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Feature Review

HIV-associated lipodystrophy: from fat injury to premature aging

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Combination antiretroviral therapy (cART) against HIV infection dramatically reduces AIDS-related morbidity. However, many patients under cART display HIV-associated lipodystrophy. Moreover, some develop early age-related comorbidities. Thymidine analog reverse transcriptase inhibitors (tRNTIs) are mainly responsible for peripheral lipoatrophy, and protease inhibitors (PIs) for fat hypertrophy and metabolic complications. Long-term HIV infection probably also causes fat alterations. Severe mitochondrial toxicity and oxidative stress cause lipoatrophy, whereas the hypertrophy of upper body fat depots could result from mild oxidative stress, cortisol activation and inflammation. The metabolic complications associated with lipodystrophy are responsible for increased cardiovascular and hepatic risks and could also participate in premature aging. We propose that adipose tissue injury by HIV and cART induces fat hypertrophy or atrophy and contributes to premature aging.

From HIV infection to treatment-related complications

In the 1980 s and 1990 s, HIV infection led to a devastating burst of morbidity and mortality. However, the introduction of nucleoside analog reverse transcriptase inhibitors (NRTIs) and viral PIs rapidly decreased virus-linked mortality and morbidity (Table 1). However, soon after the introduction of PIs in 1996, patients developed a syndrome of fat redistribution with peripheral loss and central gain, generally associated with metabolic abnormalities and insulin resistance [1]. In the early 2000 s, about half of HIV-infected patients were diagnosed as lipodystrophic (20–80%) [2]. Several *in vitro, ex vivo* and *in vivo* experiments have since shown that some drugs from the two classes are directly toxic to adipose tissue and could act in synergy to produce complex clinical and biological alterations [3–5]. Here, we provide some new hypotheses on the pathophysiology of fat redistribution and propose that mitochondrial toxicity is involved not only in lipoatrophy but also in fat hypertrophy. Even though direct studies on visceral adipose tissue (VAT) alterations in HIV-infected patients are scarce, the increased inflammation and activation of the cortisol system could be involved in VAT hypertrophy. We also propose that HIV-associated lipodystrophy is a feature of age-related fat redistribution that could amplify age-related comorbidities and lead to an earlier occurrence.

HIV-associated lipodystrophy is still a problem even though the probability of developing lipoatrophy has decreased in western countries as the pattern of cART prescription has significantly changed [6]. Indeed, fat gain is frequently observed after cART initiation [6]. Long-standing lipoatrophy is only partially and slowly reversible, and abdominal lipohypertrophy remains frequent, leading to increased cardiometabolic risks. Moreover, stavudine, considered responsible for the most severe lipoatrophies, is widely used as a first-line treatment in resource-limited settings and leads to lipodystrophic syndromes associated with a deleterious metabolic profile. Long-term-treated adolescents or young adults perinatally HIV-infected also display characteristics of lipodystrophy [7].

In western countries, long-term HIV-infected patients encounter several age-related comorbidities earlier than the general population [8]. These patients display signs of premature aging that are probably caused by HIV infection and some antiretrovirals. We consider several questions in this review: (i) what is HIV-related lipodystrophy: are lipoatrophy, lipohypertrophy and metabolic complications linked or independent, and are they reversible; (ii) how can fat injury result in opposing phenotypes: some depots are atrophic and others are hypertrophic; (iii) why do some HIV-infected patients receiving antiretrovirals display severe lipodystrophy, whereas others are spared; and (iv) is there a link between lipodystrophy and long-term complications, including premature aging?

Glossary

Hepatic steatosis: the accumulation of lipids, mainly triglycerides, in hepatocytes
Laminopathies: genetic diseases resulting from mutations in *LMNA*, the gene encoding lamin A/C. Some laminopathies alter adipose tissue, glucose and lipid metabolism and result in premature aging
Lipomatosis: localized fat tumors, affecting mainly proximal limb areas and the neck in the familial lipomatosis, that are sometimes associated with mutations in mtDNA (i.e. *MERRF*)
M1 macrophages: the stimulation of macrophages with Th1 cytokines such as interferon-γ or LPS promotes the maturation of 'classically' activated macrophages called M1 macrophages, which have high inflammatory potential
M2 macrophages: the activation of macrophages with Th2 cytokines, such as IL-4 and IL-13, promotes the 'alternative' activation of macrophages, called M2 macrophages, which function in tissue repair and remodeling

Table 1. Antiretrovirals drugs and their effects on fat and metabolism[*]

Class	Molecule	Abbreviation	Lipoatrophy	Lipohypertrophy	Dyslipidemia	Insulin resistance
NRTI	Stavudine	D4T	+++	++	++	++
	Zidovudine	AZT, ZDV	++	+	+	++
	Didanosine	ddI	+/−	+/−	+	+
	Lamivudine	3TC	0	0	+	0
	Abacavir	ABC	0	0	+	0
	Tenofovir	TDF	0	0	0	0
	Emtricitabine	FTC	0	0	0	0
NNRTI	Efavirenz	EFV	+/−	+/−	++ increased HDL	+
	Nevirapine	NVP	0	0	+ increased HDL	0
PI	Ritonavir	RTV	+/−	+	+++	++
	Indinavir	IDV	+/−	+	+	+++
	Nelfinavir	NFV	+/−	+	++	+
	Lopinavir	LPV	+/−	+	++	++
	Amprenavir Fosamprenavir	APV FPV	+/−	+	+	+/−
	Saquinavir	SQV	+/−	+	+/−	+/−
	Atazanavir	ATV	0	++	+/−	0
	Darunavir	DRV	0	+	+/−	+/−
Fusion inhibitor	Enfuvirtide	T20	?	?	0	0
CCR5 inhibitor	Maraviroc	MVC	?	?	0	0
Integrase inhibitor	Raltegravir	RAL	?	?	0	0

A combination of different classes of drugs controls HIV infection in about 90% of patients. However, it is not possible to cure HIV infection. Two widely used thymidine analogs from the first class of antiretrovirals, stavudine (or d4T) and zidovudine (or AZT or ZDV), inhibit viral multiplication; however, owing to toxicity, in particular in adipose tissue (lipoatrophy), a new generation of potent NRTIs is used. In association with NRTIs, PIs control viral infection for the long term. However, some first-generation PIs are also responsible for metabolic alterations. New-generation PIs are generally prescribed with a boosting concentration of ritonavir, an inhibitor of the liver cytochrome P450 enzyme that is responsible for PI catabolism, to maintain PI concentrations. Although metabolic toxicity of boosted PIs is far less than that of first-generation PIs, they are still considered responsible for increased cardiovascular risk. Efavirenz, a NNRTI, could be involved in fat alterations and is responsible for lipid modifications, whereas nevirapine might modify lipids but has not been shown to alter fat. The new classes of antiretrovirals, fusion inhibitors (F20), integrase inhibitors (raltegravir) or entry inhibitors (anti-CCR5, maraviroc) have not yet been shown to alter metabolic parameters or fat.
References: [6,27,35,97–99].
[*]These data should be considered with caution because discrepancies exist among studies that cannot be presented in one table.

