studies. Dietary compliance was analysed in the majority of the studies (online supplementary Table S1).

Of these nineteen studies, thirteen (21,22,24,40–42,46–51,55) presented the data that could be pooled and used in a meta-analysis. Different oils were used as a placebo: four studies (21,42,47,48) used olive oil; three (24,46,51) used sunola oil; one (22) used soyabean oil; one (55) used maize oil; two (41,50) used paraffin oil; one study(40) used a mixture of linoleic and oleic oil; one study(49) did not describe the oil type. Of these thirteen studies, only five (21,40,41,50,51) reported the dietary composition in both intervention and control groups. None of these studies showed any differences in total energy or in the proportion of macronutrient intake between the two groups. Furthermore, no studies described the consumption of n-3 and n-6 PUFA.

Studies that had analysed the effects of n-3 PUFA-rich foods on adiponectin concentrations (43,44,53) were not included in the quantitative analysis. In addition, one study (45) that combined n-3 PUFA intake with CLA supplementation in the intervention group, as well as two studies (52,54) in which data extraction was not available were excluded from the analysis. Among these six excluded studies (43–45,52–54), four did not show any

significant change in adiponectin concentrations at the end of the intervention (43–45,54). However, in two studies (52,53), an increase in adiponectin concentrations was observed at the end of the trial.

The pooled data from thirteen studies did show a modest and significant effect of n-3 PUFA supplementation on adiponectin concentrations (WMD 0.27 (95% CI 0.07, 0.47) µg/ml; I^2 79.6 %, P for heterogeneity <0.001; Fig. 2(b)). Given the high heterogeneity between the included studies, we performed a meta-regression analysis by including one variable per model. The independent variables were as follows: age (adjusted R^2 = -24.2, *P* = 0.16); sex (adjusted R^2 = -11.2, *P* = 0.11); study location (adjusted R^2 = -28.8% , *P* = 0.59); follow-up time (adjusted R^2 = -47.0%, *P* = 0.58); BMI (adjusted R^2 = -39.5%, *P* = 0.42); blinding of participants/personnel (adjusted R^2 = -103.0%, *P* = 0.71); amount of n-3 PUFA (g/day; adjusted R^2 = -64.8%, *P* = 0.85); EPA (adjusted R^2 = -65.9%, *P* = 0.83); DHA (adjusted R^2 = -87.0%, *P* = 0.60); fat type used as a placebo placebo (vegetable oil *v.* paraffin oil; adjusted R^2 = 100%, *P* = 0.04); change in body weight over the study period between the intervention and control groups (adjusted R^2 = -21.9%, *P* = 0.60).

Subsequently, we performed a sensitivity analysis with fat type as placebo (unsaturated oil or paraffin oil), which revealed that n-3 PUFA supplementation was still associated with an increase in adiponectin concentrations. Studies that had used unsaturated oil as placebo showed an effect of n-3 PUFA supplementation on adiponectin concentrations (WMD 0.23 (95% CI 0.04, 0.42) µg/ml; I^2 40.2 %, P for heterogeneity=0.09) as well as those that had used paraffin oil as placebo (WMD 1.19 (95% CI 0.24, 2.13) µg/ml; I^2

39.5 %, P for heterogeneity=0.20). Studies that had used paraffin oil as placebo showed a greater increase (0.96 µg/ml) in adiponectin concentrations than those that had used vegetable oils as placebo.

Significant evidence of publication bias was found by Egger's test (P=0.01) but not by Begg's test (P=0.95). Visual inspection of the funnel plot confirmed the existence of asymmetry (Fig. 3(b)). In fact, a theoretical pooled estimate of 0.08 (95% CI -0.13, 0.30) µg/ml (P=0.46) was obtained by using the trim-and-fill correction method after the addition of six theoretically unreported studies.

Conjugated linoleic acid supplementation

Of the total selected studies, seven (17,56–61) assessed the effect of CLA (mixture containing cis-9, trans-11 and trans-10, cis-12) supplementation on adiponectin concentrations. The median follow-up time was 13.0 weeks (8–24 weeks). These studies included twenty-eight to eighty participants, aged 18 to 80 years. The details of these studies are summarized in Table 3.

In most studies, CLA supplementation (intervention) was compared with unsaturated fatty acid supplementation (placebo) such as olive oil (56,57,60), safflower oil (58,61) or soyabean oil (17). Only one study (59) compared CLA supplementation with saturated fatty acid intake (placebo, mixture of fatty acids in capsules). The median CLA supplementation was 4.1 (range 3.0–8.0) g/d, with an equal mix of the two predominant isomers. Only two studies (58,59) described the dietary composition.

The risk of selection bias was unclear in the majority of the studies, taking into account the lack of information about random sequence generation and allocation concealment. Performance bias was low in most studies. Information about the blinding of outcome assessors was described in only two studies (58,61). Attrition bias was low in five (17,56,57,59,60) out of seven studies (17,56–61). Reporting bias was low in all studies. Dietary compliance was analysed in the majority of the studies (online supplementary Table S1).

All these seven studies (17, 56–61) were pooled in the meta-analysis. The pooled data did not show any significant effect of CLA supplementation on circulating adiponectin concentrations (WMD -0.18 (95% CI -0.84, 0.48) μg/ml; I^2 97.7 %, P for heterogeneity <0.001; Fig. 2(c)). A high level of heterogeneity was detected. The visual inspection of the funnel plot revealed the existence of asymmetry (Fig. 3(c)), suggesting a publication bias, although neither Begg's test (P=0.76) nor Egger's test (P=0.48) showed any evidence of the same. In fact, a theoretical pooled estimate of -0.64 (95% CI -1.83 to 0.55) μg/ml (P=0.29) was obtained by using the trim-and-fill correction method after the addition of one theoretically unreported study.

Given the significant heterogeneity between the included studies, we performed a meta-regression analysis by including one variable per model: age (adjusted R^2 = -21.1, *P* = 0.75); sex (adjusted R^2 = -5.6, *P* = 0.44); study location (adjusted R^2 = 41.9% , *P* = 0.07); time of follow-up (R^2 = 29.5%, *P* = 0.13), BMI (adjusted R^2 = 11.6%, *P* = 0.23); change in body weight over the study period between the intervention and control groups (adjusted R^2 = -9.0%, *P* = 0.53); amount of CLA supplementation (< 4.8 g/d *v.* > 4.8 g/d; adjusted R^2 =

11.6%, *P* = 0.23), and fat type used as placebo (unsaturated *v.* saturated fat; adjusted R^2 = 56.0%, *P* = 0.02).

Subsequently, a sensitivity analysis was performed with fat type used as a placebo (unsaturated or saturated fat). The analysis revealed a reduction in adiponectin concentrations with CLA supplementation after the removal of one study that had used saturated fat as placebo (WMD -0.74 (95% CI -1.38, -0.10) µg/ml; I^2 97.3 %, P for heterogeneity <0.001). However, the analysis showed a high level of heterogeneity among the studies that used unsaturated fat as placebo.

Other dietary lipid interventions

Among the total selected studies, three analysed the effect of fatty acid intake on adiponectin concentrations (saturated fat(63), a-lipoic acid (69) and n-6 PUFA (68)) and nine analysed the effect of the food source of lipids on adiponectin concentrations (eggs (64), partially-hydrogenated oil (20,65), nuts (66,67,71) and flaxseed (23,62,70)). The details of these studies are summarized in online supplementary Table S2. The median follow-up time was 9 weeks (4 d–48 weeks). These studies included fifteen to 160 participants, aged 20 to 80 years. However, these studies were not included in the meta-analysis due to the variability in dietary intervention.

A high consumption of saturated fat (63), n-6 PUFA (68) and a-lipoic acid (69) did not show a significant effect on adiponectin concentrations. In contrast, intake of eggs increased the circulating concentrations of adiponectin (64).

Flaxseed intake reduced adiponectin concentrations in one study (62), but did not change its concentrations in other two studies (23,70). Intake of nuts increased adiponectin concentrations in two studies (66,67), with no effect being found in one study (71).

Discussion

The present systematic review with meta-analysis analysed how different types or amounts of dietary lipids affect circulating adiponectin concentrations. Intervention studies that compared diets with low and high fat content were not associated with any differences in adiponectin concentrations. However, it was observed that n-3 PUFA supplementation modestly increased the circulating concentrations of adiponectin, whereas CLA supplementation reduced the concentrations when compared with unsaturated fatty acid supplementation used as an active placebo.

In the present meta-analysis, a difference of 18·0% of energy intake from total lipids between the intervention and control groups was not associated with changes in adiponectin concentrations, corroborating the idea that the quality of fat, rather than its amount, might have a more important role in modulating the concentrations of adiponectin. Although we found a high level of heterogeneity between the studies included in the present meta-analysis, this could not be explained by any factor in the exploratory analysis. Differences in carbohydrate content between the low-fat and high-fat dietary arms could also have an impact on adiponectin concentrations. We also performed a meta-regression analysis

by including the differences in carbohydrate content between the study arms; however, this could not explain the high level of heterogeneity found between the included studies. In addition, differences in carbohydrate content may affect insulin resistance, which is a potential modifier of adiponectin concentrations (33). However, it was unlikely to explore the aspects associated with insulin resistance due to the lack of data in most studies.

The protective effect of high intake of oily fish on the risk of type 2 diabetes has been demonstrated in a recent metaanalysis (53). Improvement in insulin sensitivity resulting from the intake of n-3 PUFA has been shown to be strongly associated with the increase in adiponectin concentrations. In fact, the utilisation of EPA and DHA in the culture medium of human and rat adipocytes increased the synthesis and secretion of adiponectin by the activation of PPARg that acts as an insulin sensitizer (72). In the present meta-analysis, n-3 PUFA supplementation modestly increased the circulating concentrations of adiponectin, suggesting the beneficial effect of this supplementation on adipocyte metabolism. Additionally, the well-known effect of n-3 PUFA intake on reducing TAG and increasing HDL-cholesterol levels (73) may be partially associated with its effect on adiponectin secretion, which also improves lipid metabolism through the modulation of insulin sensitivity and fatty acid oxidation (74).

In contrast to the study of Wu et al. (13), we found a possible explanation for the heterogeneity identified in the meta-analysis of n-3 PUFA supplementation. While updating the results published by Wu et al. (13) by the addition of three studies (50,51,55), we showed using the meta-regression

analysis that the type of the placebo oil (vegetable oil v. paraffin oil) could explain part of the heterogeneity found between the studies included in the meta-analysis. Studies that had used paraffin oil as placebo showed a greater increase in adiponectin concentrations than those that had used vegetable oils as placebo. We believe that the biological effect promoted by vegetable oils used as a placebo could reduce the difference in adiponectin concentrations between the intervention and control groups. Interestingly, even after grouping only those studies that used vegetable oils as placebo, the effect of n-3 PUFA intake remains to be significantly associated with an increase in adiponectin concentrations. However, it is likely that studies with negative results were not published. The inclusion of the studies that were not published would probably reduce the effect of n-3 PUFA intake on adiponectin concentrations, as we have already found. Therefore, caution needs to be exercised in the interpretation of the effect of n-3 PUFA intake on adiponectin concentrations.

CLA fatty acids are lipids derived from fatty tissues of ruminant animals. Some studies suggested that either the trans-10, cis-12 or cis-9, trans-11 isomer increased insulin resistance, but not a mixture of both isomers (56,75). Additionally, it was found that supplementation of the trans-10, cis-12 isomer increases C-reactive protein, a well-defined marker of subchronic inflammation associated with insulin resistance, but not the supplementation of isomeric mixture (75). A commercially prepared oil contains a 50:50 mixture of the trans-10, cis-12 and cis-9, trans-11 isomers. All studies included in the present meta-analysis assessed the effect of CLA oil as a mixture containing the cis-9, trans-11 and trans-10, cis-12 isomers compared with placebo. Although we showed

no changes in adiponectin concentrations with CLA v. placebo supplementation, data from the sensitivity analysis suggested that CLA supplementation resulted in a reduction of circulating adiponectin concentrations when compared with unsaturated fat supplementation (17,56–58,60,61). This result could be attributed to the antioxidant properties of unsaturated fatty acids that might be more effective in modulating the concentrations of adiponectin (61). The high level of heterogeneity found between these studies could not be explained by BMI, the amount of CLA supplementation, and the change in body weight over the study period between the intervention and control groups. However, we found the role of blinding of subjects/personnel to be significant in explaining this heterogeneity. As a result, we should be cautious in concluding that there is no effect of CLA supplementation on adiponectin concentrations. Further intervention studies should address the role of CLA as a dietary supplement as well as the mechanisms by which CLA acts to regulate vital steps in the modulation of insulin sensitivity and adiponectin metabolism. Although other types of fatty acid interventions (diet or supplementation) were identified, they were not included in the meta-analysis due to the lack of sufficient studies. Our data suggest that the consumption of nuts (66,67), but not flaxseed (23,62,70), is associated with increasing adiponectin concentrations; however, this effect needs to be further explored in RCT.

Although the literature search was conducted using multiple databases and was not restricted to the English language, the present meta-analysis has some limitations. First, despite several attempts to contact the authors of the published articles that had missing data by e-mail or telephone, some studies

were excluded from the meta-analysis due to the delay in response. Second, funnel plot asymmetry was apparent with n-3 PUFA and CLA supplementation and may, in part, explain the heterogeneity found between the studies included in the present meta-analysis. To better understand this issue, we performed meta-regression and sensitivity analyses. These analyses revealed that the type of oil used as a placebo (paraffin oil or vegetable oil) in the studies that had used n-3 PUFA supplementation could explain part of the heterogeneity found in the present meta-analysis. Third, the lack of data on the actual consumption of n-3 PUFA has to be taken into account because it may have an influence on adiponectin concentrations. Fourth, differences in dietary composition between the control and intervention groups were not analysed because most of the included studies had limited data, hindering the analysis of the content of other dietary components, such as n-3 PUFA, n-6 PUFA, fibre and whole grains, that have been shown to affect adiponectin concentrations. Fifth, as complete data about the presence of diabetes and the metabolic syndrome, being associated with decreased adiponectin concentrations, were not identified in most of the studies included in the meta-analysis, the results of dietary intervention on subjects with and without them may distinctly affect its concentrations. Lastly, none of the studies included in the meta-analysis presented intention-to-treat analysis, a statistical approach that is usually associated with more conservative results (76).

In conclusion, the present systematic review with meta-analysis of RCT suggests that, among the different interventions on dietary lipid intake, intake of low-fat diets were not associated with differences in adiponectin concentrations.

n-3 PUFA supplementation was associated with moderate increases in adiponectin concentrations, whereas CLA supplementation seemed to be associated with a decrease in adiponectin concentrations compared with unsaturated fat intake. Caution needs to be exercised in interpreting these results because important sources of heterogeneity were found in the meta-analyses of n-3 PUFA and CLA supplementation. Therefore, future RCT are necessary to confirm these findings.

Acknowledgements

We thank Dr Jason H. Y. Wu for his critical suggestions. We thank authors Matthias Blu¨her, Reza Rastmanesh and Marius Troseid, all of whom provided additional data for the meta-analysis. We also thank authors Charlotte Brons, Krista Varady, Nathalie Bendsen, Francesco Sofi, Sidika Karakas and Carla Taylor, all of whom answered our questions by e-mail.

The present study was supported by the Foundation for Research Support of the State of Rio Grande do Sul (FAPERGS), Hospital de Cl´nicas de Porto Alegre Research Fund (FIPE 11-226), the National Council of Technological and Scientific Development (CNPq). F. G. was supported by a scholarship from the International Scholarship Program of the Endocrine Society. The funding sources had no role in the design or conduct of this meta-analysis.

The authors' contributions are as follows: A. D v. F., F. M. S., J. C. d. A. and F. G. designed and performed the study; A. D. v. F., F. M. S., F. G., C. B. L., L. L. R. R. and D. U. analysed the data; A. D. v. F., F. M. S., F. V. d. N., V. P. and F. G. wrote the paper. All the other authors critically reviewed and improved the manuscript.

None of the authors has any conflict of interest to declare.

References

1. Ahima RS (2006) Metabolic actions of adipocyte hormones: focus on adiponectin. Obesity (Silver Spring) 14, Suppl. 1, 9S–15S.

2. Schulze MB, Shai I, Rimm EB, et al. (2005) Adiponectin and future coronary heart disease events among men with type 2 diabetes. Diabetes 54, 534–539.

3. von Frankenberg AD, do Nascimento FV, Gatelli LE, et al. (2014) Major components of metabolic syndrome and adiponectin levels: a cross-sectional study. Diabetol Metab Syndr 6, 26.

4. Rossmeislova L, Malisova L, Kracmerova J, et al. (2013) Weight loss improves the adipogenic capacity of human preadipocytes and modulates their secretory profile. Diabetes 62, 1990–1995.

5. Simpson KA & Singh MA (2008) Effects of exercise on adiponectin: a systematic review. Obesity (Silver Spring) 16, 241–256.

6. Mantzoros CS, Williams CJ, Manson JE, et al. (2006) Adherence to the Mediterranean dietary pattern is positively associated with plasma adiponectin concentrations in diabetic women. Am J Clin Nutr 84, 328–335.

7. Arvidsson E, Viguerie N, Andersson I, et al. (2004) Effects of different hypocaloric diets on protein secretion from adipose tissue of obese women. Diabetes 53, 1966–1971.

8. Keogh JB, Brinkworth GD, Noakes M, et al. (2008) Effects of weight loss from a very-low-carbohydrate diet on endothelial function and markers of

cardiovascular disease risk in subjects with abdominal obesity. Am J Clin Nutr 87, 567–576.

9. Wycherley TP, Brinkworth GD, Keogh JB, et al. (2010) Longterm effects of weight loss with a very low carbohydrate and low fat diet on vascular function in overweight and obese patients. J Intern Med 267, 452–461.

10. Trichopoulou A, Costacou T, Bamia C, et al. (2003) Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 348, 2599–2608.

11. Qi L, Rimm E, Liu S, et al. (2005) Dietary glycemic index, glycemic load, cereal fiber, and plasma adiponectin concentration in diabetic men. Diabetes Care 28, 1022–1028.

12. Silva FM, de Almeida JC & Feoli AM (2011) Effect of diet on adiponectin levels in blood. Nutr Rev 69, 599–612.

13. Wu JH, Cahill LE & Mozaffarian D (2013) Effect of fish oil on circulating adiponectin: a systematic review and metaanalysis of randomized controlled trials. J Clin Endocrinol Metab 98, 2451–2459.

14. The Cochrane Collaboration (2011) Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0. www.cochrane-handbook.org

15. Liberati A, Altman DG, Tetzlaff J, et al. (2009) The PRISMA statement for reporting systematic reviews and metaanalyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol 62, e1–34.

16. Bluher M, Rudich A, Kloting N, et al. (2012) Two patterns of adipokine and other biomarker dynamics in a long-term weight loss intervention. Diabetes Care 35, 342–349.

17. Shademan Z, Rastmanesh R & Hedayati M (2011) Effect of conjugated linoleic acid on serum leptin, adiponectin and body composition in overweight type II diabetic patients. Kowsar Med J 16, 101–107.

18. Brons C, Jensen CB, Storgaard H, et al. (2009) Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. J Physiol 587, 2387–2397.

19. Varady KA, Bhutani S, Klempel MC, et al. (2011) Improvements in vascular health by a low-fat diet, but not a highfat diet, are mediated by changes in adipocyte biology. Nutr J 10, 8.

20. Bendsen NT, Stender S, Szecsi PB, et al. (2011) Effect of industrially produced trans fat on markers of systemic inflammation: evidence from a randomized trial in women. J Lipid Res 52, 1821–1828.

21. Sofi F, Giangrandi I, Cesari F, et al. (2010) Effects of a 1-year dietary intervention with n-3 polyunsaturated fatty acidenriched olive oil on non-alcoholic fatty liver disease patients: a preliminary study. Int J Food Sci Nutr 61,792–802.

22. Vargas ML, Almario RU, Buchan W, et al. (2011) Metabolic and endocrine effects of long-chain versus essential omega-3 polyunsaturated fatty acids in polycystic ovary syndrome.Metabolism 60, 1711–1718.

