UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE – UFCSPA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Diego Olschowsky Borges

Análise da Expressão de Genes de Adipocinas no Tecido Adiposo de Pacientes Submetidos à Cirurgia Bariátrica

Porto Alegre
2013

Diego Olschowsky Borges

Análise da Expressão de Genes de Adipocinas no Tecido Adiposo de Pacientes Submetidos à Cirurgia Bariátrica

Dissertação submetida ao Programa de Pós-Graduação em Ciências da Saúde da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre em Ciências da Saúde

Orientadora: Dra.Vanessa Suñé Mattevi

Porto Alegre
2013

Catalogação na Publicação

B732a Borges, Diego Olschowsky
Análise da expressão de genes de adipocinas no tecido adiposo de pacientes submetidos à cirurgia bariátrica / Diego Olschowsky Borges. – 2013.
60 f. : il., tabelas ; 30 cm.

Dissertação (mestrado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em Ciências da Saúde, 2013.

"Orientadora: Dra. Vanessa Sune Mattevi"

1. Obesidade mórbida. 2. Cirurgia bariátrica. 3. Polimorfismo de nucleotídeo único. 4. Expressão gênica. 5. Adipocinas. Título.

CDD 616.398
CDU 616.39

Bibliotecário Vladimir Luciano Pinto - CRB 10/1112

SUMÁRIO

RESUMO

A prevalência de obesidade tem aumentado globalmente nas últimas décadas. Esta patologia é caracterizada pelo acúmulo excessivo de tecido adiposo no organismo humano. As moléculas produzidas pelo tecido adiposo são conhecidas como adipocinas e parecem exercer papeis importantes em diversas rotas metabólicas. Entretanto, os mecanismos genéticos de controle da expressão de adipocinas nos variados depósitos de tecido adiposo ainda não estão bem compreendidos. Dessa maneira, o objetivo do presente trabalho foi avaliar o efeito de polimorfismos de nucleotídeo único (SNPs) localizados em regiões regulatórias dos genes de três adipocinas, leptina, resistina e adiponectina, nos níveis de expressão desses genes em indivíduos obesos mórbidos. Tratou-se de um estudo transversal com 60 pacientes submetidos a cirurgia bariátrica. Os SNPs foram genotipados pela reação em cadeia da polimerase em tempo real ou aliada à clivagem com enzimas de restrição a partir de amostras de DNA extraídas de sangue total. As variantes investigadas foram *LEP* -2548 G>A (rs7799039), *RETN* -420 C>G (rs1862513), *ADIPOQ* -11377 C>G (rs17300539) e -11391 G>A (rs266729). Amostras frescas de tecido adiposo subcutâneo e visceral foram obtidas e os níveis de expressão gênica foram avaliados pela quantificação relativa por PCR em tempo real. Nesta população avaliada, 92% pertenciam ao sexo feminino com idade média de 42,3±8,9 anos. Todas as adipocinas apresentaram maiores níveis de expressão no tecido adiposo subcutâneo do que no tecido adiposo visceral. Além disso, a expressão tecidual da adiponectina foi diretamente correlacionada à expressão da leptina em ambos os tecidos e inversamente correlacionada à expressão da resistina no tecido subcutâneo. Por fim, a variante *ADIPOQ* -11377 C>G foi associada a um menor nível de expressão de adiponectina no tecido subcutâneo e todos os polimorfismos parecem exercer um papel na expressão diferencial entre os dois sítios do tecido adiposo de seus respectivos genes.

Palavras-chave: Obesidade Mórbida. Polimorfismo. Expressão Gênica. Adipocinas.

ABSTRACT

The worldwide prevalence of obesity has been increasing in the last decades. This pathology is characterized by excessive accumulation of adipose tissue in human body. Molecules produced by adipose tissue are known as adipokines and seem to exert important roles in several metabolic pathways. However, the genetic mechanisms that control the expression of adipokines in different adipose tissue depots are still not well recognized. Therefore, this study aimed to evaluate the effect of single nucleotide polymorphisms (SNPs) located on the regulatory regions of leptin, resistin and adiponectin genes on their adipose tissue expression levels of morbidly obese subjects. It was a cross-sectional study with 60 obese subjects that undergone bariatric surgery. SNPs were genotyped by real-time polymerase reaction or through restriction endonuclease cleavage in whole blood DNA samples. Variants investigated were *LEP* -2548 G>A (rs7799039), *RETN* -420 C>G (rs1862513), *ADIPOQ* -11377 C>G (rs17300539) and -11391 G>A (rs266729). Fresh visceral (VAT) and subcutaneous (SAT) adipose tissue was obtained and the expression levels was assessed by real-time PCR with relative quantification. Ninety-two percent of patients were female with 42.3±8.9 years old. All adipokines showed a higher level of expression in the SAT than in VAT. Furthermore, adiponectin expression was directly correlated with leptin expression in both tissues and inverse correlated with resistin expression on SAT. Finally, the *ADIPOQ* -11377 C>G variant was associated with lower expression of adiponectin in SAT and all polymorphisms seem to exert a role in differential adipose tissue expression of their respective genes.

Key Words: Morbid Obesity. Polymorphism. Gene Expression. Adipokines.

1 REVISÃO DE LITERATURA

1.1 A doença

A obesidade, juntamente com o excesso de peso, é definida como o acúmulo de gordura anormal ou excessivo que pode prejudicar a saúde (World Health Organization - WHO, 2012). É considerada uma doença crônica que resulta do balanço energético positivo associado ao ganho de peso, sendo considerada uma epidemia global (WHO, 2000). A Sociedade da Obesidade dos Estados Unidos reconhece as razões para defini-la como doença e em uma recente revisão a considerou como uma condição complexa que reduz a qualidade de vida e aumenta a mortalidade e, ainda, traz consigo a discriminação e estigmatização, mesmo tendo fatores causais que não podem ser controlados pelos acometidos (ALISSON et al., 2008).

Segundo o Centro de Controle e Prevenção de Doenças (CCPD) do governo dos Estados Unidos, a obesidade e o excesso de peso são indicadores de faixas de peso maiores do que é considerado saudável para uma determinada altura (CCPD, 2012). Assim, o principal índice para a classificação da obesidade e do excesso de peso em adultos é o índice de massa corporal (IMC), calculado pela simples divisão do peso em quilos, pelo quadrado da altura em metros. São considerados obesos os indivíduos com IMC maior ou igual a 30,0 kg/m² e excesso de peso os indivíduos com IMC entre 25,0 e 29,9 kg/m² (WHO, 2012). As demais classificações de adultos conforme seu IMC podem ser visualizadas na tabela 1.

Apesar de ser a medida mais útil a nível populacional para diagnóstico de obesidade, o IMC não diferencia se o excesso de peso está associado ganho de massa muscular ou massa de gordura (WHO, 2000). Desta forma, outra maneira de definir a obesidade seria de acordo com o percentual de gordura corporal (NATIONAL INSTITUTES OF HEALTH, 2002). Nesse contexto, podemos definir a obesidade como 25% ou mais em homens e 35% ou mais em mulheres (GRUNDY, 2004). Os métodos para avaliação do percentual de gordura incluem pesagem na água, medida da espessura das pregas cutâneas e a bioimpedância. Geralmente, seus custos e complexidades inviabilizam sua inserção na rotina clínica (GRUNDY, 2004).

Tabela 1. Classificação de indivíduos adultos de acordo com seu IMC

Classificação	IMC (kg/m²)
Baixo peso	<18,5
Normal	18,5 a 24,99
Excesso de peso:	≥25,00
Pré-obeso	25,00 a 29,99
Obeso Classe I	30,00 a 34,99
Obeso Classe II	35,00 a 39,99
Obeso Classe III	≥ 40,00

IMC = Índice de Massa Corporal
Adaptado de: Obesity: preventing and managing the global epidemic: report of a WHO consultation (2000).

Outro aspecto que diferencia o risco em obesos é onde essa gordura está localizada, visto que o excesso de gordura abdominal é maior fator de risco para doenças do que o excesso de gordura equilibrado e perifericamente distribuído (WHO, 2000). Nesse contexto, a melhor forma de estimar a obesidade abdominal é pela mensuração da circunferência abdominal (GRUNDY, 2004). Este é um importante marcador de risco e um dos critérios para diagnóstico de síndrome metabólica(GRUNDY et al., 2004).

1.2 Epidemiologia

Como já mencionado anteriormente, a obesidade tornou-se uma epidemia global (WHO, 2000). As prevalências mundiais de obesidade para ambos os sexos em adultos no ano de 2008 podem ser visualizadas na figura 1. No geral, as maiores prevalências observadas são as do continente americano, que atinge os 26%. Porém, os dados mais alarmantes são em relação ao excesso de peso, que já atinge 62,0% dos americanos e 57,4% dos europeus. Entre 1980 e 2008 a prevalência mundial da obesidade praticamente dobrou, resultando em cerca de meio bilhão de obesos. (WHO, 2012). No Brasil, o último levantamento aponta prevalências de obesidade e excesso de peso de 15,0% e 48,1%, respectivamente. A região Sul apresenta as maiores prevalências, com 49,4% para o excesso de peso e 16,4%

para obesidade. Entretanto, quando observamos as capitais da região Sul em separado, Porto Alegre apresenta a menor prevalência de obesidade, 15,4%, e a maior prevalência de excesso de peso, 50,8% (DATASUS, 2010).

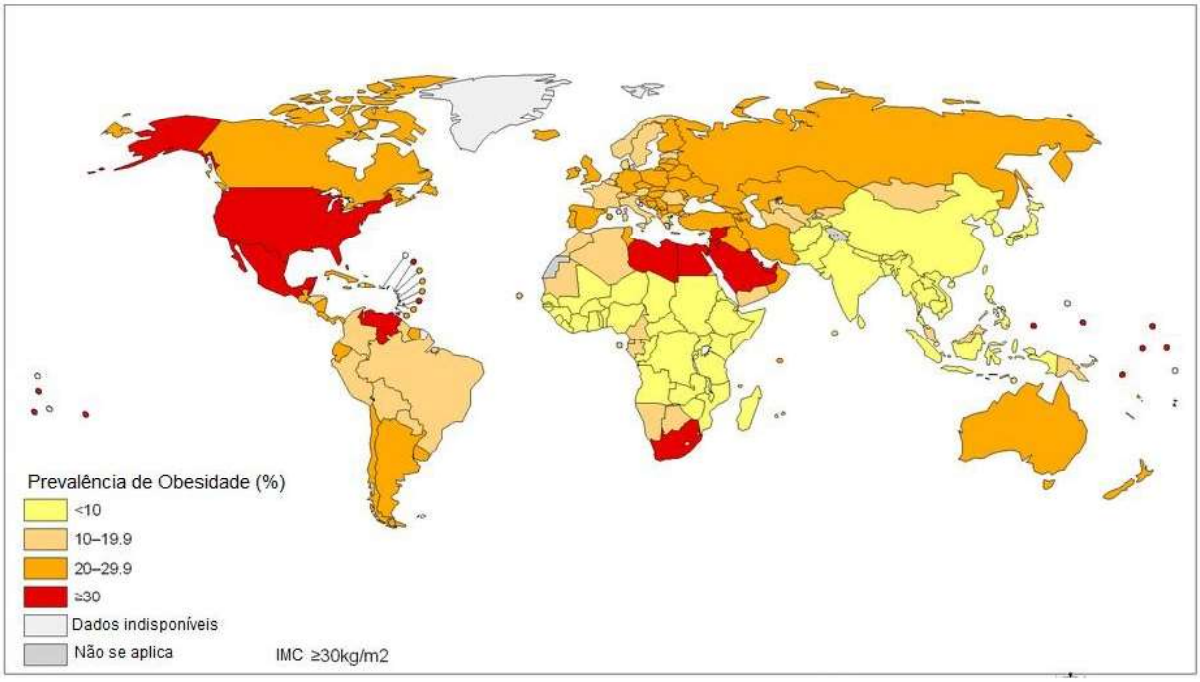

Figura 1 - Prevalência de Obesidade para ambos os sexos em adultos em 2008 (Adaptado de: World Health Organization, 2011 – IMC = Índice de Massa Corporal).

Na Europa, estima-se que a obesidade seja responsável por 80% dos casos de diabetes mellitus do tipo 2 (DM2), 35% dos casos de doença cardíaca isquêmica e 55% dos casos de hipertensão (TSIGOS et al., 2008). Sugere-se que um mínimo de 279 mil mortes anuais são causadas pelo excesso de peso (BANEGAS et al., 2003).

A obesidade tornou-se um grande problema de saúde pública, especialmente por ser considerada um fator de risco para outras doenças crônicas como DM2, doenças cardiovasculares, hipertensão e alguns tipos de câncer (POIRIER et al., 2006). Ainda, sugere-se que a epidemia atual da obesidade também é a principal responsável pelo aumento da prevalência de síndrome metabólica (SM), definindo esta síndrome como um agrupamento de complicações metabólicas da obesidade clinicamente identificada pelo incremento da circunferência abdominal (GRUNDY et al., 2004).