Adipose tissue, a complex organ controlling metabolism and insulin sensitivity

The roles of adipose tissue were once considered restricted to the storage of lipids after meals and the release of free fatty acids (FFA) in the post-absorptive state to deliver energy to most tissues. More recently, adipose tissue has emerged as an integrator of a wide array of homeostatic processes including blood pressure control (adipocytes possess a complete renin-angiotensin system, RAS) and insulin sensitivity [5,9]. Adipocytes produce several adipokines, such as leptin and adiponectin, which enhance insulin sensitivity. Non-adipocyte cells also participate in fat physiology; in particular, resident macrophages produce chemokines and cytokines prone to favor insulin resistance. However, in human fat under physiological conditions, macrophages contribute to an anti-inflammatory phenotype [10].

Although numerous works have focused on adipocyte differentiation and function as well as alterations under pathophysiological conditions, only a few studies have considered the importance of mitochondrial activity in these situations [11]. In fact, mitochondria play important roles in adipocyte differentiation and function. Pre-adipocytes mature in two steps: differentiation and then hypertrophy. During the early maturation stage, an increased number of mitochondria are required [11,12], resulting in small adipocytes, which are highly sensitive to insulin and that secrete high levels of adiponectin [12]. By contrast, older adipocytes increase in size (hypertrophy), lose their

functional activities and become resistant to insulin. They exhibit decreased numbers of mitochondria with impaired functions and secrete less adiponectin [12]. In addition, mitochondrial reactive oxygen species (ROS), generated by the respiratory chain, could have dual effects on adipocyte differentiation. At physiologically low levels, ROS could act as secondary messengers to activate adipogenesis and lipogenesis, resulting in increased adipocyte number and size. In hepatic cells, oxidative stress activates the transcription factor SREBP1c, which is highly expressed in adipocytes and increases lipogenesis and lipid accumulation [13]. At higher levels, ROS could inhibit differentiation. Finally, under physiologic conditions, most abdominal fat is subcutaneous adipose tissue (SAT) and a minor part visceral adipose tissue (VAT). To our knowledge, mitochondrial content has not been assessed in human samples of VAT and SAT, but in rats mitochondrial content is higher in visceral than subcutaneous adipocytes [14]. If this is also the case in human fat, this difference could explain the higher sensitivity of SAT to drug-induced mitochondrial dysfunction.

Adipose tissue alterations in obesity and non-HIV linked lipodystrophies

Fat expansion in the upper body, or android obesity, is highly prevalent in the general population, is associated with metabolic alterations and can result in metabolic syndrome or type-2 diabetes. Excessive VAT associates with metabolic alterations and insulin resistance that

result from increased visceral adipocyte–FFA release and also from modified adipokine and cytokine production. Indeed, activated macrophages, which have an M1 proinflammatory phenotype (Glossary) [10], invade expanded adipose tissue; upon invasion, their production of proinflammatory cytokines and chemokines increases. This could lead to the decreased secretion of adiponectin by adipocytes in response to tumor necrosis factor-α (TNF-α) [15]. Increased abdominal fat lipolysis increases levels of fatty acid derivatives within tissues (liver, skeletal muscle and heart). Subsequently, these derivatives overwhelm mitochondrial oxidative capacity and activate stress kinases, leading to insulin resistance. This situation, known as lipotoxicity, associates with ectopic depots of triglycerides in non-adipose tissues that buffer excess fatty acid derivatives [16]. Moreover, a paracrine loop is present between adipocytes and macrophages; macrophage-secreted cytokines (TNF-α and interleukin-6 {IL-6}) activate the proinflammatory nuclear factor-κB (NFκB) pathway in adipocytes, resulting in increased IL-6 and FFA production. Saturated FFA can, in turn, activate the Toll-like receptor-4 (TLR-4) on macrophages and adipocytes, thereby increasing the proinflammatory loop. This paracrine loop has been reported in obesity as a result of macrophages infiltrating fat [17].

During the onset of obesity in murine models and humans, adipocyte hypertrophy has been clearly linked to decreased mitochondrial functions and/or mitochondrial DNA (mtDNA) level [18]. In addition, some mtDNA mutations result in lipomatosis (Glossary) with increased regional fat depots [18]. Mildly increased ROS production associates with mitochondrial dysfunction and could play a role in fat hyperplasia and hypertrophy. Conversely, high ROS concentrations, which are associated with severe mitochondrial dysfunction, inhibit the expression of the adipogenic factor peroxisome proliferator-activated receptor-γ (PPARγ) [19] and induce cell apoptosis [11,18], which could result in lipoatrophy [11].

Human lipodystrophic syndromes are a heterogeneous group of diseases characterized by partial or generalized loss of fat (Box 1). Partial forms are often associated with fat hypertrophy in other depots. All forms are associated with insulin resistance, altered glucose tolerance or diabetes, dyslipidemia, increased FFA and decreased adiponectin [20]. Genetic forms are uncommon. The severe recessive generalized congenital lipodystrophies (BSCL) result, in most cases, from mutations in two genes that encode either seipin or the acyltransferase AGPAT2. Seipin is implicated in lipid metabolism, more specifically in lipid droplet formation at the level of the endoplasmic reticulum, and also in adipocyte differentiation [20,21]. In cells from patients with seipin inactivating mutations, the fatty acid desaturation activity of stearoyl-CoA desaturase-1 is decreased and this is likely to impede the formation of new lipid droplets [22]. Altered adipocyte differentiation and lipid droplet replenishment could explain the lipoatrophic phenotype displayed by the patients. AGPAT2 catalyzes the acylation of lysophosphatidic acid to phosphatidic acid in triglyceride synthesis and is also involved in adipocyte differentiation. Its absence in adipocytes could explain lipoatrophy [20]. In these cases, the complete absence of fat results in severe metabolic complications in keeping with the lack of adipokines and failure to store triglycerides in fat, which leads to their accumulation in other tissues and lipotoxicity.

Familial partial lipodystrophic syndromes (FPLD) are mainly dominantly inherited and are caused by mutations either in *LMNA*, encoding the nuclear protein lamin A/C (FPLD2), or in PPARγ (FPLD3) [21]. These patients display mixed lipodystrophy with subcutaneous lipoatrophy and central fat gain; FPLD2 patients also have an increased amount of fat in the cervico-facial area compared with individuals without these mutations. Therefore, a single protein mutation leads to two opposing fat localization phenotypes. Moreover, the phenotype is age-related, revealed in adolescents or young adults but absent during infancy. Importantly, mutations in *LMNA* are also responsible for metabolic laminopathies (Glossary) resembling metabolic syndrome and Hutchinson–Gilford progeria, a severe syndrome of premature aging [20].

