PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

IDENTIFICAÇÃO DE MARCADORES GENÉTICOS ASSOCIADOS A LIPODISTROFIA E DISLIPIDEMIA EM PACIENTES COM SÍNDROME DA IMUNODEFICIÊNCIA ADQUIRIDA SOB TERAPIA ANTIRRETROVIRAL

JUCIANE RODRIGUES TRINCA
Orientador: Profa. Dra. Silvana Almeida
Co-orientador: Profa. Dra. Vanessa S. Mattevi

Biblioteca Paulo Lacerda de Azevedo

Área de Concentração:
Métodos Diagnósticos e Epidemiologia das Doenças

2010

Dissertação de Mestrado

Livros Grátis

http://www.livrosgratis.com.br

Milhares de livros grátis para download.

JUCIANE RODRIGUES TRINCA

IDENTIFICAÇÃO DE MARCADORES GENÉTICOS ASSOCIADOS A LIPODISTROFIA E DISLIPIDEMIA EM PACIENTES COM SÍNDROME DA IMUNODEFICIÊNCIA ADQUIRIDA SOB TERAPIA ANTIRRETROVIRAL

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre para a obtenção de grau de Mestre em Ciências da Saúde.

Orientador: Profa. Dra. Silvana Almeida
Co-orientador: Profa. Dra. Vanessa S. Mattevi

PORTO ALEGRE

2010

T832i Trinca, Juciane Rodrigues

Identificação de marcadores genéticos associados a lipodistrofia e dislipidemia em pacientes com síndrome da imunodeficiência adquirida sob terapia antirretroviral / Juciane Rodrigues Trinca. – Porto Alegre, 2010.
124 f. : il.

Dissertação (Mestrado) – Programa de Pós-Graduação em Ciências da Saúde – Universidade Federal de Ciências da Saúde de Porto Alegre, 2010.
Orientador: Silvana Almeida
Co-orientador: Vanessa S. Mattevi

1. Leptina. 2. Adiponectina. 3. PPAR gama. 4. Proteínas de ligação a elemento regulador de esterol. 5. HAART. 6. Farmacogenética. 7. HIV. I. Título. II. Almeida, Silvana. III. Mattevi, Vanessa S.

CDD 616.39

Eleonora Liberato Petzhold
CRB10/1801

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO

UFCSPA
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

ATA Nº 217-2009

De acordo com o estabelecido previamente pela Comissão Coordenadora do Curso de Pós-Graduação em Ciências da Saúde da Universidade Federal de Ciências Saúde de Porto Alegre, realizou-se aos vinte e um dias de dezembro de dois mil e nove, às 10:00h, no Auditório do Centro de Pesquisa e Pós-Graduação Prof. Heitor Cirne Lima da UFCSPA, a apresentação da Dissertação de Mestrado da aluna **Juciane Rodrigues Trinca**, orientada pela Professora Silvana Almeida no PPG-Ciências da Saúde e intitulada **"IDENTIFICAÇÃO DE MARCADORES GENÉTICOS ASSOCIADOS A LIPODISTROFIA E DISLIPIDEMIA EM PACIENTES COM SÍNDROME DA IMUNODEFICIÊNCIA ADQUIRIDA SOB TERAPIA ANTIRRETROVIRAL"**, na área de concentração de Métodos Diagnósticos e Epidemiologia das Doenças. A Banca Examinadora foi composta pelos Professores Pedro Roosevelt Torres Romão (UFCSPA), Rosane Vianna Jorge (UFRJ) e José Artur Bogo Chies (UFRGS). A sessão foi aberta pela Profª Cláudia Ramos Rhoden, vice-coordenadora do PPG-Ciências da Saúde. Após a abertura da sessão a candidata dispôs de 45 minutos para expor seu trabalho. Ao término da apresentação, foi franqueada a platéia a possibilidade de dirigir perguntas ao autor.

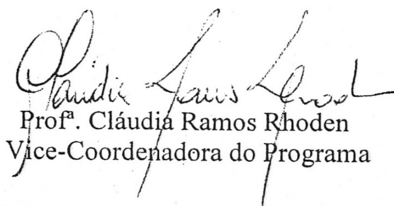
Ao término da Sessão, foram anunciadas as notas conferidas pelos examinadores:

Prof. Pedro Roosevelt Torres Romão	9,6
Profª Rosane Vianna Jorge	9,7
Prof. José Artur Bogo Chies	9,8

NOTA FINAL: 9,7

Nada mais havendo a tratar, foi encerrada a reunião e lavrada a presente ata, que após lida deverá ser assinada.

Porto Alegre, 21 de dezembro de 2009.



Profª. Cláudia Ramos Rhoden
Vice-Coordenadora do Programa

Rua Sarmento Leite, 245 • Porto Alegre, RS • CEP 90050-170 • Fone: 0 xx 51 3303 9000 • Fax: 0 xx 51 3303.8810 • www.ufcspa.edu.br

"Algo só é impossível até que alguém
duvide e acabe provando o contrário"

Albert Einstein (1879-1955)

AGRADECIMENTOS

A Dra. Vanessa Suñé Mattevi e a Dra. Silvana Almeida pela orientação, dedicação e paciência ao longo dos anos que trabalhamos juntas.

As funcionárias do Laboratório de Biologia Molecular, Grasiela Agnes e Magda Brasil, pela amizade e pelo apoio técnico sempre imprescindível.

As amigas e colegas de laboratório, Ananda, Aline, Lisiane e Luciana, pelo apoio nos momentos mais difíceis, mas principalmente pelos momentos de descontração.

Aos demais colegas e amigos do laboratório pela amizade e pelo excelente ambiente de trabalho.

Aos meus pais, Mauro e Nilva, pelo esforço e empenho inabaláveis, para que eu pudesse me dedicar integralmente aos estudos e às minhas irmãs Juliana e Juciara, por estarem sempre por perto. Ao pequeno Dimitry pelas bagunças, risadas e brincadeiras que aliviavam as tensões diárias.

Ao meu querido companheiro de todas as horas, Fabio, pelo respeito, carinho, paciência para suportar a distância e, sobretudo, por sempre acreditar e investir nas minhas capacidades.

A CAPES, pela concessão da bolsa de estudos e, a Secretaria Nacional de DST/AIDS do Ministério da Saúde e ao CNPq, patrocinadores desta pesquisa.

SUMÁRIO

ABSTRACT **8**

RESUMO **9**

Capítulo 1 – Introdução **10**
1.1. Epidemiologia da infecção pelo HIV/AIDS..11
1.2 O vírus HIV 12
1.3 Fases da infecção 14
1.4 Fundamentos da terapia antirretroviral 15
1.5 Lipodistrofia e dislipidemias 19
1.6 Estudos farmacogenômicos da TARV 20
1.7. Genes de Adipocinas e TARV 22
1.7.1. Leptina 23
1.7.2. Adiponectina 27
1.7.3. PPAR-γ 29
1.7.4 SREBP-1c 32
1.8 Justificativa e objetivos 34
1.9 Referências bibliográficas 36

Capítulo 2 – SNPs in the *APM1* gene promoter are associated with adiponectin levels in HIV-infected individuals receiving HAART 45

Capítulo 3 – A genetic variant in sterol-regulatory element-binding protein-1c is associated with HAART-related lipohypertrophy 72

Anexo 1 – Metodologia Utilizada **99**

Anexo 2 – Termos de aprovação pelos comitês de ética..**103**

Anexo 3 – Termo de consentimento livre e esclarecido **106**

Anexo 4 – Carta de confirmação da submissão do artigo *"SNPs in the APM1 gene promoter are associated with adiponectin levels in HIV-infected individuals receiving HAART"* ao periódico *JAIDS* **109**

Anexo 5 – Instruções para publicação de artigos no periódico *JAIDS* **111**

Anexo 6 - Instruções para publicação de artigos no periódico *Antiviral Therapy* ..**120**

ABSTRACT

The advent of antiretroviral therapy (HAART) against HIV led to the reduction of viral load to undetectable levels, changing the perception of this disease from a fatal disease to a chronic condition. Despite the advances accomplished by HAART, the appearance of significant adverse effects might compromise quality of life of patients and their adherence to treatment. This study aimed to investigate the association between polymorphisms in the leptin (*LEP* -2548G>A), leptin receptor (*LEPR* Gln223Arg*)*, adiponectin (*APM1* -11391G>A and -11377C>G), peroxissome proliferator-actived receptor gamma (*PPARG* Pro12Ala) and sterol regulatory element binding protein (*SREBP-1c* 3322C>G) genes and the occurrence of lipodystrophy (LD) and dyslipidemia in HIV-infected patients receiving HAART. Genotypes of 410 HIV-infected patients from Hospital de Clínicas de Porto Alegre, receiving HAART at least one year, were analyzed through polymerase chain reaction-based methods. Anthropometric (weight, height, waist circumference and skinfolds thickness) and biochemical (blood lipids and glucose) parameters were evaluated. Genotype frequencies were compared between patients with and without LD. Adjusted mean biochemical and anthropometric parameters were compared between the different genotypes. Poisson regression models were used to estimate the contribution of genetic variants to LD phenotypes. LD prevalence was 53.4%, being higher in euro-Brazilians (61.1%) than in afro-Brazilians (42.7%, *P*<0.001). *APM1* -11391A carriers and *APM1* -11377C carriers presented higher adiponectin levels compared to other genotypes, as well as carriers of the -11391A-11377C haplotype when compared to carriers of other haplotypes (AC carriers= 9.83 µg/mL; other haplotypes= 9.16 µg/mL; *P*=0.005). *SREBP-1c* 3322G allele was associated with higher risk of developing lipohypertrophy in European descendants (PR=1.41, *P*=0.039). SNPs (single nucleotide polymorphism) in *APM1* gene are associated with adiponectin levels in HIV-infected patients receiving HAART and may thus affect the occurrence of metabolic alterations in these patients. *SREBP-1c* is a significant contributor to lipohypertrophy in Euro-Brazilians. No influence of the *LEP*, *LEPR* and *PPARG* gene polymorphisms on the occurrence of LD and dyslipidemia was observed.

Key Words: leptin, adiponectin, *PPARG*, *SREBP*, HAART, pharmacogenomics, HIV.

RESUMO

O advento da terapia antirretroviral (TARV) contra o HIV permitiu a redução da sua carga viral para níveis não-detectáveis, alterando a percepção desta patologia de uma doença fatal para uma condição crônica. Apesar dos avanços alcançados pela TARV o aparecimento de efeitos adversos significativos pode comprometer a qualidade de vida do paciente e sua adesão ao tratamento. Este trabalho teve como objetivo investigar a associação entre polimorfismos nos genes da leptina (*LEP* –2548G>A), receptor da leptina (*LEPR* Gln223Arg), adiponectina (*APM1* –11391G>A e –11377C>G), receptor ativado por proliferadores de peroxissoma gama (*PPARG* Pro12Ala) e proteína de ligação a elementos regulatórios de esteróis (*SREBP* 3322C>G) e a ocorrência de lipodistrofia (LD) e dislipidemia em pacientes HIV-positivos sob TARV. Os genótipos de 410 pacientes provenientes do Ambulatório HIV/AIDS do Hospital de Clínicas de Porto Alegre, sob TARV há pelo menos um ano, foram investigados pela técnica da reação em cadeia da polimerase (PCR). Parâmetros antropométricos (peso, altura, circunferência da cintura e pregas cutâneas) e bioquímicos (colesterol, LDL, HDL, triglicerídeos, glicose, leptina e adiponectina séricos) foram avaliados. As freqüências alélicas e genotípicas foram comparadas entre os pacientes com ou sem LD. As médias ajustadas de parâmetros bioquímicos e antropométricos foram comparadas entre os diferentes genótipos. Modelos de regressão de Poisson foram utilizados para estimar a contribuição das variantes genéticas para os subtipos de LD. A prevalência de LD foi de 53,4%, sendo maior entre os descendentes de europeus (61,1%) do que entre os afro-descendentes (42,7%, *P*<0,001). Portadores do alelo A do polimorfismo *APM1* -11391G>A e do alelo C do polimorfismo *APM1* -11377C>G apresentaram níveis de adiponectina mais elevados do que portadores de outros genótipos, bem como portadores do haplótipo -11391A-11377C, quando comparados com os demais haplótipos (portadores do haplótipo AC= 9.83 μg/mL; demais haplótipos= 9.16 μg/mL; *P*=0.005). O alelo *SREBP-1c* 3322G foi associado com maior risco de desenvolvimento de lipohipertrofia em euro-descendentes (RP=1.41, *P*=0.039). SNPs (single nucleotide polymorphism) no gene *APM1* estão associados com os níveis de adiponectina em indivíduos portadores do HIV sob TARV e podem afetar a ocorrência de alterações metabólicas nestes pacientes. O polimorfismo SREBP-1c 3322C>G contribui significativamente para a ocorrência de lipohipertrofia em pacientes descendentes de europeus. Nenhuma influência dos polimorfismos estudados nos genes *LEP*, *LEPR* e *PPARG* sobre lipodistrofia e/ou dislipidemia foi observada.

Palavras-chave: leptina, adiponectina, *PPARG*, *SREBP*, HAART, farmacogenômica, HIV.

Capítulo 1 – Introdução

1. INTRODUÇÃO

1.1. Epidemiologia da infecção pelo HIV/AIDS

Cerca de 33,4 milhões de pessoas em todo o mundo estão infectadas pelo vírus da imunodeficiência humana (HIV) (Tabela 1) (UNAIDS/WHO, 2009).

Tabela 1: Resumo da epidemia de AIDS no mundo

Número de pessoas vivendo com HIV em 2008	
Total	**33,4 milhões [31,1 – 35,8 milhões]**
Adultos	31,3 milhões [29,2 – 33,7 milhões]
Mulheres	15,7 milhões [14,2 – 17,2 milhões]
Crianças (menores de 15 anos)	2,1 milhões [1,2 – 2,9 milhões]
Pessoas infectadas pelo HIV em 2008	
Total	**2,7 milhões [2,4 – 3,0 milhões]**
Adultos	2,3 milhões [2,0 – 2,5 milhões]
Crianças (menores de 15 anos)	430.000 [240.000 – 610.000]
Mortes causadas pela AIDS em 2008	
Total	**2,0 milhões [1,7 – 2,4 milhões]**
Adultos	1,7 milhões [1,4 – 2,1 milhões]
Crianças (menores de 15 anos)	280.000 [150.000 – 410.000]

Fonte: AIDS update epidemic. Joint United Nations Programme on HIV/AIDS / World Health Organization (UNAIDS/WHO), 2009.

Na América Latina, de modo geral, a epidemia de HIV/AIDS permanece estável. O número estimado de novas infecções pelo HIV no ano de 2008 foi 170.000. Estima-se que 2,0 milhões de pessoas estão vivendo com HIV nesta região e que 77.000 pessoas tenham morrido em consequência da AIDS (*acquired immune deficiency syndrome*) em 2008 (Tabela 2) (UNAIDS/WHO, 2009).

No Brasil, o número de pessoas vivendo com HIV foi estimado em 620.000, para o ano de 2005; cerca de um terço de todas as pessoas infectadas pelo HIV na América Latina. Embora a epidemia inicialmente tenha sido

concentrada entre homens que fazem sexo com homens, logo englobou usuários de drogas injetáveis e hemofílicos, e finalmente atingiu a população em geral, com um número cada vez maior de mulheres infectadas. A ampla distribuição de antirretrovirais, através de programas governamentais, aponta resultados positivos, como a redução nos casos de transmissão vertical do HIV (da mãe para o filho), nas taxas de mortalidade e no número de hospitalizações em decorrência de complicações da doença (UNAIDS/WHO, 2009).

Tabela 2: Estatísticas regionais do HIV e AIDS em 2008

	Adultos e crianças vivendo com HIV	Prevalência entre adultos (%)	Mortes devido à AIDS (adultos e crianças)
África Sub-Sahariana	22,4 milhões	5,2	1,4 milhões
Oriente Médio e Norte da África	310.000	0,2	20.000
Sul e Sudeste da Ásia	3,8 milhões	0,3	270.000
Leste da Ásia	850.000	<0,1	59.000
Oceania	59.000	0.3	2.000
América Latina	**2,0 milhões**	**0,6**	**77.000**
Caribe	240.000	1,0	12.000
Leste Europeu e Ásia Central	1,5 milhão	0,7	87.000
Oeste e Centro da Europa	850.000	0,3	13.000
América do Norte	1,4 milhão	0,6	25.000
Total	**33,4 milhões**	**0,8**	**2,0 milhões**

Fonte: AIDS update epidemic. Joint United Nations Programme on HIV/AIDS / World Health Organization (UNAIDS/WHO), 2009.

1.2 O vírus HIV

O HIV é um vírus pertencente à família *Retroviridae*, ou seja, um retrovírus (BARRE-SINOUSSI *et al.*, 1983). Compõem esta família também o

HTLV-I (vírus linfotrópico T humano tipo I) e o HTLV-II. O HIV é constituído por um genoma de RNA, um capsídeo protéico e um envoltório lipoproteico (envelope). No interior do capsídeo, adjacente ao RNA viral, encontra-se uma enzima fundamental para a replicação do vírus – a transcriptase reversa. O "envelope viral" contém duas glicoproteínas de extrema importância, pois garantem a ligação do vírus à célula hospedeira – gp120 e gp41 (ALCAMI, 2008).

Dentre as células humanas, o HIV tem tropismo para linfócitos T CD4+, macrófagos e células dendríticas. Estas células têm em comum um receptor de membrana denominado CD4. Entretanto, para a penetração celular do genoma viral após ligação do vírion ao CD4 é necessária a interação com co-receptores como CCR5 e CXCR4 (FENG *et al.*, 1996; DRAGIC *et al.*, 1996). Sem estes co-receptores, não é possível proceder ao ciclo reprodutivo viral. Assim que o RNA ganha o citoplasma da célula hospedeira (no caso o linfócito T CD4+), ele sofre ação da transcriptase reversa, enzima capaz de transcrever um DNA fita dupla a partir de um RNA fita simples. Quando o genoma viral passa a ser um DNA fita dupla pode integrar-se ao DNA nuclear, utilizando assim o maquinário enzimático do hospedeiro para produzir suas próprias proteínas e seu RNA. Durante a replicação viral, milhares de partículas virais brotam da célula hospedeira, aproveitando o material de sua membrana plasmática para formar seu envoltório protéico. Ao se replicar em alta escala, prejudica a fisiologia celular, contribuindo para sua morte (ALCAMI, 2008).

1.3 Fases da infecção

Após um período de incubação de duas a quatro semanas, inicia-se um período de intensa replicação viral, elevando a viremia a níveis muito altos. Neste momento, 50 a 90% dos pacientes apresentam sinais e sintomas de uma síndrome viral aguda, semelhante a mononucleose infecciosa, chamada por alguns de “Síndrome da Primo-infecção pelo HIV” ou “Síndrome de Soroconversão” ou simplesmente “Síndrome Retroviral Aguda” (KAHN & WALKER, 1998). Os sintomas mais frequentes são: febre, geralmente entre 38-40ºC, adenopatia cervical, axilar e occipital, faringite eritematosa, *rash* cutâneo-mucoso, mialgia e artralgia, diarreia, cefaleia, náuseas e vômitos e hepato-esplenomegalia. Estes sintomas duram cerca de uma a duas semanas e deixam uma sensação de fadiga e certa letargia por algumas semanas a meses. Em conjunto com a viremia, costuma haver uma queda abrupta da contagem periférica de células T CD4+ (KAHN & WALKER, 1998).

Após três a doze semanas desde o início da infecção, a maioria dos indivíduos “soroconverte”, isto é, apresenta títulos detectáveis de imunoglobulina G (IgG) anti-HIV. Este anticorpo permanece positivo para o resto da vida do paciente. Os anticorpos imunoglobulina M (IgM) contra o vírus geralmente não são detectados ou aparecem em títulos muito baixos. O aparecimento da imunidade humoral anti-HIV contem parcialmente a replicação viral, fazendo a viremia cair para um nível denominado *“set point”* (STEKLER & COLLIER, 2004), com conseqüente queda na contagem de linfócitos T CD4+. Após a viremia atingir o “*set point”* a contagem dos linfócitos T CD4+ volta a aumentar, atingindo níveis acima de 500/mm^3, mas geralmente não voltando totalmente ao normal. O

paciente entra então na “Fase Assintomática” ou “Latência Clínica da Infecção” (FORD *et al.*,2009).

A fase assintomática pode durar de dois a vinte anos, mas a média oscila em torno de dez anos. Durante toda a fase assintomática o vírus permanece se replicando de forma menos intensa e a contagem de células CD4+ permanece estável. Finalmente, a fase assintomática evolui para uma fase de “reativação viral”, na qual o vírus aumenta novamente sua capacidade replicativa, elevando a viremia e fazendo a contagem de células T CD4+ cair de forma mais rápida. Valores inferiores a 350/mm^3, juntamente com a ocorrência de infecções oportunistas e/ou neoplasias são, segundo o Ministério da Saúde, indicadores de imunodepressão, caracterizando a AIDS (MINISTÉRIO DA SAÚDE, 2003). Esta fase representa um estado de imunodepressão grave, cujo mecanismo principal é a queda da contagem de linfócitos T CD4+ para níveis abaixo de 20% do valor normal. Neste momento infecções oportunistas e neoplasias levam o paciente ao óbito em média depois de 18 meses. Cerca de dois anos antes da fase AIDS, a contagem começa a cair mais rápido. Ao atingir 200/mm^3, sem doença oportunista, a sobrevida média sem tratamento é de 3,7 anos. A média de contagem de CD4 no aparecimento da primeira doença oportunista é de 60-70/mm^3 (FORD *et al.*, 2009).

1.4 Fundamentos da terapia antirretroviral

Desde a aprovação da zidovudina (AZT), primeiro fármaco antirretroviral, em 1987, mais de vinte fármacos foram aprovados para o tratamento contra o HIV. A combinação destes em regimes triplos permitiu a

redução da carga viral para níveis não detectáveis, modificando a percepção desta patologia de uma doença fatal para uma condição crônica (ENGEL *et al*, 2004).

A terapia antirretroviral (TARV) tem um papel primordial no controle da doença relacionada ao HIV, tendo como principais objetivos: 1) diminuir a duração e gravidade das manifestações clínicas da doença aguda; 2) reduzir o número de células infectadas; 3) preservar a resposta imune HIV-específica; 4) reduzir o *set point* viral inicial, o qual pode afetar as taxas de progressão da doença; 5) reduzir a taxa de mutação viral como resultado da supressão da replicação viral; 6) diminuir as possibilidades de transmissão viral (MINISTÉRIO DA SAÚDE, 2006). Isso garante um controle, pelo menos momentâneo, da infecção viral e dos sintomas a ela relacionados. Infelizmente a cura ainda não foi alcançada; o tratamento atual apenas suprime ou contem a replicação viral, mas não é capaz de erradicar o vírus. A suspensão da TARV está relacionada com o retorno dos níveis prévios da viremia e o retorno da imunodepressão (ENGEL *et al*., 2004).

Atualmente cinco classes de fármacos são utilizadas contra o HIV. A primeira classe de fármacos antirretrovirais é a dos inibidores da transcriptase reversa análogos de nucleosídeos (ITRN). Estes medicamentos atuam incorporando-se a cadeia de DNA que o vírus cria através da enzima transcriptase reversa. Os ITRNs tornam essa cadeia de DNA defeituosa, impedindo que o vírus se reproduza. Outra classe de fármacos que atua no mesmo passo do ciclo vital do vírus, mas de modo diferente, é a dos inibidores da transcriptase reversa não análogos de nucleosídeos (ITRNN). Essa classe de fármacos bloqueia diretamente a ação da enzima. A terceira classe de

antirretrovirais é a dos inibidores da protease (IP). Estes fármacos impedem a produção de novas células infectadas com HIV. A quarta classe de fármacos compreende os inibidores de integrase que atuam impedindo a inserção do DNA viral no DNA do humano. Assim, impossibilita a replicação do vírus e sua capaciade de infectar novas células. Uma quinta e nova classe de antirretrovirais, ainda pouco utilizada, inclui os inibidores de fusão, que impedem a entrada do vírus na célula (ALCAMI, 2008).

A Figura 1 demonstra esquematicamente o ciclo reprodutivo do HIV e os processos que são possíveis alvos para os antirretrovirais.

