Antirétroviraux et atteintes du tissu adipeux

a) Lipodystrophie et molécules de première génération

L'utilisation des molécules ARV de première génération a conduit à l'apparition de **syndromes lipodystrophiques** caractérisés par une lipoatrophie périphérique du TASC (membres et visage) associée ou non à une accumulation tronculaire de TA. Entre 1990 et le début des années 2000, la prévalence de la lipodystrophie représentait environ 50% des personnes infectées sous ARV (Miller et al. 2003, Domingo et al. 2012). Chez certains patients, elle pouvait être associée à une accumulation de TASC dorso-cervical appelée bosse de bison (Mallon et al. 2005). La lipodystrophie est diagnostiquée en clinique grâce à une échelle permettant de mesurer la sévérité de la redistribution du TA (Carr et al. 2003).

La lipodystrophie liée au VIH a été décrite pour la première fois suite à la mise en place de la trithérapie correspondant à l'association des ARV de première génération : deux NRTI (en particulier d4T et ZDV), et d'un PI (en particulier NFV et LPV/r) (Bacchetti et al. 2005, Study of Fat and Metabolic Change in 2006, Boothby et al. 2009, Caron-Debarle et al. 2010). Cependant, certains NNRTI tel que l'EFV, ont été impliqués dans la lipoatrophie (Haubrich et al. 2009). Malgré la mise en place de nouveaux traitements moins délétères (tels que les NNRTI) favorisant une augmentation de la masse du TASC des membres, le syndrome lipodystrophique persiste (Martin et al. 2004, Ribera et al. 2013). Par ailleurs, quelques études suggèrent que la perturbation du système nerveux sympathique favoriserait la redistribution du tissu adipeux chez les patients lipodystrophiques infectés par le VIH sous ARV (Fliers et al. 2003, van Gurp et al. 2006). De nombreuses études ont été menées *in vivo* et *in vitro*, pour comprendre les mécanismes impliqués dans **l'effet des ARV de première génération** (Caron-Debarle et al. 2010, Lagathu et al. 2017).

Le **TASC abdominal lipoatrophique** présente des adipocytes en apoptose, une infiltration de cellules immunitaires (avec des *crown-like structures*) et une inflammation (sécrétion de TNFα, IL-6, IL-1β) (Kannisto et al. 2003, Jan et al. 2004, Gallego-Escuredo et al. 2013). Il est caractérisé par une diminution de l'expression des gènes impliqués dans la biogenèse des mitochondries (*PPARGC1A*), l'adipogenèse (*PPARG*, *SREBP1C*, *CEBPA*), le métabolisme lipidique (*LPL*, *FASN*, *LIPE*, *FABP4*, *CD36*) et glucidique (*GLUT4*) (Giralt et al. 2006). *In vitro,* les traitements par le d4T et l'AZT, et certains PI (LPV, SQV, APV et IDV) sont incriminés dans ces atteintes (Dowell et al. 2000, Caron et al. 2007, Lagathu et al. 2007, Gallego-Escuredo et al. 2010, Diaz-Delfin et al. 2011, Leroyer et al. 2011, Minami et al. 2011, Manente et al. 2012, Walker et al. 2014). Les PI plus récents comme l'ATV/r ou le DRV/r semblent avoir moins

d'effets délétères (Kim et al. 2006, Jones et al. 2008, Caso et al. 2010, Minami et al. 2011, Capel et al. 2012, Perez-Matute et al. 2012, Hernandez-Vallejo et al. 2013). En accord avec ces observations, le TASC abdominal des patients lipodystrophiques recevant des molécules de première génération présente des petits adipocytes (Bastard et al. 2002) , une résistance à l'insuline et une diminution de la sécrétion de leptine et de l'adiponectine en réponse à certains NRTI et PI (Cammalleri and Germinario 2003, Rudich et al. 2003, Jan et al. 2004, Hadigan et al. 2006, Caron-Debarle et al. 2010, Giralt et al. 2011, Capel et al. 2012, Klos et al. 2019). L'altération de la différenciation des adipocytes et la mort adipocytaire seraient responsables de la diminution du nombre d'adipocytes et pourraient expliquer la perte de TASC observée chez ces patients.

La **lipohypertrophie centrale** est une accumulation abdominale de TA (TASC et TAV) (de Waal et al. 2013). Elle est apparue dès le début de la mise sous ARV et est encore observée de nos jours. Jusqu'à 70% des patients infectés par le VIH sous ARV présentent une accumulation tronculaire de TA seule (Dube et al. 2007, Wohl and Brown 2008, Gelpi et al. 2019). Elle est principalement associée aux PI (Moyle et al. 2010, McComsey et al. 2016). Grâce aux études cliniques, des facteurs de risque se distinguent : l'âge, le sexe féminin et l'IMC initial des individus (Moyle et al. 2010).

Peu d'études ont été réalisées sur le **TAV des patients lipodystrophiques** notamment car il n'est pas facilement accessible. Les ARV ne semblent pas exercer d'effets délétères sur l'expression des gènes adipogéniques, la captation du glucose, la lipolyse ou l'accumulation de lipides (Hadigan et al. 2006, Gallego-Escuredo et al. 2013, Walker et al. 2014). La capacité d'expansion du TAV ne semble donc pas être altérée contrairement à celle du TASC chez les patients lipodystrophiques liés au VIH. L'hypertrophie du TAV pourrait donc être un mécanisme compensatoire pour pallier à la capacité de stockage défectueuse du TASC (Giralt et al. 2011).

Concernant la **bosse de bison**, des biopsies ont montré dans ce tissu de petits adipocytes avec un phénotype proche de celui des adipocytes bruns : une augmentation du nombre de mitochondries et de l'expression des marqueurs de TA brun (PRDM16, UCP1) et de SREBP1c et PPARγ (Guallar et al. 2008, Bereziat et al. 2011, Cereijo et al. 2015). Ce TA très particulier présente une fibrose mais pas d'inflammation car le nombre de CD68 et de *crown-like structures* semblent inchangés comparés au TASC cervical ou de personnes non infectées (Bereziat et al. 2011).

b) Prise de poids et nouveaux antirétroviraux : les inhibiteurs d'intégrase

Avec le développement des nouvelles molécules, une prise de poids est souvent observée rapidement après la prise d'ARV en parallèle de la reconstitution immunologique (Koethe et al. 2016). C'est le phénomène de « retour à la santé » (r*eturn to health*). Cependant, les individus infectés par le VIH prennent plus de poids que les individus de la population générale (Erlandson et al. 2016), avec une accumulation homogène du TA dans le corps aussi bien au niveau des membres qu'au niveau tronculaire (Debroy et al. 2019, Venter et al. 2019).