Lamin A is derived from prelamin A that gains a farnesyl membrane anchor and migrates to the inner nuclear envelope. Then, a specific protease ZMP-STE24 removes the carboxy-terminal end including the farnesyl anchor and releases free lamin A. In most cases, progeria results from a heterozygous *LMNA* mutation that creates a new splicing site, deleting 50 amino acids and precluding cleavage by ZMP-STE24. The mutated protein progerin remains associated with the membrane through the farnesyl anchor, leading to genomic instability and early cell senescence [23].

Importantly, cultured skin fibroblasts from patients with lamin mutations associated with FPLD2 and metabolic laminopathies exhibit increased oxidative stress because of farnesylated prelamin A accumulation [24]. Hypertrophied fat from these patients' neck regions exhibits mitochondrial dysfunction [25], linking fat hypertrophy with mitochondrial dysfunction and oxidative stress. Furthermore, some patients with typical FPLD2 or metabolic laminopathies show signs of early atherosclerosis resulting in cardiovascular disease. Accordingly, in long-term cultures of patients' skin fibroblasts, farnesylated prelamin A accumulation associates with signs of premature cellular senescence (the accumulation of cell-cycle

arrest proteins p16^{INK4} and p21^{WAF-1} and increased activity of senescence-associated β-galactosidase). This senescent phenotype reverts when prelamin A farnesylation is impeded [24]. These data implicate prelamin A in increased oxidative stress and in the occurrence of cellular senescence.

Apart from HIV-associated lipodystrophy, an acquired form of lipodystrophy is displayed by patients with endogenous or exogenous hypercortisolism [21]. This lipodystrophy is characterized by fat hypertrophy in the upper body depots: excess fat in the trunk and cervico-facial area, often a presence of a buffalo neck and increased VAT together with decreased limb fat. The pathophysiology of lipodystrophic syndrome remains poorly explained. Cortisol could activate adipocyte differentiation and hypertrophy, mainly in visceral fat depots, because of the higher expression of glucocorticoid receptors (GR-α) and the 11β−hydroxysteroid dehydrogenase type 1 (11β−HSD-1) that transforms inactive cortisone into active cortisol in adipocytes from central depots [26]. In addition, cortisol induces insulin resistance and increased lipolysis in adipocytes, leading to alterations in glucose and lipid metabolism [26].

Clinical characteristics of HIV-associated lipodystrophy

Peripheral lipoatrophy is easily visible in limbs, buttocks and the face. In addition, visceral, neck (buffalo hump) and breast fat depots are frequently hypertrophied [1]. About 50% of patients display mixed forms, with the loss of limb fat and marked expansion of VAT [27]. The high frequency of this association suggests that these two opposite phenotypes could be, at least in part, causally related.

Evidence for a role of antiretrovirals

Several clinical studies have reported a link between the thymidine NRTI (tNRTI) stavudine and the development of lipoatrophy [3,5,28], which led to the removal of this drug from first-line antiretroviral therapy (ART) in western countries. Zidovudine also induces lipoatrophy [28,29]. These tNRTIs, which cause mitochondrial toxicity in part by inhibiting the mtDNA polymerase γ [30,31], are also associated with fat hypertrophy in visceral depots [27,29,32]. Interestingly, most ART-naïve patients display increased amounts of both limb and visceral fat early after tNRTI initiation [1,28,29,32,33] and before the appearance of peripheral lipoatrophy, which is not the case after treatment initiation with other NRTIs or non-nucleoside analog reverse transcriptase inhibitors (NNRTIs) [29,32,34]. We propose that this initial fat gain could be related to ROS production linked to early mitochondrial dysfunction.

Some clinical studies indicate that first-generation PIs alter lipid and glucose parameters including increased triglycerides, decreased high-density lipoprotein cholesterol (HDL-C) and increased insulin resistance [35], and are probably involved in central hypertrophy and less probably in peripheral lipoatrophy. PIs could amplify the effect of tNRTIs [27,33,36,37]. Initial studies have not revealed a role for efavirenz in lipodystrophy [27] but the recent ACTG A5142 study found that lipoatrophy was more frequent with efavirenz than lopinavir/r (Table 1) when combined with stavudine or zidovudine [38]. This could suggest an additive toxicity of the long-term combination of efavirenz with tNRTI on peripheral fat. Metabolic studies have not uncovered a negative effect of the new ART belonging to other classes, the CCR5 inhibitor maraviroc or the integrase inhibitor raltegravir, on lipid or glucose parameters, and their use has not been associated with altered fat depots. Taken as a whole, although tNRTIs and first-generation PIs exhibit severe adverse effects on fat and metabolism, more recently marketed drugs have decreased toxicity, which, if present, requires longer treatment duration to be clinically observed.

Lipodystrophic phenotypes differ between men and women

The prevalence and clinical forms of lipodystrophy differ between men and women. Women frequently show fat accumulation, whereas isolated lipoatrophy is less common [39,40]. Accordingly, in longitudinal studies women gain more central fat (VAT and SAT) and lose less limb fat than men [41]. Although a satisfactory explanation for this observation has not been provided, it is important to consider that fat repartition is markedly different between men and premenopausal women with strong determinants linked to sex hormones, the total amount of body fat and, in particular, peripheral fat content in the lower body, which is higher in woman [42]. Therefore, clinical lipoatrophy would require the loss of more fat in female patients.

Antiretrovirals induce adipocyte dysfunction *in vitro* and *ex vivo*

Murine and human adipocytes treated *in vitro* with tNRTIs and first-generation PIs exhibit several alterations [4,37]. Stavudine decreases mtDNA content but maintains respiratory chain activity [43]. Thymidine analogs, but not other NRTIs, induce severe mitochondrial dysfunction [44], which can be prevented by uridine addition [45]. Stavudine and zidovudine increase oxidative stress, resulting in the reduced production of adiponectin and leptin and increased production of MCP-1 and IL-6 [44,46]. Efavirenz also inhibits adipocyte differentiation [47].

In cultured adipocytes and fibroblasts, first- or second-generation PIs in combination with ritonavir induce an accumulation of prelamin A, in accordance with their reported inhibition of ZMP-STE24, increase oxidative stress and alter adipokine and cytokine production [4,24,46]. The adverse effect of PIs could also result from the induction of endoplasmic reticulum stress or the inhibition of the proteasome [37,48].