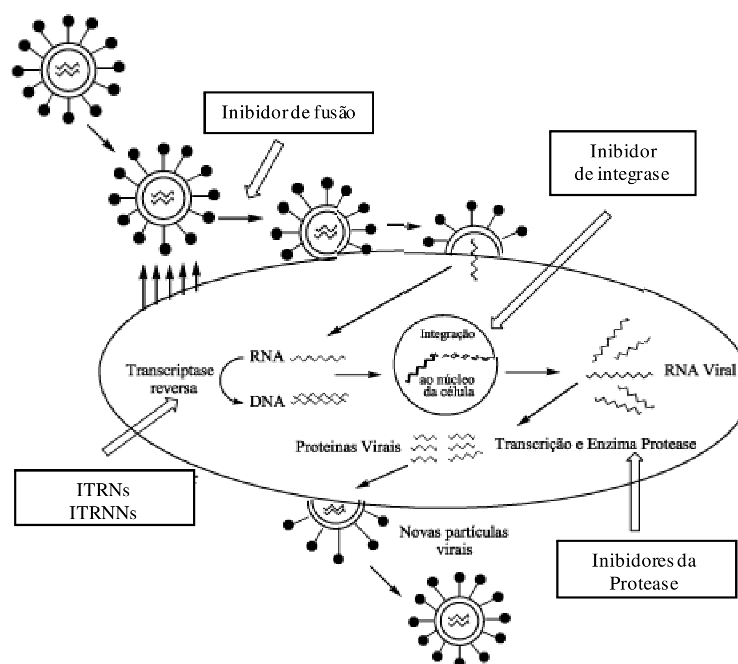


Figura 1: Esquema resumido do ciclo reprodutivo do HIV com os alvos dos fármacos antirretrovirais. Adaptado de DE SOUZA & DE ALMEIDA, 2003.

A Tabela 3 apresenta os representantes das diferentes classes de fármacos. A TARV inicial deve sempre incluir combinações de três fármacos: dois ITRNs associados a um ITRNN ou a um IP reforçado com ritonavir (IP/r).

Tabela 3: As diferentes classes de antirretovirais e seus representantes:

ITRN	Zidovudina (AZT), didanosina (ddI), estavudina (d4T), lamivudina (3TC), abacavir (ABC) e tenofovir (TDF)
ITRNN	Nevirapina (NVP) e efavirenz (EFV)
IP	Lopinavir (LPV), atazanavir (ATV), indinavir (IDV), nefinavir (NFV), ritonavir (RTV), saquinavir (SQV) e amprenavir (APV)
Inibidor de integrase	Raltegravir
Inibidores de fusão	Enfuvirtida (T-20)

Fonte: Ministério da Saúde. ITRN, inibidor da transcriptase reversa análogo de nucleosídeo; ITRNN, inibidor da transcriptase reversa não análogo de nucleosídeo; IP, inibidor da protease.

Apesar do avanço representado pela utilização da TARV, nem todos os pacientes respondem igualmente bem ao tratamento. Cerca de 10 a 20% dos pacientes que iniciam o tratamento com os esquemas atuais não conseguem suprimir a viremia de forma satisfatória após alguns meses de terapia e de 20 a 50% dos que apresentam boa resposta inicial apresentarão falha virológica após um ano de tratamento, sendo a taxa de resposta virológica aos tratamentos de resgate subsequentes progressivamente menor. Assim, o desenvolvimento de falha terapêutica, principalmente por resistência aos fármacos disponíveis, é um fenômeno esperado, e um número crescente de pacientes já se encontra sem opções terapêuticas. Além disso, o aparecimento de vários efeitos adversos significativos como neuropatias, hepatotoxicidade, pancreatite, lipodistrofia, diabetes, dislipidemias, osteoporose e acidose láctica, está entre as complicações associadas a TARV que podem piorar consideravelmente a qualidade de vida destes pacientes (MINISTÉRIO DA SAÚDE, 2006).

Numerosos esforços têm sido empregados na tentativa de melhorar os resultados clínicos da TARV. No entanto, dado o fato de que a infecção pelo HIV

permanece incurável, os fatores genéticos determinantes da variabilidade inter-individual na eficácia e toxicidade da TARV têm se tornado, mais recentemente, objetos de intensa pesquisa.

1.5 Lipodistrofia e dislipidemias

Conforme comentado anteriormente, dentre os efeitos adversos da TARV, a lipodistrofia (LD) e as dislipidemias destacam-se por sua alta prevalência. Estudos indicam que mais de 50% dos portadores de HIV sob TARV apresentam algum tipo de alteração na distribuição de tecido adiposo corporal (lipohipertrofia, lipoatrofia ou lipodistrofia mista) (SWEENEY *et al.*, 2007).

A lipodistrofia, um dos principais efeitos adversos da TARV, é caracterizada por alterações parciais ou generalizadas no desenvolvimento e/ou distribuição do tecido adiposo (perda de tecido adiposo subcutâneo nas extremidades e/ou acúmulo de tecido adiposo na região abdominal e dorsocervical). É geralmente acompanhada por anormalidades metabólicas como: resistência à insulina, hiperlipidemia e diabetes tipo 2 (CHEN *et al.*, 2002; CARR, 2003; VIGOUROUX *et al.*,2003). Pode ser classificada como: lipoatrofia, quando há perda de tecido adiposo subcutâneo nas extremidades e face; lipohipertrofia, quando ocorre acúmulo de tecido adiposo na região abdominal e dorsocervical; ou lipodistrofia mista, quando há lipoatrofia e lipohipertofia simultaneamente.

Segundo a Sociedade Brasileira de Cardiologia, as dislipidemias são caracterizadas por elevações nos níveis séricos de triglicerídeos, colesterol total e colesterol LDL, e/ou diminuição dos níveis de colesterol HDL. As dislipidemias podem ser classificadas, de forma laboratorial, conforme descrito na Tabela 4.

As dislipidemias são observadas em mais de 70% dos indivíduos tratados com antirretrovirais (CHEN *et al.*, 2002; SWEENEY *et al.*, 2007). Estas alterações estão associadas com aumento do risco de doenças cardiovasculares e diabetes tipo 2.

Tabela 4: Classificação laboratorial das dislipidemias

Hipercolesterolemia isolada	Elevação isolada do colesterol LDL (≥ 160 mg/dL)
Hipertrigliceridemia isolada	Elevação isolada dos triglicerídeos (≥ 150 mg/dL)
Hiperlipidemia mista	Valores aumentados de colesterol LDL e triglicerídeos. Nos casos com triglicerídeos ≥ 400 mg/dL, considera-se hiperlipidemia mista se o colesterol total ≥ 200 mg/dL
Colesterol HDL baixo	Redução do colesterol HDL isolada (homens <40 mg/dL e mulheres <50 mg/dL) ou em associação com aumento de colesterol LDL ou triglicerídeos

Fonte: IV Diretriz Brasileira Sobre Dislipidemias e Prevenção da Aterosclerose, Sociedade Brasileira de Cardiologia. LDL, lipoproteína de baixa densidade; HDL, lipoproteína de alta densidade.

1.6 Estudos farmacogenômicos da TARV

A farmacogênomica é um campo de pesquisa emergente, tendo como principal objetivo a análise das bases genéticas da variação interindividual na resposta aos medicamentos, dando embasamento para um tratamento individualizado.

Dentro da farmacologia, existem duas grandes áreas de pesquisa: farmacodinâmica, que descreve os efeitos farmacológicos de um princípio ativo no organismo (tanto os efeitos desejáveis quanto os indesejáveis); e farmacocinética, que descreve o curso de um fármaco e seus metabólitos,

compreendendo sua absorção, distribuição, metabolismo e eliminação. A farmacogenômica envolve o estudo da associação de variantes de genes que codificam proteínas e enzimas relacionadas a estas duas áreas da farmacologia com a resposta ao tratamento medicamentoso.

Dentro do enfoque farmacodinâmico, uma das primeiras investigações em indivíduos HIV positivos foi um estudo de associação entre polimorfismos no gene do fator de necrose tumoral alfa (TNFA) e a ocorrência de lipodistrofia em pacientes sob TARV (MAHER *et al.*, 2002). Os resultados deste estudo demonstraram que um polimorfismo na região promotora do gene TNFA (-238 G>A) estava associado com a ocorrência de lipodistrofia nos pacientes. Este tipo de polimorfismo, conhecido como SNP (do inglês "*single nucleotide polymorphism*"), é uma variação na seqüência de DNA que ocorre quando um único nucleotídeo (A, T, C ou G) difere entre membros de uma mesma espécie (ou entre cromossomos pareados em um mesmo indivíduo). Por exemplo, dois fragmentos de DNA sequenciados a partir de dois indivíduos, AAGC**C**TA e AAGC**T**TA, são diferentes em apenas um nucleotídeo. Neste caso, diz-se que há dois alelos: C e T. Os SNPs mais comuns possuem dois alelos. Dentro de uma população, SNPs podem ser designados como alelos de maior ou menor frequência, quando uma variante é mais comum que a outra. Para ser considerado um polimorfismo e não uma mutação, o alelo variante deve estar presente em, pelo menos, 1% da população estudada. É importante salientar que há variações entre populações diferentes, onde um alelo comum em determinado grupo étnico pode ser raro em outros (ALBERTS *et al.*, 2010).

O mecanismo responsável pela síndrome lipodistrófica ainda não foi totalmente esclarecido. Estudos *in vitro* demonstraram que os IPs podem inibir a diferenciação de pré-adipócitos em adipócitos maduros, modulando a expressão de fatores de transcrição como o SREBP-1c (proteína de ligação a elementos regulatórios de esteróis – *1c*) e o PPAR-γ (receptor ativado por proliferadores de peroxissoma – gama) (DOWELL *et al.*, 2000; CARON *et al.*, 2001; RUDICH *et al.*, 2001) e marcadores específicos de adipócitos (como a leptina, LEP, e a adiponectina, APM1) (PACENTI *et al*, 2006). Os ITRNs também alteram a expressão gênica de fatores de transcrição importantes para a adipogênese (PACENTI *et al*, 2006). No entanto, anormalidades metabólicas não ocorrem em todos os pacientes, apesar das similaridades demográficas, no regime terapêutico e nas respostas imunológicas e virológicas (RODRIGUEZ-NOVOA *et al.*, 2006). Dessa forma, polimorfismos nos genes envolvidos no metabolismo lipídico e na diferenciação de adipócitos poderiam explicar estas diferenças. Assim, genes codificadores de adipocinas (como *LEP* e *APM1*), do receptor da leptina (*LEPR*) e de fatores de transcrição (*SREBP-1c* e *PPARG*) tornam-se candidatos promissores na investigação etiológica da LD e das dislipidemias.

1.7. Genes de Adipocinas e TARV

Adipocinas são proteínas secretadas pelo tecido adiposo, com funções autócrinas, parácrinas e endócrinas (COSTA & DUARTE, 2006). Desempenham um papel importante na homeostase energética, sensibilidade à insulina, resposta imunológica e doença vascular (FANTUZZI, 2005), podendo ser agrupadas em adipocinas com função metabólica (adiponectina e resistina), imunológica

(interleucina-6 e TNF-α), cardiovascular (inibidor de ativação do plasminogênio tipo I) e endócrina (leptina) (PRINS, 2002).

Sabe-se que algumas adipocinas têm importante papel na homeostase de glicose, de lipídios e na sensibilidade à insulina, estando associadas com obesidade. A expressão dessas adipocinas pode ser influenciada por fatores de transcrição que agem na diferenciação dos pré-adipócitos em adipócitos, como o PPAR-γ e o SREBP-1c, o que pode levar a alterações na expressão dessas adipocinas, contribuindo para a ocorrência de alterações no tecido adiposo e obesidade (Figura 2).

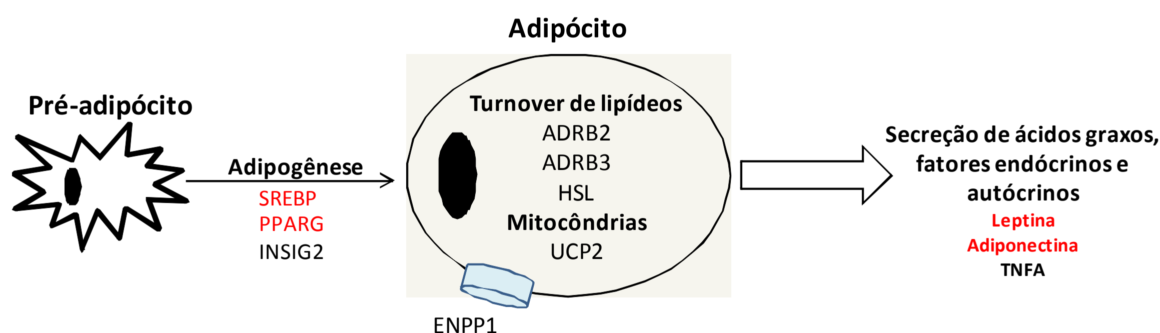


Figura 2: Genes que regulam a diferenciação e a função do tecido adiposo humano que podem predispor à obesidade. SREBP, proteína de ligação a elementos regulatórios de esteróis; PPARG, receptor ativado por proliferadores de peroxissoma gama; INSIG2, gene indutor de insulina 2; ENPP1, ectonucleotídeo pirofosfatase/fosfodiesterase; ADRB 2 e 3, adrenorreceptores beta 2 e 3; HSL, lipase hormônio-sensível; UCP 2, proteína desacopladora 2, TNFA, fator de necrose tumoral alfa. Adaptado de Dahlman & Arner, 2007.

1.7.1. Leptina

Leptina, a molécula protótipo da classe das adipocinas, apresenta níveis séricos proporcionais à quantidade de tecido adiposo corporal (MYNARCIK *et al.*, 2002). Produzida principalmente pelo tecido adiposo (também pode ser encontrada em pequenas quantidades no epitélio intestinal, placenta, leite

materno, músculo esquelético e cérebro), age regulando o gasto energético (via norepinefrina e lipólise no tecido adiposo) e a ingestão de alimentos (via hipotálamo e sistema límbico) (Figura 3). Mutações inativadoras do gene codificador da leptina (*LEP*) levam a hiperfagia e obesidade (FAROOQI, 2005). Existe uma correlação positiva dos níveis da leptina com o aumento dos níveis séricos de insulina, glicose e triglicerídeos, e uma correlação negativa com resistência à insulina (VIGOUROUX *et al.*, 2003).

Em indivíduos portadores do HIV com lipodistrofia os níveis séricos de leptina podem estar elevados (ocorrendo juntamente com lipohipertrofia) ou diminuídos (normalmente acompanhando lipoatrofia) (TSIODRAS & MANTZOROS, 2006), neste último caso havendo associação com resistência à insulina (NAGY *et al.*, 2003).

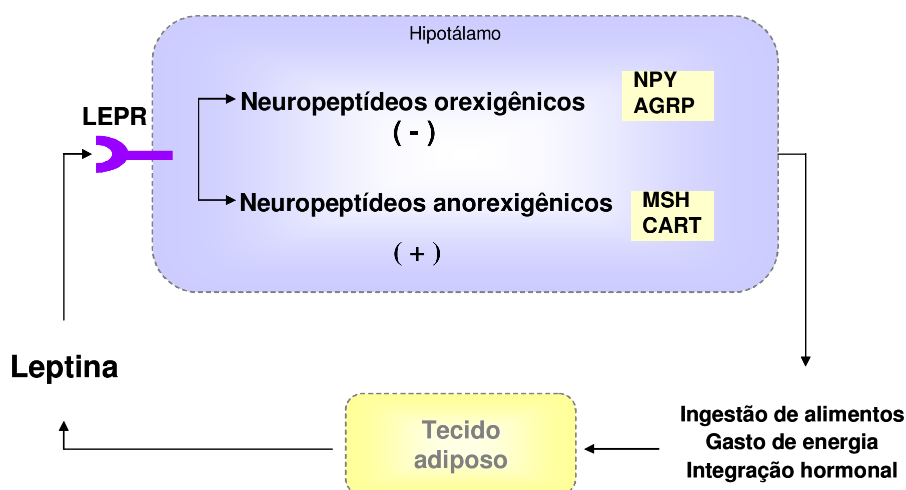


Figura 3: Liberação da leptina pelo tecido adiposo e seus efeitos na regulação de energia e na atividade neuroendócrina. LEPR, receptor de leptina; NPY, neuropeptídeo Y; AGRP, *agouti related protein*; MSH, hormônio estimulador de melanócito; CART, transcrito regulado por cocaína e anfetamina. Adaptado de Palou *et al.*, 2000.

Uma consulta, realizada em 31 de janeiro de 2010, na base de dados de SNPs mantida pelo *National Center for Biotechnology Information* (NCBI) dos Institutos Nacionais de Saúde dos Estados Unidos (disponível em http://www.ncbi.nlm.nih.gov/) revelou que 253 polimorfismos já foram identificados na região cromossômica que compreende o gene *LEP*, sendo que, destes, 12 estão citados na base de dados *Pubmed Medline*. O polimorfismo -2548G>A (rs7799039) na região promotora do gene foi associado com resposta à dieta de baixa caloria, obesidade e níveis de leptina (MAMMES *et al*., 2000; HOFFSTEDT *et al*., 2002; WANG *et al*., 2006). Em um extenso estudo sobre polimorfismos no gene da leptina e obesidade, variantes comuns a montante da região 5' têm mostrado associação com o índice de massa corporal (IMC) (DAHLMAN & ARNER, 2007).m

O receptor de leptina (LEP-R) é uma proteína transmembrana pertencente à superfamília dos receptores de citocinas classe I. A sequência de DNA para o LEP-R é codificada pelo gene *LEPR*, localizado no cromossomo 1 (1p31) (TARTAGLIA *et al*., 1995). Até o momento foram identificadas seis isoformas deste receptor (de LEP-Ra a LEP-Rf), geradas por *splicing* alternativo e processamento pós-traducional, porém apenas a isoforma longa LEP-Rb tem um domínio de sinalização intracelular, sendo a única capaz de originar a cascata de transdução de sinal. As outras isoformas parecem atuar como transportadores da leptina, sem consequente transdução do sinal (CHUNG *et al*., 1997). A sinalização intracelular induzida pela ligação da leptina ao LEP-Rb parece estar associada à ativação das vias JAK/STAT (*janus activated kinase/signal transducers and activators of transcription)*. Na ausência do hormônio, os

receptores apresentam-se como monômeros; quando a leptina se liga ao domínio extracelular do receptor, dois monômeros juntam-se formando um dímero (Figura 4) (DALLONGEVILLE *et al.*, 1998; FRUHBECK, 2006). O receptor da leptina é expresso no cérebro, principalmente no plexo coróide e em regiões hipotalâmicas como o núcleo arqueado, regiões paraventricular e ventromedial, que estão relacionadas com a regulação do balanço energético. Adicionalmente, o LEP-R é expresso em diversos órgãos periféricos, como tecido adiposo, pulmão, rins, fígado, pâncreas, músculo esquelético, células hematopoiéticas, no estômago e na placenta (KLOK *et al.*, 2007).

Já foram identificadas mutações no gene codificador do LEP-R que causam disfunção e/ou inativação dos receptores e levam à obesidade extrema. Entretanto, essas mutações são muito raras e não são causas comuns de obesidade em humanos (FAROOQI *et al.*, 2007). Por outro lado, 1650 polimorfismos foram identificados neste gene, sendo que 26 estão citados na base de dados *Pubmed Medline* (31 de janeiro de 2010). Uma variante comum no éxon 6 do gene codificador do receptor de leptina, Gln223Arg (rs1137101), foi associada com elevação do IMC em vários estudos (YIANNAKOURIS *et* al., 2001; PORTOLES *et al.*, 2006; DUARTE *et al.*, 2007), incluindo um realizado pelo presente grupo de pesquisa (MATTEVI *et al.*, 2002). Portanto, este polimorfismo pode estar de alguma forma relacionado com as modificações na formação e/ou distribuição do tecido adiposo apresentadas pelos indivíduos portadores do HIV sob TARV avaliados neste trabalho.

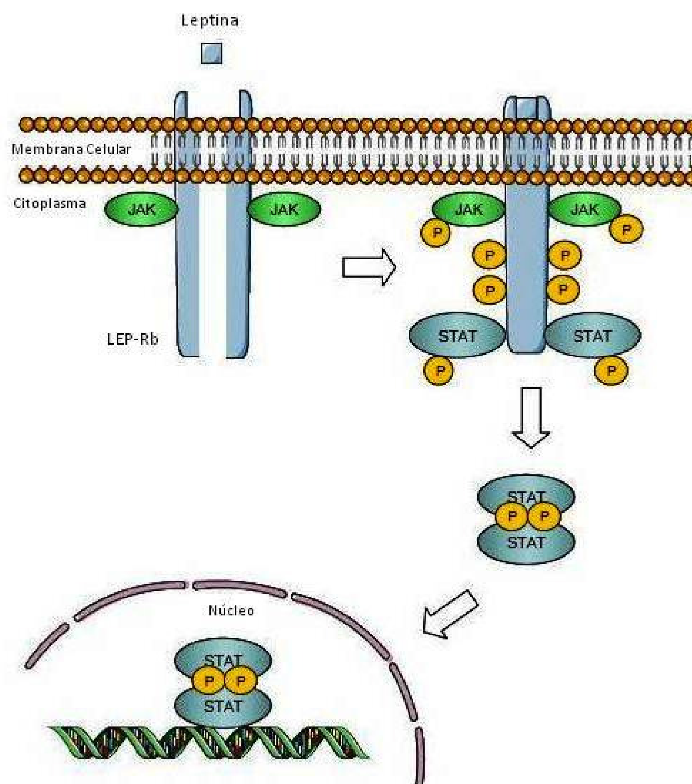


Figura 4: Efeito da ligação da leptina ao seu receptor. Quando a leptina se liga ao domínio extracelular do receptor LEP-Rb, dois monômeros juntam-se formando um dímero. A cada um dos domínios intracelulares está associada uma proteína quinase JAK2 na forma inativa. Com a dimerização do receptor duas JAKs2 são ativadas e fosforilam resíduos chave uma da outra. Após a ativação das JAKs, estas fosforilam os STATs e o próprio receptor. Duas proteínas STAT fosforiladas juntam-se formando dímeros que migram para o núcleo, ligando-se a locais específicos do DNA de forma a regular a expressão gênica. LEP-Rb, isoforma longa do receptor da leptina; P, fósforo. Retirado de Fruhbeck, 2006.

1.7.2. Adiponectina

A adiponectina, codificada pelo gene *APM1*, é uma adipocina produzida exclusivamente por adipócitos maduros (SCHERER *et al*., 1995). Tem como função a regulação sistêmica do metabolismo da glicose e de lipídeos (Figura 5). Seus níveis plasmáticos estão reduzidos na presença de obesidade, resistência à insulina (ARITA *et al*., 1999) e diabetes tipo 2 (HOTTA *et al*., 2000). Os níveis de adiponectina estão inversamente relacionados com o IMC, onde indivíduos obesos apresentam níveis de adiponectina reduzidos quando comparados com

indivíduos magros (ARITA *et al.*, 1999). Recentemente foi demonstrado que a expressão de adiponectina é maior no tecido adiposo subcutâneo do que no tecido adiposo visceral (FAIN *et al.*, 2004), o que pode explicar a ocorrência de hipoadiponectinemia em indivíduos com lipodistrofia. Além disso, análises funcionais revelaram que a adiponectina possui potencial antiinflamatório (WOLF *et* al., 2004; YAMAGUCHI *et al.*, 2005; NEUMEIER *et al.*, 2006) e anti-aterogênico (OKAMOTO *et al.*, 2000; OUCHI *et al.*, 2001).

A adiponectina sinaliza através da ligação aos receptores 1 (ADIPOR-1) e 2 (ADIPOR-2) (KOERNER *et al.*, 2005). ADIPOR-1 é um receptor comum no tecido adiposo subcutâneo, e sua expressão é diminuída em obesos (RASMUSSEN *et al.*, 2006). Polimorfismos nos receptores ADIPOR-1 e ADIPOR-2 já foram associados com diabetes tipo 2, mas não com obesidade (DAMCOTT *et al.*, 2005; KANTARTZIS *et al.*, 2006).

A expressão de *APM1* é reduzida em indivíduos obesos e em estados insulino-resistentes, mas eleva-se após perda de peso corporal (KOERNER *et al.*, 2005). Vários estudos têm analisado a associação entre polimorfismos comuns no gene *APM1* e obesidade. Foram identificados 257 polimorfismos na região do gene *APM1*, sendo que 17 estão citados no *Pubmed Medline* (31 de janeiro de 2010). Variantes como *+45 T>G* e *+ 276 G>C* têm sido associados com níveis circulantes de adiponectina, obesidade e diabetes tipo 2, mas com resultados inconsistentes entre diferentes coortes (GABLE *et al.*, 2006).

Polimorfismos na região promotora do gene da adiponectina (*APM1* -11377 G>C, rs266729, e *APM1* -11391 G>A, rs17300539) foram associados com modificações nos níveis circulantes de seus produtos (POITOU *et al.*, 2005),

sendo possível sua associação com as alterações desenvolvidas pelos indivíduos HIV positivos sob terapia antirretroviral.

