**TELMISARTAN REVERSES ANTIRETROVIRAL-INDUCED ADIPOCYTE TOXICITY AND INSULIN RESISTANCE *IN VITRO***

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**Abbreviations:**

ARB: Angiotensin receptor blocker; ARV: Antiretroviral; ATV: Atazanavir; cART: Combination antiretroviral therapy; CVD: Cardiovascular disease; HIVLD: HIV lipodystrophy; IL-6: Interleukin-6; LPIN1: Lipin1; LPV: Lopinavir; NRTIs: Nucleoside reverse transcriptase inhibitors; pAKt: phospho-Akt; PCR: Polymerase Chain Reaction; PI3K: Phosphoinositide-3-kinase; PIs: Protease inhibitors; PPARγ: Peroxisome proliferator receptor-gamma; RAS: renin angiotensin system; ROSI: rosiglitazone; RTV: Ritonavir; T2DM: Type 2 diabetes; TEL: Telmisartan; TMB: 3,3´,5,5´- tetramentyl benzidine; TNF-α: Tumor necrosis factor- α; NS: Non-significant.

**Abstract:**

**Background:** Antiretroviral therapy in HIV-positive patients leads to insulin resistance which is central to the pathogenesis of the various metabolic abnormalities and cardiovascular disease seen in this patient group. We have investigated the dose-response relationship of telmisartan, an antihypertensive, on adipocytes *in vitro* in order to determine whether it may have metabolic beneficial effects.

**Methods:** Using *in vitro* chronic toxicity models (3T3-F442A murine and primary human adipocytes) we evaluated the effects of different concentrations of telmisartan on adipocyte differentiation and adipogenic gene expression using lipid accumulation assays and Real Time PCR, respectively. Adipokine secretion and expression of insulin signalling mediators were evaluated using ELISAs.

**Results:** Telmisartan partially reversed the deleterious effects of antiretrovirals on adipocyte lipid accumulation, expression of adipogenic regulators (*PPARγ* and *LPIN1),* adipokine secretion and expression of the insulin signalling mediator, pAktSer473. The metabolic effects of telmisartan followed a non-monotone response with the maximal effect observed at 5µM in the primary human adipocyte model.

**Conclusions:** Telmisartan has beneficial metabolic effects in adipocytes *in vitro*, but its potential to reduce antiretroviral-induced cardiometabolic disease in HIV-infected individuals needs to be evaluated in a well-designed adequately powered clinical trial.

**Key words:** HIV; antiretroviral; insulin resistance; metabolic disease; telmisartan; adipocyte

**Introduction**

Combination antiretroviral therapy (cART) is the mainstay of treatment in HIV. It has improved the morbidity and mortality associated with HIV, turning it into a chronic disease. However, cART, together with the virus itself, increases the risk of various metabolic complications, including obesity,1 type 2 diabetes (T2DM), and cardiovascular disease (CVD).2 Indeed CVD is the leading cause of death in HIV-infected patients on cART with a linear increase in the incidence of myocardial infarction observed with long term cART exposure.3

Insulin resistance is central to the development of cardiometabolic disease,4 being present in 21% of HIV patients on antiretroviral (ARV) therapy.5 *In vitro* as well as single drug studies in both healthy6 and HIV-infected patients7 have shown that insulin resistance can be induced by both protease inhibitors (PIs) and nucleoside reverse transcriptase inhibitors (NRTIs). Although newer ARVs are increasingly used in clinical practice, insulin resistance very much remains an important problem; HIV patients (n=328) randomised to TDF/3TC (tenofovir disoproxil fumarate/lamivudine) with either boosted atazanavir or boosted darunavir or raltegravir showed a 1.9-fold increase in HOMA-IR within 4 weeks.8 Importantly, HIV-associated metabolic disease results in increased healthcare burden; a recent study in the US identified the management of insulin resistance/diabetes to be the biggest contributor to the cost burden and resource use amongst all HIV-related adverse events studied.9

Adipose tissue is a major determinant of insulin resistance and may therefore play a key role in cART-associated metabolic disease. Adipose tissue has also been shown to be a reservoir for HIV and a source of chronic inflammation.10 Clinical interventions to arrest or reverse cART-associated adipose-mediated insulin resistance are a potential strategy to reduce the incidence of T2DM and CVD in HIV-positive patients. To this end, insulin sensitisers such as thiazolidinediones and metformin have been trialled but results from randomised clinical trials in HIV-positive patients have been disappointing and sometimes deleterious.11-13 There is therefore a need for novel clinical interventions that can reduce cART-induced insulin resistance in HIV-positive individuals.