23. Taylor CG, Noto AD, Stringer DM, et al. (2010) Dietary milled flaxseed and flaxseed oil improve N-3 fatty acid status and do not affect glycemic control in individuals with well-controlled type 2 diabetes. J Am Coll Nutr 29, 72–80.

24. Troseid M, Arnesen H, Hjerkinn EM, et al. (2009) Serum levels of interleukin-18 are reduced by diet and n-3 fatty acid intervention in elderly high-risk men. Metabolism 58, 1543–1549.

25. Higgins JPT & Green S (2011) Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [Updated March 2011]. http://www.cochrane-handbook.org (accessed 16 April 2014)

26. Follmann D, Elliott P, Suh I, et al. (1992) Variance imputation for overviews of clinical trials with continuous response. J Clin Epidemiol 45, 769–773.

27. DerSimonian R & Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7, 177–188.

28. Peters JL, Sutton AJ, Jones DR, et al. (2007) Performance of the trim and fill method in the presence of publication bias and between-study heterogeneity. Stat Med 26, 4544–4562.

29. Begg CB & Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics 50, 1088–1101.

30. Egger M, Davey Smith G, Schneider M, et al. (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629–634.

31. Cardillo S, Seshadri P & Iqbal N (2006) The effects of a low-carbohydrate versus low-fat diet on adipocytokines in severely obese adults: three-year follow-up of a randomized trial. Eur Rev Med Pharmacol Sci 10, 99–106.

32. Ng TW, Watts GF, Barrett PH, et al. (2007) Effect of weight loss on LDL and HDL kinetics in the metabolic syndrome: associations with changes in plasma retinol-binding protein-4 and adiponectin levels. Diabetes Care 30, 2945–2950.

33. Keogh JB, Brinkworth GD & Clifton PM (2007) Effects of weight loss on a low-carbohydrate diet on flow-mediated dilatation, adhesion molecules and adiponectin. Br J Nutr 98, 852–859.

34. Al-Sarraj T, Saadi H, Calle MC, et al. (2009) Carbohydrate restriction, as a first-line dietary intervention, effectively reduces biomarkers of metabolic syndrome in Emirati adults. J Nutr 139, 1667–1676.

35. Vetter ML, Wade A, Womble LG, et al. (2010) Effect of a lowcarbohydrate diet versus a low-fat, calorie-restricted diet on adipokine levels in obese, diabetic participants. Diabetes Metab Syndr Obes 3, 357–361.

36. Yeung EH, Appel LJ, Miller ER, et al. (2010) The effects of macronutrient intake on total and high-molecular weight adiponectin: results from the OMNI-Heart trial. Obesity (Silver Spring) 18, 1632–1637.

37. Summer SS, Brehm BJ, Benoit SC, et al. (2011) Adiponectin changes in relation to the macronutrient composition of a weight-loss diet. Obesity 19, 2198–2204.

38. Heggen E, Klemsdal TO, Haugen F, et al. (2012) Effect of a low-fat versus a low-glycemic-load diet on inflammatory biomarker and adipokine concentrations. Metab Syndr Relat Disord 10, 437–442.

39. Rajaie S, Azadbakht L, Saneei P, et al. (2012) The effect of moderate substitution of dietary carbohydrates by fats on serum levels of adipocytokines, inflammatory indices, and biomarkers of endothelial function among women with metabolic syndrome. J Zanjan Univer Med Sci Health Services 20.

40. Krebs JD, Browning LM, McLean NK, et al. (2006) Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women. Int J Obes (Lond) 30, 1535–1544.

41. Kabir M, Skurnik G, Naour N, et al. (2007) Treatment for 2mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. Am J Clin Nutr 86, 1670–1679.

42. Damsgaard CT, Frokiaer H, Andersen AD, et al. (2008) Fish oil in combination with high or low intakes of linoleic acid lowers plasma triacylglycerols but does not affect other cardiovascular risk markers in healthy men. J Nutr 138, 1061–1066.

43. Kratz M, Swarbrick MM, Callahan HS, et al. (2008) Effect of dietary n-3 polyunsaturated fatty acids on plasma total and high-molecular-weight adiponectin concentrations in overweight to moderately obese men and women. Am J Clin Nutr 87, 347–353.

44. Ramel A, Martinez A, Kiely M, et al. (2008) Beneficial effects of long-chain n-3 fatty acids included in an energy-restricted diet on insulin resistance in overweight and obese European young adults. Diabetologia 51, 1261–1268.

45. Sneddon AA, Tsofliou F, Fyfe CL, et al. (2008) Effect of a conjugated linoleic acid and omega-3 fatty acid mixture on body composition and adiponectin. Obesity (Silver Spring) 16, 1019–1024.

46. Micallef MA & Garg ML (2009) Anti-inflammatory and cardioprotective effects of n-3 polyunsaturated fatty acids and plant sterols in hyperlipidemic individuals. Atherosclerosis 204, 476–482.

47. Rizza S, Tesauro M, Cardillo C, et al. (2009) Fish oil supplementation improves endothelial function in normoglycemic offspring of patients with type 2 diabetes. Atherosclerosis 206, 569–574.

48. Gammelmark A, Madsen T, Varming K, et al. (2012) Low dose fish oil supplementation increases serum adiponectin without affecting inflammatory markers in overweight subjects. Nutr Res 32, 15–23.

49. Koh KK, Quon MJ, Shin KC, et al. (2012) Significant differential effects of omega-3 fatty acids and fenofibrate in patients with hypertriglyceridemia. Atherosclerosis 220, 537–544.

50. Mohammadi E, Rafraf M, Farzadi L, et al. (2012) Effects of omega-3 fatty acids supplementation on serum adiponectin levels and some metabolic risk factors in women with polycystic ovary syndrome. Asia Pac J Clin Nutr 21, 511–518.

51. Munro IA & Garg ML (2012) Dietary supplementation with n-3 PUFA does not promote weight loss when combined with a very-low-energy diet. Br J Nutr 108, 1466–1474.

52. Simão ANC, Lozovoy MAB, Bahls LD, et al. (2012) Blood pressure decrease with ingestion of a soya product (kinako) or fish oil in women with the metabolic syndrome: role of adiponectin and nitric oxide. Br J Nutr 108, 1435–1442.

53. Zhang J, Wang C, Li L, et al. (2012) Dietary inclusion of salmon, herring and pompano as oily fish reduces CVD risk markers in dyslipidaemic middle-aged and elderly Chinese women. Br J Nutr 108, 1455–1465.

54. Guebre-Egziabher F, Debard C, Drai J, et al. (2013) Differential dose effect of fish oil on inflammation and adipose tissue gene expression in chronic kidney disease patients. Nutrition 29, 730–736.

55. Spencer M, Finlin BS, Unal R, et al. (2013) Omega-3 fatty acids reduce adipose tissue macrophages in human subjects with insulin resistance. Diabetes 62, 1709–1717.

56. Risérus U, Vessby B, Arner P, et al. (2004) Supplementation with trans10cis12-conjugated linoleic acid induces hyperproinsulinaemia in obese men: close association with impaired insulin sensitivity. Diabetologia 47, 1016–1019.

57. Syvertsen C, Halse J, Hoivik HO, et al. (2007) The effect of 6 months supplementation with conjugated linoleic acid on insulin resistance in overweight and obese. Int J Obes (Lond) 31, 1148–1154.

58. Norris LE, Collene AL, Asp ML, et al. (2009) Comparison of dietary conjugated linoleic acid with safflower oil on body composition in obese postmenopausal women with type 2 diabetes mellitus. Am J Clin Nutr 90, 468–476.

59. Zhao WS, Zhai JJ, Wang YH, et al. (2009) Conjugated linoleic acid supplementation enhances antihypertensive effect of ramipril in Chinese patients with obesity-related hypertension. Am J Hypertens 22, 680–686.

60. MacRedmond R, Singhera G, Attridge S, et al. (2010) Conjugated linoleic acid improves airway hyper-reactivity in overweight mild asthmatics. Clin Exp Allergy 40, 1071–1078.

61. Joseph SV, Jacques H, Plourde M, et al. (2011) Conjugated linoleic acid supplementation for 8 weeks does not affect body composition, lipid profile, or safety biomarkers in overweight, hyperlipidemic men. J Nutr 141, 1286–1291.

62. Nelson TL, Stevens JR & Hickey MS (2007) Adiponectin levels are reduced, independent of polymorphisms in the adiponectin gene, after supplementation with alpha-linolenic acid among healthy adults. Metabolism 56, 1209–1215.

63. Lithander FE, Keogh GF, Wang Y, et al. (2008) No evidence of an effect of alterations in dietary fatty acids on fasting adiponectin over 3 weeks. Obesity (Silver Spring) 16, 592–599.

64. Ratliff JC, Mutungi G, Puglisi MJ, et al. (2008) Eggs modulate the inflammatory response to carbohydrate restricted diets in overweight men. Nutr Metab (Lond) 5, 6.

65. Vega-Lopez S, Matthan NR, Ausman LM, et al. (2009) Substitution of vegetable oil for a partially-hydrogenated fat favorably alters cardiovascular disease risk factors in moderately hypercholesterolemic postmenopausal women. Atherosclerosis 207, 208–212.

66. Kalgaonkar S, Almario RU, Gurusinghe D, et al. (2011) Differential effects of walnuts vs almonds on improving metabolic and endocrine parameters in PCOS. Eur J Clin Nutr 65, 386–393.

67. Aronis KN, Vamvini MT, Chamberland JP, et al. (2012) Shortterm walnut consumption increases circulating total adiponectin and apolipoprotein A concentrations, but does not affect markers of inflammation or vascular injury in obese humans with the metabolic syndrome: data from a doubleblinded, randomized, placebo-controlled study. Metabolism 61, 577–582.

68. Bjermo H, Iggman D, Kullberg J, et al. (2012) Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. Am J Clin Nutr 95, 1003–1012.

69. Manning PJ, Sutherland WH, Williams SM, et al. (2012) The effect of lipoic acid and vitamin E therapy in individuals with metabolic syndrome. Nutr Metab Cardiovasc Dis 23, 543–549.

70. Kontogianni MD, Vlassopoulos A, Gatzieva A, et al. (2013) Flaxseed oil does not affect inflammatory markers and lipid profile compared to olive oil, in young, healthy, normal weight adults. Metabolism 62, 686–693.

71. Somerset SM, Graham L & Markwell K (2013) Isoenergetic replacement of dietary saturated with monounsaturated fat via macadamia nuts enhances endothelial function in overweight subjects. e-SPEN J 8, e113–e119.

72. Banga A, Unal R, Tripathi P, et al. (2009) Adiponectin translation is increased by the PPARg agonists pioglitazone and omega-3 fatty acids. Am J Physiol Endocrinol Metab 296, E480–E489.

73. Bernstein AM, Ding EL, Willett WC, et al. (2012) A meta-analysis shows that docosahexaenoic acid from algal oil reduces serum triglycerides and increases HDL-cholesterol and LDL-cholesterol in persons without coronary heart disease. J Nutr 142, 99–104.

74. Karbowska J & Kochan Z (2006) Role of adiponectin in the regulation of carbohydrate and lipid metabolism. J Physiol Pharmacol 57, Suppl. 6, 103–113.

75. Riserus U, Vessby B, Arnlov J, et al. (2004) Effects of cis-9, trans-11 conjugated linoleic acid supplementation on insulin sensitivity, lipid peroxidation, and proinflammatory markers in obese men. Am J Clin Nutr 80, 279–283.

76. Hollis S & Campbell F (1999) What is meant by intention to treat analysis? Survey of published randomised controlled trials. BMJ 319, 670–674.

77. Moher D, Liberati A, Tetzlaff J, et al. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. PLoS Med 6, e1000097.

TABLE 1
Characteristics of the studies investigating changes in adiponectin concentrations by modifying the amount of total dietary lipid intake

Author Year Reference	Study design Follow-up	Sample	Dietary Intervention and Control groups	Dietary composition (%): fat:carbohydrate:protein; total energy intake	Difference in percentage points of lipid intake between groups	Changes on adiponectin µg/mL (% of change)
Arvidsson 2004 [7]	parallel 10 weeks	80 obese women 21-49 years BMI (kg/m²) 30.9-47.7	**I:** Low-fat diet **C:** High-fat diet	**I:** 27:52:21 **C:** 41:39:20 Hypocaloric diets (- 500 kcal/d) without difference between them Ratio of saturated to monounsaturated to polyunsaturated fatty acids 2:2:1	14	**I:** 2.3 ± 2.18 (↑12.6%) **C:** 0.5 ± 1.67 (↑3.1%)
Cardillo 2006 [31]	parallel 144 weeks	132 subjects 55 ± 10 years 8.03% males BMI (kg/m²) >35	**I:** Lower fat diet **C:** Low-carbohydrate diet	**I:** 32:47:20 1940 kcal **C:** 42:39:19; 1940 kcal Total energy intake did not differ between I e C groups	10	**I:** -1.1 ± 12.5 **C:** -6.68 ± 34.8 (% of change NA)
Ng 2007 [32]	parallel 16 weeks	35 males with MetS Age not reported BMI (kg/m²) 35.2 ± 1.0	**I:** Lower fat diet **C:** Weight-maintenance diet	**I:** 25:55:20; 1668 kcal **C:** 35:40:20; 2327 kcal Total energy intake did differ between I e C groups	10	**I:** 0.7 ± 0.3* (↑17.9%) **C:** 0.1 ± 0.4 (↑2.9%)
Keogh 2008 [8]	parallel 8 weeks	107 subjects 24 - 64 years Gender not reported BMI (kg/m²) 27- 44	**I:** Low-fat, low-SFA diet **C:** High-fat, high SFA diet	**I:** 30:46:24; 1433 kcal (<8.0% SFA) **C:** 61:4:35; 1433 kcal (20.0% SFA) Total energy intake did not differ between I e C groups	31	**I:** 0.4 ± 2.26 (↑7.6%) **C:** 0.3 ± 2.16 (↑5.0%)
Al-Sarraj 2009 [34]	parallel 6 weeks	39 subjects with MetS 18-50 years 36.0% males	**I:** Lower fat diet (AHA) **C:** Low-carbohydrate diet	**I:** 28:53:19; 2046 kcal **C:** 48:25;28; 2565 kcal (mainly MUFA and PUFA, with restriction of SFA)	20	**I:** 0.04 ± 3.95 (↑0.04%) **C:** -0.42 ± 4.22 (↓3.9%)

		BMI (kg/m²) 38.7 ± 7.6		Total energy intake did not differ between I e C groups		
Brons 2009 [18]	crossover 5 days (7 weeks washout)	26 males 30-31 years BMI (kg/m²) 23.4 ± 2.4	**I:** Lower fat diet **C:** High-fat, high-calorie diet	**I:** 35:50:15; calories not reported; 1/3 MUFA, 1/3PUFA,1/3 SFA **C:** 50.0% extra energy as 60:33:8; calories not reported	8	**I:** 7.61 ± 3.9* **C:** 8.63 ± 4.3*† (% of change NA)
Wycherley 2009 [9]	parallel 52 weeks	49 subjects with MetS 50 ± 1.1 years 34.7% males BMI (kg/m²) 33.7 ± 0.6	**I:** Lower fat diet **C:** High-fat diet	**I:** 30:46:24; 1536 kcal (<8.0% SFA) **C:** 61:4:35; 1598 kcal (20.0% SFA) Total energy intake did not differ between I e C groups	31	**I:** 4.6 ± 1.9* (↑29.9%) **C:** 2.8 ± 2.8* (↑17.8%)
Vetter 2010 [35]	parallel 24 weeks	144 patients with type 2 DM 60.84 ± 10.13 years 48.6% males BMI (kg/m²) 38.2 ± 6.0	**I:** Low-fat, calorie-restricted diet **C:** Low-carbohydrate, *ad libitum* diet	**I:** 37:44:22; 1587 kcal **C:** 44:33:18; 1917 kcal Total energy intake did not differ between I e C groups	7	**I:** 2.6 ± 8.4 * (↑16.3%) **C:** 4.3 ± 14.6 * (↑32.1%)
Summer 2011 [37]	parallel 24 weeks	81 females 35-50 years BMI (kg/m²) 30 – 35	**I:** Lower fat diet (AHA) **C:** Low-carbohydrate *ad libitum* diet (Atkins)	**I:** 31:50:19; 1342 kcal **C:** 49:27:24; 1405 kcal Total energy intake did not differ between I e C groups	18	**I:** 0.9 ± 1.4 (↑9.8%) **C:** 1.9 ± 1.4* (↑19.0%)
Blüher 2012 [16]	parallel 48 weeks	322 subjects 52 years 86.0% males BMI (kg/m²) ≥ 27	**I:** Lower fat diet (AHA) **C:** Low-carbohydrate diet	**I:** 30:51:19; 1500 kcal for females and 1800 kcal for males **C:** 39:40:22; no energy restriction Total energy intake did not differ between I e C groups	9	**I:** 0.8 ± 2.9 (↑11.0%) **C:** 1.5 ± 3.5 (↑20.8%)

Heggen 2012 [38]	parallel 12 weeks	181 subjects 49.8 ± 8.1 years 41.6% males BMI (kg/m²) 33.0 ± 2.7	**I:** Lower fat diet **C:** Low-Gycemic-Load Diet	**I:** 32:45:19 **C:** 37:37:21 Hypocaloric diets (- 500 kcal/d) without difference between them	5	**I:** -0.7 ± 10.1 (↓3.7%) **C:** 0.4 ± 10.4 (↑2.3%)
Rajaie 2012 [39]	crossover 6 weeks	30 females with MetS BMI>25 kg/m 42.4 ± 7.2	**I:** Lower fat diet **C:** High-fat diet	**I:** 20-25:60-65:20 **C:** 36-40:43-47:20 Hypocaloric diets (- 350 to 700 kcal/d) without difference between them	15	**I:** -1.7 ± 2.3* (↓16.0%) **C:** -0.5 ± 2.2 (↓4.9%)

Abbreviations: I, intervention; C, control; BMI, body mass index; MetS, metabolic syndrome; SFA, saturated fatty acids; AHA, American Heart Association; DM, diabetes; MUFA, monounsaturated fat acids; PUFA, polyunsaturated fat acids; NA, not available. Adiponectin concentrations expressed as means ± SDs or median (25th – 75th percentile). *Significant change from baseline ($P < 0.05$).