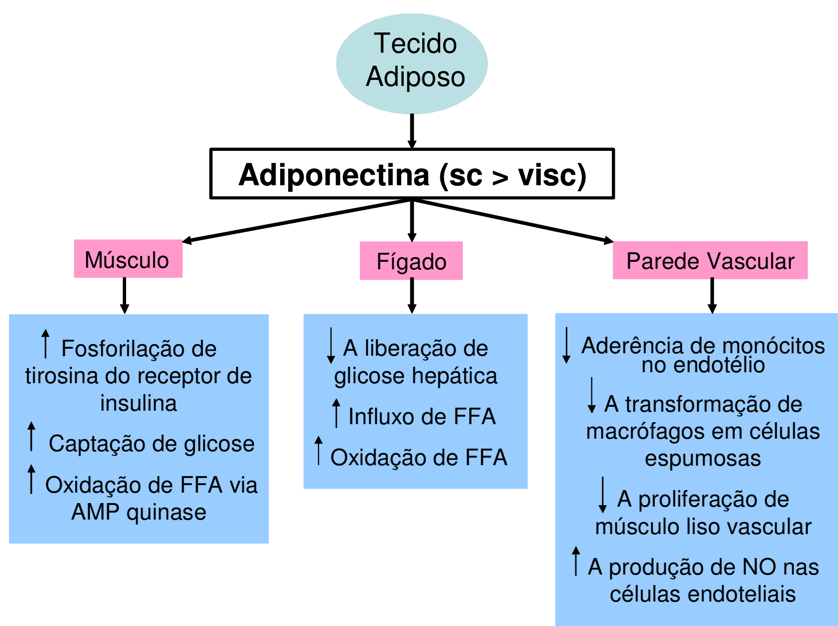


Figura 5: Ações da adiponectina no metabolismo da glicose e de lipídeos. sc, tecido adiposo subcutâneo; visc, tecido adiposo visceral; FFA, ácidos graxos livres; AMP, adenosina monofosfato; NO, óxido nítrico. Adaptado de Lacquemant *et al.*, 2003.

1.7.3. PPAR-γ

Os PPARs (receptores ativados por proliferadores de peroxissoma) são fatores de transcrição da família de receptores nucleares, caracterizados por seu padrão de distribuição nos tecidos e por sua função metabólica. São três proteínas, codificadas por genes distintos: *PPARα, PPARδ e PPARγ* (WALCZAK & TONTONOZ, 2002). O PPARγ possui três isoformas: PPARγ 1, PPARγ 2 e PPARγ 3. O PPARγ 1 é expresso em uma ampla variedade de tecidos (cardíaco, intestinal, renal, pancreático, esplênico). O PPARγ 2 é expresso exclusivamente

no tecido adiposo e o PPARγ 3 tem expressão restrita a macrófagos e intestino grosso (vide revisão em TAVARES, 2007).

O PPARγ 2 é necessário e suficiente para diferenciar adipócitos. Nos adipócitos, o PPARγ regula a expressão de numerosos genes envolvidos no metabolismo de lipídeos (TAVARES, 2007). Mutações raras no gene *PPARG*, que codifica o PPARγ, foram descritas em oito indivíduos que apresentavam um complexo fenótipo com lipodistrofia parcial, resistência à insulina severa e precoce, diabetes tipo 2, dislipidemia (triglicerídeos elevados e colesterol HDL diminuído), hipertensão arterial precoce e esteatose hepática, indicando que o PPARγ é essencial para a ação da insulina, homeostase da glicose e controle da massa adiposa (MEIRHAEGHE & AMOUYEL, 2004).

Segundo a base de dados do NCBI existem 1397 polimorfismos identificados na região do gene *PPARG*. Destes, 33 estão citados no *Pubmed Medline* (31 de janeiro de 2010). Um polimorfismo relativamente comum localizado na porção amino-terminal da isoforma PPARγ-2, substituindo a prolina por alanina no códon 12 (Pro12Ala, rs1801282) (YEN *et al.*, 1997) tem sido relacionado com resistência à insulina, diabetes tipo 2 e obesidade, entre outras patologias. Estudos analisando o efeito do alelo Ala sobre diabetes tipo 2 e melhora na resistência à insulina têm obtido resultados contraditórios. Enquanto alguns resultados indicam um efeito protetor do alelo Ala (HARA *et al.*, 2000; MORI *et al.*, 2001; POULSEN *et al.*, 2003) outros apresentam um efeito deletério (EVANS *et al.*, 2001; LINDI *et al.*, 2002) ou nenhuma influência deste polimorfismo sobre a ocorrência de diabetes (ZIETZ *et al.*, 2002; MULLER *et al.*, 2003; MALECKI *et al.*, 2003). Com relação ao IMC, estudos iniciais sugeriram

haver um efeito protetor do alelo Ala, reduzindo o IMC (PIHLAJAMAKI *et al.*, 2000; LUAN *et al.*, 2001; DONEY *et al.*, 2002; FRANKS *et al.*, 2004). Porém alguns estudos não confirmam estes achados (CLEMENT *et al.*, 2000; FREDERIKSEN *et al.*, 2002; GURNELL *et al.*, 2003; CARAMORI *et* al., 2003), enquanto outros sugerem um efeito contrário (GONZELEZ SANCHEZ *et al.*, 2002; ROBITAILLE *et al.*, 2003; MIRZAEI *et al.*, 2009). Um estudo realizado pelo presente grupo apontou em efeito gênero específico em indivíduos da população em geral, onde homens portadores do alelo Ala apresentaram médias de IMC superiores as dos portadores do alelo Pro, não ocorrendo diferenças entre o IMC de mulheres portadoras de um ou outro alelo (MATTEVI *et al.*, 2007). De acordo com uma meta-análise realizada com dados para cerca de 19.000 indivíduos, o polimorfismo Pro12Ala está associado com maior IMC em indivíduos com sobrepeso e/ou obesos (IMC > 27 kg/m^2) (MASUD & YE, 2003). Os efeitos díspares do polimorfismo Pro12Ala no IMC em indivíduos obesos e magros sugerem que esta variante genética interage com outros fatores. Considerando que o *PPARG* promove armazenamento de lipídeos, é surpreendente que o alelo Ala esteja associado à obesidade, já que apresenta reduzida ligação e transcrição de DNA *in vitro* (DEEB *et al.*, 1998) Várias explicações para estes resultados aparentemente contraditórios para o alelo Ala são possíveis: pode haver compensação ou diferentes vias pelas quais o *PPARG* regula a obesidade, ou o alelo Ala poderia estar em forte desequilíbrio de ligação com outro alelo predisponente à obesidade. Outra hipótese é que o alelo Pro seria protetor contra efeitos de outros genes que predispõem à obesidade. *PPARG* C1431T é um polimorfismo silencioso na região codificadora, exibindo estreito desequilíbrio de

ligação com Pro12Ala (GURNELL *et al.*, 2005). O alelo T tem sido associado a um aumento do IMC, mas não está claro se a associação é independente do Pro12Ala (CECIL *et al.*, 2006).

Em indivíduos portadores do HIV, o polimorfismo *PPARG* C1431T parece não estar relacionado com a ocorrência de LD (ZANONE POMA *et al.*, 2008). Outro estudo avaliando os efeitos do *PPARG* Pro12Ala no risco de desenvolvimento de lipodistrofia em pacientes sob TARV demonstrou que portadores do Ala apresentavam níveis plasmáticos de colesterol total e colesterol LDL mais elevados que os homozigotos Pro/Pro, porém, parece não haver associação deste polimorfismo com a ocorrência de LD (SAUMOY *et al.*, 2009).

Resultados tão contraditórios podem ser explicados devido a diferenças entre as populações estudadas e/ou devido ao número de participantes em cada estudo. Mesmo assim, a importância do PPARγ para a adipogênese é evidente, o que o mantém como um bom candidato para estudo em indivíduos portadores do HIV com lipodistrofia.

1.7.4 SREBP-1c

Os SREBPs (*sterol regulatory element binding proteins ou* proteínas de ligação a elementos regulatórios de esteróis) são uma família de fatores de transcrição responsável pela síntese de colesterol e ácidos graxos, triglicerídeos e fosfolipídeos em células humanas (EBERLE *et al.*, 2004b). Três isoformas estão descritas em muitas espécies de mamíferos: SREBP-1a e 1c que são produzidas a partir do mesmo gene (*SREBP1*, através de promotores específicos e *splicing* alternativo) localizado no cromossomo 17p11.2 (HUA *et al.*, 1995) e SREBP-2,

produzido por um gene diferente (*SREBP2*), localizado no cromossomo 22q13 (MISEREZ *et al.*, 1997).

A isoforma 1c é encontrada principalmente no fígado, músculo e tecido adiposo, onde é responsável pela mediação dos efeitos transcricionais da insulina em genes codificadores de enzimas envolvidas na glicólise, lipogênese e gliconeogênese (FOUFELLE & FERRE, 2002; EBERLE *et al.*, 2004a). SREBP-1c é uma peça importante na diferenciação de adipócitos, ativando a transcrição de PPARγ (FAJAS *et al.*, 1999).

Até 31 de janeiro de 2010, 253 SNPs foram identificados no gene *SREBP1*, sendo que apenas três estão citados no *Pubmed Medline*. Estudos demonstraram que o polimorfismo Gly952Gly (3322 C>G, rs2297508) no éxon 18c está associado com obesidade, diabetes e prevalência de nefropatia em alguns estudos (EBERLE *et al.*, 2004a; FELDER *et al.*, 2007), nos quais o alelo G foi mais frequente em pacientes obesos, diabéticos (independentemente de obesidade) e em pacientes com nefropatia. Por outro lado, o alelo C deste mesmo polimorfismo foi também associado com mudanças nos níveis lipídicos em homens (LAAKSONEN *et al.*, 2006).

Um estudo realizado com indivíduos HIV positivos sob TARV (MISEREZ *et al.*, 2001) demonstrou que o polimorfismo *SREBP-1c* 3322C>G estava associado com hiperlipoproteinemia. Neste estudo, aumento nos níveis plasmáticos de colesterol foram mais frequentes em portadores do alelo C.

1.8 Justificativa e objetivos

O advento da TARV permitiu a redução da carga viral do HIV para níveis não-detectáveis, modificando a percepção da AIDS, de uma doença fatal para uma condição crônica. A utilização da terapia combinada em regimes triplos reduziu drasticamente a morbi/mortalidade entre os pacientes infectados pelo HIV. No Brasil, a ampla distribuição de antirretrovirais também demonstra resultados altamente positivos. Apesar dos aspectos positivos da terapia, o aparecimento de efeitos adversos vem comprometendo a adesão ao tratamento e a qualidade de vida dos pacientes. Dentre os principais efeitos adversos da TARV, a lipodistrofia e a dislipidemia destacam-se devido a sua alta prevalência, ocorrendo em mais de 50% dos pacientes em tratamento.

A etiologia da síndrome lipodistrófica ainda não está totalmente esclarecida. A modulação da expressão de fatores de transcrição, como o PPAR-γ e o SREBP-1c, e de marcadores específicos de adipócitos (como a leptina e a adiponectina) por IPs e ITRNs parece estar envolvida. No entanto, essas alterações metabólicas e do tecido adiposo não ocorrem em todos os pacientes expostos a TARV, indício de que possa haver o envolvimento de fatores genéticos na ocorrência destes efeitos adversos.

Dessa forma, polimorfismos nos genes codificadores da adiponectina (*APM1* -11377 G>C e *APM1* -11391 G>A), da leptina (*LEP* -2548 G>A), do receptor da leptina (*LEPR* Gln223Arg) e dos fatores de transcrição PPAR-γ (*PPARG* Pro12Ala) e SREBP-1c (*SREBP-1c* 3322C>G) tornam-se candidatos plausíveis para a investigação etiológica da LD e dislipidemia.

Outro aspecto importante é que os estudos farmacogenômicos da TARV são ainda pouco numerosos, embora esta área de pesquisa seja extremamente promissora. Com a identificação de possíveis marcadores genéticos, as variações nas reações adversas e na resposta aos fármacos poderão ser esclarecidas, disponibilizando uma ferramenta de avaliação útil para a seleção de fármacos e o desenvolvimento de tratamentos personalizados, aumentando a segurança e eficácia das terapias existentes.

Dado o exposto, os objetivos específicos deste trabalho são:

1. Determinar as frequências dos polimorfismos *LEP* -2548 G>A, *LEPR* Gln223Arg, *APM1* -11377 C>G e -11391 G>A, *PPARG* Pro12Ala e *SREBP-1c* 3322C>G em pacientes HIV positivos sob terapia antirretroviral;

2. Verificar a associação desses polimorfismos com a ocorrência de lipodistrofia nestes pacientes;

3. Correlacionar os genótipos destes pacientes para os referidos polimorfismos com fenótipos relacionados a síndrome lipodistrófica, como perfil lipídico, medidas antropométricas e níveis das adipocinas leptina e adiponectina.

1.9 Referências bibliográficas

ALBERTS, B., *et al.* **Biologia Molecular da Célula**. Tradução de A. L. VANZ, et al. 5ª. ed. Porto Alegre: Artmed, 2010, 1268 p.

ALCAMI, J. [The HIV replication cycle. Established therapeutic targets and potential targets]. **Enferm Infecc Microbiol Clin**, v. 26 Suppl 12, p. 3-10, Nov 2008.

ARITA, Y., *et al.* Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. **Biochem Biophys Res Commun**, v. 257, n. 1, p. 79-83, Apr 2 1999.

BARRE-SINOUSSI, F., *et al.* Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). **Science**, v. 220, n. 4599, p. 868-871, May 20 1983.

Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Programa Nacional de DST/AIDS. Critérios para definição de casos de AIDS em adultos e crianças. Brasília (DF): 2003 Avaible online at: http://www.aids.gov.br/data/documents/storedDocuments/%7BB8EF5DAF-23AE-4891-AD36-1903553A3174%7D/%7B6C99F8D6-CC7B-4D09-BECD-1AA2C7E7B587%7D/criterios_aids_2004.pdf (accessed 28 de janeiro 2010).

Brasil. Ministério da Saúde. Coordenação Nacional DST/AIDS. Guia de tratamento: recomendações para terapia antirretroviral em adultos e adolescentes infectados pelo HIV. Brasília (DF): 2006 Avaible online at: www.aids.gov.br/final/tratamento/adulto.pdf (accessed 28 de janeiro de 2010).

CARAMORI, M. L., *et al.* The human peroxisome proliferator-activated receptor gamma2 (PPARgamma2) Pro12Ala polymorphism is associated with decreased risk of diabetic nephropathy in patients with type 2 diabetes. **Diabetes**, v. 52, n. 12, p. 3010-3013, Dec 2003.

CARON, M., *et al.* The HIV protease inhibitor indinavir impairs sterol regulatory element-binding protein-1 intranuclear localization, inhibits preadipocyte differentiation, and induces insulin resistance. **Diabetes**, v. 50, n. 6, p. 1378-1388, Jun 2001.

CARR, A. HIV lipodystrophy: risk factors, pathogenesis, diagnosis and management. **AIDS**, v. 17 Suppl 1, p. S141-148, Apr 2003.

CECIL, J. E., *et al.* Energy balance and food intake: the role of PPARgamma gene polymorphisms. **Physiol Behav**, v. 88, n. 3, p. 227-233, Jun 30 2006.

CHEN, D., *et al.* Clinical review 153: Lipodystrophy in human immunodeficiency virus-infected patients. **J Clin Endocrinol Metab**, v. 87, n. 11, p. 4845-4856, Nov 2002.

CHEN, D.*, et al.* Clinical review 153: Lipodystrophy in human immunodeficiency virus-infected patients. **J Clin Endocrinol Metab**, v. 87, n. 11, p. 4845-4856, Nov 2002.

CHUNG, W. K.*, et al.* Exonic and intronic sequence variation in the human leptin receptor gene (LEPR). **Diabetes**, v. 46, n. 9, p. 1509-1511, Sep 1997.

CLEMENT, K.*, et al.* The Pro115Gln and Pro12Ala PPAR gamma gene mutations in obesity and type 2 diabetes. **Int J Obes Relat Metab Disord**, v. 24, n. 3, p. 391-393, Mar 2000.

COSTA, J. V. and DUARTE, J. S. [Adipose tissue and adipokines]. **Acta Med Port**, v. 19, n. 3, p. 251-256, May-Jun 2006.

DAHLMAN, I. and ARNER, P. Obesity and polymorphisms in genes regulating human adipose tissue. **Int J Obes (Lond)**, v. 31, n. 11, p. 1629-1641, Nov 2007.

DALLONGEVILLE, J.*, et al.* Leptin, a pleiotropic hormone: physiology, pharmacology, and strategies for discovery of leptin modulators. **J Med Chem**, v. 41, n. 27, p. 5337-5352, Dec 31 1998.

DAMCOTT, C. M.*, et al.* Genetic variation in adiponectin receptor 1 and adiponectin receptor 2 is associated with type 2 diabetes in the Old Order Amish. **Diabetes**, v. 54, n. 7, p. 2245-2250, Jul 2005.

DE SOUZA, M. V. and DE ALMEIDA, M. V. Drogas anti-HIV: passado, presente e perspectivas futuras. **Quimica Nova**, v. 26, n. 3, p. 366-372, May 2003.

DEEB, S. S.*, et al.* A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. **Nat Genet**, v. 20, n. 3, p. 284-287, Nov 1998.

DONEY, A.*, et al.* Haplotype analysis of the PPARgamma Pro12Ala and C1431T variants reveals opposing associations with body weight. **BMC Genet**, v. 3, p. 21, Nov 13 2002.

DOWELL, P.*, et al.* Suppression of preadipocyte differentiation and promotion of adipocyte death by HIV protease inhibitors. **J Biol Chem**, v. 275, n. 52, p. 41325-41332, Dec 29 2000.

DRAGIC, T.*, et al.* HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. **Nature**, v. 381, n. 6584, p. 667-673, Jun 20 1996.

DUARTE, S. F.*, et al.* LEPR p.Q223R, beta3-AR p.W64R and LEP c.-2548G>A gene variants in obese Brazilian subjects. **Genet Mol Res**, v. 6, n. 4, p. 1035-1043, 2007.

EBERLE, D.*, et al.* SREBF-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. **Diabetes**, v. 53, n. 8, p. 2153-2157, Aug 2004a.

EBERLE, D.*, et al.* SREBP transcription factors: master regulators of lipid homeostasis. **Biochimie**, v. 86, n. 11, p. 839-848, Nov 2004b.

ENGEL, C.*, et al.* **Infectologia - Síndrome da Imunodeficiência Adquirida**. Rio de Janeiro: Editora Frattari, 2004, 107 p.

EVANS, D.*, et al.* Association between the P12A and c1431t polymorphisms in the peroxisome proliferator activated receptor gamma (PPAR gamma) gene and type 2 diabetes. **Exp Clin Endocrinol Diabetes**, v. 109, n. 3, p. 151-154, 2001.

FAIN, J. N.*, et al.* Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. **Endocrinology**, v. 145, n. 5, p. 2273-2282, May 2004.

FAJAS, L.*, et al.* Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. **Mol Cell Biol**, v. 19, n. 8, p. 5495-5503, Aug 1999.

FANTUZZI, G. Adipose tissue, adipokines, and inflammation. **J Allergy Clin Immunol**, v. 115, n. 5, p. 911-919; May 2005.

FAROOQI, I. S. Genetic and hereditary aspects of childhood obesity. **Best Pract Res Clin Endocrinol Metab**, v. 19, n. 3, p. 359-374, Sep 2005.

FAROOQI, I. S.*, et al.* Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. **N Engl J Med**, v. 356, n. 3, p. 237-247, Jan 18 2007.

FELDER, T. K.*, et al.* The SREBF-1 locus is associated with type 2 diabetes and plasma adiponectin levels in a middle-aged Austrian population. **Int J Obes (Lond)**, v. 31, n. 7, p. 1099-1103, Jul 2007.

FENG, Y.*, et al.* HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. **Science**, v. 272, n. 5263, p. 872-877, May 10 1996.

FORD, E. S.*, et al.* Immunopathogenesis of asymptomatic chronic HIV Infection: the calm before the storm. **Curr Opin HIV AIDS**, v. 4, n. 3, p. 206-214, May 2009.

FOUFELLE, F. and FERRE, P. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription

factor sterol regulatory element binding protein-1c. **Biochem J**, v. 366, n. Pt 2, p. 377-391, Sep 1 2002.

FRANKS, P. W.*, et al.* Does peroxisome proliferator-activated receptor gamma genotype (Pro12ala) modify the association of physical activity and dietary fat with fasting insulin level? **Metabolism**, v. 53, n. 1, p. 11-16, Jan 2004.

FREDERIKSEN, L.*, et al.* Comment: studies of the Pro12Ala polymorphism of the PPAR-gamma gene in the Danish MONICA cohort: homozygosity of the Ala allele confers a decreased risk of the insulin resistance syndrome. **J Clin Endocrinol Metab**, v. 87, n. 8, p. 3989-3992, Aug 2002.

FRUHBECK, G. Intracellular signalling pathways activated by leptin. **Biochem J**, v. 393, n. Pt 1, p. 7-20, Jan 1 2006.

GABLE, D. R.*, et al.* Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic syndrome and cardiovascular disease. **Atherosclerosis**, v. 188, n. 2, p. 231-244, Oct 2006.

GONZALEZ SANCHEZ, J. L.*, et al.* Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma-2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population. **Eur J Endocrinol**, v. 147, n. 4, p. 495-501, Oct 2002.

GURNELL, M. Peroxisome proliferator-activated receptor gamma and the regulation of adipocyte function: lessons from human genetic studies. **Best Pract Res Clin Endocrinol Metab**, v. 19, n. 4, p. 501-523, Dec 2005.

GURNELL, M.*, et al.* The metabolic syndrome: peroxisome proliferator-activated receptor gamma and its therapeutic modulation. **J Clin Endocrinol Metab**, v. 88, n. 6, p. 2412-2421, Jun 2003.

HARA, K.*, et al.* The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. **Biochem Biophys Res Commun**, v. 271, n. 1, p. 212-216, Apr 29 2000.

HOFFSTEDT, J.*, et al.* A polymorphism in the leptin promoter region (-2548 G/A) influences gene expression and adipose tissue secretion of leptin. **Horm Metab Res**, v. 34, n. 7, p. 355-359, Jul 2002.

HOTTA, K.*, et al.* Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. **Arterioscler Thromb Vasc Biol**, v. 20, n. 6, p. 1595-1599, Jun 2000.

HUA, X.*, et al.* Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11.2 and 22q13. **Genomics**, v. 25, n. 3, p. 667-673, Feb 10 1995.

KAHN, J. O. and WALKER, B. D. Acute human immunodeficiency virus type 1 infection. **N Engl J Med**, v. 339, n. 1, p. 33-39, Jul 2 1998.

KANTARTZIS, K.*, et al.* The -8503 G/A polymorphism of the adiponectin receptor 1 gene is associated with insulin sensitivity dependent on adiposity. **Diabetes Care**, v. 29, n. 2, p. 464, Feb 2006.

KLOK, M. D.*, et al.* The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. **Obes Rev**, v. 8, n. 1, p. 21-34, Jan 2007.

KOERNER, A.*, et al.* Adipocytokines: leptin--the classical, resistin--the controversical, adiponectin--the promising, and more to come. **Best Pract Res Clin Endocrinol Metab**, v. 19, n. 4, p. 525-546, Dec 2005.

LAAKSONEN, R.*, et al.* Genetic variant of the SREBF-1 gene is significantly related to cholesterol synthesis in man. **Atherosclerosis**, v. 185, n. 1, p. 206-209, Mar 2006.

LACQUEMANT, C.*, et al.* [Adipocytokins, obesity and development of type 2 diabetes]. **Med Sci (Paris)**, v. 19, n. 8-9, p. 809-817, Aug-Sep 2003.

LINDI, V. I.*, et al.* Association of the Pro12Ala polymorphism in the PPAR-gamma2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. **Diabetes**, v. 51, n. 8, p. 2581-2586, Aug 2002.

LUAN, J. A.*, et al.* Sample size determination for studies of gene-environment interaction. **Int J Epidemiol**, v. 30, n. 5, p. 1035-1040, Oct 2001.

MAHER, B.*, et al.* TNF-alpha promoter region gene polymorphisms in HIV-positive patients with lipodystrophy. **AIDS**, v. 16, n. 15, p. 2013-2018, Oct 18 2002.

MALECKI, M. T.*, et al.* The Pro12Ala polymorphism of PPARgamma2 gene and susceptibility to type 2 diabetes mellitus in a Polish population. **Diabetes Res Clin Pract**, v. 62, n. 2, p. 105-111, Nov 2003.

MAMMES, O.*, et al.* Association of the G-2548A polymorphism in the 5' region of the LEP gene with overweight. **Ann Hum Genet**, v. 64, n. Pt 5, p. 391-394, Sep 2000.

MASUD, S. and YE, S. Effect of the peroxisome proliferator activated receptor-gamma gene Pro12Ala variant on body mass index: a meta-analysis. **J Med Genet**, v. 40, n. 10, p. 773-780, Oct 2003.

MATTEVI, V. S.*, et al.* Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. **Int J Obes Relat Metab Disord**, v. 26, n. 9, p. 1179-1185, Sep 2002.

MATTEVI, V. S., *et al.* Effects of a PPARG gene variant on obesity characteristics in Brazil. **Braz J Med Biol Res**, v. 40, n. 7, p. 927-932, Jul 2007.

MEIRHAEGHE, A. and AMOUYEL, P. Impact of genetic variation of PPARgamma in humans. **Mol Genet Metab**, v. 83, n. 1-2, p. 93-102, Sep-Oct 2004.

MIRZAEI, H., *et al.* Polymorphism of Pro12Ala in the Peroxisome Proliferator-Activated Receptor gamma2 Gene in Iranian Diabetic and Obese Subjects. **Metab Syndr Relat Disord**, Jun 26 2009.

