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Estudo do Síndrome da Lipodistrofia e das Repercussões Endócrino-metabólicas e Cardiovasculares na Infeção por VIH

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À memória dos meus pais
À família
Aos Amigos

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De acordo com o nº2 do artigo 8º do Decreto-Lei nº 388/70, fazem parte desta dissertação as seguintes publicações:

1. Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. Nature Genetics 2001; 28: 365-370.
2. Lipodystrophy: The metabolic link of HIV infection with insulin-resistance syndrome (aceite provisoriamente para publicação como capitulo de um livro - "HIV Infection", Intech, ISBN 980-953-307-754-8).
3. Fat Mass Ratio: an objective tool to define lipodystrophy in HIV-infected patients under antiretroviral therapy. Journal of Clinical Densitometry: Assessment of Skeletal Health, vol. 13, no. 2, 197-203, 2010.
4. Assessment of body fat composition disturbances by bioimpedance analysis in HIV infected patients. J Endocrinol Invest. 2011 Jul 5. PMID: 21737998.
5. Impact of lipodystrophy on the prevalence and components of metabolic syndrome in HIV-infected patients. BMC Infectious Diseases 2011, 11: 246.
6. Prevalence of obesity and its relationship to clinical lipodystrophy in HIV-infected adults on anti-retroviral therapy. J Endocrinol Invest. 2011. Dec 16. PMID: 22186163.
7. Central/peripheral fat mass ratio is associated with increased risk of hypertension in HIV-infected patients (aceite para publicação no The Journal of Clinical Hypertension).
8. Lipodistrophy defined by Fat Mass Ratio in HIV infected patients is associated with high prevalence of glucose disturbances and insulin resistance (submetido à BMC Infectious Diseases).
9. Emerging laboratory cardiovascular risk factors in HIV-infected patients with objectively defined lipodystrophy (submetido à Lipids).
10. Adipokines, hormones related to body composition, and insulin resistance in HIV fat redistribution syndrome (submetido ao European Journal of Endocrinology).
11. Carotid intima media thickness is associated with body fat abnormalities in HIV-infected patients (submetido à AIDS).

A contribuição pessoal nestes trabalhos foi a seguinte: planeamento da investigação, recolha de dados, análise de resultados e redação de todos os manuscritos, exceção feita à primeira publicação elencada. A primeira publicação resultou da Conferência de Lipodistrofia diabética do tipo Berardinelli – Seip, na qual participou na elaboração do protocolo de diagnóstico e tratamento da doença e contribuiu com o estudo de 2 doentes. Coordenou a recolha de casos portugueses, tendo obtido informações de uma doente da Dra. Paula Bogalho incluída no estudo.

ABREVIATURAS EM INGLÊS

11β-HSD-1	11β-hydroxysteroid dehydrogenase type 1
ACS	acyl-CoA synthetase
AGPAT2	acyltransferase 1-acylglycerol-3-phosphate O- acyltransferase 2
ART	antiretroviral therapy
BMI	body mass index
BSCL	Berardinelli-Seip syndrome
C/EBPα	enhancer-binding protein-α
cART	combined antiretroviral therapy
CDC	Centers for Disease Control and Prevention criteria for staging of HIV infection
CGL	congenital generalized lipodystrophy
CL	clinical lipodystrophy
CRABP-1	cytoplasmic retinoic acid-binding protein type 1
CRP	C reactive protein
CT	computed tomography
CV	cardiovascular
d4T	stavudine
DGAT	diacylglycerol acyltransferase
DM	diabetes mellitus
DXA	dual-energy X-ray absorptiometry
FABP [pm]	plasma membrane-associated fatty acid-binding protein
FFA	free fatty acids
FMR	fat mass ratio
FMR-L	FMR defined lipodystrophy
FPL	familiar partial lipodystrophy
FXR	farnesoid X receptor
HAART	highly active antiretroviral therapy
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
HOMA	the homeostasis model assessment of insulin resistance
ICAM-1	intercellular adhesion molecule 1

IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IL	interleukins
IL-6	interleukin – 6
IMT	intima-media thickness
IR	insulin resistance
LRP	lipoprotein-receptor-related protein
MAD	mandibuloacral dysplasia
MCP-1	monocyte chemoatractant protein -1
MS	metabolic syndrome
mtDNA	mitochondrial DNA
NASH	nonalcoholic steatohepatitis
NFkB	nuclear factor- kB
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor-1
PI	protease Inhibitor
PPARγ	peroxisome proliferator-activated receptor-γ
ROS	reactive oxygen species
SAT	subcutaneous adipose tissue
SREBP-1	sterol-regulatory element binding protein-1
TLR-4	Toll-like receptor-4
TNF-α	tumor necrosis factor-α
tPA	tissue plaminogen activator
VAT	visceral adipose tissue
VCAM-1	vascular adhesion molecule 1
VLDL	very low-density lipoprotein
ZMPSTE24	zinc metalloproteinase

ABREVIATURAS EM PORTUGUÊS

VIH	virus da imunodeficiência humana
SIDA	síndrome da imunodeficiência adquirida
PCR	proteina C reactiva

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1. INTRODUÇÃO GERAL

1. INTRODUÇÃO GERAL

Esta dissertação, que apresento à Faculdade de Medicina da Universidade do Porto, resulta de uma investigação clínica na área da lipodistrofia relacionada com o vírus da imunodeficiência humana.

O meu interesse pelas lipodistrofias começou em 1997 quando o Professor Doutor José Luís Medina me convidou para o representar na Primeira Conferência de Lipodistrofia Diabética e apresentar o caso clínico de duas irmãs com síndrome de Berardinelli-Seip. Uma delas foi seguida por mim na consulta externa de Endocrinologia durante o Internato Complementar realizado sob orientação do Dr José Pedro Lima Reis.

Nesse ano fui a Bruxelas à Primeira Conferência de Lipodistrofia Diabética do tipo Berardinelli-Seip apresentar o caso clínico dessas 2 irmãs com lipodistrofia generalizada congénita do tipo Berardinelli-Seip, fui membro do Comité para o estudo da lipodistrofia diabética e participei na elaboração do primeiro protocolo de diagnóstico e tratamento desta doença. A minha participação foi emocionante por duas razões: primeiro, porque tive o grato prazer de conhecer pessoalmente o Professor Seip e segundo, porque pela primeira vez, como interna do terceiro ano do internato complementar tive o prazer e o privilégio de conhecer pessoalmente vários proeminentes investigadores na área das lipodistrofias que apenas conhecia dos artigos que lera até à exaustão para preparar a minha primeira comunicação em inglês. O stress induzido provocou uma alteração epigenética que terá condicionado todo o meu futuro.

Em 1998, o professor Leonel Van Maldergem (sendo eu também coautora) apresentou um trabalho no “European Society of Human Genetics Congress” intitulado “Berardinelli-Seip Syndrome: a new Portuguese disease?” (European Journal of Human Genetics 1998; 74: P1-160), colocando a hipótese de que no século XVI ocorrera uma mutação e os portugueses teriam “disseminado” a doença para o Brasil, Europa e Ceilão (Sri-Lanka), já que, muitos dos doentes estudados - e na altura eram menos de 100, ou tinham ancestralidade ou sobrenomes portugueses.

Lembro que nessa altura não se sabia quase nada acerca da genética deste tipo de lipodistrofia. E será, pela primeira vez em 1999, o professor Abhimanyu Garg de Dallas, Texas a descobrir um gene associado à doença: "A gene for congenital generalized lipodystrophy maps to human chromosome 9q34. J Clin Endocrinol Metab 1999; 84: 3390".

Dois anos mais tarde, a professora Jocelyne Magré de Paris identificou em vários doentes e nas nossas próprias doentes um outro gene: "Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13". O trabalho foi publicado na Nature Genetics (Nature Genetics 2001; 28: 365-370), tendo como coautores eu e o Professor JL Medina.

Anos mais tarde, ao fazer uma pesquisa bibliográfica, fiquei surpreendida ao verificar a existência de doentes com fenótipo semelhante ao dos portadores de lipodistrofia tipo Berardinelli Seip, mas que correspondiam a uma forma adquirida de lipodistrofia associada à infeção VIH. Nessa altura, ainda se desconhecia de que forma a infeção VIH se relacionava com a lipodistrofia.

Em 1999, Carr e colaboradores, descreveram pela primeira vez uma forma de lipodistrofia que se associava à terapêutica antirretrovírica "Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study" Lancet 1999; 353:2093.

Quando em 2005 o Professor Doutor José Luís Medina me convidou a iniciar um trabalho de investigação tendo como objetivo o meu doutoramento pareceu-nos interessante estudar esta forma nova de lipodistrofia.

Em 16 de fevereiro de 2006 efetuei provas de avaliação do ano probatório de doutoramento e obtive a inscrição definitiva como estudante de doutoramento.

Durante os últimos 6 anos, como estudante de doutoramento "em tempo parcial", desenvolvi em colaboração com de colegas de outras especialidades e sob indicação dos meus, orientador e coorientador, e do Professor Doutor Davide Carvalho, investigação na área da lipodistrofia VIH. Os nossos resultados refletem-se nas dez publicações que fazem parte desta tese mas também nas 62 comunicações de trabalhos originais em reuniões científicas, 45 ações de ensino pós-graduado, 4 bolsas de investigação, 5 prémios nacionais e 2 prémios internacionais.

Dos onze artigos que constituem esta tese, o primeiro é o ponto de partida da minha viagem pelo mundo das lipodistrofias, iniciada com as lipodistrofias congénitas, nomeadamente a de tipo Berardinelli-Seip. Esse artigo, de que eu e o Professor Doutor José Luís Medina somos coautores, identificou um novo gene alterado no cromossoma 11 associado à lipodistrofia congénita generalizada de tipo Berardinelli-Seip.

O primeiro e o segundo artigo fazem parte da introdução desta tese. O segundo é um artigo de revisão que se foca na etiopatogenia das lipodistrofias genéticas e adquiridas, com ênfase na forma mais comum – a lipodistrofia associada ao VIH – desde a etiologia às complicações. Nele, analisamos o papel relativo dos diferentes tipos de tecido adiposo, nomeadamente o efeito protetor cardiovascular do tecido adiposo subcutâneo. Discutimos também os fatores relacionados com a redistribuição do tecido adiposo nos doentes infetados pelo VIH, nomeadamente o impacte da terapêutica antirretrovírica, o papel da inflamação, fatores genéticos, lipotoxicidade e cortisol. Também fizemos uma revisão dos mecanismos da insulinorresistência, dislipidemia, síndrome metabólico e das sequelas associadas à lipodistrofia VIH, nomeadamente disfunção endotelial, doença e mortalidade cardiovascular.

Ao avançar com a investigação, sentimos a necessidade de um método simples e objetivo para definir lipodistrofia embora, ao longo dos anos, já tivessem surgido metodologias diferentes com essa finalidade: a autodeclaração pelo doente, a avaliação clínica subjetiva do médico baseada na sua experiência e um método definido por Carr complexo e pouco prático para uso na clínica diária, "HIV lipodystrophy case definition" Lancet 2003; 361:726.

Dessa necessidade de uma definição simples e objetiva surge o terceiro artigo – "Fat Mass Ratio: an objective tool to define lipodystrophy in HIV-infected patients under antiretroviral therapy", que nos permitiu definir de um modo objetivo, baseado na gordura corporal avaliada por densitometria, a presença de lipodistrofia, fixar pontos de corte de acordo com o género e precisar, pela primeira vez, um ponto de corte para lipodistrofia para o sexo feminino. Parece-nos que o "Fat Mass Ratio" (FMR) é uma ferramenta de diagnóstico que permitirá a comparação de diferentes estudos e que também poderá ser útil para monitorizar a progressão da lipodistrofia nesta população.

A avaliação da composição corporal dos doentes é feita na prática clínica, com medidas antropométricas. Os doentes que têm lipodistrofia associada ao VIH podem

ter vários padrões de composição corporal passível de identificação clínica, nomeadamente por antropometria, mas a bioimpedância elétrica poderá tornar-se numa ferramenta útil e fácil porque permite uma avaliação da massa gorda e da massa não gorda e nos dá um quadro da composição corporal que não é possível apenas com antropometria. Essa avaliação é descrita no quarto artigo – "Assessment of body fat composition disturbances by bioimpedance analysis in HIV infected patients".

Se por um lado, a terapêutica antirretrovírica mudou o curso da história da infeção VIH, originando reduções significativas na morbilidade e mortalidade associada à SIDA, por outro lado, está associada a alterações da composição corporal (lipodistrofia) e alterações metabólicas, incluindo insulinorresistência, dislipidemia e hipertensão arterial. O síndrome metabólico refere-se a uma constelação de fatores de risco cardiovascular e é por si, um fator de risco de morbi-mortalidade e diabetes tipo 2. O objetivo no artigo nº 5 – "Impact of lipodystrophy on the prevalence and components of metabolic syndrome in HIV-infected patients" foi avaliar a prevalência do síndrome metabólico e determinar se os doentes com ou sem lipodistrofia têm uma prevalência diferente deste síndrome.

Se nos primeiros anos da infeção pelo VIH o "wasting syndrome" era a "marca" da SIDA, com a introdução da terapêutica antirretrovírica altamente ativa e com a subsequente supressão da replicação viral e aumento dos CD4+ a sua incidência diminuiu de forma drástica. Mais recentemente, assistimos ao aparecimento de obesidade nos doentes infetados pelo VIH. Os dados relativos à obesidade nesta população eram ainda escassos e a obesidade *per se* pode também contribuir para o aumento do risco cardiovascular. A publicação nº 6 – "Prevalence of obesity and its relationship to clinical lipodystrophy in HIV-infected adults on anti-retroviral therapy" aborda esse tema.

Em contraste com outras alterações metabólicas, poucos e controversos estudos existiam sobre os efeitos da terapêutica antirretrovírica na hipertensão arterial dos doentes infetados pelo VIH. E, em alguns estudos, o risco de hipertensão arterial nos doentes infetados pelo VIH associava-se com lipodistrofia, ao contrário do que acontece nas lipodistrofias genéticas, onde a diabetes é uma característica comum, mas não a hipertensão arterial. Dada esta controvérsia, a publicação nº 7 – "Central/peripheral fat mass ratio is associated with increased risk of hypertension in HIV-infected patients" teve como objetivo estimar a associação da composição corporal,

lipodistrofia definida pela clínica e pelo FMR e insulino-resistência na prevalência de hipertensão arterial.

A terapêutica antirretrovírica combinada associa-se também à insulinorresistência e à diabetes. No artigo nº8 – "Lipodistrophy defined by Fat Mass Ratio in HIV infected patients is associated with high prevalence of glucose disturbances and insulin resistance", procuramos comparar a prevalência de alterações da homeostasia da glicose e de insulinorresistência em doentes infetados pelo VIH sob terapêutica antirretrovírica de acordo com a presença de lipodistrofia definida pela clínica e pelo FMR e em diferentes padrões de distribuição da gordura corporal e estabelecer os principais responsáveis destas alterações.

A terapêutica antirretrovírica também se associa à dislipidemia e doença cardiovascular. Recentemente, têm sido implicados na aterosclerose acelerada fatores de risco emergentes, nomeadamente, apolipoproteina A1, apolipoproteina B, Lp (a), fibrinogénio, PCR de alta sensibilidade, homocisteína, microalbuminúria e o NT-proBNP. Na publicação nº 9: "Emerging laboratory cardiovascular risk factors in HIV-infected patients with objectively defined lipodystrophy" comparamos os lípidos séricos e os fatores de risco cardiometabólicos em doentes infectados pelo VIH sob terapêutica antirretrovírica de acordo com a presença de lipodistrofia definida pela clínica e pelo FMR e quatro padrões de distribuição da gordura corporal.

Como dissemos, a terapêutica antirretrovírica nos doentes infetados pelo VIH está associada a alterações metabólicas, incluindo dislipidemia, insulinorresistência e a lipodistrofia [diminuição da gordura periférica (lipoatrofia) com ou sem acumulação de gordura abdominal/visceral (lipohipertrofia)]. As alterações hormonais causadas pelo aumento (na lipohipertrofia) ou redução da gordura subcutânea (na lipoatrofia) podem ser fulcrais para as alterações metabólicas observadas nos doentes com lipodistrofia associada ao VIH. No artigo nº 10 avaliámos a relação entre hormonas derivadas do adipócito (adiponectina, leptina, resistina, TNF-α), hormonas orexigénicas (ghrelina), fatores protrombóticos (PAI-1) e insulinorresistência em doentes infetados pelo VIH sob terapêutica antirretrovírica de acordo com a presença de lipodistrofia definida pelo FMR e quadro padrões de distribuição da gordura corporal.

A determinação da espessura da íntima média carotídea tem sido usada como um marcador precoce de aterosclerose e é um fator de risco independente para

doença cardiovascular. Na publicação 11: “Carotid intima media thickness is associated with body fat abnormalities in HIV-infected patients” estudámos a espessura da íntima média carotídea em doentes infetados pelo VIH sob terapêutica antirretrovírica, de acordo com a presença de lipodistrofia definida pelo FMR e quatro padrões de distribuição da gordura corporal.

Assim, estas onze publicações abordam inicialmente aspetos das lipodistrofias de causa genética, depois das lipodistrofias adquiridas, nomeadamente a associada ao VIH, nas vertentes da etiopatogenia, definição objetiva, avaliação da composição corporal, alterações metabólicas (obesidade, hipertensão, insulinorresistência, diabetes, dislipidemia, síndrome metabólico, fatores de risco cardiovasculares emergentes), inter-relações da composição corporal com as adipocinas e finalmente a aterosclerose prematura, avaliada pela espessura da íntima média carotídea.

2. INTRODUÇÃO

2. INTRODUÇÃO

A introdução ao tema das lipodistrofias é constituída pelas publicações 1 e 2.

Publicação 1: Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. Nature Genetics 2001; 28: 365-370.

Publicação 2: Lipodystrophy: the metabolic link of HIV and genetic forms.

PUBLICAÇÃO 1:

Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13

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Identification of the gene altered in Berardinelli–Seip congenital lipodystrophy on chromosome 11q13

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Congenital generalized lipodystrophy, or Berardinelli–Seip syndrome (BSCL), is a rare autosomal recessive disease characterized by a near-absence of adipose tissue from birth or early infancy and severe insulin resistance[1–4]. Other clinical and biological features include *acanthosis nigricans*, hyperandrogenism, muscular hypertrophy, hepatomegaly, altered glucose tolerance or diabetes mellitus, and hypertriglyceridemia. A locus (*BSCL1*) has been mapped to 9q34 with evidence of heterogeneity[5]. Here, we report a genome screen of nine BSCL families from two geographical clusters (in Lebanon and Norway). We identified a new disease locus, designated *BSCL2*, within the 2.5-Mb interval flanked by markers *D11S4076* and *D11S480* on chromosome 11q13. Analysis of 20 additional families of various ethnic origins led to the identification of 11 families in which the disease cosegregates with the 11q13 locus; the remaining families provide confirmation of linkage to 9q34. Sequence analysis of genes located in the 11q13 interval disclosed mutations in a gene homologous to the murine guanine nucleotide-binding protein (G protein), γ3-linked gene[6] (*Gng3lg*) in all *BSCL2*-linked families. *BSCL2* is most highly expressed in brain and testis and encodes a protein (which we have called seipin) of unknown function. Most of the variants are null mutations and probably result in a severe disruption of the protein. These findings are of general importance for understanding the molecular mechanisms underlying regulation of body fat distribution and insulin resistance.

To localize the gene that, when mutated, causes BSCL, we undertook a genome-wide analysis of linkage and homozygosity mapping[7] in a set of families originating from two geographical clusters in Lebanon and Norway (Fig. 1*a*). We found no evidence of linkage to the 9q34 region previously reported[5]. However, on chromosome 11q13, two adjacent markers, *D11S4191* and *D11S987*, demonstrated segregation with the disease haplotype and frequent homozygosity among patients (Fig. 1*b*). Analysis of internal markers *D11S1765* and *D11S1883* showed the presence of identical alleles in all patients from Lebanon at marker *D11S1883*. The evidence for linkage was highly significant, with a maximum multilocus lod score of 13.2 (data not shown). Therefore, we designated *BSCL2* this new locus, located within an interval of approximately 8 cM (ref. 8).

We carried out fine-mapping using additional microsatellite markers (Fig. 1*b*). All affected individuals of the five Lebanese families are homozygous for the same alleles at nine contiguous markers within an interval spanned by markers *D11S4076* to *PYGM*, probably because of a founder effect. Among families clustered in the southwest region of Norway[2,4,9], we found that affected members of families BSCL-6, BSCL-7 and potentially BSCL-8 are homozygous for four consecutive markers, *D11S1765, D11S4076*, CA10 and CA9, confirming a founder effect. The affected individual of the fourth family (patient 26, BSCL-9) is heterozygous for this haplotype, indicating that despite consanguinity, he might have inherited a different disease allele on one chromosome. As the common region of homozygosity from the Lebanese and Norwegian patients overlap at adjacent markers CA10 and CA9, we inferred that *BSCL2* resides in the *D11S4076–D11S480* interval (spanning approximately 2.5 Mb based on radiation hybrid and physical mapping data).

We then tested 20 additional families of various ethnic origins at locus 11q13 with six markers spanning an interval of approximately 10 cM (ref. 8). Haplotype analysis indicates possible cosegregation with the disease in 11 families (Fig. 2). Two affected members (monozygotic twins) of family BSCL-16 are homozygous for a null allele at microsatellite CA10. Other nearby markers amplify correctly, indicating that these patients probably have a deletion in a restricted segment of DNA encompassing CA10. In the remaining nine families, most of African origin, the disease was not linked to locus 11q13 but, in contrast, cosegregates with the 9q34 locus, giving a maximum multilocus lod score of 2.4 (data not shown) and confirming the previous report of linkage to this region[5].

We identified 27 genes in the interval of *D11D4076–D11S480* using the publicly available sequence. One of these corresponds with a human mRNA AF052149 (ref. 10), contained within the BAC from which we originally isolated the marker CA10. AF052149 is homologous to cDNA of the mouse *Gng3lg* (ref. 6), of unknown function, which is localized in the region of mouse chromosome 19 that is orthologous to human 11q. Comparison between mouse and human sequences[6,10,11] showed that the human cDNA contained an open reading frame of 1,196 nucleotides, starting at position 345. The predicted protein,

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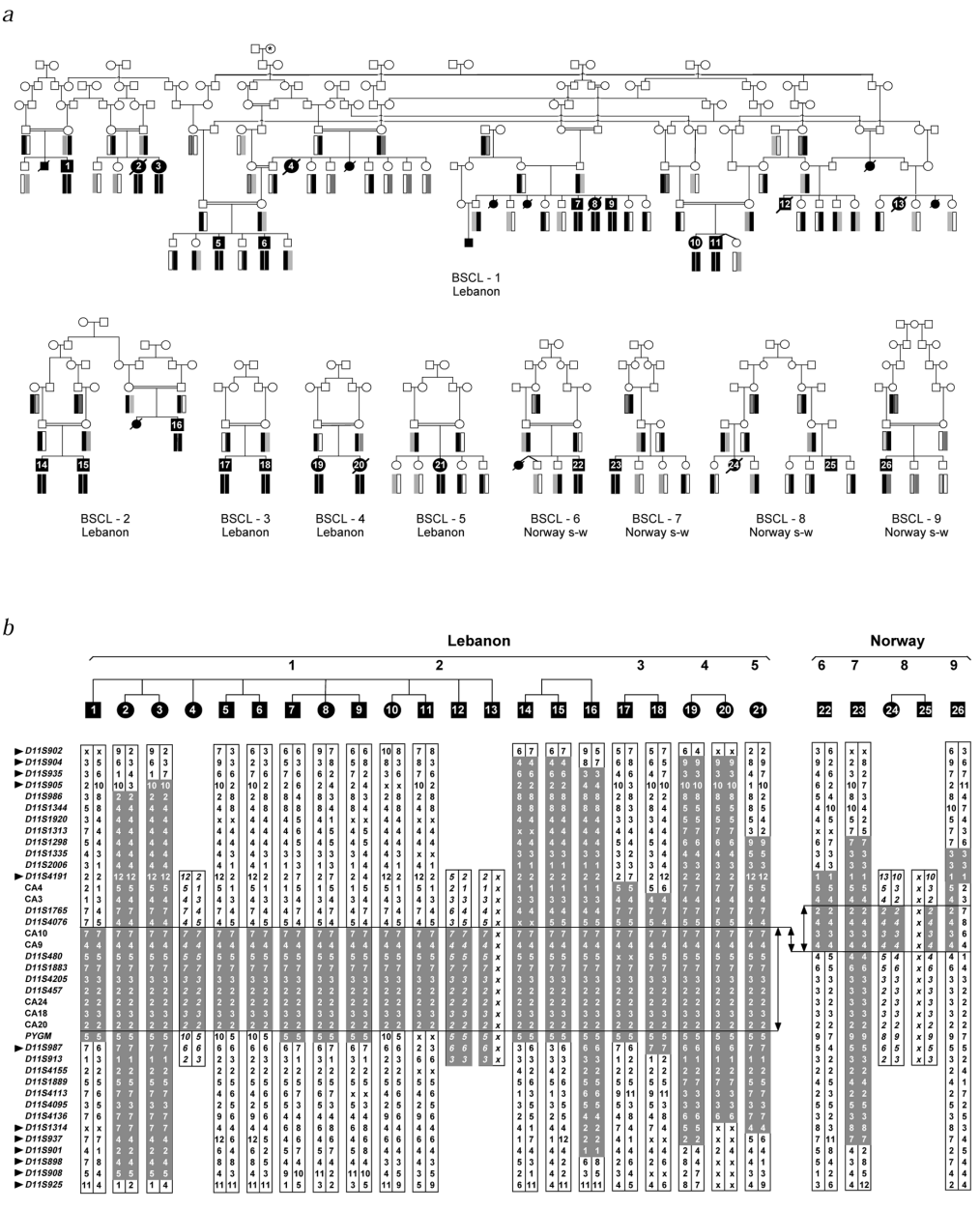

Fig. 1 BSCL patients and their families from two geographical clusters, Lebanon and Norway, used for genome-wide linkage analysis and homozygosity mapping. ***a***, The pedigrees have been simplified by showing non-affected siblings only when they were included in the genotyping. Filled symbols, affected individuals; open symbols, unaffected individuals. In family BSCL-1, the consanguinity has been inferred based on the surname of the individual indicated by the asterisk (*); this surname is common to other individuals in the pedigree. Colored bars below each individual indicate inferred 11q13 haplotypes; black indicates the haplotype that cosegregates with the disease in the two geographic clusters. ***b***, Inferred haplotypes for affected individuals based on refined mapping at locus 11q13. Grey boxes indicate regions of homozygosity. Left, microsatellite markers; arrows indicate markers used for the genome-wide scan. The numbering of affected individuals corresponds to that in *a*. Italics, genotypes inferred from relatives because of the lack of patient DNA. x, missing data.

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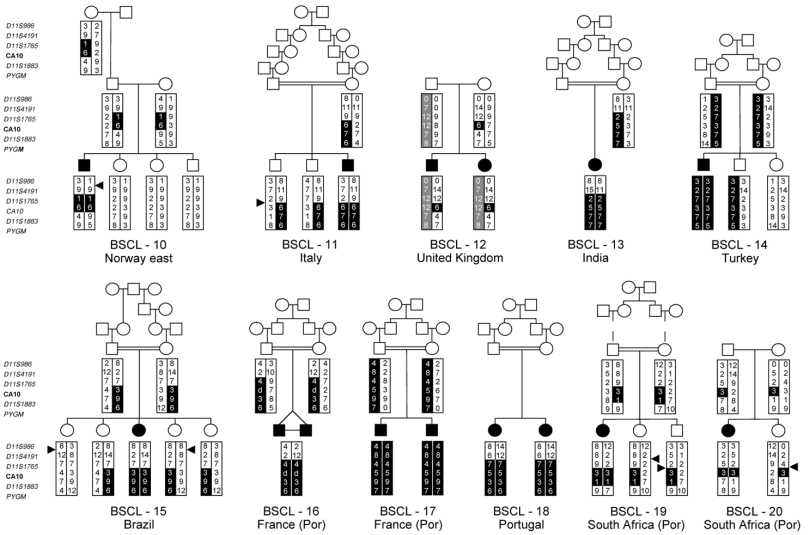

Fig. 2 Haplotype analysis in families of various ethnic origins in which the disease is inferred to cosegregate with locus 11q13. Individuals were genotyped with six markers (*D11S986*, *D11S4191*, *D11S1765*, CA10, *D11S1883* and *PYGM*) spanning an interval of ~10 cM. Portuguese origin is indicated as (Por) when this differs from the country of residence. In family BSCL-16, 'd' for the genotype of marker CA10 corresponds to a deletion. Arrows indicate recombination events.

seipin, has 398 amino acids with at least two hydrophobic amino acid stretches, indicating that it could be a transmembrane protein (Fig. 3*a*,*b*). It has 87% identity to the mouse *Gng3lg*, product and partial homology to the *Drosophila* CG9904 protein[12] (40% between residues 22 and 257). Seipin has no similarity with other known proteins or consensus motif that could predict its function.

We determined the structure of *BSCL2*. It contains 11 exons spanning at least 14 kb of DNA, with the putative initiation codon located in the second exon. The intron–exon boundaries show close adherence to 5′ and 3′ splice-site consensus sequences (Web Table A). Amplification of these exons in the two patients from family BSCL-16 revealed a deletion spanning exons 4, 5 and 6, including marker CA10 (Fig. 4*a*). We sequenced the 26 other genes in *D114076–D11S480* interval in four affected individuals of the 11q13-linked families and found no substantial molecular alterations.

We went on to analyze *BSCL2* in all the families and in 17 additional isolated BSCL individuals. We identified mutations in all the chromosome 11q13-linked families (Figs. 1*a*,*b* and 2) and in three isolated patients. Affected individuals are either homozygous for a specific mutation or are compound heterozygotes (Table 1). We analyzed the DNA of their parents whenever possible, and found it to be heterozygous with respect to *BSCL2* mutation, No unaffected siblings carry two mutations. We did

a

```
                    10          20          30          40          50
human       MVNDPPVPAL LWAQEVGQVL AGRARRLLLQ FGVLFCTILL LLWVSVFLYG
mouse       ··········· ·······H·· ·······M·· ··········· ···········
Drosophila  PAVNLGWNVY DRVRSKADEK V·TV·E·V·R L·LIAFAVV· II·LA··M·A

                    60          70          80          90         100
human       SFYYSYMPTV SHLSPVHFYY RTDCDSSTTS LCSFPVANVS LTKGGRDRVL
mouse       ··········· ·····H·· ·····A·· ··········· ·A·S·····
Drosophila  A···V···AI ··TR···MQF K·CLET··P- -·T··H·H·· ···--KQQL·

                    110         120         130         140         150
human       MYGQPYRVTL ELELPESPVN QDLGMFLVTI SCYTRGGRII STSSRSVMLH
mouse       ··········· ··········· ·········V ··········· ···········
Drosophila  ·V··A·K·IV NIDM····Q· LE····M·CA EMRDYDSMLR GH·C··A·MR

                    160         170         180         190         200
human       YRSDLLQMLD TLVFSSLLLF GFAEQKQLLE VELYADYREN SYVPTTGAII
mouse       ···Q···V·· ··L····· ·····S····· ···········
Drosophila  ···P·IR·IS ·W·L·P·YVL ·WK·EF·QVP ··IFSR·L·E RQH·I·DVYV

                    210         220         230         240         250
human       EIHSKRIQLY GAYLRIHAHF TGLRYLLYNF PMTCAFIGVA SNFTFLSVIV
mouse       ·····M···· ··········· ··········· ·····V···· ···········
Drosophila  ··Q·QK··F· TVT·H·V·D· ·····IMF·W ·VLS·IVAIS T·LF·IL·VF

                    260         270         280         290         300
human       LFSYMQWVWG GIWPRHRFSL QVNIRKRDNS RKEVQRRISA HQPGPEGQEE
mouse       ·········· AV····· ·····Q···· HHGAP····R ····Q-----·
Drosophila  ·L·WYH·SD- ---------- ---AKWLHSV QIKYA·LTKS LE··VIHSKA

                    310         320         330         340         350
human       STPQSDVTED GESPEDPSGT EGQLSEEEKP DQQPLSGEEE LEPEASDGSG
mouse       ··Q····· ··········· ·········· EKR··N···· Q·····-·-·
Drosophila  ·SLRD·DDD- ---------- --LVAYSD·S ·IADVG·DTL SDVD·D·LVL

                    360         370         380         390         398
human       SWEDAALLTE ANLPAPAPAS ASAPVLETLG SSEPAGGALR QRPTCSSS
mouse       ·--------- ··P·TS·S·· ·L·--····· ·-------·· ·------·
Drosophila  VKKSRSGKR· SPD------- ---------- --------··· K···KKTT
```

b

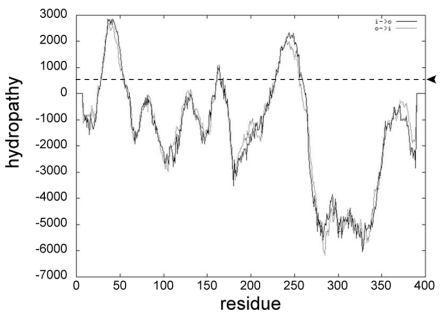

Fig. 3 Seipin characterization. ***a***, Protein alignment, showing similarities of human seipin with the *Mus Musculus Gng3lg* product and *Drosophila melanogaster* CG9904. Dots indicate conserved residues. The human amino acid sequence has high homology with the mouse sequence (87%) and partial homology to the fly sequence (40% between residues 22 and 257). ***b***, Hydropathy plot of seipin for prediction of transmembrane regions. Positive values indicate hydrophobicity. Hydrophobic amino acid stretches with a score above 500 indicate putative transmembrane domains. Two transmembrane domains are likely to be present at positions 28–49 and 237–258. A third transmembrane domain, not systematically identified in databases, might be present at residues 155–173.

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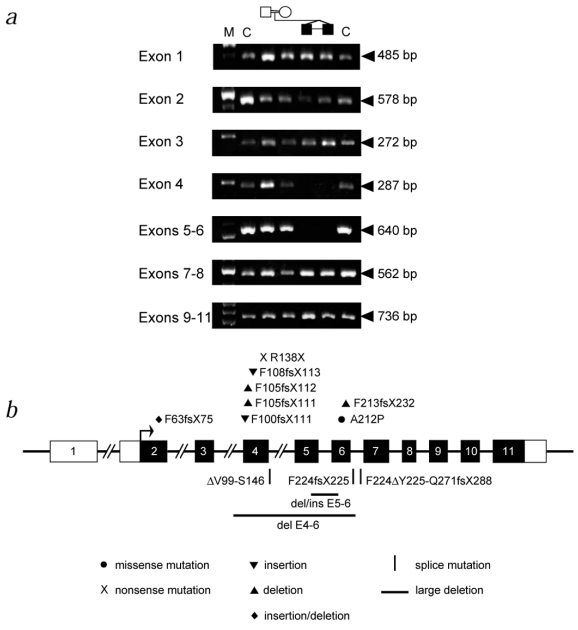

Fig. 4 Molecular alterations in *BSCL2*. ***a***, Detection of a deletion spanning exons 4–6 in individuals from the family BSCL-16. The 11 exons were amplified as 7 fragments. ***b***, Distribution of mutations along *BSCL2*. Three mutations are predicted to affect exon splicing. One is a transition at the first base of intron 4 (position +1: G→A), which belongs to a strictly conserved nucleotide consensus sequence for RNA (donor splice site). RT-PCR followed by sequencing demonstrated that this mutation induces skipping of exon 4, which results in an in-frame deletion of 48 amino acids. Another variant affects a nucleotide located next to the consensus sequence of the intron 6 acceptor splice site (position –3: G→C) and induces skipping of exon 7 and the shift of the reading frame introducing a premature stop codon. The third variant, which affects a nucleotide near the donor splice site in intron 6 (position +5: G→A), seems to induce the insertion of the first 76 nucleotides of intron 6 by activation of a cryptic splice site and the introduction of a premature stop codon.

not find any of these variants in 251 unrelated control individuals (96 CEPH family founders, 105 individuals of Lebanese origin and 50 individuals of Norwegian origin). We did not find any mutations in affected indviduals of the 9 families in which BSCL is not linked to chromosome 11q13 or in 13 additional patients who have been diagnosed with Lawrence syndrome[13] (in which lipoatrophy is not present at birth but develops at a later age). Thus, mutation of *BSCL2* causes disease in a large subset of our patients, all of whom present with the early-onset phenotype.

Identical *BSCL2* mutations in apparently unrelated families are accompanied by the same allele at marker CA10 (Figs. 1*b* and 2 and Table 1), indicating that they could have arisen from the same mutational event. All the Lebanese patients are homozygous for the same mutation, a 5-bp deletion in exon 4, which shifts the reading frame after amino acid 105, introducing a premature stop codon at position 111. Similarly, the affected members of the three families from southwest Norway (BSCL-6, -7 and -8) carry an identical missense mutation (A212P). As predicted from the marker haplotype data, the

Table 1 • Small mutations found in *BSCL2*

Gene position	Nucleotide alteration	Amino acid change[a]	Families #	Origin	Consanguinity	Patients Status	Age at onset
Exon 2	536delCCinsGGA	F63fsX75	9	Norway sw	+	het	birth
	"	"	10	Norway east	–	hom	birth
	"	"	11	Italy	+	hom	< 9 months
	"	"	12	United Kingdom	–	het	birth
Exon 4	645insAA	F100fsX111	15	Brazil	+	hom	0.5–1 month
	659delGT	F105fsX112	21	France	–	het[b]	3–6 months
	659delGTATC	F105fsX111	1	Lebanon	+	hom	0–1 month
	"	"	2	Lebanon	+	hom	birth
	"	"	3	Lebanon	+	hom	birth
	"	"	4	Lebanon	+	hom	birth
	"	"	5	Lebanon	+	hom	birth
	669insA	F108fsX113	19	South Africa (Por)	+	hom	birth
	"	"	20	South Africa (Por)	–	hom	birth
	756C→T	R138X	16	France (Por)	+	hom	birth
	"	"	17	Portugal	+	hom	~6 months
	"	"	23	Portugal	+	hom	birth
Intron 4	639/+1G→A (exon 4 skipping)	ΔV99-S146	14	Turkey	+	hom	birth
Exon 6	978G→C	A212P	6	Norway sw	+	hom	birth
	"	"	7	Norway sw	–	hom	birth
	"	"	8	Norway sw	–	hom	birth
	"	"	9	Norway sw	+	het	birth
	980delC	F213fsX232	13	India	+	hom	birth
Intron 6	1015/+5G→A (activation of a cryptic site)	F224fsX225	12	United Kingdom	–	het	birth
	1016/–3C→G (exon 7 skipping)	F224 ΔY225-Q271fsX288	22	France (Tur)	+	hom	birth

[a]Amino acid designation begins with '1' at codon 345 (translation start) of GenBank accession number AF052149. [b]Patient from family BSCL-21 is a compound heterozygote also carrying a 258-bp deletion/12-bp insertion in exons 5–6. het, compound heterozygote; hom, homozygous for the mutation; Por, Portuguese; Tur, Turkish origin; del, deletion; sw, southwest.

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affected individual of the fourth Norwegian family, BSCL-9, is a compound heterozygote carrying the mutation resulting in A212P and a frameshift mutation.

We identified 13 different mutations in *BSCL2* among 44 patients. These included two principal deletions, six small (less than 10 bp) insertions and deletions, and five single-nucleotide substitutions (Fig. 4*a*,*b* and Table 1). Nine of the mutations resulted in a frameshift or a premature stop codon. In one example, we identified a missense mutation that effects substitution of alanine with proline (A212P) and presumably alters the structure of the protein. In three patients, we found unique variants at or near a splice site that induced exon skipping and/or activation of cryptic splice sites (Fig. 4). Therefore, most of the variants carried by BSCL patients are null mutations that probably effect a severe alteration of the protein. Our data provide strong evidence that mutational events in *BSCL2* are responsible for the disease.

Tissue-specific expression of *BSCL2* reveals two RNA species of approximately 2.4 kb and 1.8 kb that are variably expressed in most tissues. Another transcript of approximately 2 kb is expressed, on its own, in brain and with other transcripts in testis (Fig. 5). We observe highest levels of expression in the brain and testis and dot blot analysis indicates that the gene is highly expressed in most regions of the central nervous system.

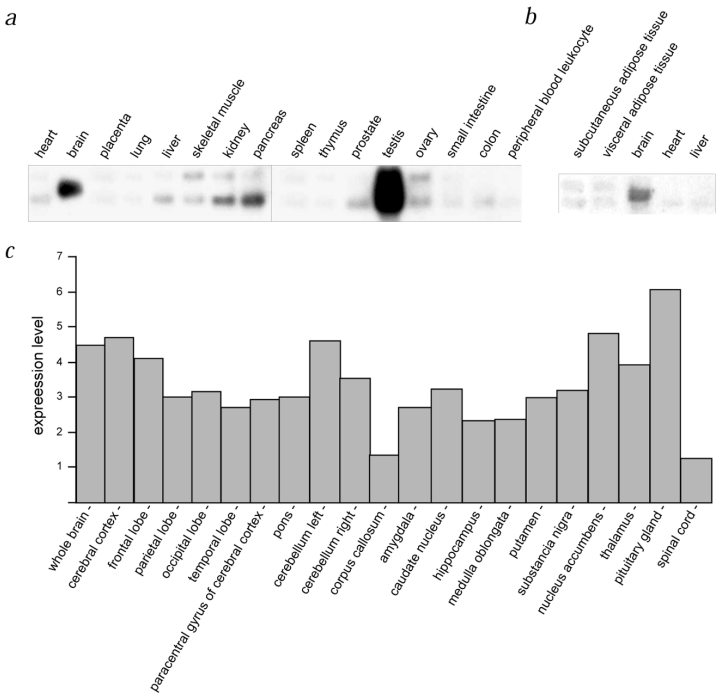

Fig. 5 Expression of *BSCL2*. Autoradiographs of northern blot analyses show hybridization with a cDNA probe covering exons 1–11. ***a***, Human poly(A)$^+$ RNA (~2 μg) of various tissues showing transcripts of ~2.4 kb, ~1.8 kb and ~2 kb. ***b***, Human total RNA (20 μg) extracted from abdominal subcutaneous and visceral adipose tissues as well as brain, heart and liver. The levels of expression varies among tissues, being strong in brain and testis, intermediate in adipose tissue (visceral and subcutaneous), pancreas, kidney, skeletal muscle, liver, ovary and prostate and weak in the remaining tissues. ***c***, Quantification of autoradiograph of a dot blot with human adult poly(A)$^+$ RNA of tissues from the central nervous system; each amount is adjusted to the level of expression of eight housekeeping genes.

The central expression of *BSCL2* is compatible with the original observations indicating a primary defect in the hypothalamic–pituitary gland axis in the etiology of the disease[1,2,4,14–16]. Moreover, all BSCL patients present with an anabolic syndrome characterized by early excessive growth and organomegaly[3,4]. They also have hypermetabolism with an increased basal metabolic rate, which is associated with excessive food intake and consumption despite the disappearance of adipose tissue[4]. The *BSCL2* region on human chromosome 11q is orthologous to a segment of rat chromosome 1 that contains some of the principal quantitative trait loci for metabolic alterations and diabetes-related phenotypes in rat disease models[17–19]. Because diabetes is reported to be frequent in patients' families, *BSCL2* is a candidate gene for involvement in metabolic disturbances and diabetes. Identification of *BSCL2* and speipin provides the basis for additional investigations that should lead to new insights into the physiology of the disease and the mechanisms regulating behavior, energy expenditure and body fat distribution which are altered in common syndromes such as diabetes, mmetabolic syndrome or obesity.

Methods

Patients. We studied 29 families and 17 additional patients (isolated or from small families) representing a total of 71 BSCL patients. Three of the Lebanese families (BSCL-1, -2 and -3) originated in the same village, which is indicative of a geographical cluster. Four families (BSCL-6, -7, -8 and -9) were clustered in the southwest region of Norway but have been shown by genealogies not to share a common ancestor over the last 400 years[9]. Patients had a generalized form of lipoatrophy with a near-absence of adipose tissue at birth or beginning in early infancy, and muscular hypertrophy. Most of the patients presented with acromegaloid features, *acanthosis nigricans*, hepatomegaly, hirsutism, insulin resistance and hypertriglyceridemia. Thirteen additional isolated patients had the delayed phenotype in which the onset of lipoatrophy occurs in later childhood or in adults. We verified the absence of mutations in the lamin A/C gene (*LMNA*) in patients to exclude familial partial lipodystrophy of the Dunnigan type[20]. Further biological and clinical characteristics of a subset of the patients group have been reported before[2,4,21–26].

Genotyping. We obtained genomic DNA from peripheral white blood cells by standard procedures[27]. We undertook a genome-wide analysis using the LMS II-MD2 panel (Perkin Biosystems) with microsatellite markers spaced, on average, ~10 cM apart and an ABI 377 DNA sequencer equipped with Genescan 3.0 and GENOTYPER software (version 2.1, Applied Biosystems). For fine-mapping, we identified new CA repeat markers from genomic sequences and tested them for polymorphism. We then used the Stanford GB3 radiation hybrid panel[28] to determine the probable order of these markers. Full information is available on request.

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Genetic analysis. We carried out parametric linkage analysis with algorithms previously described[29] as implemented in the program SimWalk2. We fixed the disease allele frequency to 0.001 and assumed a purely recessive mode of inheritance with penetrance 0.99 for the disease genotype. We also determined the most likely haplotype configurations with the SimWalk2 program and estimated the marker allele from the observed data for families used in genome screen. For the analysis of additional families, we re-estimated the marker allele frequencies from the data in those families. Re-calculation of the lod score with other marker allele frequency estimates did not modify our conclusions.

Mutation screening. We designed specific primers to amplify the exons and splice junctions using the web site of the Massachusetts Institute of Technology (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). We screened *BSCL2* exons for mutations after amplification of seven fragments (exons 1, 2, 3, 4, 5–6, 7–8 and 9–11). The sequences of these intronic primers are available (Web Table B). In Norwegian patients for whom only trace amounts of DNA were available (extracted from old stored hypophyseal tissue and serum), two sets of amplifications were necessary. We purified PCR products on Sephadex columns and sequenced them using Big Dye Terminator chemistry (Applied Biosystems). We compared sequences using Phred Phrap Consed software[30]. The nature and the allele frequencies of polymorphisms identified in CEPH family founders are presented in Web Table C. We analyzed splice site mutations using total RNA extracted from cultured cells obtained from the patients as a template and the SMART™ PCR cDNA synthesis kit (Clontech). For the first PCR, we used primers flanking the entire coding region and the conditions described below to obtain the cDNA probe, followed by nested PCR with internal primers.

Northern blot analysis. We used human multiple-tissue mRNA for dot blot and northern blot analysis, as well as total RNA from human brain, heart and liver (Clontech). In addition, we isolated total RNA from abdominal subcutaneous and visceral adipose tissues from skin and colon discarded after surgery, using the Quiagen kit. Total RNA (20 µg) was separated by electrophoresis through a 1% agarose–2.2 M formaldehyde–MOPS (3-(N-morpholino)propanesulfonic acid) gel and blotted onto a Hybond-N nylon membrane (Amersham). We hybridized blots with a probe of ~1,700 bp spanning *BSCL2* exons 1–11, including the AUG start codon and the stop codon. We obtained this probe by amplification of whole-brain cDNA with the Avantage 2 kit (Clontech) and the set of primers E1f (5′–CCCCTGCAGTGGAGTCTGTA–3′) and E11r (5′–AGTCAGGTGGGAAAGTGCTG–3′). PCR conditions were 95 °C for 1 min followed by 30 cycles of 95 °C for 30 s, 65 °C for 45 s and 72 °C for 2 min, with a final extension at 72 °C for 10 min. We confirmed the identity of the probe after re-amplification with five sets of internal primers and partial sequencing. We quantified the signals using a phosphorimager (Storm 860; Amersham Pharmacia Biotech) with the Image Quant version 5.0 program.

Web-based databases and sequence analysis. We identified the genomic sequences included within the chromosome 11q13 target interval by using the web sites http://www.ncbi.nlm.nih.gov/genome/central and http://hgrep.ims.u-tokyo.ac.jp/. We carried out homology searches using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). We created multiple alignments using CLUSTAL (http://www.infobiogen.fr/services/analyseq/cgi-bin/clustalw_in.pl) and analyzed the putative protein using the following computer programs: EXPASY Proteomic tools (http://www.expasy.ch/), which contains Profile scan; PROSITE and TMpred for search of consensus motifs and transmembrane domains; and BLOCKS for search of consensus domains (http://bioinformatics.weizmann.ac.il/blocks/).

GenBank accession numbers. Human genomic sequence containing *BSCL2*, AP001458; human mRNA AFO52149; mouse *Gng3lg* cDNA, AC069954, AB041588 and AB030196; *Drosophila melanogaster* CG9904 protein, AAF45798.

Note: Supplementary information is available on the Nature Genetics web site (http://genetics.nature.com/supplementary_info/).

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PUBLICAÇÃO 2:

Lipodystrophy: The metabolic link of HIV infection with insulin-resistance syndrome

LIPODYSTROPHY:
The metabolic link of HIV infection with insulin-resistance syndrome

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ABSTRACT
Human lipodystrophies are a heterogeneous group of diseases with generalized or partial fat loss, rare genetic or more frequent acquired forms, old or recent, such as HIV-related lipodystrophy. Lipodystrophies are associated with metabolic alterations including insulin resistance (IR), diabetes, dyslipidemia and early cardiovascular (CV) disease. Adipose tissue from different localizations (visceral and subcutaneous) has markedly divergent roles regarding metabolism. Lipoatrophy and lipohypertrophy can occur together or separately in patients with lipodystrophies.
In this review, we focus on the etiopathogenesis of genetic and acquired lipodystrophy, with the emphasis on its most common form, HIV lipodystrophy, from the etiology to complications. We analyze the relative role of the different kinds of adipose tissue, namely the protective effect of subcutaneous adipose tissue in relation to CV. We discuss the factors related to fat redistribution in HIV-infected patients, namely the impact of antiretroviral therapy, the role of inflammation, genetic factors, lipotoxicity and cortisol. We also review the mechanisms of IR, dyslipidemia, metabolic syndrome and the clinical sequelae associated with HIV and HIV-lipodystrophy, namely endothelial dysfunction and CV disease and mortality.

Keywords: Genetic lipodystrophy; HIV-lipodystrophy; etiopathogenesis; metabolic complications

INTRODUCTION

Human lipodystrophies are a heterogeneous group of diseases characterized by generalized or partial fat loss. If localized, they are often associated with fat hypertrophy in other depots, varying according to the type of lipodystrophy. Genetic lipodystrophy is generally related to severe metabolic alterations including insulin resistance (IR) and its associated complications, such as glucose intolerance and diabetes, dyslipidemia, hepatic steatosis, polycystic ovaries, acanthosis nigricans and early cardiovascular (CV) complications [1, 2]. Lipodystrophies can be genetic, which is usually uncommon, or acquired. The autosomal recessive congenital generalized lipodystrophy (CGL) and autosomal dominant familiar partial lipodystrophy (FPL) are the two most common types of genetic lipodystrophy [2]. Lipodystrophies have been reported in the medical literature for more than 100 years [2, 3]. However, about 13 years ago, new lipodystrophy syndromes were recognized that are associated with viral infection, specifically with the human immunodeficiency virus, in patients treated with combined antiretroviral therapy (cART) [4]. This has become the most frequent form of lipodystrophy [2]. Some first-generation antiretroviral drugs used in HIV patients are strongly related with peripheral lipoatrophy and metabolic alterations [1].

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Human lipodystrophies leads to severe metabolic alterations resulting in premature CV complications. On the other hand, high adiposity, such as that seen in obesity, also increases metabolic alterations and leads to increased CV risk. So, it seems that the two extremes, the absence or the excess of fat mass, are associated with the same metabolic and CV complications. The consequences of increased fat depots are markedly dependent upon their localization. Adipose tissue in the lower part of the body is able to expand and can therefore accumulate excessive energy from diet, store triglycerides, and it appears to be protective at the metabolic level [5]. By contrast, accumulation of fat in the upper part of the body is deleterious. Therefore decreased peripheral subcutaneous adipose tissue (SAT), and even more increased visceral adipose tissue (VAT), are strongly associated with metabolic alterations and IR [1].

With regards to the HIV-associated lipodystrophy, the available data suggest that this condition is caused by a complex interaction involving side effects of cART, disease related inflammation, and individual characteristics [6]. At present, HIV-infected patients are exposed to an increased metabolic risk, like the general population, resulting from ageing, increased weight and fat gain, high fat and energy food and marked sedentariness. Moreover, a number of additional factors could worsen their metabolic profile, such as the ongoing HIV infection, the presence of lipodystrophy and the continuous use of antiretroviral drugs [7]. HIV-associated lipodystrophy is also associated with premature aging [8] resembling metabolic laminopathies and progeria [1]. Premature aging of HIV-infected patients affects bone, brain, vascular wall, muscles, kidney and liver, and results of the combined effects of long-term HIV-1 infection, depleted immune responses, the toxicity of some antiretrovirals and lipodystrophy. Cellular senescence seems to result from prelamin-A accumulation induced by some antiretroviral drugs, mitochondrial dysfunction and oxidative stress. In addition, increased cytokine release in lipodystrophy further contributes to premature aging and therefore, to early CV and hepatic disease risks [9].

We focus in adipocyte dysregulation in genetic and acquired lipodystrophy, with emphasis on the most common form, HIV-lipodystrophy, from the etiology to complications.