Preliminary *in vitro* studies have suggested that telmisartan, an angiotensin II receptor blocker (ARB), reduces cART-induced adipose dysfunction by inhibition of the renin angiotensin system (RAS).14 In addition to being an ARB, telmisartan is also a partial agonist at the Peroxisome Proliferator Receptor-Gamma (PPARγ) receptor,15 a key regulator of adipose tissue metabolism.16 In this paper, we further evaluate the effect of telmisartan on cART-induced adipocyte dysfunction and insulin resistance in a novel chronic *in vitro* toxicity model, in addition to assessing its concentration-response relationship.

**Materials and methods**

**Materials**

Murine 3T3-F442A cells were a kind gift from Prof Karen Chapman (University of Edinburgh). Primary human subcutaneous preadipocytes were obtained commercially from age and sex-matched healthy donors (n=3; body mass index <25kg/m2; Promocell, Germany). Primary human adipocytes were obtained from subcutaneous adipose tissue obtained from the abdomen for all three donors. Collection of adipose tissue was approved by local ethics committee and all donors gave informed consent. None of the donors had any known medical conditions (i.e. hypertension, CVD, thyroid disorders, renal disorders, diabetes or chronic pain conditions) or were on endocrine therapy, anti-inflammatory therapy, statins, thiazolidinediones or antihypertensive therapy. Lopinavir (LPV), ritonavir (RTV), atazanavir (ATV) and rosiglitazone (ROSI) were purchased from Santacruz Biotechnology (Dallas, USA) and telmisartan (TEL) was provided by Boehringer Ingelheim GmbH (Germany). Adipocyte media were obtained from Promocell. Taqman gene expression assays (*PPARγ* and *lipin1)* and Taqman Gene Expression master mix were purchased from Life Technologies Ltd (Paisley, UK). Multiplex and singleplex ELISA for adipokines (adiponectin, IL-6, TNF-α, resistin) were obtained from Merck Millipore (Hertfordshire, UK) and Life Technologies Ltd. A colorimetric assay for free fatty acid release was obtained from Abcam (Cambridge, UK). Estimation of phospho-Akt (pAktSer473) and total Akt was performed by sandwich ELISA kits obtained from ThermoFisher Scientific (Paisley, UK).

**Methods**

***In vitro* chronic adipocyte toxicity model**

ARVs accumulate extensively within the adipocytes,10 and we thus used a chronic *in vitro* toxicity model to mimic this. Briefly, both 3T3-F442A murine cells and primary human subcutaneous adipocytes were cultured, induced to differentiate as described previously,17 and were treated with PIs with or without TEL and/or ROSI throughout adipocyte differentiation. For 3T3-F442A, cells were cultured with Dulbecco’s Modified Eagle’s medium (Sigma-Aldrich, UK) and 10% fetal calf serum followed by initiation of differentiation using 10mg/ml insulin (Sigma-Aldrich, UK). Primary human preadipocytes were cultured in a Preadipocyte Growth Medium which is a low-serum (5% v/v) medium optimised for the expansion of human preadipocytes. Once 70-80% confluent, differentiation was induced by culturing the cells in the Preadipocyte Differentiation Medium, a serum-free medium, for 3 days followed by further maintenance of differentiating adipocytes in the Adipocyte Nutrition medium. Drug treatment was started 48 hours post initiation of differentiation and carried out every 48 hours over a period of 10 days (or 12 days in the case of primary human adipocytes). The effects of PIs were tested over a wide concentration range (1-20µM) including their near-Cmax values (RTV and LPV: 10µM; ATV: 4.4µM). We initially selected 2 different concentrations for TEL (1 and 5μM) based on previous literature 14,18; but for further dose characterisation of TEL, we tested a range of concentrations (0.5-20μM). TEL was co-incubated with each of the PIs and added at the same time. ROSI (10μM), a *PPARγ* agonist, was coincubated with LPV only in the primary human adipocyte model as a comparator.

**Measurement of Cell Viability**

Viability of differentiating 3T3-F442A and primary human adipocytes was assessed using the MTT assay. MTT measure mitochondrial metabolism as a surrogate marker of cell viability19. Cells were incubated with the ARVs in serial concentrations (0.01 – 100µM) for 4 days. On day 4, cells were incubated with MTT ((3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) and absorbance of the resultant formazan product was measured at 560nm.