Des études récentes suggèrent un rôle plus important des INI par rapport aux PI ou aux NNRTI dans la prise de poids (Bhagwat et al. 2017, Bakal et al. 2018, Bourgi et al. 2019) que ce soit chez les patients infectés naïfs de traitement qui débutent une thérapie en combinaison avec un INI (Reynes et al. 2013, Young et al. 2015, McComsey et al. 2016, Menard et al. 2017, Group 2019, Venter et al. 2019, Calmy et al. 2020, Venter et al. 2020) ou chez des patients contrôlés qui changent de classe d'ARV pour un INI (Domingo et al. 2014, Norwood et al. 2017, Waters et al. 2018, Debroy et al. 2019, Gatell et al. 2019, Katlama et al. 2019, Koethe et al. 2020, Lake et al. 2020). La majorité des études cliniques sur la prise de poids sous INI montre un effet plus important du DTG par rapport au RAL et à l'EVG (Norwood et al. 2017, Bourgi et al. 2019, Sax et al. 2019, Bourgi et al. 2020). Le BIC semble avoir des effets similaires sur la prise de poids que le DTG chez les patients naïfs (Sax et al. 2019, Wohl et al. 2019). Il faut cependant rester prudent avec ces études cliniques car de multiples autres facteurs peuvent influencer la prise de poids comme le microbiote, les effets secondaires digestifs liés aux ARV, les modifications de l'humeur et autres troubles psychologiques dont souffrent d'avantage les personnes infectées par le VIH, la mauvaise qualité du sommeil ou encore l'arrêt du tabac, autant de facteurs qui ne sont pas toujours pris en compte dans ces études. L'un des principaux facteurs de risque de la prise de poids sous INI est le genre féminin. De plus, chez les patients naïfs de traitements, une origine afro-américaine, une charge virale élevée, un taux de CD4+ bas, un IMC bas et une prise de TAF sont des facteurs aggravants (Bakal et al. 2018, Bhagwat et al. 2018, Debroy et al. 2019, Hill et al. 2019). Chez les patients sous *switch* avec un INI, les facteurs aggravants sont l'âge et un IMC élevé (Lake 2019, Lake et al. 2020).

Quelques études ont été réalisées *in vitro* et montrent que RAL ne semble pas avoir d'effet sur l'adipogenèse et les adipocytes (Minami et al. 2011, Perez-Matute et al. 2011, Moure et al. 2016). De plus, une étude montre que l'EVG altère l'adipogenèse (Moure et al. 2016).

L'ensemble de ces effets et les mécanismes physiopathologiques liés aux INI seront discutés dans le chapitre 4) Article 2.

c) Atteintes du tissu adipeux et comorbidités

Les patients infectés par le VIH peuvent donc développer une lipodystrophie ou prendre du poids sous ARV. L'ensemble de ces altérations sont associées à une résistance à l'insuline, une stéatose hépatique et une augmentation du risque cardiovasculaire (Jan et al. 2004, Erlandson et al. 2017, Fourman et al. 2017). De manière générale, l'accumulation du TAV et la lipodystrophie chez les patients VIH augmentent d'avantage le risque de syndrome métabolique, de complications cardiovasculaires et de mortalité (Balasubramanyam et al. 2004, Lake et al. 2011, Scherzer et al. 2011, Langkilde et al. 2018, Srinivasa et al. 2018). Les PI ont largement été incriminés dans ces perturbations métaboliques en particulier la résistance à l'insuline, mais l'effet des autres classes d'ARV ne peut être exclu (Miller et al. 1998, Caron-Debarle et al. 2010).

3) Mécanismes physiopathologiques des antirétroviraux sur le tissu adipeux

a) Toxicité mitochondriale et stress oxydant

Une toxicité mitochondriale a été observée dans le TASC et le TAV de patients lipodystrophiques principalement avec l'utilisation des NRTI et des NNRTI. Elle se caractérise par une diminution de l'ADN mitochondrial (ADNmt), de l'expression de PGC1α, et des complexes de la chaine respiratoire mitochondriale (Shikuma et al. 2001, Walker et al. 2002, Kannisto et al. 2003, Giralt et al. 2006, De Pauw et al. 2009, Gallego-Escuredo et al. 2013, Walker et al. 2014).

In vivo et *in vitro*, les NRTI analogues de la thymidine ont été associés à une dysfonction des mitochondries (caractérisée par un défaut de potentiel membranaire associé à une augmentation de la masse mitochondriale compensatoire) et un stress oxydant qui participeraient aux altérations et à l'apoptose des adipocytes, favorisant la perte de TASC chez les patients lipodystrophiques (Anderson 2001, Walker et al. 2002, Lagathu et al. 2007, Caron et al. 2008, Boothby et al. 2009, Capel et al. 2012).

b) Inflammation

L'utilisation des PI est associée au recrutement des cellules immunitaires et à une augmentation de l'expression de cytokines pro-inflammatoires dans le TASC (Kannisto et al.

2003, Jan et al. 2004, Shikuma et al. 2014). Il a été montré que les NRTI, l'EFV et les PI de première génération augmentent l'expression de facteurs inflammatoires dans les préadipocytes, les adipocytes et les macrophages (El Hadri et al. 2004, Lagathu et al. 2007, Gallego-Escuredo et al. 2010, Diaz-Delfin et al. 2011, Capel et al. 2012). Par ailleurs, une accumulation de lymphocytes T CD8+ est observée dans le TAV chez les patients infectés et sous ARV (Couturier et al. 2015, Damouche et al. 2017, Koethe et al. 2018). Cette accumulation est d'avantage le reflet d'un recrutement au niveau du TA que le résultat de la prolifération des lymphocytes T CD8+ résidents (Palmer et al. 2005, Focosi et al. 2010, Damouche et al. 2017). Il est important aussi de noter qu'une augmentation de lymphocytes T CD8+ et des lymphocytes T CD4+ mémoires dans le TASC est associée au diabète chez les patients infectés sous ARV (Brown et al. 2005, De Wit et al. 2008, Bastard et al. 2019, Wanjalla et al. 2019). Concernant les macrophages du TA, la quantité de macrophages mesurée dans le TASC et dans le TAV varie selon les études chez les patients sous HAART (Jan et al. 2004, Gallego-Escuredo et al. 2013, Shikuma et al. 2014). L'inflammation favorise la résistance à l'insuline du TA mais aussi une fibrose *via* l'induction de TGF-β et l'apoptose des adipocytes, et contribuerait donc aux effets délétères observés chez les patients (Kannisto et al. 2003, Lihn et al. 2003, Jan et al. 2004, Johnson et al. 2004).

c) Fibrose du tissu adipeux

Très peu d'études se sont intéressées à la fibrose du TA chez les patients VIH. Une étude récente réalisée chez les patients recevant un INI ou un PI associé à un NRTI ou un NNRTI montre l'existence d'une fibrose dans le TASC abdominal avec une augmentation des collagènes I et VI, de la fibronectine (Bastard et al. 2002, Utay et al. 2018). Aucune étude ne s'est intéressée à la fibrose du TAV chez les patients contrôlés sous ARV. La fibrose du TA pourrait contribuer à l'existence de petits adipocytes observée notamment en réponse aux ARV de première génération. Elle pourrait également être secondaire aux dysfonctions des adipocytes (hypertrophie) ou l'inflammation locale qui sont observés dans le TA de certains patients infectés sous ARV.

En conclusion, les patients infectés par le VIH peuvent développer différentes redistribution et atteintes du TA selon la classe et la génération d'ARV (Tableau 2).

