Facial Lipoatrophy Research Literature Review

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Introduction

In 1998, Carr *et al* described a novel side effect of antiretroviral therapy in human immunodeficiency virus (HIV) -infected adults [1] . In a cross-sectional study, they attempted to describe and characterize a syndrome they called peripheral lipodystrophy which, at that time, was associated with the use of protease inhibitors (PIs).

Lipodystrophy syndrome is the term used to define either of the two symptoms with which the syndrome is associated: *lipoatrophy*, or localized fat wasting in the face, arms, legs, and buttocks; and *lipohypertrophy*, or fat accumulation in the abdomen, breasts, or dorsocervical region [2, 3] . These symptoms rarely appear together, nor do they seem to have any definite correlation with each other [4] , and hence likely have different pathogenic mechanisms. In addition, lipodystrophy syndrome might be associated with other serious physiological effects, such as insulin resistance, hyperglycemia, hypertriglyceridemia, hypercholesterolemia, and low levels of high-density lipoprotein (HDL) [1, 5, 6] leading to an increased risk of cardiovascular complications.

Despite considerable effort, there is still no universally accepted clinical definition of HIV-associated lipodystrophy, and its evaluation in clinical trials has been largely based on subjective assessments,made by both patients and physicians rather than on more objective measures [7]; consequently, performing the necessary research that will generate a clear resolution to this problem, remains especially challenging. The Lipodystrophy Case Definition Study attempted to establish lipodystrophy as an equation, using laboratory testing, anthropometry and radiology data, but the equation proved to be too complex to use in clinical practice [8].

The prevalence of lipodystrophy is difficult to isolate, especially given the varying incidence of lipoatrophy and/or lipohypertrophy in the HIV-infected population. Studies have thus reported different estimate measures in different patient populations: a lipoatrophy incidence of 4% per year of antiretroviral therapy (ART) (5% for lipohypertrophy, and 1% for both) [9]; a one-year incidence of lipoatrophy of 22% [7]; a lipodystrophy prevalence of 46.1% and a lipoatrophy prevalence of 23.9% in 180 HIV-infected patients on highly active ART (HAART) in India [10]; and an increase in lipodystrophy from 8.3% to 41% over a two and half-year period in 95 HIV-infected men [3], to name a few.

Lipoatrophy, the fat loss component of lipodystrophy, has been described as the hallmark of body fat changes in HIV-infected people [11], and has been shown to compare with the volume loss seen in patients with HIV-related wasting [12]. Reported risk factors for lipoatrophy include: length of exposure to thymidine analogue use (especially stavudine [d4T]), age, disease severity, gender, body mass index (BMI), and Hepatitis C (HepC) co-infection [2, 7, [13-16]. More recently, the presence of elevated triglyceride levels has shown to be correlated with the ultimate development of facial lipoatrophy (FLA) [17]. In addition to its physical effects, manifested most obviously in the face, lipoatrophy also has a strong, complex psychological effect on those who suffer from it, causing social anxiety, feelings of depression, and other symptoms [18, 19] that could lead to reduced adherence and worsening health.

Pathogenesis

Suggested mechanisms for the development of lipoatrophy include impairment of adipocyte (fat cell) differentiation, adipocyte apoptosis (mediated by proinflammatory cytokines such as tumor necrosis factor [TNF]-¤), and mitochondrial toxicity [2]. Multiple studies have been conducted to examine these effects both *in-vivo* in HIV-infected patients, and *in vitro*, specifically with respect to PI and NRTI use. Significantly, many studies do not differentiate between the mechanisms associated with the development of lipoatrophy, and of lipodystrophy development in general.

A review of the pathogenesis of HIV-related lipodystrophy was presented at the 10th Conference on Retroviruses and Opportunistic Infections [20]. PIs have been linked to inhibition of adipocyte differentiation and insulin resistant adipocytes that are prone to apoptosis. In vitro, NRTIs decrease lipid content and increase apoptosis, possibly through mitochondrial dysfunction, and when added to PIs, reduce insulin resistance and increase apoptosis. Sterol regulatory element binding protein-1 (SREBP-1) and TNF-¤ are implicated in adipose tissue apoptosis and consequently, lipoatrophy. PIs might contribute to SREBP-1 dysfunction [20, 21] and TNF-¤ overexpression [22], and stopping PIs has been associated with a consequent decrease in TNF-¤ [23]. In a genetic case control study of patients with and without lipodystrophy, there was a significant difference in the frequency of polymorphism -238 in the promoter region of the TNF-¤ gene ($p=0.01$), suggesting that this polymorphism is a factor in the development of HIV-related lipodystrophy [24].

In a substudy of the TARHEEL study, researchers postulated that one of the pathogenic mechanisms underlying lipoatrophy in patients treated with stavudine (d4T), is increased adipocyte apoptosis driven by mitochondrial toxicity, therefore researchers collected fat tissue samples from a cohort of patients in the TARHEEL study at baseline, and 48 weeks after they had switched from d4T to abacavir (ABC) or zidovudine (ZDV) [25]. These samples were compared with uninfected control samples and apoptotic cells were analyzed. Baseline patient samples had a mean of 0.27 apoptotic cells per unit area, while controls had an average of 0.03 cells per unit area ($p<0.0001$). At 48 weeks, patient cells had decreased to an average of 0.1 cells per unit area, which was no longer significantly different from controls. In addition to this evidence, further analysis of the main TARHEEL study demonstrated a significant increase in fat mitochondrial DNA levels accompanying the reduction in apoptotic cells [26].

Although NRTIs preferentially inhibit HIV reverse transcriptase, they can also inhibit other DNA polymerases, such as mitochondrial DNA (mtDNA) polymerase gamma, which plays a role in mtDNA replication [27]. Recently, investigation into the role of mtDNA in the pathogenesis of lipoatrophy has increased considerably, and a substantial amount of research has been dedicated to uncovering how and why certain ARTs appear to effect mtDNA, whether this effect is multi-factorial, and identifying the consequences for HIV-infected individuals taking these ARTs. One study, reporting on factors associated with mtDNA depletion in adipose tissue of 52 HIV-infected and 9 non-infected individuals, found that only NRTI use was associated with mtDNA depletion, and that only d4T and ddI were significantly associated [27], thus mtDNA levels appear to be independent of age, sex, BMI, HepB or C co-infection, and HIV disease severity. This finding is in contrast to literature that has shown risk factors for the development of lipoatrophy as mentioned above [2, 7, 13-16].

