What Defines HIV Lipodystrophy?

A Roundtable Organized by the Forum for Collaborative HIV Research

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Based on presentations by Andrew Carr, Carl Grunfeld, and David Nolan

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Background

Since clinicians first noticed the body shape changes collectively described as HIV lipodystrophy, much research has focused on predisposing factors and possible mechanisms behind these changes. That work had been hampered, however, by lack of an accepted case definition of lipodystrophy and a poor understanding of what distinguishes these changes in people with HIV infection from similar changes in uninfected people.

Clinical impression suggested that at least four fat abnormalities could be seen—alone or together—in people with HIV lipodystrophy:

- Loss of subcutaneous fat in the arms, legs, face, and buttocks (lipoatrophy).
- Fat gain in the abdomen and trunk, especially a gain in visceral fat (lipohypertrophy).
- Dorsocervical fat pads, or "buffalo hump" on the back of the neck.
- Breast hypertrophy, usually in women.

The first systematic attempt to define the syndrome in a precise way, the Lipodystrophy Case Definition Study (LCDS) sponsored by pharmaceutical companies represented in the Oversight Committee for the Evaluation of the Metabolic Complications of HAART (an European Agency for the Evaluation of Medicinal Products (EMEA) initiative) confirmed certain measures of hypertrophy and atrophy as distinguishing features of lipodystrophy in a case-control comparison of HIV-infected people with and without physical signs of the syndrome (Carr 2002a). Early results of the Fat Redistribution and Metabolic Change in HIV (FRAM) study, comparing fat changes in people with or without HIV infection, also identified lipoatrophy as a distinguishing trait of HIV lipodystrophy (Grunfeld 2002). But this analysis, which so far involves only a portion of the men studied and none of the women, did not find more lipohypertrophy or dorsocervical fat pads in people with HIV than in controls.

This surprising result, first presented at the XIV International AIDS Conference in July, inspired intense debate—and confusion—among people with HIV, their clinicians, and researchers who study lipodystrophy. To probe the differences between the LCDS and FRAM results, and to promote discussion toward resolving unanswered questions, the Forum for Collaborative HIV

Research convened a roundtable of experts in the field to review the LCDS and FRAM findings, as well as related evidence from the Western Australian HIV Cohort Study.

The meeting took place on September 23, 2002, in San Diego, California, with funding from Serono Laboratories, GlaxoSmithKline and Boehringer Ingelheim. After slide presentations by David Nolan (Western Australian HIV Cohort Study), Andrew Carr (Lipodystrophy Case Definition Study), and Carl Grunfeld (FRAM study), attendees discussed the findings in an open roundtable.

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Antiretroviral Therapy-Associated Lipodystrophy: From the Population Level to the Cellular Level

David Nolan, MD
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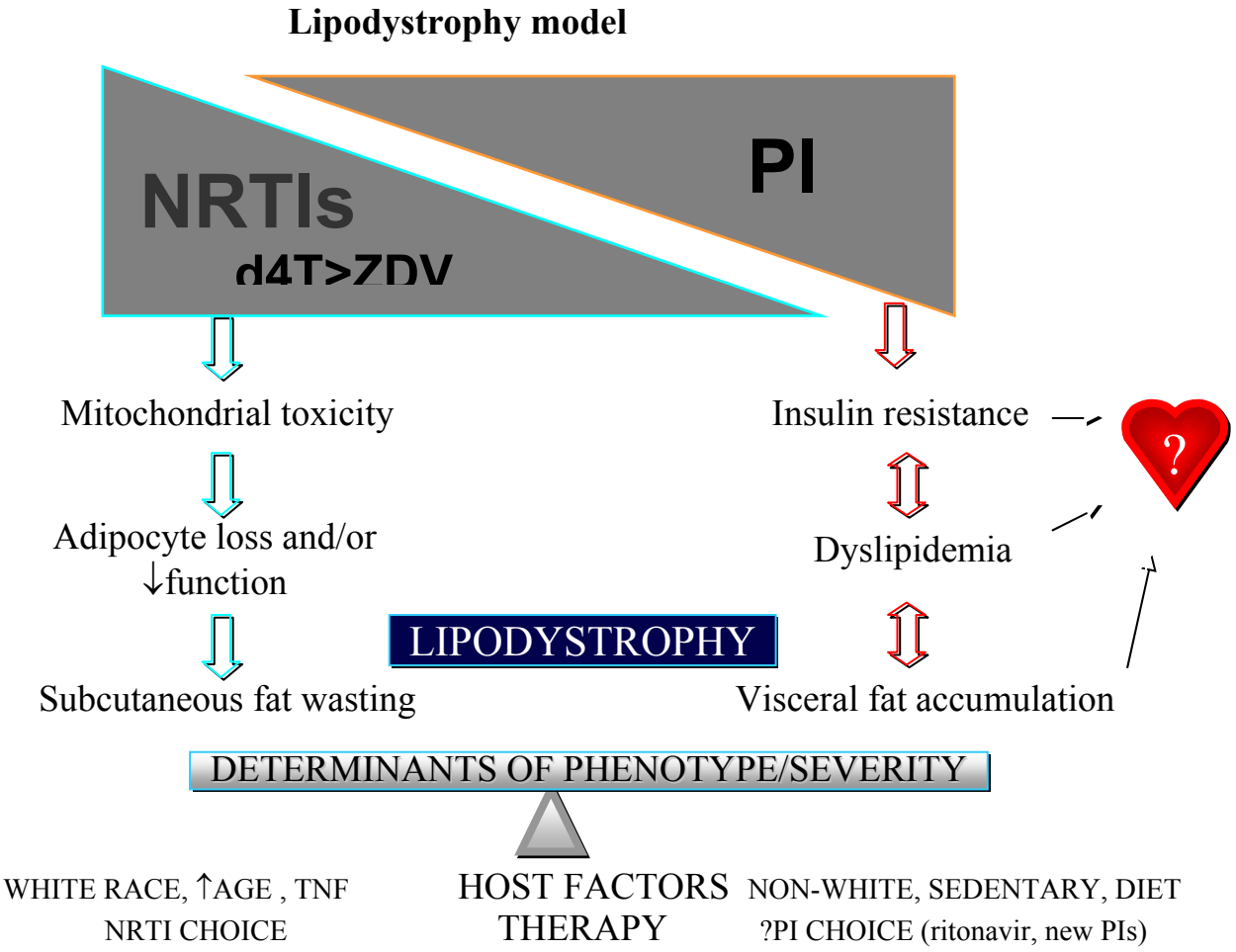
David Nolan summarized the multifaceted work of the Perth group involving the collaboration of Simon Mallal, Mina John, Ian James, Elizabeth McKinnon, Emma Hammond, Annalise Martin, and Craig Pace. The evolving understanding of HIV lipodystrophy and metabolic disorders that has emerged from this research group rests largely on intense laboratory and statistical analyses of people enrolled in the Western Australian HIV Cohort Study.