MISEREZ, A. R., *et al.* Structure of the human gene encoding sterol regulatory element binding protein 2 (SREBF2). **Genomics**, v. 40, n. 1, p. 31-40, Feb 15 1997.

MISEREZ, A. R., *et al.* A single-nucleotide polymorphism in the sterol-regulatory element-binding protein 1c gene is predictive of HIV-related hyperlipoproteinaemia. **AIDS**, v. 15, n. 15, p. 2045-2049, Oct 19 2001.

MORI, H., *et al.* The Pro12 -->Ala substitution in PPAR-gamma is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. **Diabetes**, v. 50, n. 4, p. 891-894, Apr 2001.

MULLER, Y. L., *et al.* A functional variant in the peroxisome proliferator-activated receptor gamma2 promoter is associated with predictors of obesity and type 2 diabetes in Pima Indians. **Diabetes**, v. 52, n. 7, p. 1864-1871, Jul 2003.

MYNARCIK, D. C., *et al.* Adiponectin and leptin levels in HIV-infected subjects with insulin resistance and body fat redistribution. **J Acquir Immune Defic Syndr**, v. 31, n. 5, p. 514-520, Dec 15 2002.

NAGY, G. S., *et al.* Human immunodeficiency virus type 1-related lipoatrophy and lipohypertrophy are associated with serum concentrations of leptin. **Clin Infect Dis**, v. 36, n. 6, p. 795-802, Mar 15 2003.

NEUMEIER, M., *et al.* Different effects of adiponectin isoforms in human monocytic cells. **J Leukoc Biol**, v. 79, n. 4, p. 803-808, Apr 2006.

OKAMOTO, Y., *et al.* An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. **Horm Metab Res**, v. 32, n. 2, p. 47-50, Feb 2000.

OUCHI, N., *et al.* Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. **Circulation**, v. 103, n. 8, p. 1057-1063, Feb 27 2001.

PACENTI, M., *et al.* Microarray analysis during adipogenesis identifies new genes altered by antiretroviral drugs. **AIDS**, v. 20, n. 13, p. 1691-1705, Aug 22 2006.

PALOU, A., *et al.* Obesity: molecular bases of a multifactorial problem. **Eur J Nutr**, v. 39, n. 4, p. 127-144, Aug 2000.

PIHLAJAMAKI, J., *et al.* The Pro12A1a substitution in the peroxisome proliferator activated receptor gamma 2 is associated with an insulin-sensitive phenotype in families with familial combined hyperlipidemia and in nondiabetic elderly subjects with dyslipidemia. **Atherosclerosis**, v. 151, n. 2, p. 567-574, Aug 2000.

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. **Obes Surg**, v. 15, n. 1, p. 11-23, Jan 2005.

PORTOLES, O., *et al.* Effect of genetic variation in the leptin gene promoter and the leptin receptor gene on obesity risk in a population-based case-control study in Spain. **Eur J Epidemiol**, v. 21, n. 8, p. 605-612, 2006.

POULSEN, P., *et al.* Impact of two common polymorphisms in the PPARgamma gene on glucose tolerance and plasma insulin profiles in monozygotic and dizygotic twins: thrifty genotype, thrifty phenotype, or both? **Diabetes**, v. 52, n. 1, p. 194-198, Jan 2003.

PRINS, J. B. Adipose tissue as an endocrine organ. **Best Pract Res Clin Endocrinol Metab**, v. 16, n. 4, p. 639-651, Dec 2002.

RASMUSSEN, M. S., *et al.* Adiponectin receptors in human adipose tissue: effects of obesity, weight loss, and fat depots. **Obesity (Silver Spring)**, v. 14, n. 1, p. 28-35, Jan 2006.

ROBITAILLE, J., *et al.* The PPAR-gamma P12A polymorphism modulates the relationship between dietary fat intake and components of the metabolic syndrome: results from the Quebec Family Study. **Clin Genet**, v. 63, n. 2, p. 109-116, Feb 2003.

RODRIGUEZ-NOVOA, S., *et al.* Overview of the pharmacogenetics of HIV therapy. **Pharmacogenomics J**, v. 6, n. 4, p. 234-245, Jul-Aug 2006.

RUDICH, A., *et al.* The HIV protease inhibitor nelfinavir induces insulin resistance and increases basal lipolysis in 3T3-L1 adipocytes. **Diabetes**, v. 50, n. 6, p. 1425-1431, Jun 2001.

SAUMOY, M., *et al.* PPARgamma Pro12Ala Polymorphism in HIV-1-infected Patients with HAART-related Lipodystrophy. **Curr HIV Res**, Sep 1 2009.

SCHERER, P. E., *et al.* A novel serum protein similar to C1q, produced exclusively in adipocytes. **J Biol Chem**, v. 270, n. 45, p. 26746-26749, Nov 10 1995.

STEKLER, J. and COLLIER, A. C. Primary HIV Infection. **Curr HIV/AIDS Rep**, v. 1, n. 2, p. 68-73, Jun 2004.

SWEENEY, L. L., *et al.* The role of adipokines in relation to HIV lipodystrophy. **AIDS**, v. 21, n. 8, p. 895-904, May 11 2007.

TARTAGLIA, L. A., *et al.* Identification and expression cloning of a leptin receptor, OB-R. **Cell**, v. 83, n. 7, p. 1263-1271, Dec 29 1995.

TAVARES, V. Receptor Ativado por Proliferadores de Peroxissoma Gama (PPARgama): Estudo Molecular na Homeostase da Glicose, Metabolismo de Lipídeos e Abordagem Terapêutica. **Arq Bras Endocrinol Metab**, v. 51, n. 4, p. 526-533, 2007.

TSIODRAS, S. and MANTZOROS, C. Leptin and Adiponectin in the HIV Associated Metabolic Syndrome: Physiologic and Therapeutic Implications. **Am J Infect Dis**, v. 2, n. 3, p. 141-152, 2006.

UNAIDS/WHO. AIDS epidemic update: december 2009. Avaible online at: http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009_en.pdf (accessed 28 de janeiro 2010).

VIGOUROUX, C., *et al.* Serum adipocytokines are related to lipodystrophy and metabolic disorders in HIV-infected men under antiretroviral therapy. **AIDS**, v. 17, n. 10, p. 1503-1511, Jul 4 2003.

WALCZAK, R. and TONTONOZ, P. PPARadigms and PPARadoxes: expanding roles for PPARgamma in the control of lipid metabolism. **J Lipid Res**, v. 43, n. 2, p. 177-186, Feb 2002.

WANG, T. N., *et al.* G-2548A polymorphism of the leptin gene is correlated with extreme obesity in Taiwanese aborigines. **Obesity (Silver Spring)**, v. 14, n. 2, p. 183-187, Feb 2006.

WOLF, A. M., *et al.* Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. **Biochem Biophys Res Commun**, v. 323, n. 2, p. 630-635, Oct 15 2004.

YAMAGUCHI, N., *et al.* Adiponectin inhibits Toll-like receptor family-induced signaling. **FEBS Lett**, v. 579, n. 30, p. 6821-6826, Dec 19 2005.

YEN, C. J., *et al.* Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. **Biochem Biophys Res Commun**, v. 241, n. 2, p. 270-274, Dec 18 1997.

YIANNAKOURIS, N., *et al.* The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body

weight and body composition variability. **J Clin Endocrinol Metab**, v. 86, n. 9, p. 4434-4439, Sep 2001.

ZANONE POMA, B.*, et al.* Genetic polymorphisms differently influencing the emergence of atrophy and fat accumulation in HIV-related lipodystrophy. **AIDS**, v. 22, n. 14, p. 1769-1778, Sep 12 2008.

ZIETZ, B.*, et al.* Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-gamma2 (PPARgamma2) is associated with higher levels of total cholesterol and LDL-cholesterol in male caucasian type 2 diabetes patients. **Exp Clin Endocrinol Diabetes**, v. 110, n. 2, p. 60-66, Apr 2002.

Capítulo 2 – SNPs in the *APM1* gene promoter are associated with adiponectin levels in HIV-infected individuals receiving HAART

(Artigo submetido ao periódico *JAIDS,* Fator de impacto: 4.57)

SNPs in the *APM1* gene promoter are associated with adiponectin levels in HIV-infected individuals receiving HAART

Adiponectin levels and *APM1* gene promoter

Juciane R. Trinca, BS, Eduardo Sprinz, PhD,† Rosmeri K. Lazzaretti, MSc,† Mara H. Hutz, PhD,‡ Regina Kuhmmer, MSc,† Silvana de Almeida, PhD,* Analise Tibola, BS,§ Gustavo B. Meirelles, BS,¶ Roberto C. Arena-de-Souza, MSc,¶ and Vanessa S. Mattevi, PhD**

Address for correspondence:
Prof. Dra. Vanessa S. Mattevi
Rua Sarmento Leite, 245 sala 309
CEP 90050-170 - Porto Alegre, RS - Brazil
Phone: +55- 51-33038763
Fax: +55 - 51-33038718
e-mail: vmattevi@ufcspa.edu.br

Supported by: Secretaria Nacional de DST/AIDS, Ministério da Saúde, Brasil; Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

---

* Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil.
† Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.
‡ Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.
§ Centro Universitário Feevale, Novo Hamburgo, RS, Brazil.
¶ Universidade Luterana do Brasil, Canoas, RS, Brazil.

ABSTRACT

Objective: This study aimed to investigate the association between four polymorphisms in the leptin (*LEP*), leptin receptor (*LEPR*) and adiponectin (*APM1*) genes and the occurrence of lipodystrophy and dyslipidemia in HIV-infected patients receiving highly active antiretroviral therapy (HAART).

Materials and Methods: Genotypes of 410 HIV-infected patients on HAART were investigated. Anthropometric (weight, height, waist circumference and skinfolds thickness) and biochemical (blood lipids, glucose, leptin and adiponectin levels) parameters were evaluated. Genotype frequencies were compared between patients with or without lipodystrophy. Mean biochemical and anthropometric parameters were compared between the different genotypes.

Results: Lipodystrophy prevalence was 53.4%. Genotype frequencies were not different between patients with or without lipodystrophy. Carriers of the A allele for the *APM1* -11391 G>A and of the C allele for *APM1* -11377 C>G presented higher adiponectin levels compared to other genotypes, as well as carriers of the -11391A-11377C haplotype when compared to carriers of other haplotypes.

Conclusions: SNPs in *APM1* gene are associated with adiponectin levels in HIV-infected patients receiving HAART and may thus affect the occurrence of metabolic alterations in these patients. No influence of the *LEP* and *LEPR* gene polymorphisms on the occurrence of lipodystrophy and dyslipidemia was observed.

Key Words: leptin, adiponectin, lipodystrophy, HAART, pharmacogenomics, HIV

INTRODUCTION

The emergence of highly active antiretroviral therapy (HAART) in the 1990's decade provided a large increase in life expectancy for HIV-infected patients, with a dramatic reduction on morbidity and mortality [1-2]. Despite the advances accomplished by HAART, the appearance of significant adverse events probably related to therapy might compromise quality of life of patients and their adherence to treatment. Studies indicate that over 50% of people with HIV on HAART have some kind of change in body fat distribution [3] and more than 70% have changes in lipid profile [4]. However, the mechanism responsible for the lipodystrophy syndrome has not yet been fully clarified.

It is now widely accepted that the adipose tissue (AT) is not merely a fat storage depot, but also plays roles in the regulation of energy intake and expenditure, thermal insulation, and functions as an endocrine organ [5-6]. Proteins secreted by AT, called adipokines, have important roles in energetic homeostasis, insulin sensibility, immunologic response and vascular disease [7].

The discovery of leptin and adiponectin had broadened the horizons of research in the regulation of body adiposity and energy balance [8]. Leptin circulates in levels proportional to the amount of body fat [9] and has a positive correlation with the concentration of insulin, triglycerides and glucose, with a negative correlation with insulin sensibility [10]. Produced mainly by adipose cells, leptin acts regulating energy expenditure and food intake. Inactivating mutations of the gene encoding leptin lead to hyperphagia and obesity. In individuals with HIV lipodystrophy, leptin serum levels may be high (occurring in association with lipohypertrophy) or low (usually accompanying lipoatrophy) [11]. A common single nucleotide polymorphism

(SNP) located in the promoter region of the leptin gene (*LEP* -2548 G>A) has been previously associated with changes in leptin serum levels and obesity [12-15]. A common variant in the leptin receptor gene (*LEPR* Gln223Arg) has also been associated with body mass index (BMI) in several studies [16-18], including one performed by our group [19]. Thus, we hypothesized that these polymorphisms may be involved with the modifications undertaken by HIV-infected subjects on HAART.

As leptin, adiponectin, encoded by the *APM1* gene, is another recently discovered adipokine, produced exclusively by mature adipocytes [20]. It acts in systemic regulation of glucose metabolism and lipids. Adiponectin levels are negatively correlated to obesity, insulin resistance and type II diabetes mellitus [21-22]. Polymorphisms in the promoter region of the *APM1* gene (-11377 G>C and -11391 G>A) have been associated with changes in adiponectin circulating levels in obese subjects [12].

Given the fact that the eradication of HIV is not currently possible, the study of genetic factors involved in inter-individual variation in efficacy and toxicity of HAART is now subject of intense research [23]. Considering the importance of adverse effects as factors of non-adherence to HAART and the possible contributions that pharmacogenomics can bring to the elucidation of these causes, this study had the objective to investigate the association between polymorphisms in the *LEP*, *LEPR* and *APM1* genes and the occurrence of lipodystrophy and dyslipidemia in HIV patients on HAART.

SUBJECTS AND METHODS

HIV-patients

Four-hundred and ten patients on HAART for at least one year from the South Brazilian HIV Cohort (SOBRHIV) [24], at the HIV/AIDS outpatient clinic at Hospital de Clínicas de Porto Alegre, RS, Brazil, were consecutively enrolled. All were over 18 years old, with viral load <50 copies/mL (this estimate has been used as a measure of HAART adherence). All individuals signed an informed consent form. The study protocol and consent procedure were approved by the Research Ethics Committees from the institutions involved.

Subjects from the General Population

To assess SNP frequency in our population and to evaluate genotyping assays, four-hundred samples of adults living in the metropolitan region of Porto Alegre, RS, Brazil were first tested. These samples were collected from individuals who attended in the Clinical Analysis Laboratory at the Pharmacy School of the Federal University of Rio Grande do Sul and the Biomedicine Laboratory at Feevale University Center for routine blood tests. All individuals signed an informed consent form and the use of samples for gene analyses was authorized by the Research Ethics Committees of the institutions involved.

Anthropometric Measurements

The diagnosis of lipodystrophy was performed by a physician, and patients were classified as having lipoatrophy, when there was loss of subcutaneous fat from the face and/or extremities; lipohypertrophy, when there was accumulation in the abdomen, neck and/or dorsocervical region ("buffalo hump") and mixed

pattern, when both phenotypes were present [25]. Body fat redistribution was also evaluated by measurements of weight, height, waist and hip circumferences and skinfolds from the HIV-infected subjects, performed by two nutritionists. Weight and height were used to calculate body mass index (BMI) of each participant. Measures of waist circumference were used as a measure of central fat accumulation and the sum of seven skinfolds (biceps, triceps, subscapular, axillary, suprailiac, abdominal, calf) was used as a measure of total subcutaneous fat.

Laboratory Tests

Blood samples were collected from HIV-patients after 12 hours of fasting. Levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose and viral load are part of patients' routine care. Low-density lipoprotein (LDL) cholesterol levels were estimated from Friedewald equation [26], only in individuals who had triglycerides under 400mg/dL. Serum leptin levels were measured using the Human Leptin Elisa Kit (Linco Research, St. Charles, MO, USA). Serum adiponectin levels were obtained using the Human Adiponectin Elisa Kit (Linco Research, St. Charles, MO, USA).

Genotyping

DNA was extracted from whole blood samples by a salting out standard procedure [27]. Polymorphisms were detected by PCR-RFLP analysis. PCR cycling conditions used have been previously published [12, 28-29]. Primer sequences were as follows: *LEP* -2548G>A (rs7799039): sense, 5'-

TTTCCTGTAATTTTCCCGTGAG-3'; antisense, 5'-AAAGCAAAGACAGGCATAAAAA-3' [28]; *LEPR* Gln223Arg (rs1137101): sense, 5'-ACCCTTTAAGCTGGGTGTCCCAAATAG-3'; antisense, 5'-AGCTAGCAAATATTTTTGTAAGCAATT-3' [29]; *APM1* -11391G>A (rs17300539) and -11377C>G (rs266729): sense, 5'-TGTTGAAGTTGGTGCTGGCAT-3', antisense, 5'-AGCCTGGAGAACTGGAAGCT-3' [12]. Genotyping was performed after digestion of the PCR products with specific restriction enzymes for each polymorphism (*Hha*I for *LEP* -2548G>A and *APM1* -11391 G>A; *Msp*I for *LEPR* Gln223Arg and *APM1* -11377 C>G) according to the manufacturer's instructions, electrophoresis on agarose gels (2.0% or 2.5%) and staining with ethidium bromide.

Statistical Analyses

Chi-square analysis was used to test for deviations of genotype frequencies from Hardy-Weinberg equilibrium and to compare allele and genotype frequencies among groups of patients with and without adverse reactions. Mean biochemical (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, glucose, leptin and adiponectin levels) and anthropometric parameters (BMI, waist circumference and sum of seven skinfolds) were compared among the different genotypes by analysis of variance and T test for independent samples. These anthropometric and biochemical parameters were adjusted through multiple regressions for co-variables that showed significant correlation with them in previous univariate analyses (gender, age, regular practice of physical activity, smoking, hypolipemic drug use, protease inhibitors (PI) use, BMI). Variables that did not show normal

distribution (triglycerides and leptin levels) were transformed into natural logarithm prior to statistical tests. The non-adjusted means are presented for these two parameters. Non-parametric tests were used when necessary. Poisson regression models with robust variance were used to estimate the contribution of genotypes to lipoatrophy and lipohypertrophy phenotypes separately [30]. Statistical analysis was performed in SPSS version 16.0 for Windows software.

Haplotypes were inferred using Multiple Locus Haplotype Analysis software, version 2.0 [31-32] and linkage disequilibrium value (D') was calculated using Thesias program [33].

RESULTS

Subjects Characteristics and Adipokine Levels

The main demographic, clinical, anthropometric and metabolic characteristics of the HIV-patients are shown in Table 1. The study sample consisted of 410 patients, of whom 224 (54.6%) were men and 53.4% had lipodystrophy. Mean age was 43 ± 9.4 years. The sample comprised 239 (58.3%) individuals of European ancestry and 171 of African ancestry, ascertained by skin color and morphological characteristics, as described by Zembrzuski *et al.* [34]. All patients were on at least three antiretroviral drugs, including two nucleoside reverse transcriptase inhibitors. Approximately half of the sample (202 patients) was on HAART regimen containing PI, while the remaining patients were on non-nucleoside reverse transcriptase inhibitors.

Leptin and adiponectin levels were significantly higher in women than in men in HIV-infected patients (independent samples T-test, $P < 0.001$ for both leptin and adiponectin levels, Table 1). These adipokine levels, stratified according to LD subgroups, are presented in Figure 1. Both adipokine levels were significantly different among LD subgroups ($P < 0.001$ and $P = 0.009$, respectively). Leptin levels were significantly lower in the lipoatrophy patients and patients without LD than in the lipohypertrophy and mixed pattern subgroups, as indicated by the Tukey-Kramer post-hoc tests (Figure 1). When adiponectin levels were considered, the difference observed is due to lipoatrophy and mixed pattern subgroups, which are both lower than lipohypertrophy patients' levels.

Genotype and Allele Frequencies

LEP, *LEPR* and *APM1* polymorphisms genotype frequencies in subjects of European ancestry from the general population (GP) and in HIV-infected patients are shown in Table 2 (genotype frequencies of *LEPR* Gln223Arg in the GP were previously published by Mattevi *et al.* [19]). Genotype frequencies for all SNPs were distributed as expected according to Hardy-Weinberg equilibrium. Minor allele frequencies for SNPs in the GP were: *LEP* -2548 G>A: 0.43; *LEPR* Gln223Arg: 0.40; *APM1* -11391 G>A: 0.14 and *APM1* -11377 C>G: 0.24, being not different (data not shown) from those in HIV-patients: *LEP* -2548 G>A: 0.43; *LEPR* Gln223Arg: 0.42; *APM1* -11391 G>A: 0.08 and *APM1* -11377 C>G: 0.25. Genotype and allele frequencies did not differ between European and African Brazilians from this population therefore analyses were performed in the whole sample. Nevertheless, ethnicity entered in the model as a covariate in all multivariate analyses performed herein.

Association Analyses

Table 2 also presents genotype frequencies in HIV-infected individuals according to LD status (present or absent). No associations with any of the variants analyzed with lipodystrophy were observed, as there were no differences in allele and genotype frequencies between patients with or without LD (*LEP* -2548G>A, P =0.091; *LEPR* Gln223Arg, P =0.308; *APM1* -11391 G>A, P =0.979 and *APM1* -11377 C>G, P =0.652).

Poisson regression analyses were employed to test if any of the SNPs was associated with lipoatrophy and lipohypertrophy. These analyses revealed that

none of the polymorphisms contributed significantly for these phenotypes (data not shown).

Mean adipokine (leptin and adiponectin) levels were compared between genotypes (Tables 3 and 4). Mean leptin levels were not different among *LEP* and *LEPR* genotypes (Table 3). Due to the small number of homozygotes for the two adiponectin polymorphisms, the analyses were also performed in dominant and recessive models (Table 4). Significant associations among adiponectin levels and the two *APM1* polymorphisms were observed. Carriers of the A allele for the -11391 G>A variant presented higher adiponectin levels compared with other genotypes, as well as carriers of the C allele for -11377C>G (Table 4).

The two polymorphisms in the *APM1* gene were in linkage disequilibrium (D'= - 0.795), thus the haplotype combinations were derived in HIV-infected subjects and adiponectin levels were compared among them. All four possible haplotypes (GC, GG, AC and AG) were detected. As carriers of the A allele in the -11391 position and the C allele in -11377 have the highest adiponectin levels when analyzed separately, adiponectin levels on AC haplotype carriers were significantly higher than in non-carriers (AC carriers= 9.83 µg/mL, n= 57; other haplotypes= 9.16 µg/mL, n=351; *P*=0.005; Figure 2).

Mean anthropometric parameters (BMI, waist circumference and skinfolds; Table 3) and blood lipid (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides) and glucose levels (data not shown) were also compared between genotypes. No significant differences in these parameters were observed.

DISCUSSION

To the extent of our knowledge, this is the first report about polymorphisms in *LEP*, *LEPR* and *APM1* genes in HIV individuals on HAART. In this study, we investigated the bases of two important HAART adverse reactions, lipodystrophy and dyslipidemia, by analyzing the possible association between polymorphisms in three genes involved in the signaling from adipocytes and the emergence of these side effects.

Allele frequencies for the four SNPs observed in the GP were similar to those observed in European populations (Spain, France and Greece) [12, 16-17, 35] as well as in other Brazilian studies [14, 18-19]. No differences in allele and genotype frequencies between the GP and HIV-infected samples were observed.

The relationship between *LEP*-2548G>A polymorphism and obesity-related phenotypes has been observed in several populations, with studies showing their association with leptin levels [15], BMI [14] and its relationship with weight loss [12] and hypertension [36] in obese individuals. For the *LEPR* Gln223Arg polymorphism even more studies have demonstrated its association with increased BMI and the risk of obesity in the GP [18],[19]. These associations, however, were not replicated in HIV patients studied herein. Furthermore, no associations were observed between these gene variants and lipodystrophy, suggesting that these polymorphisms in the leptin signaling pathway are not related to the occurrence of LD.

Different studies have shown [37-40] that HIV-infected patients exhibit lower leptin levels when compared to the GP, but the relationship between leptin and fat mass seems to be maintained in HIV-patients. The gender differences observed in leptin levels, with women presenting higher levels than men, are also preserved in

HIV infection. These results are in line with those reported by Kosmiski *et al.* [40], and further suggest that the leptin signaling pathway may not be involved in adipose tissue alterations that occur in LD.

APM1 gene promoter region SNPs -11391G>A and -11377C>G have been consistently associated with adiponectin levels in individuals from the GP [41-43]. The -11391A and -11377C alleles were associated with higher adiponectin levels by Poitou *et al.* [12] and Schwarz *et al.* [44], in French and German GPs, respectively. The present results demonstrated that both polymorphisms were associated with adiponectin levels in HIV patients. Therefore, we may hypothesize that HIV infection is not probably altering *APM1* gene expression.

The -11391A allele was reported to be associated with increased activity of the *APM1* gene [42]. Another report found no significant effect of both variants in the promoter activity [43], but several studies that included haplotypes, containing the -11391 G>A SNP showed that the -11391A allele is a predictor of higher serum adiponectin levels [41, 43, 45-46]. In our sample, although genotype was shown to be a determinant of adiponectin levels, there is no evidence that it is associated with the LD phenotype.