Adipose tissue biology - three different adipose tissue compartments

Adipocytes are a dynamic and highly regulated population of cells. Adipose tissue is characterized by a marked cellular heterogeneity among its cellular components: adipocytes, preadipocytes, fibroblasts, macrophages, lymphocytes, endothelial cells and multipotent stem cells, able to differentiate into several cell types. Adipose tissue can release regulatory factors (adipokines, cytokines, or chemokines) or metabolites (FFAs) capable of influencing other surrounding cells, thus establishing active cross-talk among cells within adipose tissue [9]. Overall, fat tissue consists of approximately one third mature adipocytes. The remaining two thirds are a combination of small blood vessels, nerve tissue, fibroblasts and preadipocytes in various stages of development. Preadipocytes have the ability to proliferate and differentiate into mature adipocytes, conferring a constant functional plasticity on adipose tissue [10]. Preadipocytes mature in two steps: differentiation and then hypertrophy. During the early maturation stage, an increased number of mitochondria are required [11, 12], resulting in small adipocytes, which are highly sensitive to insulin and that secrete high levels of adiponectin [12]. By contrast, older adipocytes increase in size (hypertrophy), lose their functional activities and become resistant to insulin. They exhibit decreased numbers of mitochondria with impaired mitochondrial reactive oxygen species (ROS), generated by the respiratory chain, which could have dual effects on adipocyte differentiation. New adipocytes form constantly to replace lost adipocytes, to the extent that 50 % of adipocytes in the human subcutaneous fat mass are replaced approximately every eight years. Preadipocytes are recruited to become lipid-filled mature adipocytes at the same rate that adipocytes die, and in this way the fat mass is in constant flux, and adipocyte number is kept constant. Cell death of fat cells in white adipose tissue occurs primarily by necrosis-like cell death, which involves macrophage recruitment and a subsequent inflammatory response. This has been implicated in the metabolic complications of obesity. Increased

visceral fat mass leads to IR and a low-grade inflammation status in which many adipokines and other adipocyte and macrophage factors are involved [13].

Adipose tissue is not homogeneous, but is rather a tissue with specific regional compartments with varying roles and metabolic functions [14]. Individually considered, adipose tissue compartments have stronger associations with physiological and pathological processes than does total adipose tissue mass [15-17]. The upper-body adipose tissue, including visceral fat, is involved in fat storage after meals and the release of free fatty acids (FFA) between meals to feed the liver, muscles and other organs, therefore sparing glucose for the brain. Visceral fat has a more lipolytic profile than subcutaneous fat. Peripheral lower-body fat, in the femoro-gluteal region, is mainly used for its storage capacity, thereby buffering excess fat [7]. The femoro-gluteal fat depot is relatively insensitive to lipolytic stimuli and highly sensitive to anti-lipolytic stimuli, and may play a protective role by acting as a "sink" for circulating FFA [18]. This uptake of FFA prevents ectopic fat storage in the liver, skeletal muscle, and pancreas, which causes IR and beta-cell dysfunction [19]. The excessive lipolytic capacity of visceral fat (and probably subcutaneous upper-body depots as well) results in a condition referred to as lipotoxicity (see section below on lipotoxicity) [7]. The reasons for the lower degree of expansibility of visceral adipocytes remain unknown. Visceral adipose tissue (VAT) differs histologically from subcutaneous adipose tissue (SAT): it has smaller adipocytes and a larger supply of nerves and vessels; VAT has many of the characteristics of brown adipose tissue turned into white adipose tissue [20].

Lipohypertrophy and lipoatrophy

Adipose tissue can be subject to different influences and undergo different transformations. SAT has a lower mitochondria content and this contributes to adipocyte apoptosis and therefore, to more lipoatrophy. VAT, with a higher number of resident macrophages [21] and more 11β hydroxysteroid dehydrogenase activity than SAT [22], is predisposed to hypertrophy. Hypertrophied VAT from HIV-infected patients displays mitochondrial dysfunction but not impairment of adipogenic gene expression in comparison with SAT [23]. cART can differentially alter fat development depending on the environment and physiology of the different compartments. In the case of lipoatrophy, because peripheral subcutaneous adipocytes cannot store triglycerides, non-lipoatrophic fat depots such as VAT probably buffer the increases in FFA, which worsens lipohypertrophy [8].

Subcutaneous adipocytes seem to be more susceptible to the deleterious effects of protease inhibitors (PIs) than visceral adipocytes [24]. Accordingly, studies performed on control human SAT explants reveal that some PIs increase FFA, interleukin – 6 (IL-6) and TNF-α production through the activation of the proinflammatory nuclear factor – kB (NFkB) pathway. This PI-induced deleterious paracrine loop between adipocytes and macrophages, similar to that observed in obesity, is not seen in VAT. These data indicate that SAT is more sensitive to the adverse effects of some PIs than VAT [8].

Adipogenesis

Individuals can differ remarkably in body fat distribution and the known differences in FFA uptake of the adipose tissue compartments play a role in this difference. Premenopausal female SAT takes up more FFAs than male [25] and upper-body SAT takes up FFAs more avidly than femoral fat in men, but not in women. Gene expression, mRNA transcription of FA transporters and consequently facilitated FA transport was greater in the upper body in men and in the femoro-gluteal region in women. This novel FFA disposal pathway may also play a role in the development or maintenance of body fat distribution. On the other hand, direct FFA uptake in subcutaneous fat differs from fatty acid uptake from a meal in two respects: 1) direct FFA uptake is more efficient in women than in men and 2) in men there is no preferential direct FFA uptake in upper-body subcutaneous fat compared with femoral fat in women. These gender-based differences are consistent with this process as a mechanism to develop or maintain variations in body fat distribution between men and women, both lean and obese. To the extent that message

levels relate to protein content/function, the greater direct FFA uptake in abdominal over femoral fat in men could be due to the greater facilitation of inward fatty acid transport (Figure 1). Contrary to what is generally believed, upper-body subcutaneous fat releases ~70 % of systemic FFAs in lean men and women, whereas the leg contributes only ~20%; fatty acid uptake from a meal follows a similar pattern [26]. In non-obese men, the direct uptake/storage of FFAs in upper-body and leg fat mirrors this regional difference in FFA release, whereas in lean women, direct FFA uptake was similar in upper-body and femoral adipose tissue. This imbalance between release and direct reuptake in women could redistribute fatty acids toward leg fat. In obese women, the total FFA reuptake in leg fat was also significantly greater than in upper-body fat. It may be that some populations of fat cells, such as the smaller adipocytes, take up but do not as actively release FFAs, whereas larger fat cells briskly release FFAs and do not take up FFAs under post-absorptive circumstances. In summary, there is a mechanism for adipocyte fatty acid uptake and storage that has yet to be understood, but which is independent of lipoprotein lipase and not thought to exist in the post-absorptive state [25].

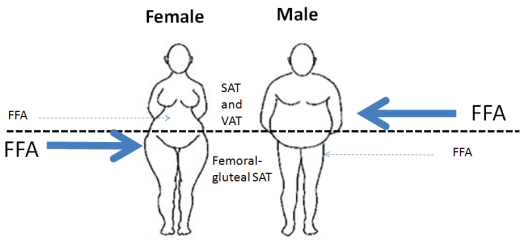

Figure 1. FFA uptake by gender (SAT – abdominal subcutaneous adipose tissue; VAT – abdominal visceral adipose tissue; FFA – free fatty acids)

Macrophage infiltration of the human adipose organ is a well-documented phenomenon that induces a low-grade chronic inflammation that is associated with IR. This reaction appears to be related mainly to macrophage-produced cytokines (TNF-α and IL-6) capable of interfering with the normal activity of insulin receptors. The greater amount of macrophages and macrophage-secreted cytokines found in visceral fat is in line with the greater morbidity associated with these depots. Subcutaneous and visceral adipocytes have cell-autonomous properties due to inherently different progenitor cells that exhibit a different gene expression pattern. Subcutaneous white adipose tissue responds better to the anti-lipolytic effects of insulin, secretes more adiponectin and less inflammatory cytokines, and is differentially affected by molecules involved in signal transduction as well as drugs, compared with visceral white adipose tissue [13].

Lipoatrophic adipose tissue is known to be characterized by smaller adipocytes, greater cell size variation, disruption of cell membranes, and signs of apoptosis as determined by immunohistochemistry staining, when compared with non-lipodystrophic adipose tissue. There is a marked difference in gene expression between dorsocervical and abdominal SAT, irrespective of the lipodystrophy status, that lies in the expression of homeobox genes involved in organogenesis and regionalization. Disparate expression of such fundamental regulators of transcription might ultimately contribute to different patterns of differentiation and affect the susceptibility of the adipose tissue depot to cART-induced toxicity, perhaps making the abdominal subcutaneous and femoro-gluteal depot more vulnerable to atrophy [27].

Morphologic alterations in lipoatrophy-prone areas of SAT have been confirmed at the level of gene expression [9]. Most studies report that these lipoatrophic areas show an abnormally low expression of the major adipogenic transcription factors peroxisome proliferator-activated receptor -γ (PPARγ), sterol-regulatory element binding protein-1 (SREBP-1), and CCAAT/enhancer-binding protein-α (C/EBPα) [28-30]. Consequently, the expression of adipogenic differentiation-related genes is also decreased. For example, there is a reduction in the expression of genes for lipoprotein lipase and for the insulin-sensitive glucose transporter GLUT 4 [28, 29], resulting in impaired fatty acid and glucose uptake, respectively, and thus leading to a deficit in the lipid-accretion capacity of SAT. Another major alteration detected, which is probably related to the impaired adipogenic gene expression, is reduced expression of the adi-

pokine genes adiponectin and leptin [28, 29]. In addition to impaired expression of adipogenetic–related genes, adipose tissue from patients with lipodystrophy, mainly those receiving NRTIs [31, 32] also show a reduction in mitochondrial DNA (mtDNA) levels. This decrease is associated with complex alterations in mitochondrial function, such as reduced expression of mtDNA-encoded transcripts and compensatory up-regulation of dysfunctional mitochondrial mass, that likely reduce the endogenous oxidative capacity of adipose tissue [33]. Moreover, increased oxidative stress and apoptosis have also been reported in lipoatrophic SAT [34]. Lipoatrophy is also accompanied by a state of chronic low-level inflammation [35]. High levels of expression of the inflammatory markers, TNF-α, IL- 6; IL-8 and IL-18 have been reported in SAT from HIV-1-infected lipoatrophic patients [28, 30, 35-37]. Expression of TNFα and IL-6 mRNA in SAT of lipodystrophic patients correlates positively with tissue apoptosis and negatively with adipogenic marker expression, which is consistent with a role for pro-inflammatory cytokines in adipocyte viability and differentiation [30]. Unlike SAT, VAT did not exhibit impaired expression of adipogenic marker genes. However, it did show some similar changes in inflammatory markers, such as induction of TNF-α, whereas others differed from that of SAT, such as, for instance, lack of monocyte chemoattractant protein -1 (MCP-1) induction. In contrast, mitochondrial dysfunction in VAT was found to be similar to that in SAT. Therefore, mtDNA depletion and signs of altered mitochondrial function are common to atrophic (subcutaneous) and hypertrophic (visceral, dorsocervical) depots in HIV-1 lipodystrophy, indicating that mitochondrial impairment cannot explain in a simple manner the final outcome for adipose depots, either in terms of lipoatrophy or lipohypertrophy [23]. Taken together, these observations indicate that different responses occurring in subcutaneous and visceral fat depots in lipodystrophic patients are likely to be related to intrinsic differences in fat physiology and/or capacity to react to the same insult. One adipose depot (visceral) enlarges in size and approaches its fat storage capacity threshold (as in obesity) merely because another adipose depot (subcutaneous) cannot [38]. A direct role for HIV-1 infection has been proposed by analyzing SAT from untreated HIV-1-infected patients ("naïve") as compared to healthy controls. Early histological studies did not indicate clear mitochondrial or inflammatory-related disturbances [39], but subsequent gene expression studies have shown a significant decrease in the expression of the adipocyte differentiation controller PPARγ and some impairment in the expression of genes encoding mitochondrial proteins and proteins specifically related to adipocyte metabolism, including adiponectin and 11β-steroid dehydrogenase type-1, the enzyme responsible for glucocorticoid activation [9, 29]. Some signs of inflammatory response have also been reported [29]. All these alterations are further enhanced in SAT from lipodystrophic patients. Therefore, it appear that HIV-1 infection initiates a first wave of alterations in adipose tissue that is amplified by cART and ultimately results in lipoatrophy [9].

Congenital generalized lipodystrophies

Generalized lipodystrophies are rare disorders that may be congenital or acquired. The genetic lipodystrophies have been reported in about 1000 patients [2]. The congenital generalized lipodystrophy (CGL) Berardinelli-Seip syndrome (BSCL), is an autosomal recessive disorder initially reported by Berardinelli [3] and Seip [40] with frequent parental consanguinity [41-44]. It has been proposed that Berardinelli-Seip syndrome could be a Portuguese disease, later spread by the Portuguese across the world [45].

Patients with CGL are recognized at birth or soon thereafter due to a near-total lack of body fat and prominent muscularity that causes a severe and striking phenotype (Figures 2A e 2B). Diabetes develops during infancy or most often during the teenage years. Hepatosplenomegaly, umbilical prominence or hernia, acanthosis nigricans, voracious appetite and accelerated growth can occur. Female patients develop hirsutism, may have clitoromegaly, oligomenorrhea and polycystic ovaries. Other uncommon manifestations include hypertrophic cardiomyopathy, mild mental retardation, and focal lytic lesions in the appendicular bones after puberty [41, 42]. Diabetes and its complications, hyperlipidemia and recurrent attacks of acute pancreatitis, hepatic steatosis and occasionally cirrhosis are the causes of morbidity and mortality [2].

Figure 2A: Two sisters with Berardinelli-Seip syndrome

Figure 2B: Women with Berardinelli-Seip syndrome

At least 4 molecularly distinct forms of congenital lipodystrophy have been defined, with the mutations of the enzyme acyltransferase 1-acylglycerol-3-phosphate O- acyltransferase 2 (AGPAT2) or (BSCL1- locus 1) and Berardinelli-Seip (BSCL2 - locus 2) being responsible for 95 % of gene mutations. AGPAT2 has been mapped in chromosomes 9q34. AGPATs are critical enzymes involved in the biosynthesis of triglycerides and phospholipids from glycerol-3-phosphate. They catalyze acylation of fatty acids at the sn-2 position of glycerol moiety and convert lysophosphatidic acid to phosphatidic acid [2, 46]. AGPAT2 is highly expressed in the adipose tissue, and its deficiency may cause lipodystrophy by limiting triglyceride or phospholipid biosynthesis [47].

Type 2 CGL is due to BSCL2 gene mutations. This gene is located in chromosome11q13 and encodes a protein called seipin [48]. Seipin appears to play a role in lipid droplet formation and may also be involved in adipocyte differentiation [49-51].

Patients with BSCL2 mutations have the most severe variety of CGL and are born without any body fat [2]. Two other genes were identified for CGL: caveolin 1 (CAV1) [52] and polymerase I and transcript release factor (PTRF) [53]. Caveolin 1 is the main component of caveolae, specialized microdomains seen in abundance on adipocyte membranes [54]. It binds fatty acids and translocates them to lipid droplets. PTRF (also known as cavin) is involved in biogenesis of caveolae and regulates expression of caveolins 1 and 3 [53].

Mortality in generalized lipodystrophies

Patients with generalized lipodystrophies are predisposed to develop acute pancreatitis, cirrhosis, endstage diabetic renal disease requiring transplantation, and blindness due to diabetic retinopathy. Many patients with FPL die of coronary heart disease or cardiomyopathy and rhythm disturbances [55-57]. Sudden death has been reported during childhood in CGL, type 4, likely due to arrhythmias [58]. Patients with HIV-lipodystrophy are predisposed to developing coronary heart disease [59]. Patients with congenital Berardinelli-Seip lipodystrophy frequently develop hypertrophic cardiomyopathy that can lead to death from cardiac failure [41]. In individuals with BSCL, ventricular dysfunction and hypertrophic cardiomyopathy are often observed. In cardiac biopsies performed in eight individuals with BSCL, the presence of subendocardial fibrosis and an abnormal architecture in the left ventricular lumen was observed [60, 61]. Hypertrophic cardiomyopathy in BSCL patients has been correlated with high plasmatic insulin levels, which activate the type 1 insulin-like growth receptors, present in large quantities in the

myocardial tissue, that stimulate cell growth [62, 63]. In addition, the presence of IR and hypertriglyceridemia in individuals with BSCL may predispose them to premature atherosclerosis. Individuals who have mutations on chromosome 11 (BSCL2) seemed to present more severe symptoms than who have mutations in BSCL1 with a high incidence of premature deaths. Cardiomyopathy was three times more frequent in those with BSCL2 mutations than in individuals with alterations in BSCL1 [42].

Mandibuloacral dysplasia (MAD)-associated lipodystrophy

MAD is characterized by skeletal abnormalities such as mandibular and clavicular hypoplasia and acroosteolysis [64], progeroid manifestations, partial or generalized lipodystrophy and metabolic complications with other clinical features [65, 66]. Patients with MAD harbor mutations in lamin A/C (LMNA) or zinc metalloproteinase (ZMPSTE24) [67, 68]. ZMPSTE24 is involved in post-translational proteolytic processing of prelamin A to mature lamin A [2].

Autoinflammatory syndromes

A syndrome of joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced (JMP) lipodystrophy was reported by Garg in three patients, belonging to two pedigrees, who were from Portugal and Mexico [69]. Three other patients have been reported from Japan [70, 71]. Mutations in PSMB8 may trigger an autoinflammatory response resulting in infiltration of adipose tissue with lymphocytes and other immune cells and adipocytes [2]. The other autoinflammatory syndrome is the chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome. The mode of inheritance seems to be autosomal recessive, but the molecular basis remains unknown [2, 72, 73].

Familial partial lipodystrophy (FPL)

FPL is characterized by the onset of fat loss from the limbs and other regions of the body, usually during childhood, adolescence or adulthood. Many regions of the body, such as the face, neck, and intra-abdominal region are spared, and patients accumulate excess body fat in the non-lipodystrophic regions [74-76]. Metabolic complications, acanthosis nigricans, oligoamenorrhea, hirsutism, polycystic ovarian syndrome, mild to moderate myopathy, cardiomyopathy, and conduction system abnormalities indicative of multisystem dystrophy can occur [2, 55, 56, 77]. The identification of the first gene for FPL, Dunnigan variety, i.e. LMNA, on chromosome 1q21-22 [2, 78-81], an integral component of nuclear lamin, was in 1998. Thereafter, four other genes were identified: PPARγ [82-84], a key transcription factor involved in adipocyte differentiation; v-AKT murine thymoma oncogene homolog 2 (AKT2) [85], involved in downstream insulin signaling; and cell death-inducing DNA fragmentation factor a-like effector c (CIDEC) [86] and perilipin 1 (PLIN1) [87], both involved in lipid droplet formation [2, 88]. Adipocyte loss is due to premature cell death resulting from disruption of the nuclear envelope [87, 89]. Defective adipocyte differentiation seems to be the cause of the lipodystrophy of PPARγ as AKT2 mutations. Fibrosis of adipose tissue with small adipocytes is responsible for the lipodystrophy of PLIN1 mutations [2, 87].

There are other rare genetic syndromes whose molecular basis remains unknown, namely atypical progeroid syndrome [90], Hutchinson-Gilford progeria syndrome [91], SHORT-associated lipodystrophy [92], and Wiedemann-Rautenstrauch syndrome [93].

Patients with FPL syndromes display mixed lipodystrophy with subcutaneous lipoatrophy and central fat accumulation. Those with FPLD2 also have an increased amount of fat in the cervico-facial area compared to patients without these mutations. Therefore, a single protein mutation leads to two opposing fat localization phenotypes [8]. Importantly, mutations in LMNA are also responsible for metabolic laminopathies resembling metabolic syndrome (MS) and Hutchinson-Gilford progeria, a severe syndrome of premature aging [94]. Prelamin A is implicated in increased oxidative stress and in the occurrence of cellular senescence [8].

ACQUIRED LIPODYSTROPHIES

Acquired generalized lipodystrophy (AGL) - Lawrence syndrome

The onset of subcutaneous fat loss in patients with AGL usually occurs during childhood [95]. The pattern and extent of fat loss is quite variable and most patients have generalized loss of fat, but a few of them have areas such as intra-abdominal and bone marrow fat spared. AGL patients are highly likely to develop severe hepatic steatosis and fibrosis, diabetes, and hypertriglyceridemia [2]. The pathogenesis of fat loss remains unknown. Panniculitis may precede loss of fat. Lipodystrophy can be associated with autoimmune diseases such as juvenile dermatomyositis [95]. Chronic hepatitis with autoimmune features and low serum complement 4 levels, suggesting involvement of the classical complement pathway in the pathogenesis of fat loss, has been reported [96].

Acquired partial lipodystrophy - Barraquer-Simons syndrome

Fat loss occurs gradually in a symmetric fashion, first affecting the face and then spreading downward. Most patients lose fat from the face, neck, upper extremities, and trunk, and subcutaneous fat from the lower abdomen and legs is spared. Many patients accumulate excess subcutaneous fat in the hips and legs. Metabolic complications are rare (Figure 3). Misra et al suggest that the fat loss involves autoimmune-mediated destruction of adipocytes because the patients have low serum levels of complement 3 and complement 3-nephritic factor, which blocks degradation of the enzyme C3 convertase [95]. It is possible that the C3-nephritic factor induces lysis of adipocytes expressing factor D [97].

Figure 3: Acquired partial lipodystrophy - Barraquer-Simons syndrome

Localized lipodystrophy

Localized lipodystrophies present with focal loss of subcutaneous fat, usually causing one or more dimples, and usually occurs due to subcutaneous injection of various drugs (Figure 4), panniculitis, pressure, and other mechanisms [98].

Lipodystrophy associated with HIV-infection

The impressive progress resulting from the discovery of drugs able to control HIV infection on a long-term basis, has offered most patients a prolonged lifespan, possibly as long as that observed in non-infected subjects [7]. Suppression of viral replication has become a treatment goal that can be reached with the use of cART. After the introduction of protease inhibitors (PIs) in 1996, as a component of highly active antiretroviral therapy, the morbidity and mortality associated with HIV dramatically reduced [99]. However, patients develop a syndrome of fat redistribution with peripheral fat loss (face, upper limbs and femoro-gluteal) and visceral fat accumulation,

Figure 4. Patient with lipohypertrophy related to subcutaneous administration of pegvisomant.

generally associated with metabolic abnormalities [4, 100-103] and increased risk of CV diseases [104, 105], similar to that which occurs in congenital lipodystrophies [4, 59]. Lipohypertrophy usually represents a central visceral fat accumulation in the abdomen and trunk, but can also be found in breasts (in women), dorsocervical region ("buffalo hump"), double chin, lipomas, and within the muscle and liver [59, 106]. Lipoatrophy and lipohypertrophy are frequently associated (mixed form), but they also can occur independently of each other [59]. This HIV-associated lipodystrophy has been proposed to be a age-related fat redistribution condition (Figure 5) that could amplify age-related co-morbidities and lead to an earlier occurrence [8]. Also, hyperlactenemia and bone demineralization can occur [107, 108].

Figure 5: Patient with mixed HIV-lipodystrophy (absence of subcutaneous peripheral fat in arms, legs and face and increased abdominal fat mass)

Owing to a lack of a consensus on the definition of lipodystrophy and lipodystrophy syndrome in HIV-infected patients, its exact prevalence is not known [6]. In the early 2000s, about half of HIV-infected patients undergoing cART were diagnosed as lipodystrophic (20-80%) [109]. Abnormalities in peripheral and central fat masses are clinically evident in 20-35 % of patients after approximately 12-24 months of cART [59, 107, 110, 111]. With the new anti-retroviral agents, the probability of developing lipoatrophy has decreased in western countries as the pattern of cART prescription has significantly changed [112]. In the study of Nguyen, lipodystrophy has become less frequent since 2003 [112]. Two widely used thymidine analogs from the first class of ART, stavudine and zidovudine, are responsible for lipoatrophy and now they have been replaced by a new generation of potent NRTIs. Although metabolic toxicity of boosted PIs is far less than that of first-generation protease inhibitors (PIs), they are still considered responsible for increased CV risk. The new classes of ART, fusion inhibitors (F20), integrase inhibitors (raltegravir) or entry inhibitors (anti-CCR5, maraviroc) have not yet been shown to alter metabolic parameters or fat distribution [8, 112-117].

HIV-infected patients can have 4 different phenotypes of body fat composition: no lipodystrophy, isolated peripheral lipoatrophy, isolated central fat accumulation and a mixed form of lipodystrophy (or redistribution syndrome) [100]. About 50% of patients with HIV-associated lipodystrophy display mixed forms, with the loss of limb fat and marked expansion of VAT [113]. The high frequency of this association suggests that these two opposite phenotypes could be, at least in part, causally related [8]. Also, HIV patients may have a picture similar to partial lipodystrophy patients with peripheral fat atrophy with hypertrophied central fat depots. Thus, there are similarities between HIV lipodystrophy and genetic forms.

HIV-lipodystrophy and related factors

There is a possible role for the virus in contributing to lipodystrophy i.e. the HIV or hepatitis infection affects fat before any ART. Monocytes are relatively resistant to HIV infection, but differentiated macrophages are highly susceptible and tissue macrophages have been found to harbor HIV-1 [118]. Infected macrophages release pro-inflammatory cytokines. Systemic inflammation associated with HIV infection might promote monocyte migration across the vascular endothelium, leading to an increased number of activated macrophages in fat [118]. Several studies reported that the severity of HIV infection is associated with an increased prevalence of lipodystrophy [59, 119], probably as a consequence of persistent HIV-infected macrophages in adipose tissue, which could enhance local inflammation.

ART-naïve HIV-positive patients have increased TNF-α expression compared with uninfected controls [29], which is consistent with increased inflammation. TNF-α alters adipocyte function and differentiation, in part through the inhibition of PPARγ expression [120]. Infected macrophages might also release viral proteins (such as Vpr and Nef) that can impact adjacent adipocytes and lead to decreased PPARγ activity and the inhibition of adipogenesis [121, 122]. Although lipodystrophy is uncommon in ART-naïve patients [113], the HIV infection of macrophages could in itself result in low-grade fat inflammation and lead to the release of viral proteins that affect neighboring adipocytes and decrease their differentiation.

The development of lipodystrophy and metabolic toxicities is partially related to the individual drugs included in cART regimens, in association with other risk factors [123], such as gender and pre-HIV-infection body composition, disease-specific factors such as the nadir levels of CD4+ lymphocytes and the duration of HIV infection [110, 123, 124]. The most significant risk factors associated with lipoatrophy are exposure to and duration of nucleoside thymidine analogues (most commonly stavudine), age, severity of disease markers (CD4 lymphocyte count and plasma HIV viral load), therapy duration, and being of Caucasian race. On the other hand, the most common statistically significant risk factors for lipohypertrophy are therapy duration, PI administration, markers of disease severity, and age. We cannot forget that along the years, patients have done multiple regimes and combinations of antiretroviral drugs, which makes it difficult to identify different risks with different drugs and studies therefore report conflicting results [6].

Protease inhibitors

PIs affect multiple metabolic pathways and are associated with lipohypertrophy and lipoatrophy, atherogenic dyslipidemia and IR, [125, 126]. Studies in-vitro reported that PIs are able to alter a number of adipocyte functions, including differentiation, expression of transcriptase factors involved in adipogenesis, cell survival, cytokine production, mitochondrial function and IR [127]. PIs may cause lipodystrophy by inhibiting ZMPSTE24, resulting in accumulation of toxic farnesylated prelamin A [128], increase oxidative stress and alter adipokine and cytokine production [89, 127, 129]. The adverse effect of PIs could also result from the induction of endoplasmic reticulum stress or the inhibition of the proteasome [130, 131].

Different PIs might affect key intranuclear genes, causing reduction in levels of RNA encoding SREBP-1, in turn affecting expression of PPAR γ [28]. The reduction in levels of intranuclear SREBP-1 leads to decreased adipocyte differentiation and altered release of adipocytokines [132]. PIs can inhibit lipogenesis and stimulate lipolysis [28, 132-135], and may induce IR by inhibiting glucose transporter 4 expression (GLUT4) [136].

Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs)

NRTIs are analogs of naturally occurring molecules and inhibit the viral reverse transcriptase enzyme. The thymidine NRTIs (zidovudine, stavudine and didanosine) cause mitochondrial toxicity by mitochondrial DNA polymerase inhibition and by causing DNA mitochondrial mutations [31, 137, 138]. These disturbances result in apoptosis of peripheral adipocytes and lead to lipoatrophy [31]. NRTIs are also associated with fat hypertrophy in visceral depots [113, 139, 140]. Nucleoside analogues may inhibit adipogenesis and adipocyte differentiation, promote lipolysis, and exert synergistic toxic effects with those of PIs [31, 141-143]. Moreover, NRTIs promote lipolysis and the subsequent efflux of non-esterified fatty acids from adipose tissue [142, 144].

Mitochondrial toxicity and fat redistribution

In western countries, long-term HIV-infected patients present several age-related comorbidities earlier than the general population [145], and display signs of premature aging [8]. New hypotheses were recently suggested on the pathophysiology of fat redistribution, proposing that mitochondrial toxicity was involved in lipoatrophy and in fat hypertrophy. HIV-associated lipodystrophy could be an age-related fat redistribution that could amplify age-related comorbidities and their earlier occurrence [8]. Mitochondria play important roles in adipocyte differentiation and function. At physiologically low levels,

ROS could act as secondary messengers to activate adipogenesis and lipogenesis, resulting in increased adipocyte number and size. In hepatic cells, oxidative stress activates the transcription factor SREBP1c, which is highly expressed in adipocytes and increases lipogenesis and lipid accumulation [146]. At higher levels, ROS could inhibit differentiation [8].

Excessive VAT releases increased visceral adipocyte-FFAs, adipokines and cytokines. Indeed, activated macrophages, which have an M1 proinflammatory phenotype, invade expanded adipose tissue; upon invasion, their production of proinflammatory cytokines and chemokines increases. This could lead to the decreased secretion of adiponectin by adipocytes in response to TNF-α [36]. Increased abdominal fat lipolysis increases ectopic fat deposition within tissues (liver, skeletal muscle and heart). Subsequently, these derivatives overwhelm mitochondrial oxidative capacity and activate stress kinases, leading to IR. This situation, known as lipotoxicity, associates with ectopic depots of triglycerides in non-adipose tissues that buffer excess fatty acid derivatives [147]. Moreover, a paracrine loop is present between adipocytes and macrophages; macrophage-secreted cytokines (TNF-α and IL-6) activate the NFkB pathway in adipocytes, resulting in increased IL-6 and FFA production. Saturated FFA can, in turn, activate the Toll-like receptor-4 (TLR-4) on macrophages and adipocytes, thereby increasing the proinflammatory loop. This paracrine loop has been reported in obesity as a result of macrophages infiltrating fat [148].

Cortisol and fat redistribution

Patients with hypercortisolism also have an acquired form of lipodystrophy [1], characterized by fat hypertrophy in the upper body with increased VAT and decreased limb fat. Cortisol could activate adipocyte differentiation and hypertrophy, mainly in visceral fat depots, because of the higher expression of glucocorticoid receptors (GR-α) and the 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) that transforms inactive cortisone into active cortisol in adipocytes from central depots [22]. In addition, cortisol induces IR and increased lipolysis in adipocytes [22]. It has been hypothesized that glucocorticoid activation is involved in cART-linked central fat hypertrophy [8]. Higher ratios of urinary cortisol:cortisone metabolites and higher subcutaneous 11β-HSD-1 expression are observed in patients with severe lipodystrophy compared with those without [149]. In cART-naïve patients 11β-HSD-1 expression increases in both abdominal and thigh SAT after 12 months of ART, only in patients with lipohypertrophy or without lipoatrophy [150]. Because TNF-α expression in fat is related to that of 11β-HSD-1 [149], inflammation is linked to glucocorticoid activation [8]. TNF-α activates 11-β-hydroxysteroid dehydrogenase (HSD)-type 1, and this enzyme's activity is higher in visceral fat compared with subcutaneous fat. Visceral fat is able to locally produce cortisol, which could act inside adipocytes and increase lipid accumulation [151]. Other hormones, such as growth hormone and testosterone, can modulate the activity of 11β-HSD-1. It is interesting to note that HIV-infected patients with lipodystrophy often present with a relative decrease in growth hormone [152] and testosterone levels. Moreover, when patients with visceral fat hypertrophy are treated with growth hormone, a reduced amount of visceral fat is reported, together with improved metabolic status (Figure 6) [153].

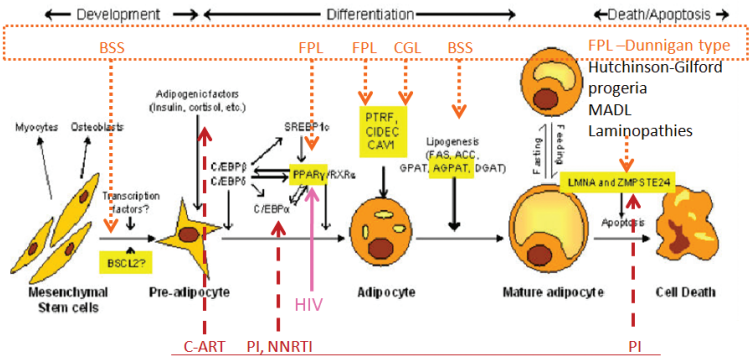

Figure 6: Genetic and adquired lipodystrophies (CGL – congenital generalized lipodystrophy; BSS – Berardinelli-Seip syndrome; cART – combined antiretroviral therapy; NRTI - nucleoside and nucleotide reverse transcriptase inhibitors ; PI – protease inhibitors; FPL – familial partial lipodystrophy; MADL – mandibuloacral dysplasia associated lipodystrophy; adapted from Agarwal & Garg. Ann Rev Genomics Human Genet 2006; 7: 175-199)

Aging

In the general population, age is associated with central fat redistribution, mitochondrial impairment and increased levels of pro-inflammatory cytokines [154]. Aging adipocytes release more pro-inflammatory cytokines than young ones [155]. Aging is physiologically associated with fat redistribution, oxidative stress and low-grade inflammation. In the general population, the proinflammatory state interacting with the genetic background potentially triggers the onset of age-related inflammatory diseases such as atherosclerosis, sarcopenia and frailty. Decreased immune response leading to immunosenescence is also probably involved in early aging [8, 156]. Aging and some cARTs result in mitochondrial dysfunction and oxidative stress, which lead to cellular senescence. Moreover, some PIs induce the accumulation of the pro-senescent protein prelamin A [8].

Genetic factors and HIV-lipodystrophy

A role for genetics is also probable in HIV-lipodystrophy. TNF-α gene promoter polymorphism is associated with lipodystrophy, but this association has not been confirmed in larger studies [157]. Interestingly, stavudine-induced lipoatrophy is linked to the HLA-B100*4001 allele, which is located in close proximity to the TNF-α gene; this observation further supports the theory that there is a role for inflammation in lipoatrophy [158]. Apo C3-455 also plays a role in lipoatrophy, and two variants of the adipogenic β2 receptor seems to be involved in fat accumulation, whereas PPARγ variants are not involved [159]. The toxicity of ART also depends on a patient's metabolism, partly genetically determined.

Role of inflammation

Even if infectivity of adipocytes by HIV has been contested, the ability of the virus to modify adipocyte phenotype has been shown in some studies [160, 161]. Moreover, macrophages and dendritic cells present in lipodystrophic adipose tissue could be infected, which could modify their characteristics (7). Macrophage infiltration is observed in adipose tissue from patients with HIV-related lipodystrophy together with decreased adipokine and increased pro-inflammatory cytokine production [162]. Some anti-retrovirals increase oxidative stress and the generation of reactive species, which results in decreased production of adiponectin and leptin by adipocytes and increased production of pro-inflammatory cytokines [7].

Lipotoxicity

Lipotoxicity is a possible explanation for the development of metabolic syndrome (MS) and diabetes in multiple pathological situations, from obesity to lipodystrophies of distinct origin. According to the lipotoxicity theory, excess availability of fatty acids or a limited capacity to metabolize them in organs and tissues elicits most of the alterations that are characteristic of MS, especially IR. The toxicity of fatty acids toward β-cells may lead to altered insulin production. Actually, high FA levels can induce a reduction in GLUT2 on β cell surfaces and induce a decrease in insulin secretion [19]. Increased availability and, ultimately, increased accumulation of fat into skeletal muscle tends to promote IR; and fatty acid levels in liver that exceed the capacity of this organ to oxidize or export them lead to the increased accumulation of fat that is often associated with MS. All of these events could result from alterations in fat metabolism or excess intake of fat-enriched nutrients, but they could also arise as a result of intrinsic alterations in the capacity of adipose tissue to store and thereby buffer against excess accumulation of fatty acids in other tissues and organs [163]. One hypothesis consistent with the appearance of MS in ART-associated lipodystrophy syndrome is lipotoxicity resulting from limitations in the capacity of subcutaneous fat to store the appropriate amounts of fat and the subsequent diversion of fatty acids to ectopic sites [164]. Several arguments in support of lipotoxicity as a major contributor to MS in distinct human pathologies come from the paradoxically common metabolic alterations found in the obesity and in lipodystrophies of genetic origin. According to the lipotoxicity theory, the fat stored in adipose tissue is biologically inert and the observed metabolic alterations are primarily caused by the increased exposure of cells to non-esterified fatty acid. Thus, in obese patients, it is not the amount of fat stored in adipose tissue that elicits metabolic dysfunc-

tions, but rather the balance between the availability of those fatty acids to tissues and organs and the capacity of the organs to eliminate them through triglyceride storage in adipose tissue, or oxidation [165]. A complementary concept is the idea that there is a threshold for adipose tissue expansibility; once reached, as in obese individuals, appropriate storage of fatty acids in their inert, esterified form inside adipocytes is impaired, determining the extent of lipotoxicity [164]. Either a total or partial lack of adipose tissue storage capacity causes an increase in circulating lipid levels and is associated with ectopic lipid accumulation in non-fat tissues such as liver, skeletal muscle and pancreas. These alterations lead to non-alcoholic fatty liver diseases, hepatic and muscle IR and development of type 2 diabetes. Adipokine deficiency further contributes to metabolic alterations (e.g. hyperphagia) owing to an associated leptin deficiency, which also contributes to positive energy balance and potential lipotoxicity through increased fat intake [94].

Insulin resistance

IR occurs in about one-third of patients on certain PI-based regimens, although the thymidine NRTIs have also been associated [166]. Asymptomatic type 2 diabetes mellitus is diagnosed in 5-10 % of patients [126]. The new-generation PIs appear to have a milder IR effect and the prevalence of diabetes is lower than the described in the early 2000s. Its prevalence, however, remains higher than in the general population at the same age. In particular, patients with severe lipodystrophy are more prone to develop IR [7]. The pathogenesis of glucose metabolism disorders is still unclear and, although a direct effect of potent antiretroviral combinations is certainly involved, it is likely that multiple factors play a role, including genetic predisposition, cytokine and hormonal alterations, changes in the immune system, non-antiretroviral drug-induced toxic effects, opportunistic diseases, and perhaps the HIV infection itself [167]. Risk factors for the development of IR in HIV-positive population include duration of ART, PI treatment, concurrent fat redistribution syndrome, dyslipidemia, increasing age, hepatitis C virus co-infection, as well as pharmacological treatment with pentamidine or megestrol acetate [100, 167-169].

PIs (including indinavir, amprenavir, nelfinavir, and ritonavir) have been shown to induce IR in vitro by reducing glucose transport mediated by the glucose transporter 4, a receptor involved in glucose uptake [136]. The deleterious impact of some PIs on adipocytes through the inhibition of GLUT4 transporters was directly demonstrated in healthy controls, given these molecules for a few weeks [169-171]. Several PIs can interfere with nuclear transcription factors, leading to decreased adiponectin levels, which also leads to IR [123]. Some PIs might also cause mitochondrial toxicity [172]. Additionally, the increase in FFA levels associated with PIs, as well as with fat redistribution, might also have a role in the development of IR (see lipotoxicity) [173].

Dyslipidemia

A number of HIV-infected patients present with dyslipidemia, increased cholesterol, low-density lipoprotein (LDL)-cholesterol and triglycerides together with decreased HDL. This profile has been associated with the presence of a MS, resulting from an increased visceral fat amount with IR [7].

In patients receiving cART the prevalence of hyperlipidemia ranges from 28 to 80% in different studies [167, 174], and it includes hypertriglyceridemia in the majority of cases [123]. Dyslipidemia is frequently, but not always, associated with fat redistribution syndrome: although lipid and glucose metabolism alterations are more common in patients with body-fat abnormalities, they are also observed in those without these morphological changes [167].

Several pathogenetic mechanisms have been proposed to explain cART-associated hyperlipidemia, which seems to result from a complex and multifactorial pathologic process: possibly HIV infection itself, leading in some cases to permanent low grade inflammation with abnormal levels of some pro-inflammatory cytokines; lipodystrophy itself, as in the genetic forms, with decreased lower-body fat depots and increased visceral fat; antiretroviral molecules (PIs, NNRTIs and some NRTIs) [7].

Various mechanisms involved in ART-related dyslipidemia have been described: reduction in the catabolism of VLDL, increased VLDL production, impaired catabolism of FFA, increased liver triglyc-

eride synthesis, increased secretion of apolipoprotein B-containing lipoproteins, reduced expression of LDL receptors, reduced degradation of SREBP, and reduction in FXR (farnesoid X receptor) [175]. The first hypothesized mechanism is based upon the structural similarity between the catalytic region of HIV-1 protease and two human proteins involved in lipid metabolism: the cytoplasmic retinoic acid-binding protein type 1 (CRABP-1) and the low-density lipoprotein-receptor-related protein (LRP). PIs probably bind to CRABP-1 and erroneously inhibit the formation of cis-9-retinoic acid, leading to increased apoptosis of peripheral adipocytes, decreased lipid storage and increased lipid release into the bloodstream. Similarly, PIs may inhibit the normal functioning of LRP and interfere with fatty acid storage in the adipocytes [167, 176]. Furthermore, data from in vitro and in vivo studies suggest that PIs may prevent proteasomal degradation of nascent apolipoprotein B, a key protein component of circulating triglycerides, leading to increased production of VLDL particles. An upregulation of metabolic pathways leading to an excessive production of VLDL can also be caused by the PI-induced intra-hepatocyte accumulation of nuclear transcription factors involved in the metabolism of apolipoprotein B, such as SRBPs. In addition, the levels of lipoprotein particles containing apolipoprotein C-III and apolipoprotein E are increased in PI-treated patients [176-179].

Metabolic syndrome

In the general population, MS is described as an association of increased upper-body fat, particularly in the visceral area, with IR responsible for and revealed by metabolic alterations, including increased LDL, decreased HDL, increased triglycerides, increased glycemia or diabetes and increased blood pressure [180-182]. All these parameters constitute a constellation of major CV risk factors [180]. This situation also includes low-grade inflammation and a prothrombotic state [7].

The pathophysiology of MS is complex – genetic factors, aging and weight gain associated with sedentariness and food habits constitute major risk factors. Also, decreased mitochondrial function related to aging [183] and an age-related redistribution of fat towards the central, visceral areas are involved, leading to IR and metabolic abnormalities [7].

The components of the HIV lipodystrophy syndrome(s) bear a striking resemblance to those of MS [123]. Both can present hyperinsulinemia, glucose intolerance, central or abdominal obesity, hypertension, atherogenic lipid profile, hyperuricemia, prothrombotic states and proinflammatory states [123].

Liver lesions of nonalcoholic steatohepatitis (or NASH) are a complication of MS, with a possible evolution towards severe liver complications such as cirrhosis. HIV-infected patients, with IR in the context of a severe lipodystrophy, present an increased risk of liver lesions [184]. Moreover, as HCV infection has been associated with steatosis and hepatic IR resulting from viral or metabolic effects, HCV co-infection could aggravate the metabolic and liver status of HIV-infected patients [185]. HIV-infected patients with MS are more likely to have higher values of carotid intima-media thickness (cIMT) and detectable coronary artery calcium score (Figure 7) [186].

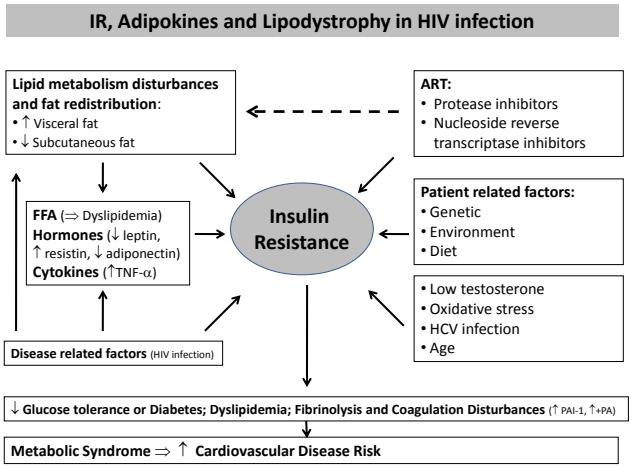

Figure 7: The relationship between HIV infection, lipodystrophy, ART, insulin resistance, adipokines, metabolic alterations, metabolic syndrome and cardiovascular disease risk

Clinical sequelae associated with HIV and HIV-lipodystrophy

Different studies suggest that the CV risk is increased among HIV-infected patients on cART and the responsible mechanisms are complex: in addition to the direct effect of the virus on the vasculature, toxicity of antiretroviral drugs and the set of metabolic alterations aggregated in the MS are involved [187]. Whether the increased CV risk is due to HIV infection itself, to ART, or to a synergistic interaction between these factors, remains to be established [167]. HIV infection itself may increase CV risk. The HIV surface glycoprotein gp120 can stimulate endothelial cell production of tissue factor, an early step in the development of atherosclerosis [188]. Both HIV infection and ART promote atherosclerosis and its clinical manifestations through inflammatory mechanisms involving endothelial cells, either directly or indirectly, and also by the lipid alterations they induce [189, 190].

Furthermore, some data suggest that endothelial dysfunction, impaired fibrinolysis, and excess inflammation are more common in HIV-positive patients than in general population, and may contribute to an increased CV risk [191]. At the same time, cIMT and coronary calcification assessments suggest increased incidence of atherosclerotic disease and premature occurrence of arterial atherosclerotic lesions among HIV-infected individuals [192]. Surrogate markers of atherosclerosis, including abnormal cIMT [193, 194], altered endothelial reactivity [195], abnormal coronary calcium scores [196], higher than expected C-reactive protein levels and abnormal proinflammatory and prothrombotic indices [197], all suggest a state of increased atherosclerotic risk [123].

Endothelial cells have been shown to be variably permissive for HIV infection. The HIV virus itself is able to penetrate coronary artery and brain microvascular endothelial cell membranes and to initiate inflammatory and biochemical intracellular reactions [198, 199]. The activation of endothelium induced by either HIV infection itself or by a leukocyte-mediated inflammatory cascade triggered by the same virus leads to the increased expression of endothelial cellular adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1), E-selectin, P-selectin, thrombomodulin, tissue plasminogen activator (tPA), and PAI-1. A significant association between increasing serum concentrations of adhesion molecules and risk of future myocardial infarction has been shown in apparently healthy men and women, and these molecules are now considered as soluble biomarkers of endothelial inflammation and early atherosclerosis [200, 201]. Increased serum levels of ICAM-1, VCAM-1, E-selectin, and thrombomodulin were demonstrated in patients with advanced HIV infection and opportunistic diseases, and a correlation between ICAM-1 concentrations and the progression of disease as well as the reduction of CD4 lymphocyte count was also reported. If circulating adhesion molecules indicate vascular endothelium injury, it seems clear that endothelium injury is associated with the progression and severity of HIV disease [202]. cART should reduce the endothelial damage by controlling HIV infection, but it would also contribute to stimulating endothelial activation by deranging both lipid and glucose metabolism [167]. Some PIs, as ritonavir and indinavir are able to directly cause endothelial dysfunction, with mitochondrial DNA damage and cell death, independently of lipid profile [203]. cART may promote atherosclerosis both by direct effects on endothelial cells and by indirect effects associated with metabolic disturbances [167].

Elevated serum levels of PAI-1, tPA, and CRP, as well as reduced serum levels of adiponectin could be considered biochemical markers of endothelial dysfunction, metabolic alterations, and CV risk in HIV-infected patients [192]. Some authors suggested that the pathogenetic mechanism responsible for carotid lesions associated with HIV infection may be more similar to an inflammatory process than the classical atherogenesis [204, 205]. Some retrospective and prospective studies have shown that the incidence of myocardial infarction in HIV-positive subjects treated with ART tends to be higher than in the general population, particularly in those receiving a PI-based treatment [206]. However, reports from large observational studies demonstrate that there is still considerable controversy regarding the association of cART, particularly PI-based combinations, with increased incidence of coronary heart disease risk [167].

Moreover, prospective studies involving large cohorts of HIV-infected patients have documented an increased incidence of myocardial infarction and cerebrovascular diseases in association with a pro-

longed exposure to cART. However, the absolute risk of CV events remains low, and should be balanced against the remarkable benefits from cART in terms of improvement in immune function and related morbidity and mortality [167].

In other words, incidence of CV disease in successfully cART-treated HIV-infected patients is low. However, the risk of CV disease is increased compared with that of uninfected people. This fact is due in great part to a higher prevalence of underlying traditional CV risk factors that are mostly host dependent. HIV may additionally contribute through immune activation, inflammation, and immunodeficiency. In a more modest way than HIV infection, the type of ART may also contribute, mainly through the impact on metabolic and body fat parameters, and possibly through other factors that are currently unclear. From the CV perspective, the benefits of ART outweigh any potential risk [207, 208].

CONCLUSIONS

Human lipodystrophies are a heterogeneous group of diseases with generalized or partial fat loss. They include rare genetic conditions or more frequent acquired forms, and may be old, or more recent, such as HIV-related lipodystrophy. Almost all lipodystrophies are associated with metabolic alterations including IR, diabetes, dyslipidemia and early CV disease. In this new context of increased aging of HIV-infected patients with high incidences of smoking status, over-nutrition and reduced exercise, lipodystrophy, dyslipidemia, altered glucose tolerance with IR, low-grade infection with the ongoing drug-induced inflammation, increased metabolic and CV complications are to be expected. It is important to be aware of these alterations and to apply risk-modification strategies to reduce CV risk in the HIV-infected patients.

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3. OBJETIVOS

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Os objetivos dos trabalhos apresentados na presente dissertação estão definidos nas publicações 3 a 11.

4. MÉTODOS

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Todos os trabalhos apresentados nesta dissertação decorreram na Consulta do Serviço de Endocrinologia do Centro Hospitalar de São João e estão detalhados no material e métodos das publicações 3 a 11.

5. PUBLICAÇÕES

PUBLICAÇÃO 3:

Fat Mass Ratio: an objective tool to define lipodystrophy in HIV-infected patients under antiretroviral therapy. Journal of Clinical Densitometry: Assessment of Skeletal Health, vol. 13, no. 2, 197-203, 2010

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Original Article

Fat Mass Ratio: An Objective Tool to Define Lipodystrophy in HIV-Infected Patients Under Antiretroviral Therapy

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Abstract

Human immunodeficiency virus (HIV) infection and its treatment with antiretroviral therapy (ART) have been associated with lipodystrophy. Different clinical methodologies have been used to define the syndrome. The aim of this study was to propose gender-specific reference values using objective measurements for defining lipodystrophy in HIV-infected patients. Using dual-energy X-ray absorptiometry (DXA), total body composition was analyzed in 221 HIV-infected patients under ART (146 men). We used fat mass ratio (FMR) as the ratio between the percent of the trunk fat mass and the percent of the lower-limb fat mass. One hundred forty patients (63.6%) presented clinically defined lipodystrophy. In men, the optimal cutoff value for the FMR was 1.961 (area under the receiver operating characteristic curve [AUC]: 0.74 [95% confidence interval (CI): 0.66–0.82], $p < 0.001$), with a sensitivity 58.3%, a specificity 83.7%, a positive predictive value (PPV) of 89.6% and a negative predictive value (NPV) of 45.5%. In women, the optimal cutoff value for the FMR was 1.329 (AUC: 0.74 [95% CI: 0.63–0.86], $p < 0.001$), with a sensitivity 51.4%, a specificity 94.6%, a PPV of 90.5%, and an NPV of 66.0%. The FMR evaluated by DXA with the gender-specific cutoffs defined here is an objective way to define HIV-related lipodystrophy.

Key Words: Fat mass ratio; gender; HIV; lipodystrophy.

Introduction

Human immunodeficiency virus (HIV) infection and its long-term treatment with combined antiretroviral therapy (ART) have been associated with morphologic abnormalities, namely, lipodystrophy. HIV-associated lipodystrophy has been linked to adverse metabolic parameters that predict increased cardiovascular disease risk. The term ''HIV-associated lipodystrophy syndrome'' refers to changes in fat distribution, including peripheral reduced fat deposition (lipoatrophy) in the face and limbs, appearance of dorsocervical fat pads and multiple lipomas, fat gain in breasts, abdominal girth, and visceral adipose tissue (VAT) *(1–4)*. It soon became clear that some patients had mainly lipoatrophy, others had increased trunk and abdominal fat accumulation, and a subset of patients had a mixed picture of both morphologic features, and the picture of the syndrome seemed to represent a clinical continuum *(5)*. The pure phenotypes (only lipoatrophy or only diffuse fat accumulation) occurred in fewer than 10% of patients with clinical diagnoses of these disorders *(6)*.

Different methodologies have been used to assess the prevalence of the syndrome: patient self-report, clinical

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evaluation, and a complex case report definition. This last definition attempted to develop an objective case definition of lipodystrophy, including age, sex, duration of HIV infection, HIV disease stage, waist to hip ratio, anion gap, serum high-density lipoprotein (HDL) cholesterol concentration, trunk to peripheral fat ratio, percentage leg fat, and intra-abdominal to extra-abdominal fat ratio. The use of such criteria has shown a sensitivity of 79% and a specificity of 80% for the diagnosis of lipodystrophy *(6)*. However, the model is complex, with limited clinical usefulness. In the Carr's model of lipodystrophy definition, patients with isolated abdominal obesity were excluded. Also, by definition, any lipoatrophy is considered as presence of lipodystrophy with or without any fat accumulation *(6)*. Most reports have defined HIV-associated lipodystrophy (clinical lipodystrophy) based on self-report of change, confirmed on examination by a health care provider *(1)*. The lack of standardized values of fat distribution in the general population (both gender) and the heterogeneity of clinical manifestations of lipodystrophy complicated this further. Dual-energy X-ray absorptiometry (DXA) is an extensively used and reproducible technique, but it is unable to distinguish between intra-abdominal and subcutaneous fat and to identify facial lipoatrophy *(7,8)*.

The detection of minimal body fat changes and, if possible, its objective measurements, may enable us to prevent or minimize further abnormalities. Therefore, there is a need for a simple, evidence-based, and validated definition of lipodystrophy *(9)*.

In 2005, Bonnet et al proposed for the first time an objective method for the definition of HIV infection lipodystrophy in men, the ''fat mass ratio'' (FMR) that seemed to allow a more accurate and earlier diagnosis *(10)*. FMR is defined as the ratio of the percentage of the trunk fat mass to the percentage of the lower-limb fat mass by DXA. It should, however, be emphasized that this cutoff point was estimated for HIV-infected men; thus, its application to women is uncertain.