Outro aspecto que acrescenta importância ao tópico da obesidade é a alta penetração em crianças e adolescentes. No ano de 2010, estimou-se que mais de 42 milhões de crianças apresentavam excesso de peso. Essas crianças tendem a

permanecer obesas e apresentar um maior risco de desenvolver doenças cardiovasculares e DM2 em estágios precoces da vida adulta, o que traz um alerta para prevenção da obesidade na infância (WHO, [S.d.]).

1.3 Causas

De acordo com a Associação Brasileira para o Estudo da Obesidade e da Síndrome Metabólica (ABESO), a obesidade é multifatorial, resultado da complexa interação entre genes, ambiente, estilos de vida e fatores emocionais (ABESO, 2009). Do ponto de vista social, ela pode ser relacionada à abundância de suprimentos e empregos sedentários resultantes do avanço da tecnologia, que fez desaparecer os trabalhos manuais e permitiu a produção de alimentos em larga escala (GRUNDY, 2004). Essa alta oferta de alimentos, associados às atuais baixas necessidades de atividade física em casa e/ou no trabalho, mantém um estado de balanço energético positivo que, após um determinado período de tempo, eleva o IMC dos indivíduos (O'RAHILLY; FAROOQI, 2006).

Do ponto de vista fisiológico, nosso organismo apresenta um gasto de energia básico para manutenção de suas funções, denominado taxa metabólica basal. Quando fazemos qualquer atividade esse gasto de energia aumenta, o que é chamado de gasto de energia ativa. A ingestão de energia é feita pelo consumo de alimentos. Devido às leis da termodinâmica, a energia não pode ser destruída e, portanto, temos a capacidade de armazenar energia. O principal armazenamento é feito em forma de gordura, já que ela é menos densa que os carboidratos e precisa de uma menor quantidade de água para a estocagem (SPEAKMAN, 2004). Assim, quando nos alimentamos, ocorre um aumento nos níveis de insulina, glicose e lipídeos que estimula a síntese de triglicerídeos e seu armazenamento no tecido adiposo. Dessa maneira, o tecido adiposo é indispensável para nossa sobrevivência, visto que participa ativamente do equilíbrio energético, armazenando gordura nos períodos de fartura e liberando ácidos graxos durante os períodos de jejum (AHIMA, 2006). Resumindo, considera-se a obesidade um problema de desequilíbrio entre o gasto e a ingestão de energia (SPEAKMAN, 2004).

1.4 Tratamento

Existem diversos tratamentos para a obesidade e sua escolha depende do grau da doença e da presença de morbidades associadas. Porém, a mudança no estilo de vida é fundamental para o sucesso da abordagem (ABESO, 2009). Ainda, os objetivos do tratamento incluem não só a perda de peso, mas também a redução do risco para o paciente e a melhora da sua saúde (TSIGOS et al., 2008).

A perda de peso é o principal objetivo, pois está relacionada à redução dos fatores de risco para diabetes e doenças cardiovasculares, como níveis pressóricos, níveis de triglicerídeos e de glicose (NHLBI, 1998). As abordagens disponíveis para causar uma redução de peso pela indução de um balanço energético negativo incluem dietoterapia, prática de exercício físico, farmacoterapia e cirurgia (POIRIER et al., 2006).

A redução na ingestão calórica é sabidamente efetiva na redução da massa corporal. Dessa maneira, as dietas são comumente utilizadas nos mais variados casos. Entretanto, a redução calórica pode ser induzida por restrição de diversos nutrientes da alimentação e os tipos de dietas podem variar como, por exemplo, dietas ricas em gorduras e escassas em carboidratos, dietas balanceadas e dietas escassas em gorduras (ABESO, 2009).

O aumento no gasto energético diário pode ser obtido pela prática de atividade física. Além disso, a extinção de comportamentos sedentários, como assistir televisão e ficar no computador, deve ser incentivada, substituindo-os por caminhadas e outras práticas esportivas, já que os benefícios do exercício físico vão além da simples perda de peso (TSIGOS et al., 2008).

As abordagens farmacológicas incluem diversos fármacos, como sibutramina, anfepramona, femproporex. Todos esses medicamentos podem apresentar efeitos colaterais consideráveis, portanto suas indicações são restritas e a sua comercialização controlada (ABESO, 2009).

A abordagem cirúrgica é considerada para casos específicos. Apenas indivíduos considerados obesos mórbidos ou severos (IMC ≥ 40 kg/m² ou ≥ 35 kg/m² com morbidades associadas) devem ser submetidos à cirurgia (NHLBI, NATIONAL HEART, 1998). Em outros casos, indivíduos que com um grau considerável de obesidade e que não conseguem sucesso nas outras formas de tratamento a cima citadas podem ser indicados à cirurgia da obesidade ou cirurgia bariátrica. Existem

técnicas cirúrgicas restritivas, associadas à diminuição do estômago e, portanto, à restrição da quantidade de alimentos ingeridos; e técnicas disabsortivas, associadas à redução do intestino e da área de absorção de nutrientes. Ainda, existem técnicas mistas, ou seja, que combinam os dois tipos (ABESO, 2009).

1.5 Tecido adiposo visceral e subcutâneo

O tecido adiposo é formado não só por células adiposas maduras, mas também por uma camada de células indiferenciadas, células endoteliais, fibras do sistema nervoso simpático e macrófagos (HAUNER, 2007). Na cavidade abdominal, o tecido adiposo tem duas formas de apresentação: o tecido adiposo visceral (TAV), que é encontrado junto aos órgãos internos no omento e no mesentério, e o tecido adiposo subcutâneo (TAB), que é localizado abaixo da pele, principalmente na região do tronco e na porção gluteofemoral (GRUNDY, 2004).

As diferenças entre TAV e TAB envolvem diferenças na expressão de diversos genes relacionados à diferenciação celular e a adipogênese (VOHL et al., 2004). Uma recente revisão sobre as características bioquímicas e estruturais de diferentes depósitos de tecido adiposo conclui que o tecido visceral apresenta um papel de tamponamento de nutrientes mais ativo metabolicamente. Já o subcutâneo teria um papel de armazenamento em longo prazo e dessa maneira seria menos ativo (WRONSKA; KMIEC, 2012). Ainda, mesmo apresentando adipócitos menores que os encontrados no tecido subcutâneo, as células do tecido visceral apresentam uma maior secreção de citocinas inflamatórias (BAMBACE et al., 2011), que parecem mediar a relação entre a obesidade visceral e seu estado pró-inflamatório e pró-trombótico (VOHL et al., 2004).

Dessa maneira, a maior atividade do TAV também o credencia como mais patogênico, porém, as pesquisas com o TAB não devem ser deixadas de lado (FOX et al., 2007). Segundo You et al., (2005) os níveis circulantes de algumas proteínas produzidas pelo tecido adiposo são mais relacionados à produção subcutânea do que à visceral. Assim, sugere-se que a quantidade de gordura visceral e as complicações da obesidade estão relacionadas aos níveis de expressão das citocinas no tecido adiposo subcutâneo (YOU et al., 2005).

1.6 Adipocinas

Devido à identificação de diversas moléculas produzidas no tecido adiposo tanto a nível local quanto na circulação, nas duas últimas décadas, ele passou a ser considerado um órgão endócrino (AHIMA, 2006; HAUNER, 2007). Os estudos destas moléculas, conhecidas como adipocinas, mostram mecanismos funcionais complexos não totalmente elucidados, mas que parecem estar relacionados à patogênese de desordens relacionadas à obesidade (KOERNER; KRATZSCH; KIESS, 2005).

Dessa maneira, a obesidade passou a ser estudada como uma doença resultante do comprometimento desta função secretória do tecido adiposo (HAUNER, 2007). O estudo da ação das adipocinas sugere que elas influenciam na sensibilidade à insulina e no processo inflamatório e, dessa maneira, se associam ao desenvolvimento da síndrome metabólica, DM2 e doenças cardiovasculares (LI; WANG; MIAO, 2011). Na área da genética, diversos estudos vêm associando diferenças nos níveis séricos de adipocinas e determinadas alterações no DNA em regiões que flanqueiam seus genes (BEN ALI et al., 2009; HAGER et al., 1998; LI et al., 1999; POITOU et al., 2005), sinalizando assim, o possível papel destas regiões na regulação da expressão das adipocinas.

As principais alterações no DNA investigadas são os polimorfismos, definidos como variações relativamente comuns da sequência de DNA (com frequência superior a 1%) e bastante úteis para estudos de ligação genética. O principal tipo de polimorfismo encontrado são os SNPs (do inglês "*single nucleotide polymorphism*") que consistem na alteração de um único par de bases nucleotídicas na sequência de DNA (MCENTYRE J; OSTELL J, 2002).

1.6.1 Leptina

A primeira adipocina descrita foi a leptina (CONSIDINE et al., 1996), sendo assim, considerada a adipocina clássica e a principal responsável pelo amplo estudo das moléculas secretadas pelo tecido adiposo (KOERNER; KRATZSCH; KIESS, 2005). Liberada pelos adipócitos, ela vai até o hipotálamo e parece estar envolvida principalmente na via de sinalização que regula a ingestão de alimentos e o gasto de energia (MATTEVI; ZEMBRZUSKI; HUTZ, 2002). Porém, funções relacionadas ao

metabolismo da glicose, à maturação sexual e à reprodução também já foram descritas (WAUTERS; CONSIDINE; VAN GAAL, 2000) e, com essa diversidade funcional, a leptina passou a ser considerada como um hormônio (HOU; LUO, 2011).

No gene da leptina (*LEP*), uma variante na região não traduzida do éxon 1 mostrou-se associada à redução nos níveis circulantes de leptina (HAGER et al., 1998) e obesidade (LI et al., 1999). Ainda, o receptor da leptina apresenta diversos sítios polimórficos em seu gene (*LEPR*) e a variante Gln223Arg mostrou-se associada a um aumento do índice de massa corporal (IMC) em diferentes populações, inclusive a brasileira (CHAGNON, 2000; MATTEVI; ZEMBRZUSKI; HUTZ, 2002). Outro polimorfismo na região promotora do gene da leptina, LEP G-2548A, foi associado à obesidade em mulheres brasileiras, revelando um risco quatro vezes maior de ter obesidade nas portadoras do alelo A (HINUY et al., 2008).

1.6.2 Resistina

A resistina foi inicialmente sugerida como uma conexão entre a obesidade e o diabetes, por ser produzida no tecido adiposo e mostrar uma relação com a resistência insulínica em camundongos (STEPPAN et al., 2001). Posteriormente, estudos em humanos foram realizados, e seus resultados indicam um papel que permanece controverso (UKKOLA, 2002; YANNAKOULIA et al., 2003).

Em se tratando do gene da resistina (*RETN*), a presença de variação na região flanqueadora do gene (-420 C>G ou -180 C>G) foi relacionada com a susceptibilidade à obesidade e síndrome metabólica (NORATA et al., 2007). Em outro estudo, entretanto, esta mesma variação apresentou uma relação inversa com o IMC e a circunferência abdominal em uma amostra de mulheres do Rio Grande do Sul (MATTEVI; ZEMBRZUSKI; HUTZ, 2004), mostrando a controvérsia que se encontra em torno da resistina e a obesidade. Esta variante igualmente mostrou-se relacionada a uma maior expressão da resistina em tecido adiposo subcutâneo e nenhuma relação com a obesidade foi encontrada (SMITH et al., 2003).

1.6.3 Adiponectina

A adiponectina, ao contrário da leptina e da resistina, parece desempenhar um papel protetor em relação ao acúmulo excessivo de gordura corporal. Seus

níveis séricos encontram-se inversamente relacionados com a obesidade, a resistência insulínica e o diabetes tipo II (ARITA et al., 1999; HOTTA et al., 2000). Ela tem sido considerada um fator protetor, pois o conjunto de suas atividades acaba inibindo a aterosclerose (BURNETT et al., 2005), e sua expressão foi inversamente relacionada com o IMC (KERN et al., 2003).

Na genética, dois polimorfismos na região promotora (-11377 G>C e -11391 G>A) de seu gene (ADIPOQ) foram associados a alterações nos níveis circulantes de adiponectina de indivíduos obesos (POITOU et al., 2005). Em acréscimo, o haplótipo derivado da combinação destas duas variantes foi associado pelo presente grupo de pesquisa a níveis mais altos de adiponectina em portadores de HIV, sugerindo que a lipodistrofia causada pela terapia antirretroviral possa estar ligada à influência deste polimorfismo, e à ação regulatória do metabolismo lipídico e glicídico exercida pela adiponectina (TRINCA et al., 2010).

1.7 Interação entre adipocinas

Conforme relatado acima, diversos estudos focaram a relação entre as adipocinas e fenótipos relacionados à obesidade separadamente. Posteriormente, autores passaram a investigá-las em conjunto, visando melhor elucidar seus efeitos. Baranova *et al.* (2006) observaram os níveis séricos de leptina, resistina e adiponectina e a expressão gênica dessas moléculas em TAV de pacientes com e sem resistência insulínica. Algumas relações entre níveis de expressão e variáveis metabólicas, como os níveis de glicose, foram descritos, mas nenhuma investigação de relação das adipocinas entre si parece ter sido observada, evidenciando que a interação entre elas parecia ser menos importante. Mais recentemente, um estudo relatou que os níveis de expressão de adiponectina em TAV se correlacionam aos níveis plasmáticos de leptina circulantes em indivíduos submetidos à cirurgia bariátrica antes e depois da cirurgia, considerando este achado a base para investigação das rotas fisiológicas que ligam estas duas moléculas e seu efeito na regulação da ingestão de alimentos (CHEN et al., 2012). Porém, poucos estudos descrevem interações entre adipocinas, o que poderia auxiliar a compreensão de seus efeitos como um todo.