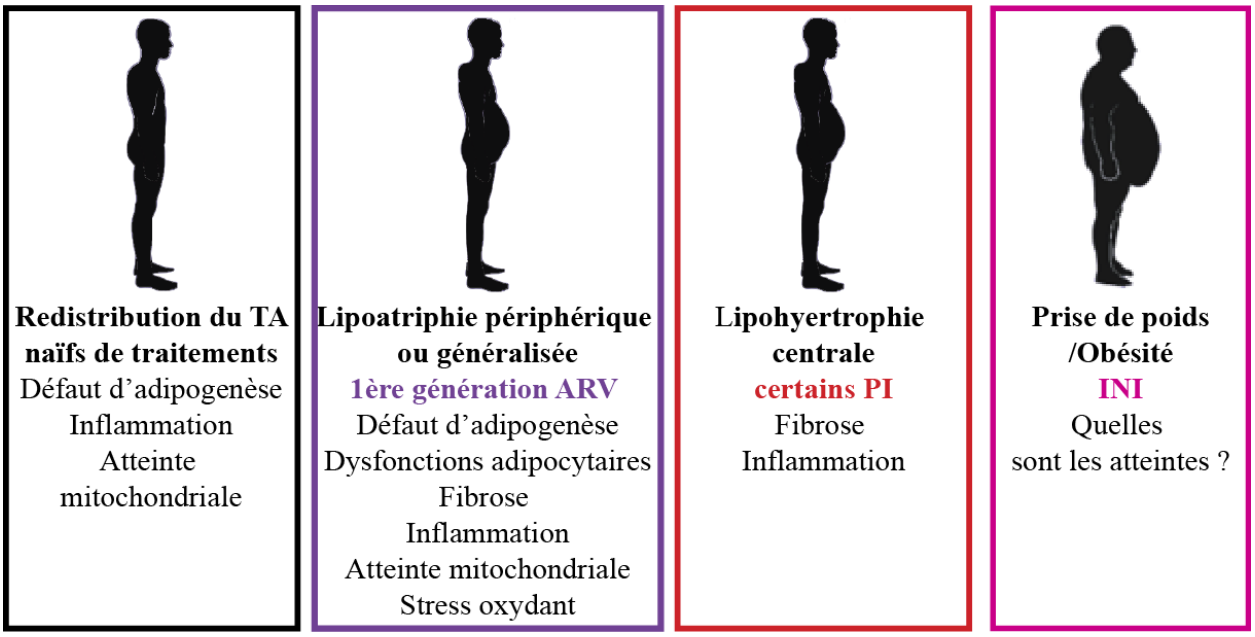

Tableau 2 : Tableau récapitulatif des atteintes décrites dans le tissu adipeux sous-cutané des patients infectés par le VIH selon la classe d'antirétroviraux et le type de redistribution.

ARV : antirétroviraux, PI : inhibiteurs de la protéase, INI : inhibiteurs de l'intégrase, TA : tissu adipeux, TASC : tissu adipeux sous-cutané.

4) Article 2: The Integrase Inhibitors Dolutegravir and Raltegravir Exert Proadipogenic and Profibrotic Effects and Induce Insulin Resistance in Human/Simian Adipose Tissue and Human Adipocytes

a) Contexte de l'étude et résumé des résultats

La prise de poids et les atteintes du TA sont des préoccupations majeures chez les patients infectés par le VIH, de par leurs rôles dans les complications cardiométaboliques. Une prise de poids est dite significative lors qu'elle est de + 5% du poids et cela est basé sur les études cliniques qui évaluent l'efficacité d'une drogue sur la perte de poids chez les patients obèses (Hill et al. 2019). Très peu d'études se sont intéressées aux mécanismes qui sous-tendent l'accumulation de TA induite par les INI. De plus, l'impact des INI sur la sensibilité à l'insuline fait débat (Young et al. 2015, Dirajlal-Fargo et al. 2016, Hulgan 2018, Langkilde et al. 2018, Offor et al. 2018, Calza et al. 2019, Katlama et al. 2019). Même si la toxicité métabolique de ces derniers n'est pas démontrée, il a été rapporté des cas de diabète chez des patients suite à un changement de combinaison thérapeutique incluant un INI (Kamei et al. 2015, Fong et al. 2017, Horikawa et al. 2018, McLaughlin et al. 2018). Le but de notre étude est donc d'évaluer l'impact des INI sur le phénotype et la fonction du TA *in vivo* et *in vitro*.

L'analyse du TASC et du TAV de patients obèses infectés par le VIH, traités ou non par des INI, a révélé une fibrose péri-adipocytaire accrue dans le TAV en présence d'INI. En accord,

dans un modèle original de macaques non infectés mais traités par des INI, nous avons observé une fibrose accrue, associée à une hypertrophie adipocytaire et une augmentation de l'expression de marqueurs adipogéniques. Une diminution de l'expression de l'adiponectine dans le TASC était en faveur de la présence d'adipocytes dysfonctionnels. *In vitro*, en réponse aux INI, les ASC humaines isolées à partir de donneurs sains et les adipocytes différenciés *in vitro* ont acquis un phénotype pro-fibrosant. En accord avec les observations faites sur le TASC et TAV, *in vitro* les INI favorisaient l'accumulation des triglycérides, augmentaient l'expression de marqueurs adipogéniques, mais diminuaient l'expression d'adiponectine suggérant une situation dichotomique dans laquelle les INI exercent un effet proadipogénique associé à une dysfonction adipocytaire.

L'ensemble de ces résultats, qui révèlent l'effet délétère des INI sur le TA, permet une meilleure compréhension des mécanismes impliqués dans l'accumulation de TA et des complications cardiométaboliques observées chez les patients infectés par le VIH sous INI.

Clinical Infectious Diseases

MAJOR ARTICLE

The Integrase Inhibitors Dolutegravir and Raltegravir Exert Proadipogenic and Profibrotic Effects and Induce Insulin Resistance in Human/Simian Adipose Tissue and Human Adipocytes

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Background. Although some integrase strand transfer inhibitors (INSTIs) promote peripheral and central adipose tissue/weight gain in people with human immunodeficiency virus (PHIV), the underlying mechanism has not been identified. Here, we used human and simian models to assess the impact of INSTIs on adipose tissue phenotype and function.

Methods. Adipocyte size and fibrosis were determined in biopsies of subcutaneous and visceral adipose tissue (SCAT and VAT, respectively) from 14 noninfected macaques and 19 PHIV treated or not treated with an INSTI. Fibrosis, adipogenesis, oxidative stress, mitochondrial function, and insulin sensitivity were assessed in human proliferating or adipocyte-differentiated adipose stem cells after long-term exposure to dolutegravir or raltegravir.

Results. We observed elevated fibrosis, adipocyte size, and adipogenic marker expression in SCAT and VAT from INSTI-treated noninfected macaques. Adiponectin expression was low in SCAT. Accordingly, SCAT and VAT samples from INSTI-exposed patients displayed higher levels of fibrosis than those from nonexposed patients. In vitro, dolutegravir and, to a lesser extent, raltegravir were associated with greater extracellular matrix production and lipid accumulation in adipose stem cells and/or adipocytes as observed in vivo. Despite the INSTIs' proadipogenic and prolipogenic effects, these drugs promoted oxidative stress, mitochondrial dysfunction, and insulin resistance.

Conclusions. Dolutegravir and raltegravir can directly impact adipocytes and adipose tissue. These INSTIs induced adipogenesis, lipogenesis, oxidative stress, fibrosis, and insulin resistance. The present study is the first to shed light on the fat modifications observed in INSTI-treated PHIV.

Keywords. integrase inhibitor; adipose tissue; fibrosis; oxidative stress; insulin resistance.

Integrase strand transfer inhibitors (INSTIs) are often prescribed as part of first-line treatment for human immunodeficiency virus (HIV) infection [1] or as a switch strategy in well-controlled older patients [2]. Recently, increased weight/fat gain in INSTI-exposed patients has been observed, and this is a worrying side effect [3–5].