Nolan explained that he and his colleagues view subcutaneous fat wasting as a process of variable rate and severity, rather than as a dichotomous clinical outcome—something that is either "present" or "not present." They focus primarily on subcutaneous adipose tissue pathology, using light and electron microscopy, mitochondrial DNA depletion analyses, and confocal microscopy.

Defining antiretroviral-associated lipodystrophy

Nolan and colleagues believe that antiretroviral-associated lipodystrophy involves treatment duration-dependent toxicities induced by two antiretroviral classes: nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs). The prominent effects of these agents on adipose tissue are likely to begin within the first days to weeks of treatment.

The Perth group distinguishes these views from a "clinical outcomes" conception of lipodystrophy, defined by a change from a baseline value that is considered pathological. A problem with the latter approach is the inherent difficulty in setting cutoff values that define body composition and metabolic abnormalities that may be considered diagnostic of the syndrome.



The lipodystrophy model conceived by Nolan and coworkers posits a close interaction between PIs and NRTIs. While PIs induce insulin resistance, dyslipidemia, and visceral fat accumulation, NRTIs promote mitochondrial toxicity, adipose tissue loss and/or dysfunction, and subcutaneous fat wasting. Research by the Perth team indicates that several therapeutic and host factors determine the phenotype and severity of lipodystrophy:

Factors involved in NRTI-related toxicities

- White race
- Older age
- Tumor necrosis factor alpha-238G/A promoter polymorphism
- Choice of NRTI (d4T > AZT)

Factors involved in PI-related toxicities

- Nonwhite race

- Sedentary lifestyle
- Diet
- Possibly, choice of PI (ritonavir > new PIs)

Effect of d4T, AZT, and PIs on subcutaneous leg fat wasting

Nolan and coworkers conducted a longitudinal analysis of subcutaneous leg fat wasting in men taking antiretrovirals. They had three aims:

- To model longitudinal profiles of objectively measured subcutaneous fat
- To investigate determinants of the rate and severity of fat wasting over time
- To identify appropriate markers of fat wasting that allow inter- and intra-individual comparisons in a study population

The 40-month study involved 25 men beginning treatment with d4T or AZT plus a nonnucleoside reverse transcriptase inhibitor (NNRTI) and 49 beginning with d4T or AZT plus a PI. Leg fat waned more among PI-treated men than among NNRTI-treated men. But regardless of whether the men took an NNRTI or a PI, they lost significantly more leg fat with d4T than with AZT.

Decrease in leg fat after 40 months of follow-up

d4T + NNRTI	*AZT + NNRTI*	*P*
-7.1% (1.9 kg)	-2.4% (0.4 kg)	<0.0005
d4T + PI	*AZT + PI*	
-10.9% (2.9 kg)	-6.2% (1.4 kg)	<0.0005

In the 49 PI-treated men, Nolan modeled the proportionality of peripheral fat compared with overall fat distribution by dividing the percentage of leg fat by body mass index (kg/m^2). That formula adjusts changes in peripheral fat for individual variation in baseline body composition. All study participants were white men who had two or more DEXA scans to determine peripheral fat mass. Measurements made after any treatment change were not included in the analysis.

After more than 50 months of follow-up, percentage of leg fat/body mass index fell significantly more in the 25 men taking d4T with a PI than in the 24 taking AZT with a PI ($P = 0.03$). Body mass index did not change significantly over time or differ between the d4T group and the AZT group. Besides treatment with d4T rather than AZT, two other factors correlated with a greater loss of leg fat/body mass index: older age, and an AIDS diagnosis at baseline. Analysis of additional men showed that those who took AZT for 30 months before starting d4T, and men who took only d4T, lost an equivalent percentage of leg fat/body mass index after 60 months of d4T therapy. But men who took AZT for 30 months and then continued AZT for another 60 months retained significantly more leg fat than either d4T group.

Nolan concluded that NRTI choice—d4T versus AZT—is the dominant determinant of long-term peripheral fat loss trends. Four other trials—OZCOMBO (Law), ALBI (Chêne), NOVAVIR (Joly), and ACTG 384 (Dubé)—also found a significantly greater risk of peripheral fat atrophy with d4T regimens than with AZT regimens.