1.8 Variantes Genéticas e os efeitos na expressão das adipocinas

Considerando que as adipocinas atuam em diversas rotas metabólicas, as variações genéticas que alteram seus efeitos também podem alterar diversos mecanismos fisiológicos e patológicos (BREITFELD; STUMVOLL; KOVACS, 2012). Ainda, o estudo da expressão dessas moléculas no tecido adiposo fornece dados importantes sobre o controle dos níveis circulantes de adipocinas e seus efeitos no processo inflamatório e na resistência insulínica (SAMARAS et al., 2010). Mesmo assim, poucos estudos avaliam o efeito de variantes genéticas nos níveis de expressão gênica das adipocinas. Dentre esses, uma publicação descreveu alterações na expressão gênica de resistina relacionadas a um polimorfismo em seu promotor, sugerindo um possível fator de transcrição que regula a expressão da resistina (CHUNG et al., 2005). A proteína Sp1 parece dobrar a atividade do promotor -420G quando comparado com o -420C. Dessa maneira, o polimorfismo é responsável por alterar a capacidade da Sp1 em se ligar ao promotor, modulando assim a atividade transcricional do gene (CHUNG et al., 2005).

No caso da adiponectina, uma variante no íntron 2 – G267T – pareceu influenciar os níveis de expressão em TAV, sugerindo uma regulação dessa alteração na deposição de gordura de indivíduos obesos (FREDRIKSSON et al., 2006).

Referências

ABESO. **Diretrizes Brasileiras de Obesidade - 3ªed**. 2009.

AHIMA, R. S. Adipose tissue as an endocrine organ. **Obesity (Silver Spring, Md.)**, v. 14 Suppl 5, n. August, p. 242S-249S, ago. 2006.

ARITA, Y. et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. **Biochemical and Biophysical Research Communications**, v. 257, n. 1, p. 79-83, 1999.

BAMBACE, C. et al. Adiponectin gene expression and adipocyte diameter: a comparison between epicardial and subcutaneous adipose tissue in men. **Cardiovascular pathology : the official journal of the Society for Cardiovascular Pathology**, v. 20, n. 5, p. e153-6, 2011.

BANEGAS, JR; LOPEZ-GARCÍA, E; GUTIÉRREZ-FISAC, JL; GUALLAR-CASTILLÓN, P; RODRÍGUEZ-ARTALEJO, F. A simple estimate of mortality attributable to excess weight in the European Union. **European Journal of Clinical Nutrition**, v. 57, p. 201-208, 2003.

BARANOVA, A. et al. Gene expression of leptin, resistin, and adiponectin in the white adipose tissue of obese patients with non-alcoholic fatty liver disease and insulin resistance. **Obesity surgery**, v. 16, n. 9, p. 1118-25, set. 2006.

BEN ALI, S. et al. Association of G-2548A LEP polymorphism with plasma leptin levels in Tunisian obese patients. **Clinical Biochemistry**, v. 42, n. 7-8, p. 584-588, 2009.

BREITFELD, J.; STUMVOLL, M.; KOVACS, P. Genetics of adiponectin. **Biochimie**, v. 94, n. 10, p. 2157-63, out. 2012.

BURNETT, M. S. et al. The potential role of resistin in atherogenesis. **Atherosclerosis**, v. 182, n. 2, p. 241-8, out. 2005.

CHAGNON, Y. C. Associations between the Leptin Receptor Gene and Adiposity in Middle-Aged Caucasian Males from the HERITAGE Family Study. **Journal of Clinical Endocrinology & Metabolism**, v. 85, n. 1, p. 29-34, 1 jan. 2000.

CHEN, J. et al. Serum leptin levels are inversely correlated with omental gene expression of adiponectin and markedly decreased after gastric bypass surgery. **Surgical endoscopy**, v. 26, n. 5, p. 1476-80, maio. 2012.

CHUNG, S. S. et al. Regulation of human resistin gene expression in cell systems: an important role of stimulatory protein 1 interaction with a common promoter polymorphic site. **Diabetologia**, v. 48, n. 6, p. 1150-8, jun. 2005.

CONSIDINE, R. V et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. **The New England Journal of Medicine**, v. 334, n. 5, p. 292-295, 1996.

DATASUS. **Ministério da Saúde, Brasil**. Disponível em: <http://tabnet.datasus.gov.br/cgi/dh.exe?vigitel/vigitel10.def>.

DOWNEY, M. et al. Obesity as a Disease : A White Paper on Evidence and Arguments Commissioned by the Council of The Obesity Society. April, p. 1-17, 2008.

FOX, C. S. et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. **Circulation**, v. 116, n. 1, p. 39-48, 3 jul. 2007.

FREDRIKSSON, J. et al. A polymorphism in the adiponectin gene influences adiponectin expression levels in visceral fat in obese subjects. **International journal of obesity (2005)**, v. 30, n. 2, p. 226-32, mar. 2006.

GRUNDY, S. M. et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. **Circulation**, v. 109, n. 3, p. 433-8, 27 jan. 2004.

GRUNDY, S. M. Obesity, metabolic syndrome, and cardiovascular disease. **The Journal of clinical endocrinology and metabolism**, v. 89, n. 6, p. 2595-600, jun. 2004.

HAGER, J. et al. A polymorphism in the 5' untranslated region of the human ob gene is associated with low leptin levels. **International journal of obesity and related metabolic disorders journal of the International Association for the Study of Obesity**, v. 22, n. 3, p. 200-205, 1998.

HAUNER, H. Secretory factors from human adipose tissue and their functional role. **Proceedings of the Nutrition Society**, v. 64, n. 02, p. 163-169, 7 mar. 2007.

HINUY, H. M. et al. Leptin G-2548A promoter polymorphism is associated with increased plasma leptin and BMI in Brazilian women. **Arquivos brasileiros de endocrinologia e metabologia**, v. 52, n. 4, p. 611-6, jun. 2008.

HOTTA, K. et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. **Arteriosclerosis thrombosis and vascular biology**, v. 20, n. 6, p. 1595-1599, 2000.

HOU, N.; LUO, J.-D. Leptin and cardiovascular diseases. **Clinical and experimental pharmacology & physiology**, v. 38, n. 12, p. 905-13, dez. 2011.

KERN, P. A. et al. Adiponectin Expression From Human Adipose Tissue: Relation to Obesity, Insulin Resistance, and Tumor Necrosis Factor-α Expression. **Diabetes**, v. 52, p. 1779-1785, 2003.

KOERNER, A.; KRATZSCH, J.; KIESS, W. Adipocytokines: leptin--the classical, resistin--the controversical, adiponectin--the promising, and more to come. **Best practice & research. Clinical endocrinology & metabolism**, v. 19, n. 4, p. 525-46, dez. 2005.

LI, W. D. et al. Sequence variants in the 5' flanking region of the leptin gene are associated with obesity in women. **Annals of Human Genetics**, v. 63, n. Pt 3, p. 227-234, 1999.

LI, Z.-Y.; WANG, P.; MIAO, C.-Y. Adipokines in inflammation, insulin resistance and cardiovascular disease. **Clinical and experimental pharmacology & physiology**, v. 38, n. 12, p. 888-96, dez. 2011.

MATTEVI, V. S.; ZEMBRZUSKI, V. M.; HUTZ, M. H. Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. **International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity**, v. 26, n. 9, p. 1179-85, set. 2002.

MATTEVI, V. S.; ZEMBRZUSKI, V. M.; HUTZ, M. H. A resistin gene polymorphism is associated with body mass index in women. **Human genetics**, v. 115, n. 3, p. 208-12, ago. 2004.

MCENTYRE J, OSTELL J, E. Bethesda (MD): National Center for Biotechnology Information (US). Glossary. In: **The NCBI Handbook [Internet].** [S.l: s.n.]. .

NATIONAL INSTITUTES OF HEALTH. **Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults**. [S.l: s.n.].

NHLBI, NATIONAL HEART, L. AND B. I. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: Executive Summary. **The American Journal of Clinical Nutrition**, v. 68, p. 899-917, out. 1998.

NORATA, G. D. et al. Effect of the -420C/G variant of the resistin gene promoter on metabolic syndrome, obesity, myocardial infarction and kidney dysfunction. **Journal of internal medicine**, v. 262, n. 1, p. 104-12, jul. 2007.

ORGANIZATION, W. H. Wordl Health Statistics 2012. p. 33-46, 2012.

O'RAHILLY, S.; FAROOQI, I. S. Genetics of obesity. **Philosophical transactions of the Royal Society of London. Series B, Biological sciences**, v. 361, n. 1471, p. 1095-105, 29 jul. 2006.

POIRIER, P. et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical. **Circulation**, v. 113, n. 6, p. 898-918, 14 fev. 2006.

POITOU, C. et al. Relationship between single nucleotide polymorphisms in leptin, IL6 and adiponectin genes and their circulating product in morbidly obese subjects before and after gastric banding surgery. **Obesity surgery**, v. 15, n. 1, p. 11-23, jan. 2005.

CENTERS FOR DESEASE CONTROL AND PREVENTION. Division of Nutrition, Phisical Activity and Obesity. **About BMI for adults**. Disponível em:

<http://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html> acessado em 31 de dezembro de 2012.

SAMARAS, K. et al. Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. **Obesity (Silver Spring, Md.)**, v. 18, n. 5, p. 884-9, maio. 2010.

SMITH, S. R. et al. A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. **Diabetes**, v. 52, n. 7, p. 1611-1618, 2003.

SPEAKMAN, J. R. Obesity : The Integrated Roles of Environment and Genetics. **The Journal of Nutrition**, v. 134, p. 2090-2105, 2004.

STEPPAN, C. M. et al. The hormone resistin links obesity to diabetes. **Nature**, v. 409, n. 6818, p. 307-12, 18 jan. 2001.

TRINCA, J. R. et al. SNPs in the APM1 gene promoter are associated with adiponectin levels in HIV-infected individuals receiving HAART. **Journal of acquired immune deficiency syndromes (1999)**, v. 55, n. 3, p. 299-305, nov. 2010.

TSIGOS, C. et al. Management of obesity in adults: European clinical practice guidelines. **Obesity facts**, v. 1, n. 2, p. 106-116, 2008.

UKKOLA, O. Resistin - a mediator of obesity-associated insulin resistance or an innocent bystander? **European journal of endocrinology**, v. 147, n. 5, p. 571-574, 2002.

VOHL, M. et al. Brief Genetic Analysis A Survey of Genes Differentially Expressed in Subcutaneous and Visceral Adipose Tissue in Men **Obesity Research**, v. 12, n. 8. p. 1217-1222 , 2004.

WAUTERS, M.; CONSIDINE, R. V; VAN GAAL, L. F. Human leptin: from an adipocyte hormone to an endocrine mediator. **European journal of endocrinology**, v. 143, n. 3, p. 293-311, set. 2000.

WHO. **Obesity and Owerweight**. Disponível em: <http://www.who.int/mediacentre/factsheets/fs311/en/> Acessado em 31 de dezembro de 2012.

WHO. **Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organization Technical Report Series**. World Health Organization, 2000. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/11234459> Acessado em 31 de dezembro de 2012.

WHO. **Childhood overweight and obesity**. Disponível em: <http://www.who.int/dietphysicalactivity/childhood/en/> Acessado em 31 de dezembro de 2012.

WRONSKA, A; KMIEC, Z. Structural and biochemical characteristics of various white adipose tissue depots. **Acta physiologica (Oxford, England)**, v. 205, n. 2, p. 194-208, jun. 2012.

YANNAKOULIA, M. et al. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. **The Journal of Clinical Endocrinology & Metabolism**, v. 88, n. 4, p. 1730-1736, 2003.

YOU, T. et al. Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. **American journal of physiology. Endocrinology and metabolism**, v. 288, n. 4, p. E741-7, abr. 2005.

2 JUSTIFICATIVA

Embora a comunidade científica admita um importante papel do componente genético na obesidade, pouco se sabe sobre as formas específicas como alguns polimorfismos predispõem ao aparecimento da doença. A relação entre as adipocinas e fenótipos intermediários relacionados à obesidade deu subsídio para escolha dos genes a serem rastreados. Sequencialmente, as investigações de polimorfismos que interfiram em seus níveis séricos esclareceram alguns aspectos, mas ainda não preenchem todas as lacunas.

Poucas evidências levam à comprovação de um papel funcional das variantes na expressão dos genes das adipocinas. A validação dos polimorfismos indicados por estudos de associação de caráter exploratório, não vem sendo acompanhada da comprovação de sua funcionalidade, lançando as bases, assim, para uma etapa posterior de aplicação clínica destas variantes no prognóstico dos pacientes. Ainda, dada as diferenças conhecidas entre os depósitos de tecido adiposo, a investigação da expressão gênica diferencial acompanhada da análise dos polimorfismos pode ser uma importante ferramenta para elucidação da fisiologia das adipocinas na obesidade.

