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Short communication

Impact of elvitegravir on human adipocytes: Alterations in differentiation, gene expression and release of adipokines and cytokines

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ABSTRACT

Elvitegravir is a recently developed integrase inhibitor used for antiretroviral treatment of HIV infection. Secondary effects, including disturbances in lipid metabolism and, ultimately, in adipose tissue distribution and function, are common concerns associated with antiretroviral treatments. Here, we provide the first study of the effects of elvitegravir (in comparison with efavirenz, a non-nucleoside analog inhibitor of reverse transcriptase; and raltegravir, another integrase inhibitor) on human adipocyte differentiation, gene expression and secretion of adipokines and cytokines. Elvitegravir impaired adipogenesis and adipocyte metabolism in human SGBS adipocytes in a concentration-dependent manner (delaying acquisition of adipocyte morphology and reducing the expression of adipogenesis marker genes such as PPARγ, glucose transporter GLUT4, lipoprotein lipase, and the adipokines adiponectin and leptin). Compared with efavirenz, the effects of elvitegravir were similar but tended to occur at higher concentrations than those elicited by efavirenz, or were somewhat less intense than those caused by efavirenz at similar concentration. Elvitegravir tended to cause a more moderate induction of pro-inflammatory cytokines than efavirenz. Efavirenz induced a marked concentration-dependent increase in interleukin-8 expression and release whereas elvitregravir had little effect. Raltegravir had totally neutral actions of adipogenesis, adipocyte metabolism-related gene expression and release of adipokines and cytokines. In conclusion, elvitegravir alters adipocyte differentiation and function and promotes induction of pro-inflammatory cytokines similarly to efavirenz, but several effects were less intense. Further assessment of lipid metabolism and adipose tissue function in patients administered elvitegravir-based regimes is advisable considering that totally neutral effects of elvitegravir on lipid homeostasis cannot be anticipated from the current study in vitro.

Antiretroviral treatment of HIV patients has been based on a combination of two nucleoside-analog reverse transcriptase inhibitors (NRTI) and either a non-nucleoside analog inhibitor of reverse transcriptase (NNRTI) or a protease inhibitor. These combinations, although usually successful in the control of disease progression, are associated with metabolic disturbances, mainly dyslipidemia, insulin resistance and, in some cases, overt lipodystrophy (Giralt et al., 2011). Some drugs, especially the NRTIs stavudine and zidovudine, are especially prone to cause lipoatrophy whereas protease inhibitors are considered to promote insulin resistance and dyslipidemia. NNRTIs have not traditionally been associated with adipose alterations. However, efavirenz, once the preferred NNRTI for use in antiretroviral regimens (Hammer et al., 2008; Gazzard et al., 2008), has been reported to favor altered patterns of circulating lipids and fat loss (Pérez-Molina et al., 2008; Riddler et al., 2008; Haubrich et al., 2009).

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Inhibitors of the HIV integrase enzyme represent the latest class of antiretroviral drugs for HIV treatment. Three drugs (raltegravir, dolutegravir and elvitegravir) that target the strand-transfer step of integration of viral DNA into the host cell chromosomal DNA have been developed (Blanco et al., 2015). These drugs are gaining a relevant role in HIV antiretroviral therapy because of their favorable clinical characteristics. Elvitegravir is an integrase inhibitor coformulated with cobicistat, a pharmacological inhibitor of elvitegravir metabolism (Cohen et al., 2011). Elvitegravir has shown high antiviral efficacy and tolerability as well as low secondary effects. Accordingly, regimens containing elvitegravir are being promoted as first-line treatment for HIV-1-infected patients. The potential effects of elvitegravir on lipid alterations have only begun to be assessed. Elvitegravir (as part of an elvitegravir-cobicistat-tenofovir-emtricitabine combination) leads to increased cholesterol levels relative to ritonavir-atazanavir but cause a less intense increase in triglycerides (Rockstroh et al., 2013). Total cholesterol, LDL cholesterol and HDL cholesterol are more moderately increased in patients under elvitegravir containing regimens than in those containing efavirenz (Sax et al., 2012).

Studies on the effects of antiretroviral drugs on human adipose cells provide initial pre-clinical information regarding the potential of drugs to alter adipose tissue and lipid metabolism. Such analyses have shown that efavirenz strongly impairs adipocyte differentiation and induces the secretion of pro-inflammatory cytokines (El Hadri et al., 2004; Gallego-Escuredo et al., 2010; Díaz-Delfín et al., 2011, 2012). The potential effects of elvitegravir on adipose cells have not been studied. Here, we analyzed the effects of elvitegravir on adipogenesis and the release of adipokines and cytokines by human adipose cells in comparison with the effects of efavirenz and raltegravir.