TABLE 2
Characteristics of the studies investigating changes in adiponectin concentrations by n-3 PUFA intake

Author Year Reference	Study design Follow-up	Sample	Dietary Intervention and Control groups	n-3 PUFA dose (EPA + DHA)	Dietary composition (%): fat:carbohydrate: protein; total energy intake	Changes on adiponectin (% of change)
Krebs 2006 [40]	parallel 24 weeks	116 hyperinsulinemic females 44.7 ± 13.2 years BMI (kg/m²) 35.0 ± 5.5	**I**: 5.0g/d of fish oil with dietary and physical activity advice **C[1]**: 5.0 g/d of placebo oil (each capsule with 2.8 g linoleic and 1.4g oleic) with dietary and physical activity advice **C[2]**: 5.0 g/d of placebo oil without dietary advice	**I:** 4.2 g/d (1.3 g EPA + 2.9 DHA g)	**I:** 35:50:15; 2500 kcal **C[1]:** 35:50:15; 2500 kcal **C[2]:** not reported	(µg/mL) **I:** 2.33 ± 6.21*† (↑22.2%) **C[1]**: 0.22 ± 6.28 † (↑1.9%) **C[2]**: 0.53 ± 6.82*† (↑5.8%)
Kabir 2007 [41]	parallel 8 weeks	26 postmenopausal females with DM2 40-60 years BMI (kg/m²) 27 – 40	**I :** 3.0g/d of fish oil **C:** 3.0 g/d of placebo oil (paraffin oil)	**I:** 1.8 g/d (1.08g EPA + 0.72g DHA)	**I:** 30:55:15; 1460 kcal **C:** 30:55:15; 1527 kcal	(µg/mL) **I :** 0.5 ± 5.3 (↑8.5%) **C:** -0.4 ± 0.78 (↓5.7%)
Damsgaard 2008 [42]	parallel 8 weeks	64 males 24.9 ± 3.9 years BMI (kg/m²) 23.1 ± 1.9	**I [1]:** 5.0 mL fish oil, sunflower oil and Becel® margarine **I [2]:** 5.0 mL fish oil, rapeseed oil and a rapeseed oil-enriched butter spread **C[1]**: 5.0 mL olive oil, sunflower oil and Becel® margarine **C [2]**: 5.0 ml olive oil, rapeseed oil and a rapeseed oil-enriched butter spread	**I [1]:** 3.1 g/d (1.8g EPA + 1.3 g DHA) **I [2]:** 3.1 g/d (1.8g EPA + 1.3 g DHA)	not reported	(µg/mL) **I[1]:** 0.4 ± 0.78 * (↑6.0%) **I [2]:** -1.3 ± 1.03 (↓18.6%) **C[1]**: 0.1 ± 0.75 (↑1.6%) **C [2]:** 0.4 ± 0.79 (↑5.9%)

Micallef 2009 [46]	parallel 3 weeks	60 hyperlipidemic subjects 55.4 ± 1.0 years 45.0% males BMI (kg/m²) 26.9± 0.5	**I [1]:** 4.0g/d of tuna oil + 2.0g/d of plant sterols **I [2]:** 4.0g/d of tuna oil **C [1]:** 4.0g/d of sunola oil + 2.0g/d of plant sterols **C [2]:** 4.0g/d of sunola oil	**I [1]:** 1.4 g/d (0.3g EPA + 1.1 g DHA) **I [2]:** 1.4 g/d (0.3 g EPA + 1.1 g DHA)	not reported	(µg/mL) **I [1]:** 0.2 ± 0.19 * (↑11.8%) **I [2]:** 0.2 ± 0.22 (↑13.3%) **C [1]:** 0.4 ± 0.2 (↑21.1%) **C [2]:** 0.5 ± 0.2 (↑23.8%)
Rizza 2009 [47]	parallel 12 weeks	50 subjects 29.9± 6.2 years 50.0% males BMI (kg/m²) 26.2 ± 4.3	**I:** 2.0g/d of fish oil **C:** 2.0g/d of refined olive oil	**I:** 1.7 g/d (in a ratio of 0.9–1.5 EPA:1 DHA)	not reported	(µg/mL) **I:** 1.7 ± 4.88 (↑22.0%) **C:** -2.2 ± 5.18 (↓20.8%)
Troseid 2009 [24]	factorial 144 weeks	563 men with righ risk of CVD 64-76 years BMI (kg/m²) 24.1 - 28.7	**I:** n-3 PUFA supplementation (2.4g/d) **C:** placebo (56% linoleic + 32% oleic + 10% palmitic – sunola oil)	**I:** 1.32 g/d (0.84 g EPA + 0.48 g DHA)	not reported	(µg/mL) **I:** 0.72 ± 3.3 (↑8.0%) **C:** 0.20 ± 3.9 (↑2.5%)
Sofi 2010 [21]	parallel 48 weeks	11 subjects with NAFLD > 18 years 80.0% males BMI (kg/m²) 29.3 ± 4.1	**I:** 6.5 ml g/d olive oil (0.83g n-3 PUFA) + dietary recommendations **C:** 6.5 g/d of olive oil + dietary recommendations	**I:** 0.71 g/d (0.47g EPA + 0.24 g DHA)	**I:** 31:49:19; 1936 kcal **C:** 29:51:18; 2135 kcal	(µg/mL) **I :** 0.3 ± 0.19 (↑30.2%) **C:** 0.08 ± 0.08 (↑6.9%)
Vargas 2010 [22]	parallel 6 weeks	51 patients with polycystic ovary syndrome 20-45 years BMI (kg/m²) 25-45	**I [1]:** 6 capsules of fish oil **I [2]:** 6 capsules of flaxseed oil **C:** 6 capsules of soybean oil	**I [1]:** 3.6 g/d (2.2 g EPA + 1.5 g DHA) **I [2]:** 3.5 g/d	**All groups analyzed together** 35: 48:17 1735 kcal	(ng/mL) **I [1]:** 0.1 ± 0.2 * (↑1.3%) **I [2]:** -0.4 ± 0.1 (↓5.0%) **C:** -0.3 ± 0.1 (↓4.6%)
Gammelmark 2012 [48]	parallel 6 weeks	49 subjects 55 years	**I:** 2.0g/d of fish oil **C:** 2.0 g/d of olive oil	**I:** 1.1 g/d (0.64 g EPA + 0.48 g	not reported	(µg/mL) **I:** 0.52 ± 5.3 (↑7.3%)

		49.0% males BMI (kg/m²) 30		DHA)		**C:** 0.02 ± 5.32 (↑0.22%)
Koh 2012 [49]	parallel 8 weeks	150 subjects 52-56 years 58.0% males BMI (kg/m²) 24 – 28	**I [1]:** 2.0g/d of fish oil **I [2]:** 160.0 mg/d of fenofibrate **C:** 2.0g/d (did not describe placebo oil)	**I [1]:** 1.7 g/d (0.93 g EPA + 0.75 g DHA)	Low-fat diet for all groups Dietary information not reported.	(µg/mL) **I [1]:** -0.3 ± 0.1 (↓12.2%) **I [2]:** 0.2 ± 0.1*† (↑8.4%) **C:** 0.06 ± 0.07 (↑2.5%)
Mohammadi 2012 [50]	parallel 8 weeks	31 PCOS female 20-35 years BMI (kg/m²) 25-40	**I:** 4 g/d of fish oil **C:** 4 capsules (500 mg each) of liquid paraffin	**I:** 1.2 g/d (720 g EPA + 480 g DHA)	**I**: 35:50:15; 1668 kcal **C**: 35:50:15; 1680 kcal	(µg/mL) **I:** 1.7 ± 3.0*† (↑14.4%) **C:** -0.3 ± 3.4 (↓2.4%)
Munro 2012 [51]	parallel 14 weeks	32 subjects 18-60 years 18.7 % males BMI (kg/m²) 30 – 40	**I:** 6 g/d of fish oil **C:** 6 g/d of sunola oil	**I:** 2.04 g/d (0.42 g EPA + 1.62 g DHA)	**I:** 16:40:40; 717 kcal for 4 weeks **C:** 16:40:40; 717 kcal for 4 weeks After 4 weeks ~1600 kcal/day	(µg/mL) **I:** 0.9 ± 1.7† (↑9.4%) **C:** 1.0 ± 4.2 (↑2.0%)
Spencer 2013 [55]	parallel 12 weeks	33 subjects with insulin resistance 51.0 years 33.3% males BMI (kg/m²) 33.0	**I:** 4 g/d of fish oil **C:** 4 g/d of corn oil (identically Packaged)	I: 3.32 g/d (1.86 g EPA + 1.46 g DHA)	Not reported	(µg/mL) **I:** -0.2 ± 0.71 (↓0.2%) **C:** -0.1 ± 0.8 (↓2.5%)

Abbreviations: C, control; BMI, body mass index; PUFA, polyunsaturated fat acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Adiponectin concentrations expressed as means ± SDs or median (25th to 75th percentile). * Significant change from baseline ($P < 0.05$); † Significant difference between intervention and control groups ($P < 0.05$).

TABLE 3
Characteristics of the studies investigating changes in adiponectin concentrations by conjugated linoleic acid (CLA) intake

Author Year Reference	Study design Follow-up	Sample	Dietary Intervention and Control groups	Dietary composition (%): fat:carbohydrate:protein; total energy intake	Changes on adiponectin µg/mL (% of change)
Risérus 2004 [56]	parallel 12 weeks	57 males 53.0 ± 10.1 years BMI (kg/m²) 30.2 ± 1.8	**I [1]:** 3.4 g/d of CLA **I [2]:** 3.4 g/d of t10c12CLA **C:** 3.4 g/d of olive oil	Dietary information not reported	**I [1]:** 0.26 ± 1.50 (↑3.5%) **I [2]:** 0.01 ± 2.50 (↑0.13%) **C:** 0.49 ± 1.50 (↑7.0%)
Syvertsen 2007 [57]	parallel 24 weeks	49 subjects 18-65 years 31.0% males BMI (kg/m²) 28-32	**I:** 3.4 g/d (37.5% cis-9 trans-11 e 38.0% trans-10 and cis-12 CLA) **C:** 4.5 g/d of olive oil	Dietary information not reported	**I:** -0.6 ± 0.4 (↓5.6%) **C:** 0.27 ± 0.3 (↑2.8%)
Norris 2009 [58]	crossover 16 weeks (4 weeks washout)	55 postmenopausal females with type 2 DM 60.1 ± 7.3 years BMI (kg/m²) 36.3 ± 6.1 kg/m²	**I:** 6.4 g/d (CLA isomers) **C:** 8.0 g/d of safflower oil	**I:** 38:44:33; 1530 kcal **C:** 38:44:33; 1592 kcal	**I:** 0.80 ± 0.6 (↑25.3%) **C:** 2.4 ± 0.8* (↑11.0%)
Zhao 2009 [59]	parallel 8 weeks	80 hypertensive subjects 59.4 ± 2.4 years 55.0% males BMI (kg/m²) 31.2 ± 1.4	**I:** 4.5 g/d of CLA (50:50 c9t11 and c10c12 CLA) **C:** control oil (SFA)	**I:** 22:64:14; 2443 kcal **C:** 33:55:12; 2777 kcal	**I:** 2.5 ± 1.35 *† (↑37.3%) **C:** 0.1 ± 0.46 (↑1.5%)
MacRedmond 2010 [60]	parallel 12 weeks	28 mild asthmatics subjects 19-40 years 50.0% males BMI (kg/m²) 27.9 (ranged 24.6 – 31.2)	**I:** 4.5 g/d of CLA (36.4% of cis-9, 37.0% of trans-11 trans-10-cis-12) **C:** 4.5 g/d of olive oil	Dietary information not reported	**I:** 0.8 ± 1.5 (↑4.6%) **C:** 0.8 ± 1.7 (↑5.2%)
Joseph	crossover	36 overweight males	**I [1]:** 3.5 g/d (50:50 of t10, c12	Dietary information not reported	**I [1]:** -0.8 ± 0.7

2011 [61]	8 weeks (4 weeks washout)	18 – 60 years BMI (kg/m²) ≥ 25	and c9, t11 CLA) **I [2]:** 3.5 g/d (c9,t11 CLA) **C:** 3.5 g/d of safflower oil		(↓6.5%) **I [2]:** -0.5 ± 0.3 (↓4.1%) **C:** 0.0 ± 0.5 (0%)
Shademan 2011 [17]	parallel 8 weeks	42 patients with type 2 diabetes 35-50 years 46.2% males BMI (kg/m²) 25 – 30	**I:** 3.0 g/d (50:50 of t10, c12 and c9, t11 CLA) **C:** 3.0 g/d of soybean oil	Dietary information not reported	**I:** -0.004 ± 0.03 (↓5.3%) **C:** -0.0005 ± 0.03 (↓0.8%)

Abbreviations: C, control; BMI, body mass index; SFA, saturated fatty acids; DM, diabetes. Adiponectin concentrations expressed in as means ± SDs.

* Significant change from baseline ($P < 0.05$); † Significant difference between intervention and control groups ($P < 0.05$).

Figure 1

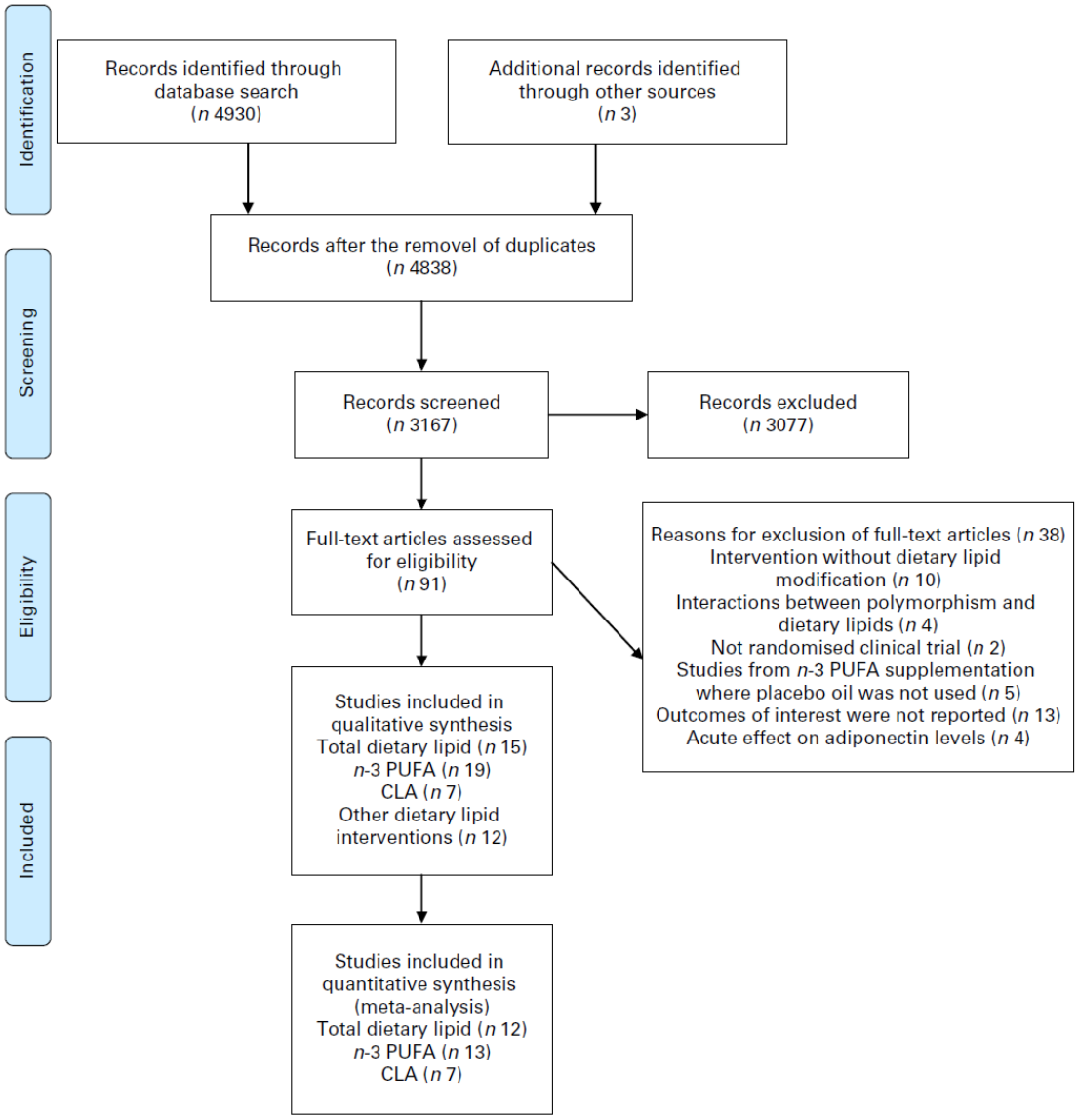


Figure 1. Flow chart of the literature search and the study selection process(77). CLA, conjugated linoleic acid.

Figure 2

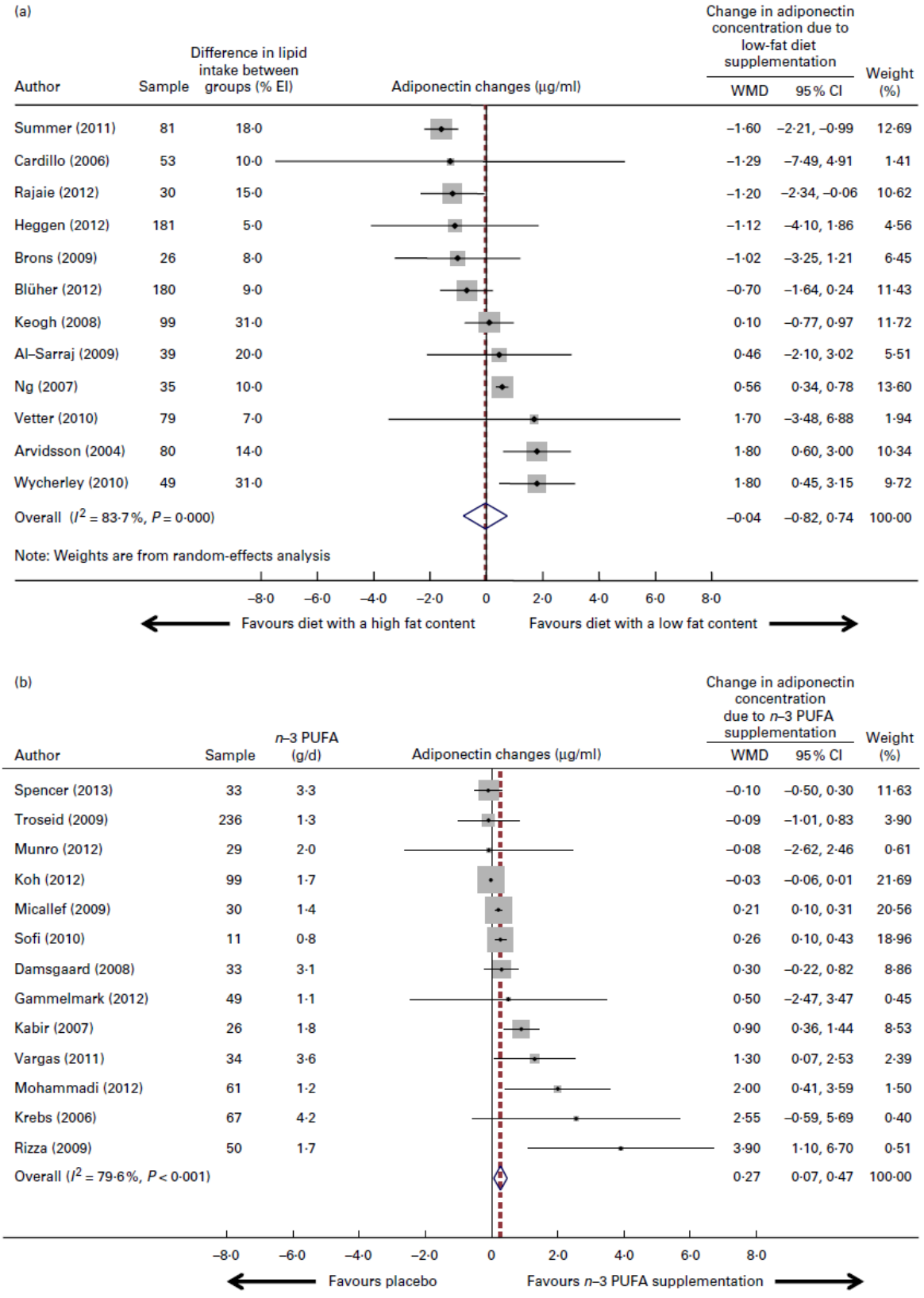

(a)
Author
Sample
Difference in lipid intake between groups (% EI)
Adiponectin changes (μg/ml)
Change in adiponectin concentration due to low-fat diet supplementation
WMD
95% CI
Weight (%)
Summer (2011) 81 18·0 −1·60 −2·21, −0·99 12·69
Cardillo (2006) 53 10·0 −1·29 −7·49, 4·91 1·41
Rajaie (2012) 30 15·0 −1·20 −2·34, −0·06 10·62
Heggen (2012) 181 5·0 −1·12 −4·10, 1·86 4·56
Brons (2009) 26 8·0 −1·02 −3·25, 1·21 6·45
Blüher (2012) 180 9·0 −0·70 −1·64, 0·24 11·43
Keogh (2008) 99 31·0 0·10 −0·77, 0·97 11·72
Al–Sarraj (2009) 39 20·0 0·46 −2·10, 3·02 5·51
Ng (2007) 35 10·0 0·56 0·34, 0·78 13·60
Vetter (2010) 79 7·0 1·70 −3·48, 6·88 1·94
Arvidsson (2004) 80 14·0 1·80 0·60, 3·00 10·34
Wycherley (2010) 49 31·0 1·80 0·45, 3·15 9·72
Overall (I^2 = 83·7%, P = 0·000) −0·04 −0·82, 0·74 100·00
Note: Weights are from random-effects analysis
−8·0 −6·0 −4·0 −2·0 0 2·0 4·0 6·0 8·0
Favours diet with a high fat content
Favours diet with a low fat content
(b)
Author
Sample
n–3 PUFA (g/d)
Adiponectin changes (μg/ml)
Change in adiponectin concentration due to n–3 PUFA supplementation
WMD
95% CI
Weight (%)
Spencer (2013) 33 3·3 −0·10 −0·50, 0·30 11·63
Troseid (2009) 236 1·3 −0·09 −1·01, 0·83 3·90
Munro (2012) 29 2·0 −0·08 −2·62, 2·46 0·61
Koh (2012) 99 1·7 −0·03 −0·06, 0·01 21·69
Micallef (2009) 30 1·4 0·21 0·10, 0·31 20·56
Sofi (2010) 11 0·8 0·26 0·10, 0·43 18·96
Damsgaard (2008) 33 3·1 0·30 −0·22, 0·82 8·86
Gammelmark (2012) 49 1·1 0·50 −2·47, 3·47 0·45
Kabir (2007) 26 1·8 0·90 0·36, 1·44 8·53
Vargas (2011) 34 3·6 1·30 0·07, 2·53 2·39
Mohammadi (2012) 61 1·2 2·00 0·41, 3·59 1·50
Krebs (2006) 67 4·2 2·55 −0·59, 5·69 0·40
Rizza (2009) 50 1·7 3·90 1·10, 6·70 0·51
Overall (I^2 = 79·6%, P < 0·001) 0·27 0·07, 0·47 100·00
−8·0 −6·0 −4·0 −2·0 0 2·0 4·0 6·0 8·0
Favours placebo
Favours n–3 PUFA supplementation