**Lipid accumulation assay**

Lipid accumulation in differentiating adipocytes was assessed on day 10 (3T3-F442A cells) or day 12 (primary human adipocytes) of differentiation using Oil Red O (Sigma, Dorset, UK) as previously described.14 Lipid bound dye was extracted using 70% isopropyl alcohol and staining was quantified at 520nm. Drug treated cells were compared against vehicle control (methanol).

**RNA extraction and gene expression**

Total RNA was isolated using the RNeasy kit (Qiagen, Manchester, UK). Total RNA was reverse transcribed using the Taqman® reverse transcription kit (Life Technologies, Paisley, UK). Gene expression of *PPARγ* and lipin1 (*LPIN1)* were assessed by Real-time PCR using Taqman Assays-on-demand gene expression assays on a 7900HT Fast Real Time PCR system (Life Technologies, Paisley UK).

**Assessment of Lipolysis**

Free fatty acid concentration in the conditioned media of primary human adipocytes was determined by using the Free Fatty Acid Quantification assay kit as per manufacturer’s instructions (Abcam, Cambridge, UK). Briefly, palmitic acid standard (1nmol/μl) was used to prepare the standard curve dilution and the reaction plates were prepared, incubated at room temperature for 30 minutes protected from light and the absorbance was measured using a microplate spectrophotometer reader (Beckman Coulter Multimode detector DTX880) at 595nm.

**Estimation of Adipokines**

Adipokine (adiponectin, IL-6, resistin and TNF-α) concentrations in the conditioned media were determined on day 10 (3T3-F442A) or day 12 (primary human adipocytes) post differentiation using bead-based Milliplex Mouse sandwich multiplex ELISA kits (Merck Millipore, Hertfordshire, UK) and Human singleplex ELISA kits (Life Technologies Ltd, Paisley, UK) respectively. The ELISA kits used had the following detection limits: Adiponectin: 5.2 pg/ml (murine), 100 pg/ml (human); IL-6: 5.3 pg/ml (murine), <1 pg/ml (human); TNFα: 11.2 pg/ml (murine), <2 pg/ml (human); and resistin: 6.1 pg/ml (murine), 10 pg/ml (human).

**Estimation of phosphorylated Akt content**

Phosphorylated Akt (Serine residue 473) as well as total Akt were quantitated by a sandwich ELISA, as recommended by the manufacturer (ThermoFisher Scientific, UK). Briefly, diluted lysates were applied to 96-well plates containing immobilized monoclonal antibodies specific for human Akt and incubated for 2 hours at room temperature. A pS473 Akt standard and samples were pipetted into the wells, followed by washing and incubation with a rabbit antibody (Cell Signalling, UK) specific for AKT phosphorylated at serine 473. Following washing, a horseradish peroxidase–labelled anti–rabbit IgG was added, washed, and a substrate solution (TMB) was added to produce colour and the absorbance was read at 450nm.

**Statistical analysis**

Data are presented as mean ± standard deviation (SD) for at least three independent experiments to ensure reproducibility. Statistical significance was determined using the non-parametric Mann–Whitney U test (IBM SPSS Statistics, Version 22). The threshold of significance was set at *P<*0.05.

**Results**

**Antiretrovirals cause adipocyte cytotoxicity**

Cytotoxicity was observed with all ARVs during differentiation of preadipocytes and followed a similar trend in both 3T3-F442A and primary human cells. In differentiating 3T3-F442A adipocytes, the rank order for cytotoxicity was LPV (IC50= 14µM) > RTV (IC50= 48µM) > ATV (IC50= 66µM) (Fig 1A)*.* In primary human adipocytes undergoing differentiation, the rank order for cytotoxicity was LPV (IC50= 28µM) > RTV (IC50= 38µM) > ATV (IC50= 84µM) (Fig 1B).

[INSERT Figure 1]

**Telmisartan reverses antiretroviral-induced inhibition in adipocyte lipid accumulation**

In 3T3-F442A adipocytes, a dose dependent reduction in lipid accumulation was observed for LPV (at 20µM, a reduction of 32% in mean absorbance, p<0.01) and RTV (at 20µM, 44% reduction, p<0.01) but not ATV (at 20µM, 4% increase, NS) in comparison with vehicle treated controls (Fig 2A; Full concentration response data are given in Supplementary Information). 1µM TEL partially reversed (p=0.01) the RTV and LPV-induced reduction in lipid accumulation in 3T3-F442A adipocytes (Fig 2A).