In non-HIV infected individuals, adiponectin levels are paradoxically decreased in obese subjects compared with lean subjects [47]. In HIV patients, this relationship seems to be more complex. The inverse relationship between adiponectin and fat mass seemed to be lost: adiponectin levels were lower in lipoatrophy and mixed pattern patients in comparison to lipohypertrophy. This finding indicates that this protein signaling pathway is altered in LD, but the disfunction probably involves post-transcriptional steps.

Kosmiski et al [40] results on adiponectin levels in LD patients are in line with the present findings. These authors suggested that the reversal in the relationship between adiponectin/fat could be more indicative of a dysfunction or loss of adipocytes rather than the simple reduction in adipose tissue size in lipoatrophy patients. In situations of severe fat depletion, such as seen in anorexia [48], high adiponectin concentrations are observed, which are in contrast to what happened in LD (including other forms of familial partial LD, which also have low adiponectin levels) [49-50]. These data suggest that low adiponectin levels produced by HIV patients with lipoatrophy are due to LD *per se* and not simply by fat mass loss.

Adiponectin undergoes post-translational modifications within the adipocyte into multimeric forms including trimers, hexamers and high-molecular-weight oligomers. The relative distribution of these multimers appears to differ between the adipose tissue and the circulation, and its multimerization seems to play an important role in its metabolic function[47]. As we and others [35, 40, 42-44] measured only total adiponectin and did not separate the different multimeric forms, it remains to be elucidated the possibility that the balance between these different forms are altered in HAART-associated lipodystrophy.

Another potential factor in the action of adiponectin, besides its oligomerization status, is its binding to two transmembrane receptors (AdipoR1 and AdipoR2), which are differently expressed in skeletal muscle and liver [47]. Thus, gene variants in these two receptors may warrant further investigation in HAART-related LD.

Some limitations of the present study are its cross-sectional design and the inclusion of patients receiving several different antiretroviral regimens, which is an

inherent difficulty to any study involving current HAART, as by definition it should include at least three different drugs. This fact might hinder the accomplishment of any pharmacogenomic study regarding this therapy. Additionally, a limited number of SNPs were analyzed. Clearly, we cannot rule out the influence of other polymorphisms in these genes on these HAART side effects.

In summary, our study brings some important findings. This is the first report on the topic of polymorphisms in *LEP*, *LEPR* and *APM1* genes in HIV-patients on HAART. Although their contribution to the LD syndrome still remains unclear, *APM1* -11391G>A and *APM1* -11377C>G were significantly associated with adiponectin levels in HIV-infected individuals receiving HAART and this fact could impact the consequences of LD- associated metabolic alterations in these patients, as adiponectin plays important roles in regulation of glucose and lipids metabolism. We did not find evidence of association of *LEP* -2548 G>A and *LEPR* Gln223Arg polymorphisms on biochemical or anthropometric parameters, indicating that these polymorphisms did not influence the development of the HAART side effects here investigated. Therefore, more studies are needed to explore the role of the adiponectin signaling pathway in the etiology of LD, expanding the applications of pharmacogenomics in the investigation of HAART-related adverse effects.

ACKNOWLEDGMENTS

This work was supported by Secretaria Nacional de DST/AIDS, Ministério da Saúde, Brasil, Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

REFERENCES

1. Bradbury RA, Samaras K. Antiretroviral therapy and the human immunodeficiency virus--improved survival but at what cost? *Diabetes Obes Metab.* 2008;10:441-450.

2. Walensky RP, Paltiel AD, Losina E, et al. The survival benefits of AIDS treatment in the United States. *J Infect Dis.* 2006;194:11-19.

3. Sweeney LL, Brennan AM, Mantzoros CS. The role of adipokines in relation to HIV lipodystrophy. *Aids.* 2007;21:895-904.

4. Carr A. HIV lipodystrophy: risk factors, pathogenesis, diagnosis and management. *Aids.* 2003;17 Suppl 1:S141-148.

5. Prins JB. Adipose tissue as an endocrine organ. *Best Pract Res Clin Endocrinol Metab.* 2002;16:639-651.

6. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab.* 2004;89:2548-2556.

7. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol.* 2005;115:911-919; quiz 920.

8. Tritos NA, Mantzoros CS. Leptin: its role in obesity and beyond. *Diabetologia.* 1997;40:1371-1379.

9. Mynarcik DC, Combs T, McNurlan MA, et al. Adiponectin and leptin levels in HIV-infected subjects with insulin resistance and body fat redistribution. *J Acquir Immune Defic Syndr.* 2002;31:514-520.

10. Vigouroux C, Maachi M, Nguyen TH, et al. Serum adipocytokines are related to lipodystrophy and metabolic disorders in HIV-infected men under antiretroviral therapy. *Aids.* 2003;17:1503-1511.

11. Tsiodras S, Mantzoros C. Leptin and Adiponectin in the HIV Associated Metabolic Syndrome: Physiologic and Therapeutic Implications. *Am J Infect Dis.* 2006;2:141-152.

12. Poitou C, Lacorte JM, Coupaye M, 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. *Obes Surg.* 2005;15:11-23.

13. Wang TN, Huang MC, Chang WT, et al. G-2548A polymorphism of the leptin gene is correlated with extreme obesity in Taiwanese aborigines. *Obesity (Silver Spring).* 2006;14:183-187.

14. Hinuy HM, Hirata MH, Forti N, et al. Leptin G-2548A promoter polymorphism is associated with increased plasma leptin and BMI in Brazilian women. *Arq Bras Endocrinol Metabol.* 2008;52:611-616.

15. Ben Ali S, Kallel A, Ftouhi B, et al. Association of G-2548A LEP polymorphism with plasma leptin levels in Tunisian obese patients. *Clin Biochem.* 2008.

16. Yiannakouris N, Yannakoulia M, Melistas L, et al. The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. *J Clin Endocrinol Metab.* 2001;86:4434-4439.

17. Portoles O, Sorli JV, Frances F, et al. Effect of genetic variation in the leptin gene promoter and the leptin receptor gene on obesity risk in a population-based case-control study in Spain. *Eur J Epidemiol.* 2006;21:605-612.

18. Duarte SF, Francischetti EA, Genelhu VA, et al. LEPR p.Q223R, beta3-AR p.W64R and LEP c.-2548G>A gene variants in obese Brazilian subjects. *Genet Mol Res.* 2007;6:1035-1043.

19. Mattevi VS, Zembrzuski VM, Hutz MH. Association analysis of genes involved in the leptin-signaling pathway with obesity in Brazil. *Int J Obes Relat Metab Disord.* 2002;26:1179-1185.

20. Scherer PE, Williams S, Fogliano M, et al. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem.* 1995;270:26746-26749.

21. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999;257:79-83.

22. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol.* 2000;20:1595-1599.

23. Rodriguez-Novoa S, Barreiro P, Jimenez-Nacher I, et al. Overview of the pharmacogenetics of HIV therapy. *Pharmacogenomics J.* 2006;6:234-245.

24. Dabis F, Balestre E, Braitstein P, et al. Cohort Profile: Antiretroviral Therapy in Lower Income Countries (ART-LINC): international collaboration of treatment cohorts. *Int J Epidemiol.* 2005;34:979-986.

25. Carr A, Emery S, Law M, et al. An objective case definition of lipodystrophy in HIV-infected adults: a case-control study. *Lancet.* 2003;361:726-735.

26. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18:499-502.

27. Lahiri DK, Nurnberger JI, Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* 1991;19:5444.

28. Mammes O, Betoulle D, Aubert R, et al. Novel polymorphisms in the 5' region of the LEP gene: association with leptin levels and response to low-calorie diet in human obesity. *Diabetes.* 1998;47:487-489.

29. Matsuoka N, Ogawa Y, Hosoda K, et al. Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. *Diabetologia.* 1997;40:1204-1210.

30. Barros AJ, Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. *BMC Med Res Methodol.* 2003;3:21.

31. *Multiple Locus Haplotype Analysis, version 2.0* [computer program]. NIAAA, National Institutes of Health, Bethesda, MD.1999.

32. Long JC, Williams RC, Urbanek M. An E-M algorithm and testing strategy for multiple-locus haplotypes. *Am J Hum Genet.* 1995;56:799-810.

33. Tregouet DA, Garelle V. A new JAVA interface implementation of THESIAS: testing haplotype effects in association studies. *Bioinformatics.* 2007;23:1038-1039.

34. Zembrzuski VM, Callegari-Jacques SM, Hutz MH. Application of an African Ancestry Index as a genomic control approach in a Brazilian population. *Ann Hum Genet.* 2006;70:822-828.

35. Vasseur F, Helbecque N, Lobbens S, et al. Hypoadiponectinaemia and high risk of type 2 diabetes are associated with adiponectin-encoding (ACDC) gene promoter variants in morbid obesity: evidence for a role of ACDC in diabesity. *Diabetologia.* 2005;48:892-899.

36. Genelhu VA, Celoria BM, Pimentel MM, et al. Association of a common variant of the leptin gene with blood pressure in an obese Brazilian population. *Am J Hypertens.* 2009;22:577-580.

37. Nagy GS, Tsiodras S, Martin LD, et al. Human immunodeficiency virus type 1-related lipoatrophy and lipohypertrophy are associated with serum concentrations of leptin. *Clin Infect Dis.* 2003;36:795-802.

38. Johnson JA, Albu JB, Engelson ES, et al. Increased systemic and adipose tissue cytokines in patients with HIV-associated lipodystrophy. *Am J Physiol Endocrinol Metab.* 2004;286:E261-271.

39. Lee JH, Chan JL, Sourlas E, et al. Recombinant methionyl human leptin therapy in replacement doses improves insulin resistance and metabolic profile in patients with lipoatrophy and metabolic syndrome induced by the

highly active antiretroviral therapy. *J Clin Endocrinol Metab.* 2006;91:2605-2611.

40. Kosmiski LA, Bacchetti P, Kotler DP, et al. Relationship of fat distribution with adipokines in human immunodeficiency virus infection. *J Clin Endocrinol Metab.* 2008;93:216-224.

41. Vasseur F, Helbecque N, Dina C, et al. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. *Hum Mol Genet.* 2002;11:2607-2614.

42. Bouatia-Naji N, Meyre D, Lobbens S, et al. ACDC/adiponectin polymorphisms are associated with severe childhood and adult obesity. *Diabetes.* 2006;55:545-550.

43. Kyriakou T, Collins LJ, Spencer-Jones NJ, et al. Adiponectin gene ADIPOQ SNP associations with serum adiponectin in two female populations and effects of SNPs on promoter activity. *J Hum Genet.* 2008;53:718-727.

44. Schwarz PE, Towers GW, Fischer S, et al. Hypoadiponectinemia is associated with progression toward type 2 diabetes and genetic variation in the ADIPOQ gene promoter. *Diabetes Care.* 2006;29:1645-1650.

45. Petrone A, Zavarella S, Caiazzo A, et al. The promoter region of the adiponectin gene is a determinant in modulating insulin sensitivity in childhood obesity. *Obesity (Silver Spring).* 2006;14:1498-1504.

46. Heid IM, Wagner SA, Gohlke H, et al. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. *Diabetes.* 2006;55:375-384.

47. Oh DK, Ciaraldi T, Henry RR. Adiponectin in health and disease. *Diabetes Obes Metab.* 2007;9:282-289.

48. Delporte ML, Brichard SM, Hermans MP, et al. Hyperadiponectinaemia in anorexia nervosa. *Clin Endocrinol (Oxf).* 2003;58:22-29.

49. Savage DB, Tan GD, Acerini CL, et al. Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-gamma. *Diabetes.* 2003;52:910-917.

50. Wong SP, Huda M, English P, et al. Adipokines and the insulin resistance syndrome in familial partial lipodystrophy caused by a mutation in lamin A/C. *Diabetologia.* 2005;48:2641-2649.

TABLE 1. Main demographic, clinical, anthropometric and metabolic characteristics of 410 HIV- infected patients on HAART

Characteristics	**HIV-infected patients (n= 410)**
Demographic	
Age, years	43.0 ± 9.4
Male sex, %	54.6
Ethnicity, %	
Euro-brazilians	58.3
Afro-brazilians	41.7
Physical activity, %	24.9
Cigarette smoking, %	27.1
Clinical	
ART duration, months	69 ± 42
Lipid-lowering drugs use, %	16.8
PI users, %	49.3
Anthropometric	
Lipodystrophy, %	53.4
Atrophy, %	38.8
Hypertrophy, %	26.5
Mixed lipodystrophy, %	34.7
Metabolic	
Leptin, ng/mL	10.6 ± 14.2 (387)
Men, ng/mL	3.9 ± 3.8 *
Women, ng/mL	19.0 ±17.5 *
Adiponectin, ug/mL	9.3 ± 7.3 (409)
Men, ng/mL	7.5 ±5.3†
Women, ng/mL	11.4 ± 8.6†

* † Independent samples T-test, $P < 0.001$
Data are mean ± standard deviation or percentage (n presented when different from total).
ART, antiretroviral therapy; PI, protease inhibitors.

TABLE 2. Genotype frequencies in the general population and in HIV-patients with and without lipodystrophy

Genotypes	*General Population*	*HIV-patients*	*Without LD*	*With LD*	*P**
LEP -2548G>A					
G/G	33.3 (132)	32.0 (131)	28.8 (55)	34.7 (76)	
G/A	47.5 (188)	49.7 (204)	55.5 (106)	44.8 (98)	0.091
A/A	19.2 (76)	18.3 (75)	15.7 (30)	20.5 (45)	
LEPR Gln223Arg					
Gln/Gln	33.2 (111)	34.3 (140)	30.9 (59)	37.3 (81)	
Gln/Arg	52.8 (177)	47.6 (194)	48.7 (93)	46.5 (101)	0.308
Arg/Arg	14.0 (47)	18.1 (74)	20.4 (39)	16.2 (35)	
APM1 -11391G>A					
G/G	74.1 (297)	85.1 (349)	85.3 (163)	84.9 (186)	
G/A	23.9 (96)	13.9 (57)	13.6 (26)	14.1 (31)	0.979
A/A	2.0 (8)	1.0 (4)	1.1 (2)	1.0 (2)	
APM1 -11377C>G					
C/C	59.9 (240)	55.9 (229)	53.9 (103)	57.5 (126)	
C/G	32.7 (131)	39.0 (160)	41.4 (79)	37.0 (81)	0.652
G/G	7.5 (30)	5.1 (21)	4.7 (9)	5.5 (12)	

* Chi-square test, with vs. without LD.
Data are percentage (n). LD, lipodystrophy.

TABLE 3. Mean anthropometric variables and leptin levels according to *LEP*, *LEPR* and *APM1* polymorphisms in HIV patients

	HIV Subjects															
	BMI (kg/cm^2)†				Waist (cm)‡				Skinfolds (mm)§				Leptin (ng/mL)¶			
LEP -2548 G>A	n	mean	sd	*P*	n	mean	sd	*P*	n	mean	sd	*P*	n	mean	sd	*P*
G/G	131	25.0	4.4		123	88.7	11.1		117	110	50		124	11.4	14.7	
G/A	204	25.1	4.1	0.717*	189	88.8	10.4	0.974*	183	104	48	0.961*	190	11.0	14.5	0.378*
A/A	75	24.6	5.3		73	88.8	13.2		70	103	51		73	8.6	12.3	
LEPR Gln223Arg																
Gln/Gln	140	25.5	4.0		132	90.4	10.8		125	105	51		133	9.7	13.0	
Gln/Arg	194	24.6	4.5	0.255*	183	87.7	10.9	0.114*	179	106	49	0.782*	183	11.2	15.1	0.573*
Arg/Arg	74	25.5	5.1		68	88.6	12.9		64	106	50		69	11.0	14.2	
APM1 -11391 G>A																
G/G	349	25.0	4.4		332	88.4	11.0		321	105	42		-	-	-	
G/A	57	25.2	4.5	0.943*	49	91.2	11.4	0.254*	45	109	49	0.445*	-	-	-	-
A/A	4	25.1	2.6		4	88.0	5.5		4	128	39		-	-	-	
APM1 -11377 C>G																
C/C	229	25.1	4.3		213	88.8	11.0		204	108	45		-	-	-	
C/G	160	25.0	4.6	0.653*	153	88.8	11.4	0.932*	147	102	39	0.989*	-	-	-	-
G/G	21	24.1	4.2		19	87.8	8.9		19	102	46		-	-	-	

* One-way analysis of variance.
† adjusted by gender and smoking.
‡ adjusted by age and smoking.
§ adjusted by gender, regular practice of physical activity and smoking.
¶ adjusted by gender and age. Values are ln transformed for statistical analysis. The non adjusted means are presented.
BMI, body mass index; sd, standard deviation.

TABLE 4. Comparisons between circulating adiponectin levels and *APM1* polymorphisms

	APM1 -11391 G>A									
	Co-dominant model				Dominant model			Recessive model		
	G/G	G/A	A/A	*P*	G/G	G/A + A/A	*p*	G/G+G/A	A/A	*P*
Adiponectin (ug/mL)	9.1 ± 7.6	9.5 ± 4.8	14.8 ± 8.3	0.003*	9.1 ± 7.6	9.9 ± 5.2	0.003**	9.2 ± 7.3	14.8 ± 8.3	0.084**
n	348	57	4		348	61		405	4	
	APM1 -11377 C>G									
	Co-dominant model				Dominant model			Recessive model		
	C/C	C/G	G/G	*P*	C/C	C/G + G/G	*p*	C/C + C/G	G/G	*P*
Adiponectin (ug/mL)	9.5 ± 7.4	9.3 ± 7.6	6.4 ± 3.6	0.095*	9.5 ± 7.4	8.9 ± 7.3	0.650**	9.4 ± 7.4	6.4 ± 3.6	0.030**
n	228	160	21		228	181		388	21	

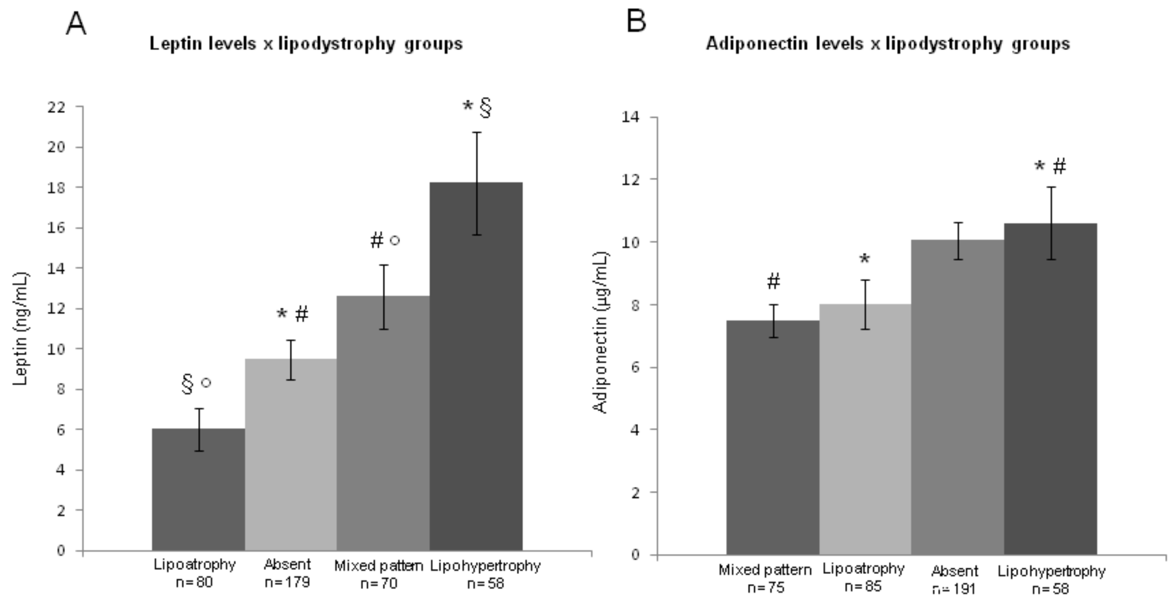
*One-way analysis of variance.
**Independent-samples T test.
Adiponectin levels are adjusted by gender, hypolipidemic drugs use, CD4 count and waist/hip relationship.
Data are mean ± SD. SD, standard deviation.

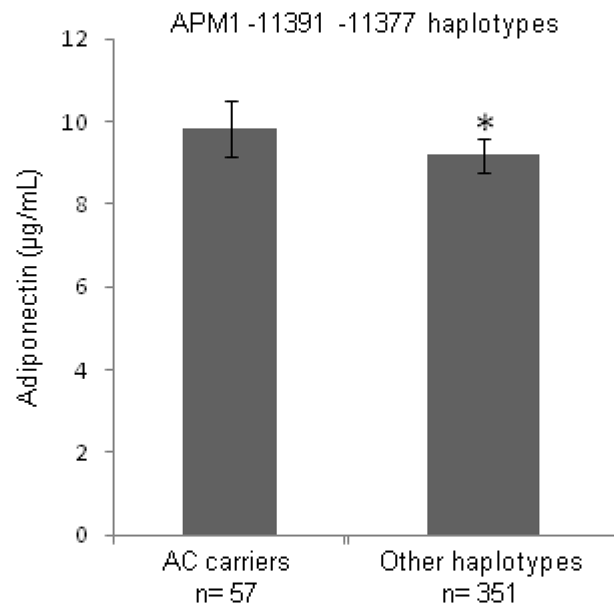
FIGURE LEGENDS

Figure 1: Circulating adipokine levels in HIV-patients separated by lipodystrophy subgroups. (A) Leptin levels. One-way analysis of variance, $P < 0.001$. Tukey-Kramer multiple comparisons test: * $P < 0.001$, # $P = 0.018$, § $P < 0.001$, ° $P<0.001$. (B) Adiponectin levels. One-way analysis of variance, $P = 0.009$. Tukey-Kramer multiple comparisons test: * $P = 0.049$, # $P = 0.048$

Figure 2: Association of -11391/-11377 haplotypes with adiponectin levels.

* Independent-samples T test, $P = 0.005$. Adiponectin levels are adjusted by gender, hypolipidemic drugs use, CD4 count and waist/hip relationship.


A
Leptin levels x lipodystrophy groups
Leptin (ng/mL)
0
2
4
6
8
10
12
14
16
18
20
22
§ ○
* #
# ○
* §
Lipoatrophy n=80
Absent n=179
Mixed pattern n=70
Lipohypertrophy n=58
B
Adiponectin levels x lipodystrophy groups
Adiponectin (µg/mL)
0
2
4
6
8
10
12
14
#
*
* #
Mixed pattern n=75
Lipoatrophy n=85
Absent n=191
Lipohypertrophy n=58


APM1 -11391 -11377 haplotypes
Adiponectin (µg/mL)
12
10
8
6
4
2
0
*
AC carriers
n= 57
Other haplotypes
n= 351

Capítulo 3 – **A genetic variant in sterol-regulatory element-binding protein-1c is associated with HAART-related lipohypertrophy**

(Manuscrito em preparação para submissão ao periódico *Antiviral Therapy*, Fator de impacto: 4.11)

A genetic variant in sterol-regulatory element-binding protein-1c is associated with HAART-related lipohypertrophy

Juciane R. Trinca[1], Eduardo Sprinz[2]; Rosmeri K. Lazzaretti[2]; Mara H. Hutz[3]; Regina Kuhmmer[2], Silvana de Almeida[1], Vanessa S. Mattevi[1]

Address for correspondence:
Prof. Dra. Vanessa S. Mattevi
Rua Sarmento Leite, 245 sala 309
CEP 90050-170 - Porto Alegre, RS - Brazil
Phone: +55- 51-33038763
Fax: +55 - 51-33038718
e-mail: vmattevi@ufcspa.edu.br

Supported by: Secretaria Nacional de DST/AIDS, Ministério da Saúde, Brasil; Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

[1] Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil.
[2] Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.
[3] Departamento de Genética, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

ABSTRACT

Background: The emergence of adverse effects such as lipodystrophy (LD) and dyslipidemia associated with highly active antiretroviral therapy (HAART) compromise patient's adherence to treatment. This study aimed to investigate the associations between polymorphisms in the *PPARG* and *SREBP-1c* genes and the occurrence of LD and dyslipidemia in HIV-infected individuals receiving highly active antiretroviral therapy (HAART).

Methods: Genotypes of 410 HIV-infected patients on HAART were analyzed. Anthropometric (weight, height, waist circumference and skinfolds thickness) and biochemical (blood lipids and glucose) parameters were evaluated. Poisson regression models were used to estimate the contribution of *PPARG* Pro12Ala and *SREBP-1c* 3322C>G genotypes to lipoatrophy and lipohypertrophy phenotypes separately.

Results: The LD prevalence was higher in euro-Brazilians (61.1%) than in afro-Brazilians (42.7%, P<0.001). *SREBP-1c* 3322C>G genotype frequencies were different between euro and afro-Brazilians (P=0.024). *SREBP-1c* 3322G allele was associated with higher risk of developing lipohypertrophy in European descendants. *SREBP-1c* 3322C>G and *PPARG* Pro12Ala polymorphisms were not significant contributors for lipoatrophy.