Many studies have quantified mtDNA content in various settings, demonstrating that mtDNA is predictably decreased in patients taking NRTIs versus no-NRTIs [28], in patients with lipoatrophy versus no lipoatrophy [28, 29], and in HIV-infected patients with lipoatrophy versus negative controls [30] by a range of 39-50%. Lower mtDNA content per adipocyte has been correlated with severity of lipodystrophy [31].

Among NRTIs, zalcitabine (ddC), stavudine (d4T), and didanosine (ddI) are most associated with mtDNA depletion [27, 32]. Zalcitabine could be the strongest inhibitor of mtDNA synthesis in human (HepG2) hepatocytes *in vitro*, causing severe changes in the size and shape of cells and increasing intracellular lipids and lactic acid, preceded by a rapid decline in mtDNA, suggesting mtDNA depletion is central to the onset of cytotoxicity in these cells [33]. Stavudine and didanosine also cause these changes; however, Walker *et al* found the effects of stavudine to be much less pronounced than either ddC or ddI, even at levels 10 times the steady state peak plasma levels (Cmax) of d4T. Considering that d4T is one of the most commonly reported antiretrovirals associated with lipoatrophy, its lack of effect is puzzling, and could be due to the type of cell studied in this case, the fact that the study was *in-vitro* (rather than *in-vivo*), or the possibility that added to one or more ARTs, d4T demonstrates significantly increased potency and/or altered mechanism of action.

Importantly, no additions or deletions in the genetic make-up of mtDNA have been noted in patients with decreased levels of mtDNA [28, 29], suggesting that the effect generated by mtDNA is quantitative, and not related to any alteration of the mtDNA genetic code. Necessary research is ongoing to elucidate the precise mechanisms of lipoatrophy, so that treatments and preventative measures can be definitively established.

Lipoatrophy Assessment Methods

Assessment of lipoatrophy in regular clinical practice is made by patients' reports and doctors' confirmation of loss of subcutaneous fat [34]. In research, patients are often asked to rate the severity of their lipoatrophy through the use of scales,such as the analogic visual scale satisfaction index (AVSI) [35-37], Carruthers lipoatrophy severity scale and QoL questionnaires [36]. Three-dimensional photos are also commonly used to assess pre- and post-treatment efficacy [36-38]. Though interesting and often indisputable, the results generated by such methods have an inherent degree of subjectivity, and cannot be accurately quantified. Because objective measurement is so crucial to the integrity of research, studies have been conducted to assess the accuracy of objective measures of calculating body or facial fat changes over time in patients with ART-associated lipoatrophy.

Ultrasound has been used in lipoatrophy studies because it is simple, safe, and inexpensive [11], however one study found that it does not correlate significantly with more established measures of lipoatrophy severity [39], therefore caution must be taken when using this method of evaluation.

Surface laser imaging is being investigated as a tool to detect minor changes in the contours of the face, and is being used by both maxillofacial and plastic surgeons [40]. This technique produces a three-dimensional image of the face, is quick, and non-invasive, and does not require exposure to radiation [40]. Seventeen, HIV-infected men (with and without FLA) were enrolled in one study, consisting of two laser scans, performed one week apart, which were then superimposed to determine reproducibility. Differences between corresponding points of the scans are illustrated using different colours. Overall, there was a moderate to substantial level of agreement for all pairs and for all areas of the face, with a maximum difference

between the 2 scans for any one area varying between -0.26mm and +0.11mm [40]. The authors of this study report that surface laser imaging is an attractive option for measuring facial contour, but acknowledge its limitations, in that this method does not directly measure fat loss in the face. Also, the potential for other causes of change in facial contour should be noted, such as hydration, facial hair, ageing, and nutritional status [40]. A second study used a mannequin and plasticine and 10 healthy subjects to determine accuracy and reproducibility of laser scanning [41]. Accuracy of the method was determined using a mannequin and a series of increasing volumes of plasticine (0-5mL in 0.5 increments) to simulate changes in facial contour, specifically the cheek, while reproducibility was established with 10 healthy volunteers, each of whom had five scans on day 1, and one scan on days 2, 4, and 7. The volume change estimated by laser scanning corresponded closely with the actual volume change (mean difference of 0.08mL), with a small trend towards underestimation by laser scanning at higher values [41]. Variation in subject scans for day 1 ranged from -0.709mL to 0.404mL with an intraclass correlation coefficient (ICC) of 0.812, and for the week ranged from -1.760 to 0.817mL with an ICC of 0.764 [41]. The authors of this study propose laser scanning as one of the few methods having the precision necessary to measure very small changes in facial contour; however, scanning procedures, the method of image manipulation for analysis, and the area used for calculation of volume change must be standardized [41].

While three-dimensional CT scans of the thighs have shown suitability in the evaluation of lipoatrophy of thighs (stratified by sex) [42] this method has limited usefulness in FLA and is costly [11]. Measurement of Bichat's fat tissue, on the other hand, permits assessment of FLA, with a clear correlation with subjective reporting measures [43]. Dual-energy x-ray absorptiometry (DEXA) has been used to measure thickness of total, truncal and limb fat [44], making it a good candidate for measuring peripheral lipoatrophy in research [21], however it may be too complex for everyday clinical use [11]. Any of the aforementioned methods are potential tools for assessing lipoatrophy severity during a clinical trial, and each has benefits and disadvantages that must be weighed appropriately so that the most suitable method is chosen to ensure accurate and worthwhile results.

Psychosocial Impact

In addition to the physiological impact of lipoatrophy, especially when manifested as FLA, there is also a growing concern for the psychological effects of this condition. Several studies have specifically examined the psychosocial and quality of life (QoL) impact of lipodystrophy and lipoatrophy syndromes in patients with HIV [18, 19, [45-48], while other studies have examined the impact of corrective treatment on these same issues [37, 49, 50]. Other studies have collectively evaluated lipodystrophy syndrome as a whole and its effects on both QoL [51-53] and adherence to ART [54-56], with varying results.