Mitochondrial DNA and subcutaneous fat wasting

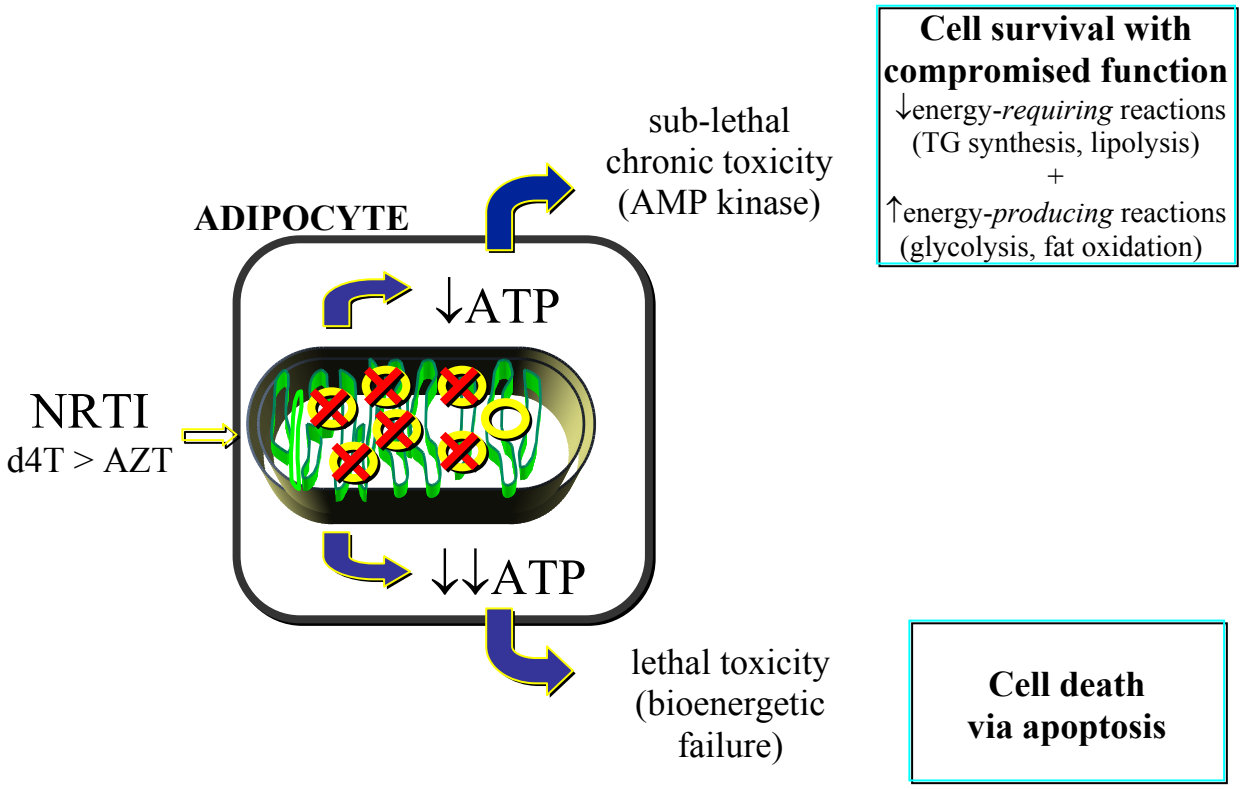
Research by the Perth group documented adipocyte cell loss through apoptosis and increased variability in adipocytes size as features of subcutaneous fat wasting (Mallal 2000a). These features are identical in people taking PIs and in those not taking PIs. Work by others showed that apoptotic indices do not improve after switching from a PI to the NNRTI nevirapine (Domingo).

Further study of NRTI effects on mitochondrial DNA content in adipocytes relied on 78 excisional subcutaneous fat biopsies (Mallal 2001). The analysis compared people taking d4T, AZT, other NRTIs, or no antiretrovirals, and HIV-seronegative controls. Counting log copies of mitochondrial DNA per cell with a real-time PCR assay, the Perth group found significantly fewer copies in people taking d4T ($P < 0.0001$) or AZT ($P = 0.002$) than in HIV-infected people taking no antiretrovirals. Mitochondrial DNA content did not differ significantly between people taking other NRTIs and untreated people with HIV infection, or between untreated people and seronegative controls. If the mitochondrial DNA content in adipocytes of untreated HIV-infected people is set at 100%, the proportional content in the treated groups equaled:

- 67.6% with other NRTIs

- 33.1% with AZT
- 14.4% with d4T

Nolan and colleagues in Melbourne and Baltimore confirmed these findings in a separate cohort. In the Western Australian Cohort patients, switching from d4T to AZT or another NRTI restored mitochondrial DNA content in adipocytes.



The model of NRTI-induced adipocyte toxicity proposed by Nolan and coworkers rests on decreased adenosine 5'-triphosphate (ATP) in these fat cells. Moderate reductions in ATP lead to sublethal chronic toxicities; greater reductions lead to cell death via apoptosis.

Switching NRTIs to reverse peripheral fat atrophy

Evidence implicating d4T in peripheral fat atrophy encouraged the Perth team and others to see if substituting another NRTI for d4T would reverse fat loss. Two 24-week studies by Andrew Carr did document modest but statistically significant rebounds in limb fat after switching from d4T (Carr 2002b; Smith 2001). A 48-week study headed by Nolan's colleague Mina John confirmed that result (John 2001). This study randomized people to continue d4T and/or a PI or to switch either or both to Combivir (AZT/3TC) and/or abacavir. Measuring peripheral fat with

DEXA scans, John found continued fat loss in the nonswitch group versus a modest gain in the switch groups ($P = 0.03$).

The Perth team has also demonstrated, however, that NRTI choice is not the only factor that favors lipoatrophy. A Cox proportional hazards analysis involving 250 people taking triple-drug therapy linked two nondrug variables, and three treatment variables, to subcutaneous fat wasting (Mallal 2000b):

Risk of subcutaneous fat wasting in 250 people taking three antiretrovirals

	P	*Relative risk*
Age	<0.0001	1.052 per year
White race	0.023	3.9
Cumulative time on nevirapine (compared with PI therapy)	0.022	0.943 per month
Cumulative time on d4T (compared with AZT)	<0.0001	1.085 per month
Cumulative time on dual NRTI therapy before triple therapy	0.0046	1.021 per month

A more recent study by Nolan and colleagues found another non-drug factor, heterozygosity for the tumor necrosis factor alpha (TNF-alpha) promoter polymorphism 238G/A (Nolan). They detected the polymorphism in 25 of 191 Caucasians (13.1%) and found a significantly greater risk of progression to clinically determined lipodystrophy among heterozygotes for the polymorphism than among people carrying the wild-type allele ($P = 0.014$). In a Cox proportional hazards model analysis involving 191 Caucasians, heterozygosity for TNF-alpha-238G/A independently raised the risk of lipodystrophy 1.7 times ($P = 0.041$). Researchers at the University of Liverpool also implicated this polymorphism in the risk of lipodystrophy (Maher).