Figure 2

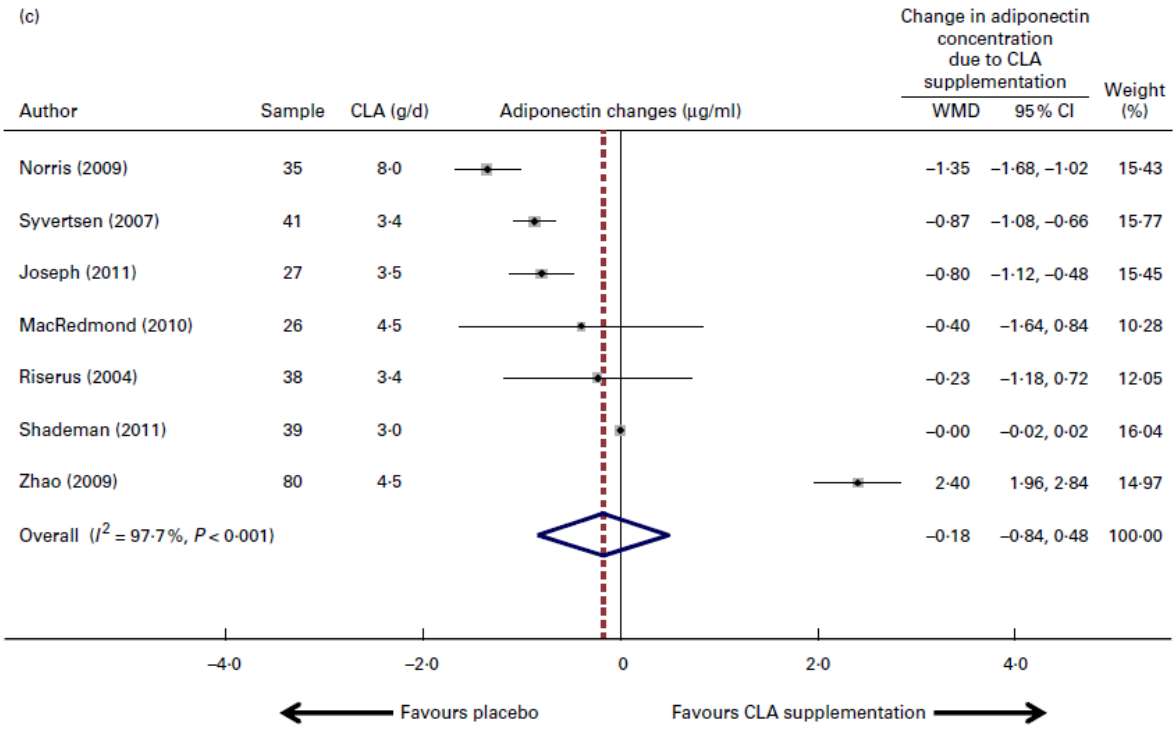


Figure 2. Forest plots (meta-analyses, random-effects models) of the effect of fatty acid interventions on circulating adiponectin concentrations (µg/ml): (a) diet with a low fat content; (b) n-3 PUFA supplementation; (c) conjugated linoleic acid (CLA) supplementation. % EI, percentage of energy intake. For the Troseid et al.(24) study, data for the main effect of fish oil intake on adiponectin concentrations were obtained directly from the authors and used in the pooled meta-analysis.

Figure 3

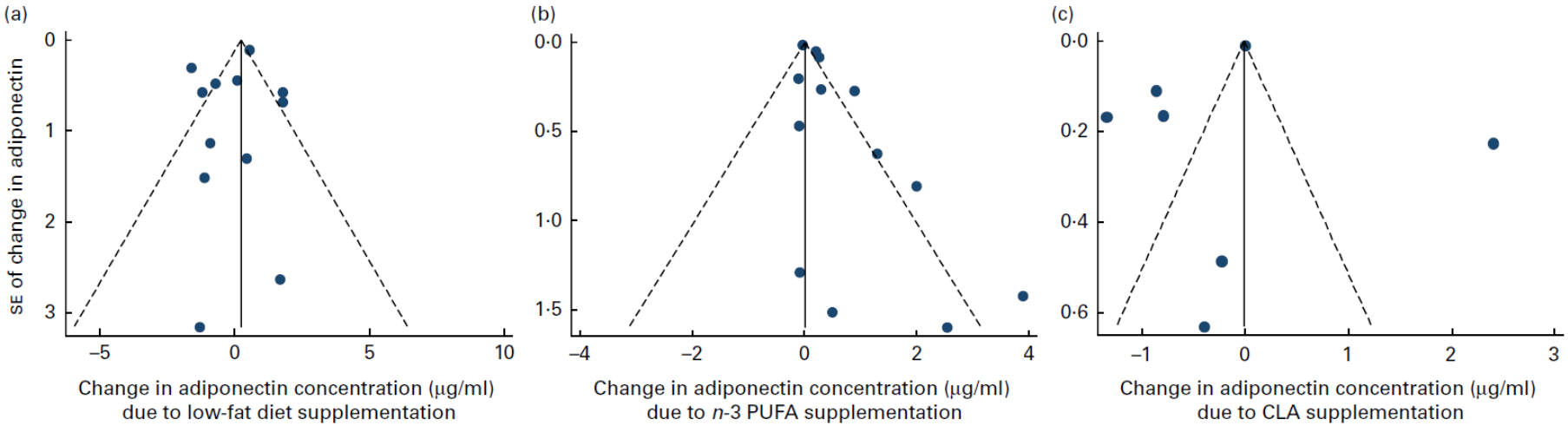


Figure 3. Funnel plots of changes in circulating adiponectin concentrations in randomised trials with (a) a diet with a low fat content, (b) n-3 PUFA supplementation and (c) conjugated linoleic acid (CLA) supplementation.

Supplementary Material. M*edline* search strategy

(((
((((((Diet[Title/Abstract]) OR Diet, Fat-Restricted[Title/Abstract]) OR Diet, Fat Restricted [Title/Abstract]) OR Fat-Restricted Diet [Title/Abstract]) OR Diets, Fat-Restricted [Title/Abstract]) OR Fat Restricted Diet [Title/Abstract]) OR Fat-Restricted Diets [Title/Abstract]) OR Diet, Low-Fat [Title/Abstract]) OR Diet, Low Fat [Title/Abstract]) OR Diets, Low-Fat [Title/Abstract]) OR Low-Fat Diet [Title/Abstract]) OR Low-Fat Diets [Title/Abstract]) OR Diet, Fat-Free [Title/Abstract]) OR Diet, Fat Free [Title/Abstract]) OR Diets, Fat-Free [Title/Abstract]) OR Fat-Free Diet [Title/Abstract]) OR Fat-Free Diets[Title/Abstract]) OR Diet Therapy[Title/Abstract]) OR Therapy, Diet [Title/Abstract]) OR Diet Therapies [Title/Abstract]) OR Therapies, Diet[Title/Abstract]) OR lipids[Title/Abstract]) OR Fats[Title/Abstract]) OR Dietary Fats [Title/Abstract]) OR Fats, Dietary [Title/Abstract]) OR Dietary Fat [Title/Abstract]) OR Fat, Dietary[Title/Abstract]) OR Cholesterol, Dietary[Title/Abstract]) OR Dietary Cholesterol[Title/Abstract]) OR Fats, Unsaturated [Title/Abstract]) OR Unsaturated Dietary Fats [Title/Abstract]) OR Dietary Fat, Unsaturated [Title/Abstract]) OR Fat, Unsaturated Dietary [Title/Abstract]) OR Fats, Unsaturated Dietary [Title/Abstract]) OR Unsaturated Dietary Fat [Title/Abstract]) OR Dietary Oils [Title/Abstract]) OR Dietary Oil [Title/Abstract]) OR Oil, Dietary [Title/Abstract]) OR Oils, Dietary[Title/Abstract]) OR Fatty Acids, Omega-3 [Title/Abstract]) OR Acids, Omega-3 Fatty [Title/Abstract]) OR Fatty Acids, Omega 3 [Title/Abstract]) OR Omega-3 Fatty Acids [Title/Abstract]) OR Omega 3 Fatty Acids [Title/Abstract]) OR ômega-3 PUFA [Title/Abstract]) OR ômega-3 Fatty Acids [Title/Abstract]) OR Fatty Acids, ômega-3 [Title/Abstract]) OR n 3 Fatty Acids [Title/Abstract]) OR ômega-3 Polyunsaturated Fatty Acid [Title/Abstract]) OR n 3 Polyunsaturated Fatty Acid[Title/Abstract]) OR alpha-Linolenic Acid[Title/Abstract]) OR alpha Linolenic Acid[Title/Abstract]) OR Linolenic Acid[Title/Abstract]) OR Docosahexaenoic Acids[Title/Abstract]) OR Acids, Docosahexaenoic [Title/Abstract]) OR Docosahexenoic Acids [Title/Abstract]) OR Acids, Docosahexenoic[Title/Abstract]) OR Eicosapentaenoic Acid[Title/Abstract]) OR Eicosapentanoic Acid [Title/Abstract]) OR Acid, Eicosapentanoic[Title/Abstract]) OR omega-3-Eicosapentaenoic Acid [Title/Abstract]) OR omega 3 Eicosapentaenoic Acid[Title/Abstract]) OR Fatty Acids, Omega-6 [Title/Abstract]) OR Acids, Omega-6 Fatty [Title/Abstract]) OR N-6 Fatty Acids [Title/Abstract]) OR Acids, N-6 Fatty [Title/Abstract]) OR Fatty Acids, N-6 [Title/Abstract]) OR N 6 Fatty Acids [Title/Abstract]) OR Omega-6 Fatty Acids [Title/Abstract]) OR Omega 6 Fatty Acids [Title/Abstract]) OR Fatty Acids, Omega 6[Title/Abstract]) OR Linolenic Acids [Title/Abstract]) OR gamma-Linolenic Acid[Title/Abstract]) OR gamma Linolenic Acid[Title/Abstract]) OR alpha Linolenic Acid[Title/Abstract]) OR Linolenic Acid[Title/Abstract]) OR Linoleic Acid[Title/Abstract]) OR Linoleic Acids, Conjugated[Title/Abstract]) OR Acids, Conjugated Linoleic [Title/Abstract]) OR Conjugated Linoleic Acids[Title/Abstract]) OR Fatty Acids[Title/Abstract]) OR Fatty

Acids, Monounsaturated[Title/Abstract]) OR Acids, Monounsaturated Fatty [Title/Abstract]) OR Monounsaturated Fatty Acids[Title/Abstract]) OR Oleic Acids[Title/Abstract]) OR Oleic Acid[Title/Abstract]) OR Acid, Oleic [Title/Abstract]) OR cis-9-Octadecenoic Acid [Title/Abstract]) OR Acid, cis-9-Octadecenoic [Title/Abstract]) OR cis 9 Octadecenoic Acid [Title/Abstract]) OR 9-Octadecenoic Acid [Title/Abstract]) OR 9 Octadecenoic Acid [Title/Abstract]) OR Acid, 9-Octadecenoic [Title/Abstract]) OR Oleate[Title/Abstract]) OR Trans Fatty Acids[Title/Abstract]) OR Acids, Trans Fatty [Title/Abstract]) OR Fatty Acids, Trans [Title/Abstract]) OR Trans-Fatty Acids [Title/Abstract]) OR Acids, Trans-Fatty[Title/Abstract]) OR Arachidonic Acids [Title/Abstract]) OR Acids, Arachidonic [Title/Abstract]) OR Eicosatetraenoic Acids [Title/Abstract]) OR Acids, Eicosatetraenoic[Title/Abstract]) OR Arachidonic Acid[Title/Abstract]) OR Fatty Acids, Essential [Title/Abstract]) OR Acids, Essential Fatty [Title/Abstract]) OR Essential Fatty Acids[Title/Abstract]) OR Oils[Title/Abstract]) OR Fish Oils[Title/Abstract]) OR Oils, Fish [Title/Abstract]) OR Fish Liver Oils [Title/Abstract]) OR Liver Oils, Fish [Title/Abstract]) OR Oils, Fish Liver[Title/Abstract]) OR Cod Liver Oil[Title/Abstract]) OR Liver Oil, Cod [Title/Abstract]) OR Oil, Cod Liver[Title/Abstract]) OR Plant Oils[Title/Abstract]) OR Oils, Plant [Title/Abstract]) OR Oils, Vegetable [Title/Abstract]) OR Vegetable Oils[Title/Abstract] Field: Title/Abstract

AND ((((((((((((Adiponectin[Title/Abstract]) OR Adipocyte Complement-Related Protein 30-kDa [Title/Abstract]) OR Adipocyte Complement Related Protein 30 kDa [Title/Abstract]) OR Adipose Most Abundant Gene Transcript 1 [Title/Abstract]) OR apM-1 Protein [Title/Abstract]) OR apM 1 Protein [Title/Abstract]) OR ACRP30 Protein [Title/Abstract]) OR Adipocyte, C1q and Collagen Domain Containing Protein[Title/Abstract]) OR Adipokines[Title/Abstract]) OR Adipocytokines[Title/Abstract]) OR Adipokine[Title/Abstract]) OR Peptide Hormones[Title/Abstract]) OR Hormones, Peptide [Title/Abstract]) OR Polypeptide Hormones [Title/Abstract]) OR Hormones, Polypeptide[Title/Abstract] Field: Title/Abstract **AND** ((((((((((randomized controlled trials as topic/)) OR (randomized controlled trial/)) OR (random allocation/)) OR (double blind method/)) OR (single blind method/)) OR (clinical trial/)) OR (exp clinical trials as topic/))) OR (((((((clinic$ adj trial$1) AND .tw.)) OR (((single OR double OR treb$ OR triple) AND adj AND (blind$3 OR mask$3)) AND .tw.)) OR (placebos/)) OR (placebo$.tw.)) OR (randomly allocated.tw.)) OR ((allocated adj2 random) AND .tw.)))) NOT ((((((case report.tw.)) OR (letter/)) OR (historical article/)) OR (review, multicase.pt.)) OR (review of reported cases.pt.)) Field: Title/Abstract

Supplementary Table S1. Assessment of risk of bias

	Selection bias		Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Dietary compliance assessed
	Amount of total dietary lipid intake						
Arvidsson, 2004	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Cardillo, 2006	Unclear	Unclear	Unclear	Unclear	High	Low	Low
Ng, 2007	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Keogh, 2008	Low	Low	Unclear	Unclear	Low	Low	Low
Al-Sarraj, 2009	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Brons, 2009	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Wycherley, 2009	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Vetter, 2010	Unclear	Unclear	Unclear	Unclear	High	Low	Unclear
Summer, 2011	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Blüher, 2012	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Hegen, 2012	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear
Rajaie, 2012	Unclear	Unclear	Unclear	Low	High	Low	Low
	n-3 PUFA intake						
Krebs, 2006	Unclear	Unclear	Low	Unclear	High	Low	Unclear
Kabir, 2007	Unclear	Unclear	Low	Unclear	Low	Low	Low
Damsgaard, 2008	Unclear	Unclear	Low	Low	Low	Low	Low

Micallef ,2009	Unclear	Unclear	Low	Unclear	Low	Low	Low
Rizza, 2009	Unclear	Unclear	Low	Unclear	Low	Low	Low
Trosseid, 2009	Unclear	Unclear	Unclear	Low	Low	Low	Low
Sofi, 2010	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
Vargas, 2010	Unclear	Unclear	Low	Unclear	High	Low	Unclear
Gammelmark 2012	Unclear	Unclear	Low	Unclear	Low	Low	Low
Kon Koh, 2012	Low	Low	High	Unclear	Low	Low	Low
Mohammadi, 2012	Unclear	Unclear	Low	Unclear	Low	Low	Low
Munro, 2012	Low	Low	Low	Low	High	Low	Low
Spencer, 2013	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
	Conjugated linoleic acid supplementation						
Risérus, 2004	Unclear	Unclear	Low	Unclear	Low	Low	Unclear
Syvertsen, 2007	Unclear	Unclear	Low	Unclear	Low	Low	Low
Norris, 2009	Low	Low	Low	Low	High	Low	Low
Zhao, 2009	Unclear	Unclear	Unclear	Unclear	Low	Low	Low
MacRedmond, 2010	Unclear	Unclear	Low	Unclear	Low	Low	Low
Joseph, 2011	Unclear	Unclear	Low	Low	High	Low	Low
Shademan, 2011	Unclear	Unclear	Low	Unclear	Low	Low	Low

Supplementary Table S2
Characteristics of the studies investigating changes in adiponectin concentrations by modifying the amount of total dietary lipid intake (qualitative analyses).

Author Year Reference	Study design Follow-up	Sample	Dietary Intervention and Control groups	Dietary composition (%): fat:carbohydrate:protein; total energy intake	Difference of percentage points between groups	Changes on adiponectin µg/mL (% of change)
Keogh 2007 [33]	parallel 12 weeks	36 subjects 20 – 65 years 32.0% males BMI (kg/m²) 27 - 44	**I:** Lower fat diet **C:** Low-carbohydrate diet	**I:** 20:60:20; 1433 kcal (4.0% SFA, 5.0% PUFA, 7.0%MUFA) **C:** 27:33:40; 1433 kcal (7.0% SFA, 6.0% PUFA, 13.0% MUFA)	7	Not reported separately by groups
Yeung 2010 [36]	crossover 18 weeks (3 weeks washout)	161 pre- and stage-1 hypertensive adults 53.5 ± 11 years 55.0% males BMI (kg/m²) 30.3 ± 6	**I[1]:** Lower fat, high-carbohydrate diet **I[2]:** Lower fat, high-protein diet **C:** High-fat diet	**I[1]:** 27:58:15; calories not reported **I[2]:**27:48:25; calories not reported **C:** 37:48:15 (8.0% MUFA)	10	Absolute values not reported
Varady 2011 [19]	parallel 6 weeks	17 subjects 18-50 years 29.3% males BMI (kg/m²) 30 – 39	**I:** Lower fat diet **C:** High-fat diet	**I:** 25:55:20; 1967kcal (9.0% MUFA, 9.0% PUFA, 299.0 mg cholesterol, 0.0% trans) **C:** 60:5:35; 1868 kcal (26.0% saturated fat, 24.0% MUFA, 9.0% PUFA, 448.0 mg cholesterol, 1.0% trans)	35	Absolute values not reported

Supplementary Table S2
Characteristics of the studies investigating changes in adiponectin concentrations by n-3 PUFA intake (qualitative analyses).