Subcutaneous adipocytes are more susceptible to the deleterious effects of PIs than visceral adipocytes [49]. Accordingly, studies performed on control human SAT explants reveal that some PIs increase FFA, IL-6 and TNF-α production through the activation of the NFκB pathway. This PI-induced deleterious paracrine loop between adipocytes and macrophages, similar to that observed in obesity, is not seen in VAT [50]. These data indicate that SAT is more sensitive to the adverse effects of some PIs than VAT.

Lipodystrophy in HIV-infected antiretroviral-naïve patients: a possible role for the virus

In addition to the well-demonstrated role of antiretrovirals, recent studies suggest that HIV infection affects fat, in particular, before any ART. Monocytes are relatively resistant to HIV infection, but differentiated macrophages are highly susceptible and tissue macrophages have been found to harbor HIV-1 [51]. Infected macrophages release proinflammatory cytokines. Systemic inflammation associated with HIV infection might promote monocyte migration across the vascular endothelium, leading to an increased number of activated macrophages in fat [51]. Accordingly, several studies report that the severity of HIV infection associates with an increased prevalence of lipodystrophy [1,40], probably as a consequence of persistent HIV-infected macrophages in adipose tissue, which could enhance local inflammation.

In fact, ART-naïve HIV-positive patients have increased TNF-α expression compared with uninfected controls [52], which is consistent with increased inflammation. TNF-α alters adipocyte function and differentiation, in part, through the inhibition of PPARγ expression [53]. Accordingly, PPARγ expression is reduced in fat from ART-naïve patients [32,52] compared with uninfected controls. Infected macrophages might also release viral proteins (such as Vpr and Nef) that can impact adjacent adipocytes and lead to decreased PPARγ activity and the inhibition of adipogenesis [54,55]. Whether mitochondrial function is altered in fat from naïve patients remains a matter of debate; either no or mild alterations have been reported in most studies [28,32,56], whereas more severe defects were observed by Giralt and colleagues [52]. Therefore, although lipodystrophy is uncommon in ART-naïve patients [27], the HIV infection of macrophages itself could result in low-grade fat inflammation and lead to the release of viral proteins that affect neighboring adipocytes and decrease their differentiation. Both increased cytokine production and decreased adipogenesis could induce limited lipoatrophy (Figure 1). Even when HIV infection is controlled by cART, the persistent infection of reservoir fat macrophages, which is probably related to the severity of the initial infection, could help maintain the lipodystrophic phenotype. In addition, initial HIV infection associates with increased bacterial translocation through the gut, leading to increased circulating levels of lipopolysaccharide (LPS) [57], which activates macrophages through the TLR-4 receptor and increases cytokine production. This shows the importance of the early treatment of HIV infection to prevent the constitution of virus reservoirs and control inflammation.

Studies of antiretroviral-treated patients' adipose tissue reveal a major role for some antiretrovirals

Although several studies have directly analyzed the cellular and molecular alterations in subcutaneous, usually lipoatrophic, adipose tissue, this is not the case for VAT for which direct analysis is scarce. These studies provide evidence of a complex set of alterations in SAT, associating mitochondrial dysfunction and increased oxidative stress, altered differentiation, increased inflammation and cortisol activation. In addition, these
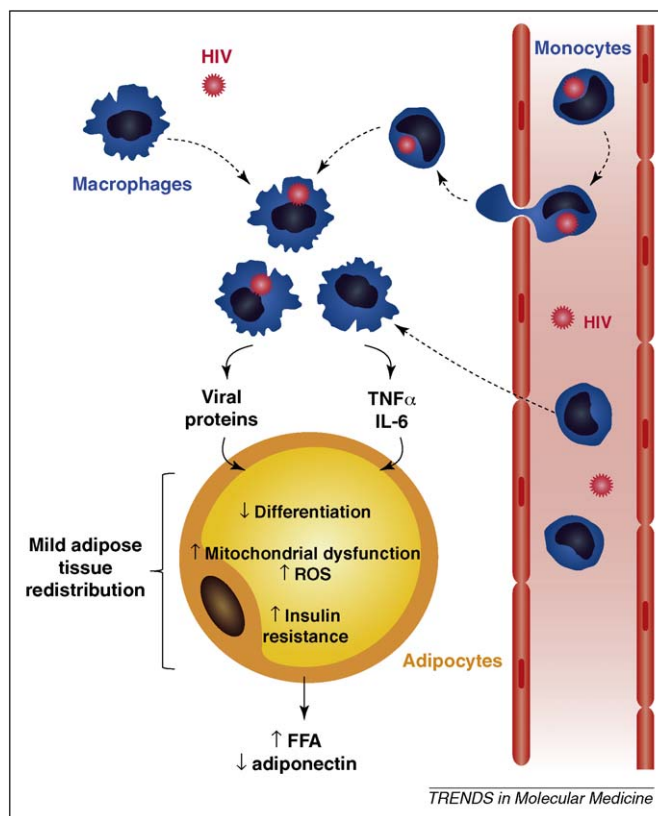
Figure 1. **Possible effects of HIV infection in adipose tissue**.
Some adipose tissue macrophages are infected by HIV and release viral proteins. When infected, macrophages secrete proinflammatory cytokines (TNF-α and IL-6). Systemic inflammation associated with HIV infection might promote monocyte migration across the vascular endothelium (right), leading to an increased number of activated macrophages in fat. Cytokines and viral proteins can inhibit adipocyte differentiation (yellow) as well as induce mitochondrial dysfunction and insulin resistance.

alterations vary according to the duration of cART and the fat depot.

Mitochondrial dysfunction and altered differentiation

In SAT from healthy volunteers treated for two weeks with tNRTIs (stavudine/lamivudine or zidovudine/lamivudine), mitochondrial pathways are affected before clinical lipodystrophy; mtRNA transcription decreases before mtDNA is depleted, adipogenesis is altered or PPARγ expression decreases [58].

Longitudinal studies comparing HIV-infected patients' SAT before and after ART initiation have provided important clues. Six months of treatment with zidovudine increases VAT and alters the expression of several key mitochondrial electron transport genes in SAT, which leads to increased oxidative stress [32]. These data are consistent with data from patients treated with tNRTIs for six to eight months who experienced increases in leg fat mass as well as a reduction in mitochondrial activity and mtDNA [28]. Zidovudine also increases the markers of adipocyte differentiation and lipid accumulation, probably resulting in fat hyperplasia and hypertrophy. The effect of tenofovir, however, is minor [32]. Accordingly, in lipoatrophic ART-treated patients, who were mostly naïve at inclusion, the expression of some adipogenic transcription factors increases in abdominal SAT after two months, whereas these same factors decrease in

thigh-level SAT [59]. Clinical lipoatrophy is seen after 12 months.

This early drug-induced mitochondrial dysfunction could lead to an initial increase in SAT before fat loss associated with more advanced and severe mitochondrial dysfunction (Figure 2). Accordingly, initial increases in limb fat and leptin levels can predict long-term peripheral lipoatrophy [60].

