UNIVERSIDADE DO ESTADO DO RIO DE JANEIRO
CENTRO BIOMÉDICO
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PÓS-GRADUAÇÃO EM FISIOPATOLOGIA CLÍNICA E EXPERIMENTAL

Relação entre a Síndrome Metabólica, Teor de Gordura Intramiocelular e os Níveis Plasmáticos da Adiponectina: papel da Rosiglitazona.

Amélio Fernando De Godoy-Matos

Rio de Janeiro
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Autor: Amélio Fernando De Godoy-Matos

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Orientadora: Profa. Dra. Eliete Bouskela
Co-orientador: Prof. Dr. Luiz Guilherme Kraemer de Aguiar

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SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

AU – arbitrary unity

ADP – adiponectina ou adiponectin

AGL – ácidos graxos livres

AMPK – activated adenosine protein kinase

BIOVASC – Laboratório de Pesquisas Clínicas e Experimentais em Biologia Vascular

BMI – body mass index

EMCL – extramyocellular

GEMC – gordura extramiocelular

GIMC – gordura intramiocelular

HDL – high density lipoprotein

HDL-c – colesterol HDL

HOMA-IR – Homeostase model assessment insulin resistance

HOMA-RI – Homeostase model assessment resistência à insulina

^{1}H-ERNM – Espectroscopia de Prótons por Ressonância Nuclear Magnética

^{1}H-NMRS – Proton nuclear magnetic resonance spectroscopy

IMC – índice de massa corporal

IMCL – intramyocellular

IR – insulin resistance

IRS-1– insulin receptor substrate 1

PAI-1 – inibidor do ativador do plasminogênio-1

PCR-US – proteína C reativa- ultra-sensível

QUICKI – Quantitative insulin sensitivity check index

RCQ – relação cintura-quadril

RI – resistência à insulina

RSG – rosiglitazona

SM – síndrome metabólica

TNF-α – fator de necrose tumoral alfa

TZD – tiazolidinediona

UA – unidade arbitrária

UCP-1– uncouple protein 1

WHR – waist-to-hip-circumference

RESUMO

Objetivos – A resistência à insulina está associada com o aumento do teor de gordura intramiocelular (GIMC) e com níveis séricos da adiponectina (ADP) diminuídos. A ADP por sua vez está envolvida na oxidação de gordura muscular. Entretanto, a relação entre ambas continua controversa. O objetivo deste estudo é explorar a relação entre a ADP e a GIMC em adultos não diabéticos, além de estudar o papel da rosiglitasona (RSG) sobre a distribuição da gordura entre os compartimentos musculares.

Desenho do estudo – Este estudo compreende duas fases: uma fase transversal (corte-transversal) e uma fase longitudinal, de intervenção terapêutica com uma droga, num desenho aberto.

Local – Laboratório de Pesquisas Clínicas e Experimentais em Biologia Vascular (Biovasc) - UERJ.

Material e métodos – Na fase transversal, 24 pacientes obesos, não diabéticos, com síndrome metabólica (SM) e 9 controles magros e saudáveis foram estudados. Foi realizada a Espectroscopia de Prótons por Ressonância Nuclear Magnética (^{1}H-ERNM) para quantificar a gordura extramiocelular (GEMC) e a GIMC. Estas, associadas à ADP e aos parâmetros antropométricos e bioquímicos, foram avaliadas e comparadas nos dois grupos. Durante a fase longitudinal, 15 destes pacientes foram re-estudados, através da ^{1}H-ERNM, após o tratamento com RSG por 6 meses. Da mesma forma, as variáveis antropométricas e metabólicas foram reavaliadas.

Resultados – Fase transversal: os pacientes com SM apresentaram maior índice de massa corporal (IMC), cintura abdominal, relação cintura-quadril (RCQ), e níveis de glicemia, insulina e triglicerídeos e menores níveis de HDL-c, quando comparados com o grupo controle. Da mesma forma o HOMA-RI [3.25 (2.58-4.13) vs 1.02 (0.73-1.29); $p<0.0001$] e a GIMC [266.1 (189.9-296.3) vs 72.85 (55.3-109.4) unidades arbitrárias-UA, $p<0.0001$]

estavam aumentados enquanto o QUICKI [0.32 (0.31-0.33) vs 0.38 (0.37-0.40); p<0.0001] e a ADP [8.6 (4.05-15.95) vs 21.1 (12.9-24.4) µg/ml; p=0.02) estavam diminuídos. O teor de GIMC associou-se diretamente com a glicose, insulina, triglicerídeos e HOMA-RI e inversamente com o HDL-c, QUICKI e, mais importantemente, com a ADP (r = -0.41; p<0.05).

Fase longitudinal: após o tratamento com RSG, o peso corporal e a circunferência do quadril aumentaram, respectivamente [100.9 (91.12-138.7) vs 107,0 (79.6-142.8) kg e 118 (107-126) cm vs 122 (110-131) cm]; enquanto a RCQ diminuiu [0.93 (0.87-1.00) vs 0.89 (0.82-0.97); P<0.001 para todos]. Adicionalmente, a glicemia, a insulina e o HOMA-RI diminuíram significativamente, enquanto a ADP aumentou mais de 3 vezes [9.7 (3.7-17.7) vs 38.0 (19.3-42.4) µg/ml]. Finalmente, a GIMC não se modificou [267.54 (213.94-297.94) vs 305.75 (230.80-424.75) UA], mas a GEMC aumentou de forma significativa [275.53 (210.39-436.66) vs 411.39 (279.92-556.59) UA; P<0.01] diminuindo a razão GIMC sobre GEMC [GIMC/GEMC; 1.07 (0.78-1.23) vs. 0.71 (0.53-0.96); p<0.01].

Conclusão - A ADP correlacionou-se inversamente com o teor da GIMC em adultos obesos não diabéticos com SM. Este achado tem possíveis implicações para o papel da ADP na oxidação da gordura muscular, na RI e na SM. O tratamento com RSG aumentou a massa corporal e a circunferência do quadril e diminuiu a RCQ. Além disso, diminuiu a razão GIMC/GEMC, por aumentar a GEMC sem alterar significativamente a GIMC. Isto sugere que este medicamento pode prevenir a deposição da gordura no compartimento intramiocelular ao aumentar os depósitos periféricos e o extramiocelular.

ABSTRACT

Study objective – insulin resistance (IR) is associated with intramyocellular lipid (IMCL) content and low serum adiponectin (ADP) levels. ADP is also involved in muscle fat oxidation but the relationship between them is still controversial. We aimed to further explore the relationship between ADP and IMCL content in non-diabetic adults and the role of rosiglitazone (RSG) in muscle fat compartment distribution in an adult population of obese non-diabetic metabolic syndrome patients.

Design – this study comprises two phases: a cross-sectional and a longitudinal, open-label, drug-interventional one.

Setting - Laboratory for Clinical and Experimental Research on Vascular Biology (Biovasc) at the State University of Rio de Janeiro.

Material and Methods – during the cross-sectional phase, 24 obese, non-diabetic patients with metabolic syndrome (MS) and 9 lean healthy controls were studied. Proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) was performed to quantify IMCL, as well as extramyocellular lipid (EMCL) content. The latter plus serum ADP, anthropometrics and biochemical parameters were evaluated and compared in these two groups. During the longitudinal phase, fifteen of the MS patients were studied by means of ^{1}H-NMRS before and after treatment with 8mg/day of RSG for 6 months. Anthropometrical and metabolic variables were assessed.

Measurements and main results – cross-sectional phase: MS patients had higher body mass index (BMI), waist, waist-to-hip ratio (WHR), glucose, insulin and triglycerides and lower HDL-c as compared to controls. HOMA-IR (3.25 [2.58-4.13] vs 1.02 [0.73-1.29]; $p<0.0001$) and IMCL content (266.1 [189.9-296.3] vs 72.85 [55.3-109.4) AU, $p<0.0001$] were higher, and QUICKI (0.32 [0.31-0.33] vs 0.38 [0.37-0.40]; $p<0.0001$) and ADP (8.6 [4.05-15.95] vs 21.1 [12.9-24.4] μg/ml; $p=0.02$) lower in MS compared to controls. IMCL content was directly associated with glucose, insulin, triglycerides and HOMA-

IR and inversely to HDLc, QUICKI and, more importantly, with ADP ($r = -0.41$; $p<0.05$).

Longitudinal phase: After RSG treatment, body weight and hip circumference increased [100.9 (91.12-138.7) vs 107,0 (79.6-142.8) kg and 118 (107-126) cm vs 122 (110-131) cm] respectively, while WHR decreased [0.93 (0.87-1.00) vs 0.89 (0.82-0.97); $P<0.001$ for all]. Additionally, fasting plasma glucose, insulin and HOMA-IR significantly decreased while adiponectin increased over 3 fold [9.7 (3.7-17.7) vs 38.0 (19.3-42.4) µg/ml]. Finally, IMCL did not change [267.54 (213.94-297.94) vs 305.75 (230.80-424.75) arbitrary units (AU)] while EMCL increased [275.53 (210.39-436.66) vs 411.39 (279.92-556.59) AU; $P<0.01$] therefore decreasing IMCL to EMCL ratio (IMCL/EMCL) [1.07 (0.78-1.23) vs. 0.71 (0.53-0.96); $p<0.01$].

Conclusions – ADP is inversely related to IMCL content in non-diabetic adults. This finding has possible implications for the role of ADP in muscle fat oxidation, IR and MS. RSG treatment increased body weight and hip circumference decreasing WHR and decreased IMCL/EMCL ratio by increasing EMCL without any significant change on IMCL, thus suggesting that this drug may prevent IMCL fat deposition by increasing EMCL and peripheral deposits.

1- INTRODUÇÃO

A Síndrome Metabólica (SM) constitui-se numa das mais importantes entidades clínico-epidemiológicas da atualidade (Eckel et al., 2005; Cornier et al., 2008). Sua importância como fator de risco para a doença aterosclerótica, infarto do miocárdio, morte por doença cardiovascular e diabetes tipo 2 tem sido documentada em diversos estudos recentes (Isomaa et al., 2001; Lakka et al., 2001; Ford et al., 2002; Hu et al., 2004; Lorenzo et al., 2007; Cornier et al., 2008).

A base fisiopatológica da SM envolve a presença da resistência à insulina (RI), que, por sua vez, está diretamente relacionada com a presença de excesso de peso ou obesidade. Mais especificamente, a quantidade aumentada de gordura na região intra-abdominal (obesidade visceral), está mais consistentemente relacionada com a RI (Banerji et al., 1999; Lebovitz & Banerji, 2005).

1.1 Gordura intramuscular e RI

O papel da gordura visceral na patogênese da RI está bem estabelecido. Este depósito de gordura pode, todavia, ser apenas um mecanismo intermediário. Este compartimento de gordura é metabolicamente muito dinâmico. Sua alta capacidade lipolítica propicia um fluxo aumentado de ácidos graxos livres (AGL) para o sistema porta e periferia, favorecendo sua deposição sob a forma de triglicerídeos (TG) em outros tecidos, como fígado e músculo, o que constitui a hipótese da deposição ectópica de gordura (Heilbronn et al., 2004; Bays et al., 2004).

No músculo, os TG podem ser depositados no compartimento extramiocelular (GEMC) ou intramiocelular (GIMC). Diversos estudos para avaliação do conteúdo intramiocelular de TG e sua relação com RI, através da Espectroscopia de Prótons por Ressonância Nuclear Magnética (^{1}H-ERNM), confirmam que o conteúdo de GIMC é o melhor preditor da RI (Jacob et al.,1999; Virkamäki et al.,2001).

1.2 Adipocinas e RI

A relação entre obesidade, gordura visceral e RI, entretanto, não se resume unicamente à presença de elevados níveis de GIMC. O papel do tecido adiposo como órgão endócrino, produtor de diversas proteínas envolvidas no metabolismo dos carboidratos e das gorduras é, indubitavelmente, relevante na fisiopatologia da RI (Ahima & Flier, 2000; Ronti et al., 2006). Entre estas substâncias, destaca-se a adiponectina (ADP), uma proteína produzida exclusivamente pelo adipócito e uma das mais abundantes no plasma (Chandran et al., 2003; Matsuzawa et al., 2004; Sheng & Yang, 2008).

Contrastando com o aumento das outras adipocinas, como o fator de necrose tumoral-alfa (TNF-α), a resistina, a leptina e a Interleucina-6 (IL-6), a ADP encontra-se diminuída em obesos e diabéticos (Weyer et al., 2001; Hotta et al., 2001; Sheng & Yang, 2008). Ainda mais, relaciona-se independente e inversamente com a sensibilidade à insulina (Chandran et al., 2003; Weyer et al., 2001; Sheng and Yang, 2008). Em animais, o declínio dos níveis plasmáticos da ADP é seguido temporalmente pelo início da RI e do diabetes (Hotta et al., 2001).

1.3 ADP e GIMC

Em músculos de roedores a ADP aumenta a expressão de genes envolvidos no transporte e oxidação de ácidos graxos e equilíbrio energético, tais como CD36, acetil-CoA oxidase e a proteína UCP-1(Yamauchi et al., 2001). Mais importantemente, a administração da ADP em roedores aumenta a oxidação dos AGL no músculo, diminuindo os TG intramusculares e melhorando a sensibilidade à insulina (Yamauchi et al., 2001). Assim, uma razoável explicação para o papel da ADP como sensibilizador da insulina, parece ser através da sua ação no depósito de gordura intramuscular (Ravussin, 2002).