In primary human adipocytes, LPV and RTV (40 and 55% decrease respectively in comparison with the vehicle control, p<0.01) but not ATV (9% increase; NS) inhibited lipid accumulation (Fig 2B). Both TEL (5µM) and ROSI (10µM) partially reversed (p<0.01) ARV-induced inhibition of lipid accumulation in primary human adipocytes (Fig 2B). The effect shown by ROSI was stronger than that of TEL.

[INSERT Figure 2]

**Telmisartan reverses antiretroviral-induced downregulation of *PPARγ and Lipin1* gene expression**

***PPARγ:*** In 3T3-F442A adipocytes, both LPV and RTV (75% and 73% downregulation respectively, p<0.01), but not ATV, downregulated *PPARγ* gene expression in comparison with vehicle control. This was partially but significantly reversed by 1µM TEL (Fig 3A). A similar result was observed in primary human adipocytes (LPV, 78% and RTV, 80% downregulation respectively) which was partially reversed by 5µM TEL (LPV+TEL, p=0.03; RTV+TEL: p=0.01) (Fig 3B).

***Lipin1:***Both LPV and RTV downregulated *LPIN1* gene expression in both 3T3-F442A adipocytes (LPV, 64%; RTV, 78%, p<0.01; Fig 3C) and primary human adipocytes (LPV, 68%; RTV, 63%, p<0.01; Fig 3D). In both models, this was partially reversed by 1 (3T3-F442A) or 5µM (primary human adipocytes) TEL (Fig 3C and 3D). ATV did not have any effect on *LPIN1* expression.

[INSERT Figure 3]

**Telmisartan reverses antiretroviral-induced changes in adipokine secretion**

***Adiponectin:*** In 3T3-F442A adipocytes, both LPV (4.0 ng/ml ± 0.4; p = 0.002) and RTV (7.0 ± 1.0; p = 0.001) but not ATV (14.2 ± 2.4) caused downregulation in secreted adiponectin protein in comparison to the vehicle control (16.5 ± 2.0) (Fig 4A). 1µM telmisartan resulted in significant but partial reversal of PI-induced downregulation of adiponectin (Fig 4A). A similar result was observed in primary human adipocytes (LPV, 88% reduction, p=0.01; RTV, 73%, p=0.01; ATV, 7.5%, NS); both TEL (5µM) and ROSI (10µM) were able to significantly reverse PI-induced downregulation of adiponectin (Fig 4B).

***IL-6:*** Both LPV and RTV but not ATV increased the secretion of IL-6 in 3T3-F442A adipocytes (LPV, 190 ng/ml ± 11.3; RTV, 243 ± 7.9; both p < 0.01; ATV: 55 ± 8.0, NS) in comparison to the vehicle control (45 ± 7.1) (Fig 4C). A similar effect was also observed in primary human adipocytes for these PIs (Fig 4D; LPV: 278% increase; RTV: 316% increase; both p<0.01). In both *in vitro* models, coincubation with TEL partially reversed PI-induced upregulation of secreted IL-6 (Fig 4C and 4D).

***TNF-α:*** LPV (3T3-F442A, 45 pg/ml ± 2.1; primary human adipocytes, 62 pg/ml ± 4.2, both p<0.001) and RTV (3T3-F442A, 57±5.0; primary human adipocytes, 78±5.7, both p<0.01) but not ATV (3T3-F442A, 30±2.0; primary human adipocytes, 45±5.0) upregulated secreted TNF-αin comparison to the vehicle control (3T3-F442A, 32±4.3; primary human adipocytes, 40±5.0) (Fig 4E and 4F). Co-incubation with either 1 (3T3-F442A) or 5µM TEL (primary human adipocytes) or 10µM ROSI (primary human adipocytes only) significantly reversed PI-induced upregulation of TNF-α.

***Resistin:***  All 3 PIs downregulated RETN in both murine (LPV: 74% decrease; RTV: 73% decrease and; ATV: 57% decrease; all in comparison to vehicle control; p<0.01; Fig 4G) and primary human adipocytes (LPV and RTV: 65% decrease; ATV: 48% decrease; all in comparison to vehicle control; p<0.01; Fig 4H). Both TEL and ROSI (in primary human adipocytes only) showed a trend to reverse the PI-induced downregulation of resistin but this was not significant in either of these models (Fig 4G and 4H).