The expression of genes involved in regulating mitochondrial functions and adipogenesis as well as the levels of mtDNA decrease in fat from lipoatrophic patients treated with stavudine and, to lesser extent, zidovudine compared with non-lipodystrophic patients or uninfected controls [61]. Conversely, the expression of genes involved in oxidative stress and apoptosis increases [61,62]. Switching patients from stavudine to tenofovir or nevirapine plus lopinavir/r improves fat mitochondrial function and decreases oxidative stress [63,64]. Thus, tNRTIs exert severe adverse effects on mitochondria, leading to increased ROS production that is probably directly involved in lipoatrophy. The replacement of these drugs with NRTIs or NNRTIs with little to no potential to induce mitochondrial dysfunction allows for the partial recovery of lipoatrophy or even causes fat hypertrophy.

Inflammation
The comparison of SAT from HIV-infected patients with and without established lipoatrophy reveals that lipoatrophy associates with markedly higher inflammation, as shown by an increase in macrophage number and the expression of TNF-α, IL-6 and IL-8 [15,61,65].

To evaluate the impact of antiretrovirals, the Lipostop study [66] compared SAT from HIV-infected patients before and six months after stopping any ART. Treatment cessation markedly improved fat function, and tNRTIs but not PIs associated with fat inflammation, whereas both tNRTIs and PIs altered adipogenesis and mitochondrial function. These data indicate that PIs boosted with ritonavir exert milder adverse effects on fat than tNRTIs.

Cortisol
Although direct VAT studies are lacking, we hypothesize that glucocorticoid activation is involved in ART-linked central fat hypertrophy. Higher ratios of urinary cortisol:cortisone metabolites and higher subcutaneous 11β−HSD-1 expression are observed in patients with severe lipodystrophy compared with those without [67]. Similarly, the increased expression of 11β−HSD-1 and GR-α are observed in SAT from zidovudine-treated patients with VAT hypertrophy compared with the level observed before ART; this increase is milder in tenofovir-treated patients without increased VAT [32]. Accordingly, in ART-naïve patients 11β−HSD-1 expression increases in both abdominal and thigh SAT after 12 months of ART only in patients with lipohypertrophy or without lipodystrophy [59]. Because TNF-α expression in fat is related to that of 11β−HSD-1 [67], inflammation is linked to glucocorticoid activation.

Lipoatrophy and lipohypertrophy preferentially affect two different fat depots
Lipoatrophy and lipohypertrophy are often associated [27] in clinical studies but are probably related to different
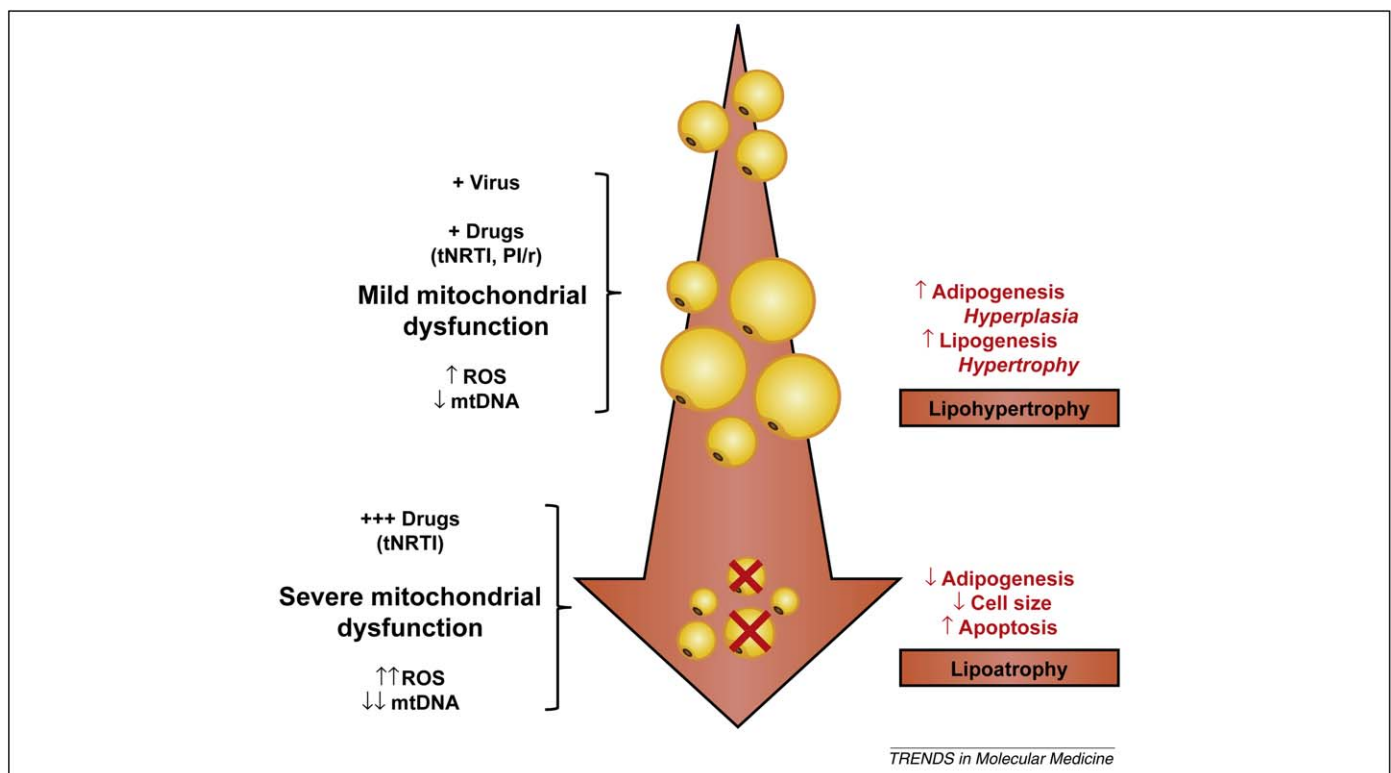
Figure 2. Proposed deleterious impact of mitochondrial dysfunction on adipose tissue.
Mitochondrial dysfunction could result from the cumulative effects of HIV and antiretroviral drugs [tNRTIs, PIs or PIs boosted with ritonavir (PI/r)]. Mild mitochondrial toxicity leads to the increased production of ROS, which probably activates mitochondrial biogenesis, adipogenesis and adipocyte hypertrophy, and results in clinical fat hypertrophy. If mitochondrial function is more severely affected, in particular through the long-term use of tNRTIs, high oxidative stress and ROS production are likely to inhibit adipogenesis, decrease lipogenesis and increase adipocyte apoptosis, which leads to clinical lipoatrophy.