Os estudos de Weiss e colaboradores (Weiss et al., 2003), em adolescentes obesos, parecem confirmar esta hipótese. Estes autores encontraram uma forte correlação inversa entre a GIMC e os níveis de ADP. Não está, porém, claramente estabelecido se a ADP tem alguma relação com o teor de GIMC em adultos, em pacientes com SM ou em diabéticos. Assim, um

dos objetivos deste estudo é aprofundar a compreensão da relação entre o conteúdo de GIMC e os níveis de ADP em adultos portadores da SM. Além disso, poderemos avaliar as possíveis correlações existentes entre a GIMC, ADP e outras variáveis metabólicas como RI e lipídeos plasmáticos.

1.4 Tiazolidinedionas (TZDs), ADP e GIMC

As TZDs são uma nova classe de agentes antidiabéticos que atuam aumentando a sensibilidade à insulina. São agonistas exógenos da isoforma *gama* dos receptores ativadores da proliferação dos peroxissomas, conhecidos pela sigla em inglês PPAR-γ (Vamecq & Latruffe, 1999; Gurnell et al., 2003; Yki-Jarvinen, 2004). Estes receptores se expressam principalmente no tecido adiposo, o que explica grande parte das suas ações.

O tecido muscular expressa pequena quantidade dos PPARγ, o que não deixa claro como os seus agonistas melhoram a sensibilidade muscular à insulina. Várias ações indiretas das TZDs, porém, podem explicar esta ação. As TZDs parecem produzir uma redistribuição da gordura corporal, ao aumentar a gordura subcutânea e/ou diminuir a gordura visceral (Kelly et al., 1999; Carey, 2002; Yki-Jarvinen, 2004). São capazes ainda de diminuir os níveis de AGL circulantes e, portanto, podem potencialmente reduzir a GIMC.

Outro importante efeito das TZDs é o de modificar os níveis plasmáticos das adipocinas associadas à RI. Em especial estas drogas são capazes de aumentar significativamente os níveis de adiponectina em humanos (Bahia et al., 2007; Maeda et al., 2001; Yang et al., 2002; Yki-Jarvinen, 2004). Como discutido acima, a ADP pode potencialmente reduzir a GIMC (Yamauchi et al., 2001).

Desta forma, um dos objetivos deste estudo é avaliar o efeito da RSG na GIMC, por um período maior de tratamento, numa população não diabética e não usuária de medicamentos que atuem no metabolismo da glicose. Além disso, objetivamos avaliar as correlações entre o provável aumento da adiponectina, o acúmulo intramuscular de gordura e as variáveis metabólicas já citadas.

2 - Objetivos

Os objetivos deste estudo, face ao exposto acima, são:

1- Estudar a relação entre a ADP e a GIMC no estado basal e as possíveis correlações desta adipocina com as variáveis metabólicas típicas da SM.

2- Estudar o efeito da RSG sobre o conteúdo dos compartimentos da gordura muscular, isto é, GIMC e GEMC.

3- Avaliar o comportamento dos níveis plasmáticos da ADP após o tratamento com RSG e determinar se existe correlação entre o seu previsível aumento e a distribuição da gordura muscular.

3 - Material e Métodos

3.1 População do estudo, avaliação clínico-antropométrica e intervenção

Este estudo consta de duas fases: uma fase basal (transversal) e uma fase longitudinal. Todos os pacientes e voluntários assinaram um termo de consentimento informado antes de iniciar o estudo. Este protocolo foi aprovado pela Comissão de Ética do Hospital Universitário Pedro Ernesto-UERJ.

Na fase transversal foram estudados 24 pacientes portadores de SM pelos critérios da ATPIII (NCEP-ATPIII, 2002)**,** dos quais 16 eram do sexo feminino e 8 do masculino. Um grupo controle composto de voluntários saudáveis, não obesos, foi convidado a participar desta fase do estudo. Este grupo era composto por 6 mulheres e 3 homens.

Foram excluídos indivíduos com diabetes, história de doenças cardíacas, renais e hepáticas, fumantes ou pacientes em uso de drogas que afetam a sensibilidade à insulina, o metabolismo dos carboidratos ou dos lipídeos.

Todos os sujeitos estudados foram submetidos a um exame clínico e inquérito demográfico para apurar dados relativos à idade, história patológica pregressa, doenças coexistentes e uso de medicamentos. No exame físico, os parâmetros antropométricos avaliados foram: altura, peso, índice de massa corporal (IMC), medida da cintura abdominal e do quadril e relação cintura-quadril (RCQ). O índice de massa corporal (IMC) foi calculado dividindo-se o peso em quilogramas pelo quadrado da altura em metros. A medida da cintura abdominal foi realizada na metade da distância entre a crista ilíaca e o rebordo inferior da última costela, medida duas vezes pelo mesmo observador. A circunferência do quadril foi medida na altura da maior circunferência entre os trocânteres maiores. Além disso, foram mensuradas a pressão arterial sistêmica e a freqüência cardíaca. A pressão arterial foi determinada pela média de duas aferições, feitas com o paciente sentado, após repouso de pelo menos 15 minutos usando um esfignomanômetro automático (Multiparameter

patient monitor - Lifewindow LW6000, USA), segundo critérios do JNC-7 (Chobanian et al., 2003).

No estudo de intervenção medicamentosa (fase longitudinal), 16 pacientes utilizaram RSG na dose de 8 mg em uma tomada diária. Quinze pacientes completaram 6 meses de tratamento e, assim, compõem o grupo final estudado nesta fase.

Com o intuito de evitar interferência da dieta ou de eventual perda de peso, o que poderia mudar o conteúdo da gordura muscular, os pacientes foram instruídos para manter suas atividades regulares e não mudarem o padrão alimentar durante a intervenção com o medicamento.

3.2 Metodologia laboratorial

Foram obtidas amostras de sangue após jejum de 12 h para determinação dos exames laboratoriais contidos na tabela 1. A glicemia pós-prandial foi colhida apenas no grupo com SM, após 2 h da administração de 75g de glicose anidra. Os pacientes foram classificados conforme a tolerância à glicose pelos critérios da ADA (2004). As variáveis bioquímicas, glicose, colesterol total, triglicerídeos e colesterol HDL-(HDL-c) foram realizadas através do método colorimétrico enzimático automático GOD-PAP (Modular Analytics PP, Roche). Outras técnicas empregadas estão descritas na tabela 1. O colesterol LDL (LDL-c) foi calculado pela equação de Friedwald. As amostras foram centrifugadas e o plasma congelado em freezer a -70^{0} C para determinação coletiva de todas as amostras.

Tabela 1: metodologia laboratorial empregada.

EXAME	MÉTODO	EXAME	MÉTODO
GLICOSE	ENZIMÁTICO	HEMOGRAMA	CONTAGEM ELETRÔNICA
INSULINA	QUIMIOLUMINESCENCIA	TGP	ENZIMÁTICO
COLESTEROL	ENZIMÁTICO	TGO	ENZIMÁTICO
TRIGLICERÍDEOS	ENZIMÁTICO	PROTEÍNA C REATIVA	NEFELOMETRIA
HDL-C	ENZIMÁTICO COLORIMÉTRICO APÓS PRECIPITAÇÃO	FIBRINOGÊNIO	COAGULOMÉTRICO
ADP	ELISA	RESISTINA	ELISA
PAI-1	ELISA		

Amostras de plasma foram enviadas para o laboratório de Endocrinologia da Universidade de Campinas (Unicamp). A ADP, a Resistina e o PAI-1, foram dosados por ELISA, utilizando um Kit comercial (Lincoplex kit CAT- HADK1-61K-A, Linco Research-St Charles, Missouri, USA). A sensibilidade e os coeficientes de variação intra e inter-ensaio foram de 145,4 pg/ml, 6,11% e 13,2%, respectivamente. Por razões técnicas, não foi possível dosar a ADP em 3 voluntários do grupo controle e em 4 dos 24 pacientes com SM. A insulina plasmática foi dosada por quimiluminescência automática (coeficiente de variação =4,68%). Com isto, a determinação da RI foi realizada através do HOMA-RI (Matthews et al. 1985) e da sensibilidade à insulina através do QUICKI (Katz et al. 2000). O cálculo do HOMA-RI obedece a seguinte fórmula: g*licose de jejum x insulina de jejum ÷ 405; e o do* QUICK: *1 ÷ log glicose de jejum x log insulina de jejum.*

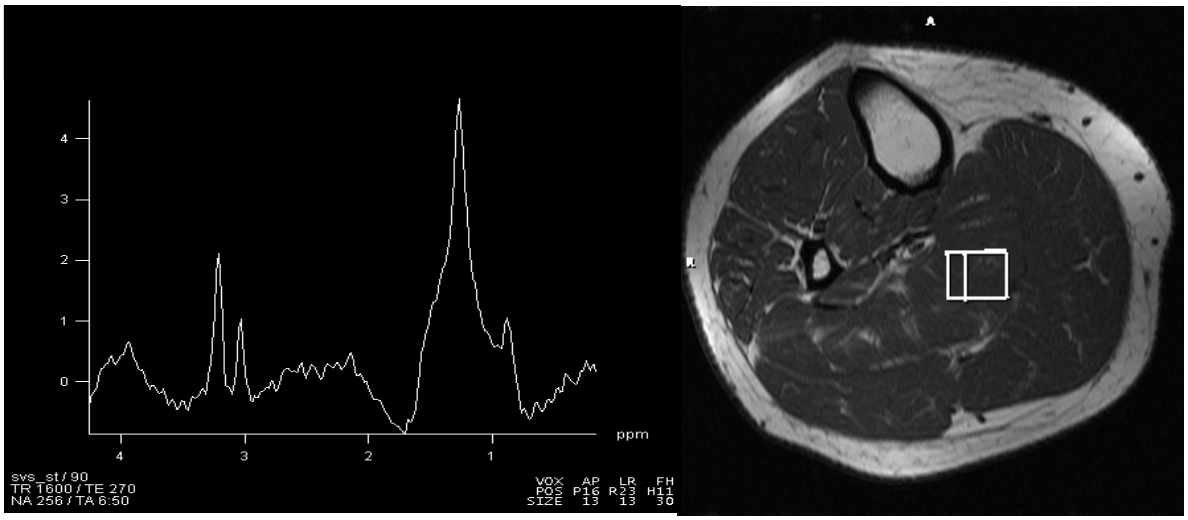
3.3 Espectroscopia de Prótons por Ressonância Nuclear Magnética

A GIMC e GEMC foram avaliadas através da ^{1}H-ERNM. Os exames foram realizados na Clinica IRM em aparelho de última geração (1.5T MR Scanner- Magneton Vision, Siemens, Erlangen, Germany). Os pacientes foram instruídos para evitar exercícios físicos intensos e o consumo de bebidas alcoólicas ou cafeína na véspera deste exame.

As imagens foram obtidas nos músculos tibial anterior e gastrocnêmico da perna direita com o paciente em posição supina. Desta forma a tíbia ficava em posição quase paralela ao campo magnético estático. O volume de interesse (13x13x30mm^{3}) foi centrado dentro do músculo gastrocnêmico de forma a evitar estruturas vasculares e depósitos grosseiros de gordura. O espectro foi adquirido pela seqüência PRESS (point resolved spectroscopy) com os seguintes parâmetros: tempo do eco 35 ms, tempo de repetição 1600 ms e 256 scans com supressão da água (figuras 1a e 1b)

O conteúdo intramiocelular foi definido pela integral do sinal obtido entre 1.2 e 1.3 ppm. O conteúdo extramiocelular foi definido pela integral do sinal obtido entre 1.4 e 1.5 ppm. O sinal da creatina foi obtido entre 2.9 e 3.1 ppm e serviu como referência para a quantificação da gordura intra e extramiocelular. Utilizamos a relação gordura/creatina para análise das espectroscopias.

Após a fase transversal do estudo, os pacientes portadores de SM foram convidados a participar da fase longitudinal. Desta forma, 16 pacientes iniciaram RSG e 15 completaram o estudo. Todas as avaliações clínicas e laboratoriais, assim como a ^{1}H-ERNM, foram repetidas 6 meses após o tratamento com RSG. É importante salientar, portanto, que para todos estes pacientes os exames basais e após 6 meses de tratamento estavam disponíveis para a análise.



A) espectroscopia de próton B) RNM do mesmo paciente

Figura 1. Modelo de um exame em um paciente com SM. Note que o maior pico na espectrometria, referente a GEMC (obtido entre 1.4 e 1.5 ppm) é seguido por um pico menor (obtido entre 1.2 e 1.3 ppm), referente a GIMC.

3.4 Análise estatística

Face ao n amostral, as variáveis têm, em sua maioria, distribuição não-normal e são apresentadas como medianas [$1^{\underline{0}}$ - $3^{\underline{0}}$ quartis]. No estudo estatístico, empregou-se o software estatístico Prism 4.01 (Graphpad Inc., San Diego, CA, USA).