Human SGBS (Simpson-Golabi-Behmel syndrome) pre-adipocytes were used (Wabitsch et al., 2001; Schlüter et al., 2002). SGBS pre-adipocytes were maintained in DMEM/F12 containing 10% fetal bovine serum. After cells had become confluent, adipogenic differentiation was initiated by incubating cells for 4 days in serum-free medium containing 20 nM insulin, 0.2 nM triiodothyronine (T_3) and 100 nM cortisol, supplemented with 25 nM dexamethasone, 500 μM 3-isobutyl-methyl-xanthine and 2 μM rosiglitazone. Subsequently, the cells were switched to differentiation medium (containing insulin, T_3, and cortisol only) and maintained for up to 16 days.

Cytotoxicity was determined after exposing differentiating preadipocytes to drugs (0.1 μM–5 μM range) for 5 days (CytoTox96, Promega). Briefly, cytotoxicity was calculated as lactate dehydrogenase activity in the cell culture medium relative to the maximum release after total cell lysis of cells. Neither elvitegravir nor efavirenz or raltegravir caused a significant cytotoxicity (Supplemental Table 2). This is similar to previous reports on other cell types, where significant cytotoxicity of elvitegravir has been reported only at concentrations higher than the maximal concentration (5 μM) tested here; for example, the 50% cytotoxic concentration (EC_{50}) of elvitegravir toward human renal proximal tubule cells is 13.7 μM (Stray et al., 2013).

For studies on the effects of drugs on adipogenic differentiation, drug treatments were initiated on day 0 and continued throughout the differentiation process. The effects of drugs on morphological differentiation of adipocytes was determined by using the Image J software to phase-contrast microscopy images to quantify the cell culture surface occupied by differentiated (lipid droplet-containing) cells. Data were expressed as percentages relative to control cultures.

RNA and DNA were extracted using an affinity column-based method (Machery-Nagel). Reverse transcription was performed using random hexamer primers (Applied Biosystems) and 0.5 μg RNA. Polymerase chain reaction (PCR) was conducted in an ABI/Prism-7700 Sequence Detector System using 25 μl of reaction mixture containing 1 μl of cDNA, 12.5 μl of TaqMan Universal PCR Master Mix, 250 nM probes and 900 nM primers (TaqMan; Applied Biosystems, see Supplemental Table 1). Controls with no RNA, primers, or reverse transcriptase were included. Each sample was run in duplicate and the mean value of the duplicate was used to calculate the relative amount of individual mRNAs. Each mean value was normalized to that of the 18S rRNA gene using the comparative ($2^{-\Delta CT}$) method. Relative mitochondrial DNA (mtDNA) levels were quantified using the primers/probe for the mtDNA-encoded cytochrome-b gene and expressed relative to nuclear DNA levels, determined by amplification of the intronless gene C/EBPα, as previously reported (Gallego-Escuredo et al., 2010).

For quantification of adipokines and cytokines released by adipocytes, 25 μl of medium collected from the last 5 days of adipocyte cultures before harvest were used. Adiponectin, leptin, MCP-1, IL-6, IL-8, total plasminogen activator inhibitor type-1 (PAI-1), hepatocyte growth factor (HGF) and nerve growth factor (NGF) were quantified using a multiplex analysis system (Linco Research/Millipore) and a Luminex100ISv2 instrument. Where appropriate, statistical analyses were performed using multivariate ANOVA and post-hoc Turkey tests for paired comparisons, significance is indicated in the text.

Effects of elvitegravir, efavirenz and raltegravir on adipogenic differentiation of human SGBS pre-adipocytes were tested over a concentration range of 0.1–5 μM (Fig. 1). Exposure of cells to elvitegravir up to 0.5 μM had no effect on the acquisition of an adipocyte morphology, as evidenced by unaltered lipid accumulation in cells. However, at higher concentrations, elvitegravir caused a significant concentration-dependent reduction in adipogenesis, strongly impairing adipocyte differentiation at 1 μM and completely abolishing it at 5 μM. Compared with elvitegravir, efavirenz was somewhat more potent, significantly inhibiting adipocyte differentiation at 0.5 μM, 1 μM (83% inhibition, versus 37% inhibition by elvitegravir, $P < 0.05$), 2 μM (94% inhibition, versus 80% inhibition by elvitegravir, $P < 0.05$) and 5 μM (Fig. 1). Raltegravir did not alter adipogenic differentiation at any concentration tested (see Fig. 2).