Em acréscimo, a insistência da literatura em descrever relações de um único polimorfismo, em um único gene parece ignorar o fato de que a obesidade é uma doença complexa. A interação entre diversos genes e diversos polimorfismos, somada ao efeito do ambiente resulta em um desafio para elucidação do processo, mas a utilização de abordagens que avalie mais de um gene, e mais de um polimorfismo parece ser razoavelmente mais importante.

3 OBJETIVOS

3.1 Gerais

Avaliar o efeito de polimorfismos em regiões regulatórias dos genes codificadores da leptina, adiponectina e resistina sobre a expressão dos mesmos no tecido adiposo de pacientes obesos mórbidos submetidos à cirurgia bariátrica.

3.2 Específicos

Verificar os níveis de expressão do RNA mensageiro dos genes da leptina, adiponectina e resistina no tecido adiposo subcutâneo e visceral de indivíduos obesos.

Avaliar o efeito da presença de diferentes polimorfismos nas regiões regulatórias dos respectivos genes sobre a expressão das adipocinas.

Correlacionar a presença dos polimorfismos investigados com características antropométricas e metabólicas dos pacientes investigados.

4 ARTIGO CIENTÍFICO

Adipokine Gene Variants and their Expression in Subcutaneous and Visceral Adipose Tissue of Patients Undergoing Bariatric Surgery

Manuscrito em preparação para submissão ao

Periódico Obesity Surgery,

Fator de impacto 3,286

Adipokine Gene Variants and their Expression in Subcutaneous and Visceral Adipose Tissue of Patients Undergoing Bariatric Surgery

Original Article

Diego O. Borges, M.Sc.[1]: borges.biomed@gmail.com

Mauricio Jacques Ramos, M.D. M.Sc. [2]: mjr.ramos@terra.com.br

Nelson Guardiola Meihnardt, M.D. [2]: nelsonmeinhardt@gmail.com

Aline Simas Gasparotto, M.Sc.[1]: lila.gasparotto@gmail.com

Grasiela Agnes, M.Sc.[1]: gragnes@gmail.com

Vanessa S. Mattevi –Ph.D. [1]: vmattevi@ufcspa.edu.br

[1] Universidade Federal de Ciências da Saúde de Porto Alegre. Programa de Pós-Graduação em Ciências da Saúde. Porto Alegre, RS, Brazil.

[2] Grupo Hospitalar Conceição. Hospital Nossa Senhora da Conceição. Centro de Atenção ao Obeso Classe III. Porto Alegre, RS, Brazil.

Correspondence to:

Prof. Vanessa Suñé Mattevi

Universidade Federal de Ciências da Saúde de Porto Alegre

Rua Sarmento Leite, 245, sala 309

CEP 90050-170, Porto Alegre, RS, Brasil

Phone: +55-51-33038763, Fax: +55-51-033038718

Email: vmattevi@ufcspa.edu.br

Short title: Adipokines Expression in Morbidly Obese

Acknowledgements: This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), PRONEX-FAPERGS/CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil.

ABSTRACT

Molecules produced by adipose tissue are known as adipokines and seem to exert important roles in several metabolic pathways. However, the genetic mechanisms that control the expression of adipokines in different adipose tissue depots are still not well recognized. Therefore, this study aimed to evaluate the effect of single nucleotide polymorphisms (SNPs) located on the regulatory regions of leptin, resistin and adiponectin genes on their adipose tissue expression levels of morbidly obese subjects. It was a cross-sectional study with 60 obese subjects that undergone bariatric surgery. SNPs were genotyped by real-time polymerase reaction or through restriction endonuclease cleavage in whole blood DNA samples. Variants investigated were LEP -2548 G>A (rs7799039), RETN -420 C>G (rs1862513), ADIPOQ -11377 C>G (rs266729) and -11391 G>A (rs17300539). Fresh visceral (VAT) and subcutaneous (SAT) adipose tissue was obtained and the expression levels was assessed by real-time PCR with relative quantification. Ninety-two percent of patients were female with 42.3±8.9 years old. All adipokines showed a higher level of expression in the SAT than in VAT. Furthermore, adiponectin expression was directly correlated with leptin expression in both tissues and inverse correlated with resistin expression on SAT. Finally, the ADIPOQ -11377 C>G variant was associated with lower expression of adiponectin in SAT and all polymorphisms seem to exert a role in differential adipose tissue expression of their respective genes.

Key Words: Morbid Obesity. Polymorphism. Gene Expression. Adipokines.

INTRODUCTION

Obesity and overweight prevalences are increasing all over the world, even in those countries historically affected by undernutrition conditions[1]. Furthermore, these conditions are considered as metabolic/genetic risk factors for cardiovascular

diseases and other complications, like type 2 diabetes mellitus (DM2) and cancer[2]. In clinical practice, obesity is defined as a body mass index (BMI) over 30 kg/m² and overweight as BMI between 25 and 29 kg/m². However, the assessment of waist circumference is considered a better surrogate for the risk of cardiovascular and metabolic complications, since abdominal obesity is more strongly associated with these co-morbidities[3].

The fat accumulation seen in obesity is situated in two major depots, visceral and subcutaneous adipose tissue. The molecules produced by those depots are known as adipokines, and their gene expression is shown to be regulated by obesity and depot localization[4]. This way, adipose tissue has been recognized as an endocrine organ, and obesity now may be considered as a disturbance of its secretory function [5,6].

Adipokines exert a role in many metabolic pathways, so the genetic variations that influence their action can also alter many physiological and pathological mechanisms[7]. Variations in regulatory regions of the genes that encode adipokines have been associated with obesity-related phenotypes in many settings, including the Brazilian population [8–13]. However, the mechanisms underlying these associations remain unclear. Most of studies performed so far have focused on the effect of one isolated adipokine [9,10,14], while it is becoming clear that an interplay among them must be important in the development of a complex phenotype such as obesity. Furthermore, visceral adipose tissue has been shown to be more metabolically active than subcutaneous adipose tissue [15] and its accumulation has been associated with more severe metabolic complications, e.g. metabolic syndrome.

Therefore, considering the complexity of obesity and the recent seen efforts to investigate the influence of isolated genes in that disorder, this study aimed to

evaluate the effect of single nucleotide polymorphisms (SNPs) located on regulatory regions of leptin, resistin and adiponectin genes on their adipose tissue-specific mRNA expression levels in morbidly obese subjects.

MATERIAL AND METHODS

This was a cross-sectional study where samples of visceral and subcutaneous adipose tissue were consecutively obtained from 60 obese subjects undergoing elective gastric bypass abdominal surgery (Roux-enY gastroenterostomy). Patients were recruited at Obese Class III outpatient clinic at the Hospital *Nossa Senhora da Conceição* (government-supported – Porto Alegre, Rio Grande do Sul, Brazil). Inclusion criteria were patients with BMI ≥ 40 kg/m² or ≥ 35 kg/m² with associated comorbidities. Exclusion criteria were serious hepatic disease, cancer, coagulation diseases and stomach diseases.

Information about birth, ethnicity, gender, physical activity, smoking, use of oral contraceptive, weight and height were obtained to sample characterization at the medical files recorded at the time of the surgery. Diagnosis of DM2 and hypertension was made according with respective guidelines[16,17]. Laboratory variables were collected to the classification of dyslipidemia[18] and metabolic syndrome[19] defined by specific criteria.

Gene Expression Analysis

Fresh subcutaneous and omental adipose tissue were excised by the surgeon, immediately immersed in Allprotect Tissue Reagent® (Qiagen, Courtabouef, France) and transported to the laboratory. Samples were maintained at -20ºC until the RNA extraction procedure. Extraction was made with RNeasy Lipid Tissue Mini kit (Qiagen; Courtabouef, France) following the manufacturer instructions. The integrity

and quality of RNA was measured with the use of by spectrophotometry and the RNA stored at -80ºC.

The cDNA was obtained with 8µL of RNA template and the utilization of the SuperScript™ First-Strand Synthesis System for RT-PCR® (Invitrogen; California, USA). Then, 25ng of cDNA were used to quantify the gene expression of leptin (*LEP*), resistin (*RETN*) and adiponectin (*ADIPOQ*) with TaqMan® Gene Expression assay (on demand primers and probes by Life Technologies, São Paulo, Brazil) and analyzed at Step One Plus™ Real-time PCR System. *B2M* and *FBXL10* were previoulsy validated as endogenous controls. The overall fold change was calculated by the $2^{-\Delta\Delta Ct}$ method using the visceral adipose tissue as calibrator. Also, the calculation of the $2^{-\Delta Ct}$ of each sample for each tissue was used as previously suggested(20).

SNPs Genotyping

Fasting whole blood samples were collected and DNA was extracted using a standard salting out technique [19]. The regions containing the polymorphisms -2548G>A for the *LEP* gene (rs7799039) and the -420C>G (rs1862513) for the *RETN* gene were amplified by polymerase chain reaction (PCR) using specific primers and reactions conditions previously described [11,20]. Then, digestion of the PCR products with the enzyme *Hha*I for the *LEP*-2548 G>A and the *Bpi*I for the RETN C-420G was conducted. Finally, the restriction fragment length analysis was performed in agarose gels (2.5%) containing ethidium bromide to determine the genotypes. The other two variants in the promoter region of the *ADIPOQ* gene (-11377 C>G; rs266729 and -11391 G>A; rs17300539) were genotyped through real-time-PCR using the TaqMan® methodology.

Statistical Analysis

Variables used for the sample characterization were expressed by mean ± standard deviation or percentage. Allele frequencies were calculated and the agreement of genotype frequencies with Hardy-Weinberg expectations was tested by a goodness-of-fit chi-square test. Correlation between the expression levels by gene and by tissue ($2^{-\Delta Ct}$) and the other variables were tested with the Spearman correlation coefficient. Expression levels ($2^{-\Delta Ct}$) were compared between tissues and between genotypes for all polymorphisms using the Mann-Whitney test and the Wilcoxon signed-rank test and expressed by mean ± standard error of the mean (SEM). All tests were two-tailed and the significance level was pre-defined at < 0.05.

Pairwise linkage disequilibrium coefficients (D´ and r2) were estimated using the Haploview software.

Ethical Considerations

The protocol of the present study was previously approved by the Research Ethics Committees of both Institutions involved. All Participants were informed about the research procedures and signed a free informed consent term.

RESULTS

From the 60 individuals analyzed, almost 92% were female and 95% were characterized as euro-descendants, with a mean age of 42.3 ± 8.9 years old. Most part of them did not smoke (93.3%) and did not make use of oral contraceptives (83.6%). Other clinical variables are described in Table 1.

The three adipokines mRNA expression, as evaluated by the $2^{-\Delta\Delta Ct}$ method using the visceral adipose tissue (VAT) as calibrator, was higher in the subcutaneous adipose tissue (SAT). Overall, mean fold change for leptin, adiponectin and resistin

were respectively 11.15 ± 3.40; 3.39 ± 0.90 and 3.87 ± 1.10, always considering VAT as calibrator. In addition, Figure 1 illustrates this increased expression of the three adipokines with significant higher mRNA levels in SAT, calculated with the $2^{-\Delta Ct}$.

As shown in Table 2, several adipokine levels were correlated. The high correlation was observed between the VAT and SAT mRNA levels for the same genes. HDL-Cholesterol showed a positive but moderate correlation with VAT mRNA of leptin (r= 0.273; P=0.042) and adiponectin (r= 0.264; P=0.049). Other clinical variables like BMI, thickness of abdominal subcutaneous fat, total cholesterol, LDL-cholesterol, triglycerides, glucose, insulin and glycosilated hemoglobin did not show any significant correlation with the adipokine levels. Also, the SAT resistin mRNA levels were the only with inverse correlation with other adipokines as seen with SAT and VAT mRNA of adiponectin (r= -0.445; P=0.001 and r= -0.293; P=0.041 respectively).

All genotype frequencies were in agreement with those expected under Hardy-Weinberg equilibrium. The minor allele frequency for *LEP* -2548 G>A was 0.38 (A); for *RETN* -420C>G was 0.31(G); for *APM1* -11391 G>A was 0.11 (A); and for *ADIPOQ* -11377 C>G was 0.31 (G).

Comparison of the expression levels in SAT and VAT according to genotypes are shown in Table 3. Leptin expression was high in the subcutaneous fat for all genotypes. Resistin expression was higher in the SAT of only for C/C homozygotes. Adiponectin expression was higher in G/G homozygotes for the SNP -11391 G>A, but for the -11377 C>G variant, both genotypes showed high expression of adiponectin in subcutaneous fat.

Table 3 also presents the comparison of expression levels for the three adipokines among genotypes in both fat depots separately. There were no

differences among genotypes of the LEP -2548 G>A, RETN -420 C>G and ADIPOQ -11391 G>A variants in both tissues. However, a significant difference was observed for the APM1 -11377 C>G SNP: carriers of the variant G-allele showed lower levels of adiponectin expression than C/C homozygotes in the SAT. This effect was not verified in the VAT.

The linkage disequilibrium was calculated for SNPs located in ADIPOQ. The D' and r-squared values were 0.208 and 0.002, respectively.