Implementation of this FMR index in studies of HIV patients will allow for more standardized estimates of lipodystrophy frequency, causes, and response to prevention or treatment strategies, at least in the mixed form of fat mass redistribution *(10)*. Thus, the aim of this study was to estimate the reference values of FMR for defining HIV-associated lipodystrophy in a population of HIV-infected patients with clinically diagnosed lipodystrophy.

Methods

Subjects

As part of a cross-sectional study, 221 noninstitutionalized, HIV-infected adult patients (146 men and 75 women), consecutively referred from the Infectious Diseases Outpatient Clinic to the Endocrinology Consultation of Hospital São João, were evaluated. Only patients on therapy were included, because lipodystrophy is related to ART. The study protocol was approved by the Hospital Ethical Committee, and all patients provided informed consent.

Clinical Assessment

Clinical lipodystrophy was defined as a peripheral lipoatrophy with or without a central fat accumulation as assessed by both patients (patients with at least 1 lipodystrophic feature, identified by lipodystrophy-specific physical examination, were asked to report whether he/she had noticed any change in fat volume in the cheeks next to the nose, lateral aspect of the face, legs, arms, and buttocks) and the clinician. Patients were classified as without clinical lipodystrophy when none of the previously described features were present. Presence of central fat accumulation or abdominal prominence was defined by the measurement of waist circumference using the International Diabetes Federation criteria for metabolic syndrome *(11)*. All clinical assessments were performed by the same practitioner (PF). The patients were analyzed in 4 different groups according to the presence or absence of either lipoatrophy or abdominal prominence.

Dual-Energy X-Ray Absorptiometry

Body composition was assessed with whole-body DXA (DXA—Lunar Expert XL, 1999). DXA measurement was performed while the patient was in a supine position, with standard positioning of the arms and feet. Markers used in this study for trunk and lower limbs that defined regions of interest were those indicated by the manufacturer. Regional fat mass values were grouped and analyzed for the following anatomical regions: arms, legs, trunk, and total body. The FMR was calculated as the ratio between the percentage of the trunk fat mass and the percentage of the lower-limb fat mass (FMR = % of the trunk fat mass/% of the lower-limb fat mass) *(10)*.

Statistical Analysis

Statistical analysis was performed using the SPSS version 17.0 software (SPSS Inc., Chicago, IL). All probabilities were 2 tailed, and p values less than 0.05 were regarded as significant.

Data were described as mean and standard deviation (SD) for quantitative variables and compared using the Student's t-test or the Mann-Whitney test, as appropriate. Furthermore, analysis of variance was used for the comparison between quantitative variables. Categorical variables were described as counts and proportions and compared using the chi-square or Fisher's exact test.

The area under the receiver operating characteristic (ROC) curve (AUC), which plots sensitivity (''true positive rate'') against 1 − specificity (''false positive rate''), was used to describe the performance of the diagnostic value of each FMR index. Additionally, the likelihood ratios (LR) were calculated for the comparison of the new FMR index threshold performance with the previously established one *(10)*.

Results

In 221 (146 male and 75 female) HIV-positive patients treated with ART, 140 (63.6%) presented clinically defined lipodystrophy. The sample characteristics according to gender are illustrated in Table 1. As it can be observed, no

Table 1
Sample Characteristics According to Gender

Sample characteristics	Men (N = 146)	Women (N = 75)	*p* Value
Age (years), mean (SD)	46.6 (11.1)	46.4 (12.5)	0.664
Duration of seropositivity from HIV diagnosis (years), mean (SD)	7.7 (3.7)	8.4 (4.1)	0.113
Duration of HART treatment (years), mean (SD)	6.7 (3.8)	6.6 (4.1)	0.802
CD4, mean (SD)	557.1 (303.2)	570.7 (315.1)	0.901
HIV RNA (<50), n (%)	110 (85.3)	63 (92.9)	0.132
HIV risk factor, n (%)			
Injecting drug user	35 (26.9)	9 (15.5)	
Homosexual contact	21 (16.2)	0 (0)	<0.001
Heterosexual contact	71 (54.6)	48 (82.8)	
Others	3 (2.3)	1 (0.5)	
Weight (kg), mean (SD)	69.4 (11.4)	64.1 (12.7)	0.001
Body mass index (kg/m^2), mean (SD)	24.5 (3.7)	26.2 (5.6)	0.035
Circumferences (cm), mean (SD)			
Waist	90.9 (10.4)	91.2 (13.4)	0.970
Hip	93.0 (6.7)	97.3 (10.5)	0.005
Thigh	47.1 (4.2)	48.8 (6.9)	0.358
Arm	27.0 (2.4)	26.8 (3.6)	0.700
Fat mass (%), mean (SD)			
Total	16.7 (8.0)	33.7 (10.1)	<0.001
Trunk	20.4 (9.7)	33.6 (10.2)	<0.001
Leg	12.2 (9.1)	32.5 (12.8)	<0.001
Arm	15.0 (8.6)	42.6 (11.4)	<0.001
Fat mass (kg), mean (SD)			
Total	12.0 (7.6)	22.2 (10.5)	<0.001
Trunk	7.8 (5.0)	11.3 (5.5)	<0.001
Leg	2.6 (2.0)	6.7 (3.8)	<0.001
Arm	1.2 (0.9)	3.3 (1.9)	<0.001
Fat mass ratio[a]	2.00 (0.97)	1.14 (0.42)	<0.001
Free fat mass (kg), mean (SD)	55.7 (7.0)	43.8 (4.1)	<0.001
Clinically defined lipodystrophy, n (%)	103 (70.5)	37 (50.0)	0.003
Abdominal prominence, n (%)	57 (39.0)	74 (79.7)	<0.001
Fat distribution, n (%)			
Without CL and without AP	20 (13.7)	4 (5.4)	
Without CL and with AP	23 (15.8)	33 (44.6)	
With CL and without AP	69 (47.3)	11 (14.9)	
With CL and with AP	34 (23.3)	26 (35.1)	<0.001

Abbr: SD, standard deviation; HIV, human immunodeficiency virus; ART; CD4, T lymphocytes; CL, clinically defined lipodystrophy; AP, abdominal prominence.

[a]Fat mass ratio = trunk % of fat mass to the lower-limb % of fat mass.

differences were observed regarding age, duration of seropositivity from HIV diagnosis, and duration of treatment between men and women. Consistent with previous results in the literature, women had significantly higher mean values of adiposity, both measured by the body mass index (BMI) and by fat mass estimated by DXA *(2)*. However, men were more frequently clinically defined as lipodystrophic and also presented a significantly higher mean FMR.

Regarding the prevalence of lipodystrophy features, we observed that 39% of the males and 79.7% of the females had abdominal fat accumulation evaluated by the measurement of abdominal circumference, and 70.5% of the males and 50% of the females had clinical lipoatrophy (Table 1). In what concerns classes of fat mass distribution (Table 1), 70.5% of men were classified as clinically lipodystrophic (CL), but only 23.3% had associated abdominal prominence

Table 2
Fat Mass Ratio Mean According to the Presence of Clinically Defined Lipodystrophy

	Without lipodystrophy	With lipodystrophy	
Fat mass ratio	Mean (SD)	Mean (SD)	*p* Value
Total	1.22 (0.53)	2.00 (0.97)	<0.001
Men	1.45 (0.60)	2.23 (1.00)	<0.001
Women	0.96 (0.24)	1.34 (0.48)	<0.001

Abbr: SD, standard deviation.

(AP) (mixed syndrome), whereas only 15.8% presented AP without CL. On the other hand, 79.7% of women presented AP, and mixed syndrome was present in 35.1% of the cases. Clinical lipodystrophy was present in 50% of women. In our sample, men had a significantly higher mean FMR than women (Table 1).

When we stratified patients according to the presence or absence of clinical lipodystrophy and by gender, those with clinically defined lipodystrophy had a significantly higher mean FMR (total and by gender) (Table 2). In this sample, a significant linear increase of the mean FMR was observed with increasing age in both genders ($p < 0.05$) (Table 3). However, this effect was not observed for BMI. Furthermore, the mean FMR increased with increasing thirds of waist circumference in men but not in women.

Table 3
Body Mass Index and Fat Mass Ratio Mean According to Demographic and Anthropometric Characteristics

	Men		Women	
Characteristics	BMI, mean (SD)	FMR, mean (SD)	BMI, mean (SD)	FMR, mean (SD)
Age range (years)				
20–29	23.3 (3.6)	1.05 (0.51)	27.4 (6.7)	0.81 (0.14)
30–39	24.1 (3.9)	1.68 (0.80)	24.0 (4.8)	0.95 (0.42)
40–49	24.0 (3.7)	1.97 (0.95)	26.5 (6.8)	1.25 (0.48)
50–59	25.8 (3.8)	2.36 (0.89)	27.3 (5.6)	1.34 (0.28)
≥60	25.0 (2.9)	2.30 (1.14)	27.9 (3.9)	1.20 (0.34)
p	0.165*	0.001*	0.275*	0.019*
Waist circumference thirds (cm)				
0–84	20.8 (2.0)	1.60 (0.82)	21.0 (2.8)	1.02 (0.41)
85–94.5	24.2 (2.0)	2.17 (1.01)	25.7 (2.0)	1.29 (0.56)
≥95	28.1 (2.8)	2.17 (1.95)	31.4 (5.0)	1.15 (0.22)
p	<0.001*	0.002*	<0.001*	0.130*

Abbr: SD, standard deviation.
**p* Value for trend.

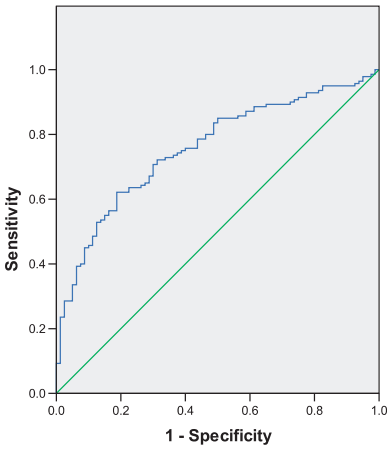

Fig. 1. Receiver operating characteristic curve for the comparison between the clinical diagnosis of lipodystrophy and fat mass ratio measured by dual-energy X-ray absorptiometry.

Fat Mass Ratio Threshold

Considering the clinical assessment of lipodystrophy as the case definition criteria, the optimal cutoff value for the FMR for the total sample was 1.501 (AUC: 0.76 [95% confidence interval (CI): 0.69–0.82], $p < 0.001$), with a sensitivity of 62.1%, a specificity of 81.3%, a positive predictive value (PPV) of 84.5%, and a negative predictive value (NPV) of 57.2% (Fig. 1).

However, as described in Table 1, there were significant differences between men and women regarding both the prevalence of clinically defined lipodystrophy and the percentage and quantity of fat mass measured by DXA. Thus, a stratified gender analysis of the optimal cutoff point of the FMR was performed.

Figures 2 and 3 illustrate the comparison between the clinical diagnosis of lipodystrophy and FMR measured by DXA by gender using ROC curves. In men, the optimal cutoff value for the FMR was 1.961 (AUC: 0.74 [95% CI: 0.66–0.82], $p < 0.001$), with a sensitivity of 58.3%, a specificity of 83.7%, a PPV of 89.6%, and an NPV of 45.5%. In women, the optimal cutoff value for the FMR was 1.329 (AUC: 0.74 [95% CI: 0.63–0.86], $p < 0.001$), with a sensitivity of 51.4%, a specificity of 94.6%, a PPV of 90.5%, and an NPV of 66.0%. Additionally, the sensitivity, the specificity, and the positive and negative LR were calculated for comparison between the present proposed thresholds with the one earlier described *(6)*. These results are presented in Table 4.

Discussion

Lipodystrophy is a general term used to describe various features and degrees of fat redistribution, including lipoatrophy and lipohypertrophy, in different body regions *(12)*. Despite the subjective nature of the clinical assessment of

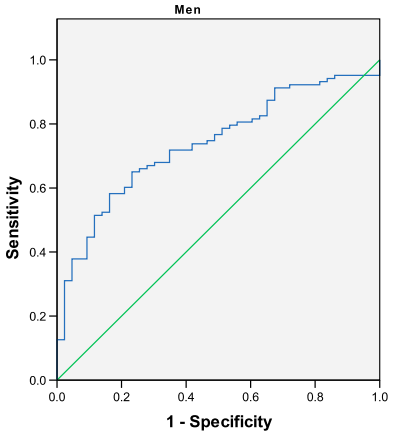

Fig. 2. Receiver operating characteristic curve for the comparison between the clinical diagnosis of lipodystrophy and fat mass ratio measured by dual-energy X-ray absorptiometry in men.

lipodystrophy, investigators report high concordance (e.g., 98%) between the subjects' and investigators' assessments of the presence of lipodystrophy, although the diagnostic reliability of those changes is highly variable *(13)*. The accuracy of the clinical definition of lipodystrophy is dependent on the skills of the observer, which means that expert observers have a higher accuracy. Hence, this method is difficult to be generalized to less-experienced, smaller centers. In this study, all the participants were evaluated by the same observer (PF).

Lipodystrophy in HIV-1 infected patients is considered to be an adverse effect of ART, not limited to a specific drug or

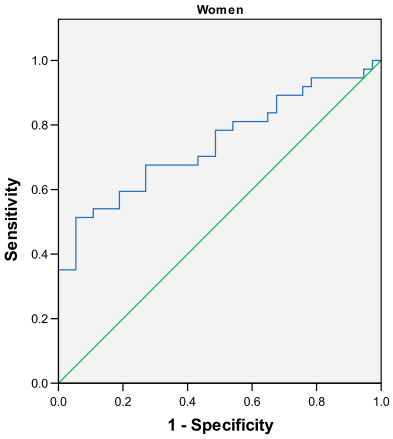

Fig. 3. Receiver operating characteristic curve for the comparison between the clinical diagnosis of lipodystrophy and fat mass ratio measured by dual-energy X-ray absorptiometry in women.

Table 4
Sensitivity, Specificity, and Positive and Negative Likelihood Ratios Using 2 Different Thresholds for FMR Index

FMR index thresholds	Sensitivity (%)	Specificity (%)	Positive likelihood ratio	Negative likelihood ratio
Men				
1.5	71.8	65.1	2.06	0.43
1.961	58.3	83.7	3.58	0.49
Women				
1.5	32.4	100	$+\infty$	0.68
1.369	51.4	94.6	9.50	0.51

Abbr: FMR, fat mass ratio.

a class of drugs *(14)*. HIV-associated lipodystrophy may affect up to half the HIV-infected patients receiving ART *(15,16)*. This is one of the reasons why we have chosen to study only patients on ART.

Bonnet et al *(10)* proposed for the first time that FMR was an easy and objective method to define lipodystrophy, based on a DXA body composition evaluation. A population of 241 negative and 162 HIV-infected men was studied, including 34 naive patients and 128 ART-treated patients, with or without clinical lipodystrophy. The 2 main reasons for the proposal of this index were that 80% of the trunk fat mass is perivisceral and 98% of limb fat mass is subcutaneous and that the HIV-associated lipodystrophy syndrome might result from a subcutaneous fat mass loss, an accumulation of perivisceral fat mass, or both. The threshold chosen for defining lipodystrophy by DXA was 1.5, corresponding to 1 SD above the mean of FMR in HIV-negative men. The FMR threshold was similar for naive HIV-infected men and controls. The FMR for HIV-treated patients with clinical lipodystrophy was very high (2.00 ± 0.97) and largely above the threshold value, and the mean of FMR for HIV-treated patients without clinical lipodystrophy was equal to the threshold value. Bonnet et al concluded that the use of the FMR should allow a more accurate and earlier diagnosis of lipodystrophy *(10)*.

In our sample of 221 (146 men and 75 women) HIV-infected patients, no significant differences were found regarding age, duration of HIV disease, and ART between both genders. Men were heavier, had lower mean BMI and lower total, trunk, leg, and arm fat mass. The FMR was significantly higher in males vs females, which led to the estimation of a gender-based threshold.

Using an ROC curve analysis to define the cutoff value of FMR for lipodystrophy, the best relationship between sensitivity and specificity of FMR for the diagnosis of lipodystrophy was obtained with a 1.961 threshold for men and 1.329 for women. If we use the 1.5 threshold, we obtain a sensitivity of 62.1 and a specificity of 81.3 for the total sample. Instead

of comparing HIV-infected with non−HIV-infected patients, we compared patients with and without clinical lipodystrophy. This decision was because of the clinical problem of distinguishing the lipodystrophic patients within an HIV-infected adult population.

We presented different thresholds of FMR for men and women, because either in the general population or in patients with or without lipodystrophy, there are significant differences between them. In his model of HIV lipodystrophy case definition, Carr et al did not present separate case definitions by gender, because it did not improve the sensitivity and specificity of the case definition *(6)*. However, some of the parameters evaluated (ratio of waist to hip circumference, HDL cholesterol, ratio of trunk to limb fat, intra-abdominal to extra-abdominal fat ratio, and percentage leg fat) already took into account gender differences.

In our data, 70.5% of the males had clinical lipoatrophy, and 39% had abdominal fat accumulation. These results are quite similar to those observed in the FRAM (Fat Redistribution And Metabolic in HIV infection) study: men had evidence of a syndrome of subcutaneous lipoatrophy, with lower-body depots affected more than upper-body depots. FRAM is a cross-sectional study of HIV-infected patients using clinical examination and magnetic resonance imaging to study regional adipose tissue distribution *(1,2)*. AP was found to be an important factor impacting the FMR, particularly in men, probably because, in our sample, men had less fat mass evaluated by DXA and women had a high AP (Tables 1 and 3). We did not observe an effect of hip circumference on the FMR. In our data, we observed a smaller number of mixed syndrome (namely in men), but this could also be ascribed to the method used to define AP.

We observed that 50% of women had clinical lipoatrophy and 79.7% had abdominal fat accumulation. These gender differences may be caused by the fact that women had more total body fat than men. In fact, our National Obesity Survey found a 17.2% prevalence of obesity and 46.1% prevalence of overweight in 40- to 49-yr-old women, mainly visceral obesity (47.8% of women with waist circumference >80 cm). In men, 51.3% were overweight and 16.8% were obese (52.1% of men with waist circumference >94 cm) *(17)*.

In FRAM study, HIV-infected women with clinical peripheral lipoatrophy had less subcutaneous adipose tissue in peripheral and central sites and less VAT than HIV-infected women without peripheral lipoatrophy. Peripheral lipoatrophy was more frequent in HIV-infected women than in the controls, whereas central lipohypertrophy was similar. These data also point out the need of a gender-specific threshold for the FMR.

Increasing age is associated with truncal abdominal fat deposition in both genders in non−HIV-infected population *(18)*. In our sample, the FMR increased with age in both genders, but this effect was not statistically significant for BMI. This is in agreement with previous reports *(10)*. For the first time, we demonstrated that the increase in FMR with age is also present in women. Previously, Snead et al demonstrated that age induces an underestimation of the upper-body adipose tissue by DXA; hence, the age-associated increase in FMR that we observed is probably not related to methodological problems *(18)*.

FMR has also been used in the evaluation of genetic lipodystrophy, and the authors discussed the need of different thresholds according to the fat mass redistribution *(19)*. Our proposed threshold values enabled a more precise identification of lipodystrophic patients.

In conclusion, FMR evaluated by DXA is an objective tool to define HIV-related lipodystrophy, allowing a more clinically useful definition for identification of patients with abnormal fat distribution. Use of gender-specific FMR thresholds as a diagnostic tool in metabolic studies of HIV-infected patients would enable the comparison of different studies and should be useful to monitor lipodystrophy progression in this population.

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Assessment of body fat composition disturbances by bioimpedance analysis in HIV-infected adults

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ABSTRACT. HIV-lipodystrophy syndrome is characterized by different patterns of body fat distribution (BFD) which are identified by clinical and body composition (BC) assessment, including bioimpedance analysis (BIA). Our aim was to compare BC in HIV-infected patients on combination antiretroviral therapy (cART) according to 4 distinct phenotypes of BFD (G1-no lipodystrophy, G2-isolated central fat accumulation, G3-lipoatrophy, G4-mixed forms of lipodystrophy) and assessed factors associated with them. Anthropometry and BIA were performed in 344 HIV-1 patients. G2 and G4 phenotype patients had significantly higher fat mass (FM) but no differences were observed in fat-free mass (FFM) and total body water among the 4 phenotypes. Significant negative associations were found between the presence of lipoatrophy and female gender, body mass index (BMI), waist (WC), hip (HC) and thigh circumferences, and total body FM estimated by BIA. After adjustment for gender, cART duration and BMI, G3 had significant lower WC [odds ratio (OR)=0.84; 0.78-0.90] and HC (OR=0.88; 0.81-0.96) mean. Independently of gender, cART duration and BMI, G2 remained significantly associated with higher WC (OR=1.11; 1.05-1.18) and HC (OR=1.15; 1.07-1.23) mean, and with FM estimated by BIA [FM as %, OR=1.17 (1.09-1.26); and FM as kg, OR=1.15 (1.06-1.25)]. There was a significant positive association between G4 and female gender (OR=1.66; 1.01-2.75), BMI (OR=1.10; 1.04-1.17) and WC (OR=1.15; 1.09-1.21). The similar FFM along the BFD spectrum describes the actual BC of these patients without sarcopenia. In a clinical setting, BIA is an easy and useful tool to evaluate fat mass and FFM and gives us a picture of BC that was not possible with anthropometry.
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INTRODUCTION

The introduction of highly active antiretroviral therapy, into clinical use in 1996 ushered in a new era of treatment for HIV disease with markedly suppressed viral replication, increased CD4+ T-cell counts, and dramatically decreased morbidity, hospitalizations, and mortality (1-9). However, long-term adverse events associated with combination antiretroviral therapy (cART) have been recognized, particularly the lipodystrophy syndrome (10, 11). Lipodystrophy is common, affecting nearly half of all patients receiving cART (12, 13). The term HIV-associated lipodystrophy syndrome refers to alterations in body fat distribution and metabolic alterations (impaired glucose or lipid metabolism). The body-fat distribution changes are typically identified by physical examination and patient self-report (14). HIV-infected patients may exhibit different patterns and varying degrees of severity of changes in body fat. Lipodystrophy studies are further hampered by the potential presence of two distinct phenotypes: peripheral lipoatrophy and central lipoaccumulation. Both can occur together and also separately. Although they are usually considered as different processes, it is unclear whether they are part of the same process or they are independent processes (15-19).

The preservation of lean body mass (primarily muscle) in lipoatrophy distinguishes this condition from HIV wasting syndrome, which is defined as the generalized loss of body fat and lean body mass with an involuntary weight loss >10% of baseline body weight (17, 20).

The diagnosis of lipodistrophy is difficult. Clinical diagnosis has been the most common way, but since it is not uniform it does not allow comparisons among different studies. There are different methods for objective measurement of body fat content, including anthropometry, bioelectrical impedance analysis (BIA), dual-energy x-ray absorptiometry (DXA), computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography. However, there are limitations with each technique (9).

BIA measures the opposition of body cells and tissues to the flow of an alternating electric current. Many studies have applied the basic technique of BIA to estimate body composition in various clinical and research settings. However, most BIA devices cannot measure segmental body fat. Anthropometry and BIA are cheap methods and they have been widespread in units assessing body composition for years. BIA is a method widely available in HIV treatment centers and it is not invasive and does not require as much training as anthropometry (21).

The aim of this study was to compare the body composition using anthropometry and BIA in HIV-infected adult

Key-words: Anthropometry, bioimpedance analysis, HIV, lipodystrophy.

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patients on cART according to the 4 distinct phenotypes of body fat distribution (no lipodystrophy, isolated central fat accumulation, lipoatrophy, and mixed forms of lipodystrophy) and assessed factors associated with them.

METHODS

Subjects

As part of a cross-sectional study, 344 HIV-infected non-institutionalized Caucasian adults, 238 men and 106 women, consecutively referred from the Infectious Diseases Outpatient Clinic, were evaluated in Endocrinology Consultation of São João Hospital. Only patients on cART were included. The study protocol was approved by the Hospital Ethics Committee and each patient provided informed consent.

Clinical assessment

For each patient the following information was collected using a standardized protocol: demographic data (age, gender), HIV infection and ART duration, HIV risk factor, and characterization of the infection. We used the "Centers for Disease Control and Prevention" (CDC) HIV staging classification. This classification considered 3 clinic stages: A [asymptomatic HIV infection, acute (primary) HIV infection with accompanying illness or a history of acute HIV infection, or persistent generalized lymphadenopathy]; B (symptomatic conditions not included among conditions listed in clinical category C and that meet at least one of the following criteria: (A) the conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or (B) the conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection); and C (conditions indicators of AIDS); and also considers laboratorial categories or subdivisions of the above categories according to the CD4+ cell counts (22).

Weight, height, circumferences of neck, waist, hip, thigh and arm were measured. Body weight was measured using TANITA (Tanita®, model TBF 300) scale, in patients wearing light clothes without shoes, and height was measured to the nearest centimeter in the standing position using a wall stadiometer (Holtain Limited Crymych, Dyfed®).

Neck circumference (NC) was measured in the midway of the neck, between midcervical spine and midanterior neck. In men with a laryngeal prominence (Adam's apple), it was measured just below the prominence. Hip circumference was measured with the subject standing erect with arms by the sides, with the feet together and the gluteal muscles relaxed. The measurer sat at the side of the subject so that the level of maximum posterior extension of the buttocks can be seen. An inelastic tape was placed around the buttocks in a horizontal plane. To ensure contiguity of the two parts of the tape from which the circumference was to be determined, the cross-handed technique of measurement was used. An assistant checked the position of the tape on the opposite side of the subject's body. The tape was in contact with the skin but did not compress the soft tissues. Fatty aprons were excluded from the hip circumference measurement. The waist was measured at the end of a gentle expiration midway between the lowest rib and iliac crest, with the patient standing, and the hips were measured at the greater trochanter level. All circumferences were taken with the subjects standing upright, with the face directed toward, and shoulders relaxed. Thigh circumference was measured around the thigh of the left leg midway between the crotch and the knee when the subject is in a standing position. Arm circumference was measured in the mid-upper arm circumference of the left arm. All measurements were performed by the same observer using standard techniques (23).

Clinical lipoatrophy (CL) was defined as a peripheral lipoatrophy assessed by both patient and practitioner. Patients with at least one light, moderate or severe subjective lipoatrophic feature, identified by lipoatrophy-specific physical examination were asked to report whether he/she had had any change in fat in the cheeks next to the nose, lateral aspect of the face, legs, arms, buttocks. Patients were classified as without CL, when none of the previously described features were present (24). Presence of central fat accumulation or abdominal prominence was defined by the measurement of waist circumference (WC) using the International Diabetes Federation criteria for metabolic syndrome (25). The clinical assessment was performed by the same practitioner (PF). The patients were categorized in 4 different groups according to the presence or absence of either lipoatrophy or abdominal prominence: 1) no lipodystrophy – patients without CL and without abdominal prominence; 2) isolated central fat accumulation – patients without CL and with abdominal prominence; 3) lipoatrophy – patients with CL and without abdominal prominence; 4) mixed forms of lipodystrophy – patients with CL and with abdominal prominence.

Body composition was assessed with BIA (TANITA®, model TBF 300). BIA measurement was performed while the patient was in an orthostatic position, with a standard positioning of the feet. The subjects abstained from strenuous exercise, excess alcohol consumption, and use of diuretic substances prior to the exam. BIA allowed the evaluation of body composition, with quantification of total body mass either as percentage or in kg of fat mass (FM) and fat-free mass (FFM) and total body water (TBW). FM, FFM, TBW were estimated from BIA data.

The CD4+ cell count was determined by flow cytometry and plasma HIV-1 RNA loads were measured by a quantitative RT-PCR (Roche Diagnostic Systems, Inc., Branchburg, NJ, USA), which had a lower limit of detection of 50 copies/ml.

Statistical analysis

Statistical analysis was performed using the SPSS version 17.0 software (SPSS Inc., Chicago, Illinois, USA). All probabilities were two-tailed and p values <0.05 were regarded as significant.

Data were described as mean and SD for quantitative variables and compared using the Student-t test or the Mann-Whitney test as appropriate. For the comparison between the 4 groups of fat distribution and the BIA evaluation parameters the Kruskall-Wallis test was used. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher's exact test.

To estimate the association between the 4 distinct phenotypes of body fat distribution and the clinical and demographic characteristics of the patients, unconditional logistic regression models were computed and odds ratios (OR) with their respective 95% confidence intervals (95% CI) estimated.

RESULTS

In the 344 HIV-1 infected patients (238 men and 106 women) on cART, included in this study, 59.6% presented CL. Table 1 shows the characteristics of the study sample according to the 4 distinct phenotypes of body fat distribution (15.1% with no lipodystrophy; 25.3% with iso-

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Table 1 - *Sample characteristic according to 4 distinct phenotypes of body fat distribution.*

	No lipodystrophy	Isolated central fat accumulation	Lipoatrophy	Mixed form of lipodystrophy	*p*
No. (%)	52 (15.1)	87 (25.3)	111 (32.3)	94 (27.3)	
Gender [no. (%)]					
Male	43 (82.7)	42 (48.3)	97 (87.4)	56 (59.6)	<0.001
Female	9 (17.3)	45 (51.7)	14 (12.6)	38 (40.4)	
Age [yr, mean (SD)]	41.0 (10.9)	46.1 (12.3)	43.4 (9.2)	51.1 (10.9)	<0.001
HIV infection duration [yr, mean (SD)]	6.6 (3.5)	6.6 (4.0)	9.4 (4.1)	8.6 (3.7)	<0.001
ART duration [yr, mean (SD)]	5.1 (3.6)	4.9 (3.7)	8.0 (3.9)	7.8 (3.5)	<0.001
Weight [kg, mean (SD)]	64.6 (8.2)	79.0 (14.1)	61.6 (9.9)	71.7 (12.9)	<0.001
Heigth [m, mean (SD)]	1.68 (0.10)	1.64 (0.09)	1.68 (0.08)	1.64 (0.10)	0.002
BMI [(kg/m^2), mean (SD)]	23.0 (2.4)	29.4 (4.6)	21.8 (2.9)	26.5 (3.4)	<0.001
Neck circumference [cm, mean (SD)]	36.2 (3.4)	37.6 (3.8)	36.0 (3.3)	38.4 (4.3)	0.002
Waist circumference [cm, mean (SD)]	84.1 (6.8)	102.1 (10.8)	83.0 (6.9)	97.4 (8.6)	<0.001
Hip circumference [cm, mean (SD)]	93.6 (4.2)	104.2 (8.5)	88.9 (5.1)	94.8 (5.8)	<0.001
Thigh circumference [cm, mean (SD)]	46.6 (4.0)	51.9 (5.3)	44.7 (3.6)	47.8 (7.8)	<0.001
Arm circumference [cm, mean (SD)]	26.0 (2.8)	29.0 (2.8)	25.4 (3.0)	27.9 (4.6)	<0.001
Waist/hip ratio	0.90 (0.08)	0.98 (0.08)	0.93 (0.06)	1.02 (0.07)	<0.001
CD4 cell count [cells/mm^3, mean (SD)]	491.1 (363.8)	515.0 (292.5)	542.6 (288.8)	634.8 (375.2)	0.011
HIV RNA (<50) [no. (%)]	39 (78.0)	71 (85.5)	102 (91.9)	85 (91.4)	0.048
HIV risk factor [no. (%)]					
Injecting drug user	16 (30.8)	14 (16.1)	48 (43.2)	11 (11.8)	<0.001
Homosexual contact	7 (13.5)	5 (5.7)	14 (12.6)	12 (12.9)	
Heterosexual contact	29 (55.8)	67 (77.0)	43 (38.7)	67 (72.0)	
Others	0 (0.0)	1 (1.1)	6 (5.4)	3 (3.2)	
CDC [no. (%)]					
A	26 (50.0)	52 (60.5)	62 (56.4)	51 (54.8)	
B	4 (7.7)	1 (1.2)	0 (0.0)	1 (1.1)	0.106
C	22 (42.3)	33 (38.4)	48 (43.6)	41 (44.1)	
ART [no. (%)]					
PI	31 (62.0)	51 (60.7)	63 (56.8)	45 (48.4)	0.296
NNRTI	22 (44.9)	37 (44.0)	53 (47.7)	48 (52.2)	0.719
NRTI	47 (95.9)	80 (95.2)	109 (98.1)	91 (98.9)	0.228
BIA					
Fat mass [%, mean (SD)]	17.6 (5.5)	31.7 (8.3)	14.9 (5.5)	24.4 (6.9)	<0.001
Fat mass [kg, mean (SD)]	11.3 (3.7)	25.1 (8.9)	9.4 (4.6)	15.9 (8.8)	<0.001
FFM [kg, mean (SD)]	53.0 (7.5)	53.3 (9.8)	52.2 (7.2)	53.4 (12.0)	0.940
TBW [kg, mean (SD)]	38.8 (5.5)	39.0 (7.2)	39.3 (5.3)	39.5 (8.0)	0.923

BMI: body mass index; ART: antiretroviral therapy; PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; BIA: bioimpedance analysis.

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lated central fat accumulation; 32.3% with lipoatrophy and 27.3% with mixed form of lipodystrophy). Patients with lipoatrophy and mixed forms of lipodystrophy were more frequently males, had significantly longer HIV infection duration and length of cART (p<0.001). Patients with isolated central fat accumulation and mixed forms of lipodystrophy were older, and presented significantly higher mean of all the anthropometric parameters (p<0.05), with the obvious exception of height. Patients with lipoatrophy had the lowest weight, body mass index (BMI), and neck, waist, hip, thigh, and arm circumferences, but not the lowest waist/hip circumference ratio.

Regarding BIA, patients with isolated central fat accumulation and mixed forms of lipodystrophy had significantly higher fat mass. There were no differences between the 4 groups of patients in FFM and TBW (Table 1).

Patients with lipoatrophy and mixed forms of lipodystrophy had significantly higher CD4+ cell count and viral suppression. No significant differences were observed between CDC classification and the pharmacologic groups of cART between the 4 groups. In what concerns the HIV risk factor, patients with no lipodystrophy and lipoatrophy were more frequently injecting drug users and patients with isolated central fat accumulation and mixed forms of lipodystrophy were more frequently belonged heterosexual contact group.

In Tables 2 to 4 results regarding the associations between the prevalence of lipoatrophy, central fat accumulation, and mixed forms of lipodystrophy and patients characteristics, namely demographic, clinical, and anthropometric information, can be observed. Crude significant negative associations were found between the presence of lipoatrophy (Table 2) and female gender, BMI, waist, hip and thigh circumferences, and total body fat mass (both in total kg and percentage) estimated by BIA. On the other hand, crude linear significant positive associations were found between the presence of lipoatrophy and HIV infection duration (p for trend <0.001) and the cART duration (p for trend <0.001) (Table 2). Af-

Table 2 - *Patients characteristics associated with lipoatrophy.*

	OR (95% CI) Crude	OR (95% CI)* Adjusted	OR (95% CI)** Adjusted
Age			
<30	1	1	1
30-39	1.38 (0.46-4.14)	0.83 (0.25-2.76)	1.15 (0.27-4.82)
40-49	1.46 (0.50-4.33)	0.85 (0.26-2.78)	1.08 (0.26-4.40)
>49	0.72 (0.24-2.18)	0.38 (0.11-1.25)	0.68 (0.16-2.87)
Gender			
Male	1	1	1
Female	0.23 (0.12-0.42)	0.22 (0.12-0.40)	0.10 (0.05-0.24)
HIV infection duration			
≤3	1	1	1
4-9	3.03 (1.14-8.09)	1.67 (0.49-5.64)	0.97 (0.24-3.94)
≥10	5.31 (1.98-14.26)	3.89 (1.02-14.8)	1.89 (0.38-9.47)
cART duration			
≤3	1	1	1
4-9	2.64 (1.38-5.04)	2.69 (1.40-5.22)	3.93 (1.76-8.78)
≥10	4.09 (2.04-8.19)	4.43 (2.16-9.08)	4.71 (1.97-11.23)
CD4			
>200	1	1	1
100-200	0.26 (0.03-2.11)	0.22 (0.03-1.89)	0.21 (0.02-2.07)
<100	1.11 (0.44-2.80)	1.14 (0.42-3.09)	1.10 (0.34-3.49)
PI	0.96 (0.85-0.91)	0.96 (0.59-1.56)	0.92 (0.51-1.66)
NNRTI	1.09 (0.70-1.70)	1.04 (0.64-1.68)	1.04 (0.58-1.86)
NRTI	2.90 (0.36-24.52)	3.37 (0.38-30.0)	4.88 (0.51-46.4)
BMI	0.69 (0.64-0.76)	0.64 (0.58-0.72)	0.64 (0.58-0.72)
Waist circumference	0.88 (0.85-0.91)	0.82 (0.78-0-86)	0.84 (0.78-0-90)
Hip circumference	0.78 (0.74-0.83)	0.77 (0.72-0.82)	0.88 (0.81-0.96)
Thigh circumference	0.83 (0.79-0.88)	0.81 (0.76-0.87)	0.97 (0.91-1.03)
% fat	0.73 (0.79-0.87)	0.83 (0.79-0.87)	0.95 (0.89-1.02)
kg fat	0.78 (0.73-0.83)	0.78 (0.72-0.83)	0.89 (0.81-1.00)

*odds ratio (OR) adjusted for gender and duration of treatment (with the exception of disease duration). **OR adjusted for gender, duration of treatment and body mass index (BMI) (with the exception of disease duration). cART: combination antiretroviral therapy; PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; CI: confidence interval.

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ter adjustment for gender, cART duration and BMI, lipoatrophy remained significantly associated with lower waist (OR=0.84; 95%CI: 0.78-0.90) and hip (OR=0.88; 95%CI: 0.81-0.96) circumferences mean. Almost fully opposite results were observed, when isolated central fat accumulation occurrence was assessed (Table 3). Isolated central fat accumulation was significantly associated with female gender (OR 3.16; 95% CI: 1.92-5.18) and all the anthropometric measures evaluated, in the crude analysis. Conversely, lower HIV infection duration and cART duration were significantly associated with isolated central fat accumulation. Independently of gender, cART duration and BMI, isolated central fat accumulation remained significantly associated with higher waist (OR=1.11; 95%CI: 1.05-1.18) and hip (OR=1.15; 95%CI: 1.07-1.23) circumferences mean, and with FM estimated by BIA [FM as %, OR=1.17 (1.09-1.26); and fat mass as kg, OR=1.15 (1.06-1.25)].

The associations observed considering the mixed forms of lipodystrophy are presented in Table 4. After the previously referred adjustments, there was a significant positive association between this phenotype and female gender (OR=1.66; 95%CI: 1.01-2.75), BMI (OR=1.10; 95%CI: 1.04-1.17), and WC (OR=1.15; 95%CI: 1.09-1.21) as it was observed when isolated central fat accumulation occurrence was assessed. Similar significant associations were observed between mixed forms of lipodystrophy and lipoatrophy regarding HIV infection and cART duration, hip and thigh circumference and FM estimated by BIA.

Finally, these same associations were estimated for patients with no lipodystrophy. As it can be observed in Table 5, the associations are opposite to the ones observed for the mixed form of lipodystrophy. Patients with no lipodystrophy were more frequently males, had lower HIV infection and cART duration, lower BMI and WC, and higher hip and thigh circumferences, independently of gender, duration of treatment, and BMI.

DISCUSSION

Body composition in HIV-infected patients and its correlation with body fat distribution is an important issue. BIA is operator-independent, repeatable, non-invasive, widely available method of body composition evaluation that requires less training than anthropometry and is less expensive than DXA (21). BIA gives us an evaluation of body composition, namely the amount of FM, FFM and TBW in a reproducible way and not operator-dependent like certain anthropometric parameters. BIA may also be an useful method in the evaluation of CL evolution (9). BIA was an established method to monitor nutritional sta-

Table 3 - *Patients characteristics associated with isolated central fat accumulation.*

	OR (95% CI) Crude	OR (95% CI)* Adjusted	OR (95% CI)** Adjusted
Age			
<30	1	1	1
30-39	1.41 (0.43-4.61)	2.84 (0.80-10.2)	2.94 (0.66-13.1)
40-49	0.80 (0.24-2.63)	1.36 (0.38-4.85)	1.14 (0.43-5.03)
>49	1.21 (0.37-3.92)	2.44 (0.69-8.63)	1.85 (0.43-7.99)
Gender			
Male	1	1	1
Female	3.16 (1.92-5.18)	3.50 (2.08-5.89)	3.41 (1.88-6.19)
HIV infection duration			
≤3	1	1	1
4-9	0.39 (0.20-0.77)	0.77 (0.32-1.87)	0.62 (0.23-1.71)
≥10	0.26 (0.12-0.53)	0.61 (0.08-0.72)	0.47 (0.13-1.68)
cART duration			
≤3	1	1	1
4-9	0.39 (0.22-0.67)	0.37 (0.21-0.66)	0.18 (0.08-0.39)
≥10	0.20 (0.09-0.42)	0.32 (0.17-0.62)	0.20 (0.08-0.49)
CD4			
>200	1	1	1
≤200	0.46 (0.16-1.36)	0.41 (0.13-1.28)	0.21 (0.02-2.07)
PI	1.19 (0.72-1.95)	1.26 (0.74-2.16)	1.65 (0.89-3.06)
NNRTI	0.89 (0.55-1.45)	0.73 (0.54-1.54)	0.76 (0.41-1.38)
NRTI	0.28 (0.07-1.15)	0.22 (0.05-1.06)	0.18 (0.03-1.10)
BMI	1.28 (1.20-1.37)	1.28 (1.20-1.37))	1.28 (1.20-1.37)
Waist circumference	1.12 (1.08-1.15)	1.13 (1.09-1.16)	1.11 (1.05-1.18)
Hip circumference	1.22 (1.17-1.23)	1.20 (1.14-1.26)	1.15 (1.07-1.23)
Thigh circumference	1.17 (1.11-1.23)	1.14 (1.08-1.21)	1.03 (0.97-1.08)
% fat	1.18 (1.14-1.22)	1.20 (1.14-1.26)	1.17 (1.09-1.26)
kg fat	1.17 (1.13-1.22)	1.16 (1.11-1.20)	1.15 (1.06-1.25)

*odds ratio (OR) adjusted for gender and duration of treatment (with the exception of disease duration). **OR adjusted for gender, duration of treatment and body mass index (BMI) (with the exception of disease duration). cART: combination antiretroviral therapy; PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; CI: confidence interval.

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tus in HIV-infected patients before the introduction of cART, when patients had almost exclusively the wasting syndrome, mainly characterized by losses of both fat and FFM (and the lipodystrophy syndrome had not been yet described) (26). By evaluating FFM, BIA allowed some estimation of the degree of sarcopenia that these patients could present. Before the highly active antiretroviral therapy (HAART) era, the patients usually presented wasting syndrome associated with sarcopenia, on the other hand, our patients regardless the categorization according to body fat distribution presented similar FFM levels.

In well-trained hands, clinical diagnosis of lipoatrophy is straightforward either in severe or in incipient cases. Besides the presence of lipoatrophy in face and limbs, patients may have accumulation of central fat. WC measurement and the waist/hip ratio had been successfully used to assess central obesity (27), but with limited sensitivity and specificity (28).

In our study, we found that patients with CL had significantly higher waist/hip ratio and lower WC (data not shown), but, when patients were categorized in the 4 independent groups according to their fat distribution, WC was higher in patients with abdominal prominence independently of the presence of lipoatrophy (i.e. in patients with isolated central fat accumulation and mixed forms of lipodystrophy) and lower in patients with CL and without abdominal prominence (AP) (i.e. patients with lipoatrophy). The higher waist/hip ratio was present in patients with AP independently of the presence of lipoatrohy (i.e. patients with isolated central fat accumulation and mixed forms of lipodysytrophy), however patients with lipoatrophy had a higher ratio than patients with no lipodystrophy. The BIA measures the electric properties of the whole body. However, several sources of variance (e.g., blood viscosity, albumin concentration, hematocrit, skin temperature, hydration status, having a recent meal, exercising, among others) may affect the whole-body impedance measurements (29, 30). The BIA methods are related to the conducting volume or mass in the body. Body fat *per se*, because it is non-conductive, is not measured by BIA, but is calculated as the difference between body weight and the derived estimate for FFM. Hence, any BIA-based estimate of body fat is subject to cumulative errors associated with the prediction of FFM. This is a limitation of all two-compartment models where fat is defined as the difference between body weight and FFM (30). This method is particularly insensitive to redistribution of body compartments (21). In our patients, those with isolated central fat accumulation and mixed forms of lipodystrophy, had a higher FM (in % and kg), and those with lipoatrophy had a lower FM, but no differ-

ences were observed when we compared the amount of FFM and TBW between the 4 groups. This means that BIA objectively quantify the amount of FM but is unable of discriminating their localization. On the other hand, body fat is the compartment measured with the least precision because of propagation error as it is calculated by subtracting FFM from body weight. Moreover, 90% of the measured whole-body impedance is determined by the impedance of the limbs which contain less than half of the FFM. Therefore, the method is particularly insensitive to redistribution of body compartments.

The AP is the great contributor for the FM and the loss of FM in the extremities in patients with mixed forms of lipodystrophy (with lipoatrophy and with AP) cannot be identified by BIA. Some authors considered that body composition empirical prediction equations from BIA may no longer be valid in populations of HIV-infected patients with a high incidence of fat redistribution (31-34). BIA estimates of body cell mass are well correlated with muscular performance (30). Suppression of the catabolic effects of viremia, decreased incidence of opportunistic infections, or increased physical activity of patients are plausible explanations for a gain in appendicular muscle mass in patients receiving successful antiretroviral treatment (35).

To evaluate the abdominal fat we used WC. In our patients, the mean WC was similar in patients with lipoatrophy and no lipodystrophy but they had a higher waist/hip ratio, which could mean that visceral fat is increased in these patients. Actually, studies using abdominal CT or MRI have demonstrated that many patients at risk for lipodystrophy have increased intraabdominal fat and decreased subcutaneous fat, even if they have not been diagnosed with lipodystrophy. And, even patients with lower WC had a higher ratio visceral fat/subcutaneous fat in CT (14, 36). The most recent BIA devices, for example, Tanita viscan analyser – TAN 2007, allows the identification of visceral and subcutaneous trunk fat.

In our analysis, we separate patients with lipoatrophy from central fat accumulation or abdominal prominence what is not always clear in previous papers. A significant number of studies have subsumed analyses of lipoatrophy and lipohypertrophy under the term lipodystrophy and therefore they may have failed to reveal associations applying to one but not the other. And, adding to the riddle is the lack of a consistent definition of lipodystrophy in the literature. We only compared our results with previous studies that made the categorization between lipoatrophy and central fat accumulation and not with those which referred lipodystrophy.

Aiming at the elucidation of the relative contribution of different risk factors – drugs, disease, and host – to fat redistribution, Lichtenstein reviewed large epidemiologic

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Table 4 - *Patients characteristics associated with mixed form of lipodystrophy.*

	OR (95% CI) Crude	OR (95% CI)* Adjusted	OR (95% CI)** Adjusted
Age			
<30	1	1	1
30-39	2.52 (0.31-20.46)	2.11 (0.25-17.56)	1.95 (0.25-16.85)
40-49	5.58 (0.72-43.50)	5.14 (0.65-40.94)	4.75 (0.57-39.41)
>49	11.31 (1.46-87.41)	10.77 (1.36-85.10)	9.82 (1.20-80.77)
Gender			
Male	1	1	1
Female	1.71 (1.06-2.78)	1.78 (1.09-2.95)	1.66 (1.01-2.75)
HIV infection duration			
≤3	1	1	1
4-9	2.60 (1.04-6.49)	2.72 (1.08-6.84)	2.65 (1.05-6.68)
≥10	2.22 (0.87-5.69)	2.19 (0.85-5.64)	2.31 (0.89-5.98)
cART duration			
≤3	1	1	1
4-9	3.48 (1.73-7.02)	3.60 (1.78-7.29)	3.96 (1.92-8.16)
≥10	2.82 (1.31-6.11)	2.88 (1.33-6.27)	3.58 (1.60-8.00)
CD4			
>200	1	1	1
≤200	1.23 (0.55-2.76)	1.30 (0.57-2.99)	1.24 (0.52-2.97)
PI	0.61(0.38-0.98)	0.54 (0.33-0.88)	0.53 (0.32-0.88)
NNRTI	1.37 (0.86-2.20)	1.51 (0.93-2.45)	1.51 (0.92-2.48)
NRTI	2.32 (0.28-19.15)	2.78 (0.33-23.68)	3.47 (0.38-31.29)
BMI	1.07 (1.02-1.12)	1.08 (1.03-1.13))	1.10 (1.04-1.17)
Waist circumference	1.05 (1.03-1.07)	1.06 (1.04-1.08)	1.15 (1.09-1.21)
Hip circumference	0.99 (0.96-1.02)	0.09 (0.96-1.02)	0.87 (0.82-0.92)
Thigh circumference	1.00 (0.96-1.04)	1.00 (0.96-1.04)	0.94 (0.88-0.99)
% fat	1.03 (1.01-1.06)	1.03 (1.00-1.07)	0.98 (0.93-1.04)
kg fat	1.02 (1.00-1.05)	1.02 (0.99-1.05)	0.90 (0.83-0.96)

*odds ratio (OR) adjusted for gender and duration of treatment (with the exception of disease duration). **OR adjusted for gender, duration of treatment and body mass index (BMI) (with the exception of disease duration). cART: combination antiretroviral therapy; PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; CI: confidence interval.

Table 5 - *Patients characteristics associated with no lipodystrophy.*

	OR (95% CI) Crude	OR (95% CI)* Adjusted	OR (95% CI)** Adjusted
Age			
<30	1	1	1
30-39	0.47 (0.16-1.40)	0.61 (0.19-1.93)	0.70 (0.21-2.40)
40-49	0.32 (0.11-0.96)	0.41 (0.13-1.29)	0.43 (0.13-1.47)
>49	0.18 (0.06-0.59)	0.21 (0.06-0.70)	0.29 (0.08-1.06)
Gender			
Male	1	1	1
Female	0.42 (0.20-0.88)	0.41 (0.19-0.88)	0.44 (0.20-0.96)
HIV infection duration			
≤3	1	1	1
4-9	0.54 (0.25-1.15)	0.51 (0.23-1.10)	0.42 (0.19-0.95)
≥10	0.22 (0.09-0.55)	0.22 (0.09-0.55)	0.14 (0.05-0.37)
cART duration			
≤3	1	1	1
4-9	0.41 (0.22-0.79)	4.18 (1.69-10.34)	0.36 (0.18-0.71)
≥10	0.25 (0.10-0.61)	1.66 (0.68-4.06)	0.17 (0.06-0.43)
CD4			
>200	1	1	1
≤200	1.52 (0.59-3.88)	1.32 (0.50-3.49)	1.33 (0.47-3.75)
PI	1.24 (0.68-2.29)	1.35 (0.72-2.53)	1.28 (0.68-2.44)
NNRTI	0.94 (0.51-1.72)	0.92 (0.50-1.71)	0.98 (0.52-1.86)
NRTI	0.44 (0.09-2.26)	0.35 (0.06-1.98)	0.34 (0.05-2.25)
BMI	0.87 (0.80-0.94)	0.83 (0.76-0.91)	0.83 (0.76-0.91)
Waist circumference	0.93 (0.90-0.96)	0.91 (0.87-0.94)	0.51 (0.79-0.92)
Hip circumference	0.97 (0.94-1.01)	0.96 (0.92-1.00)	1.11 (1.03-1.20)
Thigh circumference	0.96 (0.91-1.01)	0.95 (0.89-1.00)	1.02 (0.96-1.09)
% fat	0.93 (0.90-0.97)	0.93 (0.89-0.97)	1.02 (0.94-1.10)
kg fat	0.91 (0.86-0.95)	0.90 (0.85-0.95)	0.95 (0.85-1.06)

*odds ratio (OR) adjusted for gender and duration of treatment (with the exception of disease duration). **OR adjusted for gender, duration of treatment and body mass index (BMI) (with the exception of disease duration). cART: combination antiretroviral therapy; PI: protease inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; CI: confidence interval.

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studies using multivariate analysis. In studies assessing lipoatrophy, the most common statistically significant risk factors were: use of specific nucleoside analogues, increasing age, presence of markers of disease severity (CD4/HIV RNA), duration of therapy and white race (17). Also, in our patients, duration of therapy, in parallel with length of HIV disease, were associated with lipoatrophy. In studies assessing central fat accumulation the most common statistically significant risk factors were duration of therapy, markers of disease severity, and protease inhibitor use. Bonfaty also showed an increasing relation between the number of years of therapy and lipoatrophy, however, he found no relation between central fat accumulation and duration of ART (37). In our results, lesser duration of the disease and treatment were associated with central fat accumulation. In what concern gender, men had a significantly lower adjusted risk of presenting with any alteration than women and a significantly lower risk of central fat accumulation and mixed fat redistribution, whereas risk of lipoatrophy was similar between genders (17). Female gender was positively associated with central fat accumulation and negatively with lipoatrophy. Like us, also Shevitz found that central fat accumulation was associated with female gender and high BMI and fat depletion was associated with low BMI (38). The risk of FM redistribution has been associated with increase age. The physiological ageing process also is linked to body-fat changes that may bias the redistribution seen in HIV-1-infected patients (39). An increase of FM may be seen in both genders with increasing ageing. Additionally, a redistribution of body fat including a decrease in the size of lower limbs and an increase in truncal FM may be seen with ageing both in men and women (40). Also, Lichtenstein found that fat accumulation was associated with age, BMI gain, and viral suppression and duration cART and suggests that lipoatrophy is related to effective control of HIV-1 infection or use of drugs (namely, stavudine, and indanavir) in patients with more advanced HIV-1 disease and fat accumulation was associated with several correlates of immunologic recovery (24). With regard to the influence of HIV disease itself, there is certainly evidence that a nadir CD4+ T-cell count of $<100\times10^6$ L^{-1} is associated with increased risk of lipoatrophy while on treatment, irrespective of the virological or immunological response to therapy (41). Lichtenstein concluded that rigorous multivariate analysis controlling for numerous variables reveal multiple risk factors, suggesting that the pathogenic mechanisms for fat redistribution are likely the result of complex interactions between host, disease, and drug factors (17).