Elvitegravir caused a concentration-dependent suppression of the expression of genes involved in adipogenic differentiation, in a pattern essentially similar to that elicited by efavirenz. Both elvitegravir and efavirenz treatment significantly decreased the expression of C/EBPα, a master transcription factor controlling adipogenesis, at concentrations of 1 μM and higher. Both drugs caused a similar concentration-dependent suppression of the expression of PPARγ, the other master regulator of adipogenesis, although only efavirenz significantly reduced PPARγ expression at 1 μM. Expression of lipoprotein lipase, a marker gene encoding a key enzyme involved in lipid accumulation in adipocytes, was significantly reduced even at lower concentrations (0.1 μM) of elvitegravir and efavirenz. Notably, the expression of GLUT4, the gene encoding the insulin-dependent glucose transporter in adipocytes, was also repressed by both drugs in a concentration-dependent manner, but the extent of repression at equivalent concentrations (1 and 2 μM) was greater for efavirenz than for elvitegravir. With respect to adipokines, both elvitegravir and efavirenz inhibited expression of the adiponectin gene expression in a similar concentration-dependent manner but, at low concentrations (0.1 and 0.5 μM) only the inhibitory effect of efavirenz was statistically significant. Effects on leptin gene expression were much less pronounced, although efavirenz significantly reduced it at higher concentrations (1 and 5 μM); in contrast, elvitegravir had no effect on leptin mRNA levels. Overall, these results were in contrast with the lack of effects of raltegravir on the expression of

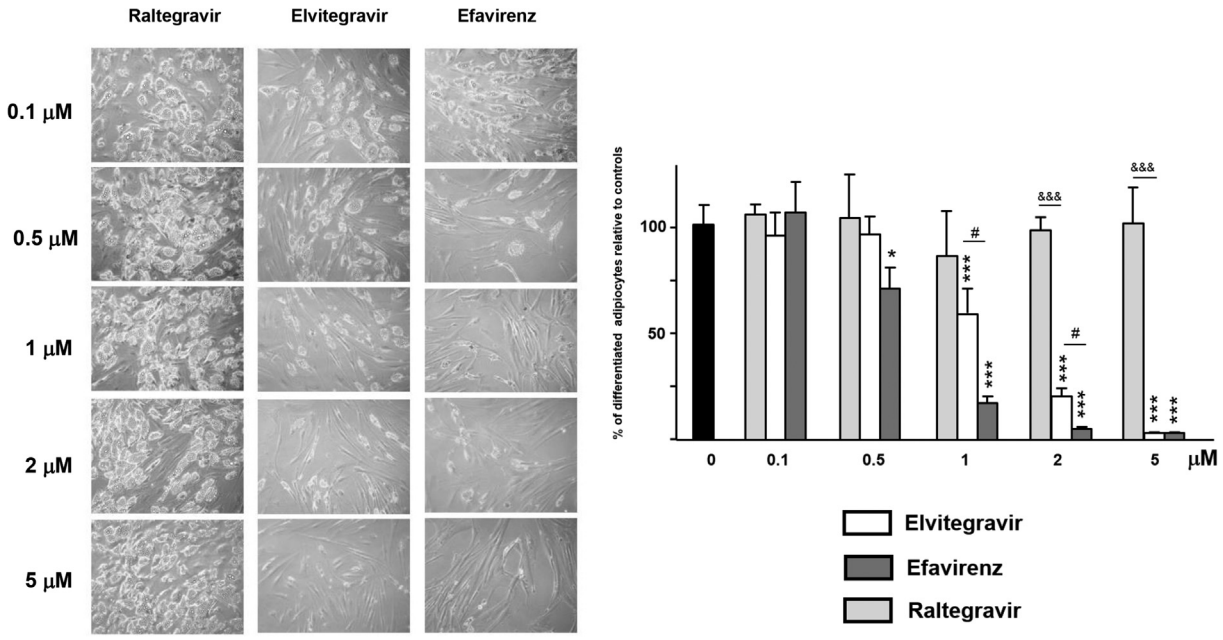

Fig. 1. Effects of elvitegravir on adipogenic differentiation of SGBS human preadipocytes in culture. SGBS human preadipocytes were differentiated in culture in the presence of the indicated concentrations of drugs. A, Representative photomicrographs of adipocyte cell cultures differentiating in the presence of the indicated concentrations of drugs. B, Bars are means ± SEM of the extent of morphological adipocyte differentiation and are expressed relative to values from untreated control cells, defined as 100% (see Methods). (*P < 0.05 and **P < 0.01 for each drug treatment vs. control; #P < 0.05 for elvitegravir vs. efavirenz treatment at the same concentration, &&&P < 0.001 for elvitegravir vs. raltegravir treatment at the same concentration).

the adipocyte-related genes and adipokine genes.