Author Year Reference	Study design Follow-up	Sample	Dietary Intervention and Control groups	n-3 PUFA dose (EPA + DHA)	Dietary composition (%): fat:carbohydrate:protein; total energy intake	Changes on adiponectin (% of change)
Kratz 2008 [43]	parallel 14 weeks	26 subjects 37.8 ± 13.6 years 38.5% males BMI (kg/m²) 30.1 ± 1.1	**I:** diet rich in n–3 PUFAs from both plant and marine sources **C:** 0.5% of energy intake from n–3 PUFAs	**I:** 3.6 ± 0.1% of energy intake (EPA and DHA not reported)	**I:** 35:50:15; 1800 kcal **C:** 35:50:15; 1800 kcal	(µg/mL) **I:** 0.59 ± 1.62 (↑14.0%) **C:** 0.15 ± 1.73 (↑3.5%)
Ramel 2008 [44]	parallel 8 weeks	324 subjects 20 – 40 years 42.6% males BMI (kg/m²) 27.5 –32.5	**I [1]:** 450.0 g /w of lean fish **I [2]:** 450.0 g/w of fatty fish **I [3]:** 3.0 g/d of fish oil, no other seafood **C:** no seafood and 3.0 g/d of sunflower oil	**I [1]:** 0.26 g/d **I [2]:** 2.1 g/d **I [3]**: 1.3 g/d (EPA and DHA not reported in all groups)	**For all groups:** 30:50:20; diet restricted of energy to 70.0% of usual diet	(µg/mL) **I [1]:** -0.2 (1.2 to -1.9) (↓1.9%) **I [2]:** -0.9 (0.7 to -2.1) (↓8.7%) **I [3]:** -0.5 (1.0 to -2.1) (↓4.6%) **C:** -0.5 (0.9 to -2.2) (↓4.0%)
Sneddon 2008 [45]	crossover 12 weeks (12 weeks washout)	59 males grouped according age (Young = 30.5 ± 4.9 years and Older = 56.3 ± 4.2	**I:** 3 capsules of fish oil + 3 capsules of CLA (760.0 mg) **C:** 6 capsules (800.0 mg palm oil and 200.0 mg	**I:** 1.53 g/d (0.9 g EPA + 0.63 g DHA)	not reported	(µg/mL) **Young lean:** **I:** -0.14 ± 0.35 (↓1.4%) **C:** -0.79 ± 0.27 (↓7.9%) **Young obese:** **I:** 0.21 ± 0.22† (↑2.8%) **C:** -0.79 ± 0.39† (↓9.0%)

		years) BMI (kg/m²) Lean = 23.6 ± 1.5 Obese = 32.1 ± 1.7	soybean oil)			**Older lean:** **I:** -0.16 ± 0.20 (↓1.5%) **C:** 0.00 ± 0.28 (0%) **Older obese:** **I:** -0.57 ± 0.37 (↓8.0%) **C:** -0.05 ± 0.33 (↑0.7%)
Simão 2012 [52]	parallel 12 weeks	65 females with the metabolic syndrome 47.9 ± 10.0 years BMI (kg/m²) 35.4	**I[1]**: 3 g/d of fish oil **I[2]:** 3 g/d of fish oil + 29 g/d of tosted ground soya bean **I[3]:** 29 g/d of tosted ground soya bean **C:** usual diet	**I[1]** e **I[2]:** 0.9 g/d (0.54 g EPA + 0.36 g DHA)	Not reported	(µg/mL) **I[1]**: 18.4* (↑27.0%) **I[2]:** 12.6 (↑17.4%) **I[3]:** 21.2* (↑22.5%) **C:** 0.4 (↑0.4%) (Δ statistical dispersion not reported)
Zhang 2012 [53]	parallel 8 weeks	127 dyslipidemic females 35-70 years BMI (kg/m²) 26.4 ± 6.1	**I[1]**: salmon group (5d/week) **I[2]:** herring group (5d/week) **I[3]:** pompano group (5d/week) **C:** pork/chicken/beef /lean fish (5d/week)	**I[1]:** 3.2 ± 0.1 g/d **I[2]:** 2.8 ± 0.1 g/d **I[3]:** 2.1 ± 0.1 g/d **C:** 1.1 ± 0.1 g/d	**I[1]**: 32:51:17; 1650 kcal **I[2]:** 32:51:17; 1676 kcal **I[3]:** 32:51:17; 1671 kcal **C:** 32:51:17; 1727 kcal	(µg/mL) **I[1]:** 0.7 ± 2.76* (↑0.7%) **I[2]:** 0.9 ± 3.15* (↑0.9%) **I[3]:** 0.6 ± 2.35 (↑0.6%) **C:** 0.4 ± 3.02 (↑5.6%)
Guebre-Egziabher 2013 [54]	parallel 10 weeks	12 chronic kidney disease patients (glomerular filtration rate <20 mL/min) 50.5 years 50% males BMI (kg/m²) <27.0	**I[1]:** 6 g/d of fish oil **I[2]:** 12 g/d of fish oil	**I[1]:** 2.04 g/d (1.08 g EPA + 0.96 g DHA) **I[2]:** 4.08 g/d (2.16 g EPA + 1.92 g DHA)	**I[1]:** 39:44:17; 1973 kcal **I[2]:** 39:42:18; 1703 kcal	(µg/mL) **I[1]:** 1.7 ± 4.7 (↑1.7%) **I[2]:** 1.3 ± 2.4 (↑1.3%)

Supplementary Table S2
Characteristics of the studies investigating changes in adiponectin concentrations by other lipid dietary interventions (qualitative analyses).

Author Year Reference	Study design Follow-up	Sample	Dietary intervention and control groups	Dietary composition (%): fat:carbohydrate:protein; total energy intake	Changes on adiponectin (% of change)
Nelson 2007 [62]	parallel 8 weeks	57 subjects 38.5 ± 11.4 years 19.0% males BMI (kg/m²) 30.3 ± 5.0	**I**: flaxseed oil capsules (increasing ALA to 5.0% of total energy intake) **C**: habitual diet	**I:** 38.6% of energy from fat; 1924.3 kcal **C:** 34.3% of energy from fat; 2171 kcal	(µg/mL) **I:** -0.89 ± 5.92† (↓11.2%) **C:** 0.17 ± 4.23† (↑1,68%)
Lithander 2008 [63]	crossover 3 weeks (4 weeks washout)	18 hyperlipidemic males 39.7 ± 13.9 years BMI (kg/m²) 25.9 ± 4.2	**$I^{1:}$** high SFA:USFA **$I^{2:}$:** lower SFA:USFA	**I^1:** 34:53:13; 3176 kcal, 18.0% saturated fat, 10.0% MUFA and 7.0% PUFA **I^2:** 34:53:13; 3176 kcal, 13.0% saturated fat, 12.0% MUFA and 8.0% PUFA	(µg/mL) **I^1:** -0.5 ± 0.4 (↓7.4%) **I^2:** 1.1 ± 0.7 (↑16.4%)
Ratliff 2008 [64]	parallel 12 weeks	28 males 40 – 70 years BMI(kg/m²) 26 – 37	**I^1:** cholesterol/fat-free eggs **I^2:** Liquid whole eggs, 640 mg additional cholesterol/d provided by eggs	**For both groups:** 57:17:26; 1900 kcal	(µg/mL) **I^1:** 0.6 ± 0.6*† (↑3.8%) **I^2:** 2.8 ± 2.8*† (↑17.7%)
Vega-López 2009 [65]	crossover 5 weeks (2 weeks washout)	37 females 64.2 ± 7.5 years BMI (kg/m²) 25.6 ± 3.6	**I^1:** Corn oil **I^2:** Partially-hydrogenated soybean oil	**I^1:** 2300 kcal; 12.1% PUFA and 0.3% trans **I^2:** 2312 kcal; 5.9% PUFA and 4.3% trans	Basal adiponectin level was not reported.

Taylor 2010 [23]	parallel 12 weeks	34 patients with type 2 diabetes 52.4 ± 1.5 years 50.0% males BMI (kg/m²) 32.4 ± 1.0	**I[1]:** bakery products containing 32.0 g/d milled flaxseed (7.4 g/d LA in weeks days) **I[2]:** bakery products containing 13.0g/d flaxseed oil (7.4 g/d LA in 6 weeks days) **C:** bakery products containing no flax	**I[1]:** 37:48:17; 1997 kcal **I[2]:** 33:46:17; 2293 kcal **C:** 38:45:18; 2052 kcal	(µg/mL) **I[1]:** 0.8 ± 4.6 (↑7.6%) **I[2]:** 0.4 ± 4.8 (↑5.8%) **C:** 0.3 ± 5.6 (↑3.1%)
Bendsen 2011 [20]	parallel 16 weeks	49 overweight postmenopausal females 45 – 70 years BMI (kg/m²) 25 – 30	**I:** 26.0 g/d of partially hydrogenated soybean oil **C:** 50.0:50.0% mixture of palm oil and high oleic sunflower oil	Dietary information not reported	(µg/mL) **I:** -0.57 ± 0.78 **C:** 0.08 ± 5.53 (% of change NA)
Kalgaonkar 2011 [66]	parallel 6 weeks	31 female with polycystic ovary syndrome 20 – 45 years BMI (kg/m²) 35.1 ± 1.8	**I[1]:** 31.0 g/d of almond-oil (19.5 g MUFA, 7.5 g LA and no ALA) **I[2]:** 31.0 g/d walnut-oil (4.5 g MUFA, 19.2 g LA and 4.3 g ALA)	**I[1]:** 39:44:19; 1717 kcal **I[2]:** 40:45:17; 1540 kcal	(ng/mL) **I[1]:** 2.1 ± 0.8* (↑20.8%) **I[2]:** 1.8 ± 0.7* (↑19.0%)
Aronis 2012 [67]	crossover 4 days (4 weeks washout)	15 subjects with MetS 58 ± 2.5 years 60.0% males BMI (kg/m²) 36.6 ± 1.7	**I:** 48.0 g/d of walnuts, incorporated into a liquid meal **C:** diet without walnuts	Diets with the same macronutrient distribution and calories. More dietary information not reported.	(µg/mL) **I:** 0.51 * (↑14.1%) **C:** -0.21 (↓6.4%) (Δ statistical dispersion not reported)
Bjermo 2012 [68]	parallel 12 weeks	31 healthy subjects 30–65 years 34% of males BMI (kg/m²) 30.3 ± 3.7	**I:** n-6 PUFA group received scones (baked-on sunflower oil), margarine, sunflower oil, and sunflower seeds **C:** SFA group received scones (baked-on butter) and butter	**I:** 38:41:17; 2052 kcal **C:** 37:44:18; 1945 kcal	(µg/mL) **I:** -0.02 (-0.36 – 0.36) (↓0.80%) **C:** -0.03 (-0.74 – 0.68) (↓0.84%) High-molecular weight adiponectin

Manning 2012 [69]	parallel 48 weeks	160 subjects with MetS 37.0% males 27 – 80 years BMI (kg/m²) 31.7 ± 6.2	**I[1]:** 600.0 mg/d α-lipoic acid **I[2]:** 100.0 UI/d vitamin E **I[1] + I[2]:** 600.0 mg/d α-lipoic acid + 100.0 UI/d vitamin E **C:** oil composition not reported	Dietary information not reported	(µg/mL) **I[1]:** 0 **I[2]:** 0.8 (↑10.5%) **I[1] + I[2]:** -0.5 (↓7.5%) **C:** -0.2 (↓3.0%) (Δ statistical dispersion not reported)
Kontogianni 2013 [70]	crossover 3 weeks	37 healthy subjects 25.6 ± 5.9 years 21.6% males BMI (kg/m²) 21.9 ± 2.5	**I:** 13.8 g/d of flaxseed oil (8 g ALA, 3 g oleic acid) **C:** 13.8 g/d of olive oil (0.13 g ALA, 11.3 g oleic acid)	**I:** 37:44.4:15.9; 1928 kcal **C:** 39:47.6:18.4; 1805 kcal	(µg/mL) **I:** 0.0 ± 1.25 **C:** -1.0 ± 1.30 (↓15.4%)
Somerset 2013 [71]	parallel 10 weeks	64 subjects 25-55 years 15.6% males BMI (kg/m²) 27-40	**I:** usual diet enriched with macadamia **C:** only usual diet	**I:** 38:36:21; 1750 kcal. MUFA = 49% **C:** 38:42:17; 1750 kcal. MUFA = 41%	(µg/mL) **I :** 0 ± 1.3 **C:** -1.0 ± 1.41 (↓10.1%)

Abbreviations: I, intervention; C, control; BMI, body mass index; MetS, metabolic syndrome; SFA, saturated fatty acids; MUFA, monounsaturated fat acids; USFA, unsaturated fatty acids; PUFA, polyunsaturated fat acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; ALA, alpha-linolenic acid; NA, not available. Adiponectin concentrations expressed as means ± SDs or median (25th – 75th percentile). *Significant change from baseline ($P < 0.05$). † Significant difference between intervention and control groups ($P < 0.05$).

CAPÍTULO 4

A high-fat, high-saturated fat diet decreases insulin sensitivity without changing intra-abdominal fat in weight-stable overweight and obese adults

Authors: Anize D von Frankenberg[1,2,5], Anna Marina[1], Xiaoling Song[3], Holly S Callahan[4], Mario Kratz[1,3] and Kristina M Utzschneider[1,5]

Affiliations:

[1]Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, University of Washington, 1959 NE Pacific Street, Seattle, WA, 98195; anize.frankenberg@gmail.com (AF) annam45@hotmail.com (AM); mkratz@fhcrc.org (MK); kutzschn@u.washington.edu (KU)

[2] Post-Graduate Endocrinology Program, School of Medicine, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

[3]Fred Hutchinson Cancer Research Center, Division of Public Health Sciences, 1100 Fairview Ave N, Seattle, WA 98109 and the University of Washington, Department of Epidemiology, 1959 NE Pacific Street, Seattle, WA 98195; mkratz@fhcrc.org (MK) Xiaoling Song (XS)

[4]University of Washington, School of Medicine, 1959 NE Pacific Street, Seattle, WA 98195; hcal@uw.edu (HSC)

[5]VA Puget Sound Health Care System, Endocrinology, 1660 S Columbian Way (151), Seattle, WA, 98108; (KU)

Address for correspondence and reprints: Kristina M. Utzschneider, M.D.; Email: kutzschn@u.washington.edu**;** Tel: 206-277-3568**;** Fax: 206-764-2164

Word Count: Abstract: 250 Text: 4,164

ClinicalTrials.gov Identifier: NCT00930371

Key words: dietary fat, saturated fat, high-fat diet, insulin sensitivity

Os resultados desse artigo foram analisados durante o estágio de doutorado sanduíche no Departamento de Metabologia, Endocrinologia e Nutrição na Universidade de Washington sob a supervisão da orientadora estrangeira Dra. Kristina Utzschneider. Este artigo foi submetido para publicação na revista European Journal of Nutrition.

ABSTRACT

Purpose: We sought to determine the effects of dietary fat on insulin sensitivity and whether changes in insulin sensitivity were explained by changes in abdominal fat distribution or very low density lipoprotein (VLDL) fatty acid composition.

Methods: Overweight/obese adults with normal glucose tolerance consumed a control diet (35% fat/12% saturated fat/47% carbohydrate) for ten days, followed by a four week low fat (LFD, n=10: 20% fat/8% saturated fat/62% carbohydrate) or high fat diet (HFD, n=10: 55% fat/25% saturated fat/27% carbohydrate). All foods were provided and adjusted for weight stability. Insulin sensitivity was measured by labeled hyperinsulinemic-euglycemic clamps, abdominal fat distribution by MRI and fasting VLDL fatty acids by gas chromatography.

Results: The rate of glucose disposal (Rd) during low- and high-dose insulin, decreased on the HFD but remained unchanged on the LFD (Rd-low: LFD: 0.12±0.11 vs. HFD: -3.67±0.15 mmol/min, mean±SE, $p<0.01$; Rd-high: LFD: 0.11±0.37 vs. HFD: -0.71±0.26 mmol/min, $p=0.08$). Hepatic insulin sensitivity did not change. Changes in subcutaneous fat were positively associated with changes in insulin sensitivity on the LFD ($r=0.78$, $p<0.01$) with a trend on the HFD ($r=0.60$, $p=0.07$), whereas there was no association with intra-abdominal fat. The LFD led to an increase in VLDL stearic, palmitoleic and palmitic acids, while no changes were observed on the HFD. Changes in VLDL 22:5n6 were strongly associated with changes in insulin sensitivity on both diets (LFD: $r=-0.77$; $p<0.01$; HFD: $r=-0.71$; $p=0.02$).

Conclusions: A diet high in fat and saturated fat adversely affects insulin sensitivity and thereby might contribute to the development of type 2 diabetes.

INTRODUCTION

Type 2 diabetes is reaching epidemic proportions worldwide with the World Health Organization estimating that worldwide 9% of adults older than age 18 have diabetes with the vast majority having type 2 diabetes [1]. Elevated hepatic glucose production, impaired insulin secretion, and insulin resistance, a typical complication of obesity, are major factors underlying the pathogenesis of type 2 diabetes [2]. Weight loss induced by lifestyle measures including diet and physical activity has been shown to decrease the risk of developing diabetes, with a 16% reduction in risk for every kilogram of weight loss [3]. Insulin sensitivity is improved by hypocaloric dietary interventions irrespective of whether they are low or high in fat content [4-6], but this effect may be attributed to weight loss itself rather than diet composition. Thus, to determine the effects of dietary macronutrient composition on insulin sensitivity it is important that they be tested in the absence of weight change.

Medium to long-term diet intervention studies have examined whether isocaloric high-fat diets modify insulin sensitivity. Two studies that compared a high-fat diet (50-55% of calories as fat) *versus* a low-fat diet (20-25% of calories as fat) demonstrated no difference in insulin sensitivity measured by clamp, in healthy adults after three and two weeks respectively [7,8]. We also observed no significant change in fasting insulin concentrations or the Matsuda index measure of insulin sensitivity after four weeks on a high fat/high-saturated fat diet (43% calories from fat/24% saturated fat) in weight-stable older subjects [9]. Additionally, after 11 days on isocaloric low-fat, intermediate-fat or high-fat diets (0%, 41%, and 83% of fat respectively) insulin sensitivity did not differ between the high- and low-fat diets [10].

These data are at odds with the preconception that high fat diets lead to insulin resistance.

In contrast, a low-fat diet with less than 10% of energy from saturated fat improved insulin sensitivity after 24 weeks [11]. We also observed an improvement of the Matsuda index after four weeks on a low-fat/low saturated fat/low glycemic index diet [9]. Low fat diets are by default higher in carbohydrate content if protein content is kept stable. Carbohydrate content alone may modify insulin sensitivity with one study showing a significant increase in this parameter after eating a very high carbohydrate diet (85%) [12]. Diets high in carbohydrates also provide substrate to stimulate *de novo* lipogenesis (DNL) in the liver [13,14] and result in production of fatty acids such as stearic acid and palmitic acid that have been shown to be related to decreased insulin sensitivity [15]. However, increases in palmitoleate after a low fat diet may promote insulin sensitivity in white adipose tissue [15]. Thus, dietary effects on fatty acid composition may influence effects on insulin sensitivity and warrant investigation.

In addition to fatty acids, body fat composition and ectopic fat storage are thought to be important factors in regulating glucose metabolism [16]. Potential mediators from adipose tissue include free fatty acids, inflammatory cytokines and adipokines such as leptin and adiponectin [17]. Studies have associated increased visceral adipose tissue [18], but not subcutaneous adipose tissue, with decreased insulin sensitivity [19], and fat accumulation in the liver has been associated with insulin resistance [20]. Visceral fat is an important source of non-esterified fatty acids in the portal circulation [21], draining them directly to the liver and thereby altering hepatic glucose and lipid metabolism [22,23] and contributing to insulin resistance

[24]. Recently, the impact of dietary fat on body fat deposition has been explored. In cross-sectional studies, diets high in saturated fat are associated with increased total body and trunk fat deposition compared to diets low in fat [25]. Two studies that examined the effect of altering diet fat quality but kept total dietary fat intake constant found decreases in SQF with diets high in PUFA [26,27]. To our knowledge, only two studies have investigated the effect of a high-fat, high-saturated fat *versus* a low-fat, low-saturated fat diet on body fat distribution in weight-stable subjects. Neither found a significant effect on IAF or SQF [9,20]. However, we observed an increase in abdominal SQF after 4 weeks on a high fat/high saturated fat diet despite weight stability [28]. Whether such changes in abdominal fat distribution contribute to effects of dietary fat content on insulin sensitivity is unclear.