Conclusions

- Subcutaneous fat wasting is a process of variable rate and severity, rather than a dichotomous (present-versus-absent) clinical outcome.

- Antiretroviral-associated lipodystrophy involves treatment duration-dependent toxicities induced by two antiretroviral classes: nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs).
- NRTI choice—d4T versus AZT—is the dominant determinant of long-term peripheral fat loss.
- Three nondrug factors that affect the development of lipoatrophy in the Western Australian Cohort are older age, white race, and heterozygosity for the TNF-alpha promoter polymorphism 238G/A.

The HIV Lipodystrophy Case Definition Study

Andrew Carr, MD

St. Vincent's Hospital

Sydney, Australia

Andrew Carr explained that a case definition of lipodystrophy would fulfill four needs:

- Improve estimates of prevalence and incidence of the syndrome with different drugs and in different patient populations
- Increase understanding of risk factors
- Improve estimates of responses to treatment and prevention
- Assist in diagnosis

But formulating a case definition for lipodystrophy is difficult because there is no "gold standard" diagnostic test, because no single physical or metabolic feature distinguishes lipodystrophy from unaffected individuals with HIV infection, and because the variable phenotype suggests there may be more than one syndrome. Already there are five tentative, nonvalidated definitions, Carr noted, but they are derived from mostly male populations at few sites and with limited body composition data.

The variable phenotype of lipodystrophy makes diagnosis difficult

	NRTI-related lipodystrophy	*PI-related lipodystrophy*
Peripheral lipoatrophy	+++	++
Abdominal distension	+	+++
Buffalo hump, lipomata	+	+
Recent weight loss, nausea, fatigue	-/++	-
Lactic acidemia	-/++	-
Liver dysfunction	-/++	-
Dyslipidemia	-/+	+++
Insulin resistance, diabetes	-/+	+++
Possible pathogenesis—inhibition of:	Mitochondria	Proteases

NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor.

The American College of Rheumatology has developed case definitions for more than 20 autoimmune syndromes, ranging from rheumatoid arthritis and fibromyalgia to Takayasu's arteritis and Wegener's granulomatosis. In defining these syndromes, Carr explained, researchers faced some of the same obstacles faced by those defining HIV lipodystrophy: limited understanding of mechanisms, clinical overlaps, lack of a diagnostic assay, and reliance on phenotypic descriptions. Carr and colleagues studied the methods used to arrive at case definitions of autoimmune disease and adopted several of them.

Design of the lipodystrophy case definition study

Aiming to arrive at an objective, sensitive, specific case definition of HIV lipodystrophy, Carr and colleagues planned a global, 32-site, case-control, cross-sectional study. They selected parameters for relevance and local availability and minimized bias by four means:

- Consecutive patient recruitment
- Recruitment at hospital and community sites
- Recruitment of equal numbers of cases and controls
- Recruitment of equal numbers of cases and controls by gender at each site

Eligible study participants had to be at least 18 years old and have no active AIDS-defining illness. Case patients had to have at least one moderate or severe feature of lipodystrophy agreed upon by both the patient and the physician. Lipodystrophy in this study was defined as lipoatrophy, diffuse accumulation or lipoma, although patients with isolated moderate to severe abdominal fat accumulation were excluded. Controls and their physicians had to agree that the control had no features of lipodystrophy and no fat changes since being diagnosed with HIV infection. Nonassigned individuals were all other eligible study subjects.

The training data set used to select distinguishing features of lipodystrophy consisted of randomly selected cases and controls. A univariate comparison of all case and control data excluded lipodystrophy assessments, symptoms, and therapy. Then a forward stepwise logistic regression analysis incorporated gender and all variables that discriminated cases from controls

in the univariate analysis. The objective models derived were tested on a validation set of cases and controls.

The training data set included 265 cases and 239 controls, while the validation data set included 152 cases and 132 controls. Among the 288 people not assigned to the case or control groups, 65% were not assigned because the patient claimed moderate to severe lipodystrophy while the physician saw no or mild lipodystrophy; 26% were not assigned because patients claimed no or mild lipodystrophy while the physician saw moderate to severe lipodystrophy; and 10% had only mild features described by the patient and/or physician.

Variables distinguishing cases from controls

Cases, controls, and nonassigned individuals were well matched for gender, ethnicity, risk factor for HIV infection, and viral load. But several other variables distinguished cases from controls:

- Older age
- Longer duration of HIV infection
- Longer follow-up
- More with an AIDS diagnosis
- Lower CD4 nadir
- Greater change in CD4 with treatment

Based on raw numbers, more cases than controls had ever used an NRTI, a PI, or a nonnucleoside reverse transcriptase inhibitor (NNRTI), and more cases than controls were using drugs from those classes when assessed. Similarly more cases than controls were taking lipid-lowering agents, antidiabetic drugs, appetite stimulants, and anabolic agents, whereas more controls than cases were taking appetite suppressants and almost all were smokers. Cases did not differ from controls in dietary intent to gain, lose, or keep the same weight, and equivalent proportions of cases and controls exercised or did not. Significantly more cases than controls had diabetes, and significantly more controls than cases had more than 14 alcoholic drinks weekly.

Compared with controls, cases reported significantly more episodes of dry skin, dry lips, ingrown nails, peripheral neuropathy, unexplained diarrhea, increased menstruation, increased bleeding, and abdominal bloating. Significantly more cases than controls lost more than 3 kg and

significantly more cases than controls gained more than 3 kg. Significantly more cases reported a loss of appetite, and significantly more cases reported a gain in appetite. Patients were asked for the presence or absence of these symptoms in the preceding three months.