biological factors. Animal studies reveal that SAT is more sensitive to zidovudine-induced mitochondrial dysfunction than VAT, because the chronic treatment of rats with zidovudine decreases mitochondrial cytochrome *c* activity and depletes mtDNA content in SAT, whereas VAT remains unaffected [68]. This difference relates to the lower mitochondria content of SAT, which is, therefore, more prone to lipoatrophy. However, in several patients VAT is also reduced in the long term by tNRTIs. Otherwise, VAT, with a higher number of resident macrophages [10] and more cortisol activity than SAT [26], is predisposed to hypertrophy. Accordingly, central fat hypertrophy in HIV-infected patients relates to TNF-α circulating levels and waist circumference [60]. Hypertrophied VAT from HIV-infected patients displays mitochondrial dysfunction together with increases in TNF-α expression but no impairment of adipogenic gene expression in comparison with SAT [18]. Therefore, antiretrovirals can differentially alter fat development depending on the environment and physiology of the different depots (Figure 3). In addition, in the case of lipoatrophy, because subcutaneous adipocytes cannot store triglycerides, non-lipoatrophic fat depots such as VAT probably buffer the increases in FFA, which worsens lipohypertrophy.

Buffalo hump in HIV: probably no role for functional brown adipose tissue (BAT)

The presence of BAT in healthy adults at the supraclavicular level was recently revealed by positron emission tomography [5]. Therefore, the causal relationship between HIV-linked buffalo hump and BAT hypertrophy owing to increased energy expenditure and postprandial thermogenesis [18] is in question. However, even if uncoupling protein-1 (UCP-1), the uncoupling protein characteristic of BAT, is expressed in the cervical samples, supraclavicular areas and buffalo hump of HIV-infected patients, the overall pattern of gene expression in these areas only partially recapitulates the gene expression features that differentiate brown fat from white fat [5]. It is likely that in the neck, abnormal brown fat-like cells with no thermogenic properties are present; these cells, however, retain the highly proliferative capacity of the brown adipocyte lineage, leading to buffalo hump. Such an explanation was proposed for the buffalo hump constitution in multiple symmetric lipomatosis [69].

Individual risk factors for lipodystrophy

Several factors probably modulate fat susceptibility to injuries. The prevalence of lipodystrophy relates to age [1,27]. Accordingly, in the general population, age associates with central fat redistribution, mitochondrial impairment and increased levels of proinflammatory cytokines. The low-grade chronic proinflammatory status associated with the aging phenotype is called inflammaging [70]. Aging adipocytes release more proinflammatory cytokines than young ones [71].

A role for genetics is also probable but has been only partly evaluated. A polymorphism in the TNF-α gene promoter associates with lipodystrophy, but this association has not been confirmed in larger studies [72]. Interestingly, stavudine-induced lipoatrophy is linked to the HLA-B100*4001 allele, which is located in close proximity to the TNF-α gene; this observation further supports a role for inflammation in lipoatrophy [73]. mtDNA variations
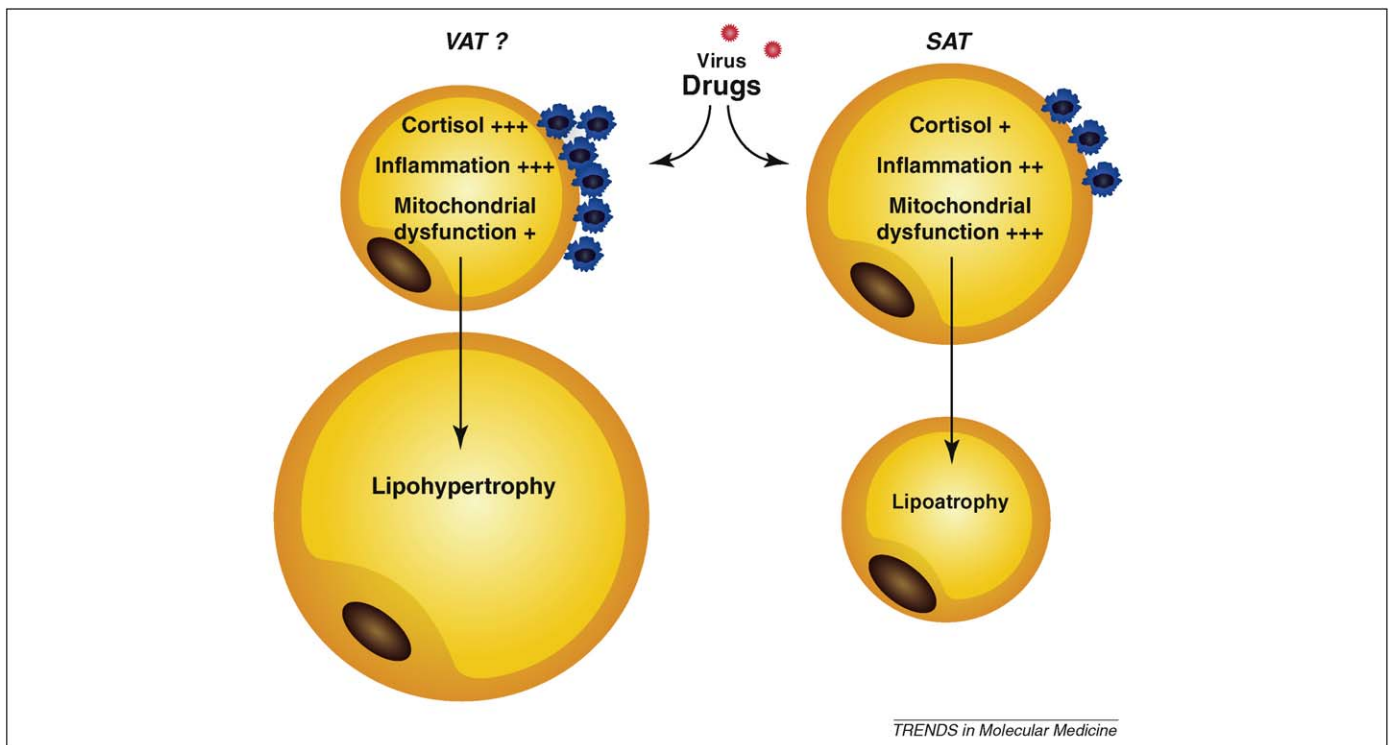
Figure 3. **Proposed differential impact of cART and HIV on VAT versus SAT**.
Owing to a probable lower mitochondrial content, SAT (right) is more sensitive than VAT (left) to drug- and virus-induced mitochondrial dysfunction. Conversely, we hypothesize that VAT has a higher level of inflammation and cortisol activation, which favors SAT lipoatrophy and VAT hypertrophy. Similar insults to the different fat depots could result in the opposite size modifications to these depots.

are probably also involved because some haplogroups associate with lipoatrophy [74]. A large study has revealed that the genetic polymorphisms of genes involved in apoptosis and adipocyte metabolism are significantly related to ART-associated lipodystrophy. In particular, ApoC3-455 plays a role in lipoatrophy, and two variants of the adipogenic β2 receptor play a role in fat accumulation, whereas PPARγ variants are not involved [75]. The toxicity of antiretrovirals also depends on a patient's metabolism, which is partly genetically determined [76]. Thus, genetic factors influence the occurrence of lipoatrophy and lipohypertrophy. The factors for each are likely to be different in accordance with the partial independent occurrences of these two phenotypes. These genetic factors regulate fat and lipid metabolism as well as inflammation and mitochondrial function, in line with the pathophysiological alterations postulated for lipodystrophy.