We observed that injecting drug users had a low prevalence of isolated central fat accumulation and mixed forms and a high prevalence of lipoatrophy. The lipoatrophy could be explain by the fact of these patients being diagnosed for a long time and used older agents of

cART, that were associated with greater toxicity. The lower prevalence of central fat accumulation, probably has to do with nutritional factors, since many times the food intake in these patients is poor, and even some inject drugs may be central anorexiant.
In our data, the CD4 count increased progressively from the phenotype "no lipodystrophy" to "isolated central fat accumulation", to "lipoatrophy" and by the end to "mixed forms of lipodystrophy". Patients with longer treatment, tend to have higher CD4 counts. Alternatively, that may also be associated with the robustness of the imumnological recovery itself.
In what concern the results of BIA, the mixed forms of lipodystrophy are in fact a mixture between the lipoatrophy and central fat accumulation phenothypes. As it happens in the patients with isolated central fat accumulation, the patients with mixed forms of lipodystrophy were more frequently women, had a higher BMI and WC, but on the other hand they are more like patients with lipoatrophy since, the mixed forms of lipodystrophy were also associated a more time of disease and length of treatment but lower hip and thigh circumference and lower fat mass evaluated by BIA. In what concern patients with no lipodsytrophy, the results are almost the opposite to the mixed forms of lipodystrophy.

Strengths
The results of BIA demonstrate an increase in FM in patients with isolated central fat accumulation and mixed forms, an objective way to evaluate body fat. Patients with lipoatrophy and no lipodystrophy had lower FM. However, the FFM is similar among the 4 groups. This picture of body composition is not possible to have with anthropometry.
As all participants were treated with cART we excluded any bias resulting from this risk factor for lipodystrophy.

Conclusions
In HIV-infected patients treated with cART for a long period of time there were no differences between the 4 phenotypes of patients (no lipodystrophy, isolated central fat accumulation, lipoatrophy, and mixed forms of lipodystrophy) in FFM and TBW, however, patients with AP (with and without lipoatrophy) had significantly higher FM, using BIA. The similar FFM along the fat distribution spectrum describes the actual body composition of these patients and excludes sarcopenia. In a clinical setting, BIA is an easy and useful tool to evaluate FM and the amount of FFM and gives us a picture of body composition that was not possible with anthropometry.

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Transparency declarations

None to declare.

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Impact of Lipodystrophy on the prevalence and components of metabolic syndrome in HIV-infected patients

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Abstract

Background: In HIV-infected patients, combination antiretroviral therapy (cART) is associated with clinical lipodystrophy (CL) and metabolic abnormalities (MA). This study aimed to evaluate the prevalence of the metabolic syndrome (MS) and its components, and to determine whether patients with or without CL had a different prevalence of MA.

Methods: We evaluated 345 HIV-infected patients on cART using two different MS definitions (NCEP-ATPIII-2005 and IDF-2005) and the Framingham risk score.

Results: CL was present in 58.7% of the patients. The prevalence of the MS was 52.2% (ATPIII) and 43.2% (IDF), and it was not significantly different between patients with (W) or without (WT) CL, regardless of the definition used (ATPIII WCL 52.9% vs WT CL 51.1%; p = 0.738; IDF WCL 41.3% vs WTCL 46.0%; p = 0.379). Moderate concordance was observed between the 2 definitions (kappa = 0.484; p < 0.001) and after gender stratification there was good concordance in women (kappa = 0.759; p < 0.001). Patients with CL had lower waist circumference and HDL-C and higher triglycerides levels. In women, CL was significantly associated with MS, hypertriglyceridemia and low HDL cholesterol independently of age, cART and BMI. Patients with CL had a significantly higher risk of coronary heart disease at 10 years, measured by the Framingham risk score, than patients without CL. Those with CL and with MS had higher frequencies of moderate and high risk categories than those without MS.

Conclusions: The prevalence of the MS was high in these HIV-infected patients with an age average of 40 years and this finding could explain why HIV patients have an increased risk for cardiovascular disease (CVD).

Keywords: Metabolic syndrome, Cardiovascular Risk, Lipodystrophy, HIV infection

Background

Combination antiretroviral therapy (cART) has changed the course of HIV infection, leading to a significant reduction in AIDS related morbidity and mortality [1]. However, cART is known to be associated with changes in fat distribution (lipodystrophy) and metabolic abnormalities, including insulin resistance, dyslipidemia, and increased blood pressure (BP) [2,3].

The Metabolic syndrome (MS), a common condition in the general population, refers to a constellation of cardiovascular disease (CVD) risk factors, including increased waist circumference (WC), glucose and lipid metabolism disorders, and hypertension [4,5]. Each of these risk factors individually increases cardiovascular risk, but the MS itself has shown to be a powerful independent risk factor for cardiovascular morbi-mortality and diabetes [6]. It is still controversial if the MS improves the identification of high risk individuals [7-9]. HIV-infected lipodystrophic patients share several components of the MS. The prevalence of the MS in HIV-infected patients has been reported to range from 4.4 to 45.4% [9-23].

HIV-lipodystrophy is considered to be an adverse effect of cART, not limited to a specific drug or class of drugs [24]. It seems to affects up to half or even more

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HIV-infected patients receiving cART [25]. However, few studies address the prevalence of the MS in patients with lipodystrophy [12,14,17].

The aim of this study was to evaluate the prevalence of the MS and of its five individual components in HIV-infected patients on cART and to determine whether patients with or without CL had a different prevalence of MA.

Methods

Subjects

As part of a cross-sectional study, 345 non-institutionalized, HIV-infected Caucasian adults (239 males), on cART referred from the Infectious Diseases to the Endocrinology Out-patient Clinic of Hospital São João due to lipodystrophy or any metabolic disorders were included. Only patients on cART were included, since lipodystrophy is mainly related to ART drugs. The study protocol was approved by the Hospital's Ethical Committee and all patients provided informed consent.

Clinical assessment

For each patient the following information was collected, using a standardized protocol: demographic data (age, gender), duration of HIV infection, history of diabetes, hypertension, use of antidiabetic, antihypertensive or lipid-lowering drugs and duration of cART. Weight, height, WC and resting BP were measured as previously described [26]. Body weight was measured using TANITA (Tanita®, model TBF 300) scale and height was measured to the nearest centimetre in the standing position using a wall stadiometer (Holtain Limited Crymych, Dyfed®).

Clinical lipodystrophy (CL) and central fat accumulation or abdominal prominence, were defined as previously described [27]. All clinical assessments were performed by the same practitioner (PF). Patients were classified in 4 different groups according to the presence or absence of either lipoatrophy or abdominal prominence: group 1- **No lipodystrophy** (patients without clinical lipoatrophy and without abdominal prominence); group 2- **Isolated central fat accumulation** (patients without clinical lipoatrophy and with abdominal prominence); group 3- **Isolated lipoatrophy** (patients with clinical lipoatrophy and without abdominal prominence); group 4- **Mixed forms of lipodystrophy** (patients with clinical lipoatrophy and with abdominal prominence).

A venous blood sample was drawn after a 12-hour overnight fast. All the samples were analyzed at the central laboratory of our hospital. Plasma glucose, total cholesterol, HDL cholesterol (HDL-C) and triglycerides were determined using automatic standard routine enzymatic methods. Hepatitis C was diagnosed by HCV-Ab serostatus. The CD4+ cell count was determined by flow cytometry and plasma RNA-VIH was measured by a quantitative reverse transcriptase polymerase chain reaction, which had a lower limit of detection of 50 copies/mL.

Metabolic Syndrome definitions

Two different MS definitions were used: the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATPIII) modified by American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement (AHA/NHLBI) in 2005 [28] and the International Diabetes Federation (IDF) definition [29].

The Framingham cardiovascular risk score was calculated for all patients using the Framingham methodology as reported by Wilson. It incorporates age, gender, systolic blood pressure, lipids, glucose, and smoking status into a model that predicts the probability of coronary heart disease risk at 10 years [30].

Statistical analysis

Data were presented as mean and standard deviation (SD) for quantitative variables when normally distributed. When the variable distribution was different than the normal, it was expressed as median and respective 25^{th} and 75^{th} percentiles. For comparison between quantitative variables Student-t test and the Mann-Whitney test were used when appropriate. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher's exact test. Kappa coefficients were estimated to analyze the statistical agreement between ATP III as modified by the AHA/NHLBI and IDF definitions of MS.

Odds ratios and their respective 95% confidence intervals (CI) were computed using unconditional logistical regression models. The association between lipodystrophy and the presence of MS and its individual components was estimated after adjustment for sex, age, cART duration and BMI, whenever appropriate.

Statistical analysis was performed using the SPSS version 17.0 software (SPSS Inc., Chicago, Illinois, USA). All probabilities were two tailed and p values < 0.05 were regarded as significant.

Results

Of the 345 HIV-1 infected patients on cART included in this study, 58.7% presented CL. Table 1 shows the characteristics of the study sample according to the presence of CL. Patients with CL were more frequently male, older, had longer duration of infection, longer duration of cART and lower weight, BMI and WC. Also, this group of patients had significantly higher CD4+ cell counts and a significantly higher prevalence of virological suppression.

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Table 1 Sample characteristic according to the presence of clinical lipodystrophy

	Without CL	With CL	P
n (%)	139 (39.6)	206 (58.7)	
Sex [n(%)]			
Male	85 (61.2)	154 (74.8)	0.007
Female	54 (38.8)	52 (25.2)	
Age [years, median (25^{th} and 75^{th} percentiles)]	42.0 (34.0-51.0)	45.0 (39.0-54.0)	0.006
Duration of HIV infection [years, median (25^{th} and 75^{th} percentiles)]	6.0 (4.0-9.0)	9.0 (6.0-11.0)	< 0.001
cART [years, median (25th and 75th percentiles)]	4.0 (2.0-7.0)	8.0 (5.0-10.0)	< 0.001
Weight [Kg, mean (sd)]	73.6 (14.1)	66.2 (12.4)	< 0.001
Height [m, mean (sd)]	1.65 (0.09)	1.66 (0.09)	0.430
BMI [(kg/m^2), mean (sd)]	27.0 (5.0)	24.0 (3.9)	< 0.001
Waist circumference [cm, mean (sd)]	95.4 (13.0)	89.5 (10.5)	< 0.001
Systolic BP [mmHg, median (25^{th} and 75^{th} percentiles)]	120.0 (110.0-135.0)	120.0 (110.0-135.0)	0.871
Diastolic BP [mmHg, median (25^{th} and 75^{th} percentiles)]	80.0 (70.0-80.0)	80.0 (70.0-80.0)	0.765
CD4 cell count [cells/mm3, median (25^{th} and 75^{th} percentiles)]	446.5 (291.5-633.8)	544.0 (362.0-749.0)	0.008
HIV RNA (< 50) [n (%)]	94 (82.5)	163 (91.1)	0.029
Hepatitis C co-infection [n (%)]	31 (25)	58 (31.4)	0.227
HIV risk factor [n (%)]			
Intravenous drug user	28 (24.6)	52 (30.6)	
Homosexual contact	10 (8.8)	19 (11.2)	
Heterosexual contact	75 (65.8)	90 (52.9)	
Others	1 (0.9)	9 (5.3)	0.073
CDC [n (%)]			
A	62 (54.9)	94 (53.4)	
B	4 (3.5)	0 (0.0)	
C	47 (41.6)	82 (46.6)	0.037
ART [n (%)]			
IP	67 (61.5)	98 (55.4)	0.310
NNRTI	47 (43.1)	83 (46.9)	0.534
NRTI	104 (95.4)	175 (98.9)	0.066
Smoking history [n (%)]			
Never	60 (43.5)	70 (34.8)	
Current	56 (40.6)	93 (46.3)	
Former	22 (15.9)	38 (18.9)	0.272
Glucose [mg/dL, median (25^{th} and 75^{th} percentiles)]	91.0 (84.0-108.0)	94.5 (863.0-117.0)	0.074
Total cholesterol [mg/dL, mean (sd)]	229.5 (59.8)	220.7 (59.5)	0.182
HDL- cholesterol [mg/dL, median (25^{th} and 75^{th} percentiles)]	48.0 (37.0-55.0)	43.0 (35.0-53.0)	0.047
Triglycerides [mg/dL, median (25^{th} and 75^{th} percentiles)]	192.0 (126.0-323.0)	231.0 (149.8-365.8)	0.016
Metabolic syndrome ATP III [n (%)]	71 (51.1)	109 (52.9)	0.738
Metabolic syndrome IDF [n (%)]	64 (46.0)	85 (41.3)	0.379
Metabolic syndrome features - ATP III			
High blood pressure	61 (43.9)	87 (42.4)	0.790
Hypertriglyceridemia	98 (70.5)	177 (85.9)	< 0.001
Low HDL cholesterol	93 (66.9)	160 (77.7)	0.027
High waist circumference	58 (41.7)	46 (22.3)	< 0.001
High fasting glucose	51 (36.7)	84 (40.8)	0.446
Number of metabolic syndrome features ATP III			
0	6 (4.3)	9 (4.4)	0.242

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Table 1 Sample characteristic according to the presence of clinical lipodystrophy *(Continued)*

1	25 (18.0)	19 (9.3)	
2	37 (26.6)	69 (33.7)	
3	31 (22.3)	52 (25.4)	
4	31 (22.3)	42 (20.5)	
5	9 (6.5)	14 (6.8)	
Metabolic syndrome features - IDF			
High blood pressure	61 (43.9)	87 (42.4)	0.790
Hypertriglyceridemia	98 (70.5)	177 (85.9)	< 0.001
Low HDL cholesterol	93 (66.9)	160 (77.7)	0.027
High waist circumference	87 (62.6)	94 (45.6)	0.002
High fasting glucose	51 (36.7)	80 (38.8)	0.687
Number of metabolic syndrome features IDF			
0	5 (3.6)	9 (4.4)	0.263
1	19 (13.7)	17 (8.3)	
2	35 (25.2)	51 (24.9)	
3	32 (23.0)	63 (30.7)	
4	35 (25.2)	39 (19.0)	
5	13 (9.4)	26 (12.7)	

Note - CL = Clinical lipodystrophy, MS = Metabolic syndrome; ATPIII = Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF = International Diabetes Federation; BMI = body mass index.

No significant differences in HIV risk factors or in cART regimen composition were found between patients with or without CL. The prevalence of hepatitis C co-infection was 28.8%, without significant differences between patients with or without CL. No significant differences were found in smoking history (never, current or former) between patients with or without CL, however, patients who were current smokers had the highest frequency of low and normal weight and those who never smoked had the highest frequency of overweight and obesity (data not shown). No significant difference was found in fasting glucose according to smoking history (data not shown). According to the presence of CL, no differences were found in systolic and diastolic BP, glucose and total cholesterol levels. However, patients with CL had lower HDL-C and higher triglycerides plasma levels (Table 1).

Regarding BMI distribution, men and women with CL, were more frequently classified as normal when compared to those without CL. Those without CL were more frequentely classified as overweight (Table 2).

Table 2 BMI by gender and clinical lipodystrophy

	Men		Women	
BMI [kg/m^2, (n (%)]	**Without CL**	**With CL**	**Without CL**	**With CL**
< 18.5	1 (1.2)	9 (5.8)	0 (0.0)	8 (15.4)
≥18.5 and < 25	38 (44.7)	88 (57.1)	16 (30.2)	25 (48.1)
≥25 and < 30	31 (36.5)	47 (30.5)	20 (37.7)	15 (28.8)
≥30	15 (17.6)	10 (6.5)	17 (32.1)	4 (7.7)
p value	0.009		< 0.001	

Note - CL = Clinical lipodystrophy; BMI = body mass index.

Metabolic syndrome

In our sample, the prevalence of MS was 52.2% and 43.2%, according to ATPIII and IDF criteria, respectively. No significant differences in the prevalence of the MS were observed in patients with or without CL, regardless of the definition used (table 3). Moderate concordance was observed between the 2 definitions (kappa = 0.484; p < 0.001). After gender stratification, moderate agreement was observed in men (kappa = 0.374; p < 0.001), and good agreement in women (kappa = 0.759; p < 0.001). Using both MS criteria, no significant differences in the prevalence of CL were found between patients with and without MS [[with MS 60.6% (109 patients) vs without MS 58.8% (97 patients); p = 0.822 for ATPIII] and [with MS 57.0% (85 patients) vs without MS 61.7%

Table 3 Metabolic syndrome prevalence according to ATP III and IDF criteria in the total of patients and by gender and presence of clinical lipodystrophy

MS Criteria	Total of patients	Without CL	With CL	P
ATP III [n, (%)]				
Total	180 (52.2%)	71 (51.1%)	109 (52.9%)	0.738
Men	130 (54.4%)	46 (54.1%)	84 (54.5%)	0.949
Women	50 (47.2%)	25 (46.3%)	25 (48.1%)	0.854
IDF [n, (%)]				
Total	149 (43.2%)	64 (46.0%)	85 (41.3%)	0.379
Men	88 (36.8%)	36 (42.4%)	52 (33.8%)	0.188
Women	61 (57.5%)	28 (51.9%)	33 (63.5%)	0.227

Note - CL = Clinical lipodystrophy MS = Metabolic syndrome; ATPIII = Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF = International Diabetes Federation; BMI = body mass index.

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(121 patients); p = 0.442 for IDF]]. No significant differences according to gender and presence of CL were observed regarding the prevalence of the MS (Table 3). However, men and women with lipodystrophy had a significantly different prevalence of the MS when stratified by BMI classes, regardless the criteria used to define the MS (Table 4). Men and women with CL presented a significantly higher prevalence of the MS in the normal and underweight class (Table 4). A significantly higher prevalence of hypertriglyceridemia, low HDL-C levels and lower prevalence of abdominal obesity were observed in patients with CL. No differences were found in the number of the MS features according to ATPIII and IDF criteria in patients with and without CL (Table 1).

Regarding the aggregate of the MS criteria using the ATP definition, the most frequent aggregate number was 2, except for men without CL. On the other hand, using the IDF definition, the most frequent aggregate number was 3, except for women without CL (table 5 and 6). No significant increase of MS prevalence with age was observed in patients with or without CL (data not shown).

Concerning the frequency of the individual ATPIII criteria for MS, we found a significantly lower prevalence of central obesity in men with CL and no other significant differences were observed. No significant differences were found in the frequency of the five IDF criteria for the MS in men (table 5).

In women with CL, using either ATPIII or IDF criteria, we found a statistically higher frequency of hypertriglyceridemia and lower HDL-C levels. According to ATPIII criteria, women with CL had a lower frequency of central obesity (Table 6).

Table 4 MS prevalence by BMI, gender and clinical lipodystrophy according to ATPIII criteria and IDF

	Men		Women	
	Without CL	**With CL**	**Without CL**	**With CL**
ATPIII criteria				
Classes of BMI [kg/m^2; (n (%)]				
< 25	14 (30.4%)	41 (48.8%)	3 (12.5%)	11 (44.0%)
≥25 < 30	19 (41.3%)	33 (39.3%)	9 (37.5%)	10 (40.0%)
≥30	13 (28.3%)	10 (11.9%)	12 (50.0%)	4 (16.0%)
p value	0.002	< 0.001	0.012	0.008
IDF criteria				
< 25	4 (11.1%)	11 (21.2%)	5 (18.5%)	16 (48.5%)
≥25 < 30	18 (50.0%)	32 (61.5%)	10 (37.0%)	13 (39.4%)
≥30	14 (38.9%)	9 (17.3%)	12 (44.4%)	4 (12.1%)
p value	< 0.001	< 0.001	0.084	0.012

Note - CL = Clinical lipodystrophy; MS = Metabolic syndrome; ATPIII = Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF = International Diabetes Federation; BMI = body mass in

No significant differences were found between the cumulative number MS features according to the presence of CL in both genders (Table 5 and 6).

In men, high blood pressure, high WC and the MS were more frequent in those with isolated central fat accumulation and mixed forms of lipodystrophy using either ATPIII or IDF criteria. High fasting glucose levels were significantly more frequent in patients with isolated central fat accumulation and mixed forms of lipodystrophy using ATPIII criteria and without any differences (p = 0.058) in the 4 groups of fat distribution using the IDF criteria (table 7).

In women, no significant differences were found in high blood pressure and high fasting glucose levels in the four groups of fat distribution (either ATPIII or IDF criteria). Hypertriglyceridemia, low C-HDL levels, high waist circumference and presence of the MS were more frequent in patients with isolated central fat accumulation and mixed forms of lipodystrophy using either ATPIII or IDF criteria (table 8).

Men and women with CL, after adjustment for age and cART, had significantly lower odds of having central obesity, as defined by the ATPIII criteria [OR = 0.403 (95% CI: 0.187-0.865) and OR = 0.306 (95%CI: 0.118-0.796), respectively].

In women, CL was significantly associated with higher odds of having MS as defined by IDF [OR = 5.290 (95% CI: 1.502-18.635)], hypertriglyceridemia [OR = 5.917 (95%CI: 1.50-23.349)] and low HDL cholesterol [OR = 3.990 (95%CI:1.252-12.716)], independently of age, cART and BMI (tables 9 and 10).

No significant differences were found in the five classes of the Framingham risk score between patients with or without CL (data not shown).

Patients with CL had a significantly higher median risk of coronary heart disease at 10 years, measured by the Framingham risk score, than patients without CL, [median (25th and 75th percentile) = 7.00 (4.00-14.00) and 6.00 (4.00-11.00); p = 0.049]. Independent of the presence of CL, those with MS had a higher median of the Framingham risk score. In patients without CL, those with MS had a higher Framingham risk score median than those without MS, [median (25th and 75th percentile) = 7.00 (4.00-12.50) and 6.00 (3.00-9.00); p = 0.007]. Also, in patients with CL, those with MS, had a higher Framingham risk score than those without MS, [median (25th and 75th percentile) = 9.00 (6.00-18.00) and 6.00 (4.00-9.00); p < 0.001].

In patients with CL, we found significant differences in the Framingham risk score classes between patients with or without MS. Patients with CL and with MS had higher frequencies of moderate and high risk categories than those without MS (table 11).

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Table 5 Frequency of each criterion of MS according to ATP III and IDF in men with and without clinical lipodystrophy

Men								
	ATPIII				IDF			
MS criteria	Total	Without CL	With CL	p value	Total	Without CL	With CL	p value
WC [n, (%)]	43 (18.0%)	21 (24.7%)	22 (14.3%)	0.045	98 (41.0%)	42 (49.4%)	56 (36.4%)	0.050
TG [n, (%)]	197 (82.4%)	65 (76.5%)	132 (85.7%)	0.072	197 (82.4%)	65 (76.5%)	132 (85.7%)	0.072
HDL [n, (%)]	181 (75.7%)	62 (72.9%)	119 (77.3%)	0.455	181 (75.7%)	62 (72.9%)	119 (77.3%)	0.455
BP [n, (%)]	111 (46.6%)	41 (48.2%)	70 (45.8%)	0.713	111 (46.6%)	41 (48.2%)	70 (45.8%)	0.713
GLU [n, (%)]	107 (44.8%)	35 (41.2%)	72 (46.8%)	0.407	103 (43.1%)	35 (41.2%)	68 (44.2%)	0.656
Number of MS criteria								
0	8 (3.3)	3 (3.5)	5 (3.3)		8 (3.4)	3 (3.5)	5 (3.3)	
1	29 (12.1)	15 (17.6)	14 (9.2)		24 (10.1)	11 (12.9)	13 (8.5)	
2	72 (30.1)	21 (24.7)	51 (33.3)		60 (25.29	19 (22.4)	41 (26.8)	
3	67 (28.5)	23 (27.1)	44 (28.8)		70 (29.4)	22 (25.9)	48 (31.4)	
4	48 (20.1)	17 (20.0)	31 (20.3)		48 (20.2)	20 (23.5)	28 (18.3)	
5	14 (5.9)	6 (7.1)	8 (5.2)	0.411	28 (11.8)	10 (11.8)	18 (11.8)	0.718

Note - CL = Clinical lipodystrophy MS = Metabolic syndrome; ATPIII = Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF = International Diabetes Federation; WC = waist circumference; TG = triglycerides; HDL = HDL cholesterol; BP = blood pressure; GLU = glucose.

Discussion

To our knowledge, this is the first study conducted in HIV-infected patients focusing specifically on the role of subcutaneous adipose tissue atrophy defined by presence of CL on the prevalence of the MS. Another particularity is that all patients were on cART. Other studies analysed the presence of the MS in naïve or on cART HIV-infected patients, with sub-analyses concerning the presence of CL in patients with the MS [12,14,17].

cART can induce a large spectrum of metabolic disturbances. On the other hand, lipodystrophy *per se*, including genetic lipodystrophy syndromes, is associated with lipid and metabolic disturbances, and hasmany similarities with the MS [2,31-35]. The effect of cART on the molecular processes of metabolic disturbances has been extensively studied [36] and its contribution to an increased risk of premature and accelerated atherosclerosis in HIV infection is well recognized [37,38]. Our aim was to determine the different patterns of metabolic alterations in patients with or without lipodystrophy.

It is unknown if the MS is more frequent in patients with lipodystrophy compared to either with patients without lipodystrophy and not HIV-infected [9,17]. We found a higher prevalence of MS compared with some studies [9,10,12-23] but similar to another [11]. We included all patients referred from the Infectious Diseases

Table 6 Frequency of each criterion of MS according to ATP III and IDF in women with and without clinical lipodystrophy

Women								
	ATPIII				IDF			
MS criteria	Total	Without CL	With CL	p	Total	Without CL	With CL	p
WC [n, (%)]	61 (57.5%)	37 (68.5%)	24 (46.2%)	0.020	83 (78.3%)	45 (83.3%)	38 (73.1%)	0.200
TG [n, (%)]	78 (73.6%)	33 (61.1%)	45 (86.5%)	0.003	78 (73.6%)	33 (61.1%)	45 (86.5%)	0.003
HDL [n, (%)]	72 (67.9%)	31 (57.4%)	41 (78.8%)	0.018	72 (67.9%)	31(57.4%)	41 (78.8%)	0.018
BP [n, (%)]	37 (34.9%)	20 (37.0%)	17 (32.7%)	0.639	37 (34.9%)	20 (37.0%)	17 (32.7%)	0.639
GLU [n, (%)]	28 (26.4%)	16 (29.6%)	12 (23.1%)	0.444	28 (26.4%)	16 (29.6%)	12 (23.1%)	0.444
Number of MS criteria								
0	7 (6.6)	3 (5.6)	4 (7.7)	0.680	6 (5.7)	2 (3.7)	4 (7.7)	0.214
1	15 (14.2)	10 (18.5)	5 (9.6)		12 (11.3)	8 (14.8)	4 (7.7)	
2	34 (32.1)	16 (29.6)	18 (34.6)		26 (24.5)	16 (29.6)	10 (19.2)	
3	16 (15.1)	8 (14.8)	8 (15.4)		25 (23.6)	10 (18.5)	15 (28.8)	
4	25 (23.6)	14 (25.9)	11 (21.2)		26 (24.5)	15 (27.8)	11 (21.2)	
5	9 (8.5)	3 (5.6)	6 (11.5)		11 (10.4)	3 (5.6)	8 (15.4)	

Note - CL = Clinical lipodystrophy MS = Metabolic syndrome; ATPIII = Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; IDF = International Diabetes Federation; WC = waist circumference; TG = triglycerides; HDL = HDL cholesterol; BP = blood pressure; GLU = glucose.

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Table 7 Metabolic syndrome and its individual components according the four different groups of fat distribution in men

	Four different groups of fat distribution				
[n (%)]	No lipodystrophy	Isolated central fat accumulation	Isolated lipoatrophy	Mixed forms of lipodystrophy	P
ATP III					
High blood pressure	17 (39.5)	24 (57.1)	36 (37.1)	33 (60.0)	0.017
Hypertriglyceridemia	32 (74.4)	33 (78.6)	79 (81.4)	52 (92.9)	0.086
Low HDL cholesterol	29 (67.4)	33 (78.6)	74 (76.3)	44 (78.6)	0.563
High waist circumference	0 (0.0)	21 (50.0)	0 (0.0)	22 (39.3)	< 0.001
High fasting glucose	13 (30.2)	22 (52.4)	40 (41.2)	32 (57.1)	0.035
Metabolic syndrome	16 (37.2)	30 (71.4)	43 (44.3)	40 (71.4)	< 0.001
IDF					
High blood pressure	17 (39.5)	24 (57.1)	36 (37.1)	33 (60.0)	0.017
Hypertriglyceridemia	32 (74.4)	33 (78.6)	79 (81.4)	52 (92.9)	0.086
Low HDL cholesterol	29 (67.4)	33 (78.6)	74 (76.3)	44 (78.6)	0.563
High waist circumference	0 (0.0)	42 (100.0)	0 (0.0)	56 (100.0)	< 0.001
High fasting glucose	13 (30.2)	22 (52.4)	38 (39.2)	30 (53.6)	0.058
Metabolic syndrome	0 (0.0)	36 (85.7)	0 (0.0)	52 (92.9)	< 0.001

Department to our Endocrinology Out-patient Clinic for metabolic abnormalities, but we cannot exclude the selection bias by the infectious disease specialist. We found a high prevalence of lipodystrophy but there was no selection bias for the presence of isolated lipoatrohy or abdominal prominence. Since our main goal was to evaluate whether the presence of lipodystrophy influences MA, only patients on cART were included. The high MS prevalence may reflect the fact that all the patients were on cART. Another important fact is the low prevalence of intravenous drug users in our study (24.6% in patients without CL and 30.6% in patients with CL) compared with Jericó study (41.3%), which concurred on the low prevalence of abdominal obesity.

Studies of prevalence of MS in HIV-infected individuals compared with control populations revealed different prevalences of MS) [9-23] and it also seems to be higher than the 22 to 24% prevalence rate reported for the general US population [39]. Other data suggest that the increased prevalence of MS among HIV-infected persons may be reflective of the burgeoning epidemic of obesity than a predominant effect of cART [40]. Cigarette smoking analysis could be important because of its frequent association with lower BMI and higher glucose

Table 8 Metabolic syndrome and its individual components according the four different groups of fat distribution in women

	Four different groups of fat distribution				
[n (%)]	No lipodystrophy	Isolated central fat accumulation	Isolated lipoatrophy	Mixed forms of lipodystrophy	P
ATP III					
High blood pressure	1 (11.1)	19 (42.2)	2 (14.3)	15 (39.5)	0.110
Hypertriglyceridemia	4 (44.4)	29 (64.4)	8 (57.1)	37 (97.4)	< 0.001
Low HDL cholesterol	5 (55.6)	26 (57.8)	8 (57.1)	33 (86.8)	0.021
High waist circumference	0 (0.0)	37 (82.2)	0 (0.0)	24 (63.2)	< 0.001
High fasting glucose	2 (22.2)	14 (31.1)	1 (7.1)	11 (28.9)	0.335
Metabolic syndrome	0 (0.0)	25 (55.6)	1 (7.1)	24 (63.2)	< 0.001
IDF					
High blood pressure	1 (11.1)	19 (42.2)	2 (14.3)	15 (39.5)	0.110
Hypertriglyceridemia	4 (44.4)	29 (64.4)	8 (57.1)	37 (97.4)	< 0.001
Low HDL cholesterol	5 (55.6)	26 (57.8)	8 (57.1)	33 (86.8)	0.012
High waist circumference	0 (0.0)	45 (100.0)	0 (0.0)	38 (100.0)	< 0.001
High fasting glucose	2 (22.2)	14 (31.1)	1 (7.1)	11 (28.9)	0.348
Metabolic syndrome	0 (0.0)	28 (62.2)	0 (0.0)	33 (86.8)	< 0.001

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Table 9 Association between lipodystrophy and the metabolic syndrome and its individual components in men

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
MS ATP III	1.017 (0.598-1.731)	0.621 (0.324-1.191)	0.931 (0.460-1.884)
High waist circumference	0.508 (0.260-0.991)	0.403 (0.187-0.865)	–
Hypertriglyceridemia	1.846 (0.940-3.624)	1.677 (0.782-3.600)	2.019 (0.899-4.531)
Low HDL cholesterol	1.261 (0.686-2.319)	1.116 (0.562-2.216)	1.304 (0.639-2.661)
High fasting glucose	1.254 (0.734-2.143)	1.004 (0.540-1.868)	1.256 (0.654-2.409)
High blood pressure	0.905 (0.532-1.540)	0.458 (0.223-0.939)	0.567 (0.270-1.191)
MS IDF	0.694 (0.402-1.196)	0.519 (0.274-0.985)	1.058 (0.473-2.365)
High waist circumference	0.585 (0.342-1.001)	0.491 (0.260-0.929)	–
Hypertriglyceridemia	1.846 (0.940-3.624)	1.677 (0.782-3.600)	2.019 (0.899-4.531)
Low HDL cholesterol	1.261 (0.686-2.319)	1.116 (0.562-2.216)	1.304 (0.639-2.661)
High fasting glucose	1.130 (0.661-1.931)	0.940 (0.506-1.743)	1.143 (0.599-2.180)
High blood pressure	0.905 (0.532-1.540)	0.458 (0.223-0.939)	0.567 (0.270-1.191)

Model 1 - OR crude.
Model 2 - OR adjusted for gender, age and cART.
Model 3 - OR adjusted for gender, age, duration of cART and BMI.

levels, however, no significant difference was found in smoking history (never, current or former) between patients with or without CL. Also, no significant difference was found in fasting plasma glucose levels according the smoking history.

In our sample, the prevalence of MS was lower using the IDF definition compared to the ATPIII criteria either in the total of the patients or in men with or without CL. On the other hand, in women it was higher when the IDF definition was used. This probably mirrors the fact that central obesity is a compulsory criterion for the IDF definition, and, women had a higher mean of WC. Since HIV patients despite lower WC had increased visceral fat, Capeau J proposed that a different WC cut-off is needed for HIV patients [34]. In fact, in our data, patients with CL after adjustment for age and cART had significantly lower odds of having central obesity.

No differences in the prevalence of ATPIII or IDF defined MS between patients with and without CL were observed, like it was previously described by Estrada [14]. In women, CL was significantly associated with MS, hypertriglyceridemia and low HDL cholesterol independently of age, cART and BMI. Analyzing the data differently, Samaras showed that the prevalence of CL was 57.2% in the total of patients, and 73% and 79% in

Table 10 Association between lipodystrophy and the metabolic syndrome and its individual components in women

	Modelo 1 OR (95% CI)	Modelo 2 OR (95% CI)	Modelo3 OR (95% CI)
MS ATP III	1.074 (0.501-2.303)	0.763 (0.298-1.952)	2.730 (0.810-9.194)
High waist circumference	0.394 (0.178-0.869)	0.306 (0.118-0.796)	–
Hypertriglyceridemia	4.091 (1.557-10.750)	3.091 (1.063-8.989)	5.917 (1.50-23.349)
Low HDL cholesterol	2.765 (1.174-6.513)	2.308 (0.885-6.020)	3.990 (1.252-12.716)
High fasting glucose	0.713 (0.298-1.701)	0.560 (0.204-1.534)	0.758 (0.240-2.390)
High blood pressure	0.826 (0.371-1.839)	0.511 (0.177-1.474)	0.917 (0.273-3.078)
MS IDF	1.613 (0.742-3.507)	1.461 (0.572-3.736)	5.290 (1.502-18.635)
High waist circumference	0.543 (0.212-1.393)	0.385 (0.127-1.164)	–
Hypertriglyceridemia	4.091 (1.557-10.750)	3.091 (1.063-8.989)	5.917 (1.500-23.349)
Low HDL cholesterol	2.765 (1.174-6.513)	2.308 (0.885-6.020)	3.990 (1.252-12.716)
High fasting glucose	0.713 (0.298-1.701)	0.560 (0.204-1.534)	0.758 (0.240-2.390)
High blood pressure	0.826 (0.371-1.839)	0.511 (0.177-1.474)	0.917 (0.273-3.078)

Model 1 - OR crude.
Model 2 - OR adjusted for gender, age and cART.
Model 3 - OR adjusted for gender, age, duration of cART and BMI.

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Table 11 Framingham risk score according clinical lipodystrophy and metabolic syndrome

	Without clinical lipodystrophy			With clinical lipodystrophy		
Framingham risk score	**Without MS**	**With MS**	**Total**	**Without MS**	**With MS**	**Total**
Low risk (< 5%) [n (%)]	27 (45.8)	20 (29.4)	47 (37.0)	36 (41.9)	23 (21.5)	59 (30.6)
Average (5%-9%) [n (%)]	21 (35.6)	22 (32.4)	43 (33.9)	31 (36.0)	31 (29.0)	62 (32.1)
Moderate risk (10-19%) [n (%)]	7 (11.9)	13 (19.1)	20 (15.7)	15 (17.4)	31 (29.0)	46 (23.8)
High risk (20-39%) [n (%)]	3 (5.1)	12 (17.6)	15 (11.8)	2 (2.3)	20 (18.7)	22 (11.4)
Very high risk (> 40%) [n (%)]	1 (1.7)	1 (1.5)	2 (1.6)	2 (2.3)	2 (1.9)	4 (2.1)
p			0.078			< 0.001

those with MS defined by respectively IDF and ATPIII criteria [17]. In our patients, we did not find any significant differences in the prevalence of lipodystrophy in patients with and without MS, according to both criteria. In the Jericó study, as in Samaras, lipodystrophy was more common among MS participants (50.4 vs 33.8; p = 0.0001) [12], and Squillace, also found that CL was strongly associated with MS [22].

In the Jericó and Mangili studies, the prevalence of MS in HIV-infected patients significantly increased with age [12]. In our sample, we did not observe any differences in the distribution of MS prevalence according to age. This may reflect the homogeneity of age strata in our sample, when compared to other studies (only 12% of our sample were 60 years or older).

Regarding gender differences, as in our study, Mangili reported that significantly more women than men had MS [10].

Concerning the number of MS components, in our study and according to IDF definition, we had 25% of patients with two features of MS which is lower when compared to other studies [12,17]. In the Samara study, 49% of patients had at least two features of MS, namely elevated lipid levels [17]. In the Jericó study, the MS prevalence was 17%, with 69.3% of patients showing one or more features of MS, 35.8% two or more, 4.5% three or more and 0.1% five features [12].

Concerning the prevalence of the individual features of MS, we found that the two least prevalent features were high fasting glucose level (similar to Bonfatti and Sobieszczyk) [18,21] and high WC, the latter being the least prevalent in the patients with CL. Lipid abnormalities, namely high triglyceride and low HDL-C levels, were present in all HIV-infected patients [9,10,12,13,16,17] and also in CL patients, the most prevalent components of MS. Obesity is an important risk factor for MS in the general population and in HIV-infected individuals [40]. We found a higher prevalence of overweight and obesity among the patients without CL. Although not proving causation, because of the cross-sectional nature of the study design, we can hypothesize that the high frequency of overweight and obesity in patients without CL may contribute to the observed absence of difference in the MS prevalence between patients with or without CL. To clarify the relation between obesity and MS as well as the alterations in the fat distribution, data was analysed according to the presence or absence of either lipoatrophy or abdominal prominence. In men, high blood pressure and MS were more frequent in those with isolated central fat accumulation and mixed forms of lipodystrophy (ATPIII and IDF criteria) and high fasting glucose levels were significantly more frequent in same two groups using ATPIII criteria. In women, hypertriglyceridemia, low C-HDL levels, high WC and presence of MS were more frequent in those with isolated central fat accumulation and mixed forms of lipodystrophy using either ATPIII or IDF criteria. The observation that patients with CL had significantly higher risk of coronary heart disease at 10 years, measured by the Framingham risk score, than patients without CL and that those with CL and with MS had higher frequencies of moderate and high risk categories than those without MS emphasizes the importance of the clinical diagnosis of this condition. The higher risk of CHD at 10 years of HIV lipodystrophic patients was previously reported by Ena [41]. In the De Socio study, a correlation between the Framingham risk score and MS was observed, and patients with lipodystrophy had a higher Framingham risk score than patients without lipodystrophy, suggesting that the presence of lipodystrophy has metabolic implications equivalent to those of MS [42].

Our study had some limitations. We did not exclude patients with diabetes or hypertension diagnosed prior to HIV infection. We also cannot discard the influence of the pre-HIV body composition, the cumulative exposure time of each drug and the nadir value of CD4 factors that could contribute to the risk lipoatrophy or abdominal prominence that were not evaluated. It is difficult to compare results on the prevalence of MS because of the heterogeneity in populations regarding past and actual cART regimens and treatment adherence. Regarding the CD4 count we only presented the values at the time of the cross sectional evaluation, because we did not have access to the nadir value. Finally, there exists the possibility of selection bias. Some subjects in this study were referred from to Endocrinology Clinics specifically for lipodystrophy or metabolic disorders related to cART,

and so we might have selected for a study population enriched for metabolic complications.

Conclusions

In women, CL was significantly associated with the metabolic syndrome, hypertriglyceridemia and low HDL cholesterol independent of age, cART and BMI.

Our data showed that among HIV patients on cART, the prevalence of MS may be remarkably high, especially when taking into account the mean age of our sample. The observation that patients with CL had a significantly higher risk of coronary heart disease at 10 years, measured by the Framingham risk score, than patients without CL and those with CL and with MS had higher frequencies of moderate and high risk categories than those with CL but without MS, emphasizes the importance of the clinical diagnosis of this condition.

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List of abbreviations

AHA/NHLBI: American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement; ATPIII: The Third Report of Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults; BP: Blood pressure; cART - Combination antiretroviral therapy; CL: Clinical lipodystrophy; CVD: Cardiovascular disease; HIV: Human immunodeficiency virus; IDF: International Diabetes Federation; MA: Metabolic abnormalities; MS: Metabolic syndrome; NCEP: National Cholesterol Education Program; WC: Waist circumference.

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Authors' contributions

PF conceived the study, participated in its design, in the acquisition of data and drafted the manuscript; DC conceived the study, participated in its design and drafted the manuscript; SS participate in the acquisition of data and drafted the manuscript; SX participate in the acquisition of data; ACS performed the statistical analysis and revised critically the manuscript; RM participated in its design and revised critically the manuscript; EM and AS revised critically the manuscript; JLM revised the study design. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Prevalence of obesity and its relationship to clinical lipodystrophy in HIV-infected adults on anti-retroviral therapy

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ABSTRACT. Background: Combination antiretroviral therapy (cART) is associated with lipodystrophy (lipoatrophy and lipoaccumulation) and several metabolic abnormalities that together can contribute to an increased cardiovascular risk. The aim of this study was to evaluate the prevalence of obesity in patients on cART according to the presence of clinical lipodystrophy (CL) and to analyse factors associated with obesity. Methods: We evaluated 368 HIV-infected adults on cART. Results: CL was present in 59.0 %. Independently of gender, patients with CL were more frequently underweight [5.7% (21/368)] and of normal weight [47.3% (174/368)], and less frequently overweight [33.2% (122/368)] or obese [13.9% (51/368)]. Mean BMI was higher in patients with abdominal prominence regardless of the presence of clinical lipoatrophy. Patients with CL had lower waist circumference, higher waist/hip and fat mass ratio and lower total and subcutaneous fat, without significant difference in visceral fat but with a higher visceral/subcutaneous fat ratio, as evaluated by CT at abdominal level. CL was significantly less frequent in overweight (OR=0.21, 95%CI: 0.05-0.92) and obese (OR= 0.05, 95%CI: 0.01-0.26) patients, when compared to underweight ones, independent of age, gender, duration of infection, cART regimen, virological suppression and HIV-infection risk factor. Conclusions: Being overweight or obese is highly prevalent in HIV-infected patients on cART. Patients with clinical lipodystrophy were more frequently under- or normally weighted, and less frequently overweighed or obese. Obesity is a condition that should be considered in HIV patients on cART.

INTRODUCTION

HIV disease and obesity are significant public health problems worldwide. In the early years of the HIV pandemic, the wasting syndrome (pronounced loss of weight, with lean body and fat mass loss) was the hallmark of AIDS. The widespread availability of combination antiretroviral therapy (cART), coming into clinical use in 1996, heralded a new era in the management of HIV infection with high rates of suppressed viral replication, increased CD4+ T-cell counts, reduced incidence of wasting syndrome and dramatically decreased morbidity, and mortality (1-9). Shortly after the introduction of cART, a syndrome consisting of metabolic and body fat abnormalities was reported, appearing to be linked with cART use (10, 11). This syndrome, lipodystrophy, included peripheral fat loss (lipoatrophy) and central fat accumulation (lipohypertrophy or abdominal prominence), which could be present separately or in combination in the same individual (mixed forms). Usually, but not invariably, these features were associated with lipid abnormalities and insulin resistance (10, 11). From the initial description of lipodystrophy, it became apparent that its individual components greatly overlapped with diagnostic criteria of the metabolic syndrome, a constellation of abnormalities leading to an increased risk of cardiovascular disease and diabetes in the general population (12). HIV-infected patients have higher risk of cardiovascular (CV) disease, due to several factors, which include traditional CV risk factors, exposure to some antiretroviral drugs, inflammatory cytokines, leading to accelerated lipolysis, dyslipidemia, lipotoxic insulin resistance, and vascular inflammation (13). Another factor that can contribute to the increased CV risk

Key-words: Lipodystrophy; HIV, obesity, fat mass ratio, abdominal level CT

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in these patients is obesity (14-16). This condition is associated with a chronic, systemic state of inflammation that may contribute to CV diseases and other obesity-related comorbidities (17-19). Although lipodystrophy in HIV-infected patients was first reported more than 10 years ago, the available information on obesity in HIV-infected patients is still scarce (10).
The aim of the current study was to evaluate the prevalence of obesity in patients on antiretroviral therapy according to the presence of clinical lipodystrophy and analyse the factors associated with obesity.

METHODS

Subjects

As part of a cross-sectional study, 368 Caucasian, non-institutionalized, HIV-1 infected adults treated with cART, who were outpatients followed at the Hospital São João, Porto, were consecutively evaluated. The study protocol was approved by the Hospital Ethics Committee and all patients provided signed informed consent.

Clinical assessment

For each patient the following data were collected using a standardized protocol: demographic data (age, gender), duration of HIV infection, HIV risk factor, duration of cART and characterisation of the infection. The "Centers for Disease Control and Prevention" (CDC) criteria for staging of HIV infection were used. Weight, height, circumferences of neck, waist, hip, thigh and arm were measured. Body weight was measured using a TANITA (Tanita®, model TBF 300) scale, with patients wearing light clothes without shoes. Height was measured to the nearest centimetre in the standing position using a wall stadiometer (Holtain Limited Crymych, Dyfed®).
Neck circumference was measured in the midway of the neck, between midcervical spine and midanterior neck. In men with a laryngeal prominence (Adam's apple), it was measured just below the prominence. Hip circumference was measured with the subject standing erect with arms by the sides, with the feet together and the gluteal muscles relaxed. An inelastic tape was placed horizontally around the buttocks. To ensure contiguity of the two parts of the tape from which the circumference would be measured, the cross-handed technique of measurement was used. An assistant checked the position of the tape on the opposite side of the subject's body. The tape was in contact with the skin but it did not compress the soft tissues. The waist circumference was measured midway between the lowest rib and iliac crest, at the end of a gentle expiration, with the patient standing. The hip circumference was measured at the greater trochanter. All circumferences were taken with the subjects standing upright, with the face directed forwards, and shoulders relaxed. Thigh circumference was measured around the thigh of the left leg midway between the crotch and the knee when the subject was in a standing position. Arm circumference was measured at the mid-upper arm circumference of the left arm. All measurements were taken by the same observer with standard techniques (20).
Clinical lipodystrophy (CL) was defined as a peripheral lipoatrophy with or without a central fat accumulation assessed by both patient and practitioner (21). Patients were classified as without CL when none of the previously described features were present. Presence of central fat accumulation or abdominal prominence was defined by the measurement of waist circumference using the International Diabetes Federation (IDF) criteria for metabolic syndrome (22). The clinical assessment was performed by the same practitioner (PF). The patients were placed into 4 different categories according to the presence or absence of either lipoatrophy or abdominal prominence: 1) No lipodystrophy - patients without clinical lipoatrophy and without abdominal prominence; 2) Isolated central fat accumulation - patients without clinical lipoatrophy and with abdominal prominence; 3) Isolated lipoatrophy - patients with clinical lipoatrophy and without abdominal prominence; 4) Mixed forms of lipodystrophy - patients with clinical lipoatrophy and with abdominal prominence.
Body composition was assessed with whole-body dual-energy X-ray absorptiometry (DXA – Lunar Expert XL). DXA measurement was performed while the patient was in a supine position, with standard positioning of the arms and feet. Markers used in this study for trunk and lower limbs that defined regions of interest were those indicated by the manufacturer. Regional fat mass values were grouped and analysed for the following anatomical regions: arms, legs, trunk and total body. The fat mass ratio (FMR) was calculated as the ratio between the percentage of the trunk fat mass and the percentage of the lower limbs fat mass (FMR= % of the trunk fat mass/ % of the lower limb fat mass) (21, 23).
The quantification of total, visceral, and peripheral fat was performed with a 64-slice CT scanner (Siemens Sensation 64 Cardiac). Based on a coronal topogram, a single 10 mm thick slice was obtained from each patient at the level of the transverse processes of L4. Fat was defined as the areas with an attenuation value between -190 and -30 Hounsfield units (24, 25). Total abdominal fat was calculated by drawing a region of interest including all the abdomen and subcutaneous tissue. Visceral fat area was calculated by manually drawing a region of interest along the internal contour of the abdominal wall muscles, anterior border of the psoas muscles, and anterior contour of the L4 vertebral body. Subcutaneous abdominal fat was obtained by subtracting the value of visceral fat from total fat. All values were expressed in cm2 rounded to the nearest decimal (24, 25).

The CD4+ cell count was determined by flow cytometry, and plasma HIV-1 RNA loads were measured by a quantitative reverse transcriptase polymerase chain reaction, which has a lower detection limit of 50 copies/mL, as performed in routine clinical care.

Statistical analysis

Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, Illinois, USA). All probabilities were two tailed and p values <0.05 were regarded as significant.

Data were described as mean and standard deviation (SD) for normally distributed quantitative variables and compared using the Student-t test or analysis of variance. When the distribution of the quantitative variables was different than the normal, they were described as median and minimum and maximum interval, and were compared using the Mann-Whitney test or the Kruskal-Wallis test as appropriate. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher's exact test. For estimating the association between clinical lipodystrophy and demographic, clinical and anthropometric measures, odds ratios (OR) and their respective 95% confidence intervals (95% CI) were computed using unconditional logistic regression.

Table 1: *Sample characteristics according to the presence of clinical lipodystrophy*.

	Without CL	With CL	
N (%)	151 (41.0)	217 (59.0)	p
Gender [n (%)]			
Male	89 (58.9)	165 (76.0)	<0.001
Female	62 (41.1)	52 (24.0)	
Age [years, median (min-max)]	42.0 (23.0-77.0)	45.0 (24.0-87.0)	0.008
Duration [years, median (min-max)]	6.0 (0.5-17.0)	9.0 (2.0-29.0)	<0.001
cART [years, median (min-max)]	4.0 (0.0-17.0)	8.0 (0.5-29.0)	<0.001
Weight [kg, mean (sd)]	74.0 (14.7)	66.3 (12.5)	<0.001
Height [m, mean (sd)]	1.65 (0.09)	1.66 (0.09)	0.421
BMI [(kg/m2), mean (sd)]	27.2 (5.2)	23.9 (3.9)	<0.001
Waist circumference [cm, mean (sd)]	95.5 (13.5)	89.5 (10.5)	<0.001
Hip circumference [cm, mean (sd)]	100.7 (9.4)	91.6 (6.2)	<0.001
Thigh circumference [cm, mean (sd)]	50.5 (6.2)	46.1 (6.1)	<0.001
Arm circumference [cm, mean (sd)]	28.2 (3.4)	26.6 (4.0)	<0.001
Neck circumference [cm, mean (sd)]	37.1 (4.0)	37.2 (4.0)	0.944
Waist/ hip ratio [mean (sd)]	0.95 (0.09)	0.98 (0.08)	0.006
CD4 cell count [cells/mm3, median (min-max)]	446.5 (2.0-1880.0)	510.5 (9.0-1840.0)	0.008
HIV RNA (<50) [n (%)]	121 (83.4)	197 (91.2)	0.026
HIV risk factor [n (%)]			
Inject	31 (20.5)	63 (29.2)	
Homosexual	13 (8.6)	30 (13.9)	
Heterosexual	106 (70.2)	113 (52.3)	
Other	1 (0.7)	10 (4.6)	0.002
CDC [n (%)]			
A	84 (56.0)	119 (55.6)	
B	6 (4.0)	1 (0.5)	
C	60 (40.0)	94 (43.9)	0.053
ART [n (%)]			
IP	88 (60.3)	117 (54.2)	0.250
NNRTI	65 (44.8)	105 (48.8)	0.445
NRTI	139 (95.9)	212 (98.6)	0.093
Body Fat Mass by Quantitative CT			
Total fat [cm2, median (min-max)]	340.5 (63.9-850.7)	215.8 (31.5-676.6)	<0.001
VAT [cm2, median (min-max)]	110.8 (14.5-381.8)	115.5 (9.2-385.5)	0.886
SAT [cm2, median (min-max)]	186.2 (30.5-690.1)	78.0 (5.0-417.9)	<0.001
VAT/SAT [median (min-max)]	0.52 (0.09-5.46)	1.31 (0.13-20.4)	<0.001
FMR by DXA [median (min-max)]	1.09 (0.40-3.45)	1.78 (0.43-4.97)	<0.001
BMI [kg/m^2, n (%)]			
<18.5	3 (2.0)	18 (8.3)	
≥18.5 <25	56 (37.1)	118 (54.4)	
≥25 <30	55 (36.4)	67 (30.9)	
> 30	37 (24.5)	14 (6.5)	<0.001

(BMI - body mass index; CDC - Centers for Disease Control and Prevention criteria for staging of HIV infection; VAT- visceral adipose tissue; SAT – subcutaneous adipose tissue; FMR - fat mass ratio; DXA - dual-energy X-ray absorptiometry; CL – clinical lipodystrophy)

Table 2: *Prevalence of BMI categories according to the presence of clinically defined lipodystrophy and by gender*

		Lipodystrophy defined by clinical						
		Men				Women		
BMI [Kg/m², n (%)]	Total (n=254)	Without CL (n=89)	With CL (n=165)	P	Total (n=114)	Without CL (n=62)	With CL (n=52)	p
< 18.5	11 (4.3)	1 (1.1)	10 (6.1)	0.005	10 (8.8)	2 (3.2)	8 (15.4)	<0.001
≥18.5 <25	133 (52.4)	40 (44.9)	93 (56.4)		41 (36.0)	16 (25.8)	25 (48.1)	
≥25 <30	84 (33.1)	32 (36.0)	52 (31.5)		38 (33.3)	23 (37.1)	15 (28.8)	
> 30	26 (10.2)	16 (18.0)	10 (6.1)		25 (21.9)	21 (33.9)	4 (7.7)	

(BMI – body mass index; CL – clinical lipodystrophy)

RESULTS

Of the 368 HIV-1 infected patients (254 men and 114 women) on cART included in this study, 217 (59.0 %) presented CL. Table 1 shows the characteristics of the population according to the presence of CL. Patients with CL were more frequently males, older, had longer duration of HIV infection, length of cART and lower means of weight, BMI, and of waist, hip, thigh and arm circumferences, but had higher mean of waist/hip ratio. Also, patients with CL had higher a FMR mean, and in quantitative CT at abdominal level, lower total fat and subcutaneous fat, without a statistical difference in visceral fat but with a higher visceral/subcutaneous fat ratio.