Anthropometric variables that distinguished cases from controls were:

- Greater decrease from peak weight to current weight
- Greater waist circumference
- Greater waist-to-hip ratio

Metabolic variables that discriminated between cases and controls were:

- Higher triglycerides
- Higher total cholesterol
- Lower HDL cholesterol
- Higher total-to-HDL cholesterol ratio
- Higher glucose
- Higher insulin
- Higher C peptide
- More insulin resistance by HOMA
- Lower glucose-to-insulin ratio
- Higher lactate levels
- Higher anion gap
- Higher ALT

Imaging showed the following significant differences of cases from controls:

- Less total fat
- Less arm and leg fat
- Higher trunk-to-limb fat ratio
- Higher visceral adipose tissue (VAT)
- Lower subcutaneous adipose tissue (SAT)

- Higher VAT-to-SAT ratio

Case definition and scoring models

The forward stepwise logistic regression analysis isolated 10 factors that define lipodystrophy:

1. Female gender
2. Age over 40 years
3. AIDS (CDC stage C)
4. HIV for more than 4 years
5. Increased waist-to-hip ratio
6. Decreased HDL cholesterol
7. Increased anion gap
8. Decreased leg fat
9. Increased VAT-to-SAT ratio
10. Increased trunk-to-limb fat ratio

The analysis assigned a score to each of these variables. A lipodystrophy score for an individual can be calculated by subtracting 43 from that person's total score.

- Final score ≥ 0 = lipodystrophy
- Final score < 0 = no lipodystrophy

As soon as the case definition and scores are published, clinicians will be able to rate individual patients with an online interactive calculator at http://www.med.unsw.edu.au/nchecr.

When tested against the validation data set, the sensitivity of the case definition when the score is ≥ 0 was 79% (95% confidence interval 70% to 85%) and the specificity 80% (95% confidence interval 71% to 87%). The sensitivity increases and the specificity decreases as scores fall below 0, whereas the sensitivity decreases and the specificity increases as scores increase above 0.

Carr and colleagues will put three models online:

1. The primary 10-variable model, with sensitivities and specificities described in the preceding paragraph
2. A model with 10 clinical and metabolic variables, which has a sensitivity of 73% and specificity of 71%
3. A model with 5 clinical variables, which has a sensitivity of 75% and a specificity of 60%

Because of concern that some or all abdominal adiposity or buffalo hump may represent age-related adiposity, Carr and colleagues eliminated from all analyses the 6% of cases with pure diffuse fat accumulation (mostly abdominal obesity and either breast hypertrophy or dorso-cervical fat accumulation). This exclusion did not alter the parameters, sensitivity, or specificity of the primary model. They could not generate lipoatrophy-specific or fat accumulation-specific models because these pure phenotypes were uncommon in the cohort (9% and 6%, respectively). Models specific for men or women did not vary much from each other or from mixed-gender models in sensitivity and specificity.

The prevalence of lipodystrophy was 5% among antiretroviral-naive people, 39% among antiretroviral-experienced but PI-naive people, and 59% among people with PI experience. A comparison of imaging parameters recorded at the central study site and at local sites disclosed no significant differences. Nor did the model's sensitivity or specificity vary if based only on local imaging data.

Summary

Carr listed the following features of the lipodystrophy case definition:

- The case definition is objective.
- It is no more complex than case definitions for many rheumatic diseases.
- Metabolic and body composition parameters are required to maximize diagnostic accuracy.
- Inclusion of overlapping body composition parameters in the model may be a result of inherent variability in DEXA and CT scans.
- The model is less sensitive for milder lipodystrophy.
- Sensitivity is similar for men and women.
- The model is unaffected by exclusion of subjects with pure fat accumulation.

- Centralized DEXA and CT analysis did not significantly change or simplify the model or alter its sensitivity or specificity.
- Lipoatrophy-specific and fat accumulation-specific models could not be developed because of the rarity of those phenotypes.

Carr proposed that the lipodystrophy case definition can serve as a model for other drug-related toxicities in people with HIV infection, such as peripheral neuropathy, symptomatic lactic acidemia, and drug-induced hepatitis.

Preliminary Data on Men From the Fat Redistribution and Metabolic Change in HIV (FRAM) Study

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Carl Grunfeld reported that all fat distribution changes noted in clinical practice and cohort studies were found in FRAM study participants, including loss of peripheral fat in the cheeks, arms, buttocks, and legs; buffalo hump; increased waist size; and increased adipose tissue. While both peripheral fat loss and central fat gain are seen in HIV infection it is important to ask whether both loss and gain are defining features of HIV lipodystrophy. And has a link between those two changes been demonstrated? The results of FRAM address those questions.

FRAM study design

Like the case definition study, FRAM is a cross-sectional (one evaluation per participant), multisite analysis. Unlike the case definition study, FRAM compares HIV-infected cases with HIV-negative controls. Grunfeld and colleagues recruited HIV-infected study participants by random selection from the database of each participating clinic. They were selected from the entire clinic database, not from among patients who made frequent clinic visits or were "typical study volunteers." The men were recruited from June 2000 through January 2002. Fewer than 20% of those contacted declined to participate.

Controls came from the CARDIA study, a representative sample of young adults stratified by race, gender, age, and education. Because CARDIA participants were selected 15 years ago for this prospective evaluation of cardiovascular disease risk, they now range in age from 33 to 45 years. CARDIA controls enrolled in FRAM were mainly people who enrolled in a substudy of visceral obesity 5 years ago. However, the prevalence of obesity in the CARDIA controls is similar to that in the larger NHANES database, which is representative of the US population. Less than 1% of the CARDIA participants report HIV infection.

The analysis that Grunfeld presented included approximately 350 HIV-infected men from among more than 800 recruited. Approximately 350 HIV-infected women will be studied. The HIV-

infected men in this preliminary analysis have the same age range as the CARDIA controls, 33 to 45 years. The HIV-infected men are 53% Caucasian, 33% African-American, and 12% of other ethnicities, while the controls are 55% Caucasian and 45% African-American. The HIV-infected men have the following characteristics:

- Risk factor: 77% men who have sex with men, 10% injection drug users, 13% other or unknown
- Median CD4 count: 347 cells/μL (range 6 to 1499 cells/μL)
- Median HIV RNA: <400 copies/mL (range <400 to 750,000 copies/mL)
- Current antiretrovirals: 14% none, 83% on NRTIs, 55% on PIs, 37% on NNRTIs

Grunfeld listed the following study principles:

Principles of FRAM

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1. We did not presume that we knew what the syndrome was.
2. HIV-infected cases and CARDIA controls underwent the same subjective assessments and objective measurements.
3. For a finding to be used in an HIV-specific syndrome, there had to be a statistically significant difference between HIV-infected cases and CARDIA controls.
4. For two findings to be combined into a single syndrome, there had to be a positive statistical association between those two findings.