No estudo transversal, as comparações entre os grupos foram feitas pelo teste do Qui quadrado corrigido por Yates ou pelo teste de Mann-Whitney. Dada a grande variação dos resultados de ADP e GIMC, utilizou-se a transformada logarítmica destas variáveis. As correlações entre estas e as variáveis antropométricas ou metabólicas foram realizadas pela análise de correlações de Spearman. Em caráter exploratório, e apesar da não-normalidade das variáveis, aquelas que apresentavam maior correlação e poder estatístico foram usadas em um modelo de regressão múltipla seguindo um padrão “stepwise backward”, onde o logGIMC foi a variável dependente. Os melhores modelos são apresentados neste estudo. Nesta análise, ao testarmos as variáveis com o grupo total (pacientes e controles), subseqüentemente

separamos aqueles pacientes com mais de 40 anos, a fim de avaliar a interferência da idade nos resultados encontrados.

No estudo longitudinal, as comparações do efeito da intervenção com o estado basal foram feitas através do teste de Wilcoxon para grupos pareados. Face ao elevado ganho ponderal de praticamente toda a amostra ao término dos 6 meses de tratamento, a variável DeltaPeso foi criada e dividida em quartis. Subseqüentemente, os pacientes foram divididos em dois subgrupos de acordo com o 4º quartil do ganho ponderal, ponto de corte de 4,1 kg. A correlação de Spearman foi novamente empregada nesta fase. Para estabelecer diferenças estatísticas significativas foi considerado o valor de $P<0,05$.

4 – Resultados

4.1 Estudo transversal

4.1.1 Parâmetros Metabólicos, antropométricos e de conteúdo de gordura dos pacientes e controles

Os pacientes com SM eram mais velhos que o grupo controle, mas não houve diferença na composição dos grupos por gênero (tabela 2). Além disso, 13 pacientes com SM eram portadores de intolerância à glicose e/ou hipertensão arterial. Os pacientes com SM apresentaram diferenças significativas para todos os parâmetros estudados quando comparados com o grupo controle, como demonstrado na tabela 2. Assim, estes pacientes eram mais pesados, concentravam gordura na região abdominal e exibiam um perfil metabólico típico da SM. Ressalte-se que a avaliação de resistência à insulina demonstra que este grupo apresentava um HOMA-RI maior [3,25 (2,58-4,13) vs 1,02 (0,73-1,29)] e, de maneira concordante, uma menor sensibilidade à insulina quando avaliada pelo QUICKI [0,32 (0,31-0,33) vs 0,38 (0,37-0,40); $p<0,0001$ para ambos]. Ainda mais interessante, a ADP foi significativamente mais baixa [8,6 (4,05-15,95) vs 21,1 (12,9-24,4) µg/ml; $p= 0,02$] e o conteúdo da GIMC maior [266,1 (189,8-296,3) vs 72,85 (55,3-109,4); $p<0,0001$] (Figuras 2a e 2b). O conteúdo da GEMC não foi diferente entre os grupos.

Tabela 2: Dados clínicos e laboratoriais dos grupos SM e controles.

	CONTROLES n = 9	SM n = 24	p
Gênero (feminine/masculino)	6 / 3	16 / 8	NS
Idade (anos)	23,0 [23-27]	41,5 [35-50]	<0,001
IMC (kg/alt^2)	20,6 [20.4-21.4]	37,4 [31,5-42,5]	<0,0001
Cintura (cm) – masc.	71,0 [68-84]	105,5 [99-116,5]	<0,001
Cintura (cm) – femin.	64,0 [64-68]	106 [99,5-111,5]	<0,001
RCQ – masc.	0,81 [0,71-0,82]	1,00 [0,94-1,1]	<0,01
RCQ – femin.	0,71 [0,70-0,71]	0,91 [0,86-0,96]	<0,01
Glicemia (mg/dl)	82,0 [72-86]	96,5 [86,5-109,0]	<0,001
Insulina (μUI/ml)	4,9 [4,4-5,8]	13,9 [11-18,4]	<0,0001
HOMA-RI	1,02 [0,73-1,29]	3,25 [2,58-4,13]	<0,0001
QUICKI	0,38 [0,37-0,40]	0,32 [0,31-0,33]	<0,0001
Colesterol total (mg/dl)	165,0 [144-174]	207,5 [177,5-239,0]	<0,001
HDL-colesterol (mg/dl)	♂ 53,0 [45-63]	♂ 40,0 [37,5-43,5]	<0,05
	♀ 61,0 [51-81]	♀ 44,0 [37-47,5]	0,01
LDL-colesterol (mg/dl)	80,0 [72-110]	128,0 [109,5-163,0]	<0,001
Triglicérides (mg/dl)	57 [48-77]	187,5 [107,5-216,0]	<0,0001
Adiponectina (μg/ml)	21,1 [12,9-24,4]	8,6 [4,05-15,95]	<0,05
LogAdiponectina	1,36 [1,28-1,39]	0,93 [0,61-1,20]	<0,05
GIMC (UA)	72,85 [55,3-109,4]	266,1 [189,8-296,3]	<0,0001
LogGIMC	1,86 [1,74-2,04]	2,42 [2,28-2,47]	<0,0001

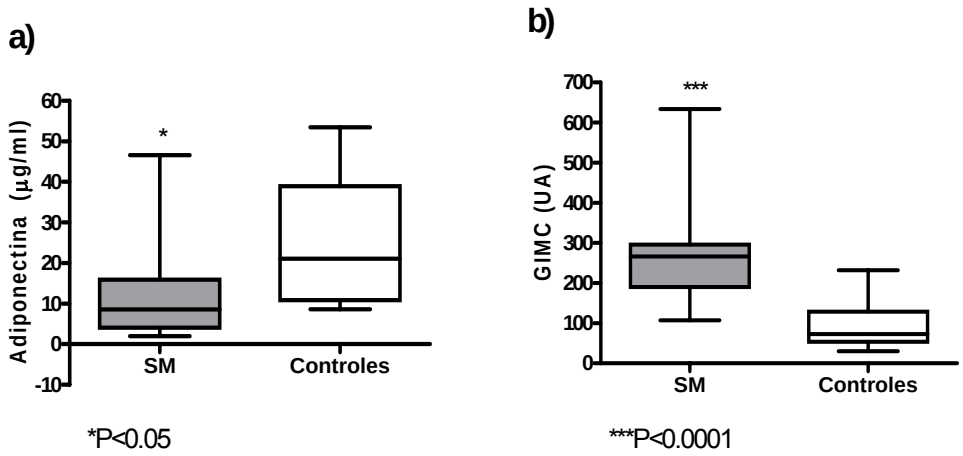


Figura 2: Valores de ADP (a) e GIMC (b) nos grupos SM e controles.

4.1.2 Correlações entre a ADP, dados clinico-laboratoriais e conteúdo muscular de gordura nos pacientes e controles

A tabela 3 descreve as correlações estudadas. Observe-se que a ADP (log) correlacionou-se inversamente com o peso ($r = -0,42$; $p<0,05$) e, de acordo com a distribuição de gordura corporal, também com a cintura abdominal e com a RCQ ($r = -0,42$ e $r = -0,48$; $p<0,05$ para ambas). Não houve relação com a medida do quadril. Além disso, se correlacionou inversamente com a insulina de jejum, HOMA-RI, triglicerídeos e, diretamente, com o QUICKI e o HDL-c (Tabela 3).

Quanto ao teor da GIMC (log), houve correlação direta com o peso e o IMC ($r = 0,74$; $p<0,001$ e $r = 0,63$; $p<0,001$ para ambas). De maior importância, observaram-se fortes correlações positivas entre a GIMC e os marcadores de deposição central de gordura, como a cintura e a RCQ ($r= 0,76$ e $r= 0,71$; $p< 0,001$ para ambas).

O conteúdo de GIMC também se correlacionou diretamente com quase todas as variáveis metabólicas, como: glicemia de jejum, insulina, triglicerídeos e; inversamente, com o HDL-c. Como esperado, houve uma forte correlação com o HOMA-RI (positiva) e o QUICKI (negativa).

Tabela 3: Correlações entre ADP, GIMC (ambas por transformação logarítmica), parâmetros antropométricos e metabólicos.

Parâmetros antropométricos	LogADP	LogGIMC
Peso	-0,42*	0,74‡
IMC	0,24	0,63‡
Cintura	-0,42*	0,76‡
Quadril	-0,23	0,54†
RCQ	-0,48*	0,71‡
Parâmetros metabólicos		
Glicemia	0,01	0,43*
Insulina	-0,55†	0,55‡
HOMA-RI	-0,50†	0,59‡
QUICKI	0,51†	-0,58‡
Colesterol total	-0,08	0,21
LDL-colesterol	-0,14	0,22
HDL-colesterol	0,54†	-0,47*
Triglicérides	-0,52†	0,49†

Dados apresentados em R. *p < 0,05; †p < 0,01; ‡p < 0,001.

Nosso principal achado desta fase do estudo foi a correlação negativa encontrada entre a ADP e a GIMC (r=-0,41;p<0,05; Figura 3) numa população de adultos não-diabéticos.

Correlação inversa entre a ADP (log) e a GIMC(log)

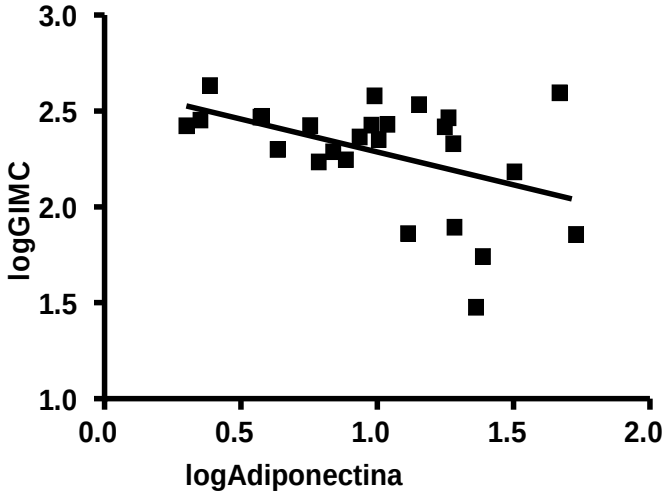


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Figura 3: Correlação entre adiponectina e GIMC, ambos com transformação logarítmica.

No grupo total, a análise multivariada mostrou que o conteúdo de GIMC foi influenciado apenas pela medida da cintura (Beta = 0,96; R2 ajustado= 0.61, p<0,001), mesmo quando os pacientes maiores de 40 anos foram excluídos da análise (Beta = 0,74; R^2 ajustado= 0,53, p<0.001). Quando analisados apenas os pacientes com SM, o teor da GIMC foi influenciado pelo IMC, RCQ, medida do quadril, glicemia de jejum, QUICKI e LDL-c, embora não independentemente dos níveis de ADP. Logo, 62% da variação da GIMC podem ser explicados por estas variáveis (tabela 4) e, parcialmente, pela ADP.

Tabela 4: Análise de regressão múltipla em obesos não-diabéticos com SM considerando a GIMC como variável dependente.

Modelo	β	P	R^2 ajustado	P
IMC	-1,07	0,003	0,62	0,03
Quadril	2,32	0,02		
RCQ	2,01	0,007		
Glicemia	-0,69	0,03		
QUICKI	-1,12	0,006		
LDL-colesterol	-1,59	0,006		
LogADP	0,93	0,006		

4.2 Estudo longitudinal

4.2.1 Parâmetros antropométricos, metabólicos e de conteúdo de gordura muscular

Dezesseis pacientes entraram na fase longitudinal de intervenção medicamentosa com RSG, porém um paciente não completou o estudo. Assim, os dados analisados referem-se àqueles que completaram o protocolo. A tabela 5 descreve os dados destes pacientes.

Após 6 meses de tratamento houve um marcante aumento do peso, do IMC e da circunferência do quadril, enquanto a medida da cintura não sofreu alterações. Logo, a RCQ diminuiu significativamente com o tratamento [0,93 (0,87-1,0) vs 0,89 (0,82-0,97); p<0,001].

Consistentemente, todas as variáveis relacionadas ao metabolismo dos carboidratos sofreram mudanças. A glicemia de jejum, os níveis plasmáticos de insulina e o HOMA-RI decresceram, enquanto o QUICKI aumentou significativamente (tabela 5). A ADP aumentou mais de 3 vezes acima do valor basal [9,7 (3,7-17,7) vs. 38,0 (19,3-42,4) µg/ml; p<0,001], enquanto a resistina manteve-se inalterada. No perfil lipídico houve apenas um discreto aumento nos níveis do colesterol total e do LDL-c. Interessantemente, a PCR-US diminuiu cerca de 70%, de maneira altamente significativa [1,0 (0,5-2,3) vs 0,3 (0,2-0,5); p<0,0001]. O fibrinogênio, mas não o PAI-1, diminuiu levemente (tabela 5).

Tabela 5: dados do grupo de obesos não-diabéticos com SM antes e depois do tratamento com rosiglitazona.