Conclusions: This a first report showing association of *SREBP-1c* 3322C>G with HAART-related lipohypertrophy in European descendants. No evidence of association between *PPARG* Pro12Ala and the development of LD was found. Further studies are needed to clarify the role of *SREBP-1c* gene on the HAART-related LD etiology.

Key Words: lipodystrophy, HAART, pharmacogenomics, HIV, *PPARG*, *SREBP*

INTRODUCTION

HIV infection was successfully controlled through the use of highly active antiretroviral therapy (HAART), suppressing the viral load and increasing the life expectancy of patients. Despite the benefits brought about by HAART, adverse effects have compromised patients' adherence to treatment. Among the adverse effects, we highlight lipodystrophy (LD) and dyslipidemia, with more than 50% of patients having some form of fat redistribution or lipid profile alteration [1]. The prevalence of LD increases with time of exposure to HAART [2-3] and is higher in HIV-patients receiving protease inhibitors-containing regimens [4-5].

Recent studies *in vitro* have demonstrated that protease inhibitors (PI) can impair adipogenesis [6-8], by inhibiting the differentiation of pre-adipocytes to mature adipocytes, and that nucleoside analogue reverse transcriptase inhibitors (NRTI) reduce the lipid content of adipocytes and modify the pattern of adipokines secretion [9].

PPAR-γ (peroxissome proliferator-actived receptor gamma) is a transcription factor belonging to the family of nuclear receptors [10]. It has three isoforms (PPARγ-1, PPARγ-2 and PPARγ-3). PPARγ-2 is expressed exclusively in adipose tissue, where it is sufficient and necessary for the differentiation of pre-adipocytes into mature adipocytes [11]. Rare mutations in the *PPARG* gene lead to the occurrence of familial partial lipodystrophy type 3 (FPLD3), characterized by loss of subcutaneous fat in the limbs and buttocks, with relative excess of adipose tissue in subcutaneous and visceral abdominal depots. Most patients also have profound metabolic complications; especially insulin resistance, early onset type 2 diabetes mellitus and marked dyslipidemia (high serum triglycerides and low levels

of HDL-cholesterol) [12-17]. A common SNP located in exon B, Pro12Ala (rs1801282) [18], has been linked to insulin resistance, type 2 diabetes and obesity[19-25].

SREBPs (sterol regulatory element binding proteins) are a family of transcription factors responsible for the synthesis of cholesterol and fatty acids, triglycerides and phospholipids in human cells [26]. Three isoforms are described in many species of mammals, SREBP-1a and 1c (produced from the same gene, *SREBP1*, through specific promoters and alternative splicing) [27] and SREBP-2, produced by a different gene (*SREBP2*) [28]. The 1c isoform is found primarily in liver, muscle and adipose tissue, which is responsible for mediating the transcriptional effects of insulin on genes encoding enzymes involved in glycolysis, gluconeogenesis and lipogenesis [29-30]. SREBP-1c plays an important role in the differentiation of adipocytes, activating PPAR-γ transcription [31]. In HIV-infected individuals under HAART, one study showed that the polymorphism *SREBP-1c* 3322C>G (rs2297508) was associated with hyperlipoproteinaemia, where PI users carrying the C allele had higher increases in plasma cholesterol levels than GG homozygotes [32].

Taking into account that not all patients receiving HAART develop adverse effects, genetic factors may be involved in the etiology of LD and dyslipidemia.

Considering the importance of adverse effects as factors of non-adherence to HAART and the possible contributions that pharmacogenomics can bring to the elucidation of these causes, this study aimed to investigate the associations between polymorphisms in the *PPARG* and *SREBP-1c* genes and

the occurrence of lipodystrophy and dyslipidemia in HIV-infected individuals receiving HAART.

SUBJECTS AND METHODS

HIV-patients

We consecutively enrolled 410 patients from the South Brazilian HIV Cohort (SOBRHIV) [33], followed in the HIV/AIDS Ambulatory of the Hospital de Clínicas de Porto Alegre, RS, Brazil. Were used as inclusion criteria in this study: to be over 18 years old, to be receiving HAART for at least one year and to have viral load <50 copies/mL, which was used as a measure of adherence to treatment. All patients signed the Free and Informed Consent Form. This study was approved by Research Ethics Committees from the institutions involved.

Anthropometric Analyses

LD diagnosis was performed by a physician, and patients were classified as follows: lipoatrophy, if they had loss of subcutaneous fat from the face and/or extremities; lipohypertrophy, if they presented fat accumulation in the abdomen, neck and/or accumulation region ("buffalo hump"), and mixed pattern, when they presented both phenotypes (lipoatrophy and lipohypertrophy) [34].

Body fat alterations were also evaluated through anthropometric measurements (weight, height, waist and hip circumferences and skinfolds), performed by two nutritionists. Waist circumference was used as a measure of central fat accumulation and the sum of seven skinfolds (biceps, triceps, subscapular, axillary, suprailiac, abdominal and calf) was used as a measure of total subcutaneous fat. Weight and height were used to calculate body mass index (BMI).

Laboratory Tests

Blood samples were collected from patients after 12 hours of fasting. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose levels and viral load were collected from patients' records. Low-density lipoprotein (LDL) cholesterol levels were estimated by Friedewald equation [35], only in patients with triglycerides below 400mg/dL.

Genotyping

DNA was extracted from whole blood samples by a salting out standard procedure [36]. The *PPARG* Pro12Ala (rs1801282) polymorphism was detected by PCR-RFLP analysis, using upstream primer 5'-GCCAATTCAAGC CCAGTC-3' and downstream primer 5'-GATATGTTTGCAGACAGTGTATCAG TGAAGGAATCGCTTTCCG-3', described by Yen et al. [18]. The PCR products were digested with 5 U of *BstU*I, electrophoresed on 2.5% agarose gels and stained with ethidium bromide. *SREBP-1c* 3322C>G (rs2297508) polymorphism was investigated by TaqMan allelic discrimination assay using primers and probes provided by Applied Biosystems (Foster City, CA, USA) in a 7300 Real-time PCR thermocycler.

Statistical Analysis

Chi-square analysis was used to test for deviations of genotype frequencies from Hardy-Weinberg equilibrium. Mean biochemical (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and glucose) and anthropometric parameters (BMI, waist circumference and sum of seven skinfolds)

were compared among the different genotypes by analysis of variance and T test for independent samples. These anthropometric and biochemical parameters were adjusted through multiple regressions for those co-variables (gender, age, regular practice of physical activity, smoking, hypolipidemic drugs use, protease inhibitors use, BMI) that showed significant correlation with them in previous univariate analyses. Triglycerides were transformed into natural logarithm prior to the adjustments and to perform the statistical tests due to the skewed distribution. Non-parametric tests were used when necessary.

General linear models were employed to verify if there was an interaction between the PPARG polymorphism and gender on the anthropometric parameters.

Poisson regression models with robust variance were used to estimate the contribution of genotypes to lipoatrophy and lipohypertrophy phenotypes separately [37]. All statistical analyses were performed in SPSS version 16.0 for Windows software.

RESULTS

Main demographic, epidemiological and laboratory characteristics of the 410 patients included in this study are summarized in Table 1. Of these subjects, 54.6% (224) were men and 53.4% (219) were found to have some form of lipodystrophy (Table 1). Mean age was 43.0±9.4 years. All patients were receiving at least three anti-retroviral drugs, including two NRTIs. Approximately half of the sample (202 patients) was receiving a HAART regimen containing PI, while the remaining patients were on non-nucleoside reverse transcriptase inhibitors.

The ethnic composition of the sample comprised 239 (58.3%) individuals of European ancestry and 171 of African ancestry, ascertained by skin color and morphological characteristics, as described in Zembrzuski *et al*. [38]. Comparison of characteristics between ethnic groups is also shown in Table 1. LD prevalence was higher in euro-Brazilians (61.1%) than in afro-Brazilians (42.7%; *P* <0.001, Pearson chi-square with continuity correction). The predominant pattern in afro-Brazilians was lipoatrophy, while in the euro-Brazilians the simultaneous occurrence of lipoatrophy and lipohypertrophy was more frequent. When comparing only individuals with LD, although patterns were not significantly different among ethnic groups (*P*= 0.074), chi-square residuals analysis showed that lipoatrophy prevalence was different among the two groups, being higher in the afro-descendants (*P*= 0.024, Table 1).

Genotype and allele frequencies of *PPARG* and *SREBP-1c* gene polymorphisms in both ethnic groups are shown in Table 2. Genotype frequencies for SNPs were distributed as expected according to Hardy-Weinberg equilibrium. *SREBP-1c* 3322C>G genotype and allele frequencies were significantly different

between euro and afro-Brazilians (*P*= 0.024 and *P*= 0.009, respectively, Table 2), while for PPARG Pro12Ala no such differences were observed.

Poisson regression multivariable models were employed to test whether the two gene variants were predictors of lipoatrophy and/or lipohypertrophy. The variables which significantly contributed to lipoatrophy were PI-use, European ancestrality and age (Table 3). *SREBP-1c* 3322C>G and *PPARG* Pro12Ala polymorphisms were not significant contributors for this outcome. For lipohypertrophy, PI-use and European ancestrality were also significant contributors; on the other hand, male gender was a protective factor (Table 4). When *SREBP-1c* 3322C>G genotype was included in the regression model, the presence of the G allele was significantly associated with higher risk (prevalence ratio 1.34, *P*= 0.046; Table 4). To verify if this allele was associated with higher risk of developing lipohypertrophy in both ethnic groups, analyses were carried out in euro-Brazilians and afro-Brazilians separately, also (Table 4). When stratified according ethnicity, it was shown that *SREBP-1c* 3322C>G influences the lipohypertrophy occurrence only in euro-Brazilians (Table 4). The *PPARG* Pro12Ala polymorphism did not contribute significantly for lipohypertrophy.

As a previous study by our group [39] showed a gender-specific association with BMI for *PPARG* Pro12Ala in the general population, we used a general linear model to verify if there was an interaction between the polymorphism and gender on the anthropometric parameters. This analysis found a significant interaction between the variant and the effect of gender on the measure of waist circumference, where women carrying the Ala allele had higher mean than those homozygous for the Pro allele (Ala allele: mean= 93.9±16.3 cm;

Pro allele: mean= 88.6±11.5 cm; *P*= 0.044). There seems to be a trend of an increase in BMI of women Ala allele carriers, but it did not reach statistical significance (Table 5).

Comparisons between lipid profile (triglycerides, total cholesterol, HDL cholesterol and non-HDL cholesterol) and glucose levels and genotypes for the two gene variants were also performed. No significant differences were observed (data not shown).

DISCUSSION

In this study, we investigated the possible association between polymorphisms in two genes involved in adipogenesis and the occurrence of LD and dyslipidemia in HIV-infected patients on HAART.

Minor allele frequencies for *PPARG* Pro12Ala observed in euro-descendants from our sample (Ala= 0.08) were similar to those observed in another study performed by our group which included only euro-descendants from the general population of the same geographical region (Ala= 0.09) [39]. Allele frequencies for this variant observed in afro-descendants from our sample (Ala= 0.07) were not different from that observed in euro-descendants, but were somewhat higher than that observed in other studies in African-Americans (Ala= 0.02) [40]. For *SREBP-1c* 3322C>G, the allele frequencies observed for euro-descendants in this report (G = 0.39) were similar to those observed in other studies conducted in French (G= 0.41) and Austrian populations (G= 0.37) respectively [29, 41]. No studies reporting allele frequencies for this variant in African-derived populations were found. In the present setting, this variant presented higher frequency in afro-descendants (G = 0.48, $P = 0.009$).

In our study, the LD prevalence was higher in euro-Brazilians than in afro-Brazilians, also with differences in LD patterns presented. In euro-Brazilians the predominant pattern was the simultaneous occurrence of lipoatrophy and lipohipertrophy, while afro-Brazilians showed as the predominant pattern only lipoatrophy. Our findings are in agreement with previous reports [42-44], suggesting that European descendants are more susceptible to the development

of fat redistribution than African descendants, even when they live in the same environment.

SREBP-1c is one of several transcription factors responsible for adipogenesis, acting possibly through the activation of PPAR-γ transcription [31]. Overexpression of SREPB-1c in adipose tissue of mice leads to lipodystrophy associated with diabetes and fatty liver [45], thus it is possible that the occurrence of LD is linked to dysregulation of the adipogenic process [46]. Studies analyzing the polymorphism 3322 C>G in exon 18c showed that the G-allele is associated with obesity and type 2 diabetes in a French case-control study [29] and type 2 diabetes and low adiponectin levels in Austrian population [41]. Our results suggest that the same allele is associated with the occurrence of lipohypertrophy in European-descendants. To our knowledge, this is the first evidence of association between this polymorphism and the LD occurrence in HIV-infected individuals. *SREBP*-1c 3322C>G is a synonymous mutation, where the nucleotide change does not result in amino acid change. Thus, it is possible that 3322C>G change exerts some effect at the mRNA level, such as its conformation, or that another SNP in linkage disequilibrium with this variant is the responsible for the observed association.

Laaksonen *et al.* [47], in a functional study of the *SREBP1c* 3322 C>G variant, showed that CC homozygotes had a cholesterol synthesis rate higher than CG or GG carriers, but they found no effect of the gene variant on plasma cholesterol levels. Another report performed by Miserez *et al*. [32], in HIV-infected individuals, found that PI users who were C allele carriers presented increases in plasma cholesterol levels. Our study found no differences in plasma cholesterol

levels between genotype groups, but these results also argue for some functional effect of this nucleotide change.

Regarding *PPARG* Pro12Ala, a study conducted by our group in individuals from the general population showed a gender specific effect for this variant, where men carriers of the Ala allele had higher BMI than those with Pro allele [39]. This polymorphism has been linked to insulin resistance, type 2 diabetes and obesity [21-22, 24-25]. However, the results regarding this gene variant have been conflicting so far. According to a meta-analysis of data from approximately 19,000 individuals, Pro12Ala polymorphism is associated with BMI in overweight and/or obese individuals (BMI above 27 kg/m^2) [48]. These results were not reproduced in HIV-infected patients studied herein, although we found some evidence that it could be associated with abdominal fat accumulation in women, which may warrant further investigations. Furthermore, no associations of PPARG Pro12Ala with LD were found. Despite being largely investigated in several populations, only two studies were found in the literature regarding the *PPARG* gene in HIV-infected patients: a study investigating the C1431T SNP [49] and other investigating the Pro12Ala polymorphism, which found no associations with LD occurrence, in agreement with our findings.

All work involved HAART is hampered by the inclusion of patients receiving several different therapeutic regimens, which can interfere on execution of pharmacogenomic study concerning this therapy. Other limitations of the present investigation are its cross-sectional design and the analysis of only one SNP in each gene. Consequently, we cannot rule out the influence of other polymorphisms in these genes on these HAART adverse effects.

In summary, this is a first report showing association of *SREBP-1c* 3322C>G with HAART-related lipohypertrophy in European descendants. We did not find evidence of association of *PPARG* Pro12Ala on LD subtypes or lipid levels, indicating that this polymorphism should not influence the development of the HAART side effects studied here. Further studies are needed to fully clarify the role of the *SREBP-1c* gene on the etiology of HAART-related LD and the application of pharmacogenetic tools on prevention and management of HAART-related adverse effects.

ACKNOWLEDGMENTS

This work was supported by Secretaria Nacional de DST/AIDS, Ministério da Saúde, Brasil, Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

REFERENCES

1. Carr A. HIV lipodystrophy: risk factors, pathogenesis, diagnosis and management. *AIDS* 2003; **17 Suppl 1:**S141-148.

2. Mallon PW, Miller J, Cooper DA, et al. Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS* 2003; **17:**971-979.

3. Martinez E, Mocroft A, Garcia-Viejo MA, et al. Risk of lipodystrophy in HIV-1-infected patients treated with protease inhibitors: a prospective cohort study. *Lancet* 2001; **357:**592-598.

4. Reus S, Arroyo E, Boix V, et al. [Lipodystrophy and hyperglycemia produced by protease inhibitors]. *An Med Interna* 2000; **17:**123-126.

5. Carr A, Samaras K, Thorisdottir A, et al. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet* 1999; **353:**2093-2099.

6. Pacenti M, Barzon L, Favaretto F, et al. Microarray analysis during adipogenesis identifies new genes altered by antiretroviral drugs. *AIDS* 2006; **20:**1691-1705.

7. Lenhard JM, Furfine ES, Jain RG, et al. HIV protease inhibitors block adipogenesis and increase lipolysis in vitro. *Antiviral Res* 2000; **47:**121-129.

8. Lagathu C, Bastard JP, Auclair M, et al. Antiretroviral drugs with adverse effects on adipocyte lipid metabolism and survival alter the expression and secretion of proinflammatory cytokines and adiponectin in vitro. *Antivir Ther* 2004; **9:**911-920.

9. Lagathu C, Kim M, Maachi M, et al. HIV antiretroviral treatment alters adipokine expression and insulin sensitivity of adipose tissue in vitro and in vivo. *Biochimie* 2005; **87:**65-71.

10. Walczak R & Tontonoz P. PPARadigms and PPARadoxes: expanding roles for PPARgamma in the control of lipid metabolism. *J Lipid Res* 2002; **43:**177-186.

11. Tavares V. Receptor Ativado por Proliferadores de Peroxissoma Gama (PPARgama): Estudo Molecular na Homeostase da Glicose, Metabolismo de Lipídeos e Abordagem Terapêutica. *Arq Bras Endocrinol Metab* 2007; **51:**526-533.

12. Agarwal AK & Garg A. A novel heterozygous mutation in peroxisome proliferator-activated receptor-gamma gene in a patient with familial partial lipodystrophy. *J Clin Endocrinol Metab* 2002; **87:**408-411.

13. Hegele RA, Cao H, Frankowski C, et al. PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy. *Diabetes* 2002; **51:**3586-3590.

14. Barroso I, Gurnell M, Crowley VE, et al. Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature* 1999; **402:**880-883.

15. Ludtke A, Buettner J, Schmidt HH, et al. New PPARG mutation leads to lipodystrophy and loss of protein function that is partially restored by a synthetic ligand. *J Med Genet* 2007; **44:**e88.

16. Ludtke A, Buettner J, Wu W, et al. Peroxisome proliferator-activated receptor-gamma C190S mutation causes partial lipodystrophy. *J Clin Endocrinol Metab* 2007; **92:**2248-2255.

17. Agostini M, Schoenmakers E, Mitchell C, et al. Non-DNA binding, dominant-negative, human PPARgamma mutations cause lipodystrophic insulin resistance. *Cell Metab* 2006; **4:**303-311.

18. Yen CJ, Beamer BA, Negri C, et al. Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. *Biochem Biophys Res Commun* 1997; **241:**270-274.

19. Hara K, Okada T, Tobe K, et al. The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 2000; **271:**212-216.

20. Mori H, Ikegami H, Kawaguchi Y, et al. The Pro12 -->Ala substitution in PPAR-gamma is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. *Diabetes* 2001; **50:**891-894.

21. Evans D, de Heer J, Hagemann C, et al. Association between the P12A and c1431t polymorphisms in the peroxisome proliferator activated receptor gamma (PPAR gamma) gene and type 2 diabetes. *Exp Clin Endocrinol Diabetes* 2001; **109:**151-154.

22. Doney A, Fischer B, Frew D, et al. Haplotype analysis of the PPARgamma Pro12Ala and C1431T variants reveals opposing associations with body weight. *BMC Genet* 2002; **3:**21.

23. Pihlajamaki J, Miettinen R, Valve R, et al. The Pro12A1a substitution in the peroxisome proliferator activated receptor gamma 2 is associated with an insulin-sensitive phenotype in families with familial combined hyperlipidemia and in nondiabetic elderly subjects with dyslipidemia. *Atherosclerosis* 2000; **151:**567-574.

24. Gonzalez Sanchez JL, Serrano Rios M, Fernandez Perez C, et al. Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor gamma-2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population. *Eur J Endocrinol* 2002; **147:**495-501.

25. Mirzaei H, Akrami SM, Golmohammadi T, et al. Polymorphism of Pro12Ala in the peroxisome proliferator-activated receptor gamma2 gene in Iranian diabetic and obese subjects. *Metab Syndr Relat Disord* 2009; **7:**453-458.

26. Eberle D, Hegarty B, Bossard P, et al. SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* 2004; **86:**839-848.

27. Hua X, Wu J, Goldstein JL, et al. Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11.2 and 22q13. *Genomics* 1995; **25:**667-673.

28. Miserez AR, Cao G, Probst LC, et al. Structure of the human gene encoding sterol regulatory element binding protein 2 (SREBF2). *Genomics* 1997; **40:**31-40.

29. Eberle D, Clement K, Meyre D, et al. SREBF-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. *Diabetes* 2004; **53:**2153-2157.

30. Foufelle F & Ferre P. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *Biochem J* 2002; **366:**377-391.

31. Fajas L, Schoonjans K, Gelman L, et al. Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. *Mol Cell Biol* 1999; **19:**5495-5503.

32. Miserez AR, Muller PY, Barella L, et al. A single-nucleotide polymorphism in the sterol-regulatory element-binding protein 1c gene is predictive of HIV-related hyperlipoproteinaemia. *AIDS* 2001; **15:**2045-2049.

33. Dabis F, Balestre E, Braitstein P, et al. Cohort Profile: Antiretroviral Therapy in Lower Income Countries (ART-LINC): international collaboration of treatment cohorts. *Int J Epidemiol* 2005; **34:**979-986.

34. Carr A, Samaras K, Burton S, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. *AIDS* 1998; **12:**F51-58.

35. Friedewald WT, Levy RI & Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18:**499-502.

36. Lahiri DK & Nurnberger JI, Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; **19:**5444.

37. Barros AJ & Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. *BMC Med Res Methodol* 2003; **3:**21.

38. Zembrzuski VM, Callegari-Jacques SM & Hutz MH. Application of an African Ancestry Index as a genomic control approach in a Brazilian population. *Ann Hum Genet* 2006; **70:**822-828.

39. Mattevi VS, Zembrzuski VM & Hutz MH. Effects of a PPARG gene variant on obesity characteristics in Brazil. *Braz J Med Biol Res* 2007; **40:**927-932.

40. Wei Q, Jacobs DR, Jr., Schreiner PJ, et al. Patterns of association between PPARgamma genetic variation and indices of adiposity and insulin action in African-Americans and whites: the CARDIA Study. *J Mol Med* 2006; **84:**955-965.

41. Felder TK, Oberkofler H, Weitgasser R, et al. The SREBF-1 locus is associated with type 2 diabetes and plasma adiponectin levels in a middle-aged Austrian population. *Int J Obes (Lond)* 2007; **31:**1099-1103.

42. Lichtenstein KA, Delaney KM, Armon C, et al. Incidence of and risk factors for lipoatrophy (abnormal fat loss) in ambulatory HIV-1-infected patients. *J Acquir Immune Defic Syndr* 2003; **32:**48-56.

43. Mutimura E, Stewart A, Rheeder P, et al. Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2007; **46:**451-455.

44. Tedaldi EM, Absalon J, Thomas AJ, et al. Ethnicity, race, and gender. Differences in serious adverse events among participants in an antiretroviral initiation trial: results of CPCRA 058 (FIRST Study). *J Acquir Immune Defic Syndr* 2008; **47:**441-448.

45. Shimomura I, Hammer RE, Richardson JA, et al. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. *Genes Dev* 1998; **12:**3182-3194.

46. Rosen ED, Walkey CJ, Puigserver P, et al. Transcriptional regulation of adipogenesis. *Genes Dev* 2000; **14:**1293-1307.

47. Laaksonen R, Thelen KM, Paiva H, et al. Genetic variant of the SREBF-1 gene is significantly related to cholesterol synthesis in man. *Atherosclerosis* 2006; **185:**206-209.

48. Masud S & Ye S. Effect of the peroxisome proliferator activated receptor-gamma gene Pro12Ala variant on body mass index: a meta-analysis. *J Med Genet* 2003; **40:**773-780.

49. Zanone Poma B, Riva A, Nasi M, et al. Genetic polymorphisms differently influencing the emergence of atrophy and fat accumulation in HIV-related lipodystrophy. *AIDS* 2008; **22:**1769-1778.

Table 1: Main demographic, clinical, anthropometric and metabolic characteristics of 410 HIV- infected patients

Characteristics	HIV-infected patients (n= 410)	Euro-Brazilians (n= 239)	Afro-Brazilians (n= 171)	*P* (euro vs. afro-Brazilians)
Demographic				
Age, years	43.0 ± 9.4	42.7 ± 9.4	43.31 ± 9.5	0.553[a]
Male sex, %	54.6	51.5	59.0	0.155[b]
Physical activity, %	24.9	21.7	29.2	0.107[b]
Cigarette smoking, %	27.1	20.9	35.7	0.001[b]
Clinical				
ART duration, months	69 ± 42	72 ± 42	65 ± 41	0.138[c]
Lipid-lowering drugs use, %	16.8	17.6	15.8	0.732[b]
PI users, %	49.3	54.0	45.0	0.092[b]
Anthropometric				
Lipodystrophy, %	53.4	61.1	42.7	<0.001[b]
Atrophy, %	38.8	33.6[e]	49.3[f]	
Hypertrophy, %	26.5	28.1	23.3	0.074[d]
Both, %	34.7	38.3	27.4	

Data are mean ± SD or percentage (n presented when different from total).
[a] Independent samples T-test.
[b] Pearson chi-square with continuity correction.
[c] Mann-Whitney's U non-parametric test.
[d] Pearson chi-square between individuals with lipodystrophy.
[e] Adjusted residual= -2.26, p= 0.024.
[f] Adjusted residual= 2.26, p= 0.024.