In people with HIV (PHIV), the body mass index (BMI) has been steadily increasing, with greater weight gain after antiretroviral therapy (ART) initiation among women and patients with a low $CD4^+$ T-cell count [6]. INSTI-based regimens have been associated with further central and peripheral weight/fat gain in ART-naive and ART-experienced patients [7–10]. This has been reported with dolutegravir (DTG) and, to a lesser extent, raltegravir (RAL) in both groups [4, 5, 9–11].

Adipose tissue (AT) gain can be due to either (*i*) adipocyte hyperplasia following the recruitment and adipogenesis of adipose stem cells (ASCs) driven by the proadipogenic transcription factors CCAAT/enhancer-binding protein α (C/EBPα) and peroxisome proliferator–activated receptor γ (PPARγ) or (*ii*) adipocyte hypertrophy linked to increased lipogenesis, driven

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mainly by the transcription factor sterol regulatory element–binding protein 1c (SREBP-1c).

The pathophysiological mechanisms involved in AT dysfunction in the context of HIV infection are still poorly understood and multifactorial. AT inflammation and fibrosis are hallmarks of metabolically challenged adipocytes. At the onset of obesity, AT fibrosis (either perilobular or periadipocyte) is characterized by abnormally high levels of extracellular matrix (ECM) deposition (mainly collagens). Periadipocyte fibrosis and elevated collagen VI levels have been associated with poor metabolic outcomes [12]. The acquisition of a profibrotic phenotype by ASCs (characterized by increased expression of ECM components and α smooth muscle actin [αSMA]) is thought to be a key mechanism in the onset of AT fibrosis [12].

The impact of INSTIs on AT morphology and function in vivo has not previously been evaluated. In vitro studies showed that RAL minimally affected adipocyte lineages [13, 14]. The impact of DTG on adipocyte biology has not previously been studied, and the mechanisms involved in INSTIs' effects have yet to be identified. Furthermore, it is not known whether INSTI-induced AT accumulation is metabolically harmful or whether these drugs have an impact on insulin sensitivity. Indeed, increased fat/weight following ART initiation has been linked to an increased risk of insulin resistance and diabetes [15–17].

The objective of the present study was to characterize the impact of INSTIs on AT morphology and function in vivo. We had access to subcutaneous and visceral adipose tissue (SCAT and VAT, respectively) biopsies from 2 unique in vivo models: treated but noninfected macaques, and obese PHIV treated with an INSTI. In vitro, we studied human proliferating ASCs and differentiating/differentiated adipocytes to determine whether DTG or RAL exerted a direct impact on ECM production, adipogenesis, oxidative stress, lipogenesis, and insulin sensitivity.

MATERIALS AND METHODS

Adipose Tissue Samples From Macaques

Cynomolgus macaques (*Macaca fascicularis*) were imported from Mauritius and housed in the Commissariat à l'Energie Atomique (CEA) animal facility (Infectious Disease Models and Innovative Therapies; government accreditation number D92–032–02). Macaques were treated or not daily for 2 weeks with an INSTI-containing regimen [18] (Supplementary Table 1). Pharmacokinetic studies were performed at first to determine the optimal doses depending on the route of administration, allowing us to obtain plasma concentrations similar to those observed in humans. The study was approved by the French Ministry of Education, Higher Education and Research and the local animal care and use committee (Comité d'Ethique en matière d'Expérimentation Animale 44, Paris, France; reference number 2015102713323361.02, Autorisation de Projet utilisant des Animaux à des Fins Scientifiques number 2453). The animal facility complied with the Standards for Human Care and Use of Laboratory of the United States Office for Laboratory Animal Welfare (number A5826–01) and the European Directive (2010/63/EU, recommendation number 9). At necropsy, SCAT and VAT samples were collected.

Adipose Tissue Samples From ART-Treated PHIV

SCAT and VAT biopsies were obtained from obese PHIV from the Study of HIV infection on an obese Cohort [19] undergoing bariatric surgery, treated or not treated with an INSTI-containing regimen (Supplementary Table 2). All patients provided written informed consent.

Adipose Tissue Histology

Samples were fixed in 4% paraformaldehyde (Sigma-Aldrich, St Louis, Missouri) for 48 hours and stained with Sirius red [20]. Adipocyte size and fibrosis index were determined using a semiautomatic image analysis system [20]. Fibrosis index is defined as the ratio of fibrosis to total AT surfaces. Periadipocyte and perilobular fibrosis were scored as 0, 1, or 2 as previously described [21].

Isolation, Culture, and INSTI Treatment of Adipose-Derived Mesenchymal Stem Cells

Human SCAT samples, used for isolation of ASCs, were obtained from 8 healthy women (BMI < 25 kg/m^2). All provided their prior written informed consent. The research complied with the tenets of the Declaration of Helsinki and was approved by the independent ethics committee [20]. During expansion, ASCs were exposed for 2 weeks to INSTIs at peak concentration (C_{max}) (DTG: 3.1 μg/mL, RAL: 2.1 μg/mL; SCBT, Dallas, Texas) [22] or to 0.1% dimethyl sulfoxide (DMSO) as a control.

Adipocyte Differentiation

Adipocyte differentiation was induced for 2 weeks [20]. ASCs were either exposed to DTG or RAL throughout differentiation and analyzed on days 7 and 14, or were first differentiated into adipocytes for 14 days and then exposed to DTG or RAL for 6 days and analyzed on day 20. To evaluate lipid accumulation, cells were stained with Oil-Red-O (Sigma-Aldrich) [20].

RNA Isolation and Quantitative Reverse-transcription Polymerase Chain Reaction

Total RNA was isolated from macaque AT samples using QIAzol reagent (Macherey-Nagel, Hoerdt, France) or from cultured cells using RNeasy mini-columns (Qiagen, Courtaboeuf, France). Messenger RNA expression was analyzed using reverse-transcription polymerase chain reaction [23, 24] (see Supplementary Tables 3 and 4 for primer sequences).

Western Blotting

After protein extraction, the samples underwent sodium dodecyl sulfate–polyacrylamide gel electrophoresis and were blotted onto nitrocellulose membranes. Specific proteins were detected using antibodies against collagen 1-α2, collagen 6-α1

(SCBT), phospho-Akt-ser473, Akt, IRβ, anti-phosphotyrosine (for phospho-IRβ) (Cell Signaling Technology), and tubulin (Sigma-Aldrich).

Quantification of Protein Secretion Into Cell Culture Media

The fibronectin concentration was determined using a Quantikine enzyme-linked immunosorbent assay kit (Bio-Techne, San Jose, California). The adipokine concentration was determined using a Proteome profiler adipokine array kit (Bio-Techne), according to the manufacturer's instructions.

Oxidative Stress and Mitochondrial Dysfunction

The production of reactive oxygen species (ROS) was assessed by the oxidation of 5–6-chloromethyl-2,7-dichlorodihydro-fluorescein-diacetate (CM-H_2DCFDA). We used tetrachloro-tetra-ethyl-benzimidazolyl-carbocyanine iodide (JC-1) to evaluate mitochondrial membrane potential, and the MitoTracker Red probe to measure mitochondrial mass [22, 25] (all from ThermoFisher Scientific). Fluorescence was measured in cells after 120 minutes at 37°C in the dark. Results were normalized to 4′,6-diamidino-2-phenylindole fluorescence [26].