The FRAM self-report recorded not only presence or absence of a morphologic change, but also the degree of severity. For example, when asked if they had noticed a change in the amount of fat on the neck, participants could answer no, yes, or don't know. If they answered yes, they had to rate the change as severely (+3), moderately (+2), or mildly increased (+1), or mildly (-1), moderately (-2), or severely (-3) decreased (with scores given in parentheses). And they had to state the month and year when they first noticed the change. The physical exam report rated (and

scored) changes at each body site as severely (+3), moderately (+2), or mildly (+1) fat, normal (0), or mildly (-1), moderately (-2), or severely (-3) wasted.

The self-report and physical examination covered five peripheral changes—cheeks (next to the nose), face shape, buttocks, legs, and arms—and five central changes—waist size (or abdominal shape), abdominal fat, neck, upper back, and chest.

Differences between HIV-infected cases and CARDIA controls

By self-report, the HIV-infected men lost peripheral fat while CARDIA control men gained peripheral fat, and the difference was significant at every peripheral site: cheeks, face, arms, legs, and buttocks. Among HIV+ and controls who reported peripheral fat loss, significantly more HIV+ than controls reported loss at each of these five sites.

Significantly fewer HIV-infected participants than controls reported increases in neck, chest, and waist fat. Equivalent proportions of HIV-infected individuals and controls reported increased abdominal and upper back fat. Significantly more HIV-infected individuals than controls reported decreases in neck, chest, waist, and upper back fat. Decreases in abdominal fat were equivalent between the two groups.

On the basis of these preliminary analyses of self-reports and physical exams of men, Grunfeld reached the following conclusions:

- On both self-report and physical exam, *peripheral lipoatrophy* distinguishes HIV-infected cases from CARDIA controls.
- The data do *not* support inclusion of central fat hypertrophy in an HIV-specific lipodystrophy syndrome.
- *Less central fat* may be an HIV-specific syndrome.

Next FRAM statisticians used the quantitative scores to look for correlations between values at one body site and values at another site. These analyses addressed the question, is loss of fat at one site associated with gain of fat at another? Grunfeld and colleagues discerned the following correlations:

- Positive correlation of fat depots among peripheral sites
- Positive correlation of fat depots among central sites
- *Positive* correlation between peripheral sites and central sites (or, to put it another way:)
- Men with peripheral lipoatrophy had less central fat

These findings contradict any link between peripheral fat atrophy and central fat hypertrophy.

Grunfeld noted that many studies of HIV lipodystrophy use concordant appraisals of patients and providers as inclusion criteria. FRAM used the *directional* concordance (loss/lesser fat or gain/greater fat change at each site) of self-reports and exams to approximate their definition. These "concordance analyses" for peripheral fat atrophy, central fat atrophy, and central fat hypertrophy allowed the FRAM investigators to ask whether peripheral lipoatrophy is associated with central fat hypertrophy or with central fat atrophy in HIV-infected men. They determined that:

- Central lipohypertrophy is not preferentially associated with peripheral lipoatrophy in HIV-infected men.
- Central lipoatrophy is associated with peripheral lipoatrophy in HIV-infected men.

Grunfeld pointed out that they did find a significant number of participants with both Central lipohypertrophy and peripheral lipoatrophy, but those without central lipohypertrophy had more peripheral lipoatrophy. He proposed several implications from FRAM findings to date: First, common findings can occur together by chance. Second, an association between such findings must be proven. The findings do not mean that peripheral fat atrophy and central fat hypertrophy cannot occur in the same person. They do say that those findings are not linked and that their etiology must be studied separately. Subcutaneous atrophy may be a more coherent syndrome. Grunfeld and colleagues will use the term "HIV-associated lipoatrophy" to avoid confusing these findings with other proposed HIV lipodystrophy syndromes.

HIV-infected individuals with lipoatrophy had significantly less limb fat by DEXA scan than did CARDIA controls. But HIV-infected cases without lipoatrophy also had significantly less limb fat by DEXA scan than did controls. That second finding, Grunfeld suggested, may explain the difficulties in determining the causes of clinical lipoatrophy.

HIV-infected men had significantly less subcutaneous abdominal fat by MRI than did controls, and HIV-infected men with clinically described lipoatrophy had less subcutaneous abdominal fat than men without lipoatrophy. HIV-infected men had less subcutaneous chest and back fat by MRI than did controls, and HIV-infected men with clinically described lipoatrophy had less chest and back fat than men without lipoatrophy. Among all HIV-infected cases, legs had the least fat compared with controls, followed by arms, abdomen, and chest and back. The finding that chests and backs of HIV-infected men have the least subcutaneous fat loss compared with controls may explain reports of central lipohypertrophy. For a given amount of fat in an HIV-infected man, more will be on the upper back and less on the legs than for controls.

HIV-infected men in FRAM had a slightly (but not significantly) lower prevalence of buffalo hump than did CARDIA controls. How can that finding be explained? Grunfeld noted that less is known about buffalo hump than about other fat changes analyzed and that its prevalence in healthy controls is unknown. When researchers were being trained to recognize buffalo hump by examining each other, he added, all were surprised to find buffalo humps in the non-infected researchers. As part of the study, researchers were shown pictures of buffalo humps from patients with HIV and were told to look for a fat pad in the center of the upper back and neck. Although buffalo hump does occur in people with HIV infection, Grunfeld concluded, the FRAM findings do not support the inclusion of its presence *per se* as a diagnostic criterion of an HIV-specific syndrome. These data do not rule out differences in the humps of those with HIV infection, such as more rapid appearance, different consistency or larger size.

Summary and implications

Grunfeld echoed David Nolan's point that subcutaneous fat wasting is not a dichotomous trait but rather a continuous variable. FRAM results to date show that the syndrome described in the clinic underestimates fat wasting. When HIV-infected men gain weight, the results show, less fat will be deposited in the legs and more in the chest and upper back compared with controls with similar weight gains. Therefore, HIV-infected men with significant central gains will have strikingly less limb fat than usual.