Elvitegravir and efavirenz exerted similar effects on the expression of the genes encoding the pro-inflammatory cytokines IL-6 and MCP-1, inducing an increase in mRNA only at the highest concentration tested (5 μM); in the case of efavirenz the increase in IL-6 expression did not reach statistical significance. Notably, elvitegravir and efavirenz differentially affected IL-8 expression. Efavirenz caused a marked concentration-dependent induction, that was statistically significant at concentrations higher than 0.5 μM, whereas elvitegravir induced a significant increase in IL-8 expression only at concentration 5 μM. In fact, at concentration 5 μM efavirenz caused a stronger induction of IL-8 expression (close to 30-fold) than elvitegravir (less than 5-fold). Raltegravir did not affect the expression of genes encoding cytokines.

Finally, we determined the effects of efavirenz and elvitegravir on mitochondrial toxicity, assessed by determining the expression of both a mtDNA-encoded transcript (cytochrome-b) and a nuclear-encoded transcript (cytochrome oxidase subunit-IVA). Both drugs impaired the expression of both transcripts, but only at concentrations of 1 μM and above. However, relative mtDNA levels were unaltered even at concentrations 2 and 5 μM efavirenz and elvitegravir (Supplemental Table 3). Raltegravir did not alter mitochondrial transcripts or mtDNA levels at the concentrations tested.

We further analyzed the effects of 0.5 and 2 μM elvitegravir and efavirenz (the concentrations at which most differential effects of the two drugs on differentiation and gen expression had been observed) on the release of adipokines and cytokines into the medium (Fig. 3). Elvitegravir and efavirenz induced a similar decrease in the levels of released adiponectin at both concentrations tested. Levels of secreted leptin were moderately reduced only by efavirenz at 2 μM, a concentration at which elvitegravir had no effect. Release of the pro-inflammatory cytokine IL-6 by adipocytes was significantly higher following efavirenz treatment than elvitegravir treatment at a concentration of 2 μM. Consistent with the results of gene expression analysis, efavirenz significantly induced the release of IL-8 at both 0.5 and 2 μM whereas elvitegravir had no effect. Similarly, efavirenz, but not elvitegravir, induced MCP-1 release at both 0.5 and 2 μM. No effects were found for raltegravir on the release of adipokines and cytokines. Other molecules that are known to be released by adipocytes and influence metabolic and inflammatory signaling such as PAI-1, HGF and NGF (Alessi et al., 2007; Bell et al., 2008; Bulló et al., 2007) were not significantly altered by any of the three drugs at any of the concentrations tested.

Collectively, our current data provide the first assessment of the action of the integrase inhibitor elvitegravir on human adipocytes. We found that elvitegravir impairs the acquisition of adipocyte morphology, inhibits the expression of genes controlling adipogenesis (PPARγ, C/EBPα) and lipid accretion (lipoprotein lipase, GLUT4), and reduces the release of adipokines. The effects of elvitegravir were qualitatively similar to those of efavirenz reported previously in closely related human cell culture models (Gallego-Escuredo et al., 2010; Díaz-Delfín et al., 2011, 2012). An analysis of the effects of efavirenz and elvitegravir at similar concentrations, especially in the range between 0.5 and 2 μM, indicated that efavirenz exerted similar but somewhat stronger deleterious effects on adipogenesis (e.g. adipose morphology, PPARγ and GLUT4 expression) than elvitegravir. The most remarkable differential effect of elvitegravir and efavirenz was related to the actions of the drugs on IL-8 expression and release, which was dramatically induced by efavirenz but less altered by elvitegravir. IL-8 is produced and secreted by human adipocytes, in addition to macrophages and monocytes, and plays a role in the induction of pro-inflammatory responses (Bruun et al., 2000, 2001). Increased expression of IL-8 in adipose tissue from HIV patients, especially under conditions of lipodystrophy, has been reported and is expected to contribute to the local pro-inflammatory environment in fat from these patients (Lihn et al., 2003). The differential effects of elvitegravir and efavirenz on the release of regulatory molecules by adipose tissue may be especially relevant in relation to the effects of