Of interest, a qualitative, in depth analytical study (of seven HIV-infected adults with Lipodystrophy) reported that the response of health care professionals is an important factor in mediating the impact of this condition [48]. Santos, *et al*. recently reported that discrepancies between self-perceived body changes and physician evaluation of body changes exist, and are important to acknowledge when discussing options for treating and/or dealing with body fat changes [57]. They suggest that listening to the patient's concerns about ART-related body changes, and engaging the patient in discussions about the available options will contribute to increased adherence and improvement in QoL [57]. The role of the health care professional, in managing the impact of lipodystrophy (and lipoatrophy in particular) on the

HIV-infected patient, is not well established in the literature, and deserves further examination.

One study found a high correlation between severity of lipoatrophy, perception of quality of life, and a patient's subsequent level of social distress and depression, as measured by a 46-item questionnaire designed to measure anxiety, depressive symptoms, social distress, social support, quality of life, body image alteration, adherence, and changes in attitude towards drugs [19, 45, 46]. Depressive symptoms were not related to AIDS diagnosis, baseline CD4 count, family or work status, or patients' beliefs. This study also found that the women (n=10) were more preoccupied with body image, reflected in greater social distress, and that they exhibited more depressive symptoms than the men in the study (n=28) [45, 46].

Another study examining the psychosocial impact of facial lipoatrophy (FLA) in 55 patients on HAART (with and without FLA) found that those with FLA experienced significantly greater state-situational anxiety, bodily self-perception, and feelings of stigmatization than non-FLA patients [18]. And, in a study of 61 patients experiencing body shape changes, 91% of whom had FLA (77% with limb atrophy and 11% truncal fat accumulation), 65% of these patients found facial changes the most disconcerting aspect of body shape changes they were experiencing [47]. Seventy-one percent stated that the body shape changes produced new problems in social interactions, with 20% reporting a severe negative impact, and 46% stating they suffered from depression since body shape changes had occurred.

A study was conducted in Toronto, Ontario, to systematically examine the relationship of lipodystrophy experienced by HIV-infected patients to a range of standardized measures of quality of life and mental health [53]. Seventy-seven patients taking HAART, who had self-identified as having one or more components of lipodystrophy syndrome completed the following instruments. Three QoL measures were evaluated: the 34-item Medical Outcomes Study HIV Health Survey (MOS-HIV); the 34-item HIV/AIDS-Targeted Quality of Life (HAT-QoL) Instrument; and the HIV Overview of Problems – Evaluation System (HOPES) Physical Summary scale, Psychosocial Summary scale, Sexual Summary scale, and a distinctive Body Image subscale within the Psychosocial domain. Three mental health instruments were also assessed: the 'state' anxiety component of the Spielberger State-Trait Anxiety Inventory (STAI); the Rosenberg Self-Esteem Scale (RSES), and the Beck Depression Inventory, 2nd edition (BDI-II). The results of this study demonstrated a negligible effect between severity of lipodystrophy syndrome on QoL and mental health when assessed by conventional measures [53]. Lipodystrophy severity did, however, have a significantly negative effect on body image. The authors of this study propose that the measures used in the study are of insufficient sensitivity and specificity for responding to lipodystrophy-related effects [53]. The authors also acknowledge that the lack of impact of lipodystrophy on QoL and mental health observed in this study could be due to the patient population studied, which consisted of older, more HIV-experienced, adequately treated patients, and not those HIV-infected people with the most concerns about the effects of HAART who might not be seeking treatment [53].

Another study, conducted in Spain, found similar results to the previously mentioned study [51]. One hundred and fifty patients, who were clinically stable, with at least one year of HAART were enrolled in the study, which involved a structured interview and standardized questionnaires. No significant difference was observed overall between QoL and patients with or without lipodystrophy syndrome (self-reported clinical evaluation). When results were further stratified, significant differences appeared. Homosexual men and patients with current psychiatric treatment who also had lipodystrophy showed a greater impairment in QoL

due to greater impact on physical capacity (physical and intellectual functioning), psychological functioning, and negative mood. This study has limitations, however, and the authors suggest further research, paying special attention to vulnerable subgroups, should be performed to better elucidate the impact of lipodystrophy on QoL [51].

The positive effects of reparatory treatments have implications that reach beyond merely repairing undesirable facial features. Studies assessing psychological parameters of HIV-infected patients with FLA undergoing corrective treatment have documented an improvement in anxiety and depression scores [22] and in health perception, mental health, transitory health, and emotional status [50] post treatment.

A growing concern related to lipoatrophy is continued adherence to antiretroviral medications in light of stigmatization [58] or other negative psychological effects. Adherence can be influenced by a variety of factors that can be categorized as: person-associated; medication-associated; and provider-associated [54]. In a study of 74 HIV-infected patients of whom 90% were on ART, 30% had changed ART due to body shape changes and/or metabolic abnormalities, and 7% stopped ART altogether [59]. Fifty-seven percent of patients had thought about changing their medications and 46% said they would change if their symptoms worsened. Of those who had already changed their medications, 83% had switched only those they thought were responsible for causing the body shape changes, and 17% had changed all drugs. Altering medications or becoming non-adherent due to unwanted body shape changes can be dangerous and limit future treatment options. To ensure that these dangers are minimized, further research into exactly how ARTs specifically affect lipodystrophy and lipoatrophy, is crucial.

A larger US study of 165 HIV-infected patients with lipodystrophy, taking at least one ART for at least six months, evaluated whether social support, QoL, and comorbid medical conditions are related to ART adherence [54]. Sixty-seven percent of study patients reported comorbidities such as depression, diabetes, hepatitis, and hypertension. Regarding adherence: ~58% reported forgetting to take their medications; ~39% said they were careless about taking their medications; ~19% stopped taking their medications when they felt better; and ~27% stopped taking their medications when they felt worse. The authors found no significant relationship between adherence and: education; time since diagnosis; or lipoatrophy, but did find a significant relationship between adherence and: having children; and QoL (social support weakly significant) [54]. A significant relationship between mixed lipodystrophic syndrome (lipoatrophy and lipohypertrophy) and QoL, but not between lipoatrophy or lipohypertrophy alone, was also found [54].