FRAM has certain limitations. Because of its cross-sectional design, FRAM could not determine:

- What path HIV-infected men followed to arrive at their current body composition
- Where the trends are going
- Whether the HIV-infected men might have had less visceral adipose tissue if they had not developed decreased subcutaneous adipose tissue

Grunfeld restated the two key conclusions of this analysis:

- Self-report, physical exam, and objective measurements clearly demonstrate that lipoatrophy is an HIV-specific syndrome that distinguishes cases from controls.
- Self-report, physical exam, and objective measurements do not support a compensatory central hypertrophy in HIV-infected men with lipoatrophy.

Discussion

Cohort selection: the weight factor

Roundtable attendees spent some time discussing the make-up the FRAM cohorts in an attempt to understand why central fat hypertrophy did not distinguish the HIV-infected population from the CARDIA controls. Stefan Mauss suggested that the CARDIA men may have over-represented heavier people in the general population. Carl Grunfeld agreed that argument can be made, but he reiterated that CARDIA controls were selected to represent the general population. Steven Grinspoon noted that waist circumference in the CARDIA cohort approaches that of the larger NHANES cohort, also selected to represent the US population. Increasing weight with age is a given that has always made abdominal hypertrophy a questionable discriminator of lipodystrophy from typical central weight gain.

Lower body weight in the FRAM HIV cohort than in the controls could have affected the findings. At the simplest level, because the HIV cohort weighed less than the CARDIA controls, they were bound to have less visceral fat. As a result, a cross-sectional analysis of this HIV cohort would not single out visceral adiposity as a morphologic trait distinguishing them from the (heavier) CARDIA controls. The more difficult questions are:

1. Did the HIV cohort weigh less because of some HIV- or drug-related pathology, or because the investigators selected a lighter cohort?
2. Would correcting the analysis for weight clarify the results, or complicate them?

Donald Kotler, a FRAM investigator who attended the Forum roundtable, addressed these issues in an online analysis:

". . . the interpretation of some [FRAM] data is based upon the assumed premorbid equivalence of the HIV-infected subjects and the CARDIA controls. . . . the matching procedure implies that the premorbid (ie, before HIV infection) body weights were the same in the subjects and controls. While it is appropriate that subjects and controls were matched by height but not by weight, this does not mean that premorbid body weights were indeed the same in the subject and control groups. If premorbid body weights and body fat contents were lower in those who were to become HIV-infected than in CARDIA controls, the depletion of [subcutaneous adipose

tissue] would be accentuated and the accumulation of [visceral adipose tissue] would be masked."

In the same article, Kotler notes the disadvantage of matching HIV-infected men with CARDIA controls by weight: "Since the development of lipodystrophy often is accompanied by weight loss, correcting for weight loss will introduce a bias in the data analysis . . . " Thus, BMI matching controls may introduce an artifact.

Can FRAM and the case definition be reconciled?

Judith Currier suggested that different findings in FRAM and the Case Definition Study may not be contradictory; they may simply reflect the different study designs. Both studies were cross-sectional, involving one-time assessments of each study participant. But the Case Definition Study compared HIV-infected people with and without signs of lipodystrophy while FRAM compared HIV-infected people with seronegative people. Because the syndrome or syndromes being seen in people with HIV involve fat loss and/or gain, and because both fat loss and fat gain change with age, a longitudinal study (involving several assessments of each study participant over time) may be the best way to define fat changes in people with HIV.

Carl Grunfeld agreed but pointed out the main difficulty of many new studies. If you begin with people newly infected with HIV, follow-up will have to be long before morphologic changes could be appreciated. Follow up is needed in the heavily treated, long-term cohorts, such as FRAM.

Donald Kotler concurred that a longitudinal study may not be the best way to explain differences seen in the cohorts described by David Nolan, Andrew Carr, and Carl Grunfeld. The goal should be to resolve those differences, even though that would be difficult. Looking at lipodystrophy as a process rather than as an endpoint, as suggested by Nolan and Grunfeld, may be one key to resolving or understanding differences.

Carl Grunfeld observed that assessing fat changes in people with HIV as "multiple processes" with "individual contributors" may also elucidate the impact of those changes on other abnormalities seen in people with HIV. For example, in people without HIV, both severe fat atrophy and severe fat accumulation lead to hypertriglyceridemia. Does that mean HIV-infected

people with both atrophy and hypertrophy have a higher risk of high triglycerides than do people with only atrophy or hypertrophy?

Andrew Carr noted that differences between the Case Definition findings and the FRAM findings are not only differences in kind, but also differences in degree: The percentage of abnormalities reported in the two studies differs markedly. Carr found the buffalo hump rate in FRAM's CARDIA controls surprisingly high and wondered if FRAM researchers who evaluated study participants may have confused lipoma with buffalo hump.

The rate of buffalo hump in the Case Definition study was only 2%, while 22% reported diffuse dorso-cervical fat accumulation. Given what has been observed in some studies, there may have been some confusion by the physician and patient as to what is diffuse and what is a localized buffalo hump. This equally applies to both FRAM and the Case Definition study.

Andrew Carr also noted that the visceral fat volume is far greater in the CARDIA patients than the HIV+, non-lipodystrophic patients. Since most of the CARDIA patients described themselves and were described by their physicians as having normal bodies, how can this enormous difference in VAT objectively be explained? One possible explanation may be that gay men in FRAM may have a very different perception of what a normal body looks like compared to the (mostly?) heterosexual population in CARDIA.

Other roundtable discussants argued that the differences between FRAM and the Case Definition Study are not as great as their similarities. Jens Lundgren noted that a case definition is, by its nature, a simplification, just as a phenotypic cutoff is a simplification of a continuous variable reflecting evolving drug resistance in a viral population.

William Powderly agreed that FRAM and the Case Definition have more in common than first meets the eye. He argued that work should now focus on what is common to these studies' results as a way to move the research forward.