Insulin Signaling and Glucose Transport

Adipocytes were serum-starved for 18 hours. Insulin sensitivity was evaluated after 7 minutes with 100 nM insulin by Western blot as the ratio of phosphorylated Akt/total Akt and of phospho-IRβ/total IRβ. Insulin-stimulated glucose uptake was assessed using the Glucose Uptake Glo assay kit (Promega, Madison, Wisconsin) after 60 minutes with insulin 100 nM. Results were expressed as the mean ± standard error of the mean fold increased luminescence.

Statistical Analysis

All in vitro experiments were performed between 3 and 10 times in triplicate samples. Differences between INSTI-treated/nontreated patients or ART-treated/nontreated macaques were probed in an unpaired parametric Student *t* test. Differences between INSTI-treated and DMSO-treated cells were probed with a parametric or nonparametric *t* test. Differences in the level of periadipocyte fibrosis were probed in a χ^2 test. The threshold for statistical significance was set to $P < .05$. All analyses were performance using Prism 5.0 software (GraphPad Software, La Jolla, California).

RESULTS

SCAT and VAT From Dolutegravir-Treated Macaques Display Elevated Fibrosis, Large Adipocytes, and High Levels of Adipogenic Markers

Fibrotic bundles and thickening of fat lobules were evidenced by Sirius red staining. The fibrosis index in SCAT was 45.5% for ART-treated vs 24.6% ($P < .0001$) for control macaques. The fibrosis index in VAT was 30.0% in ART-treated vs 14.2% in control macaques ($P < .021$). These differences were mainly due to marked periadipocyte fibrosis in the SCAT and VAT of ART-treated but not control macaques (Figure 1A and 1B). The adipocyte size distribution was homogeneous in the AT of control macaques, whereas AT from ART-treated macaques presented clusters of large adipocytes (> 5000 μm^2) (Figure 1A and 1C). Expression levels of the proadipogenic factors *CEBPA* and *PPARG* in SCAT were higher in ART-treated macaques than in controls (Figure 1D), whereas *ADIPOQ* expression was lower (Figure 1D). These data suggest that INSTIs induce fibrosis, adipocyte enlargement, and gene expression changes in both SCAT and VAT.

An INSTI-Containing Regimen Is Associated With Elevated Fibrosis in Human SCAT and VAT, Relative to an INSTI-Sparing Regimen

Nineteen PHIV underwent bariatric surgery (Supplementary Table 2). All but 1 patient had received conventional ART before being switched to an INSTI regimen (9 DTG, 3 cobicistat-boosted elvitegravir, 2 RAL), and all but 1 were obese at that time. The mean duration of INSTI treatment was 29 months and the mean weight gain was 5.2 ± 1.4 kg/year. Only 21% (3/14) of INSTI-treated and 40% (2/5) of non-INSTI-treated patients were already obese at 20 years old. Familial cases were reported in 36% (5/14) of INSTI-treated and in 100% (5/5) of non-INSTI-treated patients. The fibrosis index in SCAT and VAT were higher in the 14 INSTI-treated than in the 5 non-INSTI-treated patients (Figure 2A and 2B). In both groups, the SCAT presented perilobular and periadipocyte fibrosis (Figure 2A and 2B). However, the level of periadipocyte fibrosis was higher in the VAT of INSTI-treated than in non-INSTI-treated patients (Figure 2A and 2B).

DTG and RAL Induce a Profibrotic Phenotype, Oxidative Stress, and Mitochondrial Dysfunctions in ASCs and in Adipocytes In Vitro

We then studied the impact of DTG and RAL on proliferating ASCs. INSTI treatment led to elevated levels of collagens (Figure 3A), fibronectin (Figure 3B), and the myofibroblast marker αSMA (*ACTA2*) (Figure 3C). In adipocytes, the 2 INSTIs upregulated collagen protein expression (Figure 3D). These results strongly suggest that DTG and RAL induce AT fibrosis by promoting a profibrotic phenotype in ASCs and adipocytes. We also observed that INSTIs increased ROS production (Figure 4A and 4C) and induced mitochondrial dysfunction characterized by increased mitochondrial mass and decreased membrane potential (Figure 4B and 4D) in proliferating ASCs and, to a lower extent, in adipocytes.

DTG and RAL Promote Adipogenesis and Lipid Accumulation In Vitro

Treatments with DTG and, to a lesser extent, RAL were associated with greater lipid accumulation via elevated expression of proadipogenic markers, particularly at C_{max} (Supplementary Figure 1), during early (day 7) and late (day 14) differentiation (Figure 5A and 5B). Both INSTIs increased expression of the proadipogenic