[INSERT Figure 4]

**Telmisartan reverses antiretroviral-induced adipocyte lipolysis and inhibition of Akt phosphorylation in primary human adipocytes**

Both LPV (90% increase; p<0.03) and RTV (109% increase; p<0.01) but not ATV (23%; p=NS) resulted in an increase in free fatty acid levels in the conditioned media in primary human adipocytes suggesting enhanced lipolysis by these drugs (Fig 5A). Co-incubation with 5µM TEL reduced PI-induced lipolysis although the effect was statistically non-significant. However, 10µM ROSI showed a significant partial reversal of ARV-induced lipolysis.

A significant reduction in the expression of pAktSer473 was observed with LPV (at 20µM, 63% reduction, p<0.01) and RTV (20µM, 61% reduction; p<0.01), but not with ATV, in comparison with vehicle treated controls (Fig 5B). Both LPV and RTV reduced pAktSer473 expression in a dose-dependent manner (See Supplementary information). Co-incubation with 5µM TEL or 10µM ROSI significantly reversed PI-induced downregulation of pAktSer473 (Fig 5B).

[INSERT Figure 5]

**Characterisation of optimal telmisartan dose to elicit metabolic effect**

Using secreted adiponectin and *PPARγ* gene as exemplar markers, we evaluated the concentration-response relationship of telmisartan in the presence of LPV (20µM) in primary human adipocytes. TEL significantly reversed LPV-induced inhibition of adiponectin at 1, 5 and 10µM concentrations; for *PPARγ*, the effect of TEL was observed at 5 and 10µM only. Importantly, in both instances, the maximal response for TEL was observed at 5µM with TEL showing a non-monotone dose response (Fig 6A and 6B).

[INSERT Figure 6]

**Discussion**

We have confirmed previously reported toxic effect of PIs on adipocytes but also identified how PIs affect novel adipokines such as lipin1 and resistin. We also found that telmisartan results in partial but significant reversal of ARV-induced adipocyte toxicity, and for the first time characterised the concentration of telmisartan that elicits the maximal metabolic effect *in vitro*. We used 3T3-F442A cells as they are one of the most widely used *in vitro* models to study adipogenesis and are committed to differentiating into adipocytes20. They have also been shown to develop a homogeneous population of mature adipocytes that are morphologically and biochemically similar to adipocytes *in situ*20. The chronic drug treatment design enabled repeated drug exposure to the adipocytes over the entire period of adipocyte differentiation; this mimicked the *in vivo* situation where long term antiretroviral drug treatment may result in cumulative adipocyte toxicity.21 LPV and RTV were toxic to adipocytes in both *in vitro* models while ATV, a more lipid-friendly PI,22 was not, suggesting that accumulation of certain ARVs over time may reduce cell viability in differentiating adipocyte populations *in vivo* and potentially deleteriously affect the fat cell turnover and thereby adipose tissue distribution.

Both LPV and RTV markedly decreased lipid accumulation and mRNA expression of the adipogenic markers, *PPARγ* and *LPIN1,* consistent with their anti-adipogenic effects. By contrast, ATV did not show any effect on any the above markers of lipid metabolism even at a concentration of 20µM (>4 times its Cmax of 4.4µM). The contrasting effects of ATV support clinical data,22 which shows ATV has very little effect on body fat distribution in HIV patients. This is the first study to report an effect of PIs on Lipin1, a gene that encodes a magnesium-ion-dependent phosphatidic acid phosphohydrolase enzyme involved in triglyceride synthesis23 and a key factor in the maturation and maintenance of adipocyte differentiation.24 LPIN1 is also a key transcriptional regulator of PPARγ and various genes involved in lipid metabolism.25 Interestingly *LPIN1* mutations cause different types of severe human lipodystrophy syndromes26 although our own previous work has failed to identify any association between *LPIN1* single nucleotide polymorphisms and HIV lipodystrophy (HIVLD).27 The inhibitory effect of lipotoxic PIs on LPIN1 could potentially be one of the mechanisms involved in the transcriptional downregulation of *PPARγ* caused by these drugs.

LPV and RTV had a profound effect on the secretory characteristics of the adipocyte regardless of the model used. Our results on adiponectin, IL-6 and TNFα further highlights how certain PIs may interact with the adipokine network and regulate their transcription leading to adipocyte dysfunction and interference with insulin signalling. In addition, LPV and RTV but not ATV showed a significant reduction in the expression of phosphorylated form of Akt (Ser473); Akt is a serine/threonine kinase and a downstream target of PI3K signalling and phosphorylation of its serine residue at position 473 is an important step in the insulin signalling pathway.28