Metabolic consequences of lipodystrophy

In the general population, increased central fat associates with metabolic complications, dyslipidemia, altered glucose tolerance, insulin resistance and hypertension, which can be defined as the metabolic syndrome. In HIV-infected patients, lipoatrophy associates with increases in FFA release and decreases in adiponectin levels, leading to insulin resistance. Lipoatrophy also associates with high triglyceride levels [5,77]. Increases in central fat associate with increases in triglycerides and decreases in HDL-C [77]. Patients with lipodystrophy have metabolic alterations similar to those in the metabolic syndrome [78] and are at a higher risk of diabetes [79]. Moreover, the altered expression of 11β−HSD-1 and TNF-α in SAT relates to multiple features of insulin resistance [67], and adiponectin levels are strongly related to metabolic alterations and insulin resistance [15,67,80] (Figure 4).

HIV-linked lipodystrophy associates with subclinical atherosclerosis, hypertension and increased cardiovascular disease risk [1,81]. Some PIs can activate the RAS system in human adipocytes, which can contribute to hypertension [82]. Lipodystrophy also relates to hepatic steatosis [5] (Glossary). A high prevalence of nonalcoholic steatohepatitis (NASH) is observed in insulin-resistant lipodystrophic HIV-infected patients in the absence of HCV coinfection [83,84]. Accordingly, macrophage markers in SAT positively correlate with liver fat content [65]. Taken as a whole, both lower limb lipoatrophy, which reduces the amount of a fat depot with protective potential at the metabolic level [85], and central fat hypertrophy probably contribute to the cardiovascular and hepatic complications that lead to increased morbidity and mortality.

Lipodystrophy and aging

Aging is physiologically associated with fat redistribution, oxidative stress and low-grade inflammation. In mouse adipose tissue, the expression of proinflammatory cytokines is upregulated in adipocytes with aging as a result of NFκB activation together with a downregulation of PPARγ, which exerts anti-inflammatory properties. This inflammatory phenotype is observed in SAT but is even more marked in VAT [71]. If such modifications also occur in humans,

adipose tissue could be an important contributor to the increased cytokine levels observed in aging subjects. In the general population, the proinflammatory state interacting with the genetic background potentially triggers the onset of age-related inflammatory diseases such as atherosclerosis [86], sarcopenia (Glossary) and frailty [87], neurocognitive disorders and diabetes. Long-term HIV-infected patients display early age-related comorbidities, (i.e. dyslipidemia, diabetes, increased cardiovascular and hepatic disease risks and sarcopenia) compared with the general population [8,88,89]. The high prevalence of sarcopenia, a loss of muscle mass, is also observed in HIV-infected patients. In the general population, inflammatory factors, such as those produced by aging fat, contribute to the onset and progression of the loss of muscle mass and muscle strength as well as mobility decline [87]. Therefore, all of these alterations are linked, and fat redistribution that occurs in lipodystrophy can favor and aggravate the other disorders.

Adipose tissue, the liver and vascular wall might also be impacted by HIV-induced inflammation, both at the systemic and local levels, resulting from the activation of infected macrophages. Decreased immune response leading to immunosenescence is also probably involved in early aging [90]. Aging and some antiretroviral treatments result in mitochondrial dysfunction and oxidative stress, which lead to cellular senescence. Moreover, some PIs induce the accumulation of the pro-senescence protein prelamin A. Therefore, lipodystrophy together with metabolic alterations contributes to the phenotypes of premature aging, leading to early cardiovascular and hepatic disease risks (Figure 4).

Pathophysiological treatments

In addition to the symptomatic treatment and follow up required to limit metabolic complications, some medications are prescribed to reverse the pathophysiological processes that lead to HIV-linked lipodystrophies.

The cessation of tNRTI therapy results in a rapid recovery of mtDNA levels and fat alterations before clinical modifications [3,63,66]. Switching from tNRTIs to less aggressive molecules generally allows a slow recovery of peripheral fat (about 400–500 gm/year). However, the possible exhaustion of the fat mesenchymal stem-cell pool could limit this benefit.

PPARγ agonists or thiazolidinediones partially reverse peripheral fat loss in patients without tNRTIs [91,92], in keeping with their ability to increase mitochondria number and function [11]. Uridine, which prevents the depletion of the pyrimidine nucleotide pool, restores *in vitro* tNRTI-induced adipocyte toxicity and oxidative stress [45], but disappointing negative clinical data were presented during the 2010 Conference on Retroviruses and Opportunistic Infections [93], which do not confirm an initial small encouraging study [94].