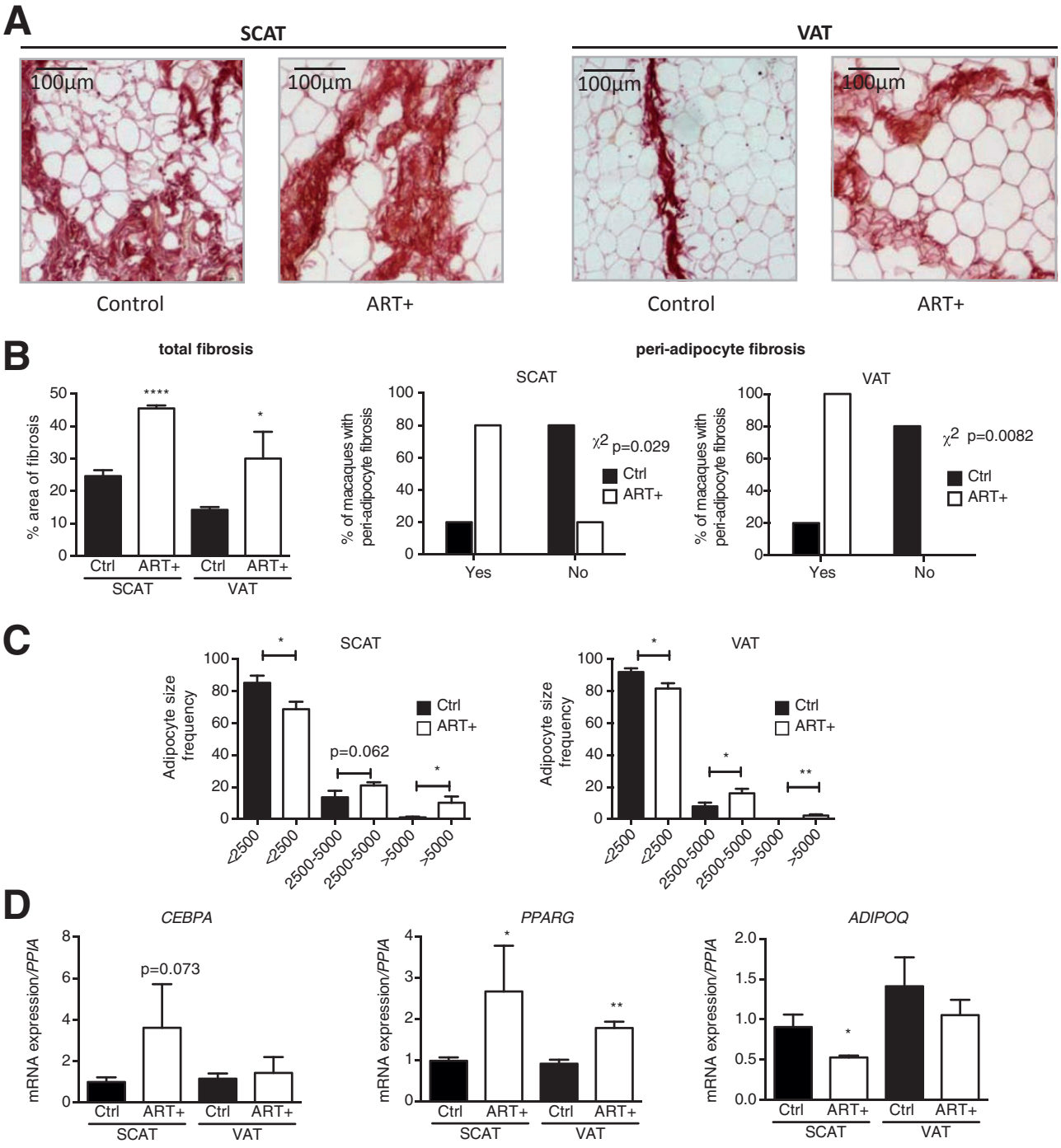

Figure 1. Subcutaneous and visceral adipose tissue from antiretroviral therapy–treated (ART+) macaques exhibits fibrosis and elevated adipogenic marker expression. *A*, Light microscopy analysis of adipose tissue depots stained with Sirius red to detect collagen fibers. Representative photographs are shown (magnification: ×10; scale bar, 100 µm). *B*, Fibrosis index in subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) was calculated (left panel) and the proportion of macaques with periadipocyte fibrosis was determined, as described in the Materials and Methods (right panel). *C*, Distribution of adipocyte size in SCAT and VAT depots in control and ART+ macaques. *D*, CCAAT/enhancer-binding protein alpha (*CEBPA*), peroxisome proliferator–activated receptor gamma (*PPARG*), and adiponectin (*ADIPOQ*) messenger RNA (mRNA) levels were measured using real-time polymerase chain reaction. The relative mRNA expression levels were normalized against cyclophilin A (*PPIA*). All results were obtained from triplicate measurements and are expressed as the mean ± standard error of the mean for the control (Ctrl) group and the ART+ group (n = 4–5 animals per group). **P* < .05, ***P* < .01, *****P* < .0001 vs Ctrl (nontreated) animals.

genes *CEBPA* and *PPARG* (Figure 5C; Supplementary Figure 1). DTG, but not RAL, was also associated with greater gene expression of the prolipogenic factor *SREBP1C* and the lipid metabolism markers *FAS* and *FABP4* (Figure 5D). It is noteworthy that DTG was associated with decreased expression and/or secretion of adiponectin and leptin (Figure 6A). We showed that DTG also

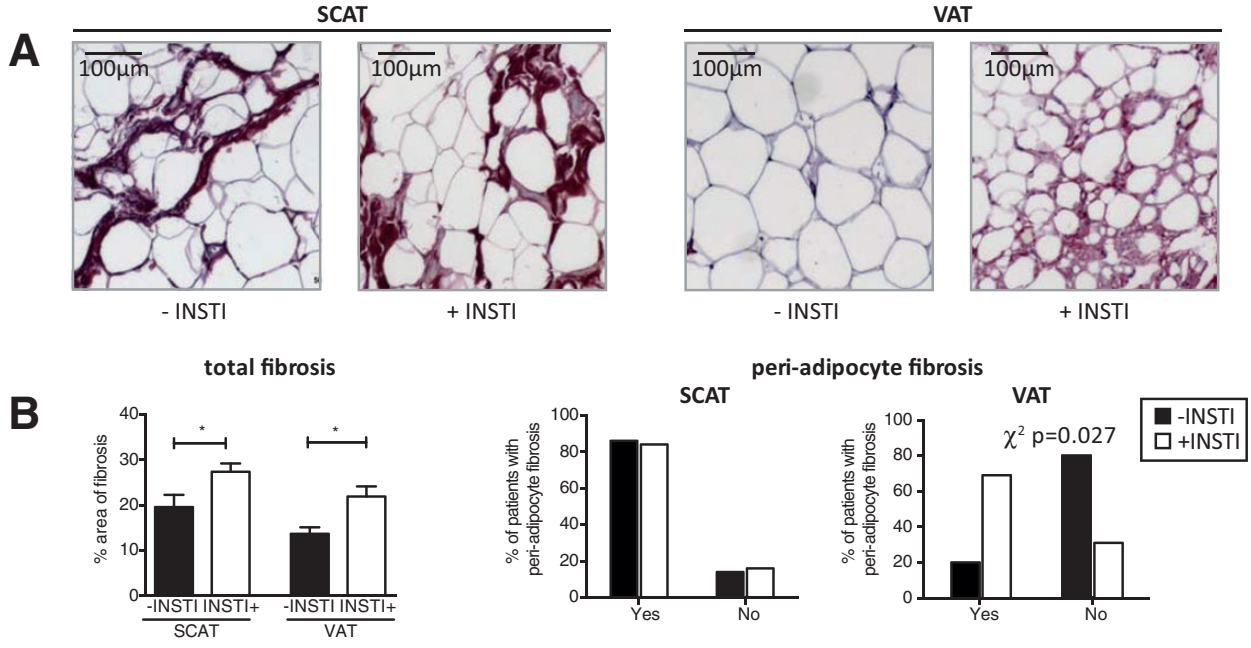

Figure 2. The level of fibrosis in subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) from integrase strand transfer inhibitor (INSTI)–treated obese patients is higher than in patients not treated with INSTIs. *A*, Light microscopy analysis of adipose tissue depots stained with Sirius red to reveal collagen fibers. Representative photographs are shown (magnification ×10; scale bar: 100 µm). *B*, The fibrosis index in SCAT and VAT was calculated (left panel), and the proportion of patients with periadipocyte fibrosis was measured, as described in the Materials and Methods (right panel). All results were obtained from triplicate experiments and are expressed as the mean ± standard error of the mean for INSTI-treated patients (n = 14) vs non–INSTI-treated patients (n = 5). **P* < .05 vs non–INSTI-treated patients.

promoted lipid accumulation when added on already differentiated mature adipocytes (Figure 6B). These results indicate that INSTIs (particularly DTG) enhance adipogenesis and lipogenesis in differentiating ASCs and mature adipocytes.

DTG and RAL Induce Insulin Resistance in Adipocytes In Vitro

In ASCs treated during (Figure 7A and 7C) or after (Figure 7B and 7D) differentiation, INSTIs inhibited acute insulin-induced phosphorylation of the insulin receptor and Akt (a key enzyme of the insulin signaling pathway) and blunted insulin-induced glucose transport (Figure 7E and 7F). These data indicate that INSTIs favor the onset of insulin resistance.

DISCUSSION

Here, we used unique in vivo models—INSTI-treated PHIV and noninfected macaques—to show for the first time that treatment with DTG or RAL is associated with elevated AT fibrosis and other adipose alterations. In a series of in vitro experiments, we demonstrated that DTG-treated and, to a lesser extent, RAL-treated ASCs and adipocytes acquired a profibrotic phenotype, enhanced oxidative stress, promoted lipid accumulation (probably through the activation of lipogenic pathways), and favored the onset of insulin resistance in adipocytes.

Several studies have highlighted increases in weight, BMI, incidence of obesity, and an overall increase in body fat with INSTI treatment, especially DTG and RAL [3–5, 7, 8] . Hence, we sought to identify the mechanisms underlying this fat gain.