DISCUSSION

Our study aimed to evaluate the effect of four SNPs on the expression levels of three adipokines in VAT and SAT and found that one of them, ADIPOQ -11377 C>G, shows a significant difference on SAT expression levels of adiponectin in morbidly obese subjects. Other SNPs did not influence the expression levels of leptin, resistin and adiponectin neither in SAT or VAT isolated, but seemed to exert a role in differential depot expression.

Furthermore, all adipokines show a high expression level on SAT in comparison with VAT in morbidly obese individuals. These high levels were also described by Samaras *et. al* [21] for leptin and adiponectin levels in obese subjects with DM2, but no differences were observed in adiponectin levels of non-diabetic obese[22]. A recently review about the different features of adipose tissue depots considered that this lower mRNA expression of adiponectin and leptin in VAT as a part of an expression profile that contributes to a systemic pro-inflammatory state[23]. Regarding resistin expression levels, few studies were performed. The only one we found described a similar expression of resistin in SAT and VAT [24].

However, this study was restricted to overweight individuals and a different expression profile may be related to the morbidly obese condition.

When we correlate the expression levels of adipokines with the clinical variables, only HDL-C show a significant correlation with the VAT levels of leptin and adiponectin. Other lipid profile measurements (total cholesterol and triglycerides) did not show any relation with the SAT levels of adipokines, as previously described[25]. However, this relation between HDL-C and VAT levels of leptin and adiponectin has not been previously described. Low HDL-C levels are a component of MS [17] and, if we consider that low levels of adiponectin and leptin in VAT relate to pro-inflammatory state as mentioned above, this positive correlation seems biologically plausible.

The interactions between adipokines have not been fully explored, and several mRNA levels of these molecules were correlated in this morbidly obese sampling, reflecting the complexity of this metabolic pathway. Our study demonstrated that expression level of SAT resistin was inversely related with SAT and VAT levels of adiponectin (Table 2). An inverse interaction between resistin and adiponectin was shown in an experimental study where resistin levels induce adhesion molecules and a state of atherogenesis, and adiponectin seems to inhibit this effect[26]. Even knowing that this is a specific experimental condition, the correlated expression levels described here indicate the crude effect of two molecules and the interplay between these different fat depots with all the complexity of the human organism. This way, we could suggest that the inhibition of resistin expression by adiponectin could start at the adipose tissue as a feedback mechanism. Also, the interaction is mainly at the SAT level, since no relation between resistin and adiponectin was observed in VAT. Also, even without using the absolute quantification, we can

observe that the resistin expression levels were quite smaller in comparison with the other adipokines. McTernan *et. al* [24] described the low resistin expression levels of fat, even showing that in comparison with other adipose depots, VAT and SAT still are the main producers of resistin.

The interaction between leptin and adiponectin is also complex. Here, we found many correlations between the expression levels of these proteins. Leptin mRNA levels in SAT were correlated with leptin's own mRNA in VAT, and adiponectin in SAT and VAT. In a sample of obese and overweight women, the subcutaneous relation between these proteins have already been described [25]. However, considering that most part of the studies investigated visceral or subcutaneous expression separately, the relation between SAT and VAT levels is novel, indicating that they must be evaluated together in both tissues.

Regarding the polymorphisms analyses, some differences must be acknowledged. For the LEP -2548 G>A variant, expression levels of leptin were the same in both tissues for A/A homozygotes (Table 3), and G-carriers exhibit increased levels of leptin in SAT compared with VAT. A previous study has shown that morbidly obese carriers of G-allele also exhibited increased levels of circulating leptin compared with the A-carriers, and these circulating levels were correlated with BMI just for the G-carriers[27]. This way, we can suppose some effect of the G variant increasing leptin SAT level in G-carriers and affecting the circulating levels of leptin and its relation with BMI, although this condition was not investigated in our study.

For the resistin gene, the results were similar of the leptin. Only the C/C homozygotes for the -420 C>G SNP show a significant high expression level of resistin in SAT in comparison with VAT (Table 3). Studies that associate resistin to conditions related to obesity have been controversial. The G variant has been

associated with high BMI and obesity prevalence[8], but C-carriers were also shown to have high with BMI and waist circumference in other study [9], this last one performed in the Brazilian population. An experimental assay described the -420G variant with a high relationship with a transcription factor of the resistin gene, increasing the transcription on two-fold basis[28]. We could endorse this hypothesis since the expression levels of G-carriers seems to be high in both tissues but, unfortunately, these differences were not significant, although with marginal significance (P = 0.062) for the VAT.

Adiponectin was the only adipokine where two polymorphisms were evaluated. The homozygotes G/G for the SNP -11391 were previously related with low levels of circulating adiponectin in obese subjects before bariatric surgery [27]. Nevertheless, we did not found difference on adiponectin expression, and G-carriers just exhibit lower levels of adiponectin in VAT compared with SAT.

Other nucleotide change on the adiponectin promoter, -11377 C>G, seems to have a more pronounced effect in the expression levels of adiponectin. Low levels of circulating adiponectin in carriers of the G allele have already been found not just in morbidly obese individuals but also in HIV-infected patients receiving highly active antiretroviral therapy [12,27]. In addition, You *et. al* [25] suggested that SAT production of adipokines could be more responsible for the circulating levels and systemic alterations produced. In line with these findings, we describe a significant difference on SAT adiponectin mRNA levels influenced by -11377 C>G polymorphism, suggesting that this alteration could affect the adiponectin pathway on a tissue-specific basis and testifying the effect of this alteration on the adiponectin metabolism.

A previous study by our group found that HIV-infected patients carriers of the -11391A-11377C haplotype presented higher adiponectin levels compared to other genotypes[12]. However, at that time, we could not predict which variant was the functional one. Although we could not demonstrate the linkage disequilibrium between these two variants in the present sample, probably due to the sample size, our expression data suggest that the -11377 variant to be the causal one, once the C/C homozygotes present higher adiponectin expression in SAT.

As limitations of the study we could cite the fact of being developed just with obese individuals, limiting the variability that could exist at the adipokines expression levels. Moreover, we failed to describe the linkage disequilibrium commonly observed for the variants -11377 C>G and -11391 G>A because of the lack of carriers of the rare homozygote genotypes in our sampling.

CONCLUSION

In conclusion, this work brings several findings. First, we could verify that the three adipokines investigated, leptin, adiponectin, and resistin, are more expressed in subcutaneous than visceral adipose tissue in morbidly obese individuals. Second, adiponectin expression is directly correlated with leptin expression in both tissues and inverse correlated with resistin expression in subcutaneous adipose tissue. Furthermore, the ADIPOQ -11377C>G variant allele is associated with lower expression of adiponectin in subcutaneous adipose tissue and, finally, all the four polymorphisms investigated seem to exert a role in differential adipose tissue depot mRNA expression of their respective genes.

CONFLICT OF INTERESSE DISCLOSURE STATEMENT

All authors declare no conflict of interest with institutions or products that is mentioned above and/or is important to the outcome of the study presented.

REFERENCES

1. Eckel RH, York D a, Rössner S, Hubbard V, Caterson I, St Jeor ST, et al. Prevention Conference VII: Obesity, a worldwide epidemic related to heart disease and stroke: executive summary. Circulation. 2004 Nov 2;110(18):2968–75.

2. Poirier P, Giles TD, Bray G a, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical. Circulation. 2006 Feb 14;113(6):898–918.

3. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. The Journal of clinical endocrinology and metabolism. 2004 Jun;89(6):2595–600.

4. Dahlman I, Elsen M, Tennagels N, Korn M, Brockmann B, Sell H, et al. Functional annotation of the human fat cell secretome. Archives of physiology and biochemistry. 2012 Jul;118(3):84–91.

5. Hauner H. Secretory factors from human adipose tissue and their functional role. Proceedings of the Nutrition Society. 2007 Mar 7;64(02):163–9.

6. Ahima RS. Adipose tissue as an endocrine organ. Obesity (Silver Spring, Md.). 2006 Aug;14 Suppl 5(August):242S–249S.

7. Breitfeld J, Stumvoll M, Kovacs P. Genetics of adiponectin. Biochimie. Elsevier Masson SAS; 2012 Oct;94(10):2157–63.

8. Norata GD, Ongari M, Garlaschelli K, Tibolla G, Grigore L, Raselli S, et al. Effect of the -420C/G variant of the resistin gene promoter on metabolic syndrome, obesity, myocardial infarction and kidney dysfunction. Journal of internal medicine. 2007 Jul;262(1):104–12.

9. Mattevi VS, Zembrzuski VM, Hutz MH. A resistin gene polymorphism is associated with body mass index in women. Human genetics. 2004 Aug;115(3):208–12.

10. Ben Ali S, Kallel A, Ftouhi B, Sediri Y, Feki M, Slimane H, et al. Association of G-2548A LEP polymorphism with plasma leptin levels in Tunisian obese

patients. Clinical Biochemistry. The Canadian Society of Clinical Chemist; 2009;42(7-8):584–8.

11. Mammès O, Betoulle D, Aubert R, Herbeth B, Siest G, Fumeron F. Association of the G-2548A polymorphism in the 5' region of the LEP gene with overweight. Annals of Human Genetics. 2000;64(Pt 5):391–4.

12. Trinca JR, Sprinz E, Lazzaretti RK, Hutz MH, Kuhmmer R, de Almeida S, et al. SNPs in the APM1 gene promoter are associated with adiponectin levels in HIV-infected individuals receiving HAART. Journal of acquired immune deficiency syndromes (1999). 2010 Nov;55(3):299–305.

13. Mattevi VS, Zembrzuski VM, Hutz MH. Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2002 Sep;26(9):1179–85.

14. Hinuy HM, Hirata MH, Forti N, Diament J, Sampaio MF, Armaganijan D, et al. Leptin G-2548A promoter polymorphism is associated with increased plasma leptin and BMI in Brazilian women. Arquivos brasileiros de endocrinologia e metabologia. 2008 Jun;52(4):611–6.

15. Arner P. OBESITY AND THE ADIPOCYTE Regional adipocity in man. 1997;155:191–2.

16. International Diabetes Federation. Global Guideline for Type 2 Diabetes - Clinical Guidelines Task Force. 2012.

17. U.S Department of Health and Human Service. Prevention , Detection , Evaluation , and Treatment of High Blood Pressure. Blood Pressure. Am Heart Assoc; 2003 p. 1206–52.

18. Cholesterol N, Program E. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106(25):3143–421.

19. Grundy SM, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation. 2004 Jan 27;109(3):433–8.

20. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nature Protocols. 2008 Jun;3(6):1101–8.

21. Lahiri DK, Nurnberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Research. 1991;19(19):5444.

22. Engert JC, Vohl M-C, Williams SM, Lepage P, Loredo-Osti JC, Faith J, et al. 5' flanking variants of resistin are associated with obesity. Diabetes. 2002;51(5):1629–34.

23. Samaras K, Botelho NK, Chisholm DJ, Lord R V. Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. Obesity (Silver Spring, Md.). Nature Publishing Group; 2010 May;18(5):884–9.

24. Fredriksson J, Carlsson E, Orho-Melander M, Groop L, Ridderstråle M. A polymorphism in the adiponectin gene influences adiponectin expression levels in visceral fat in obese subjects. International journal of obesity (2005). 2006 Mar; 30(2):226–32.

25. Wronska a, Kmiec Z. Structural and biochemical characteristics of various white adipose tissue depots. Acta physiologica (Oxford, England). 2012 Jun;205(2):194–208.

26. McTernan, P. G. McTernan, CL. Chetty, R. Jenner, K. Fisher, FM. Lauer, MN. Crocker, J. Barnett, AH. Kumar S. Increased Resistin Gene and Protein Expression in Human Abdominal Adipose Tissue. Journal of Clinical Endocrinology & Metabolism. 2002 May 1;87(5):2407–2407.

27. You T, Yang R, Lyles MF, Gong D, Nicklas BJ. Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. American journal of physiology. Endocrinology and metabolism. 2005 Apr;288(4):E741–7.

28. Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, et al. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine–endothelial cell interactions. 2004;314:415–9.

29. Poitou C, Lacorte J-M, Coupaye M, Bertrais S, Bedel J-F, Lafon N, et al. Relationship between single nucleotide polymorphisms in leptin, IL6 and adiponectin genes and their circulating product in morbidly obese subjects before and after gastric banding surgery. Obesity surgery. 2005 Jan;15(1):11–23.

30. Chung SS, Choi HH, Kim KW, Cho YM, Lee HK, Park KS. Regulation of human resistin gene expression in cell systems: an important role of stimulatory protein 1 interaction with a common promoter polymorphic site. Diabetologia. 2005 Jun;48(6):1150–8.

Table 1. Clinical characteristics of the 60 subjects analyzed.