In this study, we sought to determine the effect of diets containing either low or high amounts of fat and saturated fat on determinants of glucose tolerance, specifically insulin sensitivity, both in insulin's ability to suppress endogenous glucose production and to promote glucose uptake, and insulin secretion in weight-stable overweight/obese subjects. We hypothesized that a diet high in fat would decrease insulin sensitivity while a diet low in fat would improve insulin sensitivity. Fiber intake was standardized across the different intervention arms so that this would not be a confounding factor. Since few previous studies have looked into mechanisms by which high fat diets affect insulin sensitivity, we further investigated possible mediators of diet-induced changes in insulin sensitivity by examining changes in abdominal fat distribution, adipokines and VLDL fatty acid analysis. We have previously published that the HFD led to increases in SQF [28], but examine here

whether such changes in body fat distribution contribute to changes in insulin sensitivity.

RESEARCH DESIGN AND METHODS

Study Design

The study was a prospective, random order, crossover, controlled dietary feeding study. Details on the study design have been previously published [28]. Briefly, subjects completed a 10-day control diet followed by four weeks on either a low fat diet (LFD) or high fat diet (HFD). All food was provided to the participants during the control, HFD and LFD periods. The seven subjects who completed both the HFD and LFD underwent a 6 week washout period during which subjects ate *ad lib* at home and no food was provided. The control diet was then repeated prior to the second intervention diet.

A portion of the data have been published previously [28] but are reproduced here for ease of reference.

Subjects

The study enrolled men and women between the ages of 18-55 years with BMI >27 kg/m^2 and normal glucose tolerance based on fasting (<5.5 mmol/L or <100 mg/dl) and 2 hour glucose (<7.8 mmol/L or <140 mg/dl) levels after a standard 75 gram oral glucose tolerance test. Exclusion criteria included tobacco use, significant medical illness, reported alcohol consumption >2 alcoholic drinks/day, alanine aminotransferase (ALT) >40 U/L, serum creatinine >132.6 µmol/l (>1.5 mg/dl) in men and >123.8 µmol/l (>1.4 mg/dl) in women, hematocrit <33%, fasting triglycerides >3.4

mmol/L (>300mg/dl), fasting LDL cholesterol >5.2 mmol/L (>200 mg/dl), food allergies/intolerances, contraindications to MRI, and use of any medications affecting inflammation, insulin sensitivity or liver fat. All subjects gave written informed consent. The study was approved by the Institutional Review Boards of the Veterans Affairs Puget Sound Health Care System and the University of Washington in accordance with ethical standards on human experimentation.

Dietary Intervention

Menus were designed by a research nutritionist using ProNutra (VioCare, Inc., Princeton, NJ) to contain the following: control: 35% energy from fat (12% saturated fat), 47% energy from carbohydrate, and 18% energy from protein; LFD: 20% energy from fat (8% saturated fat), 62% energy from carbohydrate, and 18% energy from protein; and HFD: 55% fat (25% saturated fat), 27% energy from carbohydrate, and 18% energy from protein. Caloric needs were estimated using the average of the Mifflin-St. Jeor [29] and Harris-Benedict [30] equations, adjusted for physical activity. Major sources of fats in all three diets included butter and high oleic safflower oil. Soluble fiber (inulin) was added to the HFD to standardize fiber content across diets. Because fructose was limited on the HFD due to the low carbohydrate content, fructose was limited in all diets to <30 g/day based on a 2000 kcal/day. The mean fructose intake was higher on the LFD and lower on the HFD. The composition of the diets is described in Table 1.

Subjects picked up their food from the metabolic kitchen and were weighed twice weekly. Caloric intake was adjusted to achieve weight stability. Subjects were instructed to maintain regular physical activity and to eat all of the food provided, not

to eat any non-study food, and to report any deviations from the diet. To determine compliance, subjects recorded all food consumed each day using a checklist which was returned to the nutritionist. All foods that were not consumed were returned to the Nutrition Research Kitchen, and weighed to determine the actual energy intake and composition of consumed foods.

Study Procedures

Study procedures were performed at the end of the control diet and at the end of the LFD or HFD. Subjects were told to fast for at least 10 hours before undergoing study procedures.

Intravenous-glucose tolerance test

An intravenous-glucose tolerance test (IVGTT) was performed to assess the acute insulin response to glucose and glucose tolerance. An intravenous line was established in an antecubital vein and the arm was wrapped in a heating pad to "arterialize" the blood. A bolus of glucose (11.4 g/m^2) was injected over 60 seconds and blood samples were drawn at -10, -5, -1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, and 30 min relative to the start of the glucose injection.

Hyperinsulinemic-euglycemic clamp

A two-step hyperinsulinemic-euglycemic clamp with 6,6-2d glucose isotope label was performed on the following day to estimate endogenous glucose production (EGP) and insulin sensitivity. After obtaining a baseline blood sample, a primed (200 mg/m^2 x glucose/100 over 5 minutes), continuous (2 mg/m^2/minute) infusion of 6,6-2d

glucose was started and continued throughout the clamp procedure. Following a three hour basal equilibration period a three hour low dose insulin infusion (20 mU/m^2/min) followed by a two hour primed, continuous high dose insulin infusion (160 mU/m^2/min x 5 minutes then 80 mU/m^2/min) was performed. Blood glucose was measured every 5 minutes using an iStat machine and a variable rate infusion of 20% dextrose enriched with 2% 6,6-2d glucose was titrated to maintain the blood glucose concentration at 90 mg/dl. Samples were drawn for glucose and insulin every 30 minutes throughout the clamp. Samples for glucose, insulin and 6,6-2d glucose were drawn every 15 minutes during the final half hour of the basal, low dose and high dose insulin infusion periods. Samples for non-esterified fatty acids (NEFAs) were drawn into tubes containing the lipolysis inhibitor tetrahydrolipstatin (orlistat) at -30, -15, -1, 10, 20, 30 and 60 minutes relative to the start of the low dose insulin infusion and placed immediately on ice. NEFA samples were processed within 30 minutes and the plasma flash frozen.

Fat Distribution

Total fat and lean mass were determined on the first control diet by Dual Energy X-Ray Absorptiometry using the QDR® 4500A bone densitometer system (Hologic, Inc. Bedford, MA).

Abdominal fat distribution (IAF and SQF) and liver fat were measured using MRI/MRS abdominal images as previously described [9]. The inter- and intra-scan CVs were 4.9% and 2.4% for IAF and 6.2% and 3.1% for SQF respectively. MRS was used to quantify hepatic triglyceride using a Philips Achieva 3 Tesla, version

2.5.3.0 (Philips, Andover, MA) whole body scanner. The inter- and intra-scan coefficients of variation (CVs) for liver fat were 18.6% and 1.2% respectively.

Assays

The following assays were performed: glucose by glucose oxidase; insulin by automated electrochemiluminescence immunoassay (Cobas e601, Indianapolis, IN); adiponectin and leptin by radioimmunoassay (Millipore, Billerica, MA). Intra- and inter- assay CVs (%) for the adiponectin assay were respectively 6.21 and 9.25 and for the leptin assay were 3.7 and 5.2. VLDL fatty acids were isolated by gradient ultracentrifugation. Fatty acid methyl ester samples were prepared by direct trans-esterification using the method of Lepage [31] and separated using gas chromatography (Agilent 5890 Gas Chromatograph with FID detector and ChemSation software; Supelco fused silica 100m capillary column SP-2560; initial 160°C for 16 min, ramp 3.0°C/min to 240°C, hold for 15 min) (Agilent technology, Santa Clara, CA). The CV (%) ranged from 0.7 to 13.1 depending on the type of VLDL fatty acid. Levels of 6,6 2d glucose were measured by mass spectrometry as previously described [32].

Calculations

IVGTT data

The acute insulin response to glucose (AIRg) was calculated as the AUC insulin response above basal from 0 to 10 minutes. AIRg was adjusted for insulin sensitivity measured by the clamp method to estimate beta-cell function. The glucose

disappearance constant (Kg), a measure of intravenous glucose tolerance, was calculated as the slope of the natural log of glucose from 10 to 30 minutes.

Clamp data

Isotopic steady state concentrations were achieved during the final 30 min of the basal and low- and high-dose insulin periods of the clamp. The rates of glucose appearance (Ra) and disappearance (Rd) were calculated based on steady-state equations modified to include the use of a labeled dextrose infusion [33]. Endogenous glucose production (EGP) was determined in the basal state and at the end of the low dose glucose infusion. Hepatic insulin sensitivity was determined both by the percent suppression of EGP from basal at the end of the low dose insulin infusion and as the hepatic insulin resistance index (HIR index: basal EGP x fasting plasma insulin).

Statistical Analysis

Data were expressed as mean ± standard error (SE) for normally distributed data or median (interquartile range) for non-normally distributed data. Generalized Estimating Equation (GEE) analysis was performed to determine the effect of diet type on the change in each outcome variable (intervention diet - respective control diet), adjusted for diet order and type. The GEE method focuses on average changes in response over time and the impact of covariates on these changes. Unlike RM-ANOVA, GEE does not require the outcome variable to have a normal distribution and permits use of all available data (even if the subject did not complete all study phases) in an unbalanced design, leading to more efficient effect estimates.

Individuals with missing data are considered a random subset of the sample. This feature benefits crossover studies in which missing data occurred and/or data are skewed due to a small sample size [34].

The significance of the associations between changes in insulin sensitivity and changes in abdominal fat distribution, adipokines and VLDL fatty acid composition were tested using non-parametric Spearman's correlation coefficient. A $p<0.05$ was considered significant. Statistical analyses were performed using SPSS software (V19.0, SPSS Inc, Chicago,IL).

RESULTS

Subject Characteristics

This was a prospective, crossover study where a total of 13 subjects (10M/3F: 3 African Americans, 1 Asian and 9 Caucasians; age 36±2.9 years; BMI 33.6±1.3 kg/m^2; fasting glucose 5.01±0.1 mmol/l; 2-hour glucose 5.3±0.33 mmol/l) completed diets and procedures. Seven participants completed both intervention diets (control+LFD and control+HFD, n=7) and six participants completed only one of the diet interventions (control+LFD, n=3) or (control+HFD, n=3). All available data was included in the analysis. All participants except two reported consuming all food provided [28]. Removing these two subjects from all analyses did not change the results.

Response to the LFD

Compared to the control diet, body weight did not change. The glucose disappearance constant, Kg, increased significantly after the LFD (Table 2), demonstrating improved glucose tolerance, despite no significant change in AIRg. Glucose and insulin levels were well matched during the clamp (Figures 1A and 1C). There was no significant change in insulin sensitivity as measured by the rate of glucose disposal (Rd) during the low and high-level insulin infusions (Figure 1E), HIR index, basal EGP, or insulin-mediated suppression of EGP on the LFD compared to the control diet (Table 2). Additionally, during the clamp there was no difference in the ability of low dose insulin to suppress free-fatty acids (Figure 1G). As previously published [28], liver fat decreased significantly during the LFD, but there were no significant changes in fasting glucose and insulin, IAF, SQF or adipokines (Table 2). Changes in the percent fatty acid composition of VLDL are shown in Table 3. The proportion of stearic acid increased significantly whereas palmitic acid showed a trend to increase during the LFD. Among monounsaturated fatty acids, palmitoleic showed an increase during the LFD. Finally, the most abundant polyunsaturated fatty acid, linoleic acid, decreased significantly during the LFD.

Response to the HFD

Compared to the control diet, there were no significant changes in body weight, AIRg, Kg, HIR index, EGP, or EGP suppression during the HFD (Table 2). Glucose and insulin levels were well matched during the clamp (Figures 1B and 1D). There was a significant decrease in Rd but no change in the ability of low dose insulin to suppress free-fatty acids (Figures 1F and 1H). There was no significant

change in IAF or liver fat; however, there was a significant increase in SQF with the HFD. All other metabolic parameters did not change (Table 2).

There was no change in the percent fatty acid composition of VLDL during the HFD (Table 3).

Comparison of the LFD and HFD

Changes from control were compared between the LFD and HFD adjusted for diet order and type. There was a significant difference in the change in Rd-low (LFD: 0.12±0.11 vs. HFD: -3.67±0.15 mmol/min, mean±SE, $p<0.01$) with a trend for Rd-high (LFD: 0.11±0.37 vs. HFD: -0.71±0.26 mmol/min, $p=0.08$). Kg was significantly improved on the LFD compared to the HFD (LFD: 0.26±0.12 vs. HFD: -0.36±0.18 %/min, $p<0.01$). The increase in SQF on the HFD was significant compared to the change on the LFD (LFD: 9.3±42.9 vs. HFD: 156.4±42.3 cm^3, $p=0.02$) (Table 2). There were no significant differences between changes on the LFD vs. the HFD in the other metabolic variables.

Correlates of changes in insulin sensitivity

After the LFD, changes in SQF were positively associated with changes in Rd-low (Supplemental Figure 1A: $r=0.78$; $p<0.01$) and with changes in Rd-high ($r=0.83$; $p<0.01$). After the HFD, increases in SQF also tended to be positively associated with changes in Rd-low (Supplemental Figure 1B: $r=0.60$; $p=0.07$) but not with changes in Rd-high ($r=-0.52$; $p=0.13$). There was no association between changes in Rd-low or Rd-high and IAF on either the LFD (Rd-low: $r=-0.10$; $p=0.78$; Rd-high: $r=0.20$; $p=0.58$) or HFD (Rd-low: $r=-0.26$; $p=0.47$; Rd-high: $r=0.39$; $p=0.21$) (Supplemental

Figure 1C, 1D). There were no significant associations between changes in Rd-low or Rd-high and changes in the SQF/IAF ratio, liver fat, or adipokines (data not shown) after either diet.

Among VLDL fatty acids, changes in VLDL 22:5n6 were strongly negatively associated with changes in Rd-low after both diets (LFD: r=-0.77; p<0.01; HFD: r=-0.71; p=0.02) (Supplemental Figure 2). An increase of palmitic acid was associated with an increase in hepatic insulin resistance after the LFD (r=0.79; p=0.01) but not after the HFD (r=0.07; p=0.86). There were no significant associations between changes in any of the other VLDL fatty acids and changes in Rd, HIR or percent suppression of EGP.

After either LFD or HFD, there were no correlations between changes in HIR index and changes in SQF, IAF, or liver fat. Also, after both LFD and HFD there were no associations between changes in EGP suppression and changes in SQF, IAF, or liver fat.

Correlates of changes in glucose tolerance

After the LFD, changes in Kg were not associated with changes in AIRg, Rd- or Rd-high. After the HFD, changes in Kg were positively associated with changes in AIRg (r=0.82; p<0.01) but not with changes in Rd-low or Rd-high.

DISCUSSION

Four weeks on a diet high in fat and saturated fat significantly decreased insulin sensitivity in overweight/obese subjects despite the absence of weight gain. However, a diet low in fat and saturated fat did not improve insulin sensitivity. The

decrease in insulin sensitivity on the HFD could not be explained by changes in IAF, liver fat, or adipokines. However, positive correlations between changes in SQF and insulin sensitivity were observed. Intriguingly, changes in VLDL 22:5n6 were strongly negatively correlated with changes in insulin sensitivity on both the high fat and low fat diets.

We explored potential mechanisms related to the decrease in insulin sensitivity on the HFD. The strongest association we observed was a negative association between changes in insulin sensitivity and changes in VLDL 22:5n6. This association was observed on both the HFD and the LFD. This fatty acid is the end-product of n-6 PUFA desaturation and elongation. Although such a correlation does not imply a causal role, the strength of the correlation despite small changes is intriguing and further study into the role of 22:5n6 in metabolic processes is warranted. Unfortunately, data regarding the relative amounts of this fatty acid are lacking in the literature.

The VLDL fatty acid profile is strongly correlated with the fatty acid profile within the liver as assessed by liver biopsies [35]. On a balanced diet (30% as fat and 55% as carbohydrate), approximately 15% of the hepatic triglyceride is derived from the diet [35]. We anticipated that increasing the dietary contribution of saturated fatty acids would lead to increased saturated fatty acids within the liver and contribute to hepatic insulin resistance. However, we observed changes in Rd, reflecting mainly uptake of glucose into muscle, and no changes in measures of hepatic insulin sensitivity.

Other possible mechanisms whereby increased dietary fat intake decreases insulin sensitivity include decreases in cell membrane responsiveness to insulin

action through decreases in binding affinity [36]. Others have reported an exaggerated synthesis of ceramides from a high-fat diet enriched with saturated fatty acid (i.e.palmitate), which might also induce insulin resistance [37]. While others have proposed it is mediated by increases in inflammatory cytokines [38], we did not observe any changes in inflammatory markers [28] and no associations between changes in these markers and insulin sensitivity despite the very high saturated fat intake in our study (unpublished observations). This would argue against inflammation as a major underlying mechanism.

In contrast to the findings on the HFD, no improvement of insulin sensitivity was observed on the LFD. One possible explanation is that changes in insulin sensitivity on the HFD were driven by the high saturated fat content rather than total dietary fat intake. If this were the case, the lack of change in insulin sensitivity on the LFD may have been due in part to the relatively small change in saturated fat (11.9% control to 7.7% LFD). In contrast, the difference in saturated fat content between the control diet and the HFD (11.6% to 23.7%) was quite large. In a previous study, a high-saturated fat diet (17% of energy from saturated fat) reduced insulin sensitivity by 12.5% after three months of intervention in healthy subjects compared to baseline [39]. Our study was designed to compare a high fat diet with a low fat diet and was not specifically designed to determine the effect of dietary saturated fat per se. Other iso-energetic feeding studies have compared high PUFA vs. high saturated fat or high MUFA vs. saturated fat on insulin sensitivity [26,39-42]. A single liquid meal high in polyunsaturated fat (PUFA) improved postprandial insulin sensitivity as compared with a high-fat, high-saturated fat meal [41]. Additionally, after 24 hours, high-saturated fat ingestion decreased insulin sensitivity compared to both control and

high PUFA interventions [40]. Longer term studies showed increases in insulin sensitivity after 6 and 12 weeks on a diet containing large amounts of monounsaturated fat (MUFA) compared to a high-carbohydrate or a high-saturated fat diet [42,39], and after five weeks on a diet high in PUFA vs. saturated fat [26].

Another possible explanation for the finding that insulin sensitivity did not improve on the LFD compared to the control diet may be that the effect of dietary fat intake on insulin sensitivity is not linear, or that there may be a threshold effect that reduces insulin sensitivity only at very high fat intake levels. It is possible that the LFD, which by default contained higher carbohydrates, stimulated deleterious metabolic pathways that counterbalanced beneficial effects leading to no net benefit. One such pathway is hepatic *de novo* lipogenesis, which is known to be driven by high carbohydrate intake. The increase we observed in the proportion of VLDL stearic and palmitic acid, both saturated fatty acids, on the LFD likely reflects an increase in *de novo* lipogenesis [43]. There is evidence that stearic acid in the diet or as free-fatty acid induces insulin resistance [44]. Moreover, in a recent cohort study, palmitic and stearic acids, measured in plasma phospholipids, were positively associated with incident type 2 diabetes [45]. In contrast, the increase of VLDL palmitoleate observed on the LFD might mediate insulin-sensitizing effects, in part due to suppressing pro-inflammatory gene expression in white adipose tissue which has been observed in mice [46]; however, there are conflicting data in humans [47,48]. One observational study found no difference in palmitoleate content, measured in both plasma and VLDL, in insulin sensitive or resistant obese subjects, which suggests that there is no association between palmitoleate availability and insulin resistance [47]. In a prospective study, decreasing content of free fatty acid

palmitoleate was associated with improvement in insulin sensitivity after one year of a lifestyle intervention; however, this effect was not independent of lifestyle changes [48]. Therefore, any potential benefit in insulin sensitivity associated with an increase of palmitoleate could have been attenuated by increases in stearic and palmitic acid resulting in no net benefit.

Despite the lack of effect on insulin sensitivity, the LFD did result in an improvement of glucose tolerance. However, there were no changes in beta-cell function or insulin sensitivity after the LFD. Moreover, we did not find associations between improvement in glucose tolerance with changes in AIRg or Rd after the LFD to explain this finding.