This study also explored the effect of PIs on resistin, an adipocyte-secreted protein which is implicated in insulin resistance. Whilst higher circulating levels of resistin have been implicated in the development of insulin resistance29 and diabetes,30 its role in HIVLD is inconclusive. Some cross-sectional studies have reported an increase in circulating resistin levels in HIVLD patients,31 while other studies failed to find an association32 or even reported a reduction in resistin levels.33 We observed a significant reduction in the amount of resistin secreted by the adipocyte with all PIs including ATV although the ATV effect was comparatively less than other PIs. If resistin was involved in insulin resistance or adipocyte dysfunction, we would have expected its level to increase; our result suggest that secreted resistin might not be contributing directly to insulin resistance or adipocyte dysfunction in this ARV-treated cellular model. Previous clinical studies have reported a decrease in plasma resistin with TEL in diabetes patients;34 however, neither TEL nor ROSI had any effect on resistin in these *in vitro* models. It should be noted that in humans, resistin is primarily produced by cell populations other than adipocytes,35 including peripheral blood mononuclear cells, macrophages and bone marrow cells. This could potentially explain the discrepancy in resistin levels between clinical and *in vitro* studies.

TEL is widely used as an anti-hypertensive because of its ability to antagonise the effect of angiotensin II. However, TEL is highly lipophilic, and it has been suggested that its off-target effect on PPARγ could be beneficial in the treatment of metabolic and cardiovascular disease.36-38 In the current study, TEL was able to partially reverse the PI-induced inhibition in adipogenesis (lipid accumulation, expression of *PPARγ* and *LPIN1*); improve PI-induced reduction in adiponectin and expression of pAktSer473 (effect on insulin sensitivity); and reverse PI-induced upregulation in the secretion of proinflammatory markers, IL-6 and TNFα. A previous *in vitro* study had shown that TEL improves adipocyte function following incubation with ARVs through blockade of the adipose renin-angiotensin system (RAS).14 It should be noted that PPARγ is also a modulator of adipocyte RAS and activation of PPARγ using full/partial agonists like ROSI or TEL could potentially counter the effects of RAS. By testing a wide range of concentrations of TEL (0.5 - 20μM) on two exemplar markers, *PPARγ* gene and adiponectin protein, we observed TEL to show a non-monotone response with the maximal effect observed at 5μM in the primary human adipocyte model. This dose response shown by TEL here is different to that seen on blood pressure, which is linear, and mediated by the angiotensin receptor, AT1R. It might very well be that both RAS and PPARγ play an independent role in the development of PI-induced adipocyte dysfunction; given that PPARγ full agonists such as ROSI suffer from serious adverse effects, ARBs such as TEL with dual activity on both PPARγ and RAS may offer an opportunity to reduce PI-induced toxicity.

**Limitations of the study:**

This study has not investigated the effect of antiretrovirals (with/without telmisartan) in mature adipocytes; of course, the adipocyte population *in vivo* is a mixture of differentiating and differentiated adipocytes, but we felt it was important to focus on differentiating adipocytes, as harmful effects here would ultimately affect the population of differentiated adipocytes. The current study did not assess the effect of PI drug combinations as used in the clinic; relating concentration-response relationships *in vitro* to the *in vivo* situation is challenging because of differences that can occur in protein binding and drug distribution. It should be noted that we have only used three replicates (biological replicates) for each experiment in this study; therefore the results warrant replication in larger studies. Nevertheless, our findings further support the beneficial metabolic effects observed with TEL and opens up the intriguing possibility that TEL could be used to prevent the increase in insulin resistance that is seen in HIV-infected individuals treated with ARVs.

**Conclusion**

The current study has shown that TEL has beneficial metabolic effects on adipocytes when given in combination with PIs, and therefore has the potential to reverse adipocyte toxicity and insulin resistance mediated by PIs. The study also, for the first time, has characterised the dose response of TEL in human primary adipocytes. These *in vitro* findings now need to be validated in a clinical study which preferably evaluates in a randomised fashion, not only the ability of TEL to reduce insulin resistance *in vivo*, but also identifies the optimal dose. This is currently being pursued in a phase IIb adaptive design clinical trial.39

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**Author contributions**

SP, AO, DB and MP designed the research; AA, PM, GT and SK provided guidance and training with the culturing of primary human adipocytes and related work and helped with interpretation of human adipocyte data; SP carried out the experiments and collected the data; SP and MP analysed and interpreted the data; SP, MP, AA, PM, GT, SK, AO and DB wrote the manuscript.

**Declaration of conflicting interests**

The Authors declares that there is no conflict of interest.