Variables	Mean ± SD or Absolute n
Epidemiological	
Age (years)	42.3 ± 8.9
Female Gender	55
Euro-descendants	57
Smoking	4
Sedentary	50
Diabetes mellitus	32
High Blood Pressure	44
Dyslipidemia	36
Metabolic syndrome	40
Anthropometric	
BMI (kg/m²)	50.66 ± 7.77
Thickness of Abdominal	6.61 ± 1.78
Subcutaneous fat (cm)	
Laboratory	
Total Cholesterol (mg/dL)	204.2 ± 37.6
LDL-Cholesterol (mg/dL)	125.6 ± 32.0
HDL-Cholesterol (mg/dL)	48.4 ± 9.6
Triglycerides (mg/dL)	152.6 ± 73.5
Glucose (mg/dL)	129.4 ± 56.3
Insulin (μU/mL)	21.6 ± 17.5
Glycosilated Hemoglobin (%)	6.7 ± 1.8

Table 2. Correlation between the adipokines levels of mRNA and HDL-C.

	VAT LEP mRNA	VAT ADIPOQ mRNA	VAT RETN mRNA	SAT LEP mRNA	SAT RETN mRNA
HDL-C	0.273*	0.264*	0.036	0.033	-0.125
SAT LEP mRNA	0.510**	0.301*	-0.037		-0.054
SAT APM1 mRNA	0.087	0.456**	-0.210	0.433**	-0.445**
SAT RETN mRNA	-0.051	-0.293*	0.465**	-0.054	
VAT LEP mRNA		0.375**	0.115	0.510**	-0.051
VAT ADIPOQ mRNA	0.375**		-0.101	0.301*	-0.293*

Spearman's correlation coefficient; *P<0.05, **P<0.01. SAT = Subcutaneous Adipose Tissue; VAT= Visceral Adipose Tissue;

Table 3. Comparison of the adipokines expression levels by genotypes.

Polymorphism	Genotype (n)	SAT Gene Expression	VAT Gene Expression	P[b]
LEP -2548	GG (19)	2.69 ± 1.40	0.46 ± 0.16	<0.001
G>A	AG (15)	2.12 ± 1.40	0.37 ± 0.11	0.016
	AA (9)	1.01 ± 0.46	0.29 ± 0.10	0.123
	P[a]	0.604	0.989	
RETN -420	CC (22)	0.003 ± 0.001	0.002 ± 0.001	0.039
C >G	GG+CG (28)	0.030 ± 0.016	0.010 ± 0.004	0.200
	P[a]	0.590	0.062	
ADIPOQ -	GG (45)	9.46 ± 2.30	3.66 ± 0.46	<0.001
11391 G>A	AA+AG (11)	7.79 ± 3.50	3.34 ± 0.67	0.182
	P[a]	0.413	0.955	
ADIPOQ -	CC (26)	14.23 ± 4.12	5.44 ± 1.39	0.001
11377 C>G	GG+CG (31)	5.37 ± 0.69	3.12 ± 0.39	0.003
	P[a]	0.029	0.196	

Data expressed as Mean ± SEM. [a]Mann-Whitney test; [b]Wilcoxon signed-rank test. SAT = Subcutaneous Adipose Tissue; VAT= Visceral Adipose Tissue.

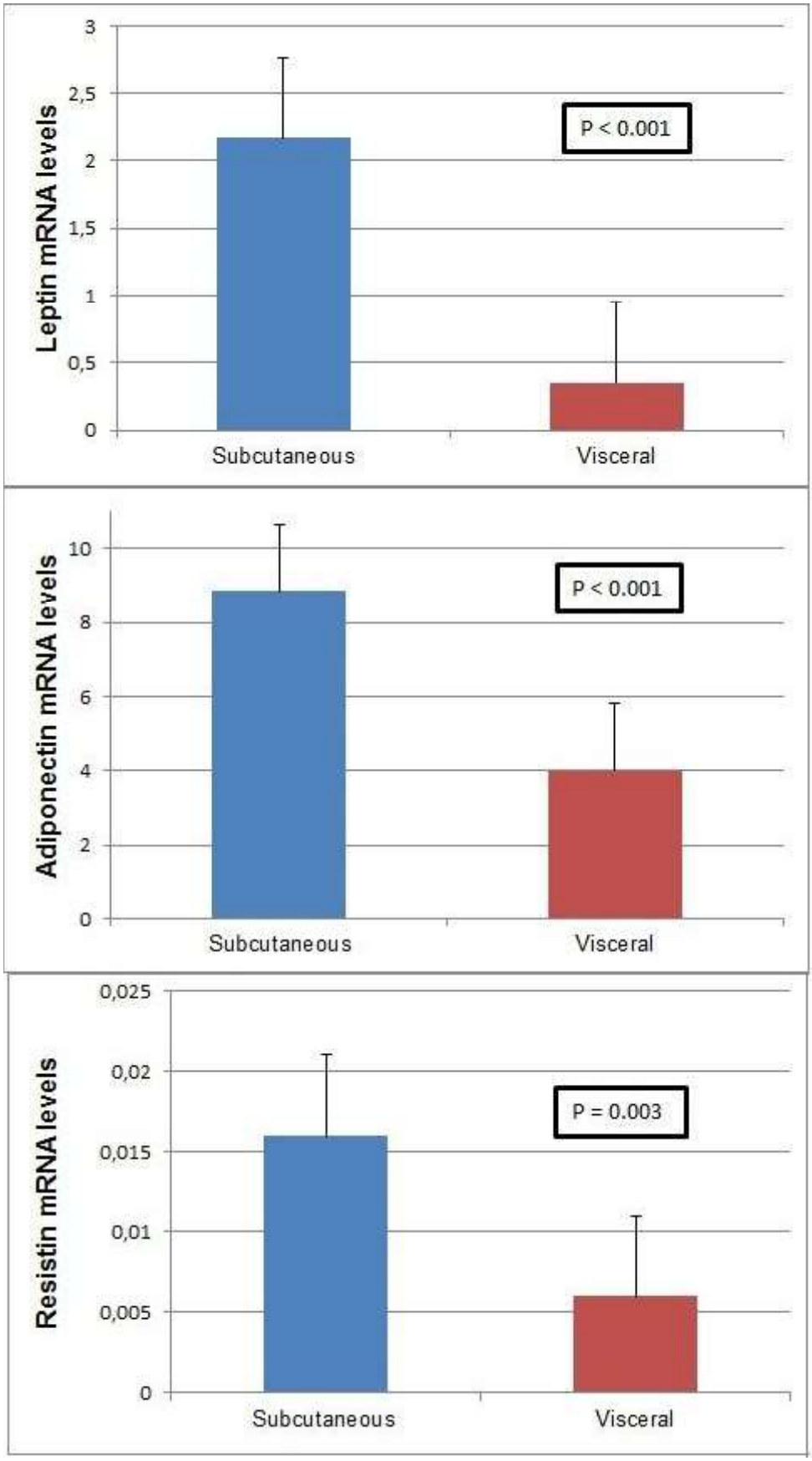

Figure 1 – Relative Expression levels of Leptin, Resistin and Adiponectin in Subcutaneous and Visceral depots. P value for Wilcoxon Signed Ranks Test.

5 CONCLUSÃO

O estudo da obesidade, assim como em outras patologias, permitiu determinados avanços no que diz respeito à fisiopatologia, ao tratamento e, especialmente, na prevenção desta patologia mundialmente considerada como uma epidemia. Nesse caso, tivemos a evolução de dois panoramas distintos: um no que diz respeito à evolução da fisiopatologia da doença e outro no que diz respeito às novas descobertas que se almejava após a descoberta da sequência do DNA humano.

Após tentativas falhas de encontrar um gene responsável pelo aparecimento desta enfermidade, hoje sabemos que se trata de uma condição complexa e que o aparecimento do fenótipo obeso não é resultante de uma simples alteração genética.

Além do componente genético, no caso da obesidade, o ambiente contribui e muito para o acúmulo de gordura observado na patologia. Sabemos que o comportamento das pessoas mudou muito com os avanços da tecnologia aumentando o consumo de alimentos ricos em energia e de preparo rápido, e aumentando também a inatividade física dos indivíduos, que sequer necessitam levantar da poltrona para mudar o canal da televisão. Ainda, a ansiedade se tornou um dos principais transtornos encontrados e também tem sua parcela na ingestão de alimentos descontrolada observada em muitos obesos.

A principal característica observada, o aumento da gordura corporal, fez com que passássemos a prestar mais atenção nesse imenso conjunto de lipídeos. Hoje sabemos que o tecido adiposo não só armazena gordura, como também participa intensamente de diversos outros processos metabólicos, podendo predispor os obesos às doenças com altos índices de morbidade e mortalidade como o *diabetes mellitus* tipo 2 e as doenças cardiovasculares.

A identificação da leptina e, posteriormente, da resistina e da adiponectina nos fizeram descobrir um importante órgão endócrino. Essas moléculas, predominantemente produzidas pelo tecido adiposo, mostraram ter efeitos em diversos outros locais do organismo, mostrando que necessitamos estudar muito mais para chegar a uma elucidação das diversas funções do tecido adiposo. Ainda, as diferenças observadas entre os diferentes depósitos de tecido adiposo, especialmente o subcutâneo e o visceral, abriram novas lacunas a serem preenchidas.

Nesse contexto, a biologia molecular vem se mostrando como uma importante ferramenta. Aprofundando o estudo dos fenômenos biológicos ao nível molecular, conseguimos vislumbrar o efeito que uma simples alteração na sequência do DNA pode causar em um determinado organismo. Essa nova ciência também nos fez perceber que essa alteração pode depender do contexto ambiental para se manifestar ou não, e que em doenças como a obesidade, diversas alterações atuam junto para a manifestação do fenótipo, dando a obesidade todos os desígnios necessários para ser considerada uma doença complexa.

Porém, quando muitos podem se desanimar com a complexidade, alguns preferem visualizá-la como um importante desafio. Assim, utilizando todos os conhecimentos acima descritos e tendo a biologia molecular como aliada, persiste o esforço para contribuir na compreensão do desenvolvimento da obesidade e da influência do componente genético nessa doença.

Com isso, concluímos que os polimorfismos em regiões regulatórias de leptina, resistina e adiponectina aqui avaliados possuem, sim, um efeito na expressão dessas moléculas em tecido adiposo subcutâneo e visceral de obesos mórbidos nesta população. Em nosso estudo, um polimorfismo pareceu influenciar o nível de expressão gênica dentro de um único compartimento adiposo, e ainda, todos os demais polimorfismos parecem ter alguma influência nas diferenças de expressão observadas entre o tecido adiposo subcutâneo e visceral.

Um posterior acompanhamento desses indivíduos pode acrescentar informação a esse achado, avaliando se essas diferenças influenciam na perda de peso e a posterior manutenção do peso no restante da vida dos indivíduos que são submetidos à cirurgia bariátrica.

6 ANEXOS

Anexo I – Carta de Aprovação do Comitê de Ética da Universidade Federal de Ciências da Saúde de Porto Alegre

UFCSPA

COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

COMITÊ DE ÉTICA EM PESQUISA - CEP
UFCSPA

O Comitê de Ética em Pesquisa da UFCSPA, registrado na Comissão Nacional de Ética em Pesquisa (CONEP) sob o nº 075/05 em 23/07/04, analisou o Projeto:

Projeto: 11-772 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
VANESSA SUÑE MATTEVI
DIEGO OLSCHOWSKY BORGES
MAURÍCIO JACQUES RAMOS
NELSON GUARDIOLA MEINHART

Título: ANÁLISE DA EXPRESSÃO DE GENES DE ADIPOCINAS NO TECIDO ADIPOSO DE PACIENTES SUBMETIDOS À CIRURGIA BARIÁTRICA.

Esse projeto foi aprovado em seus aspectos éticos e metodológicos conforme as Resoluções 196/09 e demais Resoluções complementares. Toda e qualquer alteração do projeto, assim como eventos adversos graves, deverão ser comunicados a este CEP. Os TCLE, quando necessários, somente poderão ser utilizados após prévia e explícita aprovação (carimbo) de sua redação por este CEP".

Porto Alegre, 09 de maio de 2011.

José Geraldo Vernet Taborda
Coordenador do CEP/UFCSPA

Anexo II – Carta de Aprovação do Comitê de Ética do Grupo de Ensino e Pesquisa do Grupo Hospitalar Conceição

GHC
HOSPITAL N. S. DA CONCEIÇÃO S.A. Av. Francisco Trein, 596 CEP 91350-200 - Porto Alegre - RS Fone: 3357 2300 CNPJ: 92.787.116/0001-23
HOSPITAL DA CRIANÇA CONCEIÇÃO (Unidade Pediátrica do Hospital Nossa Senhora da Conceição S.A.)
HOSPITAL CRISTO REDENTOR S.A. Rua Domingos Rubbo, 25 CEP 91040-000 - Porto Alegre - RS Fone: 3357 4100 CNPJ: 92.787.126/0001-75
HOSPITAL FÊMINA S.A. Rua Ramiro Barcelos, 17 CEP 90035-003 - Porto Alegre - RS Fone: 3314 3200 CNPJ: 92.690.134/0001-03
100% SUS
Vinculados ao Ministério da Saúde - Decreto nº 80.284/90

COMITÊ DE ÉTICA EM PESQUISA - CEP/GHC

O Comitê de Ética em Pesquisa do Grupo Hospitalar Conceição (CEP/GHC), que é reconhecido pela Comissão Nacional de Ética em Pesquisa (CONEP)/MS desde 31/10/1997, pelo Office For Human Research Protections (OHRP)/USDHHS, como Institutional Review Board (IRB0001105) e pelo FWA - Federalwide Assurance (FWA 00000378) em reunião extraordinária realizada em 27 de julho de 2011, reavaliou o seguinte projeto:

Projeto: 11-105 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
MAURÍCIO JACQUES RAMOS
NELSON GUARDIOLA MEINHARDT
DIEGO OLSCHOWSKY BORGES
VANESSA SUÑÉ MATTEVI

Título: Análise da expressão de genes de adipocinas no tecido adiposo de pacientes submetidos a cirurgia bariátrica

Documentação: Aprovados
Aspectos Metodológicos: Aprovados
Aspectos Éticos: Aprovados

Parecer final: Este projeto, bem como os documentos adicionais incluindo o Termo de Consentimento Livre e Esclarecido, por estar de acordo com as Diretrizes e Normas Internacionais e Nacionais especialmente as Resoluções 196/96 e complementares do Conselho Nacional de Saúde, obteve o parecer de APROVADO, neste CEP.