Finally, a cohort study using merged data from AdICoNA and LipoICoNA substudies of the Italian Cohort Naïve Antiretrovirals (IcoNA) study evaluated the relationship between adherence to ART and adipose tissue alterations (ATA = lipodystrophy syndrome) [56]. During a median follow-up of 45 weeks, of 207 patients from 16 clinical centres, 18% developed ATA. Thirty-one percent of the 207 patients developed ATA within 104 weeks, with a prevalence of 43% among adherent patients, and 18% among non-adherent patients. Patients who were adherent to ART at baseline were more likely to develop body shape alterations ($p=0.03$), and median time to development of ATA was five times shorter in adherent vs. non-adherent patients. Patient-reported fat accumulation (hypertrophy), but not previous clinical diagnosis of ATA, and longer duration of ART use was independently associated with an increased risk of non-adherence [56], further emphasizing the importance of perceived body shape changes and their importance to ART adherence long term.

Management Options for Treating Facial Lipoatrophy

The detection and identification of lipodystrophy syndrome initiated and drove the impetus to discover treatment methods to counter its effects. During the past several years, new treatment methods (many of which have failed)have attempted to reverse, slow, and treat the consequences of antiretroviral therapy. The following section discusses methods that have been, and continue to be, studied; antiretroviral switching to halt or delay the onset of lipodystrophy syndrome; rosiglitazone, growth hormones, and uridine to treat lipoatrophy; and cosmetic treatments such as poly-L-lactic acid (NewFill/Sculptra), polyalkylamide gel (BioAlcamid), silicone oil (e.g. Silskin), autologous fat transfer, and hyaluronic acid (e.g. Restylane) to treat facial lipoatrophy.

Antiretrovirals and Lipoatrophy

The MITOX study was the first randomized study to demonstrate statistically significant increases in limb fat after 24 weeks in 105 patients who were switched to abacavir (ABC) from either d4T or zidovudine (ZDV), versus those who remained on their NRTI regimen (control group) [16]. Quality of life, assessed by both patients and physicians, did not change significantly from baseline through 24 weeks, however, and change in limb fat mass did not correlate with change in perceived lipoatrophy severity [16]. At 104 weeks of follow-up, ABC patients had a clinically significant mean increase in limb fat of 1.26 +/- 2.02kg (35%) vs. the ZDV/d4T arm who had a clinically significant mean increase of 0.49 +/- 1.38kg (13%) [60]. In addition, the ABC group had a significant improvement in subjectively assessed lipodystrophy through 72 weeks of follow-up, while the ZDV/d4T group did not [60].

Another study involving a switch to ABC was the previously mentioned TARHEEL study, where 118 patients receiving d4T-containing HAART for at least 6 months and who had lipoatrophy were switched to either ABC or ZDV-containing HAART [44]. After 48 weeks, increases in fat percentages from baseline were: 35% in arms, 12% in legs, and 18% in trunk ($p<0.0001$), with more fat gain observable in the arms and legs of patients who switched to ABC. Although not objectively measured, 27% of patients reported, in their answers to a body image questionnaire, that they "gained some/a lot" of facial fat at both 24 and 48 weeks, while 76% stated "no change or gain" in facial fat at 48 weeks [44]. Encouragingly, viral load suppression and CD4 count increases were maintained throughout the duration of the study. In a substudy of the TARHEEL study, reversibility of mitochondrial abnormalities in 16 patients who had during three years of d4T experience at baseline were examined [28]. Mean mtDNA/cell was measured in peripheral blood mononuclear cells (PBMCs), muscle tissue, and adipose tissue at baseline and week 48. Mean mtDNA levels were 64, 2305, and 194 at baseline in PBMCs, muscle tissue, and adipose tissue, respectively, and rose at 48 weeks to 256($p<0.0001$), 3754 ($p=0.11$), and 430 ($p=0.01$) [28]. No large mitochondrial genomic deletions or rearrangements were detected.

One study reduced the dose of d4T (rather than discontinuing it altogether) in patients on HAART who had developed lipoatrophy [61]. Forty patients taking 40mg BID were reduced to 30mg BID, and 40 patients taking 30mg BID were reduced to 20mg BID. All patients showed improvement in lipoatrophy and remained suppressed virologically up to 86 weeks [61], suggesting a potential role for therapeutic drug monitoring in the minimization of lipoatrophy. Recently, a study was conducted comparing regular d4T dose, reduction of d4T dose, and switching to tenofovir (TDF) (a non-thymidine NRTI), while maintaining all other ARTs, to

determine which method had a better efficacy and safety profile [62]. Results after six months indicate that switching to TDF caused a greater increase, in total and limb fat, than either of the d4T groups, and a greater decrease in triglycerides and total cholesterol than either d4T group, while maintaining equivalent viral control.

In initial therapy, newer NRTIs, such as tenofovir, cause less lipodystrophy than d4T [21]. In a study of d4T/lamivudine (3TC) vs. TDF/3TC in 262 ART naïve patients, TDF/3TC patients had 2.9kg and 4.1kg more limb fat after 96 and 144 weeks, respectively, vs. the d4T/3TC group [63]. A recent study compared ABC to tenofovir in patients taking d4T or ZDV containing ART regimens with moderate to severe lipoatrophy [64]. One hundred and five patients were randomized to switch from their tNRTI-containing regimen to either ABC or tenofovir for 48 weeks. Both ABC and tenofovir demonstrated similar increases in body fat and maintenance of viral suppression, however tenofovir had fewer discontinuations and a greater safety profile with respect to lipid parameters than ABC [64], thus tenofovir switching might be a good alternative for patients who have hypersensitivity reactions to ABC or who have abnormal lipid levels.