Reducing excessive inflammation is an important therapeutic objective. However, no data are available for anti-inflammatory drugs except a small study, revealing that pravastatin, a hypolipemic drug thought to have anti-inflammatory action, partially reverses lipodystrophy [95]. The inhibition of local cortisol action has not yet been
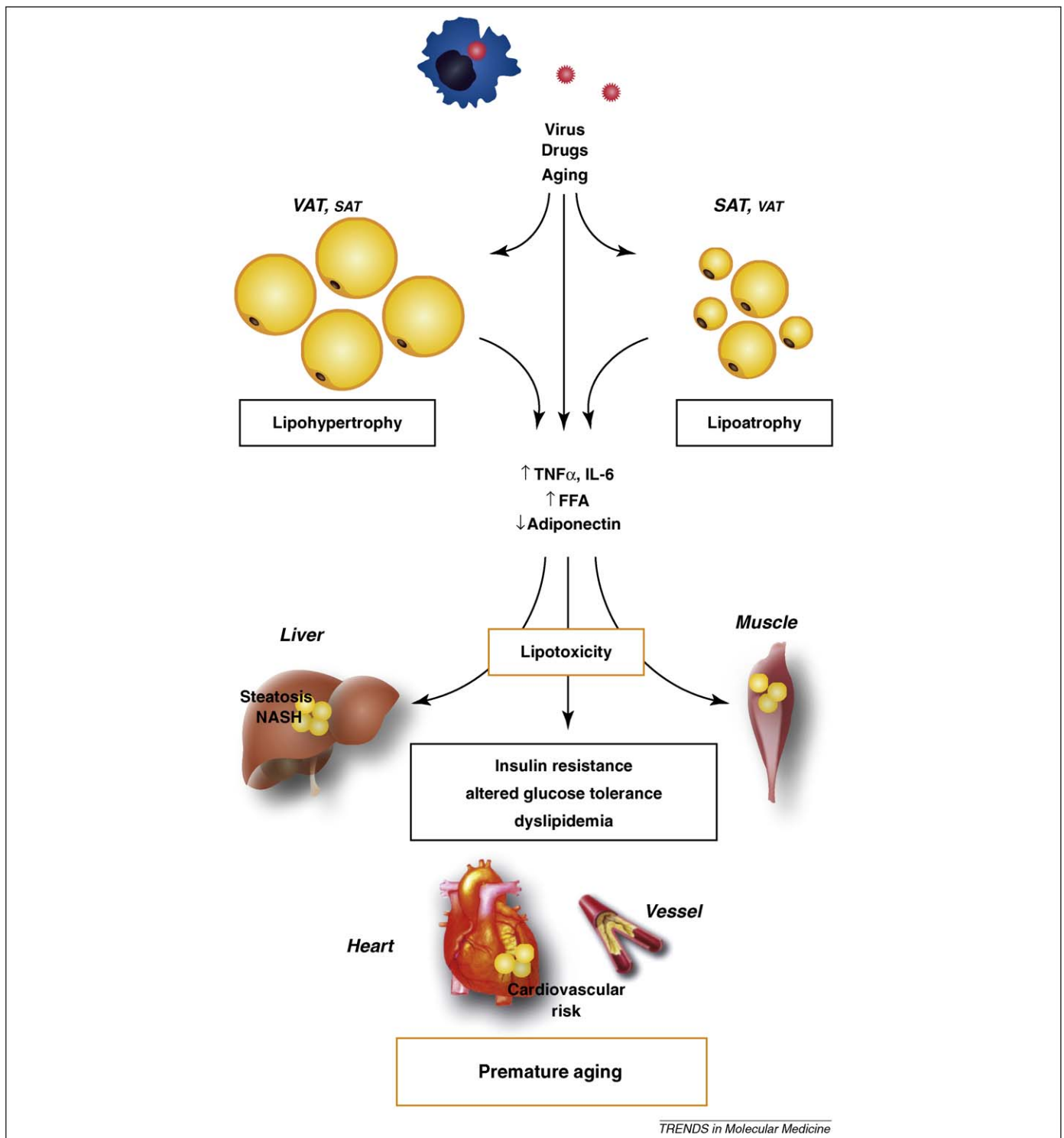
Figure 4. **Metabolic consequences of lipodystrophy and premature aging.**
Increased central fat and decreased limb fat are both involved in metabolic complications. The increased release of cytokines and FFA together with decreased adiponectin production by adipose tissue leads to insulin resistance and triglyceride depots in tissues such as the liver, skeletal muscle and heart. These changes impair glucose tolerance and cause dyslipidemia with decreased HDL-C and increased triglycerides. Metabolic complications are responsible for increased cardiovascular and hepatic disease risks. Long-term HIV infection, some antiretroviral drugs and lipodystrophy can exacerbate the normal aging processes and lead to the early occurrence of these age-related comorbidities defining premature aging.

evaluated. Otherwise, using the growth hormone-releasing factor analog tesamorelin to restore growth hormone levels decreases visceral fat hypertrophy and improves triglycerides and HDL-C levels, but has no beneficial effect on glucose values [96].

Concluding remarks

The occurrence of severe lipoatrophy together with lipohypertrophy and metabolic abnormalities constitutes a major complication in HIV-infected patients. Although lipoatrophy is now less prevalent, fat hypertrophy,

metabolic complications and early cardiovascular or hepatic diseases remain major health preoccupations.

In addition to a role for viral infection, we propose that although severe lipoatrophy is likely to result from the long-term use of tNRTIs with a high mitochondrial toxicity, lipohypertrophy could also result from mild mitochondrial dysfunction, induced by some NRTIs and PIs. Peripheral lipoatrophy and central lipohypertrophy result from the same insults (virus and antiretroviral drugs), but are likely to be related to different fat depot physiologies. Lipoatrophy is linked to severe mitochondrial dysfunction, oxidative stress and inflammation. By contrast, hypertrophy might be related to mild mitochondrial dysfunction and cortisol activation promoted by inflammation (Figure 2). Both lipoatrophy in the lower part of the body and abdominal lipohypertrophy are involved in insulin resistance and metabolic disorders, as observed in genetic lipodystrophies and the metabolic syndrome.

The proinflammatory environment, linked to ART but also to resident macrophage infection, might be an important pathophysiological factor for lipodystrophy. Aging is associated with fat redistribution, decreased mitochondrial function and increased cytokine release, all factors favoring lipodystrophy. Moreover, HIV-infected patients display features of premature aging affecting bone, brain, vascular wall, muscles, kidney and liver, which result collectively from long-term HIV infection, immune depletion and the toxicity of some antiretrovirals. These early complications represent an important challenge for the treatment of HIV-infected patients, and their reversibility might be linked to decreasing the stresses that lead to mitochondrial dysfunction and increased inflammation.

What are the challenges for the future (Box 2)? Although the pathophysiology of peripheral lipodystrophy has been intensely studied, VAT alterations remain largely unknown, so studies are required to decipher the cellular and molecular alterations affecting VAT. Peripheral and central increases in fat are generally considered a return to normal health in HIV-infected patients after treatment initiation. We propose that this increase in fat amounts, in particular at the trunk level, results from drug toxicity and contributes to the increase in metabolic risk displayed by these patients. This needs to be validated by clinical studies. Further studies searching for drug-induced altera-

tions are required for the molecules currently used to treat patients. Several studies suggest that even controlled viral infection could injure adipose tissue by inducing low-grade inflammation. The early diagnosis and treatment of infection are important to prevent this type of injury. The revelation that antiretrovirals from several classes injure adipose tissue in HIV-infected patients could be related to a previous effect of the virus on fat, inducing subclinical adipose tissue dysfunction. This possibility needs to be investigated.

HIV infection, cART and immunosenescence could all contribute to inflammation, but what are the respective roles for each component? How do HIV-related inflammation and aging exacerbate each other [90]? What is the role of HIV- or cART-associated mitochondrial toxicity and increased oxidative stress in the age-related illnesses observed in HIV-infected patients [90]? Initial recommendations to help decrease inflammation could include smoking cessation, diet prescriptions and exercise. Exercise is even more important in HIV-infected patients to prevent sarcopenia and frailty. Clinical trials to demonstrate the beneficial effects of these strategies would be interesting. New therapeutic strategies are required to protect mitochondria and decrease inflammation.

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