	Basal	Após RSG
Peso (Kg)	100,9 [91,12-138,7]	107,0 [79,6-142,8]***
IMC (Kg/m^2)	38,1 [32,8-44,3]	38,4 [34,6-48,4]***
Cintura (cm)	106,0 [98,0-112,0]	109,0 [101,0-114,0]
Circunferência do quadril	118 [107-126]	122 [110-131]***
RCQ	0,93 [0,87-1,00]	0,89 [0,82-0,97]***
GJ (mg/dl)	103 [95-109]	94 [84-101]*
Insulina (μUI/ml)	14,6 [10,6-20,3]	9,0 [7,2-12,8]***
HOMA-IR	3,30 [2,47-4,37]	2,00 [1,61-3,16]***
QUICKI	0,320 [0,307-0,333]	0,343 [0,321-0,355]***
Colesterol Total (mg/dl)	190 [172-233]	199 [190-265]**
Colesterol LDL (mg/dl)	116 [95-163]	134 [122-183]**
Colesterol HDL (mg/dl)	43 [37-46]	44 [35-48,5]
Triglicerídeos (mg/dl)	175 [98-211]	151 [119-211]
PCR	1,0 [0,5-2,3]	0,3 [0,2-0,5]***
Fibrinogênio	303,9 [254,2-364,0]	265,0 [209,7-296,5]*
Adiponectina	9,7 [3,7-17,7]	38.0 [19,3-42,4]***
PAI-1	23,8 [21,2-28,1]	22,5 [20,6-24,7]
Resistina	17,3 [17,2-18,2]	17,1 [16,9-17,8]
GEMC	275,53 [210,39-436,66]	411,39 [279,92-556,59]**
GIMC	267,54 [213,94-297,94]	305,75 [230,80-424,75]

*P< 0,05 **P<0,01 ***P<0,001

O principal objetivo desta fase do estudo foi avaliar possíveis mudanças na distribuição da gordura muscular. De fato, enquanto não se demonstrou mudança no teor da GIMC, um marcante aumento do teor da GEMC pode ser observado [275,53 (210,39-436,66) vs 411,39 (279,92-556,59); p<0,01]. Isto significou que a razão entre a GIMC e a GEMC (GIMC/GEMC) diminuiu importantemente após o tratamento com RSG [1,07 (0,78-1,23) vs. 0,71 (0,53-0,96); p<0,01 – Figura 4].

A razão GIMC/ GEMC diminuiu significativamente após 6 meses
De tratamento com RSG

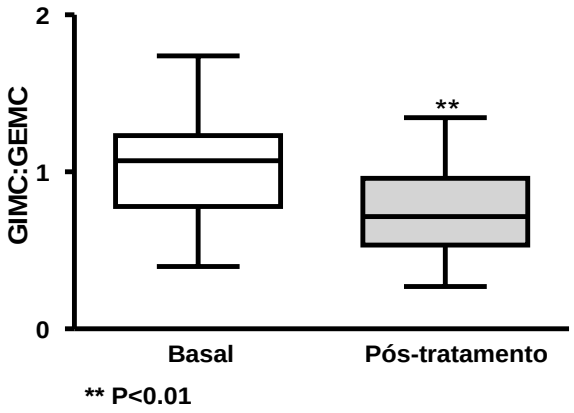

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Figura 4: Razão entre a GIMC e a GEMC basal e após tratamento com RSG em obesos não-diabéticos com SM.

Como houve um importante aumento de peso no grupo como um todo, os pacientes foram divididos por quartis, entre os que ganharam muito peso ($4^{\underline{0}}$ quartil ou ≥ 4,1kg) e aqueles que pouco aumentaram. Ainda assim, mesmo naqueles que ganharam mais de 4kg, não houve aumento no teor de GIMC. Em contraste, o teor de GEMC aumentou significativamente em ambos (Figura 5).

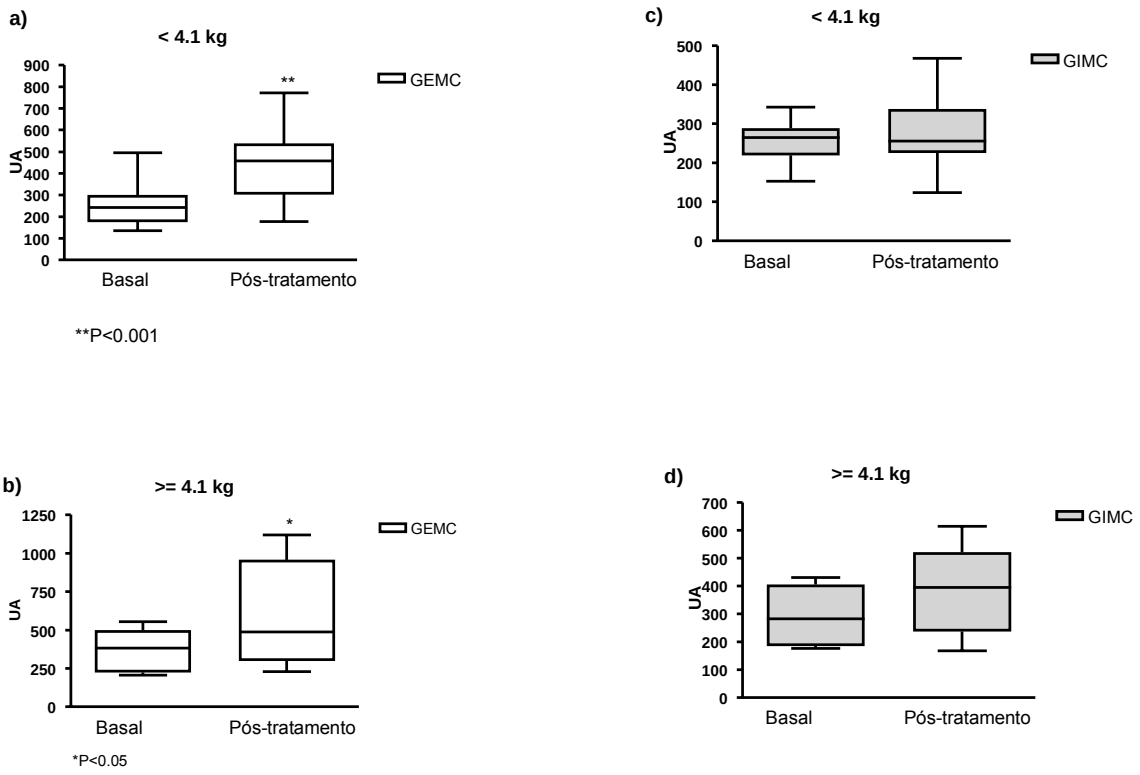

Figura 5. Diferença da GEMC e GIMC antes e após tratamento com RSG. Observa-se que, quando divididos por quartis, a GEMC aumentou em ambos os subgrupos (Figuras a e b). Em contraste, a GIMC (Figuras c e d) não se modificou significativamente naqueles que ganharam mais ou menos peso.

5- Discussão:

O principal achado da fase transversal deste estudo foi a ocorrência de uma relação inversa entre a ADP e a GIMC em uma população adulta não-diabética com SM. Weiss e colaboradores (2003) apresentaram resultados similares em uma população de adolescentes. Nesse caso, todavia, esta relação foi mais forte apenas no grupo de obesos, sugerindo que era mais proeminente acima de um determinado limite de GIMC. Da mesma forma, outros descreveram a mesma relação inversa apenas num grupo de obesos, sem qualquer correlação em magros (Kantartzis, 2006). No presente estudo, em adultos, ressalte-se, quando homogeneizamos a amostra apenas com pacientes obesos a relação da ADP com a GIMC não se tornou mais significativa. Isto talvez possa ser explicado porque nosso grupo foi selecionado estritamente pela presença da SM. Ainda mais, um estudo recente vem de encontro aos nossos achados ao observar que, independente da obesidade, a ADP correlacionou-se com a GIMC apenas na musculatura oxidativa, isto é, músculo *soleus*, mas não no músculo tibial anterior (Thamer et al., 2002). Este fato está de acordo com o provável papel da ADP na oxidação de gordura. Por outro lado, Furler e colaboradores (2006) não encontraram uma correlação simples entre estas variáveis. Todavia, quando modelaram a GIMC como uma função da ADP e da adiposidade uma forte correlação apareceu, mas, curiosamente, o modelo sugeria uma relação inversa nos magros e direta nos obesos. Estes autores estudaram apenas homens enquanto nós estudamos pacientes de ambos os sexos.

Recentemente, Perseghin e colaboradores (2007), também estudaram a relação entre as adipocinas e a gordura ectópica, em jovens magros, filhos de diabéticos. Eles mostraram que a leptina correlacionou-se fortemente com a GIMC, enquanto a ADP mostrou apenas uma tendência. De certa forma isto concorda com os nossos achados e sugere que a contradição pode estar apenas relacionada com a corpulência dos seus pacientes. Outro estudo, também em adultos, observou que níveis mais baixos de ADP ocorriam apenas em sujeitos com maior teor de gordura hepática e não naqueles com maior depósito de gordura muscular (Kotronen et al., 2008), o que está em

concordância com achados que sugerem que a hipoadiponectinemia está associada com maior acumulo de gordura no fígado e não no músculo (Koska et al., 2008). No nosso estudo não avaliamos a gordura hepática, o que poderia contribuir para esta discussão. Em suma, dois estudos em crianças (Weiss et al., 2003; Taksali et al., 2008) e dois em adultos (Kantartzis, 2006; Thamer et al., 2002), sugerem uma relação inversa entre a ADP e a GIMC, um mostrou uma tendência no mesmo sentido (Perseghin et al., 2007) e dois não confirmam tal associação (Furler et al., 2006; Kotronen et al., 2008). É, portanto, possível que diferenças metodológicas, principalmente nas populações estudadas, expliquem esta controvérsia. O fato é que o tema permanece não esclarecido e isto garante a necessidade de mais estudos.

Este estudo confirma muitos aspectos das alterações metabólicas em pacientes com SM. Um aspecto notável foi que, numa população não diabética, os níveis plasmáticos da ADP eram menos da metade dos encontrados num grupo controle saudável.

Em relação às variáveis antropométricas, a ADP se correlacionou inversamente com o peso e IMC, e, mais importantemente, com as medidas de adiposidade central como circunferência abdominal e RCQ. Tanko e colaboradores (2004), utilizando densitometria de duplo feixe de raios X, demonstraram que a ADP estava diminuída em mulheres com obesidade central, quando comparadas àquelas com obesidade periférica, ou mesmo generalizada. A gordura visceral é o principal componente, sob o ponto de vista metabólico, da adiposidade central e mostra-se preditora independente dos níveis de ADP (Cote et al.,2005). De fato, homens com o mesmo IMC, mas maior gordura visceral, exibem níveis reduzidos de ADP em comparação com aqueles com menor gordura visceral. Ainda mais, vários grupos já demonstraram que a ADP está diminuída em pacientes com obesidade, IR ou diabetes mellitus tipo 2 (Matsuzawa et al., 2004; Cote et al., 2005; Tschritter et al., 2003) e se correlaciona inversamente com o conjunto de fatores da SM (Matsuzawa et al., 2004; Conte et al., 2006; Matsubara et al., 2002), exceto com o HDL-c, com o qual se relaciona positivamente (Matsubara et al., 2002). Assim, os nossos achados estão em inteira concordância com a literatura.

Em relação à GIMC, observou-se que estava aumentada nos pacientes com SM e, além disso, se associava diretamente com as medidas de deposição central de gordura corporal, ou seja, cintura e RCQ. Da mesma forma, houve associação com as anormalidades metabólicas típicas da SM. De acordo com a teoria da deposição ectópica de gordura, o acúmulo de gordura em fígado e músculo pode ser devido a uma incapacidade parcial do tecido adiposo em estocar gordura (Heilbronn et al., 2004; Ravussin, 2002)

Estudos anteriores demonstraram que a GIMC está diretamente relacionada com a RI, além de ser um excelente preditor desta (Jacob et al., 1999; Virkamäki et al., 2001). No nosso estudo, os marcadores indiretos da RI (insulina, HOMA-IR e QUICK) também se associaram significativamente com a GIMC, o que está de acordo com os estudos citados. Além disso, a GIMC também se relacionou com todos os achados bioquímicos e metabólicos.

A relação entre a ADP e a GIMC é de capital importância, uma vez que pode explicar muitas alterações metabólicas que estão por trás da RI, SM e diabetes tipo 2. Como já discutido, o conteúdo de gordura intramiocelular está intrinsecamente relacionado a RI. Tal associação deve-se, parcialmente, a uma diminuição da fosforilação do IRS-1 estimulado pela insulina (Petersen & Shulman, 2006). Em modelos animais com grande aumento de GIMC e RI, a infusão de ADP diminuiu o triglicerídeo muscular e recuperou a transdução do sinal da insulina, exatamente por ativar a cascata de fosforilação em tirosina do receptor de insulina e do passo seguinte, ou seja, a fosforilação do IRS-1 (Yamauchi et al., 2001). Recentemente, novas avenidas de investigação foram abertas para tentar explicar a complexa relação entre o teor de GIMC e as reduzidas taxas de fosforilação mitocondrial, associadas por sua vez com diminuição da síntese de ATP, que refletem uma disfunção mitocondrial (Petersen et al., 2004).

Em conclusão, os achados da fase transversal deste estudo adicionam-se a uma literatura controversa, mas está em consonância com a sugestão de uma relação inversa entre a ADP e a GIMC. Logo, aponta para um suspeitado e provável papel da ADP na oxidação da gordura muscular.