The strengths of our study include the controlled diet intervention, weight stability, measurement of abdominal fat distribution and the use of labeled clamps to measure insulin sensitivity. There are some limitations to the present study. While the HFD was designed to be high in saturated fat, our study was designed to compare the effects of a low vs high fat diet and not specifically designed to compare saturated fat vs. other types of fat. Thus, conclusions about the effect of the high saturated fat content vs total fat content cannot be made. Second, because of the small sample size and four week time period, small effects of the LFD on insulin sensitivity might have been missed. Third, we studied subjects with normal glucose tolerance and normal liver enzymes. Thus, our findings reflect relatively healthy overweight/obese adults who may be more able to quickly adapt to changes in dietary lipid intake. The results therefore cannot necessarily be extrapolated to individuals with impaired glucose metabolism or diabetes. Additionally, the HFD contained very high fat and saturated fat, which is not typical of a diet consumed by

free living individuals. Finally, the complexity of dietary composition manipulation does not permit matching of all nutrients. While maintaining protein intake stable, as dietary fat content decreases, carbohydrate content increases and vice versa. The very low carbohydrate content on the HFD also prevented matching of fructose content, although this was limited in all diets. The higher fructose content in the LFD is unlikely to have impacted our results as the average difference between the control and LFD diet was only 12 g per day and both were relatively low in fructose. We did match fiber intake as this has been shown to affect glucose metabolism [49].

CONCLUSIONS

Based on a significant decrease in insulin sensitivity after a diet high in fat and saturated fat we conclude that such a diet may be detrimental for glucose homeostasis and could contribute to the development of type 2 diabetes. While the low fat, low saturated fat diet did improve intravenous glucose tolerance (Kg), we failed to observe any improvement in insulin sensitivity. We hypothesize that this could be related to counterbalancing effects of higher carbohydrate intake driving de novo synthesis of detrimental fatty acids.

Acknowledgements: We are grateful to the study participants for their contribution and time. This study was supported by funding and resources from the Department of Veteran Affairs, Diabetes Research Center (P30DK017047**)**, the Institute for Translational Health Sciences (UL1TR000423), the Nutrition Obesity Research Center (P30 DK035816), the Diagnostic Imaging Sciences Center at the University of Washington, and the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) (AF).

CONFICT OF INTEREST: The authors declare that they have no conflict of interest.

REFERENCES

1. Organization WH (2014) Global status report on noncommunicable diseases 2014. Geneva

2. Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444 (7121):840-846. doi:10.1038/nature05482

3. Hamman RF, Wing RR, Edelstein SL, Lachin JM, Bray GA, Delahanty L, Hoskin M, Kriska AM, Mayer-Davis EJ, Pi-Sunyer X, Regensteiner J, Venditti B, Wylie-Rosett J (2006) Effect of weight loss with lifestyle intervention on risk of diabetes. Diabetes care 29 (9):2102-2107. doi:10.2337/dc06-0560

4. Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, Greenberg I, Golan R, Fraser D, Bolotin A, Vardi H, Tangi-Rozental O, Zuk-Ramot R, Sarusi B, Brickner D, Schwartz Z, Sheiner E, Marko R, Katorza E, Thiery J, Fiedler GM, Bluher M, Stumvoll M, Stampfer MJ, Dietary Intervention Randomized Controlled Trial G (2008) Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. The New England journal of medicine 359 (3):229-241. doi:10.1056/NEJMoa0708681

5. Guldbrand H, Dizdar B, Bunjaku B, Lindstrom T, Bachrach-Lindstrom M, Fredrikson M, Ostgren CJ, Nystrom FH (2012) In type 2 diabetes, randomisation to advice to follow a low-carbohydrate diet transiently improves glycaemic control compared with advice to follow a low-fat diet producing a similar weight loss. Diabetologia 55 (8):2118-2127. doi:10.1007/s00125-012-2567-4

6. Guldbrand H, Lindstrom T, Dizdar B, Bunjaku B, Ostgren CJ, Nystrom FH, Bachrach-Lindstrom M (2014) Randomization to a low-carbohydrate diet advice improves health related quality of life compared with a low-fat diet at similar weight-

loss in Type 2 diabetes mellitus. Diabetes research and clinical practice. doi:10.1016/j.diabres.2014.08.032

7. van Herpen NA, Schrauwen-Hinderling VB, Schaart G, Mensink RP, Schrauwen P (2011) Three weeks on a high-fat diet increases intrahepatic lipid accumulation and decreases metabolic flexibility in healthy overweight men. The Journal of clinical endocrinology and metabolism 96 (4):E691-695. doi:10.1210/jc.2010-2243

8. Eckel RH, Hernandez TL, Bell ML, Weil KM, Shepard TY, Grunwald GK, Sharp TA, Francis CC, Hill JO (2006) Carbohydrate balance predicts weight and fat gain in adults. The American journal of clinical nutrition 83 (4):803-808

9. Utzschneider KM, Bayer-Carter JL, Arbuckle MD, Tidwell JM, Richards TL, Craft S (2013) Beneficial effect of a weight-stable, low-fat/low-saturated fat/low-glycaemic index diet to reduce liver fat in older subjects. The British journal of nutrition 109 (6):1096-1104. doi:10.1017/S0007114512002966

10. Bisschop PH, de Metz J, Ackermans MT, Endert E, Pijl H, Kuipers F, Meijer AJ, Sauerwein HP, Romijn JA (2001) Dietary fat content alters insulin-mediated glucose metabolism in healthy men. The American journal of clinical nutrition 73 (3):554-559

11. Jebb SA, Lovegrove JA, Griffin BA, Frost GS, Moore CS, Chatfield MD, Bluck LJ, Williams CM, Sanders TA, Group RS (2010) Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity and cardiovascular risk: the RISCK (Reading, Imperial, Surrey, Cambridge, and Kings) trial. The American journal of clinical nutrition 92 (4):748-758. doi:10.3945/ajcn.2009.29096

12. Chen M, Bergman RN, Porte D, Jr. (1988) Insulin resistance and beta-cell dysfunction in aging: the importance of dietary carbohydrate. The Journal of clinical endocrinology and metabolism 67 (5):951-957. doi:10.1210/jcem-67-5-951

13. Sjostrom L (1973) Carbohydrate-stimulated fatty acid synthesis de novo in human adipose tissue of different cellular types. Acta medica Scandinavica 194 (5):387-404

14. Angel A, Bray GA (1979) Synthesis of fatty acids and cholesterol by liver, adipose tissue and intestinal mucosa from obese and control patients. European journal of clinical investigation 9 (5):355-362

15. Hudgins LC, Hellerstein M, Seidman C, Neese R, Diakun J, Hirsch J (1996) Human fatty acid synthesis is stimulated by a eucaloric low fat, high carbohydrate diet. The Journal of clinical investigation 97 (9):2081-2091. doi:10.1172/JCI118645

16. Jazet IM, Pijl H, Meinders AE (2003) Adipose tissue as an endocrine organ: impact on insulin resistance. The Netherlands journal of medicine 61 (6):194-212

17. Mlinar B, Marc J (2011) New insights into adipose tissue dysfunction in insulin resistance. Clinical chemistry and laboratory medicine : CCLM / FESCC 49 (12):1925-1935. doi:10.1515/CCLM.2011.697

18. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE (2003) Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 46 (4):459-469. doi:10.1007/s00125-003-1074-z

19. Hsieh CJ, Wang PW, Chen TY (2014) The relationship between regional abdominal fat distribution and both insulin resistance and subclinical chronic inflammation in non-diabetic adults. Diabetology & metabolic syndrome 6 (1):49. doi:10.1186/1758-5996-6-49

20. Westerbacka J, Lammi K, Hakkinen AM, Rissanen A, Salminen I, Aro A, Yki-Jarvinen H (2005) Dietary fat content modifies liver fat in overweight nondiabetic

subjects. The Journal of clinical endocrinology and metabolism 90 (5):2804-2809. doi:10.1210/jc.2004-1983

21. Arner P (1995) Differences in lipolysis between human subcutaneous and omental adipose tissues. Annals of medicine 27 (4):435-438

22. Fisher EA, Ginsberg HN (2002) Complexity in the secretory pathway: the assembly and secretion of apolipoprotein B-containing lipoproteins. The Journal of biological chemistry 277 (20):17377-17380. doi:10.1074/jbc.R100068200

23. Rebrin K, Steil GM, Getty L, Bergman RN (1995) Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. Diabetes 44 (9):1038-1045

24. Boden G (1997) Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46 (1):3-10

25. Neville MM, Geppert J, Min Y, Grimble G, Crawford MA, Ghebremeskel K (2012) Dietary fat intake, body composition and blood lipids of university men and women. Nutrition and health 21 (3):173-185. doi:10.1177/0260106012467242

26. Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML, Moore NR, Frayn KN (2002) Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. Diabetologia 45 (3):369-377. doi:10.1007/s00125-001-0768-3

27. Tapsell LC, Batterham MJ, Teuss G, Tan SY, Dalton S, Quick CJ, Gillen LJ, Charlton KE (2009) Long-term effects of increased dietary polyunsaturated fat from walnuts on metabolic parameters in type II diabetes. European journal of clinical nutrition 63 (8):1008-1015. doi:10.1038/ejcn.2009.19

28. Marina A, van Frankenberg AD, Suvag S, Callahan HS, Kratz M, Richards TL, Utzschneider KM (2014) Effects of Dietary Fat and Saturated Fat Content on Liver

Fat and Markers of Oxidative Stress in Overweight/Obese Men and Women under Weight-Stable Conditions. Nutrients 6 (11):4678-4690. doi:10.3390/nu6114678

29. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO (1990) A new predictive equation for resting energy expenditure in healthy individuals. The American journal of clinical nutrition 51 (2):241-247

30. Harris JA BF (1919) A Biometric Study of Basal Metabolism in Man. Washington, DC, Carnegie Institution of Washington

31. Lepage G RC (1999) Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 27:114-120

32. Yarasheski KE, Cade WT, Overton ET, Mondy KE, Hubert S, Laciny E, Bopp C, Lassa-Claxton S, Reeds DN (2011) Exercise training augments the peripheral insulin-sensitizing effects of pioglitazone in HIV-infected adults with insulin resistance and central adiposity. American journal of physiology Endocrinology and metabolism 300 (1):E243-251. doi:10.1152/ajpendo.00468.2010

33. Wolfe RR (1992) Radioactive and Stable Isotope Tracers in Biomedicine. . Wiley-Liss, Nerw York

34. Ma Y, Mazumdar M, Memtsoudis SG (2012) Beyond repeated-measures analysis of variance: advanced statistical methods for the analysis of longitudinal data in anesthesia research. Regional anesthesia and pain medicine 37 (1):99-105. doi:10.1097/AAP.0b013e31823ebc74

35. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ (2005) Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. The Journal of clinical investigation 115 (5):1343-1351. doi:10.1172/JCI23621

36. Manco M, Calvani M, Mingrone G (2004) Effects of dietary fatty acids on insulin sensitivity and secretion. Diabetes, obesity & metabolism 6 (6):402-413. doi:10.1111/j.1462-8902.2004.00356.x

37. Eissing L, Scherer T, Todter K, Knippschild U, Greve JW, Buurman WA, Pinnschmidt HO, Rensen SS, Wolf AM, Bartelt A, Heeren J, Buettner C, Scheja L (2013) De novo lipogenesis in human fat and liver is linked to ChREBP-beta and metabolic health. Nature communications 4:1528. doi:10.1038/ncomms2537

38. Kennedy A, Martinez K, Chuang CC, LaPoint K, McIntosh M (2009) Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. The Journal of nutrition 139 (1):1-4. doi:10.3945/jn.108.098269

39. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, Storlien LH, Study K (2001) Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. Diabetologia 44 (3):312-319

40. Xiao C, Giacca A, Carpentier A, Lewis GF (2006) Differential effects of monounsaturated, polyunsaturated and saturated fat ingestion on glucose-stimulated insulin secretion, sensitivity and clearance in overweight and obese, non-diabetic humans. Diabetologia 49 (6):1371-1379. doi:10.1007/s00125-006-0211-x

41. Jans A, Konings E, Goossens GH, Bouwman FG, Moors CC, Boekschoten MV, Afman LA, Muller M, Mariman EC, Blaak EE (2012) PUFAs acutely affect triacylglycerol-derived skeletal muscle fatty acid uptake and increase postprandial

insulin sensitivity. The American journal of clinical nutrition 95 (4):825-836. doi:10.3945/ajcn.111.028787

42. Gadgil MD, Appel LJ, Yeung E, Anderson CA, Sacks FM, Miller ER, 3rd (2013) The effects of carbohydrate, unsaturated fat, and protein intake on measures of insulin sensitivity: results from the OmniHeart trial. Diabetes care 36 (5):1132-1137. doi:10.2337/dc12-0869

43. Aarsland A, Wolfe RR (1998) Hepatic secretion of VLDL fatty acids during stimulated lipogenesis in men. J Lipid Res 39 (6):1280-1286

44. van den Berg SA, Guigas B, Bijland S, Ouwens M, Voshol PJ, Frants RR, Havekes LM, Romijn JA, van Dijk KW (2010) High levels of dietary stearate promote adiposity and deteriorate hepatic insulin sensitivity. Nutrition & metabolism 7:24. doi:10.1186/1743-7075-7-24

45. Forouhi NG, Koulman A, Sharp SJ, Imamura F, Kroger J, Schulze MB, Crowe FL, Huerta JM, Guevara M, Beulens JW, van Woudenbergh GJ, Wang L, Summerhill K, Griffin JL, Feskens EJ, Amiano P, Boeing H, Clavel-Chapelon F, Dartois L, Fagherazzi G, Franks PW, Gonzalez C, Jakobsen MU, Kaaks R, Key TJ, Khaw KT, Kuhn T, Mattiello A, Nilsson PM, Overvad K, Pala V, Palli D, Quiros JR, Rolandsson O, Roswall N, Sacerdote C, Sanchez MJ, Slimani N, Spijkerman AM, Tjonneland A, Tormo MJ, Tumino R, van der AD, van der Schouw YT, Langenberg C, Riboli E, Wareham NJ (2014) Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study. The lancet Diabetes & endocrinology 2 (10):810-818. doi:10.1016/S2213-8587(14)70146-9

46. Yang ZH, Miyahara H, Hatanaka A (2011) Chronic administration of palmitoleic acid reduces insulin resistance and hepatic lipid accumulation in KK-Ay Mice with genetic type 2 diabetes. Lipids in health and disease 10:120. doi:10.1186/1476-511X-10-120

47. Fabbrini E, Magkos F, Su X, Abumrad NA, Nejedly N, Coughlin CC, Okunade AL, Patterson BW, Klein S (2011) Insulin sensitivity is not associated with palmitoleate availability in obese humans. J Lipid Res 52 (4):808-812. doi:10.1194/jlr.M013599

48. Corpeleijn E, Feskens EJ, Jansen EH, Mensink M, Saris WH, de Bruin TW, Blaak EE (2006) Improvements in glucose tolerance and insulin sensitivity after lifestyle intervention are related to changes in serum fatty acid profile and desaturase activities: the SLIM study. Diabetologia 49 (10):2392-2401. doi:10.1007/s00125-006-0383-4

49. Silva FM, Kramer CK, de Almeida JC, Steemburgo T, Gross JL, Azevedo MJ (2013) Fiber intake and glycemic control in patients with type 2 diabetes mellitus: a systematic review with meta-analysis of randomized controlled trials. Nutrition reviews 71 (12):790-801. doi:10.1111/nure.12076

Table 1: Diet composition

	Control LFD	LFD	Control HFD	HFD
Daily energy (kcal)	3284 ± 125	3321 ± 150	3140 ± 120	3208 ± 92
Fat (% of total energy)	35.8 ± 0.6	20.2 ± 0.003[1]	35.2 ± 0.02	54.8 ± 0.05 [2,3]
Saturated fat (% of total energy)	11.9 ± 0.4	7.7 ± 0.01[1]	11.6 ± 0.02	23.7 ± 0.05 [2,3]
MUFA (% of total energy)	16.7 ± 0.1	7.7 ± 0.01 [1]	16.6 ± 0.04	22.2 ± 0.06 [2,3]
PUFA (% of total energy)	4.7 ± 0.01	3.0 ± 0.01 [1]	4.7 ± 0.03	5.2 ± 0.04 [2,3]
n-6 PUFA (% of total energy)	2.7 ± 0.05	2.04 ± 0.02 [1]	2.8 ± 0.04	3.8 ± 0.06 [2,3]
n-3 PUFA (% of total energy)	0.12 ± 0.003	0.19 ± 0.002 [1]	0.13 ± 0.001	0.30 ± 0.002 [2,3]
Cholesterol (mg/day)	378 ± 10	492 ± 21 [1]	352 ± 11	506 ± 17 [2]
Trans-fat (% of total energy)	0.79 ± 0.04	0.53 ± 0.01 [1]	0.8 ± 0.04	1.37 ± 0.01 [2,3]
Protein (% of total energy)	17.9 ± 0.01	18.1 ± 0.02	17.8 ± 0.01	17.8 ± 0.03 [3]
Carbohydrate (% of total energy)	46.4 ± 0.6	61.7 ± 0.02	46.9 ± 0.01	27.4 ± 0.05 [2,3]
Total fiber (g/day)	47.2 ± 2.0	46.1 ± 2.1	45.8 ± 1.8	39.8 ± 1.3 [2,3]
Fructose (g/day)	34.1 ± 1.8	46.1 ± 2.1 [1]	33.1 ± 1.4	10.0 ± 0.3 [2,3]

Data for the study diet composition is inclusive of all subjects who completed the control and corresponding LFD or HFD (n = 10 for each). Mean diet composition data for the subset of subjects (n = 7) who completed both diet protocols is not listed separately here, but was similar to those who completed only one of the intervention diets. All data are reported as mean ± SEM. Abbreviations: MUFA = mono-unsaturated fatty acids, PUFA = poly-unsaturated fatty acids. [1] $p < 0.017$ compared to CONT LFD.[2] $p < 0.017$ compared to CONT HFD.[3] $p < 0.017$ HFD *vs.* LFD for the 7 subjects who completed both diet interventions.

Table 2: Effects of diets on body weight, liver fat, glucose metabolism, abdominal fat distribution and other metabolic parameters

	Low-fat diet			High-fat diet			
	Control	LFD	Change	Control	HFD	Change	Difference mean between the change on LFD vs. HFD (95% CI)
Sex (M/F)		9/1			7/3		
Weight (kg)	100.7±4.1	100.5±4.4	-0.51±0.47	104.0±5.8	104.1±5.9	0.06±0.37	-0.6 (-1.8; 0.6)
Fasting glucose (mmol/l)	5.3±0.18	5.4±0.22	0.01±0.11	5.3±0.18	5.3±0.16	-0.03±0.12	-0.003 (-0.3; 0.3)
Fasting insulin (pmol/l)	96.5±14.6	88.9±13.9	-7.6±8.3	115.3±25.7	104.2±22.9	-11.8±9.7	25.0 (-178.5; 229.2)
Rd-low (mmol/min)	1.77±0.21	1.84±0.25	0.12±0.11	1.99±0.27	1.56±0.26	-0.37±0.15 [1,2]	0.49 (0.14; 0.83)
Rd-high (mmol/min)	5.48±0.43	5.64±0.58	0.11±0.37	5.79±0.54	5.11±0.50	-0.71±0.26 [1]	0.82 (-0.09; 1.72)
AIRg (pmol/L)	6254±825	5512±1059	-251.2±482	6682±1332	6051±1083	-625±573	374 (-1605; 2353)
Kg (%/min)	1.8±0.19	2.3±0.3	0.26±0.12 [1,2]	2.4±0.3	2.1±0.3	-0.36±0.18	0.63 (0.21; 1.04)
HIR index (EGP x fasting insulin; mmol.pM.min$^{(-1)}$)	11696±1596	12610±1774	105 (-3798;4128)	15249±3548	12387±3551	-1553 (-4720;1526)	1547 (-7170; 10265)

Basal EGP (mmol/min)	176±12	182±15	4.2±13.8	173±5.4	163±12	-19.3±12.1	23.5 (-19.2; 66.1)
EGP supression (%)	59±3.7	58±6.2	-5.17±5.8	62±10.2	54±6.5	-7.7±7.8	2.6 (-14.1; 19.3)
Liver fat (%)	9.4±2.4	7.2±2.4	-2.1±0.8 [1]	8.3±2.5	7.0±2.3	-1.2±0.7	-0.9 (-2.9; 1.1)
IAF (cm^3)	1479±331	1447±321	-74.3±54.3	1179±235	1202±238	17.2±63.4	-91.5 (-229.5; 46.4)
SQF (cm^3)	2440±316	2413±301	9.3±42.9	2704±478	2861±483	156.4±42.3 [1,2]	-147 (-271.7; -22.5)
Adiponectin (µg/ml)	3.4±0.3	4.1±1.2	0.03±0.6	4.2±0.9	4.6±1.2	0.67±0.8	-0.65 (-2.9; 1.67)
Leptin (µg/l)	13.9±3.3	15.1±3.3	0.9±0.6	17.3±3.5	16.8±4.1	-0.6±1.9	-1.5 (-5.3; 2.3)

Data are presented as Mean±SEM or Median (p25;p75).