Despite early indications that PIs were mainly responsible for lipodystrophic effects, switching protease inhibitors has been shown to have little, if any, impact on body fat [11]. In the PI Induced Lipodystrophy Reversal Study (PIILR), patients were randomly assigned to either discontinue PI therapy and start ABC, nevirapine, adefovir, and hydroxyurea, or continue PI therapy for 24 weeks [65]. Patients continuing PI therapy at week 24 were offered the non-PI switch regimen after 24 weeks. Eighty patients were enrolled in the study, with 49 assigned to the switch group and 31 assigned to the PI group. Quality of life (QoL) was significantly greater in the switch group at 24 weeks, with a significant decline in the PI group's QoL [65]. No significant difference in viral load was seen between the 2 groups, however CD4 counts declined significantly in the switch group after the addition of hydroxyurea at 4 weeks. A greater decrease in total body fat, weight, and total lean mass was seen in the switch group at 24 weeks, despite an improved perception of lipodystrophy severity, including lipoatrophy, as assessed by the patients [65]. Although this study was meant to assess the effect of stopping PI-containing ART regimens, the switch group also discontinued all ART they had been taking prior to the study. Eighty-four percent of patients in the PI group and 80% of patients in the switch group, were taking d4T at the time of enrolment, therefore the potential for confounding results with the discontinuation of d4T in the switch group and continuation of d4T in the PI group is likely. In fact, in a long-term follow-up of the PIILR study group, to 120 weeks, on-study usage of d4T was independently and significantly correlated with both decreased limb fat mass and a higher lipodystrophy case definition score [66].

Although switching of antiretroviral therapy associated with lipoatrophy has shown effectiveness in some studies, especially with respect to switching d4T to ABC or TDF, it is important to weigh the risks of switching against the potential benefits. In the MITOX study, for example, 10% of patients who were randomized to switch to ABC experienced a hypersensitivity reaction [20], and TDF has been associated with renal toxicity in some cases, though the severity of this toxicity varies [67-69]. Other authors have cautioned that switching from d4T to ABC or ZDV could include a tradeoff in adverse events such as hypersensitivity reactions (ABC) or anemia (ZDV) [44]. In a study where patients were given SQV, RIT, efavirenz, and nevirapine, 9 of 21 patients discontinued treatment due to rash (NVP) or SQV intolerance [70]. Also, several authors have suggested that although lipoatrophy can be improved by modification of ART, the recovery process might be slow if it occurs at

all, especially in patients who have been taking the lipoatrophy-causing drugs longer [21, 44, 61]. Fortunately, whether progression of lipoatrophy can be stopped or not, there are treatments available that can help correct lipoatrophy, specifically FLA, one of the most distressing lipodystrophic symptoms [47].

Pharmacological Correction of Facial Lipoatrophy

Other methods that have been suggested for the treatment of lipoatrophy include: rosiglitazone [71-73], growth hormones [11, 74, 75] and uridine [76-78], though none of these methods has yet shown incontrovertible proof of safety and/or efficacy.

Rosiglitazone, an insulin-sensitizing anti-diabetic agent, has been investigated as a potential treatment for ART-associated lipoatrophy, due to its side effect of weight gain in patients with diabetes [21]. Studies conducted with rosiglitazone on patients with ART-associated lipoatrophy have not demonstrated any significant and prolonged increase in body fat [72, 73], however the lack of effect seems to be related to continued exposure to the lipoatrophy-associated ARTs [11, 73, 78]. In addition to its lack of effect, rosiglitazone has also been shown to increase serum lipids such as triglycerides and cholesterol [73], therefore even its use in patients who have switched from lipoatrophy-associated ARTs might be limited. Growth hormones, in various formulations, have also been investigated for the treatment of lipoatrophy in HIV-infected patients taking ART. One study investigating Serostim, a recombinant growth hormone, in 20 patients with moderate to severe FLA for six months noted an objective improvement in FLA (by CT scan) from 4.5mm to 5.4mm at the level of the maxillary antrum, and significant weight gain and improvement in QoL (questionnaire) in patients by month 3 of the study [74]. Side effects included mild arthralgia (40%), and enlarged mammary glands (15%). Another study investigated the use of growth hormone-releasing hormone in 31 patients in a 12 week study and found improved lipoatrophy and reduced visceral fat without glycaemic effects [75]. Despite the apparent effectiveness of Serostim and growth hormone-releasing hormone for the treatment of lipoatrophy, most studies using recombinant growth hormones have investigated their use in the treatment of fat accumulation, demonstrating efficacy, if not safety, in reducing visceral fat [79-81]. Due to the ambiguous effects of recombinant growth hormones, they are not recommended for the treatment of lipoatrophy [11].

Walker, *et al* have suggested uridine as a potential therapy for mtDNA depletion [76, 77], which could result in the correction and/or prevention of ART-related lipoatrophy, if mtDNA depletion proves to be a causative factor in the development of lipoatrophy. Uridine is a nucleoside manufactured by the mitochondria that is needed to produce glycogen and is essential for the synthesis of DNA and RNA [76]. Walker, *et al* evaluated whether uridine might be protective against mitochondrial toxicity in human liver cells (HepG2 cells) exposed to NRTIs [35]. NRTIs with or without uridine were added to HepG2 cells in concentrations corresponding to Cmax in humans during ART. Uridine + NRTI maintained normal cell function and protected against mtDNA depletion, maintaining it at about 65%, whereas NRTI exposure alone severely impaired cell function, preceded by a steep and rapid decline in mtDNA/cell. When added to NRTI-containing, mtDNA-depleted cells, uridine improved cell growth, lactate production, and mtDNA levels.

One patient taking d4T, 3TC, ABC, and efavirenz who developed myalgias, elevated CK, lactate and transaminases, and liver steatosis was given NucleomaxX, a commercially available source of uridine, threetimes a day for four days [77]. After two weeks, the

patient's liver and muscle enzymes and myalgias had improved, with no medication changes. Lactate normalized after seven weeks, and subsequently d4T was replaced with tenofovir. No subsequent liver or lab abnormalities were observed, and viral control remained adequate.