First, we observed an increase in adipocyte size in the AT of noninfected INSTI-treated macaques, suggesting that INSTIs can induce adipocyte hypertrophy. DTG has been detected within adipocytes and the stromavascular fraction in VAT [27], suggesting that it has access to ASCs and adipocytes in vivo. RAL has a high level of tissue penetration and therefore is likely to accumulate in AT [19]. Consistently, our in vitro data revealed for the first time that DTG and, to a lesser extent, RAL can directly promote lipid accumulation by enhancing both adipogenic and lipogenic pathways in adipocytes. Interestingly, in differentiating ASCs, DTG had a higher impact on lipogenic than adipogenic gene expression, suggesting that DTG-induced adipocyte hypertrophy is rather due to a prolipogenic effect. Raltegravir treatment was associated with a slight elevation in lipid accumulation but did not affect adipogenesis per se, as the expression of the proadipogenic markers *PPARG* and *CEBPA* was not elevated as compared to the control. Our findings are in line with literature data showing that RAL did not impact adipogenesis [13, 14, 28]. It has been previously shown that protease inhibitors (atazanavir and lopinavir) and efavirenz can alter adipogenesis, with low levels of adipogenic marker expression in human adipocytes (unpublished data and [13, 25]), suggesting that the INSTIs differ from other antiretrovirals with regard to their impact on adipogenesis and lipogenesis. Thus, our results indicate that DTG and RAL promote adipocyte hypertrophy, thus increasing the fat mass and weight in INSTI-treated patients.

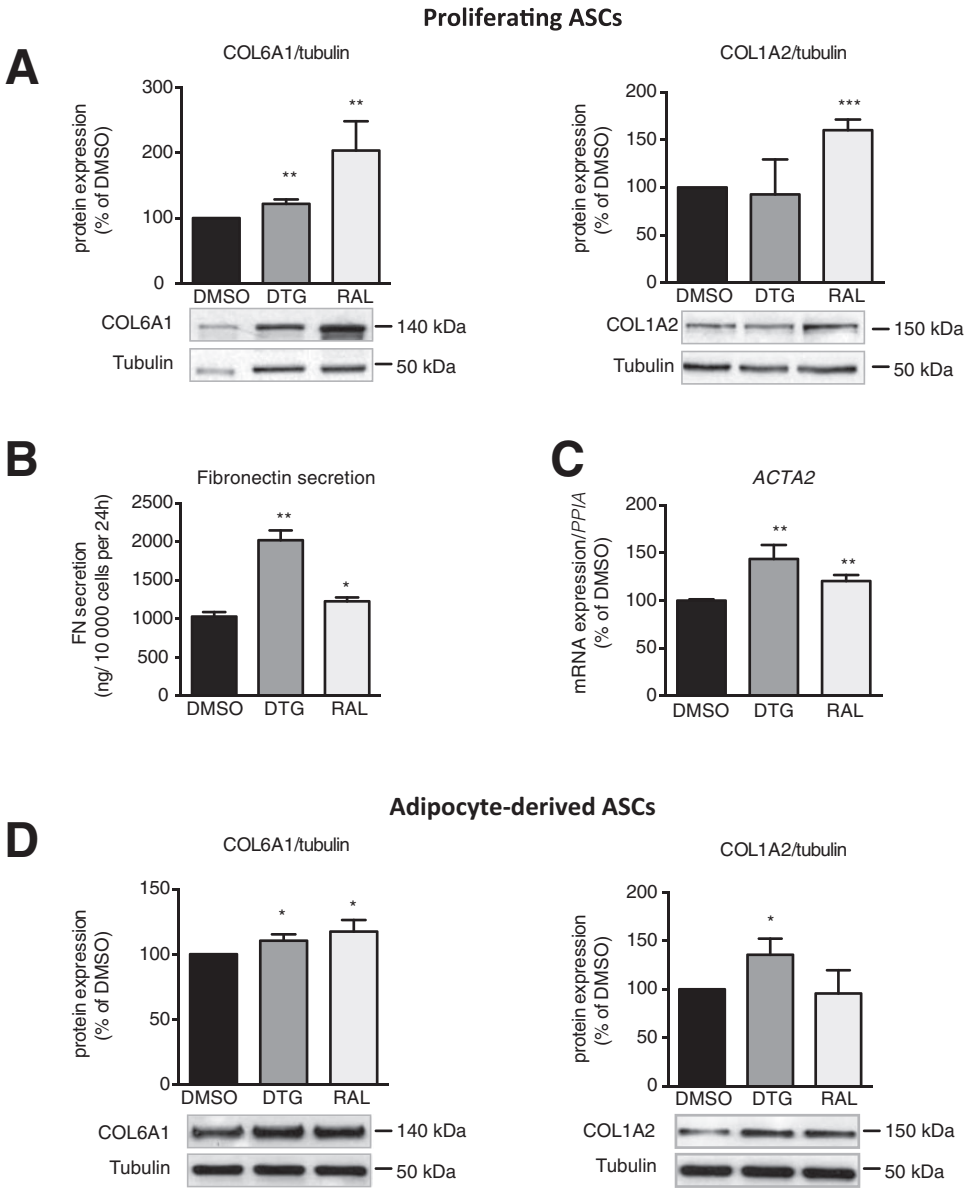

Figure 3. Long-term exposure to dolutegravir (DTG) and raltegravir (RAL) in proliferating adipose stem cells (ASCs) and in adipocytes is associated with greater expression of extracellular matrix proteins. *A–C*, ASCs were maintained in a proliferating, undifferentiated state for 15 days, treated with DTG (3.1 μg/mL) or RAL (2.1 μg/mL), and compared with cells treated with .1% dimethyl sulfoxide (DMSO). Total messenger RNA (mRNA) and whole-cell lysates were extracted and analyzed using reverse-transcription polymerase chain reaction and immunoblotting assays, respectively. *A*, Representative immunoblots for human collagen 6 (COL6A1) and 1 (COL1A2), normalized against tubulin as a loading control. *B*, Level of fibronectin (FN) in the cell culture medium was determined with an enzyme-linked immunosorbent assay. The results are expressed as nanograms per 10 000 cells per 24 hours. *C*, Human α-smooth muscle actin (*ACTA2*) mRNA levels, normalized against *PPIA* mRNA. *D*, ASC-differentiated adipocytes were treated with integrase strand transfer inhibitors after 2 weeks of differentiation. Whole-cell lysates were prepared and analyzed in immunoblots. Representative immunoblots for human COL6A1 and COL1A2 (quantified against tubulin as a loading control) are shown. The data are quoted as the mean ± standard error of the mean. All experiments were performed in triplicate. **P* < .05, ***P* < .01, ****P* < .001 vs DMSO-treated cells. Abbreviation: *PPIA*, peptidylprolyl isomerase A.

We hypothesized that this process might result from increased oxidative stress. Indeed, lipohypertrophy in PHIV has previously been linked to mitochondrial toxicity in AT [29], and DTG can induce mitochondrial dysfunction and oxidative stress in CD4 T-lymphocytes [30]. Accordingly, we found that DTG and RAL promoted ROS production and mitochondrial dysfunction.