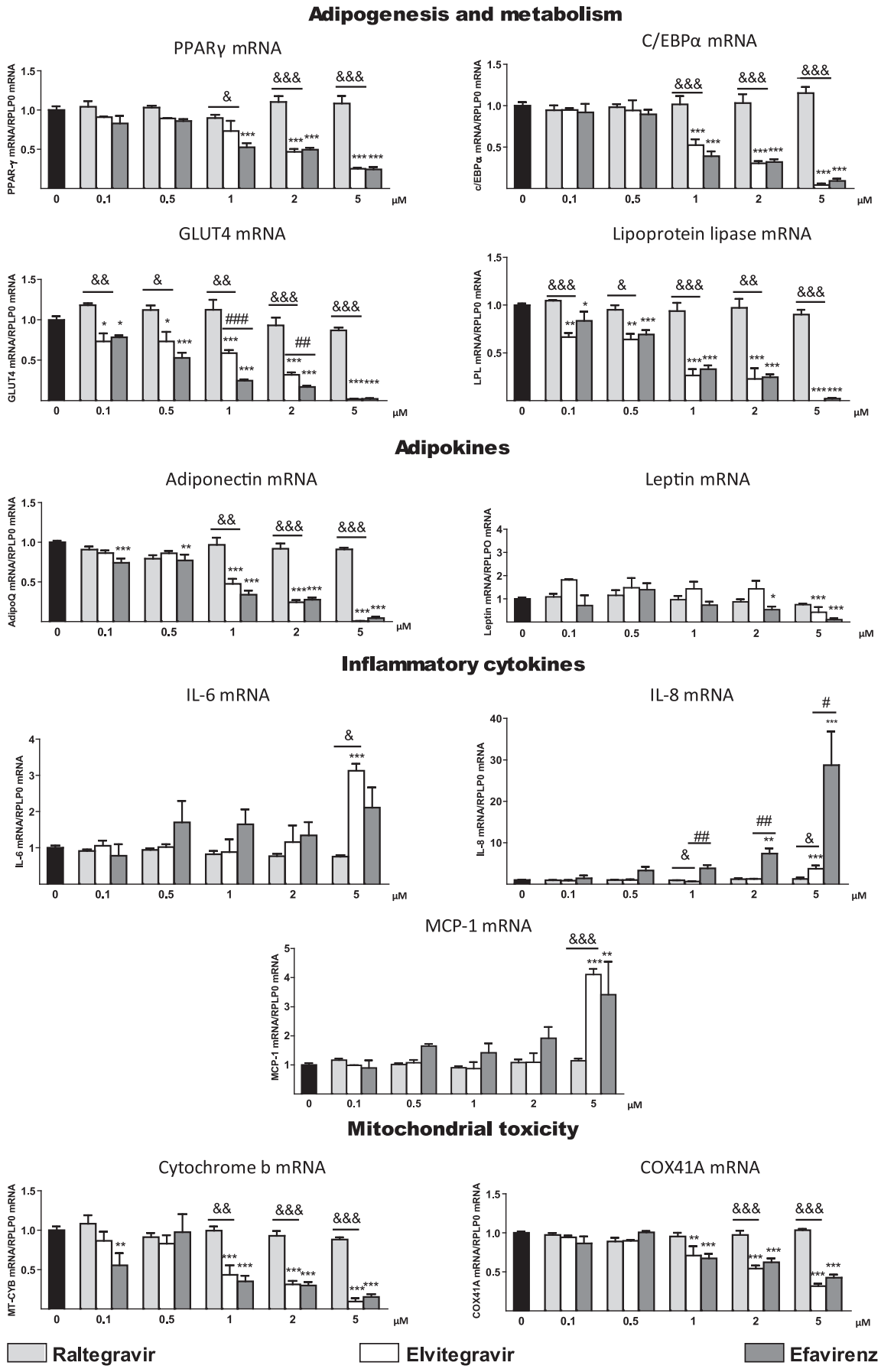

Fig. 2. Effects of elvitegravir on the expression of genes related to adipogenic function, inflammation and mitochondrial toxicity in SGBS human adipocytes differentiating in culture. SGBS human preadipocytes were differentiated in culture in the presence of the indicated concentrations of drugs. Data on mRNA levels are presented as means ± SEM from 4 to 5 independent experiments, and are expressed relative to values from untreated control cells (defined as 1). *P < 0.05, **P < 0.01, and ***P < 0.001 for each drug treatment vs. control; #P < 0.05 for elvitegravir vs. efavirenz treatment at the same concentration; &P < 0.05, &&P < 0.01, &&&P < 0.001 for elvitegravir vs. raltegravir treatment at the same concentration.