O principal achado da fase longitudinal, após intervenção terapêutica com RSG por seis meses, foi um aumento significativo da GEMC. Todavia o mesmo não foi observado em relação à GIMC, que não se alterou. Por conseguinte, a razão GIMC/GEMC diminuiu significativamente. Embora um estudo *in vitro*, utilizando músculo esquelético de pacientes diabéticos, tenha demonstrado um aumento da utilização de ácidos graxos, potencialmente reduzindo a GIMC (Cha et al., 2005), os estudos clínicos mostram resultados contraditórios. De fato, Mayerson e colaboradores (2002), ao estudar diabéticos em uso de RSG por apenas três meses, não logrou demonstrar qualquer alteração no conteúdo de GIMC. Todavia, observou um aumento de 39% na GEMC. Seus resultados estão em consonância com os nossos, quando em seis meses observamos um aumento de 49.3% na GEMC. Mesmo quando separados por categoria de ganho de peso, entre aqueles que ganharam 4,1kg ou mais (4º quartil) e aqueles que ganharam menos, a GIMC não mudou significativamente nos dois subgrupos, ao contrário da GEMC. Uma vez mais, a razão GIMC/GEMC diminuiu. Um estudo em ratos Zucker obesos mostrou o mesmo achado, ou seja, que a RSG diminuiu a razão GIMC/GEMC (Jucker et al., 2005).

Teranishi e colaboradores (2007), conseguiram demonstrar uma diminuição da GIMC com pioglitazona ou metformina num grupo de diabéticos tipo 2. Em contraste com o presente estudo, porém, os pacientes que usaram pioglitazona não ganharam peso e aqueles em uso de metformina perderam peso levemente. Isto pode sugerir que mudanças no estilo de vida (exercícios e dieta) implementadas antes e mantidas durante o estudo, como afirmado pelos autores, influenciaram os seus resultados. Rasouli e colaboradores (2005), num estudo semelhante, mas por apenas 10 semanas e utilizando biopsia muscular ao invés da ^{1}H-ERNM, mostraram uma diminuição de 34% na GIMC com a pioglitazona, mas não com a metformina. Estes autores relataram um discreto aumento de peso (2.63 ± 0.65 kg) no grupo pioglitazona. Assim, é possível que diferenças nas metodologias, no ganho de peso ou mesmo nas peculiaridades de cada droga, possam explicar nossos achados discordantes.

Algumas possíveis interpretações podem ser levantadas a partir do presente estudo: i) o excesso de gordura pode ter sido desviado para o compartimento extracelular, que se comportaria como adipócitos periféricos,

acumulando gordura sob estímulo das tiazolidinedionas (Mayerson et al., 2002); ii) o excesso de gordura, eventualmente dirigido ao compartimento intramiocelular, pode ter sido oxidado pela ativação PPAR-γ (Rasouli et al., 2005) ou iii) ocorreu um desvio dos depósitos ectópicos para o tecido subcutâneo (Mayerson et al., 2002; Rasouli et al., 2005; Kim et al., 2003; Yamauchi et al., 2002). Em reforço a estas hipóteses, no nosso grupo de pacientes o aumento de peso se acompanhou de modificações das medidas antropométricas que avaliam distribuição da gordura. Nominalmente, aumento da circunferência do quadril e decréscimo da RCQ. Além disso, a RSG aumenta a sensibilidade do adipócito à insulina e diminui a lipólise da gordura periférica (Mayerson et al., 2002).

Em animais, a ADP aumenta a expressão de genes envolvidos com o transporte e oxidação dos ácidos graxos e diminui o teor muscular de triglicerídeos (Yamauchi et al., 2002). As tiazolidinedionas ativam a AMPK, aumentam a ADP, que por sua vez aumenta a AMPK em músculo e fígado (Yamauchi et al., 2002; Fryer et al., 2002). Como a ADP aumentou substancialmente na nossa experimentação, isto poderia ser responsável por um aumento na oxidação de gorduras no músculo e assim diminuir a GIMC. Como, de fato, não houve redução da GIMC, tal relação (ΔADP versus ΔGIMC) não pode ser demonstrada. Além disso, não encontramos correlação entre a diminuição da razão GIMC/GEMC e o aumento da ADP (dados não mostrados).

Finalmente, quanto às modificações nas variáveis metabólicas como glicemia, insulinemia, perfil lipídico, HOMA-RI, QUICK, fibrinogênio e, ainda, no marcador de inflamação (PCR) este estudo está, indubitavelmente, de acordo com o espectro de ações das tiazolidinedionas (Yki-Jarvinen, 2004). É notável que, mesmo numa condição experimental livre, onde os pacientes ganharam muito peso, o tratamento com a RSG mudou todos os marcadores de resistência (ou sensibilidade) à insulina na direção correta, enquanto ao mesmo tempo reduzia a inflamação. Isto deve ser entendido como resultado de uma deposição favorável de gordura, como sugerido pelo aumento do peso, da circunferência do quadril e diminuição da RCQ. Em apoio a esta sugestão, em pacientes diabéticos a RSG diminuiu a RI, enquanto aumentava a leptina, um marcador da gordura corporal total (Kim et al., 2008). Mais interessante, o

aumento da espessura do subcutâneo, medido por ultrasonografia, correlacionou-se positivamente com o aumento da leptina e negativamente com o HOMA-RI (Kim et al., 2008). Em suma, a melhora do perfil metabólico pode ser entendida como resultado da diminuição da RI, secundária a deposição periférica de gordura.

Em conclusão, o tratamento com RSG num grupo de pacientes obesos, não diabéticos, portadores de SM, aumentou o peso corporal, mas melhorou vários parâmetros metabólicos e de inflamação. Além disso, diminuiu a razão entre a GIMC e a GEMC ao aumentar o compartimento da GEMC sem afetar o compartimento da GIMC. Desta forma, este estudo sugere que a RSG pode prevenir a deposição de GIMC através do aumento da gordura periférica e extramiocelular.

6 – Conclusões

1- A adiponectina correlaciona-se negativamente com o conteúdo de gordura intramiocelular em adultos. Isto aponta para um suspeitado e provável papel da ADP na oxidação da gordura muscular.

2- O tratamento com rosiglitazona, num grupo de pacientes obesos, não diabéticos, portadores de SM, diminuiu a razão entre a gordura intramiocelular e a extramiocelular, aumentando o depósito no compartimento extramiocelular.

3- O tratamento com rosiglitazona aumentou significativamente os níveis da adiponectina plasmática, mas este aumento não se correlacionou com a diminuição da razão entre a gordura intra e extramiocelular observada.

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8.ARTIGO 1: ADIPONECTIN IS RELATED TO INTRAMYOCELLULAR LIPID CONTENT IN NON-DIABETIC ADULTS(Journal of Endocrinological Investigation-2009, no prelo)

9.ARTIGO 2: ROSIGLITAZONE DECREASES INTRA TO EXTRAMYOCELLULAR FAT RATIO IN OBESE NON-DIABETIC ADULTS WITH METABOLIC SYNDROME (Submetido à publicação)

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Keywords:	insulin resistance, adiponectin, intramyocellular lipid content, metabolic syndrome

ADIPONECTIN IS RELATED TO INTRAMYOCELLULAR LIPID CONTENT IN NON-DIABETIC ADULTS.

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2 Figures

Abstract

Objective: insulin resistance (IR) is associated with intramyocellular lipid (IMCL) content and low serum adiponectin (ADP) levels and ADP is also involved in muscle fat oxidation. However, the relationship between ADP and IMCL content is still controversial and in this study we explored it further in non-diabetic adults.

Design: cross-sectional clinical study.

Subjects: thirty-three adult subjects, 24 obese non-diabetic patients with metabolic syndrome (MS) and 9 lean healthy controls.

Measurements: Proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) was performed to quantify IMCL content. The latter plus serum ADP, anthropometrics and biochemical parameters were evaluated and compared in these two groups.

Results: MS patients had higher body mass index, waist, waist-to-hip ratio, glucose, insulin and triglycerides and lower HDLc compared to controls. HOMA-IR (3.25 [2.58-4.13] *vs* 1.02 [0.73-1.29]; p<0.0001) and IMCL content (266.1 [189.9-296.3] *vs* 72.85 [55.3-109.4) AU, p<0.0001] were higher, and QUICKI (0.32 [0.31-0.33] *vs* 0.38 [0.37-0.40]; p<0.0001) and ADP (8.6 [4.05-15.95] *vs* 21.1 [12.9-24.4] µg/ml; p=0.02) were lower in MS subjects compared to controls. IMCL content was directly associated to glucose, insulin, triglycerides and HOMA-IR and inversely to HDLc, QUICKI and, more importantly, to ADP (r = -0.41; p<0.05). Only in the MS group, ADP partially influenced IMCL content.

Conclusion: ADP is inversely related to IMCL content in non-diabetic adults. This finding has possible implications for the role of ADP in muscle fat oxidation, IR and MS.

Key words: insulin resistance, adiponectin, intramyocellular lipid content, metabolic syndrome.

Introduction

Insulin resistance (IR) is pathophysiologically linked to obesity, metabolic syndrome (MS) and type 2 diabetes (1). Of note, the visceral compartment of central body fat is the one most related to IR (2). However, it is possible to think of an intermediary pathway facilitating fat deposition in liver and muscle (ectopic fat theory)(3,4). In this way, overflow of an excessive amount of fatty acids may be deposited as triglycerides at extramyocellular (EMCL) as well as at intramyocellular (IMCL) compartments, being IMCL lipid content the one intrinsically related to IR. Indeed, several studies utilizing proton Nuclear Magnetic Resonance Spectroscopy (1H-NMRS) have confirmed IMCL size as the main IR predictor (5,6).

The adipose tissue is capable of secreting several proteins that act as regulators of glucose and lipid metabolism. The most abundant of these adipokines is adiponectin (ADP), but in contrast to most of them, ADP is decreased in states of obesity and IR (7,8). In animals, ADP increases the expression of genes involved in fatty acid transportation and oxidation (9). More importantly, administration of ADP increased fat oxidation in muscle, decreased muscle triglycerides (TG) and ameliorated insulin sensitivity (9). Therefore, a reasonable explanation for ADP role in insulin sensitivity may be through its relationship to IMCL content. It has already been demonstrated that ADP is related to IMCL content in adolescents (10,11), although in adults, specially non-diabetic ones, there are still controversies about this association (12-16). Thus, we aimed with this study to further investigate the relationship between ADP and IMCL content in an adult non-diabetic population.

Subjects and Methods

This is a cross-sectional study. Patients from both sexes fulfilling the diagnostic of MS according to NCEP-ATPIII criteria (17) and with body mass index (BMI) of at least 30 kg/m^2 were included. Subjects with fasting or 2 hours impaired glucose tolerance, according to American Diabetes Association criteria(18) were also allowed to participate. Patients with diabetes, smoking and previous cardiovascular, kidney or liver diseases were excluded. In respect to dyslipidemia, those with total cholesterol ≥ 260 mg/dl, triglycerides ≥ 400 mg/dl or taken drugs known to affect glucose or lipid metabolism were excluded. No hypertensive patient, defined according to JCN-7 (19), was on use of angiotensin converting enzyme blockers, angiotensin II antagonists or β-blockers. In order to avoid interference on fat deposition, patients and controls were advised to maintain their usual diet and life style and had to be in a stable weight for 3 months before initiating the study (no gain or loss of more than 2 kg during this period). The control group included healthy volunteers with BMI<25 kg/m^2, not in use of any medication and non-smokers. All patients were selected during their first visit (screening) to the Cardiometabolic Clinic for Outpatient Care of the State University of Rio de Janeiro. Healthy, lean volunteers were enrolled as a control group, recruited among medical students from the State University of Rio de Janeiro.

Therefore, 33 adults were included in this study. Twenty-four were non-diabetic obese MS patients, 16 females and 17 males, being 54.2% (n=13) of them with hypertension and/or glucose intolerance, age 41.5 (35-50) years and mean BMI 37.4 (31.5-42.5) kg/m^2. The control group had 9 subjects, 6 females and 3 males, age 23.0 (23-27) years and mean BMI 20.6 (20.4-21.4) kg/m^2. Clinical and laboratory parameters of both groups are shown on table 1.

Anthropometric measurements and blood pressure

BMI was calculated dividing weight (in kilograms) by squared height (in meters). Waist circumference was obtained measuring the narrowest point midway between the iliac crest and the lower costal margin. Hip circumference was measured at the largest diameter of the gluteal region. Waist-to-hip ratio (WHR) was determined dividing the waist circumference by the hip circumference. Supine blood pressure was measured twice after a 15-min rest using an automatic sphygmomanometer (Multiparameter patient monitor - Lifewindow LW6000, USA).