Considerações Finais: Toda e qualquer alteração do projeto, deverá ser comunicada imediatamente ap CEP/GHC, bem como os Eventos Adversos ocorridos. O Pesquisador compromete-se a encaminhar dentro dos prazos estipulados, o(s) relatório(s) parcial(ais) e final ao Comitê de ética em Pesquisa do GHC.

Porto Alegre, 27 de julho de 2011.

Daniel Demétrio Faustino da Silva
Coordenador-geral do CEP/GHC

Anexo III – Termo de Consentimento Livre e Esclarecido

Termo de Consentimento Livre e Esclarecido

Título: "Análise da expressão de genes de adipocinas no tecido adiposo de pacientes submetidos à cirurgia bariátrica"

Versão 1 – 14.03.2011

Atualmente, sabe-se que a obesidade é uma doença causada por uma relação entre a má alimentação, a falta de exercícios físicos e a herança genética que recebemos de nossos pais. O fator genético vem sendo bastante estudado, buscando a identificação das pessoas que têm mais chance de desenvolver esta doença, já que, mesmo adotando um estilo de vida mais saudável, muitas pessoas acabam apresentando uma dificuldade maior em perder peso, enquanto outras apresentam piores hábitos e perdem peso com mais facilidade.

Esta pesquisa tem como objetivo avaliar o efeito da variação no código genético sobre a predisposição a obesidade de pacientes submetidos a cirurgia de redução de estômago. Os resultados deste estudo poderão ser utilizados, no futuro, para esclarecer o papel que esses genes desempenham no organismo dentro do mecanismo que resulta na obesidade. Desta forma, no futuro, os cientistas poderão focar o tratamento de cada indivíduo, evitando o desperdício de tempo e medicamentos em tratamentos que não terão sucesso.

Concordando em participar do estudo, serão coletadas informações de seu prontuário, como peso, idade, e resultados de exames de sangue (dosagens de gordura e açúcar) que são feitos na rotina do seu atendimento no ambulatório. Serão também feitas algumas perguntas no momento da sua internação para realização da cirurgia, que levarão de cinco a dez minutos. Durante a cirurgia de redução de estômago, serão coletadas amostras de sangue para identificação do código genético de cada indivíduo e de pequenos pedaços de gordura da barriga que seriam descartados. Este procedimento não causará nenhuma alteração na rotina da realização da sua cirurgia de redução do estômago. Todos as análises adicionais previstas no presente estudo serão realizadas no Laboratório de Biologia Molecular da Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA).

Pelo presente Termo de Consentimento Livre e Esclarecido, declaro que autorizo a minha participação neste projeto de pesquisa, pois fui informado de forma clara e detalhada, livre de qualquer forma de constrangimento e/ou coerção, a respeito dos objetivos, da justificativa e dos procedimentos aos quais serei submetido. Também fui informado dos riscos, desconfortos e benefícios a minha participação, todos acima listados.

Fui igualmente informado:

- da garantia de receber respostas ou esclarecimento sobre qualquer dúvida a respeito dos procedimentos, riscos, benefícios e outros detalhes relacionados com a pesquisa;
- da liberdade de retirar meu consentimento, a qualquer momento, e deixar de participar do estudo, sem que isso traga prejuízo à continuação do meu tratamento;

- da garantia que não serei identificado quando da divulgação dos resultados e que as informações obtidas serão utilizadas apenas para fins científicos vinculados ao presente projeto de pesquisa;
- do compromisso de receber informação atualizada obtida durante o estudo, ainda que possa afetar a minha vontade em continuar participando;
- de que o material biológico que será coletado será armazenado, podendo ser utilizado para posteriores estudos por apenas 5 anos;
- da utilização do material armazenado, que só ocorrerá mediante aprovação do novo projeto pelo Comitê de Ética em Pesquisa.

Caso eu tenha novas perguntas sobre este estudo, posso chamar a pesquisadora responsável por este Projeto de Pesquisa, Prof. Vanessa Suñé Mattevi, telefone: 3303.8763, endereço Rua Sarmento Leite 245, prédio central, sala 309. Também, que se houverem dúvidas quanto a questões éticas, poderei entrar em contato com Daniel Demétrio Faustino da Silva, Coordenador-Geral do Comitê de Ética em Pesquisa do Grupo Hospitalar Conceição pelo telefone 3357-2407, endereço Avenida Franscisco Trein 596, 3º andar, Bloco H, sala 11, ou ainda com o Comitê de Ética da UFCSPA (telefone: 3303.8804).

Este documento foi revisado em seus aspectos éticos e metodológicos e aprovado pelos Comitês de Ética em Pesquisa do Hospital Nossa Senhora da Conceição e da Universidade Federal de Ciências da Saúde de Porto Alegre.

Data ___/___/_____

_____	_____
Nome	Assinatura do paciente

_____
Concordo em deixar minha amostra armazenada para estudos posteriores

_____	_____
Nome	Assinatura do responsável pela obtenção do consentimento

Observação: O presente documento, baseado no item IV das Diretrizes e Normas Regulamentadoras para a Pesquisa em Saúde, do Conselho Nacional de Saúde (Resolução 196/96), será assinado em duas vias, de igual teor, ficando uma via em poder do Paciente ou seu Responsável Legal e outra com o Pesquisador Responsável.

Anexo IV – Instrução para autores do periódico Obesity Surgery

OBESITY SURGERY
INSTRUCTIONS FOR AUTHORS
1. ABOUT OBSU

Obesity Surgery is published by Springer Science+Business Media LLC and is the official journal of the International Federation for the Surgery of Obesity and metabolic disorders (IFSO). Obesity Surgery publishes concise articles on Original Contributions, New Concepts, "How I Do It," Review Articles, Brief Communications, Letters to the Editor and dedicated Video Submissions. Requirements are in accordance with the "Uniform Requirements for Manuscripts submitted to Biomedical Journals," www.icmje.org.

Articles that are accepted for publication are done so with the understanding that they, or their substantive contents, have not been and will not be submitted to any other publication.

2. IMPORTANT SUBMISSION INFORMATION

2a. SYSTEM REQUIREMENTS

Authors will need the following items in order to use Editorial Manager:
• Internet access
• A current Adobe Acrobat browser plug-in
• Electronic files of all required documents for upload.

2b. YOUR AUTHOR ACCOUNT

Authors entering the journal's Editorial Manager site for the first time can create a new account at http://www.edmgr.com/obsu/ by clicking "Login" at the top of the screen, and "Register Now" at the next screen, and then following the online prompts in order to create your account and submit a manuscript. NOTE: If you have previously logged into the system, you should *always use your existing account* for ALL subsequent submissions. If you have forgotten your Username or Password, you may use the "Send Username/Password" link at the OBSU Log In Page.

2c. ONLINE SUBMISSION

After you have logged into your account and entered your Submission Center, Editorial Manager will lead you through a step-by-step submission process. When submitting your manuscript through Editorial Manager, you will navigate through nine (9) submission steps.

The required documents for all online submissions include the main Manuscript document, and a Conflict of Interest (COI) form, which should be completed by each contributing author.

Note: Always keep copies of your word-processing, graphics, video and COI files. You may want to revise the manuscript text, figures or forms after the review process and you will need the original files if your manuscript requires revisions.

Please make sure that all required online fields are completed before attempting to submit. If you cannot finish your submission in one visit, you can save a draft and later re-enter the process at the same step by clicking on the “Incomplete Submissions” link in your Author Main Menu.

3. MANUSCRIPT PREPARATION

3a. MANUSCRIPT SECTIONS AND FILE ITEMS

When you upload your manuscript documents to OBSU, the system will ask you to indicate the manuscript file “Item.” Your manuscript should be submitted in various parts; for example, your item, “Manuscript” should be uploaded separately from the item, “Official Conflict of Interest Form.” Images should be submitted separately, as should any electronic supplementary material (or “Other”) and videos (either as supplementary videos or as dedicated video submissions).

Please use the following format guidelines.

- Use a normal, plain font (e.g., 12-point Times Roman) for text.
- Double-space the text, and set page borders at one inch.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents; do not use the space bar for indents.

i. File Item: “Manuscript” (required)

In the "Attach Files” step (final step) of your submission, the file item “Manuscript” should include a Title Page, the Main Text (which should include a Conflict of Interest Disclosure Statement), References, and Figure Legends (if any). Tables may also be included at the end of this document, or submitted separately under the File Item, “Table.”

Title Page. This should include:

- The title of the article.
- The manuscript type.
- The complete names and academic degrees for each contributing author (first name, middle initial[s], surname, degree[s]).
- The departmental and institutional affiliations with complete street or mailing addresses and email addresses for each contributing author. Include the city, state or province, and country where the work was performed.
- "Correspondence to" followed by the name and contact information for the corresponding author.
- A shortened title for use as a running head (not to exceed 30 characters in length, including spaces between words).
- At the bottom of the page, any detailed grant information, and an acknowledgment of grant support.
- Acknowledgments: Individuals, other than authors, who were of direct help in the

reported work should be acknowledged by a brief statement. Each acknowledged person should give their written consent to be named in the manuscript.

Main Text. The main text document for most submissions should include:

- Abstract (not required for Letters)
- Introduction/Purpose
- Materials and Methods
- Results
- Conclusion
- Conflict of Interest Disclosure Statement (see details below)
- References (see details below)
- If separate figures are provided, then a Figure Legend should be included in the main text document after the References.
- Any Tables that you provide should be included at the end of the text.

Additional format requirements and details for specific manuscript *types* are included in the "3b. MANUSCRIPT TYPES AND FORMATS" section below.

Conflict of Interest Disclosure Statement (in Text).
A Conflict of Interest disclosure statement is required to be included for each author within the manuscript text, and should be located just before the list of References. For each author, the statement must declare the potential conflict of interest, or "no conflict of
interest."

Note: The details provided in the COI Disclosure Statement within the manuscript text must correspond with the information provided in the required author ICMJE COI forms.

Potential conflicts of interest do not imply impropriety but could either directly or indirectly, purposely or inadvertently affect the conduct, outcome, or reporting of any scholarly activity. Potential conflicts of interest exist when an author is related to a forprofit company or institution in any of the following ways:

1. Employment
2. Consultancies in the last 3 years (please list)
3. Honoraria in the last 3 years (please list)
4. Stock ownership/ options other than mutual funds (current; please list)
5. Expert testimony in the last 3 years (please list)
6. Grants received in the last 3 years (please list)
7. Grants pending (please list)
8. Patents received
9. Patents pending
10. Royalties (describe)
11. Other relationships (please specify)

References
For the references, please:

- Use Medline®/Pubmed® Style.

• Type references double-spaced and list them in consecutive, numerical order as they appear in the text (not alphabetically).
• Identify reference citations in the text by numbers in square brackets (e.g., [1]). Once a reference is cited, all subsequent citations should be to the original number.
• Cite all references within the text or tables.
• Papers that have been accepted for publication or are in press may be listed in the References, but the Journal does not reference unpublished data and personal communications.
• If several references are available on the same subject, cite only the most recent and pertinent, giving preference to original articles over review articles or textbooks.

Journal Articles
Journal articles should be cited according to the Medline®/Pubmed® Journal Article Citation Format. An example follows:
Lee MJ, Fanelli F, Haage P, Hausegger K, Van Lienden KP. Patient safety in interventional radiology: a CIRSE IR checklist. Cardiovasc Intervent Radiol. 2012 Apr;35(2):244-6. Epub 2011 Oct 20. PMID: 22011783

Books and Other Published Material
For citation format examples of books, other monographs, other published material, and electronic material, please visit
http://www.nlm.nih.gov/bsd/uniform_requirements.html

Tables

• Any tables included in the manuscript should be provided as follows.
• Use the table function (not spreadsheets) to make tables.
• Number all tables using Arabic numerals.
• Always cite tables in the text in consecutive, numerical order.
• For each table, please supply a table heading. The table title should explain clearly and concisely the components of the table.
• Identify any previously published material by giving the original source in the form of a reference at the end of the table heading.
• Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.
• All tables should be supplied on a separate page at the end of the main document and have callouts in the text.

ii. File Item: "Official Conflict of Interest Form(s)" – (required)

Every contributing author must complete the official ICMJE Conflict of Interest (COI) form, which is available by clicking the "Conflict of Interest Form" link in the "For Authors and Editors" section of the Springer page at www.springer.com/11695, or by directly visiting http://www.icmje.org/coi_disclosure.pdf. No hand-written information

is required in this form. A completed, submitted form from each author is required for our records. The form(s) will not appear as part of the manuscript for review.