Table 2: Genotype and allele frequencies of *PPARG* and *SREBP-1c* polymorphisms in HIV-infected patients by ethnic group

Genotypes	Ethnic group % (n)			Alleles	Ethnic group %		
	Euro-Brazilians	Afro-Brazilians	*P* *		Euro-Brazilians	Afro-Brazilians	*P* §
PPARG Pro12Ala				*PPARG* Pro12Ala			
Pro/Pro	84.3 (199)	85.8 (145)	0.682	Pro	92.0	93.0	0.797
Pro/Ala	15.7 (37)	14.2 (24)		Ala	8.0	7.0	
SREBP-1c 3322 C>G				*SREPB-1c* 3322 C>G			
C/C	36.0 (85)	25.5 (42)		C	61.0	51.5	0.009
C/G	50.0 (118)	52.1 (86)	0.024	G	39.0	48.5	
G/G	14.0 (33)	22.4 (37)					

* Chi-square test.
§ Chi-square test with Yates correction.

Table 3: Poisson regression models and predicting variables for development of lipoatrophy in HIV-infected subjects

Outcome	Variable	PR	95% CI	*P*
Lipoatrophy (whole sample)	PI – use	1.40	1.12 – 1.76	0.003
	Age	1.02	1.02 – 1.04	<0.001
	Ethnic group (euro-descendent)	1.43	1.13 – 1.81	0.003

PR, prevalence ratio; 95% CI, 95% confidence interval; PI, protease inhibitor

Table 4: Poisson regression models and predicting variables for development of lipohypertrophy in HIV-infected subjects

Outcome	Variable	PR	95% CI	*P*
Lipohypertrophy (whole sample)	PI – use	1.46	1.12 – 1.90	0.005
	Sex (male)	0.73	0.56 – 0.94	0.015
	Ethnic group (euro-descendent)	1.74	1.28 – 2.36	<0.001
	SREBP-1c G-allele	1.33	0.99 – 1.77	0.054
Lipohypertrophy (euro-Brazilians)	PI – use	1.42	1.06 – 1.91	0.018
	Sex (male)	0.81	0.61 – 1.09	0.151
	SREBP-1c G-allele	1.41	1.02 – 1.96	0.039
Lipohypertrophy (afro-Brazilians)	PI – use	1.58	0.91 – 2.72	0.098
	Sex (male)	0.52	0.30 – 0.93	0.026
	SREBP-1c G-allele	1.08	0.60 – 1.93	0.792

PR, prevalence ratio; 95% CI, 95% confidence interval; PI, protease inhibitor

Table 5: Comparison between anthropometric parameters and *PPARG Pro12Ala* polymorphism by gender

	Men		Women		P_{gen}	P_{sex}	$P_{gen*sex}$
	Pro/Pro	Pro/Ala	Pro/Pro	Pro/Ala			
BMI (kg/cm^2)							
mean	24.6	24.1	25.3	27.1			
sd	3.6	3.1	5.0	6.7	0.305	0.005	0.077
n	192	29	152	32			
Waist (cm)							
mean	88.3	86.9	88.6	93.9			
sd	10.1	7.1	11.5	16.3	0.211	0.024	0.044
n	179	27	143	31			
Skinfolds (mm)							
mean	84.4	82.2	131.5	135.6			
sd	36.9	26.8	53.0	42.7	0.936	<0.001	0.571
n	175	27	135	28			

Univariate analysis of variance.
P_{gen}, *P* value for genotype; P_{sex}, *P* value for gender; $P_{gen*sex}$, *P* value for the interaction between genotype and gender; BMI, body mass index; sd, standard deviation.

Anexo 1 – Metodologia Utilizada

Metodologia utilizada

As amostras de DNA foram obtidas de leucócitos de sangue periférico através da técnica de Lahiri e Nurnberger (1991), através da precipitação de proteínas pelo excesso de sal.

O DNA extraído foi amplificado pela técnica de PCR (reação em cadeia da polimerase) para análise dos polimorfismos nos genes *LEP*, *LEPR*, *APM1* e *PPARG*. Os fragmentos que continham cada um dos polimorfismos foram amplificados de acordo com as condições descritas na tabela A-1. Para análise dos polimorfismos no gene *APM1*, somente um fragmento compreendendo os dois SNPs foi amplificado. Após a amplificação, a efetividade da reação foi averiguada pela análise dos produtos através de eletroforese em géis de agarose 1,5% corados com brometo de etídio.

A seguir, 10 µL dos produtos amplificados foram clivados com as respectivas enzimas de restrição, de acordo com a tabela A-2. A genotipagem foi realizada através de eletroforese em géis de agarose com diferentes concentrações, de acordo com o tamanho dos fragmentos produzidos pela digestão enzimática (Tabela A-2). A coloração dos géis com brometo de etídio permitiu a verificação dos genótipos através da utilização de luz ultravioleta (UV). O tamanho dos fragmentos foi estimado por comparação com marcadores de peso molecular (50 bp e 100 bp)

O polimorfismo no gene *SREBP* foi analisado pela técnica de PCR em tempo real, através do ensaio de discriminação alélica *TaqMan*, utilizando *primers* e *probes* adquiridas da empresa Apllied Biosystems.

Tabela A-1: Protocolos utilizados para amplificação através de PCR dos polimorfismos analisados no presente estudo

SNP	Sequência dos primers	Composição da reação	Ciclos de temperatura	Referências
***LEP* -2548G>A**	**L1:** 5'-TTTCCTGTAA TTTTCCCGTGA-3' **L2:** 5'-AAAGCAAAG ACAGGCATAAAAA-3'	- 2 mM de $MgCl_2$ - 187 µM de cada dNTP - 0,5 µM de cada primer - 0,75 U de *Taq* polimerase - 1 µL de DNA genômico - Volume final: 20 µL	94°C – 5 min 94°C – 30 s 50°C – 30 s } 35 X 72°C – 30 s 72°C – 5 min	Le Stunff e cols. (2000) Mammés e cols. (2000)
***LEPR* Gln223Arg**	**LR-1:** 5'- ACCCTTTAAGCT GGGTGTCCCAAATAG-3 **LR2:** 5'-AGCTAGCAAATA TTTTTGTAAGCAATT-3'	- 1,5 mM de $MgCl_2$ - 200 µM de cada dNTP - 0,3 µM de cada primer - 1,25 U de *Taq* polimerase - 1 µL de DNA genômico - Volume final: 20 µL	94°C – 5 min 94°C – 45 s 55°C – 45 s } 35 X 72°C – 1 min 72°C – 7 min	Matsuoka e cols. (1997)
***APM1* -11391G>A e** ***APM1* -11377C>G**	**A1:** 5'-TGTTGAAGTTGG TGCTGGCAT-3' **A2:** 5'-AGCCTGGAGAA CTGGAAGCT-3'	- 2 mM de $MgCl_2$ - 166 µM de cada dNTP - 0,23 µM de cada primer - 0,75 U de *Taq* polimerase - 1 µL de DNA genômico - Volume final: 30 µL	94°C – 5 min 94°C – 30 s 57°C – 30 s } 35 X 72°C – 30 s 72°C – 5 min	Poitou e cols. (2005)
***PPARG* Pro12Ala**	**P1:** 5'-GCCAATTCAAG CCCAGTC-3' **P2:** 5'-GATATGTTTGCA GACAGTGTATCAGTGA AAGGAATCGCTTTCCG -3'	- 1,5 mM de $MgCl_2$ - 200 µM de cada dNTP - 0,3 µM de cada primer - 0,75 U de *Taq* polimerase - 1 µL de DNA genômico - Volume final: 25 µL	96°C – 5 min 96°C – 30 s 58°C – 30 s } 35 X 72°C – 45 s	Yen e cols. (1997)

Tabela A-2: Condições das reações de clivagem dos polimorfismos e de eletroforese para separação dos fragmentos obtidos

SNP	**Enzima de restrição**	**Concentração da enzima**	**Condições de clivagem**	**Gel de agarose**
LEP -2548G>A	*Hha*I	4 U	1 h a 37℃	2,0 %
LEPR Gln223Arg	*Msp*I	5 U	2 h a 37℃	2,0 %
APM1 -11391G>A	*Hpa*II	5 U	2 h a 37℃	3,0 %
APM1 -11377C>G	*Hha*I	4 U	1 h a 37℃	3,0 %
PPARG Pro12Ala	*Bst*UI	5 U	2 h a 60℃	2,5 %

Anexo 2 – Termos de aprovação pelos comitês de ética

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO FACULDADE FEDERAL DE CIÊNCIAS MÉDICAS DE PORTO ALEGRE
COMITÊ DE ÉTICA EM PESQUISA
APROVADO PELA CARTA Nº 880/2004-CONEP/CNS/MS
RUA SARMENTO LEITE, 245 – FONE: (51) 3224.8822
CEP 90050-170 – PORTO ALEGRE – RS - cep@fffcmpa.edu.br

Of. 302/06-CEP

Porto Alegre, 05 de outubro de 2006.

Ilma. Sra.

Profa. Vanessa Suñé Mattevi

Nesta Faculdade

Senhora Professora

Informamos que seu projeto intitulado "Identificação de Marcadores Genéticos Associados a Efeitos Adversos em Pacientes com Síndrome da Imunodeficiência Adquirida Sob Terapia Anti-Retroviral.", Processo nº 141/06, foi avaliado pelo Comitê de Ética em Pesquisa, na reunião de 05 de outubro de 2006, sendo o projeto aprovado, conforme parecer consubstanciado nº 246-06, em anexo.

Outrossim, informamos que de acordo com o Art. 4º, letra c, do Regulamento do CEP, V. Sa. deverá nos encaminhar relatórios semestrais do desenvolvimento do projeto.

Atenciosamente,

José Geraldo Vernet Taborda
Coordenador do CEP/FFFCMPA

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
Grupo de Pesquisa e Pós-Graduação
COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

A Comissão Científica e a Comissão de Pesquisa e Ética em Saúde, que é reconhecida pela Comissão Nacional de Ética em Pesquisa (CONEP)/MS como Comitê de Ética em Pesquisa do HCPA e pelo Office For Human Research Protections (OHRP)/USDHHS, como Institucional Review Board (IRB0000921) analisaram o projeto:

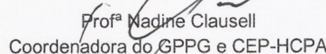
Projeto: 05-295 **Versão do Projeto:** 06/09/2005 **Versão do TCLE:** 06/09/2005

Pesquisadores:
EDUARDO SPRINZ
ROSMERI KUHMMER LAZZARETTI
VANESSA SUNE MATTEVI
JORGE PINTO RIBEIRO
SILVIA KELBERT
PATRICIA REIS PEREIRA
MARA HELENA HUTZ
REGINA KUHMER

Título: IDENTIFICAÇÃO DE MARCADORES GENÉTICOS ASSOCIADOS A EFEITOS ADVERSOS EM PACIENTES COM SÍNDROME DA IMUNODEFICIÊNCIA ADQUIRIDA SOB TERAPIA ANTI-RETROVIRAL

Este projeto foi Aprovado em seus aspectos éticos e metodológicos, inclusive quanto ao seu Termo de Consentimento Livre e Esclarecido, de acordo com as Diretrizes e Normas Internacionais e Nacionais, especialmente as Resoluções 196/96 e complementares do Conselho Nacional de Saúde. Os membros do CEP/HCPA não participaram do processo de avaliação dos projetos onde constam como pesquisadores. Toda e qualquer alteração do Projeto, assim como os eventos adversos graves, deverão ser comunicados imediatamente ao CEP/HCPA. Somente poderão ser utilizados os Termos de Consentimento onde conste a aprovação do GPPG/HCPA.

Porto Alegre, 03 de outubro de 2005.



Profª Nadine Clausell
Coordenadora do GPPG e CEP-HCPA

Anexo 3 – Termo de consentimento livre e esclarecido

Termo de Consentimento Livre e Esclarecido

Com o passar do tempo, estão sendo identificadas mudanças no sangue e no corpo antes não descritas em pessoas portadores do HIV, tais como aumento exagerado das gorduras e açúcares no sangue e modificações nas formas do corpo, que consistem no aumento de gordura no pescoço, na barriga e no peito, e uma diminuição da gordura na face, braços, pernas e nádegas, que surgem durante a utilização do coquetel.

Esta pesquisa tem como objetivo identificar várias formas dos genes que podem determinar a eficácia e a segurança do coquetel. Os resultados deste estudo poderão ser utilizados, no futuro, para definir o melhor tratamento para cada paciente de forma individualizada, evitando-se assim o uso desnecessário de fármacos com diferentes efeitos colaterais em pacientes que não se beneficiarão dos mesmos, com a conseqüente redução da desistência do tratamento e da mortalidade.

Concordando em participar do estudo, você será submetido a uma coleta de sangue que lhe causará o desconforto da picada com agulha e, às vezes, poderá deixar o local um pouco roxo. Além disso, serão realizadas medidas de peso, altura e dobras cutâneas.

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 da 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 por parte dos pesquisadores de proporcionar informação atualizada obtida durante o estudo, ainda que possa afetar a minha vontade em continuar participando;
- da disponibilidade de tratamento médico e indenizado, conforme estabelecido em legislação, caso existam danos a minha saúde diretamente causados por esta pesquisa;

- de que se existirem gastos adicionais, estes serão absorvidos pelo orçamento da pesquisa.
- de ser informado sobre os resultados e de suas implicações clínicas após o término do estudo;
- de que o material biológico que será coletado será armazenado, podendo ser utilizado para posteriores estudos;
- da utilização do material armazenado, que só ocorrerá mediante aprovação do novo projeto pelo Comitê de Ética em Pesquisa;

Os pesquisadores responsáveis por este Projeto de Pesquisa são o Prof. Dr. Eduardo Sprinz (Fone: 21018152) e a Profª Drª Vanessa Suñé Mattevi (Fone: 33038763), tendo este documento sido revisado em seus aspectos éticos e metodológicos e aprovado pelos Comitês de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre e da Fundação Faculdade Federal de Ciências Médicas de Porto Alegre.

Data ___/___/____

______________________________ Nome	______________________________ Assinatura do paciente
______________________________ Nome	______________________________ Concordo em deixar a minha amostra armazenada por 5 anos
______________________________ Nome	______________________________ Assinatura do responsável pela obtenção do presente 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 4 – Carta de confirmação da submissão do artigo *"SNPs in the APM1 gene promoter are associated with adiponectin levels in HIV-infected individuals receiving HAART"* **ao periódico *JAIDS***

--------------------------- Mensagem Original ----------------------------
Assunto: Submission Confirmation for SNPs in the APM1 gene promoter are associated with adiponectin levels in HIV-infected individuals receiving HAART
De: "JAIDS, Basic Science" <dgottwal@adarc.org>
Data: Dom, Janeiro 3, 2010 11:24 am
Para: vmattevi@ufcspa.edu.br
vmattevi@terra.com.br

Dear Prof. Mattevi,

Your submission entitled "SNPs in the APM1 gene promoter are associated with adiponectin levels in HIV-infected individuals receiving HAART" has been received by the Journal of Acquired Immune Deficiency Syndromes - Basic Science section.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an Author. The URL is http://jaids-basicscience.edmgr.com/.

Your manuscript will be given a reference number once your submission is processed and ready for review.

Thank you for submitting your work to this journal.

Kind regards,

Editorial Office
Journal of Acquired Immune Deficiency Syndromes - Basic Science

Anexo 5 – Instruções para publicação de artigos no periódico *JAIDS*

Journal of Acquired Immune Deficiency Syndromes (JAIDS)

Online Submission and Review System

SCOPE

The ***Journal of Acquired Immune Deficiency Syndromes*** is a peer-reviewed, multidisciplinary journal directed to an audience of physicians and researchers. The journal publishes original articles in the form of rapid communications, original research reports, short reviews, brief reports, and letters to the editor. ***JAIDS*** generally does not publish case reports.

MANUSCRIPT SUBMISSION

A submitted manuscript must be an original contribution not previously published (except as an abstract or preliminary report), must not be under consideration for publication elsewhere, and, if accepted, must not be published elsewhere in similar form, in any language, without the consent of Lippincott Williams & Wilkins. Each person listed as an author is expected to have participated in the study to a significant extent. Although the editors and referees make every effort to ensure the validity of published manuscripts, the final responsibility rests with the authors, not with the journal, its editors, or the publisher.

All submissions will be rigorously peer-reviewed by members of the Editorial Board, and possibly by other specially qualified individuals as well. In the interests of rapid reviewing of contributions, only one of the Editors-in-Chief will, in general, make the final determination as to the acceptability of a submission, after collecting the referee's comments. Contributors may recommend specific names of reviewers from the Editorial Board, as well as other individuals they deem especially well qualified. However, the Editors-in-Chief will not be bound to follow such suggestions.

In general, the instructions for preparation of manuscripts should follow the International Committee of Medical Journal Editors (ICMJE) Uniform Requirements for Manuscripts Submitted to Biomedical Journals. In case of questions, please feel free to contact the Editorial Office of any one of the Editors-in-Chief.

Authors must submit their manuscripts to the relevant section through the Web-based tracking system: Basic Science (**http://jaids-basicscience.edmgr.com**), Clinical Science (**http://jaids-clinical.edmgr.com**), or Epidemiology (**http://jaids-epidemiology.edmgr.com**). The site contains instructions and advice on how to use the system, guidance on the creation/scanning and saving of electronic art, and supporting documentation. In addition to allowing authors to submit manuscripts on the Web, the site allows authors to follow the progression of their manuscript through the peer review process. Authors should not send hard copies of the manuscript to the editorial office. They may, however, send to the editorial office any artwork, letters, or files that cannot be submitted electronically. Address all inquiries regarding manuscripts not yet accepted or published to the Journal's editorial office. The editorial office will acknowledge receipt of your manuscript via e-mail.

Authorship

An author is considered to be someone who has made substantive contributions to a published study. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. More specifically, authorship credit requires *a*) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; *b*) drafting the paper or revising it critically for important intellectual content; and *c*) final approval of the version to be published. Contributors must meet conditions for *a, b*, and *c*—all 3—to be eligible for authorship. All persons listed as authors must meet the 3 criteria above, and all persons who meet the above criteria must be listed as authors. Please note that acquisition of funding, collection of data, or general supervision of a research group, alone, does not justify authorship.

All papers must include a completed Mandatory Authorship Checklist at the time of submission.

For large, multicenter group studies, individuals who accept direct responsibility for the manuscript must be identified. Those individuals will be required to complete the ***JAIDS*** Copyright Transfer form.

Contributors who do not meet the criteria for authorship should be listed in the acknowledgments section. Persons providing technical help, writing assistance, or a department chair providing general support are examples of persons who should not be included as authors, but who should be listed in the Acknowledgments section.

Conflict of Interest Declaration and Mandatory Checklist

Authors must disclose all financial and personal relationships that might bias their work. The Mandatory Authorship Checklist must be completed for each submission, and it is on this form (or in an attachment) that conflicts of interest must be detailed. Conflicts that are determined to be substantial may be printed in the journal in a footnote on the first page of the article.

Studies funded by an agency with a proprietary or financial interest in the outcome must include a statement with information about the sponsor(s) involvement. Authors involved in such a study must state whether they had full access to all of the data and take full responsibility for the accuracy of the data analysis.

Copyright

All authors must complete and sign a copy of the journal's Copyright Transfer form and submit it when submitting the original manuscript online.

Compliance with NIH and Other Research Funding Agency Accessibility Requirements

A number of research funding agencies now require or request authors to submit the post-print (the article after peer review and acceptance but not the final published article) to a repository that is accessible online by all without charge. As a service to our authors, LWW will identify to the National Library of Medicine (NLM) articles that require deposit and will transmit the post-print of an article based on research funded in whole or in part by the National Institutes of Health, Wellcome Trust, Howard Hughes Medical Institute, or other funding agencies to PubMed Central. The revised Copyright Transfer Agreement provides the mechanism.

Patient Anonymity and Informed Consent

It is the author's responsibility to ensure that a patient's anonymity be carefully protected and to verify that any experimental investigation with human subjects reported in the manuscript was performed with informed consent and following all the guidelines for experimental investigation with human subjects required by the institution(s) with which all the authors are affiliated. Authors should mask patients' eyes and remove patients' names from figures unless they obtain written consent from the patients and submit written consent with the manuscript.

Protection of Human Subjects and Animals in Research

When reporting experiments involving 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.

For research involving animals, authors should indicate whether the procedures followed were in accordance with the standards set forth in the *Guide for the Care and Use of Laboratory Animals* (published by the National Academy of Science, National Academy Press, Washington, D.C.).

Permissions

Authors must submit written permission from the copyright owner (usually the publisher) to use direct quotations, tables, or illustrations that have appeared in copyrighted form elsewhere, along

with complete details about the source. Any permissions fees that might be required by the copyright owner are the responsibility of the authors requesting use of the borrowed material, not the responsibility of Lippincott Williams & Wilkins.

PREPARATION OF MANUSCRIPT

Manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review.

Online Submission

Manuscript files must be uploaded into the Editorial Manager online interface. Most word-processing file formats are acceptable. Editorial Manager will then create PDF files of the authors' submission, and the author must view and approve the files before they will be submitted to the editorial office. Please be sure that the manuscript file contains complete text for your submission (title page and abstract), as this is the file that will be downloaded by the reviewers and publisher. Please see the sections below for instructions regarding Figure and Table files.

Once the paper has been accepted for publication, and final versions of the manuscript, figures, and table files have been uploaded to the Editorial Manager interface, PDF files will not be used for typesetting. This is important to note for Table and Figure files, which may lose formatting when converted to PDF, but will remain intact in their original file format.

Title Page

A title page must be included in the manuscript file. Include on the title page: *a*) complete manuscript title; *b*) authors' full names, academic degrees, and affiliations; *c*) name and address for correspondence, including fax number, telephone number, and e-mail address; *d*) address for reprints if different from that of corresponding author; *e*) meetings at which parts of the data were presented (including title of conference, city, and date); *f*) sources of support; and *g*) a running head of no more than 40 characters.

The title page must also include disclosure of funding received for this work from any of the following organizations: National Institutes of Health (NIH); Wellcome Trust; Howard Hughes Medical Institute (HHMI); and other(s).

Abstract and Key Words

The abstract should be structured and limited to 200 words. It must be factual and comprehensive. Limit the use of abbreviations and acronyms, and avoid general statements (eg, "the significance of the results is discussed"). List 3 to 6 key words or phrases.

Text

Organize the manuscript file into sections with appropriate section headings. The sequence should be as follows: title page, abstract/key word page, introduction, methods, results, discussions, acknowledgments, references, tables, figures and figure captions.

Authors should type, whenever possible, all mathematical and chemical symbols, equations, and formulas, and identify all unusual symbols the first time they are used. Define abbreviations at first mention in text and in each table and figure. If a brand name is cited, supply the manufacturer's name and address (city and state/country).

Abbreviations

For a list of standard abbreviations, consult the Council of Biology Editors Style Guide (available from the Council of Science Editors, 9650 Rockville Pike, Bethesda, MD 20814) or other standard sources. Write out the full term for each abbreviation at its first use unless it is a standard unit of measure.

References

The authors are responsible for the accuracy of the references. Key the references (double-spaced) at the end of the manuscript. (If using End Note, set the style output to *JAMA*.) Cite references in text in order of appearance. Cite unpublished data, such as papers submitted but not yet accepted for publication, or personal communications, in parentheses in the text. If there are more than 3 authors, list only the first 3 authors and then use et al. Refer to the List of Journals Indexed in Index Medicus for abbreviations of journal names. Sample references are given below:

Journal Article

1. Schambelan M, Benson CA, Carr A, et al. Management of metabolic complications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. *J Acquir Immune Defic Syndr.* 2002;31:257–275.

Book Chapter

2. Wortmann RL, Bentzel CJ. Renal handling of uric acid. In: Massry SG, Glassock RJ, eds. *Massry and Glassock's Textbook of Nephrology*. Phiadelphia: Lippincott Williams & Wilkins, 2001;90–92.

Entire Book

3. Mandell GL, Mildvan D, eds. *Atlas of AIDS*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2001.

Software

4. Epi Info [computer program]. Version 6. Atlanta: Centers for Disease Control and Prevention, 1994.

Online Journals

5. Friedman SA. Preeclampsia: a review of the role of prostaglandins. *Obstet Gynecol* [serial online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

Database

6. CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute, 1996. Updated March 29, 1996.

World Wide Web

7. Panel on Clinical Practices for the Treatment of HIV Infection. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. Department of Health and Human Services and Henry J. Kaiser Foundation, January 28, 2000. Available at: http://www.hivatis.org/guidelines/AA599.pdf.