Patients with CL had a significantly higher mean CD4+ cell count and a higher prevalence of viral suppression. No differences were observed between patients with and without CL regarding pharmacologic groups of cART.

Considering BMI categories, 5.7% (21/368) of patients were underweight, 47.3% (174/368) were of normal weight, 33.2% (122/368) were overweight and 13.9% (51/368) were obese. Patients with CL were more frequently underweight and normally weighted (Table 1). Stratifying by gender, similar results were found, and CL was more frequent in patients with BMI <25.0 kg/m2, regardless of gender (Table 2).

Looking at the 4 groups of body fat distribution, males had a higher prevalence of either isolated lipoatrophy or no lipodystrophy while females had a higher prevalence of isolated central fat accumulation and mixed forms of lipodystrophy. Mixed forms of lipodystrophy were more prevalent in older patients. Patients with a longer duration of HIV infection and cART treatment more frequently had isolated lipoatrophy and mixed forms. Patients with isolated central fat accumulation and mixed forms had higher neck, waist, hip, thigh, arm circumferences and waist/hip ratio. As expected, patients with isolated lipoatrophy and mixed forms had higher FMR. In quantitative CT at abdominal level, patients with isolated central fat accumulation and mixed forms had higher total, visceral and subcutaneous fat, but those with isolated lipoatrophy and mixed forms of lipodystrophy had a higher visceral/subcutaneous fat ratio.

Patients with isolated lipoatrophy and mixed forms had a significantly higher mean CD4+ cell count and a higher prevalence of viral suppression. No differences were observed between patients in the 4 different fat distribution categories regarding CDC classification and pharmacologic groups of cART. Patients with isolated lipoatrophy were more frequently underweight; those without AP (patients with no lipodystrophy and patients with isolated lipoatrophy) were normally weighted; those with AP regardless the presence of clinical lipoatrophy (patients with isolated central fat accumulation and patients with mixed forms of lipodystrophy), were frequently overweight and obese (Table 3). Also, as expected, mean BMI was significantly higher in patients with AP (patients with isolated central fat accumulation and patients with mixed forms of lipodystrophy) and lower in patients with isolated lipoatrophy.

After adjustment for the demographic and clinical features that were significantly associated with CL in the univariate analysis, the presence of CL was significantly associated with male gender, older ages and cART duration. Also, a strong significant association (OR= 3.00, 95%CI: 1.28-7.00) was found between CL and virological suppression. Patients with CL were less frequently overweight (OR=0.21, 95%CI: 0.05-0.92) or obese (OR= 0.05, 95%CI: 0.01-0.26), when compared with underweight patients, and this association was independent of age, sex, duration of infection, cART regimen, virological suppression and HIV-infection risk factor (Table 4).

Table 3: *Sample characteristics according to the 4 fat distribution categories.*

	No lipodystrophy (n=52)	Isolated central fat accumulation (n=87)	Isolated lipoatrophy (n=111)	Mixed forms of lipodystrophy (n=94)	P
Gender [n (%)]					
Male	43 (82.7)	42 (48.3)	97 (87.4)	56 (59.6)	
Female	9 (17.3)	45 (51.7)	14 (12.6)	38 (40.4)	<0.001
Age [years, median (min-max)]	40 (23-77)	43 (28-73)	43 (26-72)	50 (24-72)	<0.001
Duration of disease					
[years, median (min-max)]	6.0 (0.50-16.0)	6.0 (1.0-17.0)	9.0 (2.0-22.0)	8.5 (2.0-19.0)	<0.001
cART [years, median (min-max)]	4.0 (0.0-14.0)	4.0 (0.0-15.0)	8.0 (0.5-20.0)	8.0 (1.0-17.0)	<0.001
Waist circumference [cm, mean (sd)]	84.1 (6.76)	102.1 (10.79)	83.0 (6.90)	97.4 (8.65)	<0.001
Hip circumference [cm, mean (sd)]	93.6 (4.25)	104.2 (8.49)	88.8 (5.07)	94.8 (5.77)	<0.001
Thigh circumference [cm, mean (sd)]	46.6 (4.04)	51.9 (5.30)	44.7 (3.63)	47.8 (7.82)	<0.001
Arm circumference [cm, mean (sd)]	26.0 (2.78)	29.0 (2.76)	25.4 (2.97)	27.9 (4.63)	<0.001
Neck circumference [cm, mean (sd)]	36.2 (3.45)	37.6 (3.81)	36.0 (4.34)	38.4 (4.34)	<0.001
Waist/ hip ratio [mean (sd)]	0.90 (0.08)	0.98 (0.08)	0.93 (0.06)	1.02 (0.07)	<0.001
CD4 cell count [cells/mm^3, median	386.0	466.0	509.0	573.0	
(min-max)]	(2.00-1880.0)	(119.0-1730.0)	(91.0-1657.0)	(9.00-1840.0)	0.011
HIV RNA (<50) [n (%)]	39 (78.0)	71 (85.5)	102 (91.9)	85 (91.4)	0.048
CDC [n (%)]					
A	26 (50.0)	52 (60.5)	62 (56.4)	51 (54.8)	
B	4 (7.7)	1 (1.2)	0 (0.0)	1 (1.1)	
C	22 (42.3)	33 (38.4)	48 (43.6)	41 (44.1)	0.106
ART [n (%)]					
IP	31 (62.0)	51 (60.7)	63 (56.8)	45 (48.4)	0.296
NNRTI	22 (44.9)	37 (44.0)	53 (47.7)	48 (52.2)	0.719
NRTI	47 (95.9)	80 (95.2)	109 (99.1)	91 (98.9)	0.201
Body Fat Mass by Quantitative CT					
Total fat [cm^2, median (min-max)]	188.4 (63.9-372.1)	391.9 (217.8-850.7)	136.6 (31.4-466.8)	303.1 (137.5-676.6)	<0.001
VAT [cm^2, median (min-max)]	66.4 (14.5-217.3)	126.1 (31.2-381.8)	67.2 (9.2-262.3)	172.7 (20.0-285.5)	<0.001
SAT [cm^2, median (min-max)]	100.3 (30.5-229.3)	259.7 (109.0-690.1)	58.1 (5.0-204.6)	136.0 (32.0-418.0)	<0.001
VAT/SAT [median (min-max)]	0.54 (0.22-5.46)	0.45 (0.07-2.75)	1.24 (0.17-20.43)	1.32 (0.13-6.36)	<0.001
FMR by DXA [median (min-max)]	0.95 (0.40-3.45)	1.17 (0.54-2.65)	1.71 (0.52-4.47)	1.90 (0.43-4.97)	<0.001
BMI [kg/m^2, n(%)]					
<18.5	1 (1.9)	0 (0.0)	16 (14.4)	1 (1.1)	
18.5-24.9	42 (80.8)	12 (13.8)	83 (74.8)	29 (30.9)	
24.9-29.9	9 (17.3)	43 (49.4)	11 (9.9)	51 (54.3)	
>29.9	0 (0.0)	32 (36.8)	1 (0.9)	13 (13.8)	<0.001
BMI [kg/m^2 (mean (sd)]	23.0 (2.4)	29.4 (4.6)	21.8 (2.9)	26.5 (3.4)	<0.001

(BMI - body mass index; CDC - Centers for Disease Control and Prevention criteria for staging of HIV infection; VAT – visceral adipose tissue; SAT – subcutaneous adipose tissue; FMR - fat mass ratio; DXA - dual-energy X-ray absorptiometry)

DISCUSSION

Although wasting syndrome was common in the early HIV epidemic, it is not expected to appear in patients successfully treated with cART (16). In fact, recent studies have shown that being overweight or obese is more common than wasting in these patients (Table 5). This may be partly justified by the increased survival rate in HIV patients and by the weight gain that occurs with ageing in the general population (14-16, 26, 27). Our data also showed that, regardless of gender, being of normal weight, overweight or obese is more common than being underweight in these HIV-infected patients.

In some studies, HIV-infected women and men were equally or more frequently overweight, although women were more likely to be obese than men (Table 5). When we compared our prevalence figures with other studies (Table 5), we found a lower prevalence of underweight patients (except for the Crum-Cianflone paper (16)), and a similar prevalence of overweight subjects regardless of gender (similar to Amorosa (15)), although there was more obesity among women in our population.

Comparing our data with the Portuguese National Survey for the same age group (40 to 49 years), we observed that our HIV-infected female population had a higher prevalence of being underweight or obese with a lower prevalence of being overweight (28). Our population of HIV-infected male patients had a higher prevalence of being underweight or normal, but contrary to the women, there was a lower frequency of being overweight or obese than in the general population.

In a retrospective study of HIV-infected male pa-

Table 4: *Association between clinical lipodystrophy and demographic, clinical and anthropometric characteristics.*

	Crude OR (95%CI)	Adjusted OR (95%CI)**
Sex		
Male	1*	1*
Female	0.45 (0.29-0.71)	0.42 (0.23-0.76)
Age (years)	1.02 (1.00-1.04)	1.05 (1.02-1.07)
Duration of infection	1.18 (1.11-1.25)	1.12 (0.99-1.25)
cART duration	1.24 (1.16-1.32)	1.16 (1.03-1.30)
CD4 cell count	1.00 (1.00-1.00)	--
HIV RNA (<50)	2.06 (1.08-3.91)	3.00 (1.28-7.00)
HIV risk factor		
Inject	1*	1*
Homosexual	1.14 (0.52-2.48)	0.60 (0.25-1.71)
Heterosexual	0.52 (0.32-0.87)	0.91 (0.44-1.89)
Other	4.92 (0.60-40.12)	2.17 (0.24-19.47)
BMI (Kg/m^2)		
< 18.5	1*	1*
≥18.5<25	0.35 (0.10-1.24)	0.33 (0.08-1.89)
≥25<30	0.20 (0.06-0.72)	0.21 (0.05-0.92)
> 30	0.06 (0.07-0.25)	0.05 (0.01-0.26)

*reference class.
**OR adjusted for all the variables in the table, with the exception of CD4 cell count.

Table 5: *Studies of prevalence of obesity in HIV-infected patients by author*

Author, year	Prevalences Underweight	Overweight	Obese
Amorosa V, 2005 (15)	9%	31% 14% (31% men; 30% women)	(28.3% women; 10.7% men)
Hendricks KM, 2006 (14)		39.6% men 33.9% women	12.5% men 28.6% women
Hodgson LM, 2001 (26)	12%	34%	9%
Shuter J, 2001 (27)	10.6% (11.7% men; 9.3% women)	21.7% (12.6% men; 32.5% women)	10.6%
Crum- Cianflone N, 2008 (16)	1% (men)	46% (men)	17% (men)
Jaime PC, 2004 (32)	2.3% men; 7,7% women	25.1% men; 26.9% women	3.5% men; 9.6% women
Freitas P, this paper	5.7% (4.3% men; 8.8% women)	33.2% (33.1% men; 33.3% women)	13.9% (10.2% men; 21.9% women)

tients, 54% were already overweight or obese at the time of HIV diagnosis, suggesting that the risk of becoming overweight or obese over the course of infection may not be higher when compared to the general population (16). On the other hand, weight gain may be a sign of the efficacy of cART in reducing the occurrence of AIDS and wasting. As patients with HIV live longer and the infection has been relegated to a chronic illness, HIV patients may be now facing similar problems to the general population, including excessive weight gain due to poor lifestyle habits (14, 29).

More important than diagnosing obesity is assessing the types of fat distribution. The best way to quantify the visceral adipose tissue (VAT) is through CT or MRI scanning. In our patients, those with CL, despite having a similar VAT and lower total and subcutaneous adipose tissue (SAT) at abdominal level, had a higher WHR and a higher VAT/SAT ratio by CT. The presence of a similar VAT and a higher VAT/SAT ratio results from a lower SAT. The same phenomenon is observed with WHR because they had a decrease in SAT that is the predominant adipose tissue in the hip. When we analysed the data according to the 4 groups of fat redistribution, patients with isolated lipoatrophy had a higher VAT/SAT fat ratio indicating not an increase in visceral adipose tissue, but lower subcutaneous fat. These patients with low SAT and VAT had a similar fat distribution to patients with Berardinelli-Seip congenital generalized lipodystrophy (30). We do not know if this

difference in fat distribution pattern in HIV patients is related to a different cardiovascular risk. Older age in the general population and CD4 cell count in HIV-patients were associated with increased BMI. Also, CL was associated with older age, longer HIV and cART duration, and higher CD4 cell count, most of them linked with increased BMI. Thus we would expect that CL patients would have a higher prevalence of obesity and of being overweight, which is not true. After adjustment for the demographic and clinical features significantly associated with the presence of CL, these patients were significantly less overweight or obese, and this association was independent of age, gender, duration of infection, cART regimen, virological suppression and HIV-infection risk factor. In the Amorosa study, in the multivariate analysis, a CD4 count > 200 cells/mm3, independent of gender, was associated with obesity and with being overweight. Compared with men, HIV-infected women had a higher prevalence of obesity. Female gender, CD4 cell count, and nonsmoking status correlated with obesity and with being overweight within the overall group. Women far surpassed men in prevalence of obesity, even when factoring in other risks, including the women's earlier stage of the disease as assessed by CD4+ cell count. For patients with known specific antiretroviral regimens, no correlations were seen with any of the following: BMI, being antiretroviral naïve, being on ART currently, or with PI use (15).

In the Crum-Cianflone study, multivariate analysis showed that weight gain during HIV infection was significantly related to younger age at HIV diagnosis and longer time of HIV follow-up (16). Higher CD4 cell count was associated with weight gain. cART use was not predictive of weight gain in the final model. Subjects with a CD4 cell nadir greater than 200 cells/mm3 had a higher BMI. This suggests that those who do not experience end stage HIV disease are more likely to be overweight. Patients with HIV who were overweight/obese, as well as those who gained weight during the course of their HIV infection, were more likely to have higher CD4 cell counts. Also in a cross-sectional obesity study, no association was found between weight and current cART regimen (15). Successful virological responses were also associated with weight gain (13, 31).

One of the strengths of our study is that it addresses the prevalence of obesity according to the presence of CL and this has not been reported previously. Despite having a lower prevalence of being overweight or obese when compared to patients without lipodystrophy, lipodystrophic patients had an increased proportion of VAT, as reflected by the increased VAT/SAT fat ratio determined by CT at abdominal level. This probably suggests that these patients have a comparable risk of metabolic and cardiovascular complications, in spite of the actual frequency of obesity being lower.

Limitations of our study

One of the limitations of our study is that the lipoatrophy definition is clinical, having been performed by the same experienced observer (PF), so the findings of this study may not be generalized to all HIV populations nor to less experienced centres. Also, objective measurements of body composition (FMR and CT) were not used for the definition of obesity.

CONCLUSIONS

In this HIV-infected patient population on cART, about 22% were overweight and 10% were obese. Patients with clinical diagnoses of lipodystrophy were less frequently overweight or obese. As expected, BMI was higher in patients with abdominal prominence (patients with isolated central fat accumulation and patients with mixed forms of lipodystrophy) and lower in patients with isolated lipoatrophy. Patients with CL had lower mean WC, higher waist/hip ratio and FMR and, in quantitative CT at abdominal level, lower total and subcutaneous fat, without a significantly different visceral fat mass but with a higher visceral/subcutaneous fat ratio. HIV-infected male patients were more often underweight than the general population. Obesity instead of wasting is a new reality after the introduction of anti-retroviral therapy in HIV-infected patients.

List of abbreviations

cART – combination antiretroviral therapy
HIV – human immunodeficiency virus
CL – clinical lipodystrophy
CLA – clinical lipoatrophy
CV – cardiovascular
CT – computed tomography
AP – abdominal prominence
BMI – body mass index
FMR – fat mass ratio
DXA – dual-energy X-ray absorptiometry
WHR – waist/hip circumference ratio
SAT – subcutaneous adipose tissue
VAT – visceral adipose tissue
MRI – magnetic resonance imaging

Transparency declarations
None to declare

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PUBLICAÇÃO 7:

Central/peripheral fat mass ratio is associated with increased risk of hypertension in HIV-infected patients

ORIGINAL PAPER

Central/Peripheral Fat Mass Ratio Is Associated With Increased Risk of Hypertension in HIV-infected Patients

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The data on the risk of hypertension in human immunodeficiency virus (HIV)-infected patients, particularly in those with lipodystrophy, are controversial. The authors assessed the impact of lipodystrophy on hypertension in a cohort of HIV-infected adults receiving combination antiretroviral therapy. This was a cross-sectional study in which lipodystrophy (clinically and fat mass ratio [FMR]-defined), blood pressure, and body composition (dual-energy x-ray absorptiometry and computed tomography) were evaluated in 368 HIV adults. The prevalence of hypertension in HIV patients with or without clinically or FMR-defined lipodystrophy was similar (with clinical lipodystrophy 35.3% vs without 32.9%, not significant; with FMR lipodystrophy 41.7% vs without 32.2%, not significant). When HIVinfected patients were classified into 4 categories of fat distribution (based on the presence or absence of lipoatrophy and abdominal prominence), isolated lipoatrophy was not significantly associated with hypertension, but patients with isolated central fat accumulation and mixed forms of lipodystrophy had a significantly higher prevalence of hypertension. Hypertensive HIV patients had significantly higher total fat, central, and central/peripheral mass ratio than normotensive ones. After adjustment for age, sex, smoking, and body mass index, hypertension remains significantly associated with central/peripheral fat mass ratio (odds ratio, 1.258; 95% confidence interval, 1.008-1.569). Hypertension was not more prevalent in lipodystrophic HIV-infected patients, but was significantly associated with central/peripheral fat mass ratio. *J Clin Hypertens (Greenwich)*. 2012;00:00–00©2012 Wiley Periodicals, Inc.

The widespread use of combination antiretroviral therapy (cART) has changed HIV-1 infection into a chronic manageable illness. However, together with chronic HIV infection and aging, lifelong cART is associated with adverse effects including cardiovascular disease and lipodystrophy[1-4].

In contrast with metabolic abnormalities, few and sometimes controversial studies are available on the effect of cART and lipodystrophy on blood pressure in HIV-infected patients[5-13].

Regular blood pressure monitoring to rule out hypertension in HIV-infected patients has been recently recommended [14], and some authors have suggested a high prevalence of hypertension among these patients[11,15-18], which was not confirmed by others [12,16,19,20]. In some studies, the higher risk for hypertension in HIV-infected patients is associated with lipodystrophy[21-25], which is in opposition to data from uninfected HIV patients with genetic lipoatrophies, for whom diabetes is a common feature but not hypertension[21-25]. Given this controversy, the aim of this study was to estimate the association between lipodystrophy [clinically and Fat Mass Ratio (FMR) defined] and body composition with the prevalence of hypertension in HIV-1 patients on antiretroviral therapy.

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METHODS

Subjects

As part of a cross-sectional study, we evaluated all 364 non-institutionalized, HIV-1 infected Caucasian adult patients, consecutively referred from the Infectious Diseases Clinic to the Endocrinology Outpatient Clinic. Patients 1The study protocol was approved by the Hospital Ethics Committee and all patients provided informed consent.

For each patient, the following information was collected using a standardized protocol: demographic data (age, gender), duration of HIV infection, probable transmission route of HIV infection, duration of cART, anti-hypertensive drug therapy and characterization of the infection using the "Centers for Disease Control and Prevention" (CDC) staging criteria [26].

A blood sample was collected and CD4 cells, HIV-1 RNA, and fasting glucose and insulin were determined. Insulin resistance was defined by the homeostatic model

assessment [27]. Body weight was measured using TANITA (Tanita®, model TBF 300) scale, with patients wearing light clothes without shoes, and height was measured to the nearest centimetre in the standing position using a wall stadiometer (Holtain Limited Crymych, Dyfed®). Neck, hip, waist, thigh and arm circumferences were measured by the same observer with standard techniques[28].

Blood pressure (BP) was measured after the subject had remained seated for 5 minutes, with the elbow flexed at the heart, using a standard aneroid sphygmomanometer with the cuff on the upper right arm. Two blood pressure readings were taken and the mean of the two readings was calculated. Hypertension was defined as systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥ 90mmHg, or self-reported medication for high blood pressure[29].

Clinical lipodystrophy was defined as a peripheral lipoatrophy with or without a central fat accumulation assessed by both practitioner and patient as we previously described[30-33]. Central fat accumulation or abdominal prominence was defined using the last International Diabetes Federation (IDF) criteria for metabolic syndrome[34], as having waist circumference ≥94 cm in men and 80 cm in women[35]. The clinical assessment was performed by the same practitioner (PF). Patients were classified into 4 different groups according the presence or absence of either lipoatrophy or abdominal prominence: group 1- No lipodystrophy (patients without clinical lipoatrophy and without abdominal prominence); group 2- Isolated central fat accumulation (patients without clinical lipoatrophy and with abdominal prominence); group 3- Isolated lipoatrophy (patients with clinical lipoatrophy and without abdominal prominence); group 4- Mixed forms of lipodystrophy (patients with clinical lipoatrophy and with abdominal prominence).

Body composition and laboratory definitions

Body composition was assessed with whole-body dual-energy X-ray absorptiometry (DXA) (DXA – Lunar Expert XL, 1999). DXA measurements were performed while the patient was in a supine position, with standard positioning of the arms and feet. Cut lines separating regions of interest were those indicated by the manufacturer. Regional fat mass values were grouped and analysed for the following anatomical regions: arms, legs, trunk and total body. The fat mass ratio (FMR) was calculated as the ratio between the percentage of the trunk fat mass and the percentage of the lower limb fat mass (FMR= % of the trunk fat mass/ % of the lower limb fat mass)[36]. For the definition of lipodystrophy using FMR, we used the cut-off values of 1.961 for men and 1.329 for women[30].

The quantification of total, visceral, and peripheral fat was performed using a 64-slice CT scanner (Siemens Sensation 64 Cardiac). Based on a coronal topogram, a single 10 mm thick slice was obtained in all patients at the level of the transverse processes of L4. Radiological evidence of fat was considered as any area with an attenuation value comprised between -190 and -30 Hounsfield units[37,38]. Total fat area was calculated by drawing a region of interest (ROI) including all the abdomen and subcutaneous tissue. Visceral fat area was calculated by manually drawing a ROI along the internal contour of the abdominal wall muscles, anterior border of the psoas muscles, and anterior contour of the L4 vertebral body. Peripheral fat area was obtained by subtracting the value of visceral fat area from total fat area. All values were expressed in cm^2 rounded to the nearest centesimal[37,38]. The CD4+ cell count was determined by flow cytometry and plasma HIV-1 RNA load was measured by a quantitative reverse transcriptase polymerase chain reaction, which has a lower limit of detection of 50 copies/mL.

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 software (SPSS Inc., Chicago, Illinois, USA). All probabilities were two tailed and p values <0.05 were regarded as significant. Data were described as mean and standard deviation (SD) for quantitative variables and compared using the Student-t test or the Mann-Whitney test as appropriate. Kruskal-Wallis test was used for the comparison between the four groups of fat distribution. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher's exact test.

Odds ratio (OR) and respective 95% confidence intervals (95% CI) were computed using unconditional logistic regression, for estimating the association between hypertension and anthropometric measures and insulin resistance.

RESULTS

Characteristics of the population

In this sample of 364 HIV-1 infected patients (250 men and 114 women) on antiretroviral therapy (cART), 59.1% patients presented clinical lipodystrophy (CL). Table 1 shows the characteristics of the study sample according to the presence of CL. Patients with CL were more frequently males, older, had longer HIV infection duration, length

TABLE I. Sample Characteristics According to the Presence of Clinical Lipodystrophy

	Without CL	With CL	p
N (%)	149 (40.9)	215 (59.1)	
Sex [n(%)]			
Male	87 (58.4)	163 (75.8)	<0.001
Female	62 (41.6)	52 (24.2)	
Age [years, mean (sd)]	44.4 (12.0)	47.0 (11.0)	0.010
Duration [years, mean (sd)]	6.7 (3.9)	9.2 (4.1)	<0.001
cART [years, mean (sd)]	5.0 (3.8)	8.0 (4.0)	<0.001
Weight [Kg, mean (sd)]	73.7 (14.3)	66.2 (12.5)	<0.001
Height [m, mean (sd)]	1.65 (0.09)	1.66 (0.09)	0.383
BMI [(kg/m^2), mean (sd)]	27.1 (5.1)	24.0 (3.9)	<0.001
Waist circumference [cm, mean (sd)]	95.5 (13.3)	89.5 (10.6)	<0.001
Hip circumference [cm, mean (sd)]	100.7 (9.4)	91.6 (6.2)	<0.001
Thigh circumference [cm, mean (sd)]	50.4 (6.2)	46.1 (6.1)	<0.001
Arm circumference [cm, mean (sd)]	28.2 (3.4)	26.6 (4.0)	<0.001
Neck circumference [cm, mean (sd)]	37.0 (3.9)	37.2 (4.0)	0.856
Waist/ hip circumference ratio [mean (sd)]	0.95 (0.09)	0.98 (0.08)	0.006
CD4 cell count [cells/mm^3, mean (sd)]	503.5 (311.6)	573.4 (324.0)	0.010
HIV RNA (<50) [n (%)]	121 (83.4)	197 (91.2)	0.026
HIV risk factor [n (%)]			
Inject	31 (20.8)	63 (29.4)	0.002
Homosexual	12 (8.1)	30 (14.0)	
Heterosexual	105 (70.5)	111 (51.9)	
Other	1 (0.7)	10 (4.7)	
CDC [n (%)]			
A	82 (55.4)	118 (55.7)	0.056
B	6 (4.1)	1 (0.5)	
C	60 (40.5)	93 (43.9)	
ART [n (%)]			
IP	88 (61.1)	116 (54.2)	0.263
NNRTI	63 (44.1)	104 (48.8)	0.438
NRTI	137 (95.8)	210 (98.6)	0.064
Body Fat Mass by Quantitative CT			
Total fat [cm^2, mean (sd)]	345.8 (163.2)	230.9 (133.1)	<0.001
Central fat [cm^2, mean (sd)]	131.2 (86.5)	127.1 (79.9)	0.886
Peripheral fat [cm^2, mean (sd)]	214.6 (125.0)	103.8 (82.9)	<0.001
Central/peripheral [mean (sd)]	0.82 (0.84)	2.06 (2.41)	<0.001
FMR by DXA [mean (sd)]	1.24 (0.53)	1.98 (0.98)	<0.001
Systolic blood pressure [mean (sd)]	120.6 (19.0)	120.5 (17.2)	0.978
Diastolic blood pressure [mean (sd)]	75.6 (13.3)	75.6 (10.9)	0.945
Taking anti-hypertension medications [n (%)]	21 (14.1%)	44 (20.6%)	0.149
Smoking history [n (%)]			
Never	66 (45.2)	71 (34.5)	0.103
Current	59 (40.4)	94 (45.6)	
Former	21 (14.4)	41 (19.9%)	

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention criteria for staging of human immunodeficiency virus (HIV) infection; CL, clinical lipodystrophy; CT, computed tomography; DXA, dual-energy x-ray absorptiometry; FMR, fat mass ratio; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.; SD, standard deviation.

of cART and presented a lower mean of all the anthropometric measures, with the exception of height and neck circumference. In addition, patients with CL had higher FMR. Patients with CL had a significantly higher mean CD4+ cell count and viral suppression rate. No differences were observed between patients with and without CL regarding the cART regimens. Also, no differences between patients with or without clinical lipodystrophy were observed regarding the frequency of anti-hypertensive medication use.

TABLE II. Prevalence of HTN According to Lipodystrophy Defined Clinically and by FMR

	Lipodystrophy defined clinically				Lipodystrophy defined by FMR			
	Total	Without CL	With CL	*P value*	Total	Without L	With L	*P value*
HTN	n=364	n=149	n=215		n=221	n=118	n=103	
Without HTN, No. (%)	239 (65.7)	100 (67.1)	139 (64.7)	0.708	140 (63.3)	80 (67.8)	60 (58.3)	0.184
With HTN, No. (%)	125 (34.3)	49 (32.9)	76 (35.3)		91 (36.7)	38 (32.2)	43 (41.7)	
No.	250	87	163		147	57	90	
Men								
Without HTN, No. (%)	157(62.8)	55 (63.2)	102 (62.6)	1.000	92 (62.6)	41 (71.9)	51 (56.7)	0.091
With HTN, No. (%)	93 (37.2)	32 (36.8)	61 (37.4)		55 (37.4)	16 (28.1)	39 (43.3)	
No.	114	62	52		74	61	13	
Women								
Without HTN, No. (%)	82 (71.9)	45 (72.6)	37 (71.2)	1.000	48 (64.9)	39 (63.9)	9 (69.2)	1.000
With HTN, No. (%)	32 (28.1)	17 (27.4)	15 (28.8)		26 (35.1)	22 (36.1)	4 (30.8)	

Abbreviations: CL, clinical lipodystrophy; FMR, fat mass ratio; HTN, hypertension; L, lipodystrophy.

TABLE III. Prevalence of Hypertension by Body Composition According to 4 Groups of Fat Distribution and Sex

HTN	No Lipodystrophy	Isolated Central Fat Accumulation	Isolated Lipoatrophy	Mixed Forms of Lipodystrophy	P Value
Total					
Without HT [n (%)]	39 (76.5)	54 (52.8)	80 (72.7)	53 (56.4)	0.029
With HT [n (%)]	12 (23.5)	32 (37.2)	30 (27.3)	41 (43.6)	
Men					
Without HT [n (%)]	31 (73.8)	23 (56.1)	67 (69.8)	29 (51.8)	0.049
With HT [n (%)]	11 (26.1)	18 (43.9)	29 (30.2)	27 (48.2)	
Women					
Without HT [n (%)]	8 (88.9)	31 (68.9)	13 (92.9)	24 (63.2)	0.119
With HT [n (%)]	1 (11.1)	14 (31.1)	1 (7.1)	14 (36.8)	

Abbreviation: HTN, hypertension.

Prevalence of hypertension

Of the 364 patients 34.3% were hypertensive. No significant differences were observed regarding the prevalence of hypertension in patients with and without lipodystrophy, regardless of gender or the definition that was used (Table 2). Also, no differences in mean SBP or DBP between patients with or without lipodystrophy were found (Table 1), defined either by clinical criteria or by FMR [SBP: with lipodystrophy 123.7 mmHg (16.8) vs. without 119.4 mmHg (18.3); p=0.068 and DBP: with lipodystrophy 76.8 mmHg (9.9) vs. without 76.0 mmHg (12.5); p=0.411].

When patients were classified into 4 categories of fat distribution as previously described, the presence of hypertension was more prevalent in patients with isolated central fat accumulation and mixed forms of lipodystrophy (patients with abdominal prominence regardless of the presence or absence of lipoatrophy) both in men and in the total sample (Table 3).

Patient characteristics according the presence of hypertension

Patients with hypertension were older, had higher mean weight, BMI, waist, arm and neck circumferences and higher waist/hip circumference ratio. They also had higher total and central fat and central/peripheral fat ratio without difference in the peripheral fat on CT at abdominal level (Table 4). There was no difference between patients with or without hypertension in gender, duration of HIV infection, length of cART, hip and thigh circumferences, CD4 cell count and presence of lipodystrophy (clinical or FMR-defined).

Anthropometric characteristics and the presence of hypertension

After adjustment for age, gender, smoking history and BMI, hypertension was not associated either with insulin resistance or the presence of clinical or FMR-defined lipodystrophy. Also, total abdominal, central and periph-

TABLE IV. Sample Characteristics According to the Presence of Clinical Lipodystrophy

	Without HTN	With HTN	*P Value*
N (%)	239 (65.7)	125 (34.3)	
Sex [n(%)]			
Male	157 (65.7)	82 (34.3)	0.114
Female	93 (74.4)	32 (25.6)	
Age [years, mean (sd)]	42.0 (9.3)	53.5 (11.6)	<0.001
Duration [years, mean (sd)]	8.2 (4.1)	8.0 (4.6)	0.394
ART [years, mean (sd)]	6.8 (4.0)	6.7 (4.4)	0.739
Weight [Kg, mean (sd)]	67.8 (13.3)	72.1 (14.2)	0.009
Height [m, mean (sd)]	1.66 (0.09)	1.65 (0.08)	0.388
BMI [(kg/m2), mean (sd)]	24.6 (4.5)	26.5 (4.8)	<0.001
Waist circumference [cm, mean (sd)]	89.9 (11.9)	96.0 (8.9)	<0.001
Hip circumference [cm, mean (sd)]	94.9 (8.8)	96.2 (8.9)	0.151
Thigh circumference [cm, mean (sd)]	48.3 (6.7)	47.0 (6.0)	0.214
Arm circumference [cm, mean (sd)]	27.0 (4.1)	27.8 (3.3)	0.031
Neck circumference [cm, mean (sd)]	36.5 (3.8)	38.2 (3.9)	0.002
Ratio waist/ hip circumference [mean (sd)]	0.95 (0.08)	1.00 (0.08)	<0.001
CD4 cell count [cells/mm3, mean (sd)]	554.2 (326.1)	526.8 (309.8)	0.402
HIV risk factor [n (%)]			
Inject	85 (35.7)	9 (7.2)	<0.001
Homosexual	23 (9.7)	19 (15.2)	
Heterosexual	122 (51.3)	94 (75.2)	
Other	8 (3.4)	3 (2.4)	
CDC [n (%)]			
A	126 (53.4)	74 (59.7)	0.031
B	6 (2.5)	1 (0.8)	
C	104 (44.1)	49 (39.5)	
ART [n (%)]			
IP	140 (59.8)	64 (51.6)	0.167
NNRTI	106 (45.3)	61 (50.0)	0.464
NRTI	228 (97.9)	119 (96.4)	0.664
Body Fat Mass by Quantitative CT			
Total fat [cm^2, mean (sd)]	241.9 (139.4)	329.0 (165.7)	<0.001
Central fat [cm^2, mean (sd)]	107.2 (72.7)	167.4 (72.7)	<0.001
Peripheral fat [cm^2, mean (sd)]	134.7 (102.6)	161.5 (129.7)	0.184
Central/peripheral [mean (sd)]	1.30 (1.36)	2.16 (2.90)	0.005
Clinical lipodystrophy [n (%)]			
Without	100 (41.8)	49 (39.2)	0.708
With	139 (58.2)	76 (60.8)	
Lipodystrophy FMR defined [n (%)]			
Without	80 (57.1)	38 (46.9)	0.184
With	60 (42.9)	43 (53.1)	

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention criteria for staging of human immunodeficiency virus (HIV) infection; CL, clinical lipodystrophy; CT, computed tomography; DXA, dualenergy x-ray absorptiometry; FMR, fat mass ratio; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.; SD, standard deviation.

eral fat mass evaluated by CT scan were not associated with hypertension. On the other hand, central/peripheral fat mass ratio was significantly associated with hypertension, even after adjustment for the previously mentioned variables (OR=1.26; 95%CI: 1.01-1.57). Total body, arm and trunk fat mass, assessed by DXA, were not associated with hypertension, independent of age, gender and smoking history (Table 5).

TABLE V. Association Between Anthropometric Characteristics and the Presence of Hypertension

	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	Adjusted OR (95% CI)[b]
Homa score			
< 4	1	1	1
≥ 4	2.13 (1.26-3.60)	1.95 (1.07-3.56)	1.80 (0.97-3.31)
Clinical lipodystrophy	1.12 (0.72-1.74)	0.86 (0.51-1.46)	1.12 (0.64-1.96)
Lipodystrophy defined by FMR	1.51 (0.87-2.62)	0.95 (0.46-1.94)	0.97 (0.47-2.01)
Body Fat Mass by Quantitative CT			
Total fat [cm^2, mean (sd)]	1.00 (1.00-1.01)	1.00 (1.00-1.01)	1.00 (0.99-1.00)
Central fat [cm^2, mean (sd)]	1.01 (1.01-1.01)	1.01 (1.00-1.01)	1.00 (0.99-1.00)
Peripheral fat [cm^2, mean (sd)]	1.00 (1.00-1.01)	1.00 (1.00-1.01)	1.00 (0.99-1.00)
Central/peripheral ratio [mean (sd)]	1.27 (1.06-1.52)	1.15 (0.94-1.40)	1.26 (1.01-1.57)
DXA			
Total body fat mass %	1.03 (1.00-1.05)	1.05 (1.01-1.09)	1.02 (0.96-1.08)
Leg body fat mass %	1.01 (0.99-1.03)	1.02 (0.99-1.05)	1.00 (0.96-1.04)
Arm body fat mass %	1.01 (0.98-1.02)	1.04 (1.01-1.08)	1.03 (0.98-1.07)
Trunk body fat mass %	1.04 (1.10-2.00)	1.04 (1.01-1.08)	1.01 (0.96-1.07)
FMR by DXA [mean (sd)]	1.48 (1.10-2.00)	1.17 (0.79-1.74)	1.16 (0.78-1.73)

Abbreviations: CI, confidence interval; CT, computed tomography; DXA, dual-energy x-ray absorptiometry; FMR, fat mass ratio; HOMA, Homeostatic Model Assessment; SD, standard deviation. [a]Odds ratio (OR) adjusted for age, sex, and smoking history. [b]OR adjusted for age, sex, smoking history and body mass index (BMI).

DISCUSSION

Prevalence of hypertension in HIV-infected patients

In this study, 34.3 % of the HIV-1 infected patients [with a mean age of 45.9 (11.5) years] on cART were hypertensive. This prevalence is lower than the 42.1% (18 to 90 years of age) and 47.1% (35 to 64 years of age) in our country's general population[39].

There are few and discrepant studies on hypertension in HIV-infected patients. In Bergersen's study, the prevalence of hypertension was 21% in patients on cART, 13% (p=0.20) in ART-naïve patients and 24% in HIV-negative controls (p=0.28)[19]. Similar results were observed in patients on cART and in HIV-negative controls. These observations led to the perception that influence of cART on the prevalence of hypertension appears to be minor. Bergersen argued that the most probable explanation for those findings was that HIV-positive patients in general, have a lower BMI and thus have a lower blood pressure than the general population[19].

Gazzaruso studied 287 HIV-positive patients on cART, with a mean age of 41.1 years, and the prevalence of hypertension was similar (34.2 %) to our results (34.3%). By using multiple logistic regression analysis, it was shown that family history of hypertension, metabolic syndrome, lipodystrophy and HOMA were predictors of hypertension in HIV-infected patients[16].

Hypertension, insulin resistance and lipodystrophy

There is scarce information available on hypertension in HIV-infected patients according to the presence of lipodystrophy[10-13]. We aimed to evaluate if the loss of subcutaneous adipose tissue shown by lipodystrophy has any effect on blood pressure. In our study, the prevalence of hypertension in patients with or without lipodystrophy clinically or FMR defined was similar, but in men and using the four groups of body composition we observed an increase in prevalence of hypertension in those with lipoatrophy when compared to those without any fat distribution abnormality, despite patients with central fat accumulation and mixed forms had higher prevalence.

Hadigan[10] and Sattler[11] showed that HIV patients with lipodystrophy had high blood pressure. Also, Crane found that lipoatrophy and lipohypertrophy were significantly associated with hypertension in HIV-infected patients[40]. Gazzaruso showed that lipodystrophy and HOMA were predictors of hypertension in HIV-infected patients[16], and that insulin resistance could be involved in the development both lipodystrophy and hypertension, suggesting that the presence or the development of lipodystrophy in an HIV-infected patient should be an indication to check for hypertension. In our sample after adjustment for age,

gender and smoking history, hypertension remained associated with insulin resistance but after including BMI in the model, no significant association remained. Total body, arm and trunk fat mass, assessed by DXA were not associated with hypertension, independent of age, gender and smoking history. Also, total abdominal, central and peripheral fat mass evaluated by CT scan were not associated with hypertension, but, central/peripheral fat mass ratio was significantly associated with hypertension, even after adjustment for the above mentioned variables. Patients with hypertension had higher total, central and central/peripheral fat mass ratio. Adipose tissue produces angiotensinogen, which could lead to an activation of renin-angiotensin-aldosterone, an important endocrine blood pressure regulatory system [41,42]. But, the loss of subcutaneous peripheral adipose tissue can also contribute to hypertension through insulin resistance16. The presence of hypertension was more prevalent in patients with isolated central fat accumulation and mixed forms of lipodystrophy (patients with abdominal prominence regardless of the presence or absence of lipoatrophy). This could mean that abdominal prominence, as in the general population, is more important in determining hypertension, thus the central/peripheral fat mass ratio combining the effect of central fat accumulation with loss of subcutaneous adipose tissue is more strongly associated with hypertension.

Another important aspect is the association between hypertension and smoking. As in our data smokers had significantly lower prevalence of hypertension[43-47]. However after adjustment for age, no significant differences remained in the occurrence of hypertension according to smoking history.

Our study has some limitations. Although, all patients referred by the Infectious Diseases Department to our Department were included, bias in the referral cannot be excluded, since some patients could have been referred because they had some degree of glucose intolerance or dyslipidemia. Nevertheless, the aim of our study is to evaluate the influence of HIV-lipodystrophy on the prevalence of hypertension. It was not our aim to determine the national prevalence of hypertension in the HIV population, because these patients are not representative of the general HIV population in our country. Our results are cross-sectional and therefore to not permit causal inference.

Some aspects of this study should be highlighted.

The study was performed in a unit that is highly experienced in the assessment of metabolic and body fat abnormalities in HIV-infected patients; the clinical assessment of lipodystrophy was performed by the same investigator (PF); an objective definition of lipodystrophy was used (Fat Mass Ratio by DXA), visceral fat by CT, and consistent results with both definitions of lipodystrophy were observed.

CONCLUSION

In summary, the prevalence of hypertension was not higher in HIV-cART lipodystrophic patients compared with patients without lipodystrophy. HIV isolated lipoatrophy was not significantly associated with hypertension, but patients with isolated central fat accumulation and mixed forms of lipodystrophy had a significantly higher prevalence of hypertension. Hypertensive HIV patients had significantly higher total fat, central and central/peripheral mass ratio than normotensive. After adjustment for age, gender, smoking and BMI, hypertension remained significantly associated with central/peripheral fat mass ratio.

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PUBLICAÇÃO 8:

Lipodystrophy defined by Fat Mass Ratio in HIV infected patients is associated with high prevalence of glucose disturbances and insulin resistance

Title: Lipodystrophy defined by Fat Mass Ratio in HIV-infected patients is associated with a high prevalence of glucose disturbances and insulin resistance

Abbreviated title: HIV lipodystrophy and insulin resistance

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Abstract

Introduction: Combined antiretroviral therapy (cART) in the treatment of HIV-1 infection has been associated with complications, including lipodystrophy, hyperlipidaemia, insulin resistance (IR) and diabetes. **Aims:** To compare the prevalence of glucose homeostasis disturbances and IR in HIV patients on cART according to the presence of lipodystrophy (defined clinically and by Fat Mass Ratio) and different patterns of fat distribution and to establish their associations. **Design:** Cross-sectional cohort study. **Methods:** We evaluated body composition and IR and insulin sensitivity indexes in 345 HIV-infected adults. **Results:** Patients with clinical lipodystrophy (CL) had higher plasma glucose levels than patients without CL, without significant differences in plasma insulin levels, A1c, HOMA-IR, HOMA-B, QUICKI, or MATSUDA index. Patients with lipodystrophy defined by FMR had higher plasma glucose and insulin levels, A1c, HOMA-IR, QUICKI and MATSUDA than patients without lipodystrophy, without differences in HOMA-B. Higher insulin resistance (HOMA-IR ≥ 4) was present in patients with FMR-defined lipodystrophy. Patients with FMR-defined lipodystrophy had a higher prevalence of IFG, IGT and DM than patients without lipodystrophy. Significant associations between HOMA-IR and total, central and central/peripheral fat evaluated by CT at abdominal level were found and no association between HOMA-IR and peripheral fat. Association between HOMA-IR and total and trunk fat but no association with leg and arm fat (evaluated by DXA) was found. **Conclusions:** IR and glucose disturbances were significantly increased in patients with FMR-defined lipodystrophy. FMR lipodystrophy definition seems to be a more sensitive determinant of insulin resistance and glucose disturbances than clinical definition.

Keywords: Lipodystrophy, insulin resistance, HIV, glucose homeostasis disturbances

Introduction

Combined antiretroviral therapy (cART) in the treatment of HIV-1 infection confers significant survival benefit and has altered the natural history of this disease [1]. However, cART has been associated with metabolic complications, including hyperlipidemia, insulin resistance (IR), diabetes mellitus (DM) and lipodystrophy, with resultant increased risk of cardiovascular disease [2].

DM is a known complication of several antiretroviral therapies, being associated with both short [3, 4] and long-term [5, 6] exposure to some of these drugs [7]. The reported prevalence of DM in HIV-infected populations ranges from 2% to 14%, with differences in prevalence explained by differences in demographic characteristics, lifestyle, and antiretroviral exposure [7-11]. Some studies have suggested an increased risk of premature cardiovascular disease in HIV-infected individuals, and have highlighted the need to understand the relationship of HIV infection and cART with the risk of DM, a primary cardiovascular risk factor [12, 13]. Diabetes is associated with IR, and IR among treated HIV-infected patients is multifactorial: in addition to the common contributors to IR (e.g. obesity, physical inactivity and genetic influences), antiretroviral drugs and lipodystrophy or alterations in body fat distribution are also involved [5]. The term "HIV-associated lipodystrophy syndrome" was coined, but it soon became clear that some patients have pure lipoatrophy, while others have central fat accumulation, and a subset of patients have a mixed picture of both morphologic features. As in congenital lipodystrophy, lipodystrophy related to HIV-infected patients is also associated with IR and overt DM [12, 14, 15].

The aim of this study was to compare the prevalence of glucose homeostasis disturbances and IR in HIV-infected adult patients on cART according the presence of lipodystrophy [clinically defined and FMR-defined determined by whole-body dual-energy X-ray absorptiometry (DXA)] and to different patterns of fat distribution, and to establish the main contributors to these alterations in HIV-infected adults.

Methods

Subjects

As part of a cross-sectional cohort study, 345 HIV-infected Caucasian adults, 239 men and 106 women, who were non-institutionalized, were evaluated in the Endocrinology Outpatient Department of São João Hospital, consecutively referred from the Infectious Diseases Clinic. Patients were included on the first visit, and all were on cART. The study protocol was approved by the Hospital Ethics Committee for Health and all patients provided informed consent.

Clinical assessment

For each patient the following information was collected using a standardized protocol: demographic data (age, gender), duration of HIV infection, HIV infection risk factors, duration of cART and characterization of the infection. We used the "Centers for Disease Control and Prevention" (CDC) criteria for classifying the degree of infection [16]. Clinical history of diabetes, hypertension and use of anti-diabetic, anti-hypertensive and lipid-lowering drugs, as well as duration of cART, were also evaluated. Weight, height, circumferences of neck, waist, hip, thigh and arm were measured as previously published [17-20]. Blood pressure (BP) was measured after 5 minutes seated, with the elbow flexed at the heart, using a standard aneroid sphygmomanometer with the cuff on the upper right arm. Two blood pressure readings were taken and the mean of the two readings was calculated. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Clinical lipodystrophy was defined as a peripheral lipoatrophy with or without central fat accumulation assessed by both patient and practitioner, as we have previously described [17-20]. Presence of central fat accumulation or abdominal prominence was defined by the measurement of waist circumference using the International Diabetes Federation (IDF) criteria for metabolic syndrome [21].

Patients were classified into 4 different groups according the presence or absence of either clinical lipoatrophy or abdominal prominence: **no lipodystrophy** - patients without clinical lipoatrophy and without abdominal prominence; **isolated central fat accumulation** - patients without clinical lipoatrophy and with abdominal prominence; **isolated lipoatrophy** - patients with clinical lipoatrophy and without abdominal prominence; **mixed forms of lipodystrophy** - patients with clinical lipoatrophy and

with abdominal prominence. The clinical assessment was performed by the same practitioner (PF).

Evaluation of body composition

Body composition was also assessed with whole-body dual–energy X-ray absorptiometry (DXA – Lunar Expert XL). DXA measurement was performed while the patient was in a supine position, with standard positioning of the arms and feet. Markers used in this study for trunk and lower limbs that defined regions of interest were those indicated by the manufacturer. Regional fat mass values were grouped and analysed for the following anatomical regions: arms, legs, trunk and total body. The fat mass ratio (FMR) is the ratio of the percentage of the trunk fat mass to the percentage of the lower limb fat mass (FMR= % of the trunk fat mass/ % of the lower limb fat mass) [22]. We define lipodystrophy by FMR using the cut-off value of 1.961 threshold for men and 1.329 for women [17].The quantification of total, visceral, and peripheral fat was performed with a 64-slice computed tomography (CT) scanner (Siemens Sensation 64 Cardiac) with the same technique as previously described [23, 24]. All values were expressed in cm^2 rounded to the nearest centesimal.

Laboratory analysis

A venous blood sample was drawn after a 12-hour overnight fast. All the samples were analysed at the central laboratory of our hospital. Patients without a previous diagnosis of diabetes were submitted to a glucose tolerance test (OGTT). The test was performed as described by the World Health Organization using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.

Hepatitis C was diagnosed by HCV-Ab serostatus. The CD4 cell count was determined by flow cytometry and plasma HIV-1 RNA loads were measured by a quantitative reverse transcriptase polymerase chain reaction (Roche Diagnostic Systems, Inc., Branchburg, NJ, USA), which has a lower limit of detection of 50 copies/mL.

Definition of the alterations of glucose metabolism

Diabetes was defined if two consecutive measurements of fasting plasma glucose were ≥126 mg/dL and if 2 h plasma glucose was ≥200 mg/dL during the oral glucose tolerance test (OGTT).

Alterations of glucose metabolism were defined by American Diabetes Association (ADA) 2009 criteria [25]. We classified patients into 4 different categories of glycaemic profile (normoglycaemia – NG, impaired fasting glucose – IFG, impaired glucose tolerance – IGT and diabetes mellitus type 2 – DM), according to plasma glucose levels at 0' and 120' measured during OGTT.

Measurements of insulin resistance

IR was defined by the homeostasis model assessment of insulin resistance (HOMA) and insulin sensitivity by the quantitative insulin sensitivity check index (QUICKI) and Matsuda index. These indexes were calculated using the following formulas:

HOMA-IR index = (fasting insulin 0 x fasting glucose 0)/22.5 [26].

HOMA B index = (20x insulin 0 (mU/mL))/(glucose 0 (mMol/L) -3.5) [26].

QUICKI = 1/(log [fasting insulin in mU/l] +log [fasting plasma glucose in mg/dL][27].

Matsuda Index = 10 000/sqrt [glyc 0x insulin 0 x (glyc mean x insulin mean)] [28].

Glucose expressed in mmol/L and insulin in μUI/mL.

IR was defined when the value of HOMA >4 [29, 30] .

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 software (SPSS Inc., Chicago, Illinois, USA). All probabilities were two tailed and p values <0.05 were regarded as significant.

Data were described as mean and standard deviation (SD) for quantitative variables and compared using the Student-t test or the Mann-Whitney test as appropriate. Furthermore, analysis of variance or the Kruskal-Wallis test were used for the comparison between quantitative variables. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher's exact test.

Spearman correlation coefficients were calculated to estimate the associations between HOMA-IR and body fat mass evaluated either by CT or DXA.

After log transformation, linear regression models were computed for estimating the association between FMR and HOMA score, fasting and 2 hours glucose and A1C levels. Models were adjusted for age, sex, current antiretroviral therapy [protease

inhibitor (PI), nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI)], CD4 count cells and plasma HIV-1 RNA load.

Results

Baseline characteristics

A total of 345 HIV-infected patients on cART were evaluated with a mean age of 45.0±11.3 years. 69.3% were males. The demographic and clinical characteristics of the patients included in this study, according to the presence of lipodystrophy are shown in Table 1. Patients with clinical lipodystrophy (CL) were older, had higher duration of HIV infection and length of cART. Regarding anthropometric measures, patients with CL had lower BMI, waist, hip, thigh, and arm circumferences. Patients with CL had higher CD4+ cell count and percentage of viral suppression. With regards to the risk of HIV transmission in patients with CL, heterosexuality was responsible for 52.9%, followed by drug addiction in 30.6%, homo-bisexuality in 11.2%. In the remaining cases, haemophilia, transfusions and unknown causes were responsible for 1.2%, 1.2% and 2.9%, respectively. Similarly, in patients without CL, heterosexuality was also the leading cause with 65.8%, followed by drug addiction, homo-bisexuality and unknown causes in 24.6%, 8.8% and 0.9%, respectively. No significant difference was observed in lipodystrophic patients according to HIV risk transmission. The prevalence of co-infection was similar in patients with and without lipodystrophy [clinically- (Table 1) or FMR-defined (data not shown)]. Nevertheless, patients with no lipodystrophy and with isolated peripheral lipoatrophy exhibited a higher proportion of hepatitis C co-infection when compared to the other two groups (Table 2).

There was a significant difference in the different stages of CDC criteria between patients with or without lipodystrophy, with the majority of patients being in groups A or C. No differences were observed in the type of cART between the two groups of patients, nor in hypoglycaemic therapy (oral anti-diabetic drug and insulin). FMR evaluated by DXA was higher in patients with CL. When body fat mass was evaluated using quantitative CT, patients with CL had lower total and peripheral fat, but higher central/peripheral fat ratio than patients without CL.

No differences in smoking status between patients with or without lipodystrophy [clinically- (Table 1) or FMR-defined (data not shown)] were found. Patients with no

lipodystrophy and with isolated peripheral lipoatrophy were more frequently current smokers when compared to the other two groups (Table 2).

Insulin resistance

No significant differences in the means of HOMA-IR, QUICKI, MATSUDA, insulin and A1c were observed between patients with and without CL. In fact, regarding the alterations of glucose metabolism, only for fasting glucose was there a trend for significantly higher values in CL. On the other hand, when lipodystrophy was defined by FMR, all indicators of insulin resistance and glucose metabolism were significantly associated with lipodystrophy with the obvious exception of QUICKI and MATSUDA indices (Table 3).