1. Atrophy is prevalent in both study populations (as well as in the Western Australian Cohort), and FRAM suggests that it may be clinically underestimated.

2. Both the Case Definition Study and FRAM found fat gains in people with HIV. The issue is what those gains should be compared with. Control groups allow researchers to decide whether these fat gains are HIV-specific and what is multifactorial and harder to define.
3. The Case Definition model does not include fat accumulation as a parameter. Of the four body composition parameters, three are ratios of peripheral to central measures, and the other is percentage limb fat.

Powderly believes the studies have not sorted out whether fat accumulation and fat loss occur completely independently or in parallel. That will require further study, but Powderly acknowledged his bias is "that we're looking at separate syndromes." Because fat changes are a dynamic process, clinical trials should incorporate objective measures of these changes at baseline and at planned follow-ups.

David Nolan added that therapeutic variables, and especially treatment duration, have an impact on fat changes and must be accounted for in any analysis. FRAM investigators plan to analyze the impact of antiretrovirals on their findings by chart review. Only 14% of the HIV cohort in FRAM was not being treated.

Norma Muurahainen and Donald Kotler proposed a way to compare the Case Definition data with the FRAM HIV data. Because FRAM collected most of the data needed for the Case Definition equation, that equation can be applied to people with and without atrophy in FRAM. That exercise would tell which FRAM participants have higher or lower lipodystrophy scores according to the Case Definition and so determine whether the two data sets are truly irreconcilable. If the data sets are irreconcilable, they would have to be viewed as disparate approaches to the same issue, with the Case Definition including central fat accumulation as one component of lipodystrophy and FRAM excluding it.

Conclusions and Recommendations

- Fat atrophy is a distinguishing feature of HIV lipodystrophy and may be underestimated clinically.

- Fat hypertrophy occurs in people with HIV infection, but research results conflict on whether it can be used as a defining trait of HIV lipodystrophy.

- Fat atrophy and fat hypertrophy may have different causes and may need to be studied separately.

- Clinical trials can help define the dynamics of fat changes in people with HIV by objectively measuring fat at baseline and regular follow-ups.

- A longitudinal study of people with and without HIV could elucidate the evolution of fat changes with aging, HIV duration, and treatment. But such a study would take years.

- A better understanding of what defines HIV lipodystrophy may be reached by using the Case Definition Study lipodystrophy equation to evaluate FRAM participants with and without lipoatrophy.

References

Carr A. An objective case definition of HIV lipodystrophy. 9th Conference on Retroviruses and Opportunistic Infections. February 24-28, 2002a. Seattle. Abstract 31.

Carr A, Workman C, Smith DE, et al. Abacavir substitution for nucleoside analogs in patients with HIV lipoatrophy: a randomized trial. *JAMA* 2002b;288:207-215.

Chêne G, Angelini E, Cotte L, et al. Role of long-term nucleoside-analogue therapy in lipodystrophy and metabolic disorders in human immunodeficiency virus-infected patients. *Clin Infect Dis* 2002;34:649-657.

Domingo P, Matias-Guiu X, Pujol RM, et al. Switching to nevirapine decreases insulin levels but does not improve subcutaneous adipocyte apoptosis in patients with highly active antiretroviral therapy-associated lipodystrophy. *J Infect Dis* 2001;184:1197-1201.

Dubé MP, Zackin R, Tebas P, et al. Prospective study of regional body composition in antiretroviral-naive subjects randomized to receive zidovudine + lamivudine or didanosine + stavudine combined with nelfinavir, efavirenz, or both: A5005s, a substudy of ACTG 384. 4th International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV. September 22-25, 2002. San Diego. Abstract 27.

Grunfeld C. Basic science and metabolic disturbances. XIV International AIDS Conference. July 7-12, 2002. Barcelona. Presentation TuOr158.

John M, James I, McKinnon E, et al. A randomized, controlled, open-label study of revision of antiretroviral regimens containing stavudine (d4T) and/or a protease inhibitor to zidovudine/lamivudine/abacavir to prevent or reverse lipoatrophy: 48-week data. 9th Conference on Retroviruses and Opportunistic Infections. February 24-28, 2002. Seattle. Abstract 700.

Joly V, Flandre P, Meiffredy V, et al. Assessment of lipodystrophy in patients previously exposed to AZT, ddI, or ddC, but naive for d4T and protease inhibitors, and randomized between

d4T/3TC/indinavir and AZT/3TC/indinavir (NOVAVIR trial). 8th Conference on Retroviruses and Opportunistic Infections. February 4-8, 2001. Chicago. Abstract 539.

Kotler DP. Update on lipodystrophy . . . or is it just lipoatrophy? Medscape HIV/AIDS. 2002. http://www.medscape.com/viewarticle/439480.

Law M, Emery S, French M, et al. Lipodystrophy and metabolic abnormalities in a cross-sectional study of participants in randomized controlled studies of combination antiretroviral therapy. 2nd International Workshop on Adverse Drug Reactions and Lipodystrophy. September 13-15, 2000. Toronto. Abstract O28.

Maher B, Alfirevic A, Javier Vilar F, et al. TNF-alpha promoter region gene polymorphisms in HIV-positive patients with lipodystrophy. *AIDS* 2002;16:2013-2018.

Mallal S, Nolan D. Light and electron microscopy findings in subcutaneous fat in antiretroviral treated and naive HIV-infected patients. XIII International AIDS Conference. July 9-14, 2000. Durban. Abstract LpPeB7054.

Mallal SA, John M, Moore CB, et al. Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV infection. *AIDS* 2000b;14:1309-1316.

Nolan D, Moore C, Castley A, et al. Tumour necrosis factor alpha gene -238G/A promoter polymorphism associated with more rapid onset of lipodystrophy. 4th International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV. September 22-25, 2002. San Diego. Abstract 26.

Smith D, Carr A, Law M, et al. A randomized trial of thymidine analogue withdrawal in lipoatrophic HIV patients, virologically controlled on protease sparing therapy—the PIILR extension study. 1st IAS Conference on HIV Pathogenesis and Treatment. July 8-11, 2001. Buenos Aires. Abstract 96.