The results of two studies evaluating the efficacy and safety of uridine, supplied in the form of NucleomaxX, were recently presented at the 10th European AIDS Conference in Ireland [82, 83]. The first study was a three-month, double-blind, placebo-controlled trial to evaluate uridine vs. placebo in patients taking a stable d4T- or AZT containing regimen [82]. Uridine, supplied in the form of NucleomaxX, or placebo, was given to 18 patients for three months, for the first 10 days of each month, at a dose of 36g three times a day [17]. Limb fat in the NucleomaxX group rose from 3370g (+/-890g) to 4260g (+/-940g) ($p<0.05$), though patients did not subjectively notice a change [17]. Total body fat and portentous intra-abdominal fat also increased significantly ($p<0.01$, $p<0.05$ respectively) [17]. The second study involved 16 people taking a d4T-containing regimen, and was an open label uncontrolled study [83].
Patients took NucleomaxX, 36g three times a day, every other day for 16 weeks, and then were followed without NucleomaxX for a further 16 weeks [17]. Fourteen people completed the trial, and all patients subjectively reported improvements in lipoatrophy ($p<0.05$) and lipohypertrophy at weeks 16 and 32 [17]. Physicians reported similar findings, however limb and visceral fat changes were not objectively measured during the study [17]. NucleomaxX was not found to significantly affect total cholesterol, non-HDL cholesterol, lactates, glucose, insulin, CD4 count, or viral load [17].

Uridine, in the form of NucleomaxX, has shown improvement in peripheral, abdominal, and total body fat, though is relatively expensive; thus, research will continue to determine whether a lower dose will generate equivalent results [17].

Cosmetic Correction of Facial Lipoatrophy

Several treatment options have been proposed for the correction of facial lipoatrophy. Two of the methods used most often because there is evidence of both safety and efficacy are permanent and non-permanent fillers. Permanent fillers are non-biodegradable so they do not diminish over time and may or may not be removable in the event they are no longer needed, are improperly injected, or in case of allergic reaction [21]. Non-permanent fillers are biodegradable and hence diminish over time, requiring occasional retouching, but do not form permanent foreign body reactions or granulomas and misplaced injections will not cause permanent damage [21].

Poly-L-lactic Acid

Poly-L-lactic acid (PLA), known as New-Fill in Europe, and Sculptra in the U.S., has been approved in Europe since 1999 for the cosmetic correction of scars and wrinkles [21], and was recently approved by the FDA for the treatment of HIV-related facial lipoatrophy [84]. PLA is a biodegradable, bioabsorbable, aliphatic polyester produced by carbohydrate fermentation of corn dextrose [85]. It is also immunologically inert and free from toxicity [86]. When injected, fibrous connective tissue forms around it, causing cutaneous thickening over a period of months. Initially, the effect seen is due to the water injected along with the PLA, but over the first week the water absorbs, leaving the connective tissue to build up and produce the final results [85].

A report on one physician's five-year experience with PLA cautions that complications, such as granulomas, are due mainly to the superficial nature of the injections; the deeper the injection, the fewer complications there are [86]. This author also suggests diluting with 5mL rather than 3mL for increased safety, and avoiding injections around the lip, lower eyelid, and glabella. Both the safety and efficacy of poly-L lactic acid has been evaluated in short and long-term studies [22, 36-38, 85, [87-89] in more than 350 HIV-infected patients (mainly male) with facial lipoatrophy, with positive results. The benefits of this treatment method could be limited by the fact that the procedure is costly, multiple treatment sessions are required to achieve the desired results, and results are temporary [90], which might also serve as a benefit in the event lipoatrophy is reversible, at least in some part.

In a small study, 30 HIV-infected patients with FLA were randomized to immediate (week 0, 2 and 4) or delayed (week 12, 14 and 16) treatment with PLA, with a followup period of 24 weeks [91]. The delayed study design was intended to assess temporal association between treatment and improvement in both objective and subjective outcomes. Evaluations included fasting bloodwork, standardized facial photography, facial ultrasound, visual analogue scale (designed specifically for the study), and the Hospital Anxiety and Depression Scale (HADS), to assess anxiety and depression, at weeks 0, 12, and 24 [91]. Ultrasound studies demonstrated increased dermal thickness in the treated areas at week 12 for the immediate and week 24 for the delayed treatment group, and median visual analogue scores increased with treatment, from 2 at baseline to 7 at week 12 and 6 at week 24 for the immediate treatment group, and 1 at baseline and week 12 to 7 at week 24 for the delayed treatment group [91]. Anxiety and depression scores decreased during the study, with a trend towards a difference between the two groups at week 12 ($p=0.056$). Photograph scores also trended towards a difference at week 12, but no difference was noted between baseline and week 24. Two adverse events were noted in this study; bruising and superficial local cellulitis not requiring antibiotic therapy [91].

The VEGA study was an open-label, single-arm, pilot study to evaluate the efficacy, safety, and durability of PLA in the correction of FLA in HIV-infected patients over 96 weeks [89]. This study included 50 patients with severe lipoatrophy who had been taking antiretroviral therapy for more than 3 years. Patients were injected with PLA over 6 weeks, at 2 week intervals. Evaluations included clinical examination, facial ultrasonography, and photographs at screening, and at week 0, 24, 48, 72 and 96. Patient quality of life (QoL) was measured by visual analog scale (VAS) at the same time points. The proportion of patients with a total cutaneous thickness of at least 10mm at week 96 was 43%, down from 61% at week 48 and 52% at week 72 [89]. Increases were significant at all time points. Quality of life increased throughout the study, but was particularly high at week 24 and 48 (+0.8). No serious adverse events were observed, and no interruptions in treatment occurred due to side effects [89].