Higher prevalence of insulin resistance, defined as HOMA-IR ≥ 4, was observed in patients with lipodystrophy defined by FMR (p=0.019) but not when lipodystrophy was clinically defined. Similar results were observed when we compared the prevalence of HOMA score thirds according to the definition of lipodystrophy. Again, only when lipodystrophy was defined by the FMR were the differences between HOMA score thirds statistically significant (p=0.002) (Table 4).

Glucose homeostasis abnormalities

When we classified patients into the 4 ADA categories of glycaemic profile, no significant differences were found between these categories in patients with or without CL. However, patients with lipodystrophy defined by FMR had a higher prevalence of IFG, IGT and DM when compared to patients without lipodystrophy (Table 5). When patients were stratified into 4 groups of fat distribution (presence or not of clinical lipoatrophy and abdominal prominence), no differences were observed in glycaemic profile. However, when we divided patients according to the 4 categories of fat distribution (presence or not of lipodystrophy defined by FMR and abdominal prominence), patients with abdominal prominence independent of the presence of lipodystrophy had higher IGT. Moreover, the highest prevalence of DM was observed in patients with lipodystrophy and abdominal prominence (Table 6).

Association between HOMA-IR and body fat mass evaluated by CT and DXA

After log transformation, significant associations were found between total fat, central fat and central/peripheral fat ratio evaluated by CT at abdominal level and HOMA-IR. These associations remained significant after adjustments for age, gender, PI, NRTI, NNRTI, CD4 cell count and HIV-1 RNA viral load. On the other hand, no significant association was found between IR and peripheral fat. Similar results were observed

when fat distribution was evaluated by DXA. Significant associations were found between total, trunk and arm fat mass evaluated by DXA and HOMA-IR. Also, these associations remained significant after adjustments for age, gender, PI, NRTI, NNRTI, CD4 cell count and HIV-1 RNA viral load (Table 7).

Association between FMR and glycaemic **parameters and insulin resistance**

After log transformation, a significant positive linear association was observed between FMR and insulin resistance evaluated by HOMA. This association was independent of age, gender, IP, NRTI, NNRTI, CD4 cell count and HIV-1 RNA viral load. No significant association was observed with the glycaemic parameters (Table 8). We performed linear regression models of the association between FMR and the variables described above, after excluding patients that were on anti-diabetic or insulin therapy, and similar results were obtained (data not shown).

Discussion

We compared the prevalence of glucose homeostasis disturbances and IR in HIV-infected patients on cART, according to the presence of lipodystrophy (clinically and FMR defined) and according to the four different patterns of body fat distribution previously described. In our study, all the participants were evaluated by the same observer (PF) to increase the accuracy in the clinical definition of lipodystrophy [22]. We also used an objective method to evaluate lipodystrophy to overcome the different and heterogeneous methodologies for the diagnosis of lipodystrophy of previous studies [31]. Since lipodystrophy in HIV-1 infected patients is considered to be an adverse effect of cART, not limited to a specific drug or a class of drugs [32], we chose to study only patients on cART, since our main goal was to compare the prevalence of glucose homeostasis disturbances and IR according to the presence of lipodystrophy. No differences were observed between the class of drugs used in patients with or without CL, defined either clinically or by FMR (data not shown). Also, similar results were observed when the comparison was performed in patients with and without lipodystrophy defined by FMR (data not shown). Despite the euglycaemic-hyperinsulinaemic clamp being the gold standard technique for estimation of IR [33], we used simple methods that have shown a good correlation with the gold standard method (HOMA-IR, QUICKI and MATSUDA) [28, 34]. There are studies comparing the prevalence of DM in HIV patients and the general population, and comparing ART-

naïve HIV-infected patients with the general population, but fewer compared this prevalence between patients with or without lipodystrophy.

When patients were classified as being lipodystrophic or not, according to FMR, we observed that patients with lipodystrophy had higher IR (higher HOMA and lower QUICKI and Matsuda values). Matsuda index seems to have a greater ability to predict diabetes than its HOMA equivalents [34]. They also had higher fasting plasma glucose, insulin and A1C levels, and higher % of IFG, IGT and DM. When we categorised patients into 4 categories of body fat distribution using FMR-defined lipodystrophy and waist circumference, those patients with lipodystrophy and abdominal prominence had higher prevalence of DM and IGT. Patients without FMR-defined lipodystrophy but with abdominal prominence only had a high prevalence of IGT. It seems that the loss of peripheral adipose tissue is less important than the presence of abdominal prominence in the occurrence of IR. However, the role of peripheral adipose tissue cannot be completely precluded, since patients with abdominal prominence only and without lipodystrophy, defined by FMR, had less marked glucose disturbances i.e. they only had increased prevalence of IGT. The discrepancy observed between the results obtained using the 2 different lipodystrophy definitions (Tables 3, 5 and 6) could result from the higher accuracy of the objective method in detecting slight losses of peripheral adipose tissue that were not detected by clinical inspection, as has been previously proposed by Bonnet [22].

Significant associations between IR and total fat, central fat and central/peripheral fat ratio and no association with peripheral fat at abdominal level evaluated by CT were observed, emphasizing the contribution of the central fat mass to IR. We found an association between IR and total and trunk fat evaluated by DXA. As in our results, De Wit et al showed that clinical lipodystrophy was significantly associated with new-onset diabetes and the abnormal body fat distribution in HIV-positive individuals is strongly associated with IR and/or glucose intolerance, with excess trunk or visceral fat being, as in the general population, an important risk factor for IR among those with HIV infection. In addition, De Wit observed that IR is itself independently associated with fat loss in HIV-positive individuals [5].

In our sample, no association was observed between peripheral fat mass and IR (Table 7). This result is contrary to that of the De Wit study, where patients had a marked peripheral adipose tissue loss that was clinically evident. Also, our patients may have had less evident loss of fat mass, which was detected by FMR. In fact, according to De

Wit's conclusions, information on lipodystrophy was rarely collected since the study was carried out in 11 centres, which may suggest that only marked lipodystrophies were reported.

The dual contribution of peripheral fat loss and increased abdominal fat has also been described by other authors [35-38]. Furthermore, hyperinsulinaemia seems to be more severe among the patients with a combined fat redistribution syndrome.

In our HIV-infected sample, we had a high prevalence of diabetes (20.2%), of IGT (22.5 %) and IFG (8.3%). This high prevalence could not be extrapolated for the total HIV population since we could have a referral bias, but we would like to emphasise that our aim was to evaluate the prevalence of glucose homeostasis disturbances and IR according to the presence of lipodystrophy. In HIV-infected patients, some studies showed a low prevalence of diabetes, around 3.0%, but without performing OGTT, which could have resulted in some under-identification of glucose disturbances [39]. Others suggested that the prevalence and incidence of diabetes was higher (10-14%) [7, 8, 40-42] but still lower than that which we observed. Concerning HIV-associated lipodystrophy, Carr found a baseline diabetes prevalence of 2% [43] and an over threefold increase after 14 months of follow-up [44], which suggests that lipodystrophy strongly promotes the progression to hyperglycaemia. The overall prevalence of all glucose disturbances (DM, IFG and IGT) was 25% [43]. In the Lipodystrophy Case Definition Study, diabetes prevalence was 7% in those with lipodystrophy and 3% in those without [45].

It is extremely difficult to determine which drug is responsible for the risk of diabetes because they are always used in combination and therapeutic changes in individual participants often occur during the course of the disease. The results are generally consistent, indicating a higher risk of diabetes with use of NRTI and NNRTI [46, 47]. Also, others have shown that regimens containing PI are associated with new-onset diabetes and IR [48, 49]. cART has direct effects on glucose metabolism, or exerts its effect on glucose metabolism indirectly by affecting changes in body composition. PIs directly and/or indirectly alter body composition, lipid profile, adipokine levels, and mediators of inflammation. The newer cART regimens may be less detrimental to insulin action than older regimens.

In our study, FMR as a continuum was significantly associated with insulin resistance evaluated by HOMA, even after adjustment for gender, age, IP, NRTI, NNRTI, CD4

cell count and HIV RNA viral load. No association between clinical and FMR-defined lipodystrophy and glycaemic parameters was observed.

The association between fat redistribution, glucose disturbances, IR and cART, might be explained through several mechanisms: lipoatrophy per se; lipotoxicity (cART or visceral fat-induced or both, demonstrated by increased lipolysis and circulating free fatty acids) [2]; inflammation (resulting from the infection and demonstrated by increased TNF-alpha production, increased serum concentrations of the soluble type 2 receptor of TNF-alpha [38] and other interleukins), and hormonal factors (leptin, resistin or adiponectin).

We should keep in mind that IR and diabetes are associated with age, so increased life expectancy with the use of cART is likely to increase the prevalence of diabetes [7].

The net risk of diabetes mellitus is determined by a complex interplay of individual factors, combining the traditional risk factors and newer risk factors associated with HIV-infected patients on cART. The clinical implications and message should be the need to aggressively screen for, prevent, and treat diabetes mellitus among HIV-infected patients because the presence of diabetes increases the risk of a future CHD event by almost 2.5 times [7].

Our study has some limitations. Although all patients referred from Infectious Diseases to our department were included, bias in the referral cannot be excluded, since some patients could have been referred because they had some degree of glucose intolerance or dyslipidaemia. It was not our aim to determine the prevalence of glucose homeostasis disturbances in the HIV population, because these patients are not representative of the general HIV population in our country. Our aim was to compare the prevalence of glucose homeostasis disturbances and IR in HIV-infected patients according the presence of lipodystrophy and to different patterns of fat distribution.

Also, information regarding other important risk factors for DM (e.g. family history, physical inactivity) was not collected and could not be analysed. Some drugs used for managing complications of HIV infection may be associated with worsening IR or DM; we believe that if there was any effect it was very small, since none of our patients were on niacin, megestrol acetate or steroids, and only some of them were on thiazides for the management of hypertension.

Some aspects of this study should be highlighted.

The study was performed in a unit that is highly experienced in the assessment of metabolic and body fat abnormalities in HIV-infected patients; the clinical assessment of lipodystrophy was carried out by the same investigator (PF); and an objective definition of lipodystrophy (Fat Mass Ratio by DXA), visceral and subcutaneous fat mass by CT was used.

Conclusions

Insulin resistance and glucose homeostasis abnormalities significantly increased with increasing FMR. Abdominal prominence, measured by abdominal circumference, waist/hip ratio, visceral and trunk fat defined by CT, was also associated with glucose homeostasis disturbances.

Although no association between glucose disturbances and IR was observed in clinically defined lipodystrophy, when this condition was defined by FMR, a significant positive association was observed.

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Transparency declarations

None to declare

Authors' contributions

PF conceived the study, participated in its design, in the acquisition of data and drafted the manuscript; DC conceived the study, participated in its design and drafted the manuscript; ACS performed the statistical analysis and revised critically the manuscript; JM participated in the acquisition of data and drafted the manuscript; MJM participated in the acquisition of data; AJM performed the CT scan and reviewed the data; EM and AS critically revised the manuscript; JLM revised the study design. All authors read and approved the final manuscript.

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Table 1: Sample characteristics according to the presence of clinical lipodystrophy.

	Without CL	With CL	*P*
n (%)	139	206	
Gender [n (%)]			
Male	85 (61.2)	154 (74.8)	0.007
Female	54 (38.8)	52 (25.2)	
Age [years, mean (sd)]	44.2 (12.1)	46.9 (10.7)	0.007
Duration of HIV infection [years, median (IQR)]	6.0 (5.0)	9.0 (5.0)	<0.001
cART [years, median (IQR)]	4.0 (5.0)	8.0 (5.0)	<0.001
Weight [Kg, mean (sd)]	73.7 (14.1)	66.2 (12.4)	<0.001
Height [m, mean (sd)]	1.65 (0.09)	1.66 (0.09)	0.368
BMI [(kg/m^2), median (IQR)]	26.3 (5.9)	23.8 (5.1)	<0.001
Waist circumference [cm, median (IQR)]	94.0 (17.0)	89.0 (14.5)	<0.001
Hip circumference [cm, median (IQR)]	99.0 (11.0)	91.0 (9.0)	<0.001
Thigh circumference [cm, mean (sd)]	50.0 (5.5)	46.1 (6.1)	<0.001
Arm circumference [cm, mean (sd)]	28.1 (3.1)	26.6 (4.1)	<0.001
Neck circumference [cm, mean (sd)]	37.2 (3.7)	37.1 (4.0)	0.940
CD4 cell count [cells/mm3, median (IQR)]	446.5 (342.2)	544.0 (387.0)	0.008
HIV RNA (<50) [n (%)]	93 (81.6)	163 (91.1)	0.017
Smoking history [n (%)]			
Never	61 (44.2)	70 (34.8)	
Current	55 (39.9)	93 (46.3)	
Former	22 (15.9)	38 (18.9)	0.219
HIV risk factor [n (%)]			
Injecting drug user	28 (24.6)	52 (30.6)	
Homosexual contact	10 (8.8)	19 (11.2)	
Heterosexual contact	76 (65.8)	90 (52.9)	
Others	1 (0.9)	9 (5.3)	0.247
Hepatitis C co-infection [n (%)]	31 (25.2)	58 (31.4)	0.300
CDC [n (%)]			
A	62 (54.9)	94 (53.4)	

B	4 (3.5)	0 (0)	
C	47 (41.6)	82 (46.6)	0.037
ART [n (%)]			
IP	67 (61.5)	98 (55.4)	0.310
NNRTI	47 (43.1)	83 (46.9)	0.534
NRTI	104 (95.4)	175 (98.9)	0.110
Hypoglycemic therapy [n (%)]			
Oral anti-diabetic drugs	10.1% (14)	14.6% (30)	0.132
Insulin	4.3% (6)	7.3% (15)	0.261
Body fat mass by quantitative CT			
Total fat [cm^2, median (IQR)]	340.5 (196.6)	213.6 (175.2)	<0.001
Central fat [cm^2, median (IQR)]	110.8 (111.4)	114.8 (120.5)	0.800
Peripheral fat [cm^2, median (IQR)]	186.2 (172.0)	78.7 (104.9)	<0.001
Central/peripheral fat ratio [cm^2, median (IQR)]	0.51 (0.55)	1.34 (2.15)	<0.001
FMR by DXA [median (IQR)]	1.06 (0.57)	1.79 (1.37)	<0.001

(CL- clinical lipodystrophy; CDC - Centers for Disease Control and Prevention criteria for staging of HIV infection -; cART- combined antiretroviral therapy; BMI- body mass index; FMR – fat mass ratio; DXA –dual-energy X-ray absorptiometry ; CT – computed tomography; PI – protease inhibitor; NNRTI – non-nucleoside reverse transcriptase inhibitor; NRTI – nucleoside reverse transcriptase inhibitor).

Table 2 - Smoking history and hepatitis C co-infection according to the four groups of body fat distribution

	No lipodystrophy	Isolated central fat accumulation	Isolated peripheral lipoatrophy	Mixed forms of lipodystrophy	p
Smoking history [n (%)]					
Never	14 (28.0)	46 (52.9)	24 (22.4)	45 (48.4)	
Current	30 (60.0)	25 (28.7)	67 (62.6)	26 (28.0)	
Former	6 (12.0)	16 (18.4)	16 (15.0)	22 (23.7)	<0.001
Hepatitis C co-infection [n (%)]	19 (45.2)	12 (14.8)	46 (47.9)	11 (12.5)	<0.001

Table 3: Insulin resistance indices according to lipodystrophy defined clinically and by FMR

		Lipodystrophy defined clinically			Lipodystrophy defined by FMR		
	Total	Without CL	With CL	P	Without L	With L	P
HOMA-IR [median (IQR)]	1.9 (2.8)	1.7 (2.4)	2.1 (3.0)	0.229	1.6 (2.6)	2.8 (2.8)	<0.001
HOMA-B [median (IQR)]	92.3 (106.4)	92.3 (91.1)	91.8 (117.5)	0.855	92.3 (96.1)	85.4 (120.8)	0.633
QUICKI [median (IQR)]	0.34 (0.06)	0.35 (0.06)	0.34 (0.06)	0.229	0.36 (0.07)	0.33 (0.05)	<0.001
MATSUDA [median (IQR)]	4.8 (5.1)	5.0 (4.2)	4.7 (5.4)	0.735	5.9 (5.3)	4.1 (4.4)	0.002
Glucose [mg/dL, median (IQR)]	93.5 (26.0)	90.0 (23.0)	97.0 (35.5)	0.046	90.0 (23.0)	97.0 (35.5)	0.002
Insulin [μU/mL, median (IQR)]	8.1 (9.6)	7.9 (8.2)	9.0 (10.2)	0.365	7.5 (8.1)	10.1 (9.9)	0.003
A1c [% median (IQR)]	5.3 (0.7)	5.2 (0.6)	5.3 (0.9)	0.091	5.2 (0.6)	5.5 (0.9)	0.002

(CL- clinical lipodystrophy; L- lipodystrophy).

Table 4: Insulin resistance according to lipodystrophy defined clinically and by FMR

	Lipodystrophy defined clinically			Lipodystrophy defined by FMR		
	Without CL	**With CL**	**P**	**Without L**	**With L**	**p**
HOMA –IR						
< 4 [n (%)]	93 (80.9)	121 (71.6)		94 (80.3)	58 (65.9)	0.019
≥ 4 [n (%)]	22 (19.1)	48 (28.4)	0.075	23 (19.7)	30 (34.1)	
Thirds of HOMA-IR						
1	40 (34.8)	55 (32.5)		51 (43.6)	18 (20.5)	
2	43 (37.4)	51 (30.2)	0.222	34 (29.1)	33 (37.5)	0.002
3	32 (27.8)	63 (37.3)		32 (27.4)	37 (42.0)	

(CL- clinical lipodystrophy; L- lipodystrophy).

Table 5: Prevalence of glucose homeostasis abnormalities according to lipodystrophy defined clinically and by FMR

	Lipodystrophy defined clinically				Lipodystrophy defined by FMR		
	Total (n=228)	Without CL (n=89)	With CL (n=129)	P	Without L (n=100)	With L (n=70)	p
NG [n (%)]	117 (49.1)	44 (49.4)	63 (48.8)		62 (62.0)	29 (41.4)	
IFG [n (%)]	18 (8.3)	10 (11.2)	8 (6.2)		5 (5.0)	11 (15.7)	
IGT [n (%)]	49 (22.5)	21 (23.6)	28 (21.7)	0.364	19 (19.0)	15 (21.4)	0.021
DM [n (%)]	44 (20.2)	14 (15.7)	30 (23.3)		14 (14.0)	15 (21.4)	

(NG- normal glucose; IFG – impaired fasting glucose: IGT – impaired glucose tolerance; DM – diabetes mellitus; CL – clinical lipodystrophy; L – lipodystrophy; L-lipodystrophy)

Table 6: Prevalence of glucose homeostasis abnormalities according to body composition categorised into 4 groups of fat distribution

	Categories of fat distribution by clinical lipoatrophy and WC					Categories of fat distribution by FMR and WC				
	CLA- AP-	**CLA-AP+**	**CLA+ AP-**	**CLA+AP+**	P	**L- AP-**	**L-AP+**	**L+ AP-**	**L+AP+**	p
NG [n (%)]	17 (56.7)	27 (45.8)	41 (58.6)	22 (37.3)		39 (61.9)	32 (50.8)	19 (51.4)	17 (30.9)	
IFG [n (%)]	1 (3.3)	9 (15.3)	4 (5.7)	4 (6.8)		0 (0.0)	7 (11.1)	5 (13.5)	6 (10.9)	
IGT [n (%)]	5 (16.7)	16 (27.1)	10 (14.3)	18 (30.5)	0.064	9 (14.3)	17 (27.0)	6 (16.2)	17 (30.9)	0.002
DM [n (%)]	7 (23.3)	7 (11.9)	15 (21.4)	15 (25.4)		15 (23.8)	7 (11.1)	7 (18.9)	15 (27.3)	

(NG- normal glucose; IFG – impaired fasting glucose: IGT – impaired glucose tolerance; DM – diabetes mellitus; CLA – Clinical lipoatrophy; AP – abdominal prominence defined by waist circumference >80cm in women and >94cm in men – IDF 2005; L- lipodystrophy FMR defined; CLA-AP-: no lipodystrophy; CLA-AP+: isolated central fat accumulation; CLA+AP-: isolated lipoatrophy; CLA+AP+: mixed forms of lipodystrophy)

Table 7: Association between HOMA-IR and body fat mass evaluated by CT and DXA

	Crude model		Model 1*		Model 2**	
	β	P	B	p	β	p
Body Fat Mass by Quantitative CT						
Total Fat	0.384	0.001	0.419	0.001	0.415	0.001
Central Fat	0.516	<0.001	0.486	<0.001	0.490	<0.001
Peripheral Fat	0.050	0.530	0.127	0.168	0.114	0.222
Central/peripheral fat ratio	0.258	0.001	0.239	0.015	0.262	0.008
Body fat mass by DXA						
Total	0.247	0.024	0.374	0.002	0.420	0.001
Trunk	0.403	<0.001	0.467	<0.001	0.502	<0.001
Leg	-0.043	0.625	0.049	0.653	0.087	0.438
Arm	0.162	0.058	0.352	0.001	0.366	0.001

*-Mode1: adjusted for age and gender
**-Model 2: adjusted for age, gender, IP, NRTI, CD4 cell count and HIV-1 RNA viral load
(DXA –dual-energy X-ray absorptiometry; CT – computed tomography)

Table 8: Association between FMR and glycaemic parameters and insulin resistance

	Crude model		Model 1*		Model 2**	
	β	P	B	p	β	p
HOMA	0.589	<0.001	0.595	0.001	0.266	0.002
Glucose 0´	0.124	0.002	0.041	0.367	0.098	0.222
Glucose 2h	0.079	0.142	0.086	0.139	0.149	0.104
A1C	0.053	0.015	0.007	0.765	0.083	0.314

*-Mode1: adjusted for age and gender

**-Model 2: adjusted for age, gender, IP, NRTI, CD4 cell count and HIV-1 RNA viral load

PUBLICAÇÃO 9:

Emerging laboratory cardiovascular risk factors in HIV-infected patients with objectively defined lipodystrophy

Title: Emerging laboratory cardiovascular risk factors in HIV-infected patients with objectively defined lipodystrophy

Abbreviated title: HIV lipodystrophy and emerging cardiovascular risk factors

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Abstract

Background: Combined antiretroviral therapy (cART) is very effective in the reduction of HIV disease progression and mortality, but it is associated with lipodystrophy, lipid abnormalities and cardiovascular disease. Emerging risk factors may also predispose HIV-infected patients to accelerated atherosclerosis, namely apolipoprotein A1, apolipoprotein B, Lp (a), fibrinogen, hs-CRP, homocysteine, microalbuminuria and NT-proBNP serum levels. **Aims:** To compare serum lipids and cardiovascular risk factors in HIV-infected patients on cART according to the presence of lipodystrophy [defined clinically (CL) and by Fat Mass Ratio (FMR-L)] and four different patterns of fat distribution. **Methods:** We evaluated body composition and emerging cardiovascular risk factors in 181 HIV-infected adults. **Results:** Patients with CL had lower total cholesterol, LDL-C, HDL-C and Apo A1 and higher triglyceride levels, with no differences in the other parameters analysed. Patients with FMR-L had higher triglycerides and uric acid and lower Lp(a) levels than patients without FMR-L. Patients with abdominal prominence, independent of the presence of lipoatrophy (isolated central fat accumulation and mixed forms of lipodystrophy), had higher levels of total cholesterol, LDL-C and Apo B. Patients with isolated lipoatrophy and mixed forms of lipoatrophy had higher triglycerides and the lowest Apo A1 levels. Significant positive linear associations were observed between FMR and triglycerides and Apo B/Apo A ratio, and a negative one with Apo A1. Theses associations, with the exception of Apo A1, were independent of age, gender, IP and NRTI. **Conclusions:** The emerging cardiovascular risk factors did not vary according to fat redistribution. In HIV-infected patients, the 2 components of body fat redistribution syndrome seemed to induce different deleterious effects – central fat accumulation induced increased total cholesterol, LDL cholesterol and Apo B – and isolated lipoatrophy induced low Apo A1 and high triglycerides. In the analysis of the components of adipose tissue redistribution syndrome, abdominal prominence seemed to be more important than lipoatrophy in this association.

Keywords: Lipodystrophy; HIV, Lp(a), apolipoprotein A1, apolipoprotein B, cholesterol and triglycerides, homocysteine, fibrinogen, hs-CRP, microalbuminuria, NT-proBNP; cardiovascular risk factors

Introduction

Genetic lipodystrophies are rare disorders associated with dyslipidemia and increased cardiovascular risk [1]. Lipodystrophy associated with HIV or with its treatment is a common manifestation of the disease. Combined antiretroviral therapy (cART) was a major advance in the management of HIV infection, being highly effective in the reduction of disease progression and mortality [2]. However, cART has been associated with changes in serum lipids and with inherent increases in cardiovascular risk, which has important implications in HIV therapeutic decision making and long term patient management. cART is also associated with changes in body habitus due to fat redistribution (lipodystrophy): peripheral lipoatrophy and central fat accumulation. These two distinct phenotypes can occur together or separately. There is a suggestion that emerging non-traditional risk factors may predispose HIV-infected patients to accelerated atherosclerosis. Apolipoprotein A1, apolipoprotein B, lipoprotein (a) [Lp (a)], fibrinogen, high sensitivity C-reactive protein (hs-CRP), homocysteine, microalbuminuria and NT-proBNP serum levels have also been associated with increased cardiovascular risk, and some of them are related to inflammation [3-18].

The aims of this study were to compare the serum lipids, metabolic and cardiovascular risk factors in HIV-infected patients on cART according to the presence of lipodystrophy [defined clinically (CL) and by Fat Mass Ratio (FMR)] and four different defined patterns of fat distribution and to assess the relationship of these parameters with FMR.

Methods

Subjects

As part of a cross-sectional study, consecutive clinically stable HIV-infected adults receiving cART were evaluated at the Endocrinology Outpatient Department. Of the 368 HIV-1 infected patients (254 men and 114 women) on cART included in our cohort, we excluded 187 patients from this study because they were on lipid lowering drugs (statins and/or fibrates). The study protocol was approved by the Hospital Ethics Committee and all patients provided informed consent.

Clinical assessment

For each patient the following information was collected using a standardized protocol: demographic data (age, sex), duration of known HIV infection, HIV infection risk factors, duration of cART and disease stage. The "Centers for Disease Control and Prevention" (CDC) HIV staging classification was used [19]. Weight, height, circumferences of neck, waist, hip, thigh and arm were measured, as previously described [20, 21] All measurements were performed by the same observer (PF) using standard techniques [22]. Clinical lipodystrophy (CL) was defined as a peripheral lipoatrophy with or without a central fat accumulation as assessed by both patient and practitioner as we previously described [20, 21, 23]. Presence of central fat accumulation or abdominal prominence was defined by the measurement of waist circumference using the International Diabetes Federation (IDF) criteria for metabolic syndrome [24]. The clinical assessment was performed by the same practitioner (PF). Patients were distributed into 4 different groups according to the presence of fat redistribution features: 1) **No lipodystrophy**- patients without clinical lipoatrophy and without abdominal prominence; 2) **Isolated central fat accumulation** - patients without clinical lipoatrophy and with abdominal prominence; 3) **Isolated lipoatrophy** - patients with clinical lipoatrophy and without abdominal prominence; 4) **Mixed forms of lipodystrophy** - patients with clinical lipoatrophy and with abdominal prominence.

The quantification of total, visceral, and peripheral fat was performed with a 64-slice computed tomography (CT) scanner (Siemens Sensation 64 Cardiac), as previously described [25, 26].

Body composition was assessed with whole-body dual-energy X-ray absorptiometry (DXA – Lunar Expert XL). DXA measurement was performed while the patient was in

a supine position, with standard positioning of the arms and feet. Markers used in this study for trunk and lower limbs that defined regions of interest were those indicated by the manufacturer. Regional fat mass values were grouped and analysed for the following anatomical regions: arms, legs, trunk and total body. The fat mass ratio (FMR) is the ratio of the percentage of the trunk fat mass to the percentage of the lower limb fat mass (FMR= % of the trunk fat mass/ % of the lower limb fat mass) [27]. We used the cut-off value for lipodystrophy defined by FMR for men of 1.961 and for women of 1.329 [23].

The blood samples for biological and inflammatory parameters were drawn in fasting patients. The measurements of total cholesterol (TC), low density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol , triglycerides, apolipoprotein A1, apolipoprotein B, lipoprotein (a) [Lp (a)], fibrinogen, high sensitivity C-reactive protein (hs-CRP), homocysteine, uric acid, lactate, and NT-proBNP serum levels were determined using commercial kits in the Central Laboratory at our hospital. "Non HDL-C" was defined as the difference between total cholesterol and HDL. Microalbuminuria was determined in a 24 hour urine sample. hs-CRP was divided into three groups: < 1.0; $\geq 1.0 < 3.0$ and ≥ 3.0 mg/dL. The presence of an abnormal urine albumin excretion was defined as albuminuria in 24 hour urine $\geq$ 30.0 mg (microalbuminuria). Hyperfibrinogenaemia was deemed present if plasma fibrinogen was $\geq$ 400 mg/dL and hyperhomocysteinaemia if homocysteine was ≥ 12.44 μmol/L. The CD4+ cell count was determined by flow cytometry and plasma HIV-1 RNA loads were measured by a quantitative reverse transcriptase polymerase chain reaction, which has a lower limit of detection of 50 copies/mL.

Statistical analysis

Statistical analysis was performed using SPSS version 18.0 software (SPSS Inc., Chicago, Illinois, USA). All probabilities were two tailed and p values <0.05 were regarded as significant.

Data were described as mean and standard deviation (SD) for quantitative variables and compared using the Student-t test or the Mann-Whitney test as appropriate. For lipid profile and non-traditional cardiovascular risk factor according to the four groups of fat distribution, Kruskal-Wallis test was used. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher's exact test. Linear regression models were computed for estimating the association between FMR and lipid

profile and non-traditional cardiovascular risk factors, after log transformation whenever necessary. Models were adjusted for age, sex, PI, NNRTI and NRTI.

Results

Of the 181 HIV-1 infected patients (112 men and 69 women) on cART included in this study, 50.3 % were classified as having clinical lipodystrophy (CL). Table 1 shows the characteristics of the study sample according to the presence of CL. Patients with CL were more frequently male, had longer known duration of HIV infection and higher cumulative exposure to cART, and lower weight, BMI and waist, hip, thigh and arm circumferences. No differences in age, neck circumference or waist/hip ratio circumference were found according to the presence of CL. On abdominal fat mass CT evaluation, patients with CL had less total and peripheral fat but had a higher central/peripheral ratio. No differences in central fat accumulation between patients with or without CL were observed on abdominal CT scan. No differences were observed between patients with and without CL regarding CD4+ cell count, viral suppression, pharmacologic groups of ARTs and CDC classification.

Compared to patients without CL, patients with CL had lower total cholesterol, LDL-C, HDL-C and Apo A1 mean levels and higher triglyceride mean levels. No significant differences were observed in the other parameters analysed. Patients with lipodystrophy defined by FMR had higher triglyceride and uric acid mean levels and lower Lp(a) levels (Table 2).

When patients were classified according to the four groups of fat distribution, patients with abdominal prominence, independently of the presence of lipoatrophy (patients with isolated central fat accumulation and with mixed forms of lipodystrophy) had higher mean levels of total cholesterol, LDL-C and Apo B (Table 3). Patients with isolated lipoatrophy and mixed forms of lipoatrophy had higher mean levels of triglycerides and the lower Apo A1 levels.

FMR was positively associated with triglycerides and Apo B/Apo A1 ratio. A negative linear association was observed between FMR and Apo A1 levels. The positive association between FMR and triglyceride levels and Apo B/Apo A1 ratio remained even after adjustment for age, gender, PI, NRTI and NNRTI ($p=0.006$ and $p=0.053$ respectively). The negative linear association observed between FMR and Apo A1 remained significant after adjustment for age and gender, but lost its statistical significance after adjustment for cART treatment. No significant association was observed with the other parameters analysed (Table 4).

No significant associations were observed between hs-CRP and cART (r=0.017; p=0.863) and CD4 cell count (r=0.009; p=0.929). Also, there was no difference in the median values of hs-CRP according to viral load (Table 5).

Discussion

Lipid profile

Disturbances in lipid metabolism, namely elevated cholesterol and often markedly elevated triglyceride levels, have been increasingly recognised among HIV-infected subjects after the introduction of cART [28]. In the cART era, body fat partitioning changes have appeared early after cART initiation and have been progressive [29]. In many ways, this HIV-related lipodystrophy syndrome resembles the rare congenital and autoimmune disease lipodystrophy syndromes that are also associated with abnormalities in lipid profile [30]. The extent of fat redistribution has been described by some but not all as related to the severity of metabolic alterations [5, 31-34].

An atherogenic lipid profile characterised by lower HDL–C and Apo A1 and higher triglyceride levels was observed in our HIV-infected patients on cART with CL. Patients with isolated lipoatrophy showed a similar pattern. The lowest Apo A1 levels were observed in patients with isolated lipoatrophy and mixed forms of lipodystrophy. Moreover, higher triglyceride levels were also found in those with FMR-defined lipodystrophy. Patients with abdominal prominence, either isolated central fat accumulation or mixed forms of lipodystrophy, had an atherogenic lipid profile characterised by high total cholesterol, LDL–C and Apo-B. In the general population, abdominal prominence is also associated with high total cholesterol, LDL–C and Apo B, and reduced Apo A1 [4]. Thus, it seems that patients with abdominal prominence and isolated lipoatrophy have a significantly different lipid profile. In Carvalho's study [35], high Apo B was not associated with abdominal fat accumulation (evaluated through waist circumference and waist-to-hip ratio), or lipodystrophy, while others reported higher levels of Apo B in lipodystrophy [36-38]. So, measuring serum Apo B may be especially useful among patients with hypertriglyceridemia in which the cardiovascular risk is underestimated with the use of LDL [35].

In our study, Apo A1 was found to be lower in those patients with CL and in those with isolated lipoatrophy and mixed forms of lipodystrophy, and it was higher in patients with isolated central fat accumulation. A significant positive linear association was observed between FMR and triglycerides. This association was independent of age, gender, PI, NNRTI and NRTI. A negative linear association was observed between FMR and Apo A1 (adjusted for gender and age), but it was not significant after adjustment for IP, NNRTI and NRTI. This means that HIV fat redistribution syndrome

evaluated by FMR is linked to an atherogenic profile that includes low Apo A1 and high triglycerides.

Patients with lipodystrophy defined by FMR had lower Lp(a). Lp(a) is an independent risk factor for CVD, mainly resulting from genetic background. It may be of particular interest in the context of PI-associated dyslipidemia as the atherogenic potential of Lp(a) appears to be enhanced in the presence of elevated concentrations of LDL [39]. In our data, the presence of FMR lipodystrophy seems to be protective.

Emerging laboratory cardiovascular risk factors

No significant difference in hs-CRP between patients with or without lipodystrophy (clinical and FMR defined) and the four groups of fat redistribution were observed. hs-CRP, an inflammatory biomarker, was proposed to be a valuable adjunct to traditional risk factors in estimating overall cardiovascular risk in HIV-infected patients on cART, but it remains a controversial issue [6-9, 40-42].

We found that patients with lipodystrophy defined by FMR (but not clinically defined) had higher uric acid levels but we found no differences among the four defined groups of fat distribution. Data regarding serum uric acid levels in HIV-infected patients are scarce. Walker confirmed the known factors for hyperuricaemia are relevant in HIV-infected patients (gender, age, renal function and BMI) and found a quantitatively important additional influence of some antiretroviral agents on urate [10]. Urate may also be increased due to the mitochondrial toxicity associated with some NRTIs. Mitochondrial dysfunction may increase the production of lactate, which competes with urate for tubular secretion in the kidney. Respiratory chain failure causes ATP depletion, which increases urate production in the purine nucleotide cycle [10, 11]. This mechanism may provide an explanation for the association between some dideoxynucleoside analogues (stavudine, didanosine or zalcitabine) and elevated urate [10].

No differences in lactate levels were observed in patients with or without lipodystrophy (clinical and FMR-defined) and in the four groups of fat redistribution. Lactate is a sign of anti-retroviral mitochondrial toxicity [43]. So, cART is associated with mitochondrial toxicity and related to peripheral lipoatrophy [43, 44].

We did not find differences in microalbuminuria measurement between patients with or without lipodystrophy (clinical or defined by FMR) or in the four defined groups of fat distribution. However, it should be noted that patients with known diabetes mellitus or

hypertension were not excluded in our study, which is a limitation. However, in a previous study we found no differences in the prevalence of hypertension between patients with or without lipodystrophy, either clinically or FMR-defined [45]. To our knowledge no study has been done evaluating the influence of lipodystrophy in microalbuminuria. The pathophysiological mechanism underlying urinary albumin excretion and the increased risk of CVD is not fully understood, although endothelial dysfunction and inflammation have been implicated [46, 47], factors known to be relevant in HIV infection**.**

No differences in homocysteine levels between patients with or without lipodystrophy (clinical or defined by FMR) or in the four defined groups of fat distribution were observed. Although a substantial body of epidemiological evidence suggests an association between cardiovascular risk and moderately increased plasma homocysteine levels [16, 48], and elevated plasma homocysteine levels confer an independent risk of vascular disease, the exact meaning of hyperhomocysteinemia remains unclear [17, 49, 50].

We found no differences in NT-proBNP measurement between patients with or without lipodystrophy (clinical or defined by FMR) and in the four groups of fat distribution. NT-proBNP has been shown to be an indicator of cardiac function. Increased levels of B-type natriuretic peptides (BNP and/or NT-proBNP) have been associated with an increased risk of cardiovascular events, namely myocardial infarction, heart and cardiac death in different populations, as well as with the presence of diastolic and systolic dysfunction as assessed by echocardiography [51]. No other study has evaluated the impact of lipodystrophy on NT-proBNP levels.

We did not find any differences in fibrinogen levels between patients with or without lipodystrophy (clinical or defined by FMR) or in the four defined groups of fat distribution. Plasma fibrinogen is a major determinant of blood viscosity and blood flow. Increasing evidence from epidemiological studies suggests that elevated plasma fibrinogen levels are associated with an increased risk of cardiovascular disorders, including ischemic heart disease, stroke, and other thromboembolic manifestations [52].

Our study had some limitations. Although we included all patients referred from the Infectious Diseases Department to our Endocrinology Outpatient Clinic for metabolic abnormalities, we cannot exclude selection bias by the infectious disease specialist. Some subjects in this study were referred to the Endocrinology Clinic specifically for lipodystrophy or metabolic disorders related to cART, and so we might have selected a

study population enriched with metabolic complications. We found a high prevalence of lipodystrophy but there was no selection bias for the presence of isolated lipoatrophy or abdominal prominence since our main goal was to evaluate whether the presence of lipodystrophy influences the serum lipids, metabolic and emerging cardiovascular risk factors in HIV-infected patients on cART. Finally, this is a study with a cross-sectional design and so causality cannot be attributed.

Some aspects of this study should be highlighted.

Clinical assessment of lipodystrophy was carried out, and a clinical separation of patients into 4 patterns of fat distribution was done. Additionally, the presence of lipodystrophy was objectively defined using FMR assessed by DXA. The presence of visceral fat was objectively measured by CT. This study was performed in a unit that is highly experienced in the assessment of metabolic and body fat abnormalities in HIV-infected patients, and the clinical assessment of lipodystrophy was carried by the same investigator (PF).

We assessed a range of traditional and non-traditional or emerging cardiovascular risk factors in relation to lipodystrophy, including some that have not been previously reported in HIV-infected patients on cART.

Conclusions

We concluded that patients with CL had lower total cholesterol, LDL-C, HDL-C, Apo A1 and higher mean levels of triglycerides. Patients with lipodystrophy defined by FMR had higher triglycerides and lower Lp(a). When patients were classified according to the four groups of fat distribution, patients with abdominal prominence, independent of the presence of lipoatrophy, had higher mean levels of total cholesterol, LDL-C, and Apo B. Low Apo A1 is a known risk indicator of coronary atherosclerosis [53] and we found the lowest Apo A1 plasma levels in patients with isolated lipoatrophy and mixed forms of lipodystrophy. Significant positive linear associations were observed between FMR and triglycerides and Apo B/Apo A ratio. These associations remained significant after adjustment for age, gender, PI and NRTI. Apo A1 had a significant negative linear association with FMR that remained significant after adjustment for gender and age, but it lost significance after adjustment for cART. The emerging cardiovascular risk factors did not vary according to fat redistribution. In HIV infected patients, the 2 components of body fat redistribution syndrome seemed to induce different deleterious effects: central fat accumulation induced increased total cholesterol, LDL cholesterol and Apo B; and isolated lipoatrophy induced low Apo A1 and high triglycerides. In the analysis of the components of adipose tissue redistribution syndrome, abdominal prominence seems to be more important than lipoatrophy in this association.

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Transparency declarations

None to declare

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Table 1: Sample characteristics according to the presence of clinical lipodystrophy

	Without CL	With CL	
N (%)	90 (49.7)	91 (50.3)	p
Gender [n (%)]			
Male	45 (50)	67 (73.6)	
Female	45 (50)	24 (26.4)	0.002
Age [years, mean (sd)]	41.5 (10.1)	44.0 (10.6)	0.078
Duration [years, mean (sd)]	7.2 (4.1)	9.6 (3.8)	<0.001
cART [years, mean (sd)]	4.9 (3.9)	7.8 (3.6)	<0.001
Weight [Kg, mean (sd)]	72.7 (15.5)	65.6 (13.2)	0.001
Height [m, mean (sd)]	1.65 (0.09)	1.68 (0.09)	0.094
BMI [(kg/m^2), mean (sd)]	26.8 (5.7)	23.2 (3.8)	<0.001
Waist circumference [cm, mean (sd)]	93.6 (14.4)	87.6 (11.6)	0.004
Hip circumference [cm, mean (sd)]	101.0 (10.4)	91.8 (6.5)	<0.001
Thigh circumference [cm, mean (sd)]	50.5 (6.8)	46.5 (6.8)	<0.001
Arm circumference [cm, mean (sd)]	28.5 (3.8)	26.9 (5.2)	0.001
Neck circumference [cm, mean (sd)]	36.3 (3.9)	36.6 (4.2)	0.998
Ratio waist/ hip circumference [mean (sd)]	0.93 (0.09)	0.95 (0.08)	0.121
CD4 cell count [cells/mm3, mean (sd)]	499.6 (306.1)	545.9 (299.4)	0.131
HIV RNA (<50) [n (%)]	72 (85.7)	80 (89.9)	0.544
HIV risk factor [n (%)]			
Inject	21 (24.4)	36 (41.9)	
Homosexual	11 (12.8)	8 (9.3)	
Heterosexual	53 (61.6)	37 (43.0)	
Other	1 (1.2)	5 (5.8)	0.017
CDC [n (%)]			
A	35 (55.6)	38 (53.5)	
B	3 (4.8)	0 (00.0)	
C	25 (39.7)	33 (46.5)	0.187
cART [n (%)]			
PI	50 (59.5)	53(59.6)	1.000
NNRTI	37 (44.0)	40 (44.9)	1.000
NRTI	81 (97.6)	88 (98.9)	0.610
Body Fat Mass by Quantitative CT			
Total fat [cm^2, mean (sd)]	332.0 (171.9)	195.7 (131.4)	<0.001
Central fat [cm^2, mean (sd)]	106.5 (73.4)	102.8 (80.4)	0.445
Peripheral fat [cm^2, mean (sd)]	225.5 (136.6)	92.7 (77.1)	<0.001
Central/peripheral [mean (sd)]	0.60 (0.56)	1.62 (1.51)	<0.001

FMR by DXA [mean (sd)]	1.12 (0.44)	1.71 (0.98)	0.002

(CL – clinical lipodystrophy; cART – combined antiretroviral therapy; BMI - body mass index; CDC - Centers for Disease Control and Prevention criteria for staging of HIV infection; PI – protease inhibitor; NNRTI –non-nucleoside reverse transcriptase inhibitor; NRTI – nucleoside reverse transcriptase inhibitor; CT – computed tomography; FMR - fat mass ratio; DXA - dual-energy X-ray absorptiometry.)

Table 2 – Lipid profile and non-traditional risk factors according the presence of clinical lipodystrophy and lipodystrophy defined by FMR.

	Clinical lipodystrophy			Lipodystrophy defined by FMR		
	Without CL (n= 88)	With CL (n=91)	p	Without L (n= 133)	With L (n=89)	p
Total cholesterol [mg/dL, mean (sd)]	213.8 (51.5)	195.2 (48.7)	0.021	203.9 (47.1)	199.7 (44.7)	0.520
LDL-cholesterol [mg/dL, mean (sd)]	134.6 (41.0)	117.9 (40.0)	0.012	125.6 (39.9)	116.0 (39.9)	0.264
HDL-cholesterol [mg/dL, mean (sd)]	50.3 (14.6)	44.9 (14.2)	0.004	48.7 (15.7)	45.0 (12.6)	0.262
Non-HDL cholesterol [mg/dL, mean (sd)]	163.4 (47.2)	150.4 (44.3)	0.079	155.2 (41.8)	154.7 (38.4)	0.925
Triglycerides[mg/dL, mean (sd)]	159.6 (83.8)	209.5 (124.4)	0.005	166.6 (114.4)	206.8 (93.5)	0.009
Apo A1 [mg/dL, mean (sd)]	124.1 (18.9)	115.5 (23.2)	0.005	109.0 (103.7)	111.8 (18.8)	0.084
Apo B [mg/dL, mean (sd)]	100.3 (24.9)	95.2 (22.5)	0.210	100.4 (23.5)	98.1 (18.3)	0.784
Ratio Apo B/Apo A1 [n (%)]						
< 0.90	51 (68.0)	46 (62.2)		39 (60.9)	13 (52.0)	
≥ 0.90	24 (32.0)	28 (37.8)	0.565	25 (39.1)	12 (48.0)	0.596
Lp(a) [mg/dL, mean (sd)]	29.2 (29.1)	21.7 (25.8)	0.333	29.0 (28.6)	19.9 (24.1)	0.047
Homocysteine [µmol/L, mean (sd)]	9.1 (3.1)	9.9 (3.6)	0.168	8.8 (2.90)	9.6 (4.0)	0.544
Hyperhomocysteinaemia [≥ 12.44 [µmol/L, n (%)]	11 (14.9)	22 (29.3)	0.054	9 (14.1)	8 (32.0)	0.072
hs-CRP [mg/dL, mean (sd)]	0.39 (0.46)	0.29 (0.38)	0.343	0.33 (0.53)	0.37 (0.28)	0.092
hs-CRP [n, (%)]						
<1.0	9 (30.0)	9 (31.0)		11 (35.5)	2 (18.2)	
≥1.0 <3.0	8 (26.7)	11 (37.9)		13 (41.9)	3 (27.3)	
≥3.0	13 (43.3)	9 (31.0)	0.579	7 (22.6)	6 (54.5)	0.245
Lactate [mmol/L, mean (sd)]	1.11 (0.42)	1.20 (0.49)	0.488	1.11 (0.41)	1.29 (0.52)	0.102
NT-ProBNP [pg/mL, mean (sd)]	25.8 (22.9)	41.5 (74.8)	0.604	22.1 (18.6)	44.7 (70.6)	0.256

Fibrinogen [mg/dL, mean (sd)]	345.8 (83.4)	341.3 (104.2)	0.518	339.0 (98.5)	345.0 (103.8)	0.923
Hyperfibrinoogenaemia [≥ 400 mg/dL, n (%)]	14 (24.6)	9 (16.7)	0.429	12 (20.0)	5 (20.0)	1.000
Microalbumin [mg/L, mean (sd)]	37.7 (123.8)	62.7 (187.0)	0.536	42.0 (126.5)	18.8 (24.4)	0.327
Microalbumin [≥ 30 mg/dL, n (%)]	3 (9.4)	7 (22.6)	0.184	4 (12.9)	3 (23.1)	0.404
Uric acid [mg/L, mean (sd)]	46.4 (16.3)	48.7 (15.7)	0.143	41.5 (13.0)	54.8 (12.2)	<0.001
Hyperuricaemia [≥ 82 mg/L, n (%)]	4 (4.5)	4 (4.4)	1.000	1 (1.4)	0 (0.0)	1.000

Table 3 – Lipid profile and non-traditional additional risk factors according to the four groups of fat distribution

	No lipodystrophy (n=33)	**Isolated central fat accumulation** (n=49)	**Isolated lipoatrophy** (n=52)	**Mixed forms of lipodystrophy** (n=32)	p
Total cholesterol [mg/dL, mean (sd)]	204.2 (50.4)	219.6 (52.7)	190.1 (50.3)	208.0 (43.4)	0.036
LDL-cholesterol [mg/dL, mean (sd)]	125.6 (42.1)	140.0 (40.7)	113.0 (39.6)	126.2 (40.5)	0.021
HDL-cholesterol [mg/dL, mean (sd)]	47.9 (14.4)	51.4 (15.2)	44.8 (16.6)	45.9 (10.9)	0.056
Non-HDL cholesterol [mg/dL, mean (sd)]	156.3 (50.3)	168.2 (46.0)	145.2 (45.1)	162.0 (40.0)	0.079
Triglycerides [mg/dL, mean (sd)]	174.6 (102.8)	153.4 (68.4)	204.0 (133.1)	233.5 (112.3)	0.007
Apo A1 [mg/dL, mean (sd)]	123.1 (23.8)	124.3 (16.1)	114.0 (26.7)	116.1 (18.1)	0.035
Apo B [mg/dL, mean (sd)]	92.9 (21.7)	105.6 (25.5)	92.0 (25.1)	100.4 (16.0)	0.028
Ratio Apo B/Apo A1 [n (%)]					
≥0.90	6 (21.4)	18 (40.0)	14 (33.3)	13 (46.4)	0.226
Lp(a) [mg/dL, mean (sd)]	33.8 (33.9)	27.3 (26.3)	24.9 (29.8)	18.7 (19.8)	0.724
Uric acid [mg/L, mean (sd)]	48.9 (15.6)	44.6 (14.7)	46.7 (15.8)	51.3 (16.7)	0.191
Hyperuricemia [> 82mg/L, n (%)]	1 (3.0)	2 (4.1)	2 (3.8)	2 (6.3)	0.869
Homocysteine [µmol/L, mean (sd)]	9.50 (2.8)	8.7 (3.2)	10.2 (3.60)	9.2 (3.7)	0.124
Hyperhomocysteinaemia [≥ 12.44µmol/L, n (%)]	4 (14.8)	6 (13.3)	13 (30.2)	7 (25.0)	0.195
hs-CRP [mg/dL, mean (sd)]	0.36 (0.29)	0.39 (0.49)	0.26 (0.45)	0.33 (0.28)	0.362
hs-CRP [mg/dL, n (%)]					
< 1.0	1 (25.0)	8 (30.8)	6 (37.5)	3 (23.1)	
≥1.0 < 3.0	1 (25.0)	7 (26.9)	7 (43.8)	4 (30.8)	
≥ 3.0	2 (50.0)	11 (42.3)	3 (18.8)	6 (46.2)	0.723

Lactate [mmol/L, mean (sd)]	1.10 (0.53)	1.13 (0.36)	1.15 (0.48)	1.32 (0.51)	0.268
NT-proBNP [pg/mL, mean (sd)]	19.9 (22.1)	26.9 (20.9)	39.8 (61.5)	46.8 (96.1)	0.068
Fibrinogen [mg/dL, mean (sd)]	330.0 (83.6)	354.4 (83.2)	333.4 (102.3)	355.7 (109.0)	0.603
Hyperfibrinogenaemia [≥ 400mg/dL, n (%)	4 (20.0)	10 (27.0)	5 (14.3)	4 (21.1)	0.626
Microalbumin [mg/L, mean (sd)]	55.1 (131.1)	32.8 (124.1)	19.4 (32.0)	115.3 (272.0)	0.723
Microalbumin [≥ 30 mg/L, n (%)]	1 (14.3)	2 (8.0)	4 (23.5)	3 (21.4)	0.480

Table 4: Linear regression models of the association between FMR and the described variables

FMR	Crude model		Model 1*		Model 2**	
	β	p	B	p	β	p
Total Cholesterol	0.0005	0.712	0.0002	0.832	0.0005	0.683
LDL-C	-0.0001	0.310	0.0002	0.823	0.0004	0.973
HDL-C	-0.005	0.167	-0.004	0.153	-0.004	0.213
Non-HDL-C	0.001	0.932	0.001	0.447	0.001	0.383
Triglycerides***	0.250	0.007	0.208	0.006	0.225	0.006
Apo A1***	-0.700	0.008	-0.464	0.039	-0.420	0.082
Apo B***	-0.031	0.894	0.255	0.212	0.234	0.269
Apo B/Apo A1 ratio	0.392	0.076	0.394	0.026	0.374	0.053
Lp (a)***	-0.063	0.209	-0.064	0.121	-0.061	0.165
Homocysteine	0.026	0.114	-0.006	0.682	-0.008	0.612
hs CRP***	0.010	0.885	0.016	0.753	0.028	0.598
Lactate	0.189	0.112	0.145	0.141	0.128	0.213
NT-proBNP***	0.114	0.139	0.044	0.517	0.052	0.476
Microalbuminuria***	0.011	0.847	-0.030	0.492	-0.008	0.859
Fibrinogen	-0.003	0.550	-0.001	0.254	-0.001	0.148

*-Mode1: adjusted for age and gender; **-Model 2: adjusted for age, gender, PI, NNRTI and NRTI; ***after log transformation

Table 5: hs-CRP according to viral load

HIV RNA	hs-CRP median (IQR)	P
<50	0.15 (0.33)	
>50	0.18 (0.41)	0.448

(IQR – interquartile range)

PUBLICAÇÃO 10:

Adipokines, hormones related to body composition, and insulin resistance in HIV fat redistribution syndrome

Title: Adipokines, hormones related to body composition, and insulin resistance in HIV fat redistribution syndrome

Abbreviated title: Adipokines and HIV

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Abstract

Background: Lipodystrophies are characterized by adipose tissue redistribution, insulin resistance (IR) and metabolic complications. Adipokines and hormones related to body composition may play an important role linking these alterations. **Objectives:** To evaluate adipocyte-derived hormones (adiponectin, leptin, resistin, TNF-α, PAI-1), ghrelin and IR in HIV-infected patients according to the presence of Fat Mass Ratio defined lipodystrophy (FMR-L) and four categories of fat redistribution. **Design:** Cross-sectional study. **Methods:** Anthropometric and metabolic parameters, HOMA-IR, body composition by DXA and CT, and adipokines were evaluated in 217 HIV-infected patients on cART and 74 controls. **Results:** Leptin levels were lower in patients with FMR-L and isolated lipoatrophy, and higher in those with isolated central fat accumulation (ICFA) and mixed forms (MXF) of lipodystrophy. Positive correlations were found between leptin and total, trunk, leg, arm fat evaluated by DXA, and total, Visceral Adipose Tissue (VAT), Subcutaneous Adipose Tissue (SAT), VAT/SAT ratio by CT regardless of FMR-L, and with HOMA-IR in patients with FMR-L. Adiponectin correlated negatively with VAT, and its mean levels were lower in patients with ICFA and higher in those with no lipodystrophy. Resistin was positively correlated with HOMA-IR in FMR-L patients. MXF-patients had higher PAI-1 levels and these were positively correlated with VAT in those with FMR-L. Ghrelin was higher in HIV-infected patients than controls despite BMI-matching. **Conclusion:** Different degrees of peripheral fat atrophy and central fat accumulation characteristics of HIV lipodystrophy induces changes in adipokines, hormones related to body composition that maintain a complex interplay related to IR and metabolic abnormalities.