Assessment of insulin sensitivity and biochemical analysis

All patients underwent an oral glucose tolerance test using 75 g anhydrous glucose. The 2-hour blood glucose test was used to classify glucose tolerance. Patients with type 2 diabetes mellitus (T2DM) were excluded. Fasting plasma glucose (FPG), total cholesterol, triglycerides (TG) and HDL-cholesterol (HDLc) were measured by enzyme-colorimetric GOD-PAP automated method (Modular Analytics PP, Roche). LDL-cholesterol was calculated by Friedwald equation. Plasma insulin was measured by automated chemoluminiscence (coefficient of variation – CV=4.68%). Blood samples were centrifuged and stored at -70^{o}C for further analysis of ADP by Human Serum Adipokine using ELISA (panel A, Lincoplex kit CAT- HADK1-61K-A, Linco Research-St Charles, Missouri, USA). Sensitivity and intra and inter-assay CVs were 145.4 pg/ml, 6.11% and 13.2%, respectively. Adiponectin was not measured in 3 out of 9 controls and in 4 out of 24 MS patients. HOMA-IR and QUICKI indexes were calculated to assess insulin resistance (20,21).

Assessment of muscle lipids

After an overnight fast, proton nuclear magnetic resonance spectroscopy (^{1}H-NMRS) of the right *soleus* muscle was performed using a 1.5T MR Scanner (Magneton Vision, Siemens, Erlangen, Germany). Subjects were instructed to avoid strenuous physical exercise for at least 2 days before the exam, and during the exam, they were positioned in supine position with their right lower leg in the center of the coil. As such, the tibia was oriented nearly parallel to the static magnetic field. The coil center was about 10-15 cm below the knee joint. The volume of interest (13x13x30 mm^3) was centered within the *soleus* muscle, placed to avoid vascular structures and gross adipose tissue deposits. Spectra were acquired by PRESS (point resolved spectroscopy) sequence with the following parameters: echo time 135 ms, repetition time 1600 ms and 256 scans with water suppression. The IMCL content was calculated from the peak areas of IMCL CH_2 (methylene) between 1.2 and 1.3 ppm. The extramyocellular lipid content (EMCL) was calculated from EMCL CH_2 between 1.4 and 1.5 ppm. The creatin signal between 2.9 and 3.1 ppm served as an internal reference for quantification of IMCL and EMCL contents.

Statement of Ethics

This study was approved by the Ethics Committee of the Pedro Ernesto University Hospital of the State University of Rio de Janeiro and all subjects gave their written informed consent. The authors certify that all applicable and governmental regulations concerning the ethical use of humans were followed during this research.

Statistical Analysis

Data non-normally distributed were expressed as median (1^{st} – 3^{rd}). Comparisons between groups were made by Yates corrected Qui squared and Mann-Whitney test, as indicated. ADP and IMCL were Log transformed and associations between these variables and anthropometrical/metabolic parameters were performed by Spearman correlation analysis. Despite non-normally distributed data, multiple regression analysis was done by stepwise backward procedure as data mining, testing IMCL content (log transformed) as dependent variable. Significant differences were assumed to be present at $p<0.05$.

Results

Metabolic, anthropometrics and muscle lipids content parameters in controls and MS patients

As reported on Table 1, MS patients had significant differences for all studied parameters compared to controls: higher BMI, waist, WHR, glucose, insulin and TG and lower HDLc. Markers of insulin resistance like HOMA-IR [3.25 (2.58-4.13) vs 1.02 (0.73-1.29)] and QUICKI [0.32 (0.31-0.33) vs 0.38 (0.37-0.40)] were also significantly different for MS patients ($p<0.0001$).

Circulating plasma ADP level was lower [8.6 (4.05-15.95) *vs* 21.1 (12.9-24.4) µg/ml; $p=0.02$] and IMCL content higher [266.1 (189.8-296.3) vs 72.85 (55.3-109.4) AU, $p<0.0001$] in MS patients compared to controls (figures 1 a and b).

Adiponectin, muscle lipid content and clinical-laboratorial relationships in controls and MS patients

All controls and MS subjects were subjected to ^{1}H-NMRS. ADP (log transformed) levels were inversely related to weight ($r = -0.42$; $p<0.05$) and according to fat distribution, also inversely related to waist and WHR ($r = -0.42$; $p<0.05$ and $r = -0.48$, $p<0.05$, respectively) but not to hip circumference (Table 2). Concerning to metabolic parameters, ADP (log transformed) was negatively related to insulin, HOMA-IR, TG and positively related to QUICKI and HDLc (Table 2). IMCL content (log transformed) was directly associated with weight and BMI ($r = 0.74$; $p<0.001$ and $r = 0.63$; $p<0.001$, respectively). More importantly, it was positively associated to central fat deposition, measured by waist and WHR (table 2).

IMCL content was also related to almost all biochemical and metabolic variables, as demonstrated on table 2. It was directly associated with FPG, insulin, TG and inversely with HDLc. As expected, there was a strong relationship between IMCL content and markers of IR (HOMA and QUICKI), as well as between ADP and IMCL content ($r = -0.41$; $p<0.05$; figure 2). Exclusively within MS patients, we observed associations between Log IMCL and weight ($r = 0.50$, $p < 0.05$), waist ($r = 0.56$, $p < 0.01$) and WHR ($r = 0.51$, $p < 0.05$) and also with LDL-cholesterol ($r = -0.46$, $p < 0.05$) and total cholesterol ($r = -0.45$, $p < 0.05$).

In the pooled group, only waist influenced levels of IMCL content (Beta = 0.96; adjusted $R^2 = 0.61$, $p<0.001$), even when patients older than 40 years were excluded (Beta = 0.74; adjusted $R^2 = 0.53$, $p<0.001$). Solely in MS group, BMI, WHR, hip, FPG, QUICK and LDL-cholesterol influenced IMCL content, although not independently of ADP levels. Therefore, 62% (table 3) of IMCL variation was explained by those cited variables and partially by ADP levels.

Discussion

Our main finding was an inverse relationship between ADP and IMCL content in non-diabetic adults. Weiss and co-workers in an adolescent population (10) have already observed a similar relationship. In their study, however, this relationship was stronger only in the obese group, suggesting that it was more prominent above a certain threshold of IMCL content. Such findings have also been observed by others showing a strong negative association between ADP and IMCL content only in the obese group, without any relationship in lean subjects (12). Our data on non-diabetic adults did not reproduce these findings, and instead when the sample was homogenized to include only non-diabetic MS adults, all of them obese patients, the association between ADP and IMCL content did not become more significant. This could be explained by age differences (22) and/or possible selection of patients strictly according to MS criteria. In accordance with our own data, it was noticed that independently of obesity measurements, ADP was negatively correlated with IMCL content, but only in the oxidative *soleus* muscle and not in the non-oxidative *tibialis* anterior one, suggesting a role for ADP on lipid oxidation (13). On the other hand, Furler and co-workers could not find a simple correlation between cited variables (15). However, when they modeled IMCL content as a fully factored function of ADP level and adiposity, a strong relationship appeared between them. Curiously, the model predicted an inverse relationship only in lean subjects but a direct one in obese ones. These authors studied a cohort of men only with a broad range of adiposity, whereas we studied subjects of both sexes. Recently, Perseghin and co-workers (23) also studied the relationship between adipokines and ectopic fat accumulation in young adults, non-obese, offsprings of T2DM parents and showed that serum leptin strongly correlated with IMCL content, but ADP and retinol binding protein-4 (RBP4) showed only a weak trend, which somehow confirms our findings. However, only leptin

and RBP4, but not ADP, predicted IMCL content according to them (23). On a similar viewpoint, in non-diabetic adults, it was observed that lower ADP occurred mainly in subjects with higher liver fat content rather than in those with higher muscle fat content (16), in accordance with findings that associated increased fat accumulation in the liver, and not in the muscle, with hypoadiponectinemia (14). Differences in studied populations could partially explain observed controversial associations. Taken together, two studies in children (14) and two in adults (including the present study), suggested an inverse relationship between ADP and IMCL, whereas three others (14) could not confirm such a relationship. This clearly warrants more research.

This study confirms many aspects of metabolic disarrangement in MS patients. One notable finding was that in non-diabetic MS patients, serum ADP was less than half the level found in the healthy group. In respect to anthropometrical variables, ADP was inversely related to weight but more importantly, it was strongly associated with measurements of central adiposity, as waist and WHR. Some studies have associated low ADP levels to central adiposity. Indeed, by means of densitometry evaluation, Tankó and co-workers (24) demonstrated that the ADP level was decreased in centrally obese women, compared to peripherally or even generalized obese counterparts. Metabolically, visceral fat is the key component of central adiposity and an independent predictor of ADP levels (25). In this way, men with similar BMI but with higher visceral fat exhibited reduced ADP levels compared with those with low visceral fat accumulation (25). Several groups have demonstrated diminished levels of ADP in patients with obesity, IR or T2DM (8,25,26). More importantly, ADP has been inversely related to all features of the MS cluster (8,26,27) and directly to HDL-c (27). Therefore, our findings are entirely in agreement with the literature.

We measured the IMCL content by ^{1}H-NMRS and demonstrated that it was increased in MS patients and associated with observed metabolic abnormalities. In the same way, IMCL content was associated to central fat deposition, measured by waist and WHR. According to the ectopic fat deposition theory, in obesity muscle and liver fat deposition could be due to a partial incapacity of fat storage into the adipose tissue (3,28). Adipocytes from central body regions are larger and less proliferative than ones from peripheral regions; therefore, central obesity would be a putative model to increased fat shunt from central adipose tissue to liver and muscle deposition. In fact, the relationship between visceral fat and liver fat content measured by MRI has already been demonstrated (29) but the existence of a direct relationship between visceral and muscle fat deserves further investigation.

Previous studies have demonstrated that IMCL content is directly related to IR in muscle (5,6). In our study, surrogate markers of IR were also significantly related to IMCL content in agreement with previous findings. IMCL content was also linked to almost all biochemical and metabolic measurements.

The relationship between ADP and IMCL content has capital importance as it could explain many metabolic alterations behind IR, MS and T2DM. As discussed previously, IMCL content is intrinsically associated to IR. This association has been partially attributed to a decrement in insulin-stimulated activity of IRS-1 phosphorylation (30). In animal models of increased IMCL content and IR, however, ADP infusion decreased muscle triglyceride and recovered insulin signal transduction by increasing insulin-induced tyrosine phosphorylation of insulin receptor and IRS-1 phosphorylation (9). To understand the complex relationship between decreased rates of mitochondrial oxidative phosphorylation and increased IMCL content, recent new avenues of investigation have been opened, related to decreased rate of

mitochondrial ATP synthesis, expressing mainly mitocondrial dysfunction (31) and also novel pathway mechanisms in skeletal muscle (32) by which ADP would quantitatively and functionally increase mitoconchondrial function, finally exerting its anti-diabetic effects.

Limitations of our study warrant mention. We only studied lean healthy adults as a control group and the lack of another group of obese subjects without MS would probably empower our results. Unfortunately, ADP was not tested in 3 controls and 4 patients due to problems with sample collection, what limits our conclusions. Ideally, groups should be matched for age and gender and this was not the case in this study. Our study is descriptive in nature and its cross-sectional design does not allow us to infer a causal relationship to establish definitive conclusions.

In conclusion, data of this study adds to a contradictory literature and is in agreement with an inverse relationship between ADP and IMCL, so pointing to a suspected and probable role of ADP in muscle fat oxidation.

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Titles and legends to figures

Figure 1: a- ADP in MS patients and controls; b- IMCL content in MS patients and controls

Figure 2: Correlation between ADP (log transformed) and IMCL content (log transformed).

Table1: Clinical and laboratory parameters of investigated subjects.

	CONTROLS n = 9	MS n = 24	p
Gender (female/male)	6 / 3	16 / 8	NS
Age (years)	23.0 [23-27]	41.5 [35-50]	<0.001
BMI (kg/height^2)	20.6 [20.4-21.4]	37.4 [31.5-42.5]	<0.0001
Waist (cm) - males	71.0 [68-84]	105.5 [99-116.5]	<0.001
Waist (cm) - females	64.0 [64-68]	106 [99.5-111.5]	<0.001
WHR - males	0.81 [0.71-0.82]	1.00 [0.94-1.1]	<0.01
WHR - females	0.71 [0.70-0.71]	0.91 [0.86-0.96]	<0.01
FPG (mg/dl)	82.0 [72-86]	96.5 [86.5-109.0]	<0.001
Insulin (µUI/ml)	4.9 [4.4-5.8]	13.9 [11-18.4]	<0.0001
HOMA-IR	1.02 [0.73-1.29]	3.25 [2.58-4.13]	<0.0001
QUICKI	0.38 [0.37-0.40]	0.32 [0.31-0.33]	<0.0001
Total cholesterol (mg/dl)	165.0 [144-174]	207.5 [177.5-239.0]	<0.001
HDL cholesterol (mg/dl)	♂ 53.0 [45-63]	♂ 40.0 [37.5-43.5]	<0.05
	♀ 61.0 [51-81]	♀ 44.0 [37-47.5]	0.01
LDL cholesterol (mg/dl)	80.0 [72-110]	128.0 [109.5-163.0]	<0.001
Triglycerides (mg/dl)	57 [48-77]	187.5 [107.5-216.0]	<0.0001
Adiponectin (µg/ml)	21.1 [12.9-24.4]	8.6 [4.05-15.95]	<0.05
LogAdiponectin	1.36 [1.28-1.39]	0.93 [0.61-1.20]	<0.05
IMCL (AU)	72.85 [55.3-109.4]	266.1 [189.8-296.3]	<0.0001
LogIMCL	1.86 [1.74-2.04]	2.42 [2.28-2.47]	<0.0001

MS = Metabolic Syndrome, WHR = waist-to-hip ratio

Data are expressed by median [1st - 3rd quartiles].