Two of the larger studies using PLA treatment in HIV-infected patients with FLA involved almost 100 patients each [36, 87]. The first study had a follow-up period of 6 months and included 96 patients [36], while the second had a 12 month (average) follow-up of 94 patients [87]. Patients in the 6-month study rated the severity of their FLA at baseline from 1 (mild) to 5 (severe). The mean rating pre-treatment was 3.4. Post-treatment, the mean rating was 1.3. Six months after the final treatment (average 1-6 injections per patient, given every 3 weeks), the severity rating was maintained at 1.6. Patients rated their satisfaction with the outcome on a scale from 1 (very dissatisfied) to 5 (very satisfied), with the mean being 3.9. Adverse events included mild to moderate pain (28%), mild transient bruising (15%), and small, nonvisible palpable subcutaneous nodules (56%). There were no serious adverse events

(SAEs). Efficacy in the 12-month study was more elaborate, and involved a quality of life (QoL) questionnaire, analogic visual scale satisfaction index, and 3-D photos of 50 of the patients' faces [87]. The median number of injections per patient was 5, with injections given every 15 days. The median analogic visual scale improved from 3.4 at baseline to 6.8 after completion of injections, and was maintained at 7 seven-and-a-half months later. Median dermal thickness increase in both cheeks was 2.3mm 7 months after the last injection. Interestingly, no significant variation in QoL was observed. Adverse events included malaise after the 1st injection (7%), grade 1 or 2 pain (80%), non-inflammatory small nodules (11.7%), and minor bleeding (4%). One patient suffered an anaphylactic reaction. New injections were performed in 17/87 patients during follow-up (after a minimum of 3 months post-treatment), and the probability of requiring additional injections up to 15 months post-treatment was 45% [88]. Results lasted an average of 18 months [85].

A more recent article details results of a revised method of preparing and delivering PLA, in order to reduce nodule formation, as noted in the aforementioned trials [92]. This trial involved only 14 patients, but tested PLA using a deeper subcutaneous injection and greater dilution. Results demonstrated similar efficacy as previous studies, but showed markedly improved tolerability and minor nodule formation [92]. The positive effects of PLA injections were especially marked in patients who had mild lipoatrophy, and who had suffered from it for a comparatively short period of time. In this study, treatment intervals were recommended every 2-3 weeks [92].

Polyalkylamide Gel

Bio-Alcamid™ (Polymekon), or polyalkylimide gel (PAG), is a non-reabsorbable polymer derived from acrylic acid that has been shown to have no significant effects on cell morphology [93]. It is biocompatible, non toxic [94], and non allergenic [95]. It is anchored to tissue by connective fibres, as with PLA, and is removable from tissue months or years after implantation [96]. Its implantation involves the use of a 3mm luer-lock syringe with a 2-3mm diameter injection needle, local anaesthetic, and injection of Bio-Alcamid using a 2-3mm diameter cannula and the retrograde technique [97]. Bio-Alcamid is a novelty in the field of reconstructive and cosmetic surgery, and can be described as an "injectable endoprosthesis" [95]. Its biocompatibility, physicochemical, and mechanical properties make it a candidate for wider applications, such as drug delivery system or tissue engineering [94].

The Clinic'estetica™ in Mexico has used Bio-Alcamid in more than 450 HIV-infected patients since its introduction at the clinic in December 2002 [98]. Extended follow-up of 53 patients has shown ongoing product safety, with no evidence of rejection, migration, toxicity, chronic inflammation or granuloma formation. Removal of Bio-Alcamid is possible, and can be performed for worsening lipoatrophy and consequently visible implants, or for objective or subjective overcorrection [98].

Plastic surgeons in France, with four years of experience using Bio-Alcamid, suggest that the product is of great importance in patients with HIV-related FLA [97]. A multicenter study in Italy, where Bio-Alcamid was developed, of 2000 patients with serious aesthetic defects (not related to HIV medication) demonstrated excellent results, with no migration, dislocation, granuloma, allergic response or intolerance [95]. In addition, only 12 of the 2000 patients had post-operative staphylococcus infections, and only 3 of those 12 cases could be attributed to the implant [95]. A second study was performed by some of the same investigators, of 73 patients with HIV-related FLA, and 3 years of follow-up has demonstrated excellent aesthetic

results, as assessed by both patients and physicians [99]. Encouragingly, again, no implant dislocation, migration, granuloma, allergic reactions, or intolerance has been reported in these patients [99].

A small pilot study was conducted in Toronto, Ontario, at the Maple Leaf Medical Clinic, to assess the safety, efficacy, and impact on QoL of Bio-Alcamid in the treatment of HIV-positive patients with Grades 1-4 facial lipoatrophy (based on the Carruther's Scale) [100]. Five males (one Grade 1, one Grade 3, and three Grade 4 FLA) were treated one to two times with Bio-Alcamid within a sixweek period. Seven days after the first treatment, three patients were rated a Grade 0 (no lipoatrophy), and two patients were rated a Grade 1 (slight lipoatrophy). Prior to the week sixtouch-up treatments, four patients were rated a Grade 0, and one was graded a Grade 1. Adverse events noted in all patients were pain and swelling lasting a maximum of three days, and in four patients were bruising and redness lasting a maximum of six and two days respectively. There were no infections. Improvement in QoL approached, but did not reach, significance due to the small number of patients in the study [100].

Autologous Fat Transfer

Autologous fat transfer has been used as an FLA corrective method. This procedure does, however, have several limitations in that transferred fat can be lost by the same method as the initial fat loss, and lumpiness or "hamster cheeks" can become a problem [84, 101]. Fat transplantation is also more invasive than injectable fillers, requiring general anaesthesia, hospitalization and prolonged recovery [21].

Coleman lipostructure is a method of performing autologous fat transfer for the enhancement of facial contours [102], first proposed by Sydney R. Coleman in 1987 [103]. An open-label study of 33 consecutive HIV-infected patients undergoing Coleman lipostructure was designed, to evaluate the efficiency of the technique in HIV-infected patients with FLA 1 year after surgery [102]. Approximately 40ml of abdominal subcutaneous adipose tissue was harvested where possible, centrifuged (purified), and transferred to a 1mL luer-lock syringe for implantation [102]. Patients left the hospital after six hours of observation without a facial bandage, with analgesic medications, but with no antibiotic prophylaxis. Lipostructure success was assessed by agreement of three independent specialists blinded to patient information and a self-administered quality of life questionnaire oneyear after surgery. FLA was improved in 12 patients, as judged by all three evaluators, 17 patients by at least two evaluators, and 25 patients by at least one evaluator [102]. Quantity of fat (higher) injected and baselines triglyceride levels (lower) were significantly associated with improvement of FLA, as well as age (younger) and gender (women), that tended to have more positive, though non-significant, results [102]. Eighty-five percent of patients completed the questionnaire, with 43% being very satisfied, 50% being partly satisfied, and 7% being dissatisfied with the results. The technique was, overall, deemed to be both safe and reliable, however cannot be used for all patients with FLA because it is dependent on fat availability [102].