Keywords: lipodystrophy, HIV, adipokines, body composition, insulin resistance

Introduction

Changes in body fat distribution are a common finding in patients with HIV infection being treated with combined antiretroviral therapy (cART) and this condition has many similarities to rare, congenital and acquired lipodystrophies, which are associated with depletion of subcutaneous fat, increased triglycerides and profound insulin resistance (IR) [1]. Adipose tissue was traditionally considered an energy storage organ, but over the last decade, it has emerged as a metabolically active endocrine organ secreting multiple bioactive peptides, collectively called "adipokines", other proteins including inflammatory mediators such as tumor necrosis factor alpha (TNF-α) and plasminogen activator inhibitor-1 (PAI-1), which not only influence adipocyte function in an autocrine and paracrine fashion, but also affect more than one metabolic pathway through the bloodstream's systemic circulation [2]. All adipose tissue products seem to contribute significantly in various metabolic processes both at local and systemic levels and may induce a proinflammatory state and oxidative damage, leading to initiation and progression of atherosclerosis[3]. On the other hand, these patients have marked changes in circulating levels of adipocyte secreted hormones, including leptin and adiponectin, which may contribute to metabolic abnormalities, and hormones related to body composition, such as ghrelin[4]. Ghrelin is an orexigenic gut peptide that is the endogenous ligand for the GH secretagogue receptor. Low ghrelin may be a marker of metabolic dysregulation and altered body fat without specifically contributing to low GH [5].

cART in HIV infection produces widespread metabolic complications, including hyperlipidemia, IR, lipodystrophy [peripheral fat wasting (lipoatrophy) with or without abdominal/visceral fat accumulation (lipohypertrophy)] [6]. The hormonal changes caused by increased (in lipohypertrophy) or reduced subcutaneous fat (in lipoatrophy) may be central to the metabolic abnormalities observed in HIV-lipodystrophy [5].

The aim of this study was to evaluate the relationship between fat mass, lipodystrophy defined by the Fat Mass Ratio (FMR-L) and the four different categories of fat distribution, and adipocyte derived hormones (adiponectin, leptin, resistin, TNF-α), orexigenic hormones (ghrelin), prothrombotic factors (PAI-1) and IR in HIV-infected patients on cART.

Methods

Subjects

As part of a cross-sectional study, consecutive clinically stable HIV-infected adults receiving cART were evaluated at the Endocrinology Outpatient Department. Seventy four age-, gender- and BMI-matched HIV-uninfected controls were also evaluated. These controls were selected from a previously assembled cohort of non-institutionalized community adults, the EPI-Porto study [7]. The study protocol was approved by the Hospital Ethics Committee and all patients provided informed consent.

Clinical assessment

For each patient the following information was collected using a standardized protocol: demographic data (age, gender), duration of HIV infection, HIV infection risk factors, duration of cART and characterization of the infection. We used the "Centers for Disease Control and Prevention" (CDC) criteria for classifying the degree the infection [8]. Weight, height, circumferences of neck, waist, hip, thigh and arm were measured as previously published[9-12]. Blood pressure (BP) was measured using the established recommendations[13].

Clinical lipodystrophy was defined as a peripheral lipoatrophy with or without a central fat accumulation assessed by both patient and practitioner, as previously described[9-12]. Presence of central fat accumulation or abdominal prominence was defined by the measurement of waist circumference using the International Diabetes Federation (IDF) criteria for metabolic syndrome[14]. Patients were classified into four different categories according the presence or absence of either clinical lipoatrophy or abdominal prominence: **no lipodystrophy** - patients without clinical lipoatrophy and without abdominal prominence; **isolated central fat accumulation** - patients without clinical lipoatrophy and with abdominal prominence; **isolated lipoatrophy** - patients with clinical lipoatrophy and without abdominal prominence; **mixed forms of lipodystrophy** - patients with clinical lipoatrophy and with abdominal prominence. The clinical assessment was performed by the same practitioner (PF).

Evaluation of body composition

Body composition was assessed with whole-body dual-energy X-ray absorptiometry (DXA – Lunar Expert XL). DXA measurement was performed while the patient was in

a supine position, with standard positioning of the arms and feet. Markers used in this study for trunk and lower limbs that defined regions of interest were those indicated by the manufacturer. Regional fat mass values were grouped and analysed for the following anatomical regions: arms, legs, trunk and total body. The fat mass ratio (FMR) is the ratio of the percentage of the trunk fat mass to the percentage of the lower limb fat mass (FMR= % of the trunk fat mass/ % of the lower limb fat mass)[15]. We define lipodystrophy by FMR using the cut-off value of 1.961 for men and 1.329 for women[9]. The quantification of total, visceral, and peripheral abdominal fat was performed with a 64-slice computed tomography (CT) scanner (Siemens Sensation 64 Cardiac) with the same technique as previously described [16, 17]. All values were expressed in cm^2 rounded to the nearest centesimal.

Laboratory analysis

A venous blood sample was drawn after a 12-hour overnight fast. All the samples were analysed at the central laboratory of our hospital. Patients without a previous diagnosis of diabetes were submitted to a glucose tolerance test (OGTT). The test was performed as described by the World Health Organization using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.

The CD4 cell count was determined by flow cytometry and plasma HIV-1 RNA loads were measured by a quantitative reverse transcriptase polymerase chain reaction (Roche Diagnostic Systems, Inc., Branchburg, NJ, USA), which has a lower limit of detection of 50 copies/mL.

Adipokines and hormones related to body composition

Leptin (Human Leptin RIA Kit, Linco Research), adiponectin (Human Adiponectin RIA Kit, Linco Research), resistin (Human Resistin ELISA Kit, Linco Research), TNF-α (TNF-α IRMA Kit, BioSource), ghrelin (Ghrelin Active RIA Kit, Linco Research) and PAI-1 (Human PAI-1 ELISA Kit, IBL International GMBH) were measured in the Nobre Laboratory of the Porto Medical School. For leptin, the intra-assay precision is 4.6 % and inter-assay precision 5.0 %. For adiponectin, the intra-assay precision is 1.78 % and inter-assay precision 9.25 %. For resistin, the intra-assay precision is 3.2 % and inter-assay precision 7.1 %. For TNF-α, the intra-assay precision is 3.1% and inter-assay precision 5.7 %. For ghrelin, the intra-assay precision is 6.7 % and inter-assay precision 9.6 %. The overall intra-assay coefficient of variation for PAI-1 was 4.7 %.

Insulin resistance evaluation

Insulin resistance was defined by the homeostasis model assessment of insulin resistance (HOMA) and insulin sensitivity by the quantitative insulin sensitivity check index (QUICKI). These indexes were calculated by the following formulas:

HOMA-IR index = (insulin 0 x glucose 0)/22.5 [18].

QUICKI = 1/[log (fasting insulin in mU/l) +log (fasting plasma glucose in mg/dL)][19].

Glucose was expressed in mmol/L and insulin in μUI/mL.

Statistical analysis

Data were described as mean and standard deviation (SD) for quantitative variables and compared using the Student-t test or the Mann-Whitney test as appropriate. For the comparison between the four groups of fat distribution and adipokine parameters the Kruskal-Wallis test was used. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher's exact test.

For estimating the association between adipokine levels and anthropometric, metabolic and body composition, Spearman correlation coefficients were calculated. Statistical analysis was performed using SPSS version 18.0 software (SPSS Inc., Chicago, Illinois, USA). All probabilities were two tailed and p values <0.05 were regarded as significant.

Results

Sample characteristics, adipokines and hormones related to body composition in HIV-infected patients and HIV-uninfected controls

HIV-infected patients had significantly higher waist/hip ratio, glucose, triglyceride, insulin, HOMA, and ghrelin levels, and adiponectin/leptin ratio. However, they had lower weight, hip and arm circumferences, HDL-cholesterol levels, QUICKI, resistin, adiponectin and leptin levels. No differences were observed between HIV-infected patients and HIV-uninfected controls regarding age, gender, BMI, waist circumference, systolic and diastolic blood pressure and total cholesterol, LDL-cholesterol, uric acid, hsCRP and TNF-α levels (Table 1).

Adipokines and hormones related to body composition in HIV-infected patients according to gender

HIV-infected females had a higher hip circumference, total fat mass and subcutaneous adipose tissue (SAT) on abdominal CT scan evaluation and total, trunk, leg and arm mass fat by DXA. They also had lower visceral adipose tissue (VAT) and VAT/SAT ratio on CT scan. Also, females had higher leptin levels and lower TNF-α and adiponectin/leptin ratios. No gender-related differences were observed in adiponectin, resistin, ghrelin and PAI-1 levels (Table 2).

Adipokines and hormones related to body composition according to the four categories of body composition

Patients with isolated central fat accumulation and mixed forms of lipodystrophy were older and had higher BMIs. Patients with no lipodystrophy had higher levels of adiponectin and patients with isolated central fat accumulation had the lowest levels of adiponectin. The levels of leptin were higher in patients with isolated central fat accumulation and mixed forms of lipodystrophy and lower in those with isolated lipoatrophy. No significant differences were found in resistin, ghrelin and TNF-α between the four groups of body composition. Patients with no lipodystrophy and mixed forms of lipodystrophy had higher levels of PAI-1, and the lowest levels of PAI-1 were found in those with isolated central fat accumulation. The adiponectin/leptin ratio was

higher in patients who had no lipodystrophy and lower in patients with isolated central fat accumulation, followed by the patients with mixed forms of lipodystrophy (Table 3).

Sample characteristics, body composition, metabolic parameters, adipokines and hormones related to body composition, according to the presence lipodystrophy defined by FMR

In this sample of 217 HIV-1 infected patients (144 men and 73 women) on cART, 41 % of patients presented lipodystrophy defined by FMR (FMR-L). Table 4 shows the characteristics of the study sample according to the presence of FMR-L. Patients with FMR-L were more frequently males, older and had a longer duration of HIV infection and length of cART. No significant differences were found in weight, BMI, waist, arm and thigh circumferences among patients with or without FMR-L. Patients with FMR-L had a higher neck circumference and waist/hip ratios, but a lower hip circumference. Also, they had significantly a higher mean CD4+ cell count, but no significant difference in viral suppression rate was observed. No differences were observed between patients with and without FMR-L regarding the cART regimens and CDC classification. Also, no differences were observed regarding diastolic blood pressure, hs-CRP, total cholesterol, LDL and HDL cholesterol levels. The patients with FMR-L had higher systolic blood pressure, uric acid, triglycerides, HOMA, fasting glucose and insulin levels than those without FMR-L. In relation to OGTT, glucose and insulin at 2 hours were higher in patients with FMR-L. QUICKI was lower in patients with FMR-L than in those without FMR-L.

On DXA evaluation, patients with FMR-L had lower total, leg and arm fat mass (either in % or Kg) than patients without FMR-L with no significant difference in trunk fat mass. On CT evaluation at abdominal level, patients with FMR-L had higher VAT and lower SAT with a higher central/peripheral fat ratio, with no difference in total fat at this level.

Leptin levels were lower in patients with FMR-L. No significant differences were found in the levels of adiponectin, resistin, ghrelin, PAI-1, TNF-α and adiponectin/leptin ratio among patients with or without FMR-L (Table 4).

Adipokines, body composition hormones and metabolic parameters

We evaluated the correlations between leptin, resistin, TNF-α and adiponectin with age, waist/hip circumference ratio, arm, waist, hip circumferences, BMI, fasting glucose and

insulin, HOMA, triglycerides and total cholesterol in all the HIV-infected patients and HIV-uninfected controls. Leptin was positively associated with arm, waist, hip circumferences, BMI and fasting insulin both in the HIV-infected patients and in HIV-uninfected controls. In HIV-uninfected controls, leptin was also positively correlated with HOMA. A negative correlation was found between resistin and hip circumference and BMI in the HIV-infected patients, while a positive correlation between resistin and triglycerides was found in the HIV-uninfected controls. Also, a negative correlation was observed between TNF-α and total cholesterol in HIV-infected patients, and between adiponectin and arm circumference in controls (Table 5).

Regarding the effect of lipodystrophy on the association between anthropometric and metabolic parameters in HIV-infected patients, a significant positive correlation was found between leptin and: insulin at 2 hours on OGTT; total, trunk, leg, arm fat evaluated by DXA; and total, SAT, VAT/SAT ratio on abdominal level evaluated by CT scan in these patients, regardless of the presence of FMR-L. Leptin was also positively associated with arm, thigh, waist, hip circumferences, BMI, glucose at 2 hours on OGTT, total cholesterol, and was negatively associated with length of cART in patients without FMR-L. Patients with FMR-L also exhibited positive associations between resistin levels and duration of HIV-infection, as well as between PAI-1 and VAT. A negative association was found between adiponectin and arm fat mass measured by DXA in FMR-L patients. A positive correlation was observed between TNF-α, fasting glucose and A1c in patients without FMR-L (Table 6).

The adiponectin/leptin ratio was not associated with IR [HOMA (r=-0.148, p=0.066) or Quicki (r=0.148, p=0.066)] in HIV patients overall, or in men [HOMA (r=-0.138, p=0.164) or Quicki (r=0.126, p=0.206)]. However, a positive correlation between this ratio and Quicki was observed [HOMA (r=-0.228, p=0.101); Quicki (r=0.277, p=0.044] in women.

Discussion

In acquired or genetic lipodystrophies, altered fat distribution *per se* is a strong determinant of IR and metabolic alterations, and the loss of fat likely precedes the metabolic complications[20-23]. Increased amounts of visceral fat accompanied by decrease in peripheral adipose tissue are associated with an increase of lipolysis and free fat acid fluxes, which together can alter adipocytokine production[24].

Regarding body fat composition, our data showed that there were differences in body fat distribution between HIV-infected patients and HIV-uninfected controls, males and females with HIV-infection and patients with or without FMR-L despite similar BMI. Actually, HIV-infected patients had higher waist/hip ratio and lower hip and arm circumferences. Females had a higher fat mass (hip circumference, total fat mass and SAT on abdominal CT evaluation and total, trunk, leg and arm mass fat on DXA) and men had higher VAT and VAT/SAT ratios on CT scan.

FMR-L patients had lower hip circumference, fat mass (total, leg and arm) by DXA and SAT at abdominal level by CT, and higher waist/hip ratio, VAT and central/peripheral fat ratio evaluated by abdominal CT scan.

Leptin

Leptin levels were higher in HIV-uninfected controls and lower in patients with FMR-L, indicating an association with body fat, demonstrated by the significant positive correlation between leptin and arm, thigh, waist, hip circumferences, BMI, total, trunk, leg, arm fat mass evaluated by DXA, and total, VAT, SAT, VAT/SAT ratio on abdominal level evaluated by CT scan. Like other authors, we also observed that leptin concentrations were higher in HIV-infected women compared to HIV-infected men and, therefore, the usual gender distribution for this adipocytokine remains unchanged in HIV infection[25, 26]. In genetic lipodystrophy syndromes, very low plasma leptin concentrations were found when fat mass was profoundly reduced [27] and that correlated positively with total adiposity [28].

Our HIV-infected patients with isolated central fat accumulation and mixed forms of lipodystrophy had higher BMI and leptin levels. Leptin levels were lower in those with isolated lipoatrophy, positively proportional to the BMI. Most studies, which have not differentiated between subjects with predominant lipoatrophy, lipohypertrophy, and mixed patterns of fat redistribution, have failed to find significantly lower leptin levels in subjects with HIV lipodystrophy [23, 29, 30], but when subjects with distinctive

lipoatrophy have been studied separately, lower leptin levels in this subgroup of HIV lipodystrophy patients has been demonstrated. Also, significant associations between leptin secretion and subcutaneous fat has been reported and decreased subcutaneous fat correlated with reduced leptin levels after controlling for visceral fat quantity[31]. Total and regional adiposity were strongly and positively associated with leptin in healthy controls and in HIV-infected patients, both in men and women, suggesting that adiposity remains the major determinant of plasma leptin levels in HIV infection [28].

In a prospective study, there was a significant positive correlation between the appearance of lipoatrophy and the leptin levels[30, 32]. In our sample, leptin was positive and significantly associated with fasting insulin both in the HIV-infected patients and in HIV-uninfected controls. In controls, leptin was also positive and significantly correlated with HOMA. We also observed a significant positive correlation between leptin and glucose and insulin at 2 hours on OGTT in patients without FMR-L and fasting and 2 hours insulin on OGTT and HOMA in patients with FMR-L.

The role of leptin in the modulation of insulin sensitivity is complex. Extremely low levels of leptin in congenital generalized lipoatrophies were associated with severe IR, and significantly improved with replacement of leptin in mice and humans[33].

In conclusion, leptin levels appear to be primarily determined by total adiposity in HIV-infected individuals, independently of the presence of lipodystrophy[28].

Adiponectin

Adiponectin levels were higher in HIV-uninfected controls than in HIV-infected patients. Patients with no lipodystrophy had higher levels of adiponectin and patients with isolated central fat accumulation had the lowest levels of adiponectin. Contrary to other authors, who found higher adiponectin levels in HIV-infected women[25, 26], no such gender difference was observed in our patients. Also, no significant differences were found in the levels of adiponectin among patients with or without FMR-L.

We found a significant negative correlation between adiponectin and total and trunk fat mass evaluated by DXA in the HIV-infected patients, and with arm fat mass in patients without FMR-L. A negative significant correlation was found between adiponectin and arm circumference in HIV-uninfected controls.

Kosmiski et al, observed that both total and regional adiposity were significant and negatively correlated with adiponectin levels in a control group, similar to other reports of general populations[28]. Also, in that same study, HIV-infected patients with leg SAT

below the 10th percentile of controls had significantly lower adiponectin levels than controls with similar leg SAT volumes [28]. In some studies, no association between adiponectin and total adiposity was found in HIV-infected patients, while adiponectin was positively correlated with both extremity and abdominal SAT[34, 35]. This relationship suggests that the remaining SAT in HIV-infected patients with moderate to severe lipoatrophy is dysfunctional rather than simply reduced in size, since in anorexia nervosa, a condition characterized by low body weight and severe global fat depletion (although not isolated subcutaneous adipose tissue), adiponectin is higher[36-38]. Findings of abnormal function or loss of adipose tissue in partial lipodystrophy syndromes (due to lamin A/C or to PPAR-γ receptor mutations) and low adiponectinemia also support the theory that low adiponectin concentrations in HIV-infected subjects with low levels of SAT are due to lipodystrophy *per se* [37, 38]. Thus, lipodystrophies are characterized not only by decreased fat but also by dysfunction or loss of adipocytes [28]. In HIV-infected men and women, with or without lipodystrophy, the normal negative relationship between visceral adiposity and adiponectin was maintained[28, 34, 35, 39].

In HIV-infected patients, adiponectin levels correlated significantly with the percentage of body fat distributed to the limbs and also correlated significantly with insulin sensitivity [23, 40], although some authors state that adiponectin predicts insulin sensitivity independently of the amount and repartition of body fat [41], no such association was found between adiponectin and markers of IR in our sample.

It has been reported that in HIV lipodystrophic patients, adiponectin levels are significantly lower in patients with fat redistribution and correlate inversely with serum triglycerides with the lowest levels, in peripheral lipoatrophic and central lipohypertrophic patients, representing a central role in glucose, lipid metabolism and IR in these patients [35, 41-43]. These findings were independent of age, leptin levels, HIV medications and severity of disease[35, 41]. As we observed for leptin levels, in our sample adiponectin levels were lower in patients with lipodystrophy and patients with isolated central fat accumulation were the ones with the lowest level of adiponectin.

Ratio adiponectin/leptin

Adiponectin/leptin ratio is considered to be a better predictor of insulin sensitivity and cardiovascular risk, and is usually considered independent of body mass. Adiponectin/leptin ratio has been negatively associated with IR, metabolic defects and cardiovascular risk markers[41]. We found a positive correlation between this ratio and

Quicki in women. We also showed that the adiponectin/leptin ratio was lower in HIV-uninfected controls than in HIV-infected patients. This ratio was higher in patients with no lipodystrophy and lower in patients with isolated central fat accumulation, followed by the patients with mixed forms of lipodystrophy. Women had higher levels of leptin and lower adiponectin/leptin ratio. No significant differences were found between patients with or without FMR-L.

Resistin

Elevated resistin levels have been observed in obese and diabetic subjects, and increased resistin has been associated with IR in lean and obese subjects [44]. However, other studies found no association between circulating resistin levels and IR [45, 46].

In our data, resistin was higher in HIV-uninfected controls and no significant differences were found in the four categories of body composition, or according to gender and presence of FMR-L in HIV-infected patients, similar to previously published data[47]. Resistin was positively associated with duration of HIV-infection and with HOMA in patients with FMR-L. Kamin also demonstrated that HIV-infected subjects with lipodystrophy and IR had higher circulating resistin levels[48]. A negative correlation was found between resistin and hip circumference and BMI in the HIV-infected patients and a positive one with triglycerides in the controls.

TNF- α

HIV lipodystrophy, familial partial lipodystrophy and obesity have been associated with high circulating levels of TNF-α [37, 49, 50]. No significant differences in TNF-α levels were found between HIV-infected patients and controls, either according to the presence of FMR-L, or in the four categories of fat composition. However, we found a positive correlation between TNF-α and fasting glucose and A1c in patients without FMR-L.

TNF-α homeostasis is profoundly altered by HIV-1 infection itself and it has been shown that HIV-1 infected patients have elevated pre-treatment levels of TNF-α and that these levels dramatically decrease when cART is initiated. However, concentrations of the components of TNF-α system are not normalized when cART is effective. We did not observe any correlation between TNF-α and adiponectin levels (r=0.145; p=0.060) although that has previously been described [51].

PAI-1

Patients with no lipodystrophy and mixed forms of lipodystrophy had higher levels of PAI-1 and the lowest levels of PAI-1 were found in those with isolated central fat accumulation. No differences in PAI-1 levels between patients with or without FMR-L and according to gender were found, but a significant positive association between PAI-1 and VAT was observed in patients with FMR-L.

PAI-1 concentrations are increased in conditions characterized by increased VAT or SAT, hypertriglyceridemia, and hyperinsulinemia. Both VAT and SAT produce PAI-1, but data are controversial as to whether VAT produces more, similar, or less amounts of PAI-1 than SAT. Also, it has been suggested that PAI-1 originates from stromal cells in adipose tissue rather than from adipocytes. Patients with cART-associated lipodystrophy had elevated plasma PAI-1concentrations[52]. HIV infected patients with fat redistribution had significant elevations in PAI-1, being waist–to-hip ratio the only significant predictor of PAI-1 in a univariate regression analysis. Elevated levels of PAI-1 were associated with hyperinsulinemia and central adiposity, placing HIV infected patients at substantial risk of cardiovascular disease via impaired fibrinolysis[53].

Contrary to our data, He et al, found significantly increased plasma PAI-1 level in patients with lipodystrophy as compared with patients without lipodystrophy. They also found a positive correlation between plasma PAI-1 level and BMI, waist circumference, VAT, plus an inverse correlation with the percentage of limb fat. In a stepwise multiple linear regression model, VAT turned out to be the main predictor of plasma PAI-1[54].

Ghrelin

Ghrelin was higher in HIV-infected patients than in controls. No significant differences were found in ghrelin levels by gender, by four categories of body composition or between patients with or without FMR-L. Fasting serum ghrelin level has been negatively correlated with BMI, and it is decreased in obesity and increased in cachexia[55]. Ghrelin is a peptide involved in energy homeostasis, mainly inducing food intake and GH secretion. In humans, ghrelin stimulates appetite by inducing hunger, which leads to increased food consumption and consequent metabolic alterations, with increasing body weight, body fat mass, and serum triglycerides[55]. Contrary to our data, lower levels of ghrelin have been reported in HIV-infected lipodystrophic patients[4, 56].

Falasca et al, observed higher levels of ghrelin in patients with hypertriglyceridemia, as well as a positive correlation between serum ghrelin and triglyceride levels in patients

with hypertriglyceridemia. No such association between ghrelin and triglyceride levels were observed in our sample (r=0.064, p=0.424). Falasca concluded that adipokines may have crucial roles in the pathogenesis of hypertriglyceridemia and, thus, monitoring of serum ghrelin levels could be important to identify subjects with high cardiovascular risk[55].

Limitations

Several limitations of this study should be noted. A cross-sectional design cannot prove causality between cART use, lipoatrophy, and alterations in adipokine levels. It is not possible to determine whether adipokine abnormalities are due to reduced hormone expression in adipose tissue, decreased differentiation or decreased adipose cell numbers.

Some aspects of the current study should be highlighted.

The study was performed in a unit that is highly experienced in the assessment of metabolic and body fat abnormalities in HIV-infected patients; the clinical assessment of lipodystrophy was performed by the same investigator (PF); and objective definitions of lipodystrophy (Fat Mass Ratio by DXA), visceral and subcutaneous fat mass by CT were used.

Conclusion

Leptin levels were lower in patients with lipodystrophy, indicating its close relationship with total body fat. A significant positive correlation between leptin and HOMA in patients with lipodystrophy demonstrated the role of this adipokine in insulin sensitivity. Adiponectin was lower in patients with isolated central fat accumulation and negatively correlated with visceral adipose tissue. The adiponectin/leptin ratio was lower in patients with isolated central fat accumulation. Patients with mixed forms of lipodystrophy had higher levels of PAI-1. Resistin was positively associated with HOMA in patients with FMR-defined lipodystrophy. Patients with no lipodystrophy and mixed forms of lipodystrophy had higher levels of PAI-1 and the lowest levels of PAI-1 were found in those with isolated central fat accumulation. Ghrelin was higher in HIV-infected patients than in controls despite the two groups being BMI-matched. Our results add to the evidence on the complexity of the relationship between adipokines,

hormones related to body composition, and insulin resistance in the HIV redistribution syndrome, with different degrees of peripheral fat atrophy and central fat accumulation.

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Transparency declarations

None to declare

Authors' contributions

PF conceived the study, participated in its design, in the acquisition of data and drafted the manuscript; DC conceived the study, participated in its design and drafted the manuscript; ACS performed the statistical analysis and revised critically the manuscript; AJM performed the CT scan and reviewed the data; EM critically revised the manuscript; JP performed the DXA and reviewed the data; AS critically revised the manuscript and JLM revised the study design. All authors read and approved the final manuscript.

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Table 1: Sample characteristics, adipokines and hormones related to body composition in HIV-uninfected controls and HIV-infected patients

	HIV-uninfected controls	HIV-infected patients
Age [years, mean (sd)]	47.2 (9.7)	46.6 (11.6)
Gender [n(%)]		
Male	40 (54.1)	144 (66.9)
Female	34 (45.9)	73 (33.6)
Weight [Kg, mean (sd)]	71.5 (13.4)	67.8 (12.3)
BMI [(kg/m2), mean (sd)]	26.3 (4.3)	35.1 (4.5)
Waist circumference [cm, mean (sd)]	90.7 (11.6)	91.2 (11.6)
Hip circumference [cm, mean (sd)]	99.8. (7.1)	94.5 (8.4)
Waist/hip ratio[mean (sd)]	0.91 (0.08)	0.96 (0.08)
Arm circumference [cm, mean (sd)]	30.6 (3.5)	27.0 (3.0)
Systolic blood pressure [mmHg, mean (sd)]	122.2 (17.3)	121.9 (17.5)
Diastolic blood pressure [mmHg, mean (sd)]	78.9 (10.1)	76.7 (11.3)
Glucose [mg/dL, mean (sd)]	91.7 (12.2)	107.7 (41.5)
Insulin [μU/mL, mean (sd)]	5.5 (4.9)	11.2 (10.6)
HOMA [mean (sd)]	1.3 (1.4)	3.1 (3.2)
QUICKI [mean (sd)]	0.39 (0.05)	0.36 (0.08)
Total cholesterol [mg/dL, mean (sd)]	217.2 (43.6)	224.2 (58.0)
LDL cholesterol [mg/dL, mean (sd)]	134.9 (38.8)	128.5 (49.9)
HDL cholesterol [mg/dL, mean (sd)]	57.4 (14.9)	46.1 (13.6)
Triglycerides [mg/dL, mean (sd)]	119.8 (57.6)	267.5 (182.8)
Uric acid [mg/dL, mean (sd)]	49.6 (15.4)	46.8 (15.0)
hs-CRP [mg/dL, mean (sd)]	0.36 (0.54)	0.45 (0.73)
Resistin [ng/mL, mean (sd)]	15.5 (11.0-22.2)	10.4 (8.0-13.9)
Adiponectin [ng/mL, mean (sd)]	5177.5 (2795.0-7737.5)	2676.5 (1192.1-5458.6)
TNF-α [pg/mL, mean (sd)]	45.3 (29.9-60.5)	44.6 (30.1-73.6)
Ghrelin [pg/mL, mean (sd)]	18.6 (10.5-25.4)	75.0 (47.7-139.5)
Leptin [ng/mL, mean (sd)]	11.8 (6.3-21.3)	3.4 (1.9-7.6)
Adiponectin/leptin ratio [mean (sd)]	998.5 (1949.9)	2291.8 (5870.5)

Table 2: Body composition, adipokines and hormones related to body composition in HIV-infected patients, according to gender

	Male	Female	P
Age [years, mean (SD)]	46.8 (11.2)	46.2 (12.5)	0.724
BMI [(kg/m2), mean (SD)]	24.7 (3.8)	26.1 (5.6)	0.050
Waist circumference [cm, mean (SD)]	91.2 (10.6)	91.2 (13.5)	0.980
Hip circumference [cm, mean (SD)]	93.1 (6.7)	97.2 (10.5)	0.003
Total fat mass CT [cm3, mean (SD)]	243.4 (136.2)	356.0 (166.7)	<0.001
VAT CT [cm3, mean (SD)]	140.7 (86.2)	113.6 (69.9)	0.020
SAT CT [cm3, mean (SD)]	102.6 (76.9)	242.4 (125.7)	<0.001
VAT/SAT ratio	2.2 (2.4)	0.55 (0.40)	<0.001
Total fat mass DXA [%, mean (SD)]	16.8 (8.1)	33.3 (10.0)	<0.001
Trunk fat in DXA [%, mean (SD)]	20.6 (9.8)	33.4 (10.3)	<0.001
Leg fat mass DXA [%, mean (SD)]	12.2 (9.1)	31.9 (12.5)	<0.001
Arm fat mass DXA [%, mean (SD)]	15.2 (8.9)	42.3 (11.4)	<0.001
Adiponectin [ng/mL, median (25th and 75th percentile)]	3341.8 (1790.0-6594.4)	3000.0 (1359.0-6550.0)	0.391
Leptin [ng/mL, median (25^{th} and 75^{th} percentile)]	3.0 (1.8-5.8)	11.2 (5.7-18.0)	<0.001
Resistin [ng/mL, median (25^{th} and 75^{th} percentile)]	11.2 (8.4-17.0)	11.2 (8.9-16.8)	0.421
Ghrelin [pg/mL, median (25^{th} and 75^{th} percentile)]	55.5 (26.9-99.2)	44.0 (21.4-79.2)	0.069
PAI-1 [pg/mL, median (25^{th} and 75^{th} percentile)]	61.3 (38.5-104.3)	43.4 (30.8-95.8)	0.368
TNF-α [pg/mL, median (25^{th} and 75^{th} percentile)]	49.9 (31.6-72.4)	39.7 (27.2-60.0)	0.028
Adiponectin/leptin ratio, median (25^{th} and 75^{th} percentile)	944.8 (344.1-2035.3)	244.6 (94.1-630.0)	<0.001

Table 3: Adipokines and hormones related to body composition according to four categories of body composition

	No lipodystrophy N=29	Isolated central fat accumulation N=62	Isolated lipoatrophy N=86	Mixed forms of lipodystrophy N=67	P
Age [years, mean (SD)]	38.8 (10.7)	46.1 (12.3)	43.8 (9.6)	51.9 (10.7)	<0.001
BMI [kg/m^2, mean (SD)]	22.7 (2.4)	29.1 (4.5)	21.8 (2.9)	26.3 (3.4)	<0.001
Adiponectin [ng/mL, median (25th and 75th percentile)]	4735.5 (2495.8-9451.5)	2005.0 (896.0-4246.8)	2635.0 (1185.2-7589.0)	2760.0 (1150.5-5404.0)	0.026
Leptin [ng/mL, median (25th and 75th percentile)]	2.8 (2.0-5.6)	9.5 (5.2-14.8)	1.9 (1.4-3.2)	4.7 (2.6-7.9)	<0.001
Resistin [ng/mL, median (25th and 75th percentile)]	9.7 (8.2-12.8)	10.3 (7.2-12.0)	9.5 (8.2-14.5)	10.8 (7.8-15.9)	0.621
Ghrelin [pg/mL, median (25th and 75th percentile)]	77.9 (52.8-116.1)	67.8 (40.6-100.9)	78.9 (44.8-176.2)	78.8 (52.4-112.9)	0.388
PAI-1 [pg/mL, median (25th and 75th percentile)]	111.9 (64.8-162.8)	40.4 (30.6-45.8)	61.3 (28.3-96.4)	68.7 (34.6-109.6)	0.047
TNF-α [pg/mL, median (25th and 75th percentile)]	43.0 (35.0-62.1)	43.0(28.9-84.1)	49.8 (30.1-74.7)	43.4 (28.3-80.9)	0.980
Adiponectin/leptin ratio median (25th and 75th percentile)	1485.5 (972.5-2674.5)	208.2 (73.5-408.0)	1258.7 (438-4-5224.9)	691.4 (195.8-1380.9)	<0.001

Table 4: Sample characteristic, body composition, adipokines and hormones related to body composition according to the presence of lipodystrophy defined by FMR

	Without lipodystrophy	With lipodystrophy	P
n (%)	128 (59.0)	89 (41.0)	-
Gender [n (%)]			
Male	76 (59.4)	68 (76.4)	
Female	52 (40.6)	21 (23.6)	0.014
Age [years, mean (sd)]	44.7 (12.2)	49.4 (10.2)	0.003
Duration of HIV infection [years, mean (sd)]	7.4 (3.9)	8.6 (3.6)	0.007
cART [years, mean (sd)]	5.8 (3.8)	8.0 (3.4)	<0.001
Weight [Kg, mean (sd)]	67.4 (13.0)	68.3 (11.1)	0.599
BMI [(kg/m^2), mean (sd)]	25.0 (4.8)	25.3 (4.5)	0.684
Waist circumference [cm, mean (sd)]	90.2 (12.2)	92.7 (10.5)	0.102
Hip circumference [cm, mean (sd)]	95.9 (9.2)	92.5 (6.7)	0.002
Thigh circumference [cm, mean (sd)]	48.3 (5.6)	46.9 (4.8)	0.066
Arm circumference [cm, mean (sd)]	26.7 (3.2)	27.2 (2.7)	0.275
Neck circumference [cm, mean (sd)]	36.4 (3.8)	37.9 (3.7)	0.010
Waist/hip circumference ratio	0.94 (0.08)	1.00 (0.07)	<0.001
CD4 cell count [cells/mm3, mean (sd)]	498.9 (289.4)	644.6 (354.1)	0.001
HIV RNA (<50) [n (%)]	109 (85.2)	81 (91.0)	0.448
HIV risk factor [n (%)]			
Injecting drug user	36 (28.6)	17 (19.1)	
Homosexual contact	7 (5.6)	16 (18.0)	
Heterosexual contact	81 (64.3)	54 (60.7)	
Others	2 (1.6)	2 (2.2)	0.017
CDC [n (%)]			
A	67 (52.8)	57 (64.0)	
B	2 (1.6)	0 (0)	
C	58 (45.7)	32 (36.0)	0.155
ART [n (%)]			
IP	65 (52.0)	48 (53.9)	0.888
NNRTI	62 (49.6)	45 (50.6)	0.890
NRTI	121 (96.8)	86 (96.6)	1.000
Total Cholesterol [mg/dL, mean (sd)]	218.1 (56.4)	233.1 (59.5)	0.061
LDL-cholesterol [mg/dL, mean (sd)]	125.4 (50.2)	133.0 (49.4)	0.272
HDL- cholesterol [mg/dL, mean (sd)]	47.2 (14.6)	44.4 (11.7)	0.136
Triglycerides [mg/dL, mean (sd)]	244.8 (117.6)	300.1 (195.3)	0.028
Uric acid [mg/L, mean (sd)]	43.8 (14.3)	51.0 (15.1)	<0.001
Systolic blood pressure [mmHg, mean (sd)]	120.0 (18.6)	124.8 (15.6)	0.039
Diastolic blood pressure [mmHg, mean (sd)]	75.7 (11.8)	78.1 (10.5)	0.138

Glucose 0 [mg/dL, mean (sd)]	103.0 (39.5)	114.4 (43.7)	0.002
Glucose 2h at OGTT [mg/dL, mean (sd)]	123.9 (42.9)	139.7 (49.0)	0.030
Insulin 0 [μU/mL, mean (sd)]	9,96 (10.50)	12.96 (10.55)	0.005
Insulin 2h at OGTT [μU/mL, mean (sd)]	49. 8 (40.1)	88.3 (142.2)	0.005
HOMA [mean (sd)]	2.65 (3. 01)	3.70 (3.28)	<0.001
QUICKI [mean (sd)]	0.37 (0.10)	0.34 (0.4)	<0.001
hsCRP [mg/dL, mean (sd)]	0.50 (0.90)	0.36 (0.27)	0.275
Adiponectin [ng/mL, mean (sd)]	5634.6 (7393.4)	3953.9 (4491.7)	0.228
Leptin [ng/mL, mean (sd)]	7.3 (8.5)	3.9 (3.3)	0.008
Resistin [ng/mL, mean (sd)]	12.8 (13.4)	12.8 (7.4)	0.373
Ghrelin[pg/mL, mean (sd)]	117.4 (133.6)	118.1 (106.7)	0.446
PAI-1 [pg/mL, mean (sd)]	62.6 (43.6)	102.1 (110.1)	0.262
TNF-α [pg/mL, mean (sd)]	61.9 (50.3)	52.3 (33.9)	0.425
Adiponectin/leptin ratio [mean (sd)]	2721.5 (7207.8)	1691.4 (3132.2)	0.418
Fat mass [%, mean (sd)] DXA			
Total	24.8 (13.1)	19.3 (8.5)	0.002
Trunk	25.7 (13.3)	24.3 (9.0)	0.532
Leg	24.4 (14.7)	11.1 (7.4)	<0.001
Arm	27.2 (17.1)	20.3 (13.6)	0.001
Fat mass [Kg, mean (sd)] DXA			
Total	17.3 (11.1)	13.2 (7.3)	0.016
Trunk	9.3 (6.0)	9.0 (4.8)	0.686
Leg	5.2 (3.6)	2.1 (1.6)	<0.001
Arm	2.1 (1.9)	1.5 (1.2)	0.008
Body Fat Mass by Quantitative CT			
Total [cm^2, mean (sd)]	282.9 (174.9)	276.5 (126.6)	0.917
VAT [cm^2, mean (sd)]	111.2 (85.4)	139.4 (68.7)	<0.001
SAT [cm^2, mean (sd)]	171.7 (125.9)	117.0 (92.4)	0.001
VAT/SAT ratio [mean (sd)]	1.07 (1.46)	2.44 (2.60)	<0.001

Note: cART – combined antiretroviral therapy; BMI. Body mass index; OGTT- oral glucose tolerance test; DXA - dual-energy X-ray absorptiometry CT – computed tomography; VAT- visceral adipose tissue; SAT – subcutaneous adipose tissue.

Table 5: Associations between adipokines and anthropometric and metabolic parameters in HIV patients and HIV-uninfected controls

Correlation Between	Leptin				Resistin				TNF-α				Adiponectin			
	Total HIV patients		Controls		Total HIV patients		Controls		Total HIV patients		Controls		Total HIV patients		Controls	
	r	P	R	p	r	p	r	P	R	p	r	p	r	P	r	P
Age	0.045	0.552	-0.097	0.436	-0.016	0.836	-0.149	0.209	-0.007	0.924	-0.267	0.023	-0.048	0.516	-0.033	0.779
Wais/hip ratio	0.091	0.236	0.103	0.410	0.049	0.520	0.130	0.274	0.057	0.444	0.078	0.511	-0.090	0.229	-0.114	0.336
Waist circumference	0.429	<0.001	0.356	0.003	-0.083	0.272	0.068	0.567	0.052	0.482	0.083	0.487	-0.097	0.192	-0.160	0.175
Hip circumference	0.411	<0.001	0.537	<0.001	-0.151	0.044	0.034	0.776	0.005	0.950	0.097	0.413	-0.048	0.519	-0.108	0.364
Arm circumference	0.292	<0.001	0.328	0.007	-0.105	0.205	-0.008	0.946	-0.048	0.472	0.043	0.718	-0.085	0.292	-0.237	0.044
BMI	0.507	<0.001	0.489	<0.001	-0.169	0.024	0.012	0.920	0.048	0.521	0.119	0.317	-0.060	0.422	-0.180	0.127
Glucose at 0	-0.016	0.838	0.025	0.846	0.105	0.162	-0.039	0.744	0.101	0.174	-0.203	0.087	0.041	0.583	-0. 067	0.576
Insulin at 0	0.165	0.034	0.347	0.005	0.119	0.124	0.090	0.455	0.044	0.565	0.206	0.083	-0.123	0.107	0.020	0.869
HOMA	0.125	0.111	0.313	0.015	0.145	0.061	0.035	0.779	0.051	0.508	0.114	0.361	-0.110	0.149	0.012	0.922
Triglycerides	-0.038	0.615	0.160	0.210	0.067	0.373	0.305	0.009	-0.125	0.093	-0.054	0.620	0.027	0.720	0.099	0.408
Total cholesterol	0.140	0.065	0.077	0.541	-0.012	0.870	0.097	0.420	-0.159	0.032	0.029	0.810	-0.028	0.708	0.043	0.140

Note: BMI. Body mass index

Table 6: Associations between adipokines and anthropometric and metabolic parameters in patients with and without FMR defined lipodystrophy

Correlation	Leptin				Resistin				PAI-1				TNF-α				Adiponectin		
	Without L		With L		Without L		With L		Without L		With L		Without L		With L		Without L		With
Between	R	P	R	p	R	p	r	p	r	p	R	p	r	p	r	P	R	p	r
Age	0.101	0.325	-0.014	0.909	-0.001	0.990	-0.102	0.385	-0.091	0.645	0.245	0.237	0.117	0.243	-0.189	0.109	0.037	0.713	-0.116
Duration of HIV-infection	-0.064	0.534	0.383	0.001	-0.012	0.911	0.234	0.045	0.175	0.374	-0.114	0.586	0.111	0.270	0.113	0.341	0.127	0.210	0.161
Duration of cART	-0.238	0.020	0.314	0.008	0.040	0.702	0.139	0.237	0.123	0.533	-0.162	0.440	0.045	0.657	0.054	0.650	0.041	0.687	0.209
Wais/hip ratio	0.115	0.268	0.147	0.224	0.052	0.616	0.024	0.839	0.103	0.604	0.138	0.509	0.149	0.139	-0.006	0.962	0.062	0.545	-0.193
Thigh circumf.	0.476	<0.001	-0.022	0.858	-0.174	0.092	-0.124	0.292	-0.004	0.985	0.059	0.778	-0.056	0.579	-0.020	0.867	0.070	0.551	0.070
Cervical circumf.	-0.058	0.613	-0.242	0.056	-0.019	0.871	-0.175	0.156	0.168	0.394	0.111	0.605	0.208	0.059	-0.027	0.829	-0.080	0.513	-0.080
Waist circumf.	0.530	<0.001	0.199	0.098	-0.114	0.269	0.031	0.791	-0.058	0.770	0.181	0.387	0.083	0.407	-0.011	0.927	-0.186	0.109	-0.186
Hip circumf.	0.654	<0.001	0.151	0.213	-0.198	0.054	-0.025	0.830	-0.065	0.742	0.059	0.779	-0.043	0.672	-0.035	0.770	-0.039	0.742	-0.039
Arm circumf.	0.471	<0.001	-0.057	0.660	-0.021	0.854	-0.208	0.097	-0.130	0.535	0.117	0.596	0.012	0.916	-0.208	0.094	-0.071	0.562	-0.071
CD4 cell count	-0.085	0.410	0.017	0.886	-0.011	0.913	-0.105	0.376	0.285	0.141	0.210	0.314	0.032	0.752	0.019	0.874	-0.085	0.401	-0.117
BMI	0.656	<0.001	0.167	0.166	-0.187	0.069	-0.091	0.441	-0.007	0.971	0.040	0.849	0.079	0.433	-0.019	0.872	-0.026	0.797	-0.129
Glucose at 0	0.072	0.485	-0.034	0.778	0.147	0.152	0.006	0.960	0.081	0.683	0.092	0.662	0.216	0.030	0.002	0.986	0.124	0.221	0.018
Glucose at 2h on OGTT	0.253	0.032	0.117	0.398	0.117	0.333	0.119	0.380	-0.204	0.361	-0.122	0.598	0.062	0.598	-0.024	0.862	-0.021	0.858	-0.060
Insulin at 0	0.173	0.104	0.312	0.009	0.050	0.644	0.224	0.057	-0.099	0.630	0.030	0.887	0.093	0.372	0.116	0.330	-0.077	0.464	-0.119
Insulin at 2h on OGTT	0.364	0.002	0.389	0.004	0.063	0.597	0.174	0.200	-0.213	0.328	-0.319	0.158	0.106	0.359	0.190	0.164	-0.082	0.457	-0.137
HOMA	0.176	0.098	0.267	0.027	0.081	0.453	0.252	0.031	-0.029	0.889	0.027	0.898	0.114	0.273	0.107	0.369	-0.064	0.539	-0.105
A1c	0.147	0.163	0.008	0.945	0.190	0.071	0.114	0.341	-0.338	0.078	-0.023	0.912	0.240	0.018	0.000	0.997	0.140	0.174	-0.015
Triglycerides	-0.004	0.053	0.034	0.782	0.055	0.596	0.170	0.147	-0.168	0.393	-0.184	0.379	-0.131	0.192	-0.167	0.157	0.078	0.445	-0.031
Total cholesterol	0.250	0.014	-0.011	0.925	-0.012	0.907	-0.063	0.593	-0.291	0.133	0.085	0.685	0.179	0.073	-0.168	0.157	-0.128	0.208	0.093
Total fat mass DXA %	0.877	<0.001	0.561	<0.001	-0.142	0.168	0.074	0.529	-0.054	0.784	-0.047	0.824	-0.069	0.490	-0.124	0.295	-0.167	0.098	-0.185

Trunk fat mass DXA %	0.858	<0.001	0.547	<0.001	-0.144	0.162	0.072	0.540	-0.051	0.796	0.001	0.996	-0.021	0.833	-0.102	0.390	-0.151	0.137	-0.198
Leg fat mass DXA%	0.825	<0.001	0.464	<0.001	-0.153	0.137	0.016	0.891	-0.064	0.745	-0.070	0.741	-0.085	0.400	-0.132	0.267	-0.147	0.147	-0.189
Arm fat mass DXA %	0.857	<0.001	0.650	<0.001	-0.115	0.265	0.077	0.512	-0.076	0.702	-0.187	0.371	-0.089	0.376	-0.095	0.423	-0.208	0.039	-0.058
Total fat mass in CT	0.774	<0.001	0.515	<0.001	-0.188	0.086	0.146	0.193	-0.068	0.731	0.073	0.728	0.125	0.238	-0.042	0.721	-0.078	0.467	-0.144
VAT in CT	0.483	<0.001	0.119	0.337	-0.175	0.109	0.139	0.248	0.053	0.787	0.409	0.042	0.050	0.635	-0.037	0.756	-0.071	0.507	-0.189
SAT in CT	0.829	<0.001	0.587	<0.001	-0.181	0.096	0.121	0.314	-0.102	0.605	-0.0139	0.507	0.084	0.427	-0.062	0.600	-0.090	0.400	-0.033
VAT/SAT ratio	-0.421	<0.001	-0.475	<0.001	0.099	0.366	-0.039	0.745	0.037	0.852	0.306	0.137	0.002	0.984	0.066	0.581	0.003	0.977	0.004

Note: L – lipodystrophy; cART – combined antiretroviral therapy; BMI. Body mass index; OGTT- oral glucose tolerance test; DXA - dual-energy X-ray absorptiometry CT – computed tomography; VAT- visceral adipose tissue; SAT – subcutaneous adipose tissue

PUBLICAÇÃO 11:

Carotid intima media thickness is associated with body fat abnormalities in HIV-infected patients

Title: Carotid intima media thickness is associated with body fat abnormalities in HIV-infected patients

Abbreviated title: cIMT and body fat in HIV-infected patients

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Abstract

Background: HIV-infected patients may be at increased risk of cardiovascular (CV) events, and lipodystrophy is generally associated with proatherogenic metabolic disturbances. The carotid intima-media thickness (cIMT) has been used as a surrogate marker for atherosclerosis and it has been shown to be an independent risk factor for CV disease. **Aims:** To evaluate the cIMT in HIV-infected patients on combined anti-retroviral therapy (cART) with and without lipodystrophy defined by fat mass ratio (L-FMR) and according to 4 categories of fat distribution (based on the presence or absence of lipoatrophy and abdominal prominence) and to determine the association of lipodystrophy with cIMT. **Methods:** Cross-sectional study of 199 HIV-infected patients. Body composition by DXA and abdominal CT, lipids, blood pressure, inflammatory markers, and cIMT by ultrasonography were performed. **Results:** L-FMR was present in 41.2 % patients. cIMT is higher in patients with L-FMR than patients without [0.81 (0.24) vs. 0.76 (0.25); p=0.037)]. Lipodystrophic patients had higher central fat mass (FM), lower peripheral FM and higher central/peripheral FM ratio. Patients with isolated central fat accumulation and with mixed forms of lipodystrophy had a higher cIMT. cIMT is associated with total and central FM and with central/peripheral FM ratio. No association was found between cIMT and the leg FM but patients with isolated peripheral lipoatrophy had a higher cIMT than patients with no lipodystrophy. C**onclusions:** Central fat accumulation had a major impact on cIMT in HIV cART treated patients. Nevertheless, despite lack of statistical significance, the role of isolated lipoatrophy should not be disregarded.

Keywords: lipodystrophy; HIV, carotid intima media thickness, fat mass ratio, body composition

Introduction

The development of atherosclerosis lesions is a slow and progressive process. The use of surrogate markers of atherosclerosis that are obtainable noninvasively may improve the prognostic stratification of HIV-infected patients with CV risk factors and may clarify some of the pending issues about this disease in HIV infection. Ultrasound is a safe, inexpensive and rapid technique, which allows an accurate evaluation of atherosclerosis (1). Intima-media thickness (IMT) of the carotid arteries has been used as a surrogate marker for atherosclerosis and it has been shown to be an independent risk factor for CV disease. The role of age, prolonged survival, traditional and non-traditional risk factors, direct effect of HIV infection and cART, in relation to surrogate markers of atherosclerosis are under continuing debate (1). Thus, due to the long term survival associated with cART, deleterious effects of the treatment and the chronic inflammatory state emerge. However, HIV itself may also promote atherosclerosis by activating the vascular endothelium directly or indirectly by systemic cytokine stimulation by the virus (2). The higher rates of sub-clinical atherosclerosis seen in HIV-1-infected patients, although controversial, are usually also attributed to classic CV risk factors and to side effects of cART (3-5).

Although the disease can cause alterations in several metabolic pathways, such as lipid and glucose metabolism, the lipodystrophic patients *per se* might also be at an even higher risk of atherosclerosis, since the fat redistribution is associated with presence of several known metabolic risk factors for CVD (6). It has been previously shown that patients with clinical lipodystrophy have a significantly higher risk of coronary heart disease at 10 years, measured by the Framingham risk score, than patients without clinical lipodystrophy and those with clinical lipodystrophy and with metabolic syndrome were more frequently in moderate and high risk categories than those without metabolic syndrome (7).

The aims of this study were to evaluate IMT in HIV-infected patients on cART with and without lipodystrophy, objectively defined by FMR, and in four groups of fat distribution, and to evaluate the association of lipodystrophy with cIMT.

Methods

Subjects

As part of a cross-sectional study, clinically stable HIV-infected non-institutionalized Caucasian adults receiving antiretroviral therapy, consecutively referred from the Infectious Diseases Outpatient Clinic, were evaluated. Only patients on cART were included. The study protocol was approved by the Hospital Ethics Committee for Health and each patient provided informed consent.

Clinical assessment

For each patient the following information was collected using a standardized protocol: age, gender, known duration of HIV infection and duration of cART exposure, HIV risk factor, characterization of the infection, smoking history (past, current, or never), family history of CV diseases, and use of anti-hypertensive, anti-diabetic, or lipid lowering drugs.

We used the "Centers for Disease Control and Prevention" (CDC) HIV staging classification (8). Weight, height, circumferences of neck, waist, hip, thigh and arm were measured as previously described (9) . All measurements were performed by the same observer using standard techniques (10).

Clinical lipoatrophy was defined as a peripheral lipoatrophy assessed by both patient and practitioner as previously described (9, 11). Presence of central fat accumulation or abdominal prominence was defined by the measurement of waist circumference using the IDF criteria for metabolic syndrome (12). The clinical assessment was performed by the same practitioner (PF). Resting blood pressure (BP) in a supine position was measured in a standardized fashion as previously described (7).

Each patient was categorized into one of 4 groups according to the presence or absence of either lipoatrophy or abdominal prominence: 1) **No lipodystrophy** - patients without clinical lipoatrophy and without abdominal prominence; 2) **Isolated central fat accumulation** - patients without clinical lipoatrophy and with abdominal prominence; 3) **Isolated lipoatrophy -** patients with clinical lipoatrophy and without abdominal prominence; 4) **Mixed forms of lipodystrophy** - patients with clinical lipoatrophy and with abdominal prominence.