Table 2: Correlations between adiponectin (log transformed), IMCL content (log transformed), anthropometrical and metabolic parameters in adults.

Anthropometrics	**LogADP**	**LogIMCL**
Parameters		
Weight	-0.42*	0.74‡
BMI	0.24	0.63‡
Waist	-0.42*	0.76‡
Hip	-0.23	0.54†
Waist-to-hip ratio	-0.48*	0.71‡
Metabolic Parameters		
FPG	0.01	0.43*
Insulin	-0.55†	0.55‡
HOMA-IR	-0.50†	0.59‡
QUICKI	0.51†	-0.58‡
Total cholesterol	-0.08	0.21
LDL-cholesterol	-0.14	0.22
HDL-cholesterol	0.54†	-0.47*
Triglycerides	-0.52†	0.49†

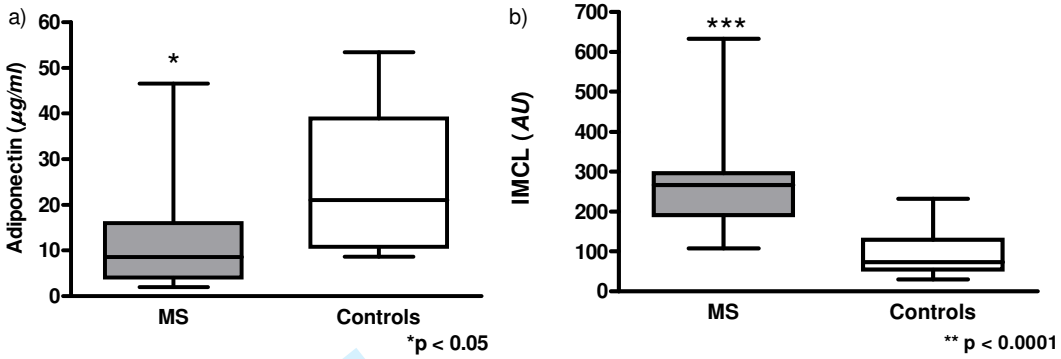
Data expressed as R. *$p < 0.05$.

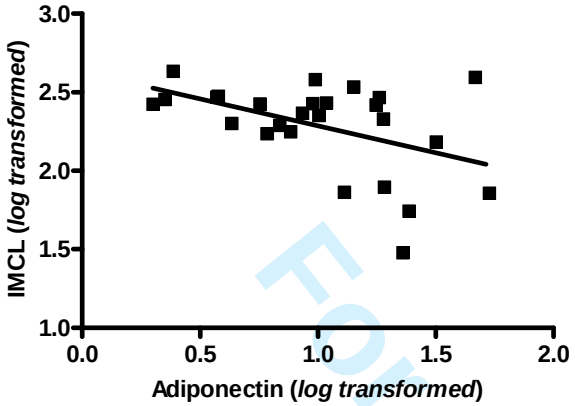
†$p < 0.01$.

‡$p < 0.001$.

Table 3: Multiple regression analysis in MS patients considering IMCL content (log transformed) as dependent variable.

Model	β	P	Adjusted R^2	P
BMI	-1.07	0.003	0.62	0.03
Hip	2.32	0.02		
WHR	2.01	0.007		
FPG	-0.69	0.03		
QUICKI	-1.12	0.006		
LDL-cholesterol	-1.59	0.006		
LogADP	0.93	0.006		





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Rosiglitazone Decreases Intra to Extramyocellular Fat Ratio in Obese Non-Diabetic Adults with Metabolic Syndrome.

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Keywords:	insulin resistance, metabolism, drug treatment

Rosiglitazone Decreases Intra to Extramyocellular Fat Ratio in Obese Non-Diabetic Adults with Metabolic Syndrome.

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Abstract

Background: insulin resistance is intrinsically related to intramyocellular (IMCL) rather than extramyocellular (EMCL) triglyceride content. Conflicting results have been reported on the ability of insulin sensitizer agents, such as thiazolidinediones, to modify muscle fat distribution. The aim of this study was to investigate the role of rosiglitazone (RSG) on muscle fat compartment distribution in an adult population of obese non-diabetic metabolic syndrome patients.

Patients and Methods: fifteen obese, non-diabetic, metabolic syndrome patients were studied by means of ^{1}H Nuclear Magnetic Resonance (^{1}H-NMRS) before and after treatment with 8mg/day of RSG for 6 months. Anthropometrical and metabolic variables were assessed.

Results: After RSG, body weight and hip circumference increased [100.9 (91.12-138.7) vs 107.0 (79.6-142.8) kg and 118 (107-126) cm vs 122 (110-131) cm]; while waist-to-hip ratio (WHR) decreased from 0.93 (0.87-1.00) to 0.89 (0.82-0.97) ($p<0.001$ for all). Additionally, fasting plasma glucose, insulin and HOMA-IR significantly decreased while adiponectin increased over 3 fold [9.7 (3.7-17.7) vs 38.0 (19.3-42.4) µg/ml] without any changes on resistin. Finally, IMCL did not change [267.54 (213.94-297.94) vs 305.75 (230.80-424.75) arbitrary units (AU), $p=0.15$] while EMCL increased [275.53 (210.39-436.66) vs 411.39 (279.92-556.59) AU; $P<0.01$] therefore decreasing IMCL to EMCL ratio (IMCL/EMCL) [1.07 (0.78-1.23) vs. 0.71 (0.53-0.96); $p<0.01$].

Conclusion: RSG treatment increased body weight and hip circumference decreasing WHR. More importantly, it decreased IMCL/EMCL ratio by increasing EMCL without any significant change on IMCL.

Key words: insulin resistance, metabolism, drug treatment.

Abreviations

- IMCL — Intramyocellular trygliceride content
- EMCL — Extramyocellular trygliceride content
- RSG — Rosiglitazone
- WHR — Waist-to-hip ratio
- HOMA-IR — Homeostasis assessment model for insulin resistance
- MS — Metabolic syndrome
- DM2 — Type 2 diabetes mellitus
- TG — Triglycerides
- LDL — Low density lipoprotein
- IECV — Interassay coefficient of variation
- AMPK — Monophosphate-activated protein kinase
- IR — Insulin resistance
- ADP — Adiponectin
- PPAR-γ — Peroxisome proliferator-activated receptor-γ
- NCEP-ATPIII — National cholesterol education treatment program – adult treatment panel III
- ADA — American Diabetes Asssociation
- BMI — Body mass index
- FPG — Fasting plasma glucose
- HDL — High density lipoprotein
- CRP — C-reactive protein
- IMCL/EMCL — IMCL-to-EMCL ratio
- AU — Arbitrary Units

Introduction

The metabolic syndrome (MS) is considered as a highly prevalent and important clinical entity related to type 2 diabetes (DM2) and cardiovascular risk [1-5]**.** Formerly, it was also known as insulin resistance syndrome, due to the seminal importance of insulin resistance (IR) as its main pathophysiological basis. IR may, however, be bystander of more complex mechanisms involving visceral obesity, ectopic fat deposition and dysfunctional adipose tissue [6]. Liver and muscle fat deposits are the main sites for IR-related pathophysiology [7].

Overflow of an excessive amount of fatty acids could be deposited as triglycerides at extramyocellular (EMCL) as well as at intramyocellular (IMCL) compartments, being IMCL one intrinsically related to IR. Indeed, several studies utilizing ^{1}H Nuclear Magnetic Resonance (^{1}H-NMRS) spectroscopy have confirmed the size of IMCL as the main IR predictor [8,9].

The adipose tissue is now considered as a dynamic endocrine organ, producing several proteins (adipokines). Among them, adiponectin (ADP), an adipokine almost exclusively produced by the adipose tissue, is directly related to insulin sensitivity [10-12] and to IMCL fat deposition in obese adolescents [13]. We as well as other authors have recently demonstrated an inverse relationship between IMCL size, but not EMCL, and ADP in adults. [14,15].

Thiazolidinediones, insulin sensitizer agents acting as peroxisome proliferator-activated receptor-γ (PPAR- γ) agonists, may decrease circulating fatty acids and ameliorate fat distribution, including liver fat reduction [16]. In culture of muscle cells from diabetic patients it has been demonstrated that troglitazone, formerly marketed PPAR-γ agonist, increased fatty acid oxidation [17] potentially reducing intramyocellular

triglycerides. Therefore, a plausible mechanism for its action as insulin sensitizer may be through reduction on muscle fat deposition. Few studies in humans have looked at this subject and their results are somehow conflicting, showing increases in EMCL after rosiglitazone (RSG) [18], decreasing levels in IMCL after pioglitazone and metformin [19] and finally, decrements in IMCL after pioglitazone, but not after metformin [20].Therefore, it is possible that some methodological differences may have contributed to reported results.

Therefore, our aim was to investigate if RSG used for a longer period of time in a group of non-diabetic, MS patients, not in use of drugs acting on carbohydrate pathway, would influence muscle fat distribution.

Patients and Methods

Sixteen obese adults (BMI 38.0 ± 5.7 kg/m^2; aged 41.4 ± 8.7 years; 11 females), MS patients, defined by NCEP-ATPIII [21] were selected at Cardiometabolic Clinic for outpatient care of the State University of Rio de Janeiro. RSG in a dose of 8mg QAD was administered to all volunteers for a 6 months period. In order to avoid interference on fat deposition, they were advised to maintain their usual diet and life style throughout the study.

Exclusion criteria included diabetes, smoking and previous cardiovascular, kidney or liver diseases. Patients taken drugs known to affect glucose or lipid metabolism were excluded. All subjects gave their written informed consent and the local Ethical Committee approved the protocol.

Anthropometric measurements and blood pressure

Height, weight, waist and hip circumferences, as well as blood pressure were collected by the same trained examiner as previously reported [22]. Body mass index (BMI) was defined as the ratio between weight in Kg and squared height in meters.

Waist circumference was obtained by measuring the narrowest point midway between the iliac crest and the lower costal margin. Hip circumference was measured at the largest diameter of the gluteal region. Waist-to-hip ratio (WHR) was determined by dividing the waist by the hip circumference. Supine blood pressure was measured twice after a 15-minute rest using an automatic sphygmomanometer (Multiparameter patient monitor - Lifewindow LW6000, USA).

Assessment of insulin sensitivity and biochemical analysis

All patients underwent an oral glucose tolerance test using 75g anhydrous glucose. Results were used to classify glucose tolerance state. As stated before, patients with type 2 diabetes mellitus were excluded, but those with impaired glucose tolerance (IGT) were allowed to participate. Blood samples were collected after 12-hour overnight fasting. All laboratory measurements were performed in duplicate using an automated method (Modular Analytics PP, Roche, Basel, Switzerland). Fasting plasma glucose (FPG), total cholesterol, triglycerides (TG) and high-density lipoprotein (HDL) cholesterol were measured respectively, by enzyme-colorimetric GOD-PAP (inter-assay coefficient of variation (IECV) = 1.09%;), enzymatic GPO-PAP (IECV = 2.93%), enzymatic GPO-PAP (IECV = 1.29%) and enzyme-colorimetric without pre-treatment (IECV = 3.23%). Plasma low-density lipoprotein (LDL) cholesterol was calculated according to Friedwald equation. Fasting plasma insulin was measured by automated chemoluminiscence (IECV 4.68%). Fibrinogen (IECV = 4.33%) and high-sensitivity C-reactive protein (CRP) (IECV = 2.66%) were measured respectively by coagulometric

and imunoturbidimetry methods on modular Analytics P (ROCHE®). Blood samples were centrifuged and stored at −70ºC for further analysis of adipokines. Adiponectin, resistin and PAI-1 were measured by Human Serum Adipokine (panel A, Lincoplex kit CAT, HADK1, 61K-A, Linco Research, St. Charles, MO, USA). Intra and inter-assay CVs were 6.11 and 13.2, 7.26 and 9.12, 4.37 and 20.8%, respectively [22]. HOMA-IR [23] and QUICK [24] were calculated to assess insulin resistance.

Assessment of muscle lipids

After an overnight fast, ^{1}H-NMRS of the right soleus muscle was performed using a 1.5T MR Scanner (Magneton Vision, Siemens, Erlangen, Germany). Subjects were instructed to avoid strenuous physical exercise for at least 2 days before the exam. They were positioned in supine position with their right lower leg at the center of the coil. Thus, the tibia was oriented nearly parallel to the static magnetic field. The coil center was about 10–15 cm below knee joint. Volume of interest (13x13x30mm^3) was centered within the soleus muscle and placed to avoid vascular structures and gross adipose tissue deposits. Spectra were acquired by PRESS (point resolved spectroscopy) sequence with the following parameters: echo time 135 OR 270 ms, repetition time 1600 ms and 128 OR 256 scans with water suppression. IMCL content was calculated from peak areas of IMCL CH2 (methylene) between 1.2 and 1.3 ppm. EMCL was calculated from EMCL CH2 between 1.4 and 1.5 ppm. The creatin signal between 2.9 and 3.1 ppm served as an internal reference for IMCL and EMCL quantification.