Combined Treatment Evaluations

Several studies have used a combination of treatment methods to compare materials for the treatment of FLA in HIV-infected patients. One study compared autologous fat transplantation (AFT), PLA, and polyalkylimide gel (PAG) in 91 patients with moderate to severe HIV-associated FLA [104]. Eleven patients received AFT, 31 received PLA, and 49 received PAG. Forty-seven percent of patients had reached week 48 at the time of presentation at the 15th

International AIDS Conference. Seventy-five percent of the AFT group, 75% of the PLA group, and 20% of the PAG group needed additional infiltrations at 48 weeks. No SAEs were reported, and QoL improved significantly in all groups relative to baseline. All methods were considered safe, however PAG showed better clinical results by week 48. No further follow-up of this cohort is currently available.

Another study involved autologous fat transfer (N=24), injections of reabsorbable PLA (N=20), and non-reabsorbable polyacrylamide hydrogel (PAAG) (N=15) [101]. Fat transfer patients were allocated to that arm because of sufficient residual subcutaneous fat in the abdomen or dorso-cervical region to accommodate such a transfer, while patients were blindly assigned to either PLA or PAAG. The primary endpoint was measurement of Bichat's fat pad region 24 weeks after the last treatment. Secondary endpoint included body image evaluation (ABCD questionnaire), facial aesthetic satisfaction (Visual Analog Scale), and aesthetic pre- and post-picture comparisons by independent reviewers. Mean change in dermal and subcutaneous thickness was similar across groups, but body image evaluation was poorer and there were four SAE's in the fat transfer arm [101]. The authors recommend longer follow-up to determine the most durable and suitable treatment.

Recently, at the 7th International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV, Guaraldi, *et al* presented interim results of a study comparing PLA with PAAG for the treatment of FLA [105]. At the time of presentation, 41 patients were 1 year post-treatment (n=17 PLA, n=24 PAG). Visual analog scale satisfaction had increased in the PLA group from 3.42 (+/-3.15) to 7.16 (+/-2.29) ($p<0.0001$), and from 2.59 (+/-1.94) to 7.04 (+/-1.58) ($p<0.0001$) in the PAG group, with no significant differences between the two groups observed. Depression scores and psychological and social distress measures had improved significantly, and body image satisfaction improved (non-significantly) in both groups as well, with no significant differences observed between groups. Quality of life measures of role function, vitality, health distress, and cognitive function improved non-significantly in both groups, and the authors suggest that new instruments are needed to better analyze the role of lipodystrophy [105].

Hyaluronic Acid

Hyaluronic acid (Restylane), which is approved for the treatment of wrinkles, has been used in five patients at the Sunnybrook Department of Dermatology in Toronto, Ontario, for the treatment of grade 2-3 FLA [106]. Patients received approximately 5-6cc total in the malar area of the face via intradermal injection, with no adverse events experienced, and high patient satisfaction up to 6 months. Studies with nonanimal hyaluronic acid for the treatment of HAART-associated lipodystrophy are being planned in Europe [21].

Silicone Oil

Silicone oil, approved by the FDA for retinal reattachment in 1994, has been investigated in the treatment of FLA in the US and Canada, though it is not approved for this indication in either country [107]. Only one published trial of HIV-related FLA is available for review. Patients with non-HIV related FLA have been treated with injectable liquid silicone, with good results [108]. In the authors' practices, 415 patients have been treated with silicone oil during a four-year period, prompting further evaluation of 77 of these patients [108]. Patients were given topical anesthetics prior to each treatment session, and then microdroplets of silicone oil (0.01ml) were injected at 2 – 4mm intervals into the subdermal plane or deeper.

Treatments were given at a minimum of one month apart, to allow time for connective fibers to develop around each silicone droplet. Patients were considered "complete" when both physician and patient deemed the patient's appearance to be similar to their pre-lipoatrophy state. No adverse events (i.e. post-treatment pain, erythema, or edema lasting more than three days or ecchymosis lasting longer than two weeks) were noted after any treatment. Most patients experienced mild to moderate discomfort, with only 5% or less requiring pre-treatment analgesia beyond topical anesthetics. Patients continue to be followed for side effects associated with injectable silicone, such as cellulitis, nodule formation, ulceration, or migration, so far none of which have occurred.

In patients who have had temporary augmentation, permanent silicone correction is preferred by both patient and physician [108]. In this study, the authors were able to determine a method of predicting the amount of silicone necessary to correct the effects of FLA, using the Carruthers lipoatrophy severity scale rating [107]. For each point of severity on the Carruthers scale (1-4), a patient will need, on average, three treatments utilizing 2ml of injectable silicone per treatment [108], and though this is only a guideline, it could assist in estimating the cost of such treatments were they to become widely available.

Conclusions

Lipoatrophy has a strong, complex psychological effect on those who suffer from it, causing social anxiety, feelings of depression, and other symptoms [18, 19] that could lead to reduced adherence and worsening health.

Though it has been shown that the progression of lipoatrophy can be slowed or stopped by switching from lipoatrophy-associated antiretrovirals, it is unclear whether there is an irreversible component to it [34]. Reversal of the symptoms of lipoatrophy appears to be a slow and most likely incomplete process, therefore avoidance of lipoatrophy would be preferable to treating it, however the pathological process still needs to be definitively established [21]. In light of the incomplete understanding of the pathology of lipoatrophy, new treatments have become available to cosmetically correct the facial abnormalities produced by lipoatrophy with fillers [20]. These fillers can improve both the physical disfigurement and psychological damages caused by lipoatrophy; [22] and until effective prevention or reversal treatments are available, they are currently the most promising treatment method [20].

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