The corresponding/submitting author is responsible for collecting ALL participating authors' completed ICMJE COI forms and uploading them with the manuscript submission. It is recommended that each ICMJE COI form be saved and renamed as the author's name. If any contributing author's COI form is incomplete or missing from the submission, the submission will be returned to the author for correction prior to review. Each author must complete the form even if no conflict of interest exists.

Note: Details provided in the ICMJE COI forms must correspond with the required COI Disclosure Statement that the authors include in the manuscript text.

iii. File Item: "Figure" (optional)

Along with uploading main text document, you can also upload separate figure and graphic image documents. Common graphics files such as GIF, JPEG, EPS, TIFF and many others are supported. *Please do not upload figures as PDF files, or in PowerPoint; we also recommend that figures not be embedded in the main text of your article.*

For vector graphics, the preferred format is EPS; for halftones, TIFF format is preferred. Very large figure files should be compressed as much as possible before uploading figures to the website. If the figures will be printed in black and white, do not refer to color in the captions. All figures are to be numbered using Arabic numerals. Figure parts should be denoted by lowercase letters. Figures should always be cited in text in consecutive numerical order. For each figure, include the figure legends at the end of the manuscript text. Make sure to identify all elements found in the figure in the caption.

Photographs of patients in which the subject is identifiable must either have the face masked out, or be accompanied by written permission from the individual in the photograph for publication.

Image Size

- Actual size of submitted image(s) should be as follows:
- Width: 39 mm, 84 mm, 129 mm or 174 mm wide.
- Height: No higher than 234 mm.
- The following open source image-conversion software is available in Mac and

  Windows format to assist you in standardizing your images:

  - GraphicsMagick - www.graphicsmagick.org
  - Image Magick - www.imagemagick.org
  - Xn Convert - www.xnconvert.com

For detailed submission guidelines regarding Line Art, Halftone Art, Combination Art, Color Art, and other artwork details, please click here: ARTWORK INSTRUCTIONS

iv. File Item: "Other" (optional)

If you want to provide a file with your submission that does not fit any of the above file designations, you may submit it under "Other."

v. File Item: "Multimedia Article (video)" (optional)

We invite contributing authors to publish additional, article-related materials on the website that complement and reinforce information published in the print journal, as well as dedicated Multimedia articles. If any multimedia is submitted, it will be reviewed along with the submission, and if accepted will be published as received from the author in the online version only. All standard instructions for manuscript and video submission should be followed (see "Videos" below).

Multimedia Articles may consist of:

- Information that cannot be printed: animations, video clips, sound recordings
- Information that is more convenient in electronic form: sequences, spectral data, etc.
- Large original data, e.g. additional tables, illustrations, etc.
- If supplying any multimedia, the text must make specific mention of the material as a citation, similar to that of figures and tables (e.g., ". . . as shown in Animation 3."

Supplementary Videos

Upon submission of articles that include supplementary video, the author(s) will be required to submit the video according to the following specifications:

- To accommodate user downloads, keep to the recommended upper limit for the size of the different file types. Larger-sized files may require very long download times, and some users may experience other problems during downloading.
- Video clips should not exceed one minute or 2MB. Anything exceeding 1 minute must be submitted in separate videos.
- Always use either .mp4 or .mov files.
- The content of these files must be identical to that reviewed and accepted by the editor-in-chief.
- All narration should be in English.

Note: For any articles *already published* on Springer.com, authors may submit follow-up or supplementary videos related to the article via **videos.springer.com.**

Dedicated Video Submissions

Upon submission of dedicated video submissions, author(s) will be required to submit the video according to the following specifications:

- Always use either .mp4 or .mov files.
- Please note that larger-sized files may take extra time to upload, and may require very long download times for reviewers and other users; some users may experience other problems during downloading, so please make sure to consider the optimal size of your video when submitting it.
- Dedicated video submissions should not exceed 10 minutes in duration. Invited videos or contributions with specific topics may be longer (at the discretion of the editors).
- Narration of the procedure is required; all narration should be in English.
- An Abstract of up to 500 words in Word or RTF must be provided along with the video. It should contain the title; authors and affiliations; a conflict of interest statement as described above, and a structured Abstract that includes an introduction (including a description of the video and the run-time), materials and methods, results and conclusions, and references.
- If applicable, provide references (as detailed above) at the end of the Abstract.

3b. MANUSCRIPT TYPES AND FORMATS

The manuscript types for submission include Review Articles, Brief Communications, New Concepts, How I Do It, Original Contributions, Letters, and Video Submissions. Each of these manuscript types requires a specific format. You may submit your manuscript in either Format I or Format II, as detailed below. Please type all manuscript text (including references) doublespaced with one-inch wide margins. Number the pages consecutively and organize the manuscript in the order indicated below.

i. Manuscript Format I (Full Articles)

- **Original Contribution**
- **New Concept**
- **How I Do It**

Format: The main body text in Original Contributions, New Concepts, and "How I Do It" submissions, is limited to eight double-spaced, typewritten pages (2,400 words, excluding references, figure legends and Abstract), and up to six figures/images. The following format should be followed:

Title Page

Manuscript Text For Review

- Abstract - The Abstract in full manuscripts must not exceed 250 words, and should consist of five (5) structured paragraphs: Introduction/Purpose, Materials and Methods, Results, and Conclusion.
- Key Words
- Introduction/Purpose - Should convey the background and purpose of the report.

• Materials and Methods, Results, and Discussion - Should follow the Introduction. When required by the nature of the report, manuscripts that do not follow this specific format may be accepted. Please include a statement in the Methods section indicating approval (or exemption) by your Institutional Review Board for the conduct of your research.
• Conflict of Interest Disclosure Statement – Is required to be included within the manuscript text for each author, and should be located just before the list of References.
• References (not included in word count)
• Tables (not included in word count)

Figures (Illustrations) - Submitted separately from the main text.

Official ICMJE Conflict of Interest forms - Must be completed and submitted from each contributing author, and are not viewable to reviewers.

3c. ADDITIONAL SUBMISSION DETAILS

i. Language Editing Services

If you would like your manuscript language edited by a scientific expert before submission or upon revision, Springer recommends using Edanz Group. Edanz provides scientific editing and related services that raise the quality of manuscripts to the standard necessary for ease of peer review. As the only international editing service centralized in China and Japan, Edanz understands the publication challenges faced by scientists whose first language is not English.

For more information and a price quotation, please contact:
http://www.edanzediting.com/springer

ii. Special Characters

The Journal does not assume responsibility for errors in conversion of customized software, newly released software, and special characters. Please indicate any special characters used in the file (e.g., Greek, math symbols) by using a symbol code (e.g., ‹ga› for Greek alpha), and defining these codes at the end of your paper.

iii. Abbreviations, Drug Names, Digits

Please use the standard abbreviations and units listed in Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers, Sixth Edition (Reston, Va., Council of Biology Editors, 1994). The first time an uncommon abbreviation appears in the text, it should be preceded by the full name for which it stands. Generic names for drugs and chemicals should be used the first time the drug or chemical is mentioned in the text and, preferably, thereafter. If an author wishes, the trade name may be inserted in parentheses following the generic name the first time the generic name appears, and the manufacturer name and city should also be included. Please express digits as numerals except when they are the first word in a sentence, and decimals should be written in North American

format. Express units of measurement in the metric system whenever possible, and abbreviate them when used with numbers.

iv. Permissions

If you use text passages, illustrations, or tables from other works, you must in each case obtain permission from the copyright holder for both the print and the online format. A written statement must accompany materials that are taken from other sources and included in the submitted manuscript. Please be informed that Springer will not be able to refund any costs that may have occurred in order to receive these permissions from other publishers. Please be aware that some publishers do not grant electronic rights for free. In these cases we kindly ask you to use figures from other sources. **To access the Rights and Permissions Instructions, go to**: http://www.springer.com/rights?SGWID=0-12212-372399-0

v. Statement of Human and Animal Rights

When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach, and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study. When reporting experiments on animals, authors should be asked to indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

vi. Statement of Informed Consent

Patients have a right to privacy that should not be infringed without informed consent. Identifying information, including patients' names, initials, or hospital numbers, shouldnot be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication. Informed consent for this purpose requires that a patient who is identifiable be shown the manuscript to be published. Authors should identify Individuals who provide writing assistance and disclose the funding source for this assistance. Identifying details should be omitted if they are not essential. Complete anonymity is difficult to achieve, however, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of patients is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning and editors should so note.

vii. Other Required Forms

Copyright forms and color publication payment details are now handled online *after* an accepted article is accepted for publication. When proofs are ready for viewing, the author is contacted via e-mail by the typesetter, and sent a website address that will provide the author with forms/orders/proofs procedures.

viii. Note regarding plagiarism and self-plagiarism:
Manuscripts are evaluated via iThenticate for signs of any potential plagiarism. For information on the concept of self-plagiarism, visit the following online link: **www.ithenticate.com/self-plagiarism-free-white-paper/**

4. MANUSCRIPT SUBMISSION

4a. SUBMISSION STEPS

i. Submission Checklist

Please view a copy of the SUBMISSION CHECKLIST here. We recommend that you have all items listed in the checklist complete and ready for upload before starting your online submission.

ii. Review Your Submission

After uploading the files for your submission, the system will convert the files to PDF. You will see the result of the conversion with the Acrobat plug-in in your browser. Make sure to review the PDF of your submission before you confirm your submission. Once you have reviewed your PDF document for completeness, click "Submit" and all contributing authors will receive an emailed confirmation.

After the manuscript is submitted, the Editors will inspect the submission before assigning reviewers. If any part of the manuscript is not complete, the manuscript will be unsubmitted and returned to your Submission Center, with an e-mail notification sent to the authors indicating the need for additional information and/or correction. Once a complete manuscript is correctly submitted, the OBSU editors will assign reviewers to your submission.

4b. KEEPING TRACK

After submission, you may monitor the progress of your submission through the review process. Only the submitting author can view the submission. In order to view your submission details and current status, you must enter the same User Name and Password that you originally used to submit your manuscript.

4c. EDITORIAL REVIEW AND ACTION

The editorial staff will examine submitted manuscripts for accuracy and completeness, and will customarily send initial manuscript submissions to two or three reviewers, depending on the manuscript type. We aim for quick reviewer turnaround times, and rely on the promptness and thoroughness of our volunteer reviewers and Editors. Authors will be notified as to the acceptability of a manuscript as rapidly as possible. The decision categories are: Accept; Immediate Reject; Reject (after review); Accept Pending Minor Revisions, and Reject but Encourage Resubmission After Major Revisions. Suggestion for resubmission does not guarantee acceptance upon resubmission.

If the manuscript is accepted pending minor revisions, or suggested for resubmission after major revisions, we emphasize the importance of authors providing their revisions as promptly as possible, and providing a point-by-point reply to all reviewer comments. The annotated version of the revised manuscript should identify all changes and include each reviewer point in parentheses, e.g., "(Reviewer 1, Comment 2)."

5. AFTER ACCEPTANCE

If your article is accepted, you will receive a link to the special Springer web page with questions related to:

5a. AUTHOR PROOFS

After a submission is accepted and processed, the author will receive e-mailed notification from the Production Office, and a proof of the article is made available to the author. You should check the proof for typesetting errors, completeness, and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor. Any such changes would require a written request and written approval/agreement from all contributing authors to the Editorial Office and to Production for their consideration.

The article will be published online after receipt of the corrected proofs. Online publication is the official first publication of the article, and citable with the DOI. After online publication, further changes can only be made in the form of an Erratum, which would be hyperlinked to the article. After release of the printed version, the paper can also be cited by issue and page numbers.

5b. OPEN CHOICE

In addition to the normal publication process (whereby an article is submitted to the journal and access to that article is granted to customers who have purchased a subscription), Springer now provides an alternative publishing option: Springer Open Choice. A Springer Open Choice article receives all the benefits of a regular subscription-based article, but in addition is made available publicly through Springer's online platform SpringerLink. We regret that Springer Open Choice cannot be ordered for published articles. Please go to: http://springer.com/openchoice or click on the link below for more information.
OPEN CHOICE

5c. PUBLICATION OF COLOR FIGURES

Color figures may be used without charge for the electronic version of the journal that is published online via SpringerLink. However, color figures will appear in the print version of the Journal at the author's expense at $1,150 per article. You may provide your choice at the Springer web page.

5d. OFFPRINTS/ REPRINTS

Can be ordered via the Springer web page.

6. SUPPORT AND ASSISTANCE

When entering your data during the submission process, support for special text characters is available. If you have questions at any point during your submission process, or you need assistance submitting your manuscript online, please contact the OBSU Managing Editor:

ATTN: Deana Rodriguez
Managing Editor, OBSU Editorial Office
Phone: +001 (562) 961-9928
E-mail: obsu.rodriguez@gmail.com