Statistical Analysis

All data group are reported as median [1st - 3rd quartiles], unless otherwise stated, and were analyzed by Prism 4.01 (Graphpad Inc., San Diego, CA, USA). The pooled

group was divided into two subgroups, according to quartiles of weight gain. Comparison r within group at baseline and after treatment period was performed using Wilcoxon matched pair test. Significant differences were assumed to be present at $p<0.05$.

Results

After six months, fifteen patients ended the treatment period and had ^{1}H-NMRS performed. Therefore, data included are for those who completed the whole study protocol.

Table 1 depicts data for all patients included in the final analysis. After 6 months of RSG treatment there was a significant increase in weight, BMI and hip circumference without changes on waist circumference. Consequently, WHR decreased after treatment [0.93 (0.87-1.0) vs 0.89 (0.82-0.97); $p<0.001$].

Changing patterns of muscle triglyceride distribution after RSG was our main objective. In accordance with this view, IMCL fat content did not change but EMCL significantly increased (table 1). There was also an important increase in body weight and the pooled group was subsequently divided into higher (4^{th} quartile; i.e. ≥ 4.1kg) and lower weight gainers during treatment. Once again, even in those who gained more than 4.0kg there was no increase on IMCL (figure 1). In contrast, EMCL significantly increased on both groups. IMCL to EMCL ratio (IMCL/EMCL) was then calculated in the pooled group at baseline and after treatment. This ratio significantly decreased after RSG [1.07 (0.78-1.23) vs. 0.71 (0.53-0.96); $p<0.01$ – figure 2]. All studied variables related to carbohydrate metabolism showed a consistent modification, where FPG, insulin and HOMA-IR decreased and QUICKI increased. Adiponectin increased over 3 fold above basal level [9.7 (3.7-17.7) vs. 38.0 (19.3-42.4) µg/ml; ($p<0.001$)] while resistin levels were kept unchanged (table 1). Lipid profile demonstrated a slight increment in

total and LDL-cholesterol, but HDL-cholesterol and triglycerides did not change (table 1). Finally, a significant ~70% reduction in CRP level was achieved [1.0 (0.5-2.3) to 0.3 (0.2-0.5) mg/dl; p<0.0001], while fibrinogen, but not PAI-1, slightly decreased (table 1).

RSG was well tolerated and only one patient presented minor pre-tibial edema. In fact, weight gain was the major side-effect noticed.

Discussion

The main finding of this study was an increase in fat deposition in the extramyocellular muscle compartment after 6 months of treatment with an agonist of the peroxisome proliferator-activated receptor-γ (PPAR-γ), RSG. However, this was not observed at the intramyocellular compartment where triglyceride content was kept unchanged. Therefore, the ratio IMCL/EMCL significantly decreased (figure 2). Although augmentation of fatty acid disposal in skeletal muscle from diabetic patients has been demonstrated *in vitro* [17], potentially reducing intramyocellular triglyceride content, clinical studies in humans have shown contradictory results. Indeed, Mayerson and co-workers [18] studying diabetic patients under RSG for only three months could not demonstrate any improvement on IMCL content. Instead, a 39% increase in EMCL was achieved. Their results are in accordance with our findings, i.e. a 49.3% increase on EMCL content. In contrast to the present study, there was no weight gain by their group. Due to massive weight gain achieved by our patients, they were divided into those who gained 4.1kg or more (4^{th} quartile of weight gain) and those who did not. It was then noticed that IMCL did not change substantially on either group while EMCL did, even in those who gained less than 4.1kg eliciting a significant decrease of the ratio IMCL/EMCL (figure 2). One study in Zucker fat rats also showed similar results of RSG, i.e. a decrease on IMCL/EMCL ratio [25].

Teranishi and co-workers [19], succeeded in demonstrating a decreased IMCL fat content after pioglitazone or metformin in a group of type 2 diabetic patients. In contrast to our study, however, patients on pioglitazone did not increase weight and a slight decrease was observed in the metformin group. This may suggest that lifestyle modification, including exercises and dietary changes, implemented before and maintained during the intervention period, as stated by the authors, influenced their results. Rasouli and co-workers [20] used muscle biopsy rather than ^{1}H-NMRS in a group of glucose intolerant otherwise healthy subjects treated with pioglitazone or metformin for 10 weeks. There was a 34% decrease on IMCL with pioglitazone but no change within the metformin group. A modest increase in body weight (2.63 ± 0.65 kg) was accompanied by a decrease in visceral-to-subcutaneous fat ratio. Therefore, differences in methodology (muscle biopsy), in weight changes, periods of treatment or even in drug characteristics may explain our contradictory results.

A few possible interpretations can be raised from the present findings i) the excess fat was shunted to extramyocellular compartment which may behave as peripheral adipocytes accumulating triglyceride under thiazolidinediones [18]; ii) the excess fat eventually driven into intramyocellular compartment have been oxidized by PPAR-γ activation [20] or iii) fat diversion from ectopic lipid deposits into the subcutaneous one [18,20,26,27]. Moreover, RSG therapy increases adipocyte sensitivity to insulin thus decreasing peripheral fat lipolysis [18]. In accordance, in our group of patients, the massive increase of body weight was accompanied by modification on indirect measures of body fat distribution, namely an increase on hip circumference and a decrease in WHR.

In animals, ADP increases expression of genes involved on fatty acid transportation and oxidation, like acyl-CoA oxidase, CD36 and uncoupled protein [28]. More importantly, administration of ADP increased fat oxidation in muscle, decreased

muscle triglycerides (TG) and ameliorated insulin sensitivity [28]. Thiazolidinediones activate adenosine monophosphate–activated protein kinase (AMPK) and increase serum concentration of adiponectin, that also activates AMPK in both muscle and liver [28,29]. The adiponectin level substantially increased during our experiment and could be responsible for an increased muscle fat oxidation. However, as IMCL fat content did not decrease, such relationship could not be demonstrated. Moreover, there was no correlation between IMCL/EMCL ratio decrement and adiponectin increment (data not shown).

Regarding the modification of the entire profile of metabolic variables, such as FPG, insulin, HOMA-IR, QUICK and fibrinogen, as well as of an inflammatory marker (PCR) and the lipid profile, this study is entirely in accordance with the spectrum of action of thiazolidinediones [16]. It is noteworthy that even in an experimental free living situation, where patients gained substantial amount of weight, treatment with RSG modified all those markers of insulin sensitivity into the right direction while ameliorating inflammatory markers. This may be understood as a result of a favorable fat deposition, as suggested by the observed increase of body weight and hip circumference while decreasing WHR. Accordingly, in type 2 diabetic patients, RSG decreased insulin resistance while increasing leptin, a marker of total body fat. Interestingly, increase on maximum subcutaneous fat thickness after RSG, measured by sonography, correlated positively with leptin augmentation and negatively with HOMA-IR [30]. Taken together, amelioration of metabolic parameters may be understood as a result of decreased IR secondary to peripheral fat deposition.

This study has a number of limitations: i- ideally, when a drug intervention is tested, a control, placebo-treated group should be included. However, in such a group of MS patients this could not be performed. However, the robust amelioration observed in the majority of metabolic parameters, adiponectin and CRP, as well as a remarkable

(235%) improvement in endothelial function previously published in the same group [22] support such assumption. Additionally, a before-and-after approach means that the treated group served as their own control. Finally, our data should be viewed restrictly to the population studied due to the small sample size, tested by non-parametric methods. ii- Our patients gained a huge amount of weight, beyond what could be expected by RSG treatment. Therefore, an isocaloric diet could have minimized the weight gain.

This study has also a number of advantages. This is the longest (6 months) study among those looking at TZD intervention and muscle fat. We also investigated a homogeneous population of obese MS adults, not using drugs that could interfere with glucose or fat metabolism.

In conclusion, in a group of obese non-diabetic metabolic syndrome patients, treatment with RSG for six months increased body weight but improved several metabolic and inflammatory markers and decreased IMCL to EMCL ratio by increasing extramyocellular without affecting intramyocellular fat compartment.

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Declaration of Competing Interests: AFG-M, LRB, MT and BG are members of the Advisory Board for GSK in Brazil and has received fees for giving talks. For all the others authors, there is nothing to declare.

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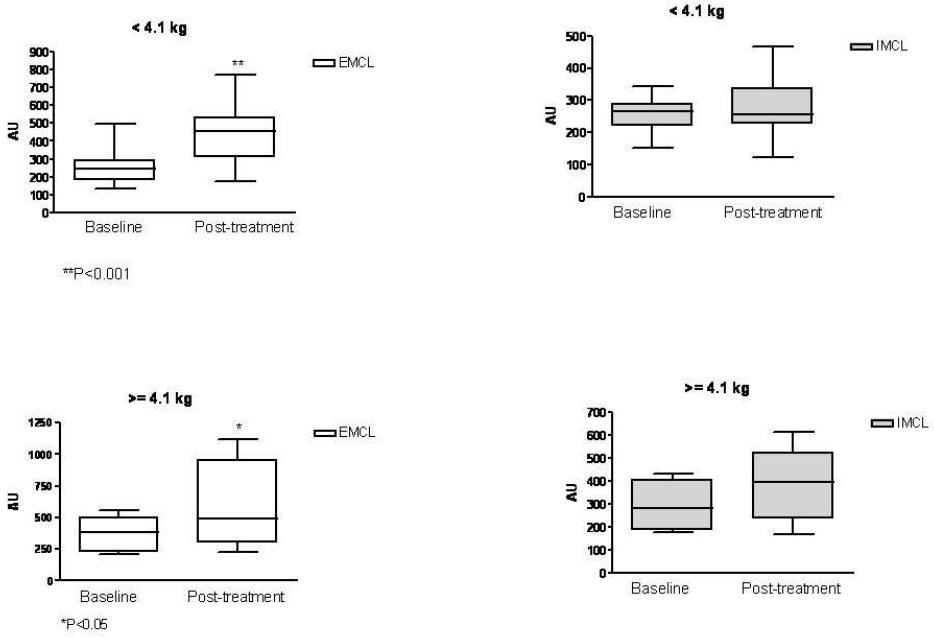
Figure 1: Intra (IMCL) and Extramyocellular (EMCL) lipid content at baseline and after six months of rosiglitazone treatment (post-treatment) divided according to weight gain during follow-up.

Figure 2: Intra (IMCL) to Extramyocellular (EMCL) ratio decrement after rosiglitazone treatment in non-diabetic obese adults with metabolic syndrome.

Table 1: ***Clinical and laboratory parameters of investigated subjects.***

	Baseline	After RSG treatment
Weight (Kg)	100.9 [91.12-138.7]	107,0 [79.6-142.8]***
BMI (Kg/m^2)	38.1 [32.8-44.3]	38.4 [34.6-48.4]***
Waist (cm)	106.0 [98.0-112.0]	109.0 [101.0-114.0]
Hip (cm)	118 [107-126]	122 [110-131]***
WHR	0.93 [0.87-1.00]	0.89 [0.82-0.97]***
FPG (mg/dl)	103 [95-109]	94 [84-101]*
Insulin (μUI/ml)	14.6 [10.6-20.3]	9.0 [7.2-12.8]***
HOMA-IR	3.30 [2.47-4.37]	2.00 [1.61-3.16]***
QUICKI	0.320 [0.307-0.333]	0.343 [0.321-0.355]***
Systolic BP (mmHg)	141 [127.5-170]	134 [128.5-157.5]
Diastolic BP (mmHg)	85 [80-95]	83 [79.5-90]
Total Cholesterol (mg/dl)	190 [172-233]	199 [190-265]**
LDL-cholesterol (mg/dl)	116 [95-163]	134 [122-183]**
HDL-cholesterol (mg/dl)	43 [37-46]	44 [35-48.5]
Triglycerides (mg/dl)	175 [98-211]	151 [119-211]
C-reactive protein (mg/dl)	1.0 [0.5-2.3]	0.3 [0.2-0.5]***
Fibrinogen (mg/dl)	303.9 [254.2-364.0]	265.0 [209.7-296.5]*
Adiponectin (μg/ml)	9.7 [3.7-17.7]	38.0 [19.3-42.4]***
PAI-1 (ng/ml)	23.8 [21,2-28.1]	22.5 [20.60-24.7]
Resistin (ng/ml)	17.3 [17.2-18.2]	17.1 [16.9-17.8]
EMCL (AU)	275.53 [210.39-436.66]	411.39 [279.92-556.59]**
IMCL (AU)	267.54 [213.94-297.94]	305.75 [230.80-424.75]

RSG, rosiglitazone; *P< 0.05 **P<0.01 ***P<0.001



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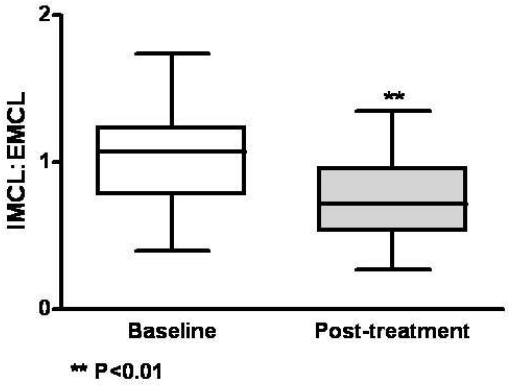

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