Fibrosis is a major feature of AT dysfunction [12]. We observed a significant elevation of the fibrosis index in SCAT and VAT in ART-treated macaques. Accordingly, we found that total fibrosis was elevated in the SCAT and VAT of obese INSTI-exposed PHIV, when compared with patients on an INSTI-sparing regimen. In the context of obesity, elevated fibrosis is mainly due to periadipocyte fibrosis; the latter has been linked to metabolic disorders [12] and was observed in SCAT and VAT of INSTI-treated macaques and in VAT of INSTI-treated patients.

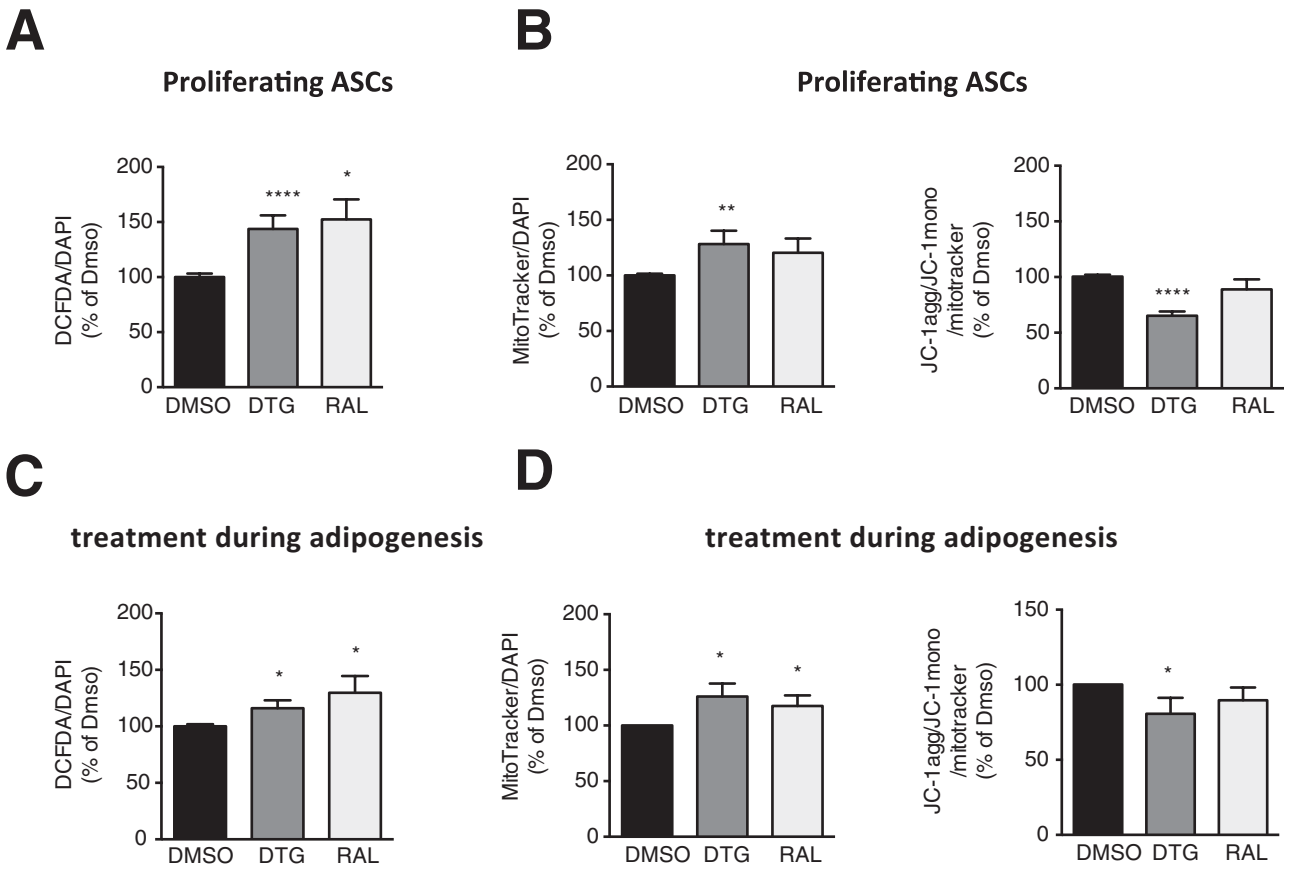

Figure 4. Long-term exposure of proliferating adipose stem cells (ASCs) and adipocytes to dolutegravir (DTG) and raltegravir (RAL) is associated with elevated reactive oxygen species (ROS) levels and mitochondrial dysfunction. ASCs were maintained in a proliferating, undifferentiated state (*A* and *B*) or were induced to differentiate into adipocytes (*C* and *D*). The cells were treated for 2 weeks with DTG (3.1 μg/mL) or RAL (2.1 μg/mL) and compared with cells treated with dimethyl sulfoxide (DMSO). ROS production was assessed by CM-H_2DCFDA in proliferating ASCs (*A*) or in ASC-differentiated adipocytes (*C*), as described in the Materials and Methods. Mitochondrial mass and membrane potential were assessed by Mitotracker and JC-1 aggregation, respectively, in proliferating ASCs (*B*) or in ASC-differentiated adipocytes (*D*), as described in the Materials and Methods. The data are expressed as the mean ± standard error of the mean. All experiments were performed in triplicate. $^*P < .05$, $^{**}P < .01$, $^{****}P < .0001$ vs DMSO-treated cells. Abbreviations: CM-H2-DCFDA, chloromethyl- diacétate de dichlorodihydrofluorescéine 2′,7′; DAPI, 4′,6-diamidino-2-phenylindole; JC, tetrachlorotetraethyl-benzimidazolyl-carbocyanine iodide.

There are several possible explanations for these differences between the macaques and the patients, including the duration of INSTI treatment, the species, the sex (the female/male ratio was 1:13 for the macaques and 15:4 for the patients), the metabolic state (morbidly obese patients vs chubby macaques), and the infection status. Moreover, it has recently been shown that tenofovir alafenamide (TAF) promoted more weight gain than tenofovir disoproxil fumarate (TDF) in combination with INSTIs and that TDF could have a protective effect in that setting [5, 31]. All of our treated macaques received TDF. In the ObeVIH study, 2 of the 14 INSTI-treated patients were taking TAF and 2 TDF, whereas 4 of the 5 non-INSTI-treated patients were taking TDF and none TAF. Hence, we cannot rule out an influence of TDF or TAF, in addition to INSTI, on the differences in adipose profiles between the 2 groups of macaques and of patients.

In vitro, we found that both DTG and RAL induced a profibrotic phenotype in ASCs and adipocytes, suggesting that these cells are key players in the onset of AT fibrosis. Fibrosis can result from AT inflammation [12]; this is rather unlikely in the present context, since RAL has no effect on cytokine secretion in adipocytes and is associated with a decrease in inflammation in endothelial cells [13, 22]. Moreover, an assessment of dorsocervical AT in HIV-linked lipodystrophy revealed that the elevation in AT fibrosis was not associated with inflammation [23]. Taken as a whole, these data indicate that INSTIs have a profibrotic effect, especially at the periadipocyte level, but the mechanisms involved remain speculative.

The impact of INSTIs on insulin sensitivity is still subject to debate. Some researchers have not observed any impact of INSTIs on the homeostatic model assessment of insulin resistance or glycemia [32, 33]. Nonetheless, other researchers have found that DTG and RAL promoted insulin resistance and lowered circulating adiponectin levels [8, 15, 34–36]. Accordingly, adiponectin expression was low in the SCAT of INSTI-treated macaques. Moreover, despite DTG's