Abbreviations: AIR = acute insulin response, Kg = glucose disappearance constant, EGP = endogenous glucose production, IAF = intra-abdominal fat, SQF = subcutaneous fat, Rd-low = rate of glucose disposal during low-level insulin infusion, Rd-high = rate of glucose disposal during high-level insulin infusion, Fasting glucose = average of (-10 min), (-5 min), (-1 min) during the IVGTT, Fasting insulin = average of (-10 min), (-5 min), (-1 min) during the IVGTT

[1]$p<0.05$ vs. control; [2] $p<0.05$ LFD vs. HFD.

Table 3: The effect of a HFD and a LFD on VLDL fatty acids

Abbreviation	Trivial names	Control (%)	LFD (%)	Change (%)	Control (%)	HFD (%)	Change (%)	Difference Mean (95% CI)
Saturated fat								
16:0	Palmitic	24.65±0.77	27.31±1.15	2.7±1.1[1]	25.43±0.62	25.57±0.51	0.5±0.6	2.26 (-0.07;1.59)
18:0	Stearic	3.58±0.39	4.33±0.42	0.9±0.23[2]	4.54±0.42	4.60±0.33	0.08±0.22	0.77 (0.23; 1.36)
22:0	Behenic	0.04±0.01	0.04±0.00	-0.01±0.002	0.05±0.01	0.06±0.01	-0.01±0.01	-0.01 (-0.04; 0.01)
MUFA								
16:1n7c	Palmitoleic	3.19±0.27	3.49±0.28	0.55±0.12[2]	3.27±0.31	2.98±0.29	-0.26±0.21	0.81 (0.36; 1.26)
18:1n7c	Vaccenic	2.15±0.13	2.19±0.10	0.06±0.05	2.23±0.13	2.22±0.09	-0.0006±0.07	0.06 (-0.08; 0.20)
18:1n9c	Oleic	35.88±1.34	32.28±0.93	-2.9±1.3	35.57±1.16	34.45±1.18	-0.4±0.9	-2.47 (-6.43; 1.48)
20:1n9	Eicosenoic	0.29±0.02	0.26±0.02	-0.03±0.01	0.29±0.02	0.29±0.02	-0.004±0.02	-0.04 (-0.09; 0.01)
n-6 PUFA								
18:2n6	Linoleic	19.49±1.49	18.15±1.64	-2.0±0.7[2]	17.34±1.30	18.30±1.30	0.92±1.1	-2.88 (-5.27; -0.5)
18:3n6	γ-linoleic	0.42±0.04	0.37±0.03	-0.06±0.03	0.39±0.04	0.39±0.05	0.0002±0.06	-0.06 (-0.19; 0.07)
20:4n6	Arachidonic	1.32±0.10	1.30±0.10	0.02±0.09	1.22±0.07	1.31±0.11	0.09±0.12	-0.06 (-0.20; 0.08)
22:5n6	Dpan-6	0.14±0.01	0.15±0.02	-0.01±0.01	0.13±0.02	0.15±0.02	0.02±0.02	-0.04 (-0.10; 0.02)
22:2n6	Eicosadienoic	0.24±0.00	0.02±0.00	0.002±0.002	0.04±0.01	0.04±0.01	-0.004±0.005	0.01 (-0.00; 0.02)

20:3n6	Homo-γ-linoleic	0.40±0.03	0.40±0.03	-0.002±0.02	0.38±0.02	0.39±0.03	0.02±0.02	-0.02 (-0.09; 0.04)
n-3 PUFA								
18:3n3	α-linolenic	1.01±0.14	1.15±0.09	0.05±0.17	1.04±0.11	0.99±0.13	-0.04±0.06	0.09 (-0.30; 0.48)
20:5n3	Eicosapentaenoic	0.16±0.03	0.19±0.03	-0.006±0.04	0.16±0.02	0.17±0.02	0.009±0.02	-0.02 (-0.11; 0.08)
22:5n3	Docosapentaenoic	0.32±0.04	0.34±0.04	0.06±0.03	0.31±0.03	0.38±0.06	0.07±0.03	-0.01 (-0.04; 0.02)
22:6n3	Docosahexaenoic	0.46±0.05	0.48±0.09	0.01±0.03	0.35±0.06	0.43±0.07	0.06±0.04	-0.06 (-0.28; 0.16)
Trans-fat								
18:2n6ct	9-cis 12-trans octadecaenoic	0.18±0.01	0.18±0.02	0.006±0.006	0.19±0.01	0.19±0.02	-0.002±0.02	0.00 (0.00; 0.00)

FIGURE 1

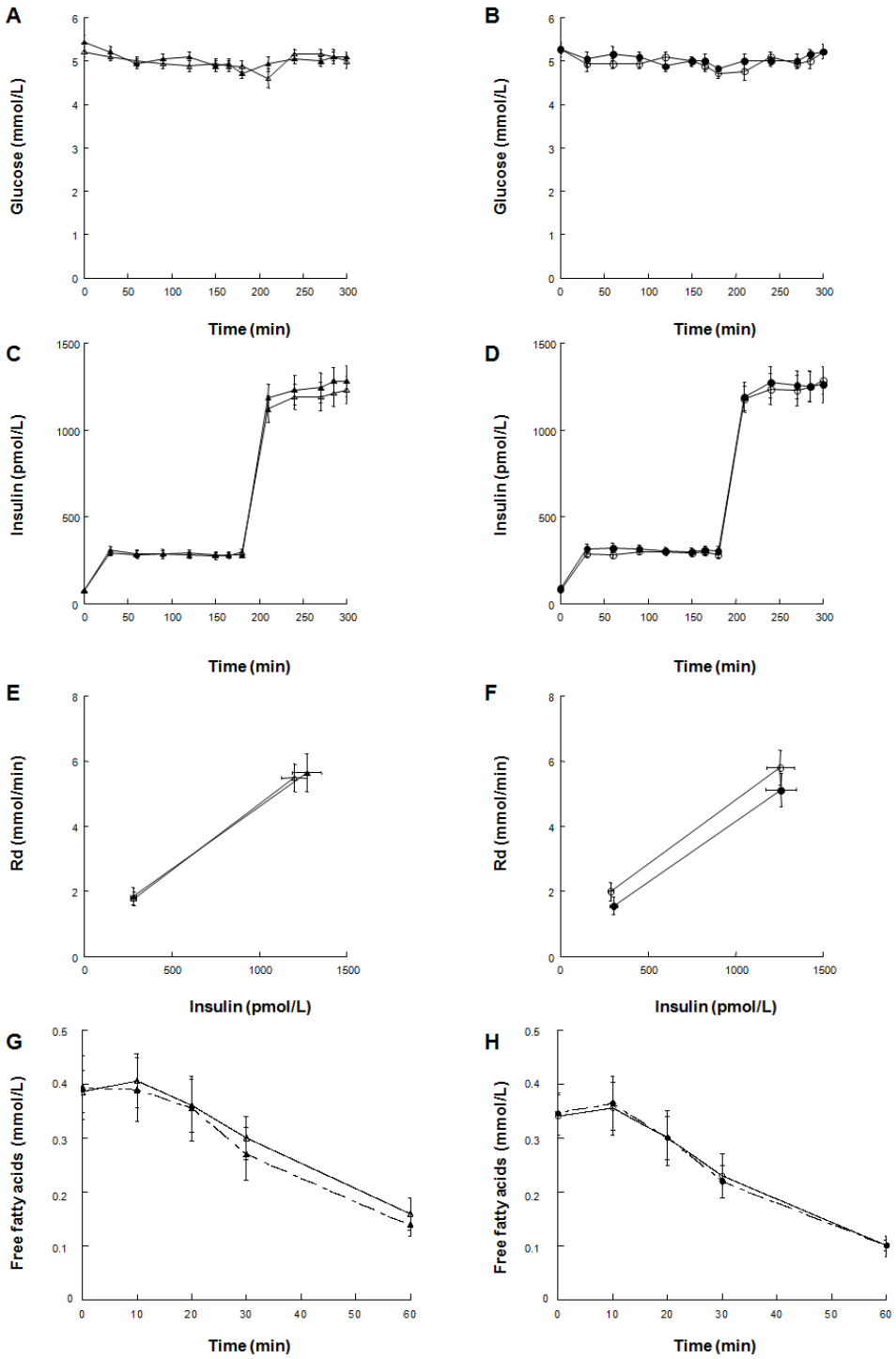
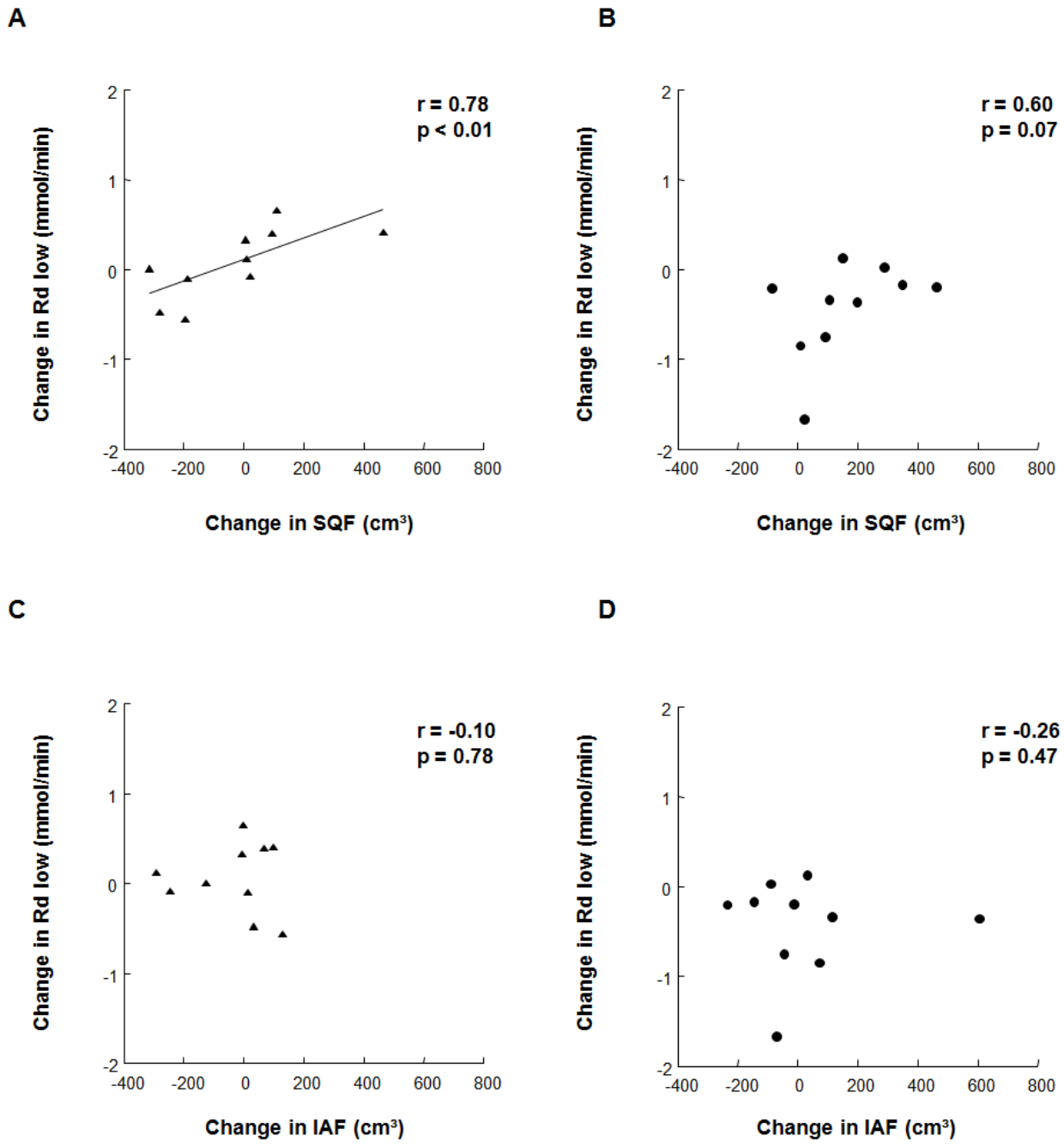


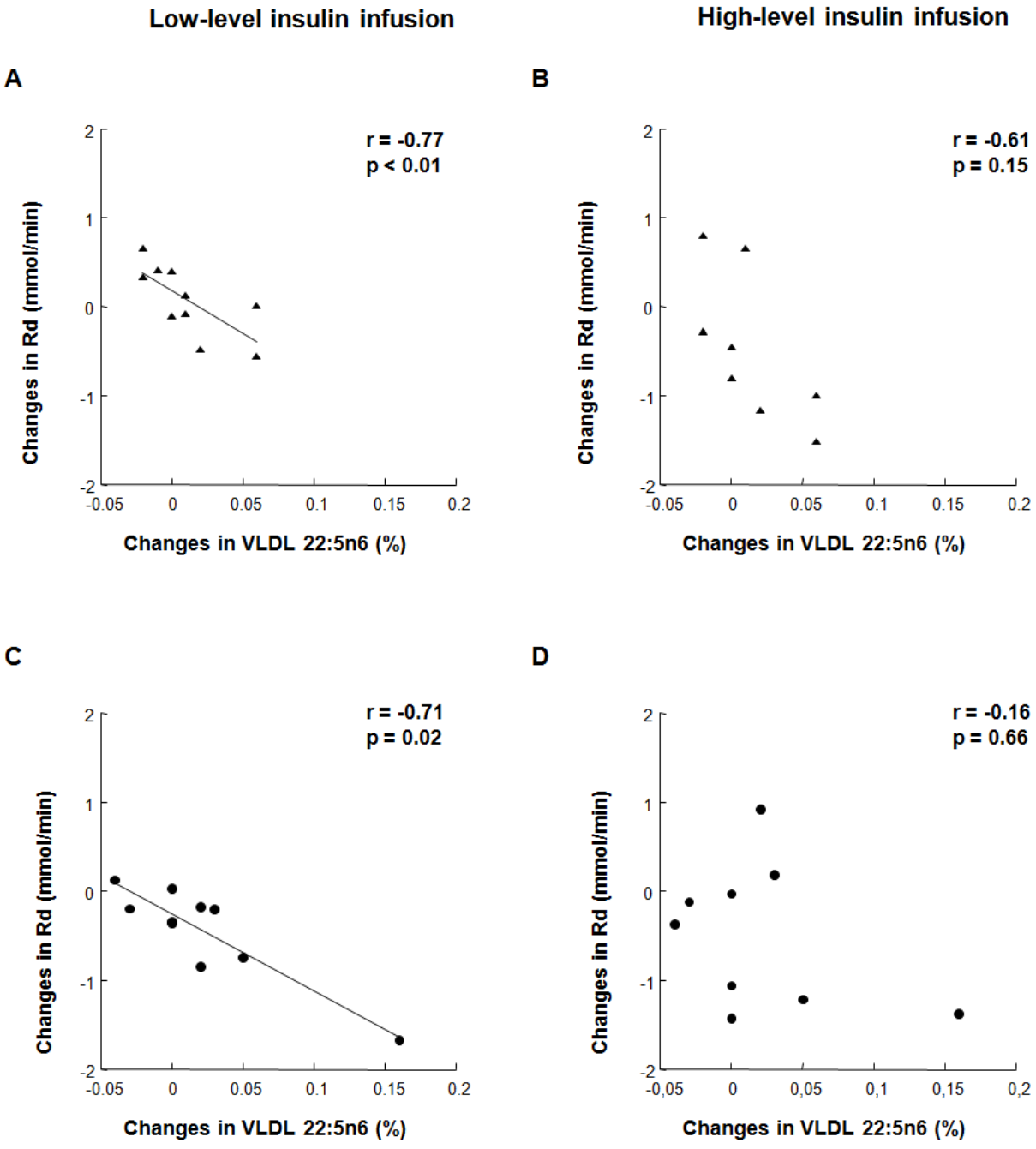
Fig 1. Glucose (A and B) and insulin (C and D) levels were well matched during the clamps. The rate of glucose disposal (Rd) did not change on the LFD (E) but decreased on the HFD (F). There was no difference in free-fatty acid suppression after the LFD (G) and the HFD (H). Symbols: control prior to LFD open triangle and solid line; LFD solid triangle and dashed line; and control prior to HFD open circle and solid line; HFD solid circle and dashed line. Mean±SEM.

SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE 1. Association between changes in insulin sensitivity (Rd = rate of glucose disposal during the low-level insulin infusion clamp) and changes in subcutaneous fat (A and B) and intra-abdominal fat (C and D). Symbols: LFD solid triangle; HFD solid circle.

SUPPLEMENTAL FIGURE 2



SUPPLEMENTAL FIGURE 2. Association between changes in insulin sensitivity (Rd = rate of glucose disposal) and changes in VLDL 22:5n6. Rd during the low-level insulin infusion clamp (A and C). Rd during the high-level insulin infusion clamp (B and D). Symbols: LFD solid triangle; HFD solid circle.

CAPÍTULO 5

CONSIDERAÇÕES FINAIS

Os níveis de adiponectina circulante estão reduzidos na presença da síndrome metabólica e, ainda, diminuem com o aumento do número de critérios para síndrome metabólica. A relação da adiponectina com o colesterol HDL, os triglicerídeos e a gordura abdominal podem explicar em parte os menores níveis de adiponectina encontrados nos indivíduos que apresentam síndrome metabólica. Ainda, sugerimos que a inflamação subclínica e a resistência à insulina podem ser mecanismos que contribuem para a redução dos níveis de adiponectina.

Com base nos achados da revisão sistemática e meta-análise, dentre as intervenções dietéticas avaliadas, as dietas com restrição de lipídios totais não demonstraram efeito na modificação da adiponectina circulante. Já a suplementação de ácido graxo poliinsaturado ômega-3 foi associada com aumento moderado da adiponectina. Em contrapartida, o ácido linoléico conjugado parece reduzir os níveis de adiponectina quando comparado ao grupo controle suplementado com gordura insaturada. Os achados em relação à suplementação de ômega-3 e ácido linoléico conjugado devem ser interpretados com cautela, visto que foi observada heterogeneidade entre os estudos. Outros ensaios clínicos randomizados devem ser conduzidos a fim de confirmar os achados desse estudo para que se possa se estabelecer intervenções dietéticas que aumentem os níveis de adiponectina e que tenham

impacto na saúde cardiovascular de indivíduos com pré-diabetes, diabetes e síndrome metabólica. Estes achados permitem uma melhor compreensão dos diferentes mecanismos pelos quais intervenções que modificam o teor e a qualidade da gordura da dieta, tais como o aumento da ingestão de ômega-3 através do consumo de peixe e inclusão das oleaginosas na dieta usual, resultam em uma proteção para doença cardiovascular.

Por fim, a dieta hiperlipídica rica em ácidos graxos saturados induz a resistência à insulina e relaciona-se ao desenvolvimento do diabetes tipo 2 através de mecanismos independentes dos relacionados a variações nos níveis de adiponectina. Em indivíduos com excesso de peso e peso estável, a dieta restrita em lipídios, apesar de promover melhora da tolerância à glicose intravenosa, não melhorou a sensibilidade à insulina em quatro semanas de intervenção. Por conter elevado teor de carboidratos, a dieta restrita em lipídios possivelmente proporciona substrato para produção de ácidos graxos saturados através da lipogênese *de novo* o que pode contrabalançar os efeitos positivos para a sensibilidade à insulina desse padrão alimentar.