Paper Presented at a Conference

8. Koenig L, Ellerbrock T, Pratt-Palmire M, et al. Prospective predictors of medication adherence: a study of the first six months of highly active antiretroviral therapy (HAART) using electronic monitoring [WePeB5818]. Presented at: XIV International AIDS Conference; 2002; Barcelona.

Figures

Cite figures consecutively in the text, and number them in the order in which they are discussed. We encourage authors to submit their figures through Editorial Manager, but if this is not possible, authors may send hard copies of the figures to the editorial office for scanning. On the hard copies,

be sure to write the first author's last name, the figure number and figure part (1A, 1B, 1C), and an arrow to indicate the top edge of the figure on a label pasted to the back of each figure. Submit all artwork in camera-ready form; illustrations should be glossy prints or high-quality, laser-printed illustrations. Photocopies are unacceptable. Authors who submit manuscripts through Editorial Manager may submit figures as separate electronic files. High-quality hard copies may be requested once the manuscript has been accepted for publication. Lettering should be large enough that it will remain legible after figure reduction; typewritten or unprofessional lettering is unacceptable. Figure parts (A, B, C) may be left unlabeled (but clearly marked on back) for professional placement by the journal's printer.

Figure Legends

Legends must be submitted for all figures. They should be included in the manuscript file, should be brief and specific, and should appear on a separate manuscript page after the references. Use scale markers in the image for electron micrographs, and indicate the type of stain used.

Color Figures

The journal accepts for publication color figures that will enhance an article. Authors who submit color figures will receive an estimate of the cost for color reproduction. If they decide not to pay for color reproduction, they can request that the figures be converted to black and white at no charge.

Digital Figures

Electronic art should be created/scanned and saved and submitted as either a TIFF (tagged image file format), an EPS (encapsulated postscript) file, or a PPT (Power Point) file. Line art must have a resolution of at least 1200 dpi (dots per inch), and electronic photographs—radiographs, CT scans, and so on—and scanned images must have a resolution of at least 300 dpi. If fonts are used in the artwork, they must be converted to paths or outlines or they must be embedded in the files. Color images must be created/scanned and submitted as CMYK files. Files should be submitted electronically through the Editorial Manager interface. Please note that artwork generated from office suite programs such as Corel Draw and MS Word and artwork downloaded from the Internet (JPEG or GIFF files) cannot be used.

Supplemental Digital Content

Supplemental Digital Content (SDC): Authors may submit SDC via Editorial Manager to LWW journals that enhance their article's text to be considered for online posting. SDC may include standard media such as text documents, graphs, audio, video, etc. On the Attach Files page of the submission process, please select Supplemental Audio, Video, or Data for your uploaded file as the Submission Item. If an article with SDC is accepted, our production staff will create a URL with the SDC file. The URL will be placed in the call-out within the article. SDC files are not copy-edited by LWW staff, they will be presented digitally as submitted. For a list of all available file types and detailed instructions, please visit http://links.lww.com/A142.

SDC Call-outs

Supplemental Digital Content must be cited consecutively in the text of the submitted manuscript. Citations should include the type of material submitted (Audio, Figure, Table, etc.), be clearly labeled as "Supplemental Digital Content," include the sequential list number, and provide a description of the supplemental content. All descriptive text should be included in the call-out as it will not appear elsewhere in the article.

Example:
We performed many tests on the degrees of flexibility in the elbow (see Video, Supplemental Digital Content 1, which demonstrates elbow flexibility) and found our results inconclusive.

List of Supplemental Digital Content

A listing of Supplemental Digital Content must be submitted at the end of the manuscript file. Include the SDC number and file type of the Supplemental Digital Content. This text will be removed by our production staff and not be published.

Example:
Supplemental Digital Content 1. wmv

SDC File Requirements

All acceptable file types are permissible up to 10 MBs. For audio or video files greater than 10 MBs, authors should first query the journal office for approval. For a list of all available file types and detailed instructions, please visit http://links.lww.com/A142.

Tables
Create tables using the table creating and editing feature of your word-processing software (eg, Word, Word-Perfect). Do not use Excel or comparable spreadsheet programs. Group all tables at the end of the manuscript, or supply them together in a separate file. Cite tables consecutively in the text, and number them in that order. Key each on a separate sheet, and include the table title, appropriate column heads, and explanatory legends (including definitions of any abbreviations used). Do not embed tables within the body of the manuscript. They should be self-explanatory and should supplement, rather than duplicate, the material in the text.

Style
Pattern manuscript style after the *American Medical Association Manual of Style* (9th edition). Stedman's Medical Dictionary (28th edition) and Merriam-Webster's Collegiate Dictionary (11th edition) should be used as standard references. Refer to drugs and therapeutic agents by their accepted generic or chemical names, and do not abbreviate them. Use code numbers only when a generic name is not yet available. In that case, supply the chemical name and a figure giving the chemical structure of the drug. Capitalize the trade names of drugs and place them in parentheses after the generic names. To comply with trademark law, include the name and location (city and state in USA; city and country outside USA) of the manufacturer of any drug, supply, or equipment mentioned in the manuscript. Use the metric system to express units of measure and degrees Celsius to express temperatures, and use SI units rather than conventional units.

Obligation to Register Clinical Trials

JAIDS has adopted the standards of the International Committee of Medical Journal Editors with regard to the registration of clinical trials. As a condition of consideration for publication, data from research projects "prospectively assigning human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome" must be registered in a public trials registry. The Protocol Registration System (http://prsinfo.clinicaltrials.gov/) offered through the U.S. National Institutes of Health is one such registry.

GenBank Accession Numbers

When manuscripts include or describe original nucleotide or amino acid sequence data, the sequence must be submitted to the GenBank/EMBL/DDBJ sequence database and an accession number obtained from them. This accession number must be returned to the journal, where it will be placed after the Key Words on the title page in the printed article. URLs for the 3 members of the International Nucleotide Sequence Database Collaboration (GenBank/EMBL/DDBJ) are as follows (respectively): http://www.ncbi.nlm.nih.gov/BankIt/, http://www.ebi.ac.uk/embl/, http://www.ddbj.nig.ac.jp/.

ARTICLE TYPES

Original Articles

The above guidelines apply to the original article format. There is no length limitation for original articles, but authors are encouraged to be succinct, as papers that are overlong do not fare well in peer review.

Rapid Communications

Articles accepted as Rapid Communications will normally be published within 8 weeks of acceptance. When submitting a paper for consideration as a Rapid Communication, please adhere to the following guidelines:

- Submit your paper to Editorial Manager and designate the article type as "Rapid Communication." Please indicate to the Editor in a cover letter file why the paper merits special attention.
- If figures cannot be submitted to Editorial Manager, authors must send them by overnight express.
- The paper should not exceed 6 printed pages (approximately 24 double-spaced typewritten manuscript pages, including illustrations, tables, and references).
- The paper should include an abstract, key words, methods, results, discussion, and reference sections.
- The title page should include the corresponding author's telephone and fax numbers and e-mail address.
- Authors will receive proofs of their article for review by e-mail and will be expected to return corrections by fax within 24 hours of receipt. Changes received after this deadline will not be accepted.

Papers that are not accepted as Rapid Communications may be considered as full-length articles.

Critical Reviews

Papers reviewing the literature on a particularly timely and interesting topic will be considered for publication in ***JAIDS***. Authors are encouraged to keep review articles to less than 24 double-spaced manuscript pages, including references, tables, and figure legends. In general, review articles written as work-for-hire by industry employees will not be considered for publication. All funding, writing assistance, and other relationships to possibly conflicted sources must be fully disclosed at the time of submission.

Brief Reports

Brief Reports are short versions of clinical studies. They represent observations that are preliminary, speak for themselves, or offer new insight into a recognized condition. Submissions should not exceed 10 double-spaced manuscript pages, including references and table. Manuscripts that are too long for this category will be shortened at the editorial office or returned to the author for shortening.

Letters to the Editor

Letters to the Editor can provide additional comment on an article published in *JAIDS*, or can be a very concise report on study findings. Letters should be no more than 3 typeset pages (6 manuscript pages double-spaced, including references and either 1 figure or 1 table [but no abstract]).

Page Proofs and Corrections

Portable document format (PDF) files of the copyedited, typeset pages and support documents (eg, reprint order form) will be sent to the corresponding author via e-mail. Complete instructions will be provided with the e-mail for downloading and printing the files and for faxing the corrected pages to the publisher. It is the author's responsibility to ensure that there are no errors in the proofs. Changes that have been made to conform to journal style should be allowed to stand if they do not alter the authors' meaning. Only critical changes improving the accuracy of the content will be made. Changes that are stylistic or are a reworking of previously accepted material will not be allowed. The publisher reserves the right to deny any changes that do not affect the accuracy of the content. Authors may be charged for alterations to the proofs beyond those required to correct errors or to answer queries. Proofs must be checked carefully and corrections faxed within 24 to 48 hours of receipt, as requested in the cover letter accompanying the page proofs.

Reprints

Reprint orders should be submitted to the Reprints Department (1-800-341-2258 or reprints@LWW.com). Payment for reprints or a purchase order number must accompany your order form. Orders without these cannot be fulfilled.

Anexo 6 - Instruções para publicação de artigos no periódico *Antiviral Therapy*

Antiviral Therapy
Guidelines for preparation of manuscripts

EDITORIAL POLICY

Antiviral Therapy welcomes the submission of high-quality research on the clinical development and use of antiviral agents and vaccines, and the treatment of all virus diseases.

Manuscripts submitted to *Antiviral Therapy* are considered for publication on the understanding that the work contained therein has not been submitted simultaneously to another journal. Copies of related manuscripts submitted elsewhere or in press should accompany the submitted manuscript.

All submissions must be accompanied by a covering letter (a letter template is available on the online submission website), signed by all the authors (or the corresponding author on behalf of all others) stating that all authors have contributed to the paper and are familiar with the contents of the final draft, and that all authors meet the criteria for authorship as established by the International Committee of Medical Journal Editors. The letter should also state whether any author has any conflict of interest. You must declare sources of funding, any influence the funding source may have had on the analysis and reporting of the results and any related interest in the Acknowledgements section. Illustrations and other material obtained from other sources must be acknowledged and permission for reproduction must be obtained from the publisher.

All manuscripts should be submitted via the online submission site. Manuscripts should not be submitted via e-mail or post.

Papers will be peer reviewed and assessed statistically before acceptance. Priority and time of publication of accepted material will be decided by the editors. The editors retain the right to shorten material accepted for publication. This can include subediting the text for style. The editors endorse the guidelines on good publication practice from the Committee on Publication Ethics (COPE).

Ethics

Papers based on clinical investigation must conform to ethical standards as set out in the Declaration of Helsinki. Reports describing data obtained from experiments performed in animals must clearly indicate that humane standards were adhered to.

For experiments on isolated tissues the paper must indicate precisely how the donor tissue was obtained. The NIH *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publications) gives guidelines for the acquisition and care of animals.

Randomized controlled trials

Authors are requested to report these in accordance with the CONSORT (Consolidated Standards of Reporting Trials) statement (Hopewell S, *et al.* 2008 *PLoS Med* **5**(1): e20 doi:10.1371/journal.pmed.0050020). This ensures that enough information is provided for editors, peer reviewers and readers to see how the study was performed and to judge whether the findings are likely to be reliable. For behavioural and public health evaluations involving nonrandomized designs, authors should include with their submission a complete checklist from the TREND statement.

Observational studies

Observational studies (cohort, case-control or cross-sectional designs) should be reported according to the STROBE recommendations (see www.strobe-statement.org).

Systematic reviews

Authors are requested to report these in accordance with the QUOROM (Quality of Reporting of Meta-analyses) statement (*Lancet* 1999; **354:**1896–1900) and the Cochrane Collaboration guidelines. When conducting any literature review, it is important that there is complete transparency concerning the choice of material included. All literature reviews must therefore contain a brief section entitled *Search strategy and selection criteria*. This should state

clearly the sources (databases, journals, or book reference lists, etc) of the material covered and the criteria used to include or exclude studies; for example, English language only or studies conducted after a specific date.

SUBMISSION CATEGORIES

Case reports

1,500 words; ≤ 20 references; ≤ 3 display items

A case report should cover the details of an unusual case or any case that warrants discussion in the journal. However, case reports can be published as an article if the editor feels that the author has adequately extended the report into a topic discussion that meets the criteria for an article, or if the case report itself is used as an example of a point in the author’s article.

Correspondence

1,000 words; ≤ 15 references

The correspondence section is for letters that are addressing issues or exchanging views on topics arising from published articles in *Antiviral Therapy*. Correspondence should not exceed 1,000 words.

Meeting report

1,500 words; ≤ 20 references

Antiviral Therapy encourages submissions on written reports from relevant and recent workshops and conferences. An abstract is not required.

Original articles

≤ 4,000 words; ≤ 50 references; ≤ 5 display items

Review articles

3,000–5,000 words; ≤ 100 references; ≤ 5 display items

Reviews are usually commissioned, but unsolicited reviews may occasionally be considered.

Articles will be assessed in-house and those considered suitable will be peer reviewed before an editorial decision is made. Reviews should either be definitive overviews of a major topic connected with antiviral therapies or updates of knowledge in a somewhat narrower field of current interest. The word count will depend on the breadth of the topic. All reviews should be prefaced by a summary of 100–120 words. The summary is important: it should contain sufficient information for the reader to be able to appreciate the relevance of the full article when read alone. Summaries are used by abstracting services and many users of these services read only the summary. It should include background information and specific examples of recent advances. References should not be included and abbreviations should be avoided as far as possible in the summary. References selected for publication in the article should be chosen for their importance, ease of access, and for the ‘further reading’ opportunities they provide. *Antiviral Therapy* welcomes systematic reviews (see Systematic reviews, above).

Short communication

1,500 words; ≤ 20 references; ≤ 3 display items

Original research findings that do not require a full paper, but are completed studies, may be submitted as a short communication. All short communications should contain an introduction, methods, results and discussion section.

MANUSCRIPT FORMAT

Manuscripts should be prepared in accordance with 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals' (International Committee of Medical Journal Editors).

All manuscripts should be submitted using editable files; manuscripts will be converted to PDFs for peer review. No page charges will be made for standard text, diagrams and black & white photographs. The use of colour figures will incur a page charge, due to the additional printing costs involved.

The manuscript should contain the following:

Title

Use of abbreviations should be avoided in the title and it should not exceed three typeset lines (~150 characters, including spaces).

Authors

Forenames and brief addresses should be included. If an author's current affiliation address differs from the one at which the research was conducted this may also be indicated. Authors (including members of study groups, working group and the like) included in the byline must satisfy the criteria of authorship given by the International Committee of Medical Journal Editors. All individuals who qualify for authorship must be included. A multicentre group can be credited with sole authorship; however, the group must identify and include in their manuscript a corresponding author on the title page and the principle investigators who accept direct responsibility for the manuscript in a separate section before the references. These principle investigators should fully meet the criteria for authorship defined by the International Committee of Medical Journal Editors and be willing to complete relevant journalspecific forms on behalf of the group. A full membership list will be published as an additional file.

Corresponding author details

Name, address, telephone and fax numbers, and e-mail address should be supplied. Unless instructed otherwise the editorial office will send page proofs of the article to this e-mail address.

Running head

A running header of up to 75 characters should be supplied. This will appear at the top of each right-hand page.

Structured abstract

The headings Background, Methods, Results, Conclusions should be used. The abstract must not exceed 250 words. All abbreviations should be defined at first mention. References and display item citations must not appear in the abstract, and the abstract must be clear and comprehensible in its own right.

Main text

Methods

The supplier (with brief address e.g. town, state and country) must be given for all laboratory equipment and materials.

Acknowledgements

Acknowledgements should be made to those, not including the authors, who have made a substantial contribution to the study. Authors are responsible for obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions. Details of sources of funding, editorial support and previous presentation of should be placed in this section, when appropriate. A summary of the role of each author on a collaborative paper may also be included.

Disclosure statement

All conflicts on interest relevant to the article should be disclosed in this section. If there are no conflicts of interest, a sentence to this effect should be included.

References

The accuracy of references is essential and this remains the responsibility of the author. As formatting information (italics, special characters and subscript and superscript text) is often lost on PubMed and other websites, the original versions should be consulted. References must be cited numerically by order of appearance in the text and listed in the bibliography. References should be cited in square brackets, for example, [3] or [1,3–5]. The manuscript bibliography must present full references in the formats given below. In the full list of references give the names and initials of all authors. If there are more than six, cite only the first three, followed by *et al*. The authors' names are followed by the title of the article, the title of the journal (italics) abbreviated according to the style of Index Medicus, the year of publication; the volume number (in bold) and the first and last page numbers in full followed by a full stop. Titles of books should be followed by the city of publication, the publisher, the year and inclusive page numbers. See the following examples:

Standard journal article

1. Bar S, Alizon M. Role of the ectodomain of the gp41 transmembrane envelope protein of human immunodeficiency virus type 1 in late steps of the membrane fusion process. *J Virol* 2004; **78:**811–820.

More than six authors

2. Hirsch MS, Brun-Vézinet F, Clotet B, *et al*. Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society-USA Panel. *Clin Infect Dis* 2003; **37:**113–128.

Translated journal title

3. Schäfer W. Vergleichende sero-immunologische Untersuchungen über die Viren der Influenza und klassichen Geflügelpest [Comparative sero-immunological investigations on the viruses of influenza and classical fowl plague]. Zeitschrift für Naturforschung 1955; **10b:**81–91. German.

Book

4. Glantz SA. *Primer of Biostatistics*. 3rd edn. New York: McGraw-Hill 1997; pp. 440.

Chapter in a book

5. Jilbert AR, Burrell CJ, Triatni M, Kann M. Hepatitis B virus replication. In *Human Virus Guide: Hepatitis B Virus*. Edited by CL Lai and S Locarnini. London: International Medical Press; 2002. pp. 43–53.

Abstract

6. Torriani F, Rockstroh J, Rodriguez-Torres M, *et al*. Final results of APRICOT: a randomized, partially blinded, international trial evaluating peginterferon-alfa-2a + ribavirin vs. interferon-alfa-2a + ribavirin in the treatment of HCV in HIV/HCV co-infection. *11th Conference on Retroviruses & Opportunistic Infections*. 8–11 February 2004, San Francisco, CA, USA. Abstract 112.

Website

7. Panel on Antiretroviral Guidelines for Adult and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services (Updated 10 October 2006. Accessed 3 August 2007.) Available from http://aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf

Prescribing information

8. Viread (tenofovir disoproxil fumarate). *Package insert* 2005. Gilead Sciences, Foster City, CA, USA.

Patents

9. Hurst DN, Jones PS, Parkes KEB, Parratt MJ, Wilson FX, inventors; Hoffmann-La Roche Inc., assignee. Inhibitors of HPV E1 helicase enzyme. United State patent US 6703387. 2004 March 9.

Display items

References to figures and tables should be made in order of appearance in the text and shouldbe in Arabic numerals in parentheses, e.g. (Figure 2). Any abbreviation used in a figure or table must be defined, even if it has already been defined in the main text. Units should be stated after a comma, for example ‘Time, years’ or ‘HCV RNA, log_{10} copies/ml’.

If a figure or table has been published before, in total or in part, the original source must be acknowledged and written permission from the copyright holder for both print and electronic formats should be submitted with the material. It is the responsibility of the author to obtain this permission, and failure to do so may delay publication. Permission is required regardless of authorship or publisher, except for documents in the public domain.

Tables

Create tables using the table editor of a word processing package. Do not embed tables as images in the manuscript file or upload tables in image or PDF formats. Each piece of data needs to be contained in its own cell in the table. Vertical rules should not be used. Avoid creating tables using spaces or tabs. Do not align cells with hard returns or extra spaces. Furthermore, no cell should contain a hard return or tab. Although individual empty cells are acceptable, be sure there are no empty columns. Tables should not be split into separate sections and should not exceed one typeset page in size.

Each table should be assigned an Arabic numeral and a brief title. Identify all statistical measures, along with all units. Place explanatory matter in footnotes, not in the heading or within the table. The footnote symbols used in the journal are *a*, *b*, *c*, *d*, *e*, *f* and so on.

Please note, accepted manuscripts not following these table guidelines must be retyped during the editing process, which may result in delays and opportunities for error.

Figures

Figures may be reduced, cropped or omitted at the discretion of the editor. Colour illustrations are acceptable, but authors will be expected to cover the extra reproduction costs (for current charges, contact the publisher). Chemical schemes should be supplied as standard figures and be called figures. Where a figure has several parts these should be labelled with upper-case letters. No lettering should be placed directly over images. Amino acid sequences should be given in Courier or a similar monospaced font.

For ease of review, clear, complete figures should be submitted in .jpg or .pdf.

Should the article be accepted for publication, the authors should be ready to supply print-quality images in cases of electron micrographs, images of blots and gels, computer-generated protein structures and the like. This will ideally be in .eps format for computer-generated images (all programs have the facility to print to an .eps file) or .tif format for photographic images. Images intended to be printed across the width of one column should be a minimum of 600 pixels (5 cm wide at 300 dpi) in width. Those intended to occupy two columns should be a minimum of 1,300 pixels (11 cm at 300 dpi) in width. It is also worth remembering that RGB colour profile figures will necessarily be converted to CMYK for printing – therefore if possible authors should supply figures as CMYK, to ensure that the alteration in colour is satisfactory.

Graphs, schemes and simple diagrams will be redrawn by in-house illustrators so the initially submitted images will usually be sufficient.

Supplementary material

Supplementary online data can be published as part of the online journal. It should be referred to within the text. Lists of members of study groups and the like can also be incorporated into the supplementary material. Supplementary material will be reproduced as submitted and will not be subedited or styled by the editorial office.

GENERAL POINTS ON MANUSCRIPT PREPARATION

Drug names

International non-proprietary names for drugs should be used throughout the text.

Spelling

Spelling should follow the Oxford English Dictionary.

Units of measurement

SI units of measurement should be used wherever applicable. Temperatures should be given in degrees Celsius. Blood pressures should be given in millimetres of mercury.

Abbreviations and symbols

The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement. Abbreviations which have been defined in the abstract must be defined again at first mention in the main text. Abbreviations should only be used when helpful to the reader, as an improvement in clarity. As a guideline, use abbreviations for terms appearing three or more times in the article. Use only standard abbreviations. Avoid abbreviations in the title and abstract. All abbreviations must be redefined in each table and figure in which they are used.

PROOFS

Page proofs of articles will be sent to the corresponding author shortly before publication and these should be corrected and returned within 2 days. For this reason please ensure that the email address supplied is checked regularly. Only corrections and essential changes should be made and the cost of additional changes will be charged to the authors. However, in some cases information that has become available since acceptance of the manuscript may be included as an addendum in proof. This is at the discretion of the editors. The editors reserve the right to make minor modifications to the manuscripts. Material changes will be submitted to the authors for approval at the proof stage.

COPYRIGHT ASSIGNMENT

To facilitate effective dissemination of the article, copyright must be transferred to the publisher before publication. Copyright assignment forms are available from the editorial office, but will usually be supplied with the page proofs of the article.

OFFPRINTS

The corresponding author of each published article may obtain a PDF of the final version of the article from the editorial office. Authors can purchase offprints at reduced rates and a form will be sent along with the page proofs.

EDITORIAL OFFICE

International Medical Press
36 St Mary at Hill
London
EC3R 8DU
UK
Tel: +44 (0) 20 7398 0700
Fax: +44 (0) 20 7398 0701
E-mail: info@intmedpress.com
Web: http://www.intmedpress.com

Livros Grátis

(http://www.livrosgratis.com.br)

Milhares de Livros para Download:

Baixar livros de Administração
Baixar livros de Agronomia
Baixar livros de Arquitetura
Baixar livros de Artes
Baixar livros de Astronomia
Baixar livros de Biologia Geral
Baixar livros de Ciência da Computação
Baixar livros de Ciência da Informação
Baixar livros de Ciência Política
Baixar livros de Ciências da Saúde
Baixar livros de Comunicação
Baixar livros do Conselho Nacional de Educação - CNE
Baixar livros de Defesa civil
Baixar livros de Direito
Baixar livros de Direitos humanos
Baixar livros de Economia
Baixar livros de Economia Doméstica
Baixar livros de Educação
Baixar livros de Educação - Trânsito
Baixar livros de Educação Física
Baixar livros de Engenharia Aeroespacial
Baixar livros de Farmácia
Baixar livros de Filosofia
Baixar livros de Física
Baixar livros de Geociências
Baixar livros de Geografia
Baixar livros de História
Baixar livros de Línguas

Baixar livros de Literatura
Baixar livros de Literatura de Cordel
Baixar livros de Literatura Infantil
Baixar livros de Matemática
Baixar livros de Medicina
Baixar livros de Medicina Veterinária
Baixar livros de Meio Ambiente
Baixar livros de Meteorologia
Baixar Monografias e TCC
Baixar livros Multidisciplinar
Baixar livros de Música
Baixar livros de Psicologia
Baixar livros de Química
Baixar livros de Saúde Coletiva
Baixar livros de Serviço Social
Baixar livros de Sociologia
Baixar livros de Teologia
Baixar livros de Trabalho
Baixar livros de Turismo