Evaluation of body composition

Body composition was assessed with whole-body dual-energy X-ray absorptiometry (DXA) (DXA – Lunar Expert XL, 1999). DXA measurements were performed while the patient was in a supine position, with standard positioning of the arms and feet. Markers used in this study for trunk and lower limbs that defined regions of interest were those indicated by the manufacturer. Regional fat mass values were grouped and analyzed for the following anatomical regions: arms, legs, trunk and total body. The fat mass ratio (FMR) was calculated as the ratio between the percentage of the trunk fat mass and the percentage of the lower limb fat mass (FMR= % of the trunk fat mass/ % of the lower limb fat mass) (13). We used the cut-off value for lipodystrophy defined by FMR for men of 1.961 and for women of 1.329 (11).

The quantification of total, visceral, and peripheral fat was performed with a 64-slice abdominal computed tomography (CT) scanner (Siemens Sensation 64 Cardiac) with the same technique as previously described (14, 15). All values were expressed in cm^2 rounded to the nearest centesimal.

Laboratory analysis

Biological and inflammatory parameters: a venous blood sample was drawn after a 12-hour overnight fast. All the samples were analyzed at the central laboratory of our hospital. The measurements of total cholesterol (TC), low density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, apolipoprotein A1 (apo A1), apolipoprotein B (apo B), lipoprotein (a) [Lp (a)], fibrinogen, high sensitivity C-reactive protein (hsCRP), homocysteine, uric acid, lactates, NT-proBNP, glucose, insulin, A1c serum levels were determined using commercial kits. Non HDL-C was defined as TC-HDL. Microalbuminuria was determined in a 24 hour urine sample. Patients without a previous diagnosis of diabetes were submitted to an oral glucose tolerance test (OGTT). The test was performed as described by the World Health Organization (16).

The CD4+ cell count (x106 cell/L) was determined by flow cytometry and plasma HIV-1 RNA loads were measured by a quantitative reverse transcriptase polymerase chain reaction (Roche Diagnostic Systems, Inc., Branchburg, NJ, USA), which had a lower limit of detection of 50 copies/mL.

Measurements of insulin resistance

Insulin resistance was defined by the homeostasis model assessment of insulin resistance (HOMA) and insulin sensitivity by the quantitative insulin sensitivity check index (QUICKI). These indexes were calculated using the following formulas:

HOMA-IR index = (insulin 0 x glucose 0)/22.5 (17).

QUICKI = 1/(log [fasting insulin in mU/l] +log [fasting plasma glucose in mg/dL])(18).

Carotid IMT measurement

High-resolution B-mode and Doppler ultrasonography of the carotid arteries was performed on a Philips iU22 machine (Philips Medical, The Netherlands), equipped with a 17 - 5 MHz high-frequency linear-array transducer. Patients were examined in the supine position, with the head in a neutral position or slightly turned away from the side being scanned. The left and right common carotid arteries (CCA) were examined in multiple directions and measurements were performed using the images with the best wall definition. The IMT was measured 3 cm below the carotid bulb in a longitudinal image. Three measurements were obtained on each side and the mean IMT of these values was calculated. All studies were performed by a single radiologist with 12 years of experience in vascular ultrasound, and were carried out using the same machine.

The presence of subclinical carotid atherosclerosis was defined as IMT > 0.80 mm, presence of plaque, or both (19-21).

Statistical analysis

Data were described as mean and standard deviation (SD) for quantitative variables and compared using Student-t or Mann-Whitney tests as appropriate. Categorical variables were described as counts and proportions, and compared using the chi-square or Fisher's exact test. To study the association between cIMT and clinical and metabolic characteristics, Spearman correlation coefficients were estimated. Means of cIMT (adjusted for age and body fat distribution measured by central vs. peripheral fat ratio estimated by quantitative CT) were computed using generalized linear models.

Statistical analysis was performed using SPSS version 17.0 software (SPSS Inc., Chicago, Illinois, USA). All probabilities were two tailed and p values <0.05 were regarded as significant.

Results

Patient characteristics

In this sample of 199 HIV-1 infected patients (132 men and 67 women) on cART, 41.2 % patients presented lipodystrophy defined by FMR. Table 1 shows the characteristics of the study sample according to the presence of lipodystrophy defined by FMR. Patients with lipodystrophy were older, had longer duration of HIV infection and length of cART. There were no differences in weight, height, BMI, waist and arm circumference among patients with or without lipodystrophy. Patients with lipodystrophy had lower hip and thigh circumferences but higher neck circumference and a higher waist/hip circumference ratio than those without lipodystrophy. Patients with lipodystrophy had higher systolic blood pressure and leukocyte count than patients without lipodystrophy.

Patients with lipodystrophy had a significantly higher mean CD4+ cell count. No differences were observed between patients with and without lipodystrophy regarding viral suppression rate, HIV risk factor, CDC classification and ART regimens that were used, smoking history, family history of CV disease and medication history (statins, fibrates, oral anti-diabetics, insulin and anti-hypertension drugs).

Body composition by DXA

In the evaluation of body composition by DXA, patients with lipodystrophy had lesser total, leg and arm fat mass, both in % and Kg, than patients without lipodystrophy. No difference was found in trunk fat mass (in % and Kg) between the two groups.

Metabolic parameters

Patients with lipodystrophy had higher glucose and insulin at 0 minutes and insulin at 120 minutes on OGTT, HOMA, non-HDL cholesterol, triglycerides, uric acid, and lactates than patients without lipodystrophy. QUICKI index was lower in patients with lipodystrophy. No significant differences were found in the 2-hour value of glucose on OGTT, total cholesterol, LDL-cholesterol, HDL-cholesterol, apo A1 , apo B, ratio Apo B/Apo A1, Lp(a), homocysteine, CRP, hsCRP, NT-ProBNP, fibrinogen and microalbumin urinary excretion between the two groups.

Carotid IMT measurements

Carotid IMT was higher in patients with lipodystrophy than patients without lipodystrophy [mean (SD) 0.81 (0.24) vs. 0.76 (0.25); p=0.037)].

When patients were classified into 4 categories of fat distribution, cIMT mean was similar in patients with isolated central fat accumulation [0.80 (0.29)] and with mixed forms of lipodystrophy [0.80 (0.28)] and significantly higher than patients with no lipodystrophy [0.68 (0.15)] and isolated lipoatrophy [0.72 (0.17)] (p<0.001).

Carotid IMT correlated positively with age, waist/hip ratio, neck circumference, trunk fat mass evaluated by DXA, systolic blood pressure, glucose at 0 and 120 minutes on OGTT, A1c, non-HDL cholesterol, uric acid, CRP, hsCRP and homocysteine. No significant correlations were found between IMT and duration of HIV infection, length of cART, thigh circumference, total, leg and arm fat mass evaluated by DXA, CD4 cell count, leukocyte, insulin at 0 and 120 minutes on OGTT, HOMA, triglycerides, and lactates (Table 2).

Using generalized linear models, cIMT means were adjusted for age and body fat distribution measured by central vs. peripheral fat ratio estimated by quantitative CT. No significant differences remained after this adjustment when patients with and without lipodystrophy were compared. The adjusted mean of cIMT was 0.776 (95%CI: 0.739-0.812) in patients without lipodystrophy and 0.777 (95%CI: 0.734-0.820) in those with lipodystrophy (p=0.968).

Discussion

It was our aim to evaluate the association of lipodystrophy *per se* in cIMT. Prior studies evaluating the effect of lipodystrophy on cIMT have had conflicting results (22-24). We found that cIMT is higher in patients with lipodystrophy defined by FMR than in patients without lipodystrophy [0.81 (0.24) vs. 0.76 (0.25); p=0.037)]. Coll et al showed that the presence of lipodystrophy defined by clinical parameters increased by 3-fold the risk of sub-clinical carotid atherosclerosis in patients with HIV-infection (23). Mercié et al showed a significant association between cIMT and lipodystrophy in a univariate analysis of 161 HIV-1-infected patients with lipodystrophy (38.1% of the patients), but the effect disappeared in the multivariate model (22). Coll et al suggest that this discrepancy with his results may be attributed to the Mercié et al sample being a younger and healthier population, since the mean cIMT was 0.54 mm (range 0.50-0.60), while in the Coll´s samples the mean cIMT was 0.80 (range 0.40-1.80). In Coll's study the mean age of the patients with lipodystrophy was 42.38 (1.09) years while in Mercié's it was 43.7 (8.3) years. The mean age of Coll's patients without lipodystrophy was 40.59 (0.75), while in the Mercié et al study, it was 39.4 (8.7) years. Our patients either with lipodystrophy [49.2 (10.2) years] or without lipodystrophy [44.8 (12.1) years] were older. In our results the mean cIMT was similar to that found by Coll [with lipodystrophy cIMT 0.88 (0.04) vs. without lipodystrophy 0.76 (0.02) mm; p=0.007], despite our patients being older than those of Coll et al.

Visceral obesity is another independent risk factor for CVD in the background population (25) and independent associations of obesity and abdominal adiposity with cIMT were observed (26). However, the mechanisms by which the obesity is linked to early carotid atherosclerosis are not clearly established, even though the role of metabolic factors, such as insulin resistance and altered plasma adiponectin levels, has been proposed. Yet, body composition and fat distribution may influence systemic hemodynamics, both systemic blood pressure and total blood volume (27). When our patients were divided into 4 categories of fat distribution, those with abdominal prominence, independently of having clinical lipoatrophy (patients with isolated central fat accumulation and patients with mixed forms of lipodystrophy) had a higher cIMT. We also found that cIMT was positively correlated with waist/hip ratio, neck circumference, and trunk fat mass evaluated by DXA.

Insulin resistance has been independently associated with vascular disease and accelerated CV disease in HIV-infected adults (28). In our results, patients with lipodystrophy had higher glucose and insulin at 0 and 120 minutes on OGTT and HOMA than patients without lipodystrophy, and lower QUICKI without significant differences in glucose at 120 min on OGTT. Carotid IMT correlated positively with glucose at 0 and at 120 minutes on OGTT and A1c. Despite the higher insulin resistance evaluated by HOMA and lower insulin sensitivity evaluated by QUICKI, no significant correlations were found between cIMT and insulin at 0 and 120 minutes on OGTT and HOMA.

Some studies have demonstrated that traditional CV risk factors overshadow the role of HIV infection and/or cART and are the major determinants of cIMT progression (4, 29-32) and another has shown that HIV infection and/or cART were associated with increased cIMT independent of traditional CV risk factors (33), and yet another has pointed to the combination of all parameters (34). Older age is a traditional CV risk factor associated with higher mean cIMT and higher prevalence of carotid lesions (32, 35). A growing body of evidence indicates that cIMT below certain levels may not reflect atherosclerotic disease, but rather vascular aging and adaptive response to hemodynamic changes leading to alterations in shear and/or tensile stress (27, 36). We also found that cIMT correlated positively with age in our patients. In the Mercié study, cIMT was significantly associated with age and also with male gender, BMI, waist- to-hip ratio, systolic BP, total cholesterol, glucose disorders and homocysteine, smoking, alcohol consumption, lipodystrophy and cART. However, after adjustment for other CV risk factors the effects of lipodystrophy and cART disappeared, leading the authors to conclude that only conventional CV risk factors (age, BMI and smoking) are independently associated with increased cIMT in HIV-infected patients (22). In another study with 77 patients (23 with lipodystrophy) cIMT was significantly higher in HIV patients, but after adjustment for the presence of classical CV risk factors, no independent association was found between lipodystrophy and cIMT (24).

In addition, adverse lipid and other metabolic changes associated with HIV infection or cART exposure may be important pro-inflammatory factors (37). Plasma lipid alterations have also been associated with an increased cIMT (38). Increasing levels of HDL-C and apo A-I have been associated with a decreased cIMT, but this association lost significance on multivariate analysis (33). We also found that cIMT was positively correlated with non-

HDL cholesterol. Contrary to the literature on non-HIV patients (39), total cholesterol and blood pressure were not associated with IMT measures in the multivariate analysis model of Mercié et al. However, we found that cIMT was positively correlated with systolic blood pressure. Mercié proposed a possible explanation for this discrepancy: the mean age of their population was low (41 years). Like Mercié et al, we also observed an association between total plasma homocysteine and cIMT in HIV-infected patients on cART, as was demonstrated in non-infected patients (22).

It should be noted that certain "classical" vascular risk factors are over-represented in the HIV-infected population e.g. smoking. However, we observed no differences between patients with and without lipodystrophy regarding smoking history and so cannot ascribe the differences between the groups to smoking habits. Also, we observed no differences between patients with and without lipodystrophy regarding viral suppression rate, HIV risk factor, CDC classification and ART regimens that were used, family history of CV disease, and medications taken (oral anti-diabetic drugs, insulin and anti-hypertension drugs). Statins are known to have beneficial effects on the arterial wall (40) but we observed no differences in the taking of medications (statins and fibrates) between patients with or without lipodystrophy.

In HIV-negative patients, both leukocyte counts and CRP and hsCRP have been linked to endothelial dysfunction and future CV events (41, 42). Like Hsue, we found that hsCRP was associated with cIMT. However, in Hsue's study, after adjusting for all traditional risk factors, the association between hsCRP and cIMT was no long significant. In our results, no significant differences were found in CRP and hsCRP according to the presence of lipodystrophy, although lipodystrophic patients had higher leukocyte counts but no significant correlation was found between cIMT and leukocyte count. The hsCRP levels were not consistently associated with cIMT after adjusting for other factors (43). Mangili found that hsCRP independently predicted cIMT and that this correlation might become more evident with prolonged HIV disease and prolonged exposure to treatment (1). Van Wijk observed elevated blood leukocyte counts and elevated CRP levels in these patients. In HIV-infected patients, the endothelium could be activated either directly by HIV or by a leukocyte-mediated inflammatory cascade triggered by HIV infection (41).

Study limitations

The limitations of this study are mainly related to the observational design and cross-sectional nature of the current analyses. The results reported herein are only associations from which no causality conclusions can be drawn. Subclinical atherosclerosis evaluated by carotid IMT reflects the cumulative effects of risk factors acting over many years, whereas we measured risk factors at one time point only. Thus, blood lipid concentrations, blood pressure, and the other CV risks estimated at one time point may not accurately represent lifetime exposure. Use of cART, CD4 + T-cell count, HIV viral load, and CVD risk mediators are dynamic variables that change over time.

Another important point to be noted is that up to 38 % of the cIMT variability might be attributed to genetic conditions, and those were not studied here.

Some aspects of the present study should be highlighted.

To evaluate body composition we used objective methods: the presence of lipodystrophy was defined by FMR by DXA and visceral fat by CT scan.

Conclusions

Patients with lipodystrophy had a higher central fat mass, a lower peripheral fat mass and a higher central/peripheral fat mass, and a higher cIMT. cIMT is associated with total and central fat mass and with the ratio central/peripheral fat. Patients with abdominal prominence, independently of having clinical lipoatrophy (patients with isolated central fat accumulation and patients with mixed forms of lipodystrophy) had a higher cIMT, but patients with isolated peripheral lipoatrophy had a higher cIMT than patients with no lipodystrophy. Thus, it seems that central fat accumulation had a major impact on cIMT but the role of isolated lipoatrophy cannot be disregarded.

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Transparency declarations

None to declare

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Table 1: Sample characteristics according to the presence of lipodystrophy defined by FMR

	Without L	With L	*P*
n (%)	117	82	-
Gender [n(%)]			
Male	71 (60.7)	61 (74.4)	0.063
Female	46 (39.3)	21 (25.6)	
Age [years, mean (sd)]	44.8 (12.1)	49.2 (10.2)	0.002
Duration of HIV infection [years, mean (sd)]	7.2 (3.8)	8.5 (3.5)	0.005
cART [years, mean (sd)]	5.7 (3.7)	7.9 (3.3)	<0.001
Weight [Kg, mean (sd)]	68.0 (14.3)	68.0 (10.6)	0.805
Height [m, mean (sd)]	1.64 (0.09)	1.64 (0.08)	0.518
BMI [(Kg/m^2), mean (sd)]	25.2 (5.0)	25.3 (4.0)	0.637
Waist circumference [cm, mean (sd)]	90.6 (12.7)	92.6 (10.2)	0.174
Hip circumference [cm, mean (sd)]	96.1 (9.4)	92.5 (6.4)	0.005
Thigh circumference [cm, mean (sd)]	48.5 (5.5)	47.0 (4.9)	0.025
Arm circumference [cm, mean (sd)]	26.7 (3.2)	27.2 (2.6)	0.285
Neck circumference [cm, mean (sd)]	36.6 (3.8)	37.8 (3.8)	0.045
Waist/ hip circumference ratio [mean (sd)]	0.94 (0.09)	1.00 (0.07)	<0.001
CD4 cell count [cells/mm3, mean (sd)]	502.7 (265.0)	631.8 (340.8)	0.006
HIV RNA (<50) [n (%)]	102 (87.2)	74 (90.2)	0.660
HIV risk factor [n (%)]			
Injecting drug user	32 (27.8)	15 (18.3)	0.050
Homosexual contact	7 (6.1)	14 (17.1)	
Heterosexual contact	74 (64.3)	51 (62.2)	
Others	2 (1.7)	2 (2.4)	
CDC [n (%)]			
A	63 (54.3)	53 (64.6)	0.209
B	1 (0.9)	0 (0)	
C	52 (44.8)	29 (35.4)	
ART [n (%)]			
PI	59 (51.3)	48 (58.5)	0.390
NNRTI	58 (50.4)	38 (46.3)	0.673
NRTI	112 (97.4)	79 (96.3)	0.695

Smoking history [n (%)]			
Never	44 (37.9)	36 (43.9)	
Current	55 (47.4)	27 (32.9)	
Former	17 (14.7)	19 (23.2)	0.092
Familial history of CVD [n (%)]	51 (43.6)	40 (48.8)	0.563
Taking medications [n (%)]			
Statins	22 (19.0)	25 (30.5)	0.088
Fibrates	39 (33.6)	39 (47.6)	0.067
Oral anti-diabetics	13 (11.2)	16 (19.5)	0.154
Insulin	6 (5.2)	4 (4.9)	1.000
Anti-hypertensive drugs	21 (17.9)	19 (23.5)	0.442
Fat mass [%, mean (sd)] DXA			
Total	24.8 (13.1)	19.3 (8.5)	0.003
Trunk	25.7 (13.3)	24.3 (9.0)	0.446
Leg	24.4 (14.7)	11.1 (7.4)	<0.001
Arm	27.2 (17.1)	20.3 (13.6)	0.007
Fat mass [Kg, mean (sd)] DXA			
Total	17.3 (11.1)	13.2 (7.3)	0.013
Trunk	9.3 (6.0)	9.0 (4.8)	0.918
Leg	5.2 (3.6)	2.1 (1.6)	<0.001
Arm	2.1 (1.9)	1.5 (1.2)	0.019
Body Fat Mass by Quantitative CT			
Total fat [cm^2, mean (sd)]	282.2 (174.5)	276.6 (124.0)	0.865
Central fat [cm^2, mean (sd)]	111.5 (85.8)	158.2 (63.2)	<0.001
Peripheral fat [cm^2, mean (sd)]	170.8 (125.7)	118.3 (93.1)	0.002
Central/peripheral Fat [cm^2, mean (sd)]	1.08 (1.46)	2.43 (2.62)	<0.001
Systolic blood pressure [mmHg, mean (sd)]	120.0 (18.9)	124.6 (15.8)	0.039
Diastolic blood pressure [mmHg, mean (sd)]	75.8 (11.9)	78.0 (10.6)	0.139
Leukocytes [10^9/L, mean (sd)]	5.66 (1.67)	6.39 (1.88)	0.007

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Glucose 0 min [mg/dL, mean (sd)]	104.4 (45.1)	114.7 (44.2)	0.003
Glucose 2 hours [mg/dL, mean (sd)]	123.6 (42.9)	139.2 (50.3)	0.051
Insulin 0 min [μU/mL, mean (sd)]	9,49 (10.28)	13.00 (10.68)	0.002
Insulin 2 hours [μU/mL, mean (sd)]	52.43 (46.65)	91.32 (147,24)	0.007
HOMA [mean (sd)]	2,54 (2.90)	3.73 (3.31)	<0.001
QUICKI [mean (sd)]	0.38 (0.10)	0.34 (0.04)	<0.001
A1c [% mean (sd)]	5.50 (1.06)	5.77 (0.06)	0.001
Total cholesterol [mg/dL, mean (sd)]	218.6 (53.2)	233.6 (61.0)	0.093
LDL-cholesterol [mg/dL, mean (sd)]	126.1 (47.1)	132.3 (50.7)	0.461
HDL-cholesterol [mg/dL, mean (sd)]	47.0 (14.4)	44.3 (12.0)	0.262
Non-HDL cholesterol [mg/dL, mean (sd)]	171.6 (48.9)	189.3 (56.5)	0.019
Triglycerides[mg/dL, mean (sd)]	247.5 (170.9)	307.2 (198.5)	0.006
Apo A1 [mg/dL, mean (sd)]	118.8 (25.8)	114.3 (18.3)	0.204
Apo B [mg/dL, mean (sd)]	104.7 (26.7)	106.1 (25.4)	0.827
Ratio apo B/apo A1 [mean (sd)]	0.91 (0.27)	0.95 (0.25)	0.297
Lp(a) [mg/dL, mean (sd)]	30.2 (31.2)	31.1 (39.7)	0.421
Homocysteine [μmol/L, mean (sd)]	9.6 (4.1)	9.8 (3.7)	0.435
CRP [mg/L, mean (sd)]	5.4 (12.9)	4.0 (4.2)	0.913
hsCRP [mg/dL, mean (sd)]	0.48 (0.91)	0.37 (0.28)	0.185
Lactates [mmol/L, mean (sd)]	1.12 (0.47)	1.36 (0.58)	0.001
NT-ProBNP [pg/mL, mean (sd)]	39.1 (67.3)	28.6 (27.1)	0.414
Fibrinogen [mg/dL, mean (sd)]	344.8 (98.3)	336.2 (92.1)	0.463
Microalbumin [mg/L, mean (sd)]	49.1 (150.6)	26.0 (67.7)	0.435
Uric acid [mg/L, mean (sd)]	44.4 (15.2)	51.2 (15.4)	<0.001
Carotid IMT [mm, mean (sd)]	0.76 (0.25)	0.81 (0.24)	0.037

(L- Lipodystrophy FMR defined; CDC - Centers for Disease Control and Prevention criteria for staging of HIV infection; cART- combined antiretroviral therapy; BMI- body mass index; FMR – fat mass ratio; CVD – cardiovascular disease; DXA – dual-energy X-ray absorptiometry; CT – computed tomography; PI – protease inhibitor; NNRTI –non-nucleoside reverse transcriptase inhibitor; NRTI – nucleoside reverse transcriptase inhibitor, IMT – intima-media thickness).

Table 2: Correlations between cIMT and body composition and metabolic parameters.

Correlations between cIMT and	R	P
Age	0.709	<0.001
Duration of HIV infection	-0.019	0.779
cART	0.024	0.727
Waist/ hip ratio	0.387	<0.001
Thigh circumference	-0.095	0.169
Neck circumference	0.202	0.007
CD4 cell count	-0.027	0.701
Systolic blood pressure	0.419	<0.001
Leukocytes	-0.049	0.478
Glucose 0 min	0.284	<0.001
Glucose 2 hours OGTT	0.220	0.006
Insulin 0 min	0.026	0.711
Insulin 2 hours OGTT	-0.089	0.271
HOMA	0.094	0.185
A1c	0.285	<0.001
Triglycerides	0.109	0.113
Non-HDL cholesterol	0.254	<0.001
CRP	0.196	0.005
hsCRP	0.334	0.003
Homocysteine	0.150	0.032
Uric acid	0.146	0.034
Lactates	0.081	0.253
Fat mass Total	0.084	0.236
Trunk	0.168	0.018
Leg	-0.037	0.604
Arm	0.029	0.686
Total fat on abdominal CT scan	0.218	0.002

Central fat on abdominal CT scan	0.418	<0.001
Peripheral fat on abdominal CT scan	-0.009	0.901
Central/peripheral fat ratio on abdominal CT scan	0.290	<0.001

(IMT – intima-media thickness; cART – combined antiretroviral therapy; CDC - Centers for Disease Control and Prevention criteria for staging of HIV infection; cART – combined antiretroviral therapy; OGTT – oral glucose tolerance test; HOMA – the homeostasis model assessment of insulin resistance; HDL-high density cholesterol; CPR –C protein reactive; CT – computed tomography)

6. DISCUSSÃO

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A more detailed discussion is made in each of the articles. In this section a more general discussion of the results is proposed.

Human lipodystrophies are a heterogeneous group of diseases with generalized or partial fat loss, with rare genetic etiology or more frequent acquired forms, old or more recently described as for example the lipodystrophy related with retrovirus [1]. We focused on the most recent - the lipodystrophy associated to HIV-infection. Lipodystrophy is a general term used to describe various features and degrees of fat redistribution, including lipoatrophy and lipohypertrophy, in different body regions [2-5]. Some patients present mainly lipoatrophy, others mixed form of lipoatrophy and trunk/abdominal fat accumulation while others have isolated trunk/ abdominal fat accumulation, and the global picture of the syndrome seems to represent a clinical continuum [6]. Different methodologies have been used to assess the prevalence of this syndrome: patient self-report, clinical evaluation and a complex case report definition. This last definition attempted to develop an objective case definition of lipodystrophy, including age, sex, duration of HIV infection, HIV disease stage, waist to hip ratio, anion gap, serum high-density lipoprotein (HDL) cholesterol concentration, trunk to peripheral fat ratio, percentage leg fat, and intra- to extra-abdominal fat ratio [7]. However, the model is complex and with limited clinical usefulness. Therefore, the need for a simple, evidence-based, and validated definition of lipodystrophy was experienced by researchers working in this area. We proposed a ratio of trunk fat mass evaluated by DXA with different thresholds for men and women, because it was our believe that gender differences in body composition are present either in the general population or in patients with or without lipodystrophy. Our proposed threshold values enabled a more precise identification of lipodystrophic patients. FMR is an objective tool to define HIV-related lipodystrophy, allowing a more clinically useful definition for identification of patients with abnormal fat distribution. Use of gender-specific FMR thresholds as a diagnostic tool in metabolic studies of HIV-infected patients would enable the comparison of different studies and should be useful to monitor lipodystrophy progression in this population [8].

Bioelectrical impedance (BIA) was an already established method to monitor nutritional status in HIV infected patients before the introduction of cART, when patients had almost exclusively the wasting syndrome with sarcopenia, mainly characterized by losses of both fat and free fat mass (before the description of lipodystrophy syndrome) [9]. The results of BIA demonstrated similar fat-free mass along the fat distribution spectrum and describing a precise body composition of the patients and enables exclude sarcopenia. BIA, an objective way to evaluate body fat, also showed an increase in fat mass in patients with isolated central fat accumulation and mixed forms of lipodystrophy. Patients with lipoatrophy and no lipodystrophy presented lower fat mass, being this picture of body composition impossible to show using more traditional anthropometric measures [10]. In a clinical setting, BIA is an easy and useful tool to evaluate fat mass and the amount of fat-free mass. BIA is operator-independent, repeatable, non-invasive, widely available method of body composition evaluation that requires less training than anthropometry and is less expensive than DXA [11]. BIA may also be a useful method in the evaluation of clinical lipodystrophy (CL) evolution [12].

Despite cART has changed the course of HIV infection, leading to a significant reduction in AIDS related morbidity and mortality [13], it was associated with changes in fat distribution (lipodystrophy) and metabolic abnormalities, including insulin resistance (IR), dyslipidemia, and increased blood pressure (BP) [14, 15]. The metabolic syndrome (MS), a common condition in the general population, refers to a constellation of cardiovascular disease (CVD) risk factors, including increased waist circumference (WC), glucose and lipid metabolism disorders, and hypertension [16, 17]. Each of these risk factors, individually, increase the risk of cardiovascular disease, but MS itself has shown to be a powerful independent risk factor for cardiovascular morbidity and mortality and for type 2 diabetes [18]. It is still controversial if the MS improves the identification of high risk individuals [19-21].

To our knowledge, our paper "Impact of lipodystrophy on the prevalence and components of metabolic syndrome in HIV-infected patients" was the first study conducted in HIV-infected patients focusing specifically on the role of subcutaneous adipose tissue atrophy defined by presence of clinical lipodystrophy (CL) on the prevalence of the MS. Another particularity is that all patients were on cART. Other studies analyzed the presence of the MS in naïve or on cART HIV-infected patients, with sub-analyses concerning the presence of CL in patients with the MS [22-24]. In men, high blood pressure and MS were more frequent in those with isolated central fat accumulation and mixed forms of lipodystrophy (ATPIII and IDF criteria) and high fas-

ting glucose levels were significantly more frequent in same two groups using ATPIII criteria. In women, hypertriglyceridemia, low C-HDL levels, high WC and presence of MS were more frequent in those with isolated central fat accumulation and mixed forms of lipodystrophy using either ATPIII or IDF criteria. The observation that patients with CL had significantly higher 10 year-risk of coronary heart disease , measured by the Framingham risk score, than patients without CL and that those with CL and with MS had higher frequencies of moderate and high risk categories than those without MS emphasizes the importance of the clinical diagnosis of this condition [25]. Our data showed that among HIV patients on cART, the prevalence of MS may be remarkably high, especially when taking into account the mean age of our sample and the observation that patients with CL had a significantly higher 10 year-risk of coronary heart disease, measured by the Framingham risk score.

Another factor that can contribute to the increased CV risk in HIV-infected patients is obesity [26-28]. This condition is associated with a chronic, systemic state of inflammation that may contribute to CV diseases and other obesity-related co-morbidities [29-31]. Although, lipodystrophy in HIV-infected patients was first reported more than 10 years ago, the available information on obesity in HIV-infected patients is still scarce [14].

In fact, recent studies have shown that being overweight or obese is more common than wasting in these patients, and this may be partly justified by the increased survival rate in HIV patients and by the weight gain that occurs with ageing, as it happens in the general population [26-28, 32, 33]. Our data also showed that, regardless of gender, being of normal weight, overweight or obese is more common than being underweight in these HIV-infected patients. As patients with HIV live longer and the infection has been relegated to a chronic condition, HIV patients may be now facing similar problems to the general population, including excessive weight gain due to poor lifestyle habits [26, 34]. But, more important than the obesity diagnose, is the assessment of the fat distribution. The best way to quantify the visceral adipose tissue (VAT) is through CT or MRI scanning. In our patients, those with CL, despite having a similar VAT and lower total and subcutaneous adipose tissue (SAT) at abdominal level, had a higher WHR (wais/hip ratio) and a higher VAT/SAT ratio by CT. The presence of a similar VAT and a higher VAT/SAT ratio resulted from a lower SAT. The same phenomenon was observed with WHR because they had a decrease in SAT that was predominantly adipose tissue in the hip. When we analyzed the data according to the 4 groups of fat redistribution, patients with isolated lipoatrophy had a higher VAT/SAT fat ratio indicating not only an increase in visceral adipose tissue, but also

lower subcutaneous fat. These patients with low SAT and VAT had a similar fat distribution to patients with Berardinelli-Seip congenital generalized lipodystrophy [35]. One of the strengths of our study is that it addressed the prevalence of obesity according to the presence of CL and this has not been reported previously. Despite having a lower prevalence of overweight or obesity when compared to patients without lipodystrophy, lipodystrophic patients had an increased proportion of VAT, as reflected by the increased VAT/SAT fat ratio determined by CT at abdominal level. This probably suggests that these patients have a comparable risk of metabolic and cardiovascular complications, in spite of the actual frequency of obesity being lower.

There are few and discrepant studies on hypertension in HIV-infected patients and scarce information is available on hypertension in HIV-infected patients according to the presence of lipodystrophy [36-39]. There is a perception that cART influence on the prevalence of hypertension appears to be minor. Bergersen argued that the most probable explanation for these findings was that HIV-positive patients usually have lower BMI, thus having a lower blood pressure than the general population [40].

In our study, hypertension was not associated with lipodystrophy: only in men, with lipoatrophy presented an increased prevalence of hypertension when compared to those without any fat distribution abnormality, when lipodystrophy was clinically defined. Gazzaruso stated that the strong association of lipodystrophy with hypertension appears to be of interest for two reasons. Firstly, it confirms that IR could be involved in the development of both lipodystrophy and hypertension. Secondly, this association suggests that the presence or the development of lipodystrophy in an HIV-infected patient should be a precise indication to check for hypertension. They concluded that hypertension was frequent in HIV-infected patients on cART and that hypertension appears to be linked to IR [39]. In our patients, after adjustment for age, gender, smoking history and BMI, hypertension was not associated either with IR or the presence of clinically or FMR-defined lipodystrophy. Also, total abdominal, central and peripheral fat mass were not associated with hypertension. On the other hand, central/peripheral fat mass ratio evaluated by CT scan was significantly associated with hypertension, even after adjustment for the above mentioned variables. Total body, arm and trunk fat mass, assessed by DXA, were not associated with hypertension, independent of age, gender and smoking history. However, patients with isolated lipoatrophy (patients with lipoatrophy and without abdominal prominence) presented more frequently absence of hypertension and the presence of hypertension was more prevalent in patients with isolated central fat accumulation and mixed forms of lipodystrophy (patients with abdominal prominence regardless of the presence or ab-

sence of lipoatrophy). This could mean that abdominal prominence, as in the general population, is more important in determining hypertension than lipoatrophy and may in fact, overcome the effect of lipoatrophy. This could also help in the explanation of the apparent paradox of lipodystrophy being associated with insulin resistance, insulin resistance being associated with hypertension, but hypertension not being associated with lipodystrophy. In summary, hypertension was not associated with IR, lipodystrophy or fat mass (total abdominal, central and peripheral), but was associated with central/peripheral fat mass ratio.

Combined antiretroviral therapy in the treatment of HIV-1 infection has been associated with other complications, including IR and diabetes. In our population, no differences were observed between IR or categorization of the glucose homeostasis according to clinical lipodystrophy classification, in spite of the well-established association between visceral fat and IR. Also, when patients were classified according to 4 categories of fat distribution by clinical lipoatrophy and waist circumference, again no differences were found. When patients were classified as being lipodystrophic or not according to FMR, we observed that patients with lipodystrophy had higher IR (higher HOMA and lower QUICKI and Matsuda values). They also had higher fasting plasma glucose, insulin and A1C levels, and higher % of IFG, IGT and DM. When we divided the patients into 4 categories of body fat distribution by using FMR-defined lipodystrophy and waist circumference, those patients with lipodystrophy and abdominal prominence had a higher prevalence of DM and IGT. Patients without FMR-defined lipodystrophy but with abdominal prominence only had a high prevalence of IGT. It seems that the loss of peripheral adipose tissue is less important than the presence of abdominal prominence in the occurrence of IR, but we cannot completely rule out its role, since patients with only abdominal prominence and without lipodystrophy, defined by FMR, had less marked glucose disturbances i.e. they only had increased prevalence of IGT. As previously proposed by Bonnet, the discrepancy observed between the results using the 2 different lipodystrophy definitions, could be the result from the higher accuracy of the objective method in reporting small losses of peripheral adipose tissue that may have been undetected by clinical inspection [41]. Significant associations between IR and total fat, central fat and central/peripheral fat ratio and no association with peripheral fat at abdominal level evaluated by CT were observed, emphasizing the contribution of the central fat mass to IR. We also found an association between IR and total and trunk fat evaluated by DXA. As in our results, De Wit et al, showed that clinical lipodystrophy was significantly associated with new-onset diabetes and the abnormal body fat distribution in HIV-positive indivi-

duals is strongly associated with IR and/or glucose intolerance, with excess trunk or visceral fat being, as in the general population, a risk factor for IR in patients with HIV infection. In addition, De Wit observed that IR is itself was independently associated with fat loss in HIV-infected individuals [42].

In our sample, no association was observed between peripheral fat mass with IR. This result is contrary to the De Wit study, where patients had a marked peripheral adipose tissue loss that was clinically evident. Also, our patients had less evident low fat mass, detected only by FMR. In fact, according to De Wit statement, information on lipodystrophy was rarely collected since the study was carried out in 11 centers, which suggests that only marked lipodystrophies were reported. In our study, FMR as a continuum was significantly associated with IR evaluated by HOMA, even after adjustment for gender, age, IP, NRTI, CD4 cell count and HIV RNA viral load. In conclusion, insulin resistance and glucose homeostasis abnormalities significantly increased with increasing FMR. Abdominal prominence, an important factor impacting the FMR, expressed by abdominal circumference, waist/hip ratio, visceral and trunk fat defined by CT, was also associated with glucose homeostasis disturbances.

In addition, being associated with lipodystrophy, cART is also related to lipid abnormalities and cardiovascular disease. And, there is a suggestion that emerging non-traditional risk factors may predispose HIV-infected patients to accelerated atherosclerosis. Apolipoprotein A1, apolipoprotein B, lipoprotein (a) [Lp (a)], fibrinogen, high sensitivity C-reactive protein (hs-CRP), homocysteine, microalbuminuria and NT-proBNP serum levels also have been associated with increased cardiovascular risk and some of them are related to inflammation [43-58]. In our cohort, patients with CL had lower total cholesterol, LDL-C, HDL-C, Apo A1 and higher mean levels of triglycerides. Patients with lipodystrophy defined by FMR had higher triglycerides and lower Lp(a). When patients were classified according to the four groups of fat distribution, patients with abdominal prominence, independent of the presence of lipoatrophy, had higher mean levels of total cholesterol, LDL-C, and Apo B. Low Apo A1 is a known risk indicator of coronary atherosclerosis [59] and we found the lowest Apo A1 plasma levels in patients with isolated lipoatrophy and mixed forms of lipodystrophy. Significant positive linear associations were observed between FMR and triglycerides and Apo B/Apo A ratio. These associations remained significant after adjustment for age, gender, PI and NRTI. Apo A1 had a significant negative linear association with FMR that remained significant after adjustment for gender and age, but it lost significance after adjustment for cART. The emerging cardiovascular risk factors did not vary according to fat redistribution. In HIV infected patients, the 2 components of body fat

redistribution syndrome seemed to induce different deleterious effects: central fat accumulation induced increased total cholesterol, LDL cholesterol and Apo B; and isolated lipoatrophy induced low Apo A1 and high triglycerides. In the analysis of the components of adipose tissue redistribution syndrome, abdominal prominence seems to be more important than lipoatrophy in this association.

Lipodystrophies are characterized by redistribution of adipose tissue, IR, and metabolic complications and adipokines may play an important role as an intermediary, linking these alterations. Adipose tissue has emerged as a metabolically active endocrine organ secreting multiple bioactive peptides, collectively called "adipokines" and other proteins including inflammatory mediators such TNF-α and PAI-1 [60]. Ghrelin, an orexigenic gut peptide, is a marker of metabolic dysregulation and altered body fat distribution [61]. Our aim was to evaluate the relationship between adipocyte derived hormones (adiponectin, leptin, resistin, TNF-α), orexigenic hormones (ghrelin), protrombotic factors (PAI-1) and IR in HIV-infected patients on cART according to the presence of Fat Mass Ratio defined lipodystrophy (FMR-L) and four categories of fat distribution. In our data, leptin levels were lower in patients with lipodystrophy, indicating its close relationship with total body fat. A significant positive correlation of leptin with HOMA in patients with lipodystrophy demonstrated the role of this adipokine in insulin sensitivity. Adiponectin was lower in patients with isolated central fat accumulation and negatively correlated with visceral adipose tissue. The adiponectin/leptin ratio was lower in patients with isolated central fat accumulation. Patients with mixed forms of lipodystrophy had higher levels of PAI-1. No significantly differences were found in resistin, PAI-1 and ghrelin levels in patients with or without lipodystrophy defined by FMR.

HIV-infected patients may be at increased risk of cardiovascular (CV) events, and lipodystrophy is generally associated with proatherogenic metabolic disturbances. The carotid intima-media thickness (cIMT) has been used as a surrogate marker for atherosclerosis and it has been shown to be an independent risk factor for CV disease [62]. The role of age, prolonged survival, traditional and non-traditional risk factors, direct effect of HIV infection and cART, in relation to surrogate markers of atherosclerosis are under continuing debate [62]. Thus, due to the long term survival associated with cART, deleterious effects of the treatment and the chronic inflammatory state emerge [63]. Although the disease can cause alterations in several metabolic pathways, such as lipid and glucose metabolism, as we demonstrated, the lipodystrophic patients *per se* might also be at a higher risk of atherosclerosis, since the fat redistribution is associated with presence of several known metabolic risk factors for

CVD [64]. Previously, we demonstrated that patients with clinical lipodystrophy have a significantly higher risk of coronary heart disease at 10 years, measured by the Framingham risk score, than patients without clinical lipodystrophy and those with clinical lipodystrophy and with metabolic syndrome had higher frequencies of moderate and high risk categories than those without metabolic syndrome [65]. Also, we demonstrated that carotid IMT was higher in patients with lipodystrophy than patients without lipodystrophy. Patients with lipodystrophy had a higher central fat mass, a lower peripheral fat mass and a higher central/peripheral fat mass. cIMT is associated with total and central fat mass and with the ratio central/peripheral fat. Patients with abdominal prominence, independently of having clinical lipoatrophy (patients with isolated central fat accumulation and patients with mixed forms of lipodystrophy) had a higher mean cIMT. Nevertheless, patients with isolated peripheral lipoatrophy had a higher cIMT when compared to patients with no lipodystrophy. It was concluded that central fat accumulation had a major impact on cIMT in HIV cART treated patients, however, despite lack of statistical significance, the role of isolated lipoatrophy should not be disregarded.

7. CONCLUSÕES

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HIV-infected patients can have different patterns of body composition with several fat distribution spectrums, but with similar fat-free mass (excluding the presence of sarcopenia). Some patients had mainly lipoatrophy, others had increased trunk and abdominal fat accumulation, and a subset of patients had mixed forms of lipodystrophy. Lipodystrophic patients had an increased proportion of VAT, as reflected by the increased VAT/SAT fat ratio determined by CT at abdominal level. This probably suggests that these patients have a comparable risk of metabolic and cardiovascular complications, in spite of the actual frequency of obesity being lower in patients with lipodystrophy.

In this sample of HIV-infected patients, hypertension was not associated with IR, lipodystrophy or fat mass (total abdominal, central and peripheral), but was associated with central/peripheral fat mass ratio.

IR and glucose homeostasis abnormalities significantly increased with increasing FMR. Abdominal prominence, an important factor impacting the FMR, expressed by abdominal circumference, waist/hip ratio, visceral and trunk fat defined by CT, was also associated with glucose homeostasis disturbances. Also, it seems that lost of peripheral adipose tissue was less important than the presence of abdominal prominence in the occurrence of IR, but one cannot completely disregard its role, since patients with isolated abdominal prominence and without lipodystrophy, defined by FMR, had less marked glucose disturbances i.e. they only had increased prevalence of IGT.

Again, in HIV infected patients, the 2 components of body fat redistribution syndrome seemed to induce different deleterious effects: central fat accumulation induced increased total cholesterol, LDL cholesterol and Apo B; and isolated lipoatrophy induced low Apo A1 and high triglycerides levels. In the analysis of the components of adipose tissue redistribution syndrome, abdominal prominence seems to be more important than lipoatrophy in this association.

In what concern adipokines and body composition hormones, leptin levels were lower in patients with lipodystrophy, indicating its close relationship with total body fat. A significant positive correlation of leptin with HOMA in patients with lipodystrophy could add to the importance of this adipokine in insulin sensitivity. Adiponectin

was lower in patients with isolated central fat accumulation and negatively correlated with visceral adipose tissue. The adiponectin/leptin ratio was lower in patients with isolated central fat accumulation. Patients with mixed forms of lipodystrophy had higher levels of PAI-1. No significantly differences were observed in resistin, PAI-1 and ghrelin levels in patients with or without lipodystrophy defined by FMR. The HIV redistribution syndrome, with different degrees of peripheral fat atrophy and central fat accumulation, had a complex relationship with adipokines, body composition related hormones and IR.

Patients with CL had a significantly higher risk of coronary heart disease at 10 years, measured by the Framingham risk score, than patients without CL. Patients with CL and with MS were more frequently classified in the moderate and high risk categories than those with CL but without MS.

Carotid IMT, a surrogate marker for atherosclerosis, was higher in patients with lipodystrophy than patients without lipodystrophy. cIMT was associated with total and central fat mass and with the ratio central/peripheral fat. Patients with abdominal prominence, independently of having clinical lipoatrophy had a higher cIMT, but patients with isolated peripheral lipoatrophy had a higher cIMT than patients with no lipodystrophy. Central fat accumulation had a major impact on cIMT in HIV cART treated patients; nevertheless, the role of isolated lipoatrophy should not be disregarded.

In conclusion, lipodystrophy had a greatest impact in metabolic and cardiovascular abnormalities in these HIV-infected patients, being the patients with higher central/peripheral fat ratio (patients with central fat accumulation and mixed forms of lipodystrophy) those with more alterations, however, one should not overlook the role of peripheral lipoatrophy, as a contributor to these changes.

8. RESUMO/SUMMARY

8. RESUMO

A infeção VIH e a terapêutica antirretrovírica combinada (TARc) têm sido associadas a alterações morfológicas (lipodistrofia), metabólicas e endócrinas que podem estar ligadas a aumento do risco cardiovascular. Os doentes com lipodistrofia têm vários graus de redistribuição da gordura, incluindo lipoatrofia, lipohipertrofia e formas mistas de lipodistrofia. O nosso objetivo foi estudar a composição corporal, as alterações metabólicas e endócrinas e a doença cardiovascular de acordo com a presença de lipodistrofia. Conduzimos vários estudos clínicos observacionais, em doente infetados pelo VIH e sob TARc, de forma a responder a estas questões.

Apesar das diferentes metodologias utilizadas previamente na definição de lipodistrofia, havia a necessidade de um método de definição que fosse simples, objetivo e especifico para o género. Utilizando a "razão massa gorda tronco/membros" (RMGTM = % de gordura do tronco/% gordura dos membros inferiores avaliada por densitometria), definimos novos pontos de corte, tendo sido pela primeira vez definidos pontos de corte específicos para o género feminino.

A avaliação da composição corporal através de impedância bioelétrica (BIA) permite-nos ter um quadro da composição corporal que não é possível apenas com a antropometria. Os resultados da BIA demonstram que nestes doente, a massa não gorda é semelhante nos vários tipos de redistribuição da gordura, que há ausência de sarcopenia e que existe um aumento da massa gorda nos doentes com acumulação isolada da gordura central e nas formas mistas de lipodistrofia. Independentemente do género, ter peso normal, sobrecarga ponderal ou obesidade é mais comum do que ter peso baixo nos nossos doentes infetados pelo VIH. Apesar dos doentes com lipodistrofia terem uma menor prevalência de sobrecarga ponderal ou obesidade, estes têm um aumento do tecido adiposo visceral, tal como refletido no aumento da razão do tecido adiposo visceral/tecido adiposo subcutâneo determinado por tomografia computadorizada (TC) a nível abdominal.

A prevalência de síndrome metabólica (SM) nestes doentes é elevada, especialmente tendo em conta a média de idade da nossa amostra. Observámos que os doentes com lipodistrofia clínica tinham um risco aos 10 anos de doença coronária significativamente maior, determinado pelo score de Framingham. Os doentes simultaneamente com lipodistrofia clínica e com SM tinham uma maior frequência de ca-

tegorias de risco moderado e elevado do que aqueles com lipodistrofia clínica mas sem SM, enfatizando a importância do diagnóstico clínico desta condição.

Na nossa coorte, a hipertensão arterial não estava associada com insulino-resistência, lipodistrofia ou massa gorda (abdominal total, central ou periférica), mas estava associada com a razão da massa gorda central/periférica.

A insulino-resistência e as alterações da homeostase da glicose aumentavam significativamente com o aumento do RMGTM. A presença de proeminência abdominal, é um importante fator com impacte na RMGTM, expressa pelo perímetro abdominal, razão cintura/anca, razão da gordura visceral/subcutânea a nível abdominal definida por TC, e estava também associada a alterações da homeostase da glicose. Não encontrámos qualquer associação entre a massa gorda periférica com insulino-resistência.

Nos doentes infectados pelo VIH, os 2 componentes do síndrome de redistribuição da gordura corporal parecem induzir efeitos deletérios diferentes: a acumulação de gordura central induz aumento do colesterol total, C-LDL e Apo B; e a lipoatrofia isolada induz baixa das Apo A1 e aumento dos triglicerídeos. Na análise dos componentes do síndrome de redistribuição do tecido adiposo, a proeminência abdominal parece ser mais importante do que a lipoatrofia nesta associação.

No que concerne a relação entre os níveis de adipocinas e composição corporal, os níveis de leptina eram menores nos doentes com lipodistrofia, indicando sua relação com a gordura corporal total. Encontrámos uma correlação significativa positiva da leptina com HOMA nos doentes com lipodistrofia, o que demonstra o papel desta adipocina na insulino-sensibilidade. A adiponectina é menor nos doentes com acumulação isolada de gordura central e correlaciona-se negativamente com o tecido adiposo visceral. A razão adiponectina/leptina era menor nos doentes com acumulação isolada de gordura central. Os doentes com formas mistas de lipodistrofia tinham níveis mais elevados de PAI-1. Não encontrámos diferenças significativas nos níveis de resistina, PAI-1 e ghrelina nos doentes com ou sem de lipodistrofia definida pela RMGTM.

A espessura da íntima média carotídea (EIMC), um marcador precoce de aterosclerose, era maior nos doentes com lipodistrofia do que naqueles sem lipodistrofia. A EIMC estava associada com a massa gorda total, central e com a razão da gordura central/periférica a nível abdominal. Os doentes com proeminência abdominal, independentemente de terem lipoatrofia clínica tinham uma maior EIMC, mas os doentes com lipoatrofia periférica isolada tinham uma maior EIMC do que doentes sem lipodistrofia. A acumulação da gordura central tinha um maior impacte na EIMC

nos doentes VIH tratados sob TARc; não obstante, o papel da lipoatrofia isolada não deve ser desconsiderado.

Em conclusão, a lipodistrofia tem um importante impacte nas alterações metabólicas e cardiovasculares. No entanto, deverá ser realçado, que os doentes com maior razão da gordura central/periférica (doentes com acumulação da gordura central e formas mistas de lipodistrofia) eram aqueles que apresentavam mais alterações. Porém, não podemos ignorar o papel da lipoatrofia periférica como possível contribuinte para estas alterações metabólicas e cardiovasculares.

SUMMARY

HIV infection and combined antiretroviral therapy (cART) have been associated with morphologic abnormalities (lipodystrophy) and linked through metabolic and endocrine alterations to increased cardiovascular risk. Patients with lipodystrophy had various features and degrees of fat redistribution, including lipoatrophy, lipohypertrophy or mixed forms of lipodystrophy. We aimed to study body composition, metabolic, endocrine alterations and cardiovascular disease according to the presence of lipodystrophy. We conducted several observational clinical studies in order to answer to above research questions. Despite different methodologies have been used to define lipodystrophy, there was a need of a simple, objective and gender-specific definition. We defined new gender-specific cutoffs to FMR (FMR=% of trunk/% of lower limbs fat mass by DXA) and for the first time in female patients. Body composition evaluation through bioelectrical impedance (BIA) gave us a picture of body composition that is not possible to have with anthropometry. The results of BIA demonstrated a similar fat-free mass along the fat distribution spectrum, absence of sarcopenia and increase in fat mass in patients with isolated central fat accumulation and mixed forms of lipodystrophy. Regardless of gender, being of normal weight, overweight or obese is more common than being underweight in our HIV-infected patients. Despite having a lower prevalence of overweight or obesity, lipodystrophic patients had an increased proportion of VAT, as reflected by the increased VAT/SAT fat ratio determined by CT at abdominal level. The prevalence of MS was high, especially taking into account the mean age of our sample and it was also observed that patients with CL had a significantly higher risk of coronary heart disease at 10 years, measured by the Framingham risk score. Also, those with CL and with MS had higher frequencies of moderate and high risk categories than those with CL but without MS, emphasizes the importance of the clinical diagnosis of this condition.

In our cohort, hypertension was not associated with IR, lipodystrophy or fat mass (total abdominal, central and peripheral), but was associated with central/peripheral fat mass ratio.

IR and glucose homeostasis abnormalities significantly increased with increasing FMR. Abdominal prominence, an important factor impacting the FMR, expressed by abdominal circumference, waist/hip ratio, visceral and trunk fat defined by CT, was also associated with glucose homeostasis disturbances. No association was observed between peripheral fat mass with IR.

In HIV infected patients, the 2 components of body fat redistribution syndrome seemed to induce different deleterious effects: central fat accumulation induced increased total cholesterol, LDL cholesterol and Apo B; and isolated lipoatrophy induced low Apo A1 and high triglycerides. In the analysis of the components of adipose tissue redistribution syndrome, abdominal prominence seems to be more important than lipoatrophy in this association.

In what concern adipokines and body composition hormones, leptin levels were lower in patients with lipodystrophy, indicating its close relationship with total body fat. A significant positive correlation of leptin with HOMA in patients with lipodystrophy demonstrates the role of this adipokine in insulin sensitivity. Adiponectin was lower in patients with isolated central fat accumulation and negatively correlated with visceral adipose tissue. The adiponectin/leptin ratio was lower in patients with isolated central fat accumulation. Patients with mixed forms of lipodystrophy had higher levels of PAI-1. No significantly differences in resistin, PAI-1 and ghrelin levels in patients with or without lipodystrophy defined by FMR were found.

Carotid IMT, a surrogate marker for atherosclerosis, was higher in patients with lipodystrophy than patients without lipodystrophy. cIMT was associated with total and central fat mass and with the ratio central/peripheral fat. Patients with abdominal prominence, independently of having clinical lipoatrophy had a higher cIMT, but patients with isolated peripheral lipoatrophy had a higher cIMT than patients with no lipodystrophy. Central fat accumulation had a major impact on cIMT in HIV cART treated patients; nevertheless, the role of isolated lipoatrophy should not be disregarded.

In conclusion, lipodystrophy had an important impact in metabolic and cardiovascular abnormalities, nonetheless, patients with higher central/peripheral fat ratio (patients with central fat accumulation and mixed forms of lipodystrophy) were those that presented more alterations. One should not however ignore the role of peripheral lipoatrophy as a contributor to these metabolic and cardiovascular changes.

9. BIBLIOGRAFIA

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