**References:**

1. Koethe JR, Jenkins CA, Lau B, et al. Rising Obesity Prevalence and Weight Gain Among Adults Starting Antiretroviral Therapy in the United States and Canada. *AIDS research and human retroviruses* 2016; 32: 50-8.

2. Galescu O, Bhangoo A, Ten S. Insulin resistance, lipodystrophy and cardiometabolic syndrome in HIV/AIDS. *Reviews in endocrine & metabolic disorders* 2013; 14: 133-40.

3. Worm SW, Sabin C, Weber R, et al. Risk of myocardial infarction in patients with HIV infection exposed to specific individual antiretroviral drugs from the 3 major drug classes: the data collection on adverse events of anti-HIV drugs (D:A:D) study. *The Journal of infectious diseases* 2010; 201: 318-30.

4. Samaras K. Prevalence and pathogenesis of diabetes mellitus in HIV-1 infection treated with combined antiretroviral therapy. *J Acquir Immune Defic Syndr* 2009; 50: 499-505.

5. Araujo S, Banon S, Machuca I, Moreno A, Perez-Elias MJ, Casado JL. Prevalence of insulin resistance and risk of diabetes mellitus in HIV-infected patients receiving current antiretroviral drugs. *European journal of endocrinology / European Federation of Endocrine Societies* 2014; 171: 545-54.

6. Lee GA, Rao M, Mulligan K, et al. Effects of ritonavir and amprenavir on insulin sensitivity in healthy volunteers. *Aids* 2007; 21: 2183-90.

7. Bernal E, Masia M, Padilla S, Ramos JM, Martin-Hidalgo A, Gutierrez F. [Insulin resistance in HIV-infected patients receiving long-term therapy with efavirenz, lopinavir/ritonavir and atazanavir]. *Medicina clinica* 2007; 129: 252-4.

8. Dirajlal-Fargo S, Moser C, Brown TT, et al. Changes in Insulin Resistance After Initiation of Raltegravir or Protease Inhibitors With Tenofovir-Emtricitabine: AIDS Clinical Trials Group A5260s. *Open forum infectious diseases* 2016; 3: ofw174.

9. Dekoven M, Makin C, Slaff S, Marcus M, Maiese EM. Economic Burden of HIV Antiretroviral Therapy Adverse Events in the United States. *Journal of the International Association of Providers of AIDS Care* 2016; 15: 66-76.

10. Damouche A, Lazure T, Avettand-Fenoel V, et al. Adipose Tissue Is a Neglected Viral Reservoir and an Inflammatory Site during Chronic HIV and SIV Infection. *PLoS pathogens* 2015; 11: e1005153.

11. Sheth SH, Larson RJ. The efficacy and safety of insulin-sensitizing drugs in HIV-associated lipodystrophy syndrome: a meta-analysis of randomized trials. *BMC infectious diseases* 2010; 10: 183.

12. Kohli R, Shevitz A, Gorbach S, Wanke C. A randomized placebo-controlled trial of metformin for the treatment of HIV lipodystrophy. *HIV medicine* 2007; 8: 420-6.

13. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *The New England journal of medicine* 2007; 356: 2457-71.

14. Boccara F, Auclair M, Cohen A, et al. HIV protease inhibitors activate the adipocyte renin angiotensin system. *Antiviral therapy* 2010; 15: 363-75.

15. Schupp M, Clemenz M, Gineste R, et al. Molecular characterization of new selective peroxisome proliferator-activated receptor gamma modulators with angiotensin receptor blocking activity. *Diabetes* 2005; 54: 3442-52.

16. Hsueh WA, Law R. The central role of fat and effect of peroxisome proliferator-activated receptor-gamma on progression of insulin resistance and cardiovascular disease. *The American journal of cardiology* 2003; 92: 3j-9j.

17. Jones SP, Janneh O, Back DJ, Pirmohamed M. Altered adipokine response in murine 3T3-F442A adipocytes treated with protease inhibitors and nucleoside reverse transcriptase inhibitors. *Antiviral therapy* 2005; 10: 207-13.

18. Moriuchi A, Yamasaki H, Shimamura M, et al. Induction of human adiponectin gene transcription by telmisartan, angiotensin receptor blocker, independently on PPAR-gamma activation. *Biochem Biophys Res Commun* 2007; 356: 1024-30.

19. Kepp O, Galluzzi L, Lipinski M, Yuan J, Kroemer G. Cell death assays for drug discovery. *Nature reviews Drug discovery* 2011; 10: 221-37.

20. Sarjeant K, Stephens JM. Adipogenesis. *Cold Spring Harbor perspectives in biology* 2012; 4: a008417.

21. Janneh O, Hoggard PG, Tjia JF, et al. Intracellular disposition and metabolic effects of zidovudine, stavudine and four protease inhibitors in cultured adipocytes. *Antiviral therapy* 2003; 8: 417-26.

22. Jemsek JG, Arathoon E, Arlotti M, et al. Body fat and other metabolic effects of atazanavir and efavirenz, each administered in combination with zidovudine plus lamivudine, in antiretroviral-naive HIV-infected patients. *Clin Infect Dis* 2006; 42: 273-80.

23. Fang Z, Wang S, Du X, Shi P, Huang Z. Phosphatidate phosphatase-1 is functionally conserved in lipid synthesis and storage from human to yeast. *Acta biologica Hungarica* 2014; 65: 481-92.

24. Koh YK, Lee MY, Kim JW, et al. Lipin1 is a key factor for the maturation and maintenance of adipocytes in the regulatory network with CCAAT/enhancer-binding protein alpha and peroxisome proliferator-activated receptor gamma 2. *J Biol Chem* 2008; 283: 34896-906.

25. Kim HE, Bae E, Jeong DY, et al. Lipin1 regulates PPARgamma transcriptional activity. *The Biochemical journal* 2013; 453: 49-60.

26. Reue K. The lipin family: mutations and metabolism. *Curr Opin Lipidol* 2009; 20: 165-70.

27. Pushpakom SP, Owen A, Vilar FJ, et al. Adipogenic gene variants in patients with HIV-associated lipodystrophy. *Pharmacogenet Genomics* 2011; 21: 76-83.

28. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nature reviews Molecular cell biology* 2006; 7: 85-96.

29. Norata GD, Ongari M, Garlaschelli K, Raselli S, Grigore L, Catapano AL. Plasma resistin levels correlate with determinants of the metabolic syndrome. *European journal of endocrinology / European Federation of Endocrine Societies* 2007; 156: 279-84.

30. Chen BH, Song Y, Ding EL, et al. Circulating levels of resistin and risk of type 2 diabetes in men and women: results from two prospective cohorts. *Diabetes care* 2009; 32: 329-34.

31. Arama V, Munteanu DI, Streinu Cercel A, et al. Lipodystrophy syndrome in HIV treatment-multiexperienced patients: implication of resistin. *Journal of endocrinological investigation* 2014; 37: 533-9.

32. Barb D, Wadhwa SG, Kratzsch J, et al. Circulating resistin levels are not associated with fat redistribution, insulin resistance, or metabolic profile in patients with the highly active antiretroviral therapy-induced metabolic syndrome. *The Journal of clinical endocrinology and metabolism* 2005; 90: 5324-8.

33. Escote X, Miranda M, Veloso S, et al. Lipodystrophy and insulin resistance in combination antiretroviral treated HIV-1-infected patients: implication of resistin. *J Acquir Immune Defic Syndr* 2011; 57: 16-23.

34. Derosa G, Fogari E, D'Angelo A, et al. Metabolic effects of telmisartan and irbesartan in type 2 diabetic patients with metabolic syndrome treated with rosiglitazone. *Journal of clinical pharmacy and therapeutics* 2007; 32: 261-8.

35. Fain JN, Cheema PS, Bahouth SW, Lloyd Hiler M. Resistin release by human adipose tissue explants in primary culture. *Biochem Biophys Res Commun* 2003; 300: 674-8.

36. Michel MC, Brunner HR, Foster C, Huo Y. Angiotensin II type 1 receptor antagonists in animal models of vascular, cardiac, metabolic and renal disease. *Pharmacology & therapeutics* 2016; 164: 1-81.

37. Takagi H, Niwa M, Mizuno Y, Goto SN, Umemoto T. Telmisartan as a metabolic sartan: the first meta-analysis of randomized controlled trials in metabolic syndrome. *Journal of the American Society of Hypertension : JASH* 2013; 7: 229-35.

38. Takagi H, Umemoto T. Telmisartan improves insulin sensitivity: a meta-analysis of randomized head-to-head trials. *International journal of cardiology* 2012; 156: 92-6.

39. Pushpakom SP, Taylor C, Kolamunnage-Dona R, et al. Telmisartan and Insulin Resistance in HIV (TAILoR): protocol for a dose-ranging phase II randomised open-labelled trial of telmisartan as a strategy for the reduction of insulin resistance in HIV-positive individuals on combination antiretroviral therapy. *BMJ open* 2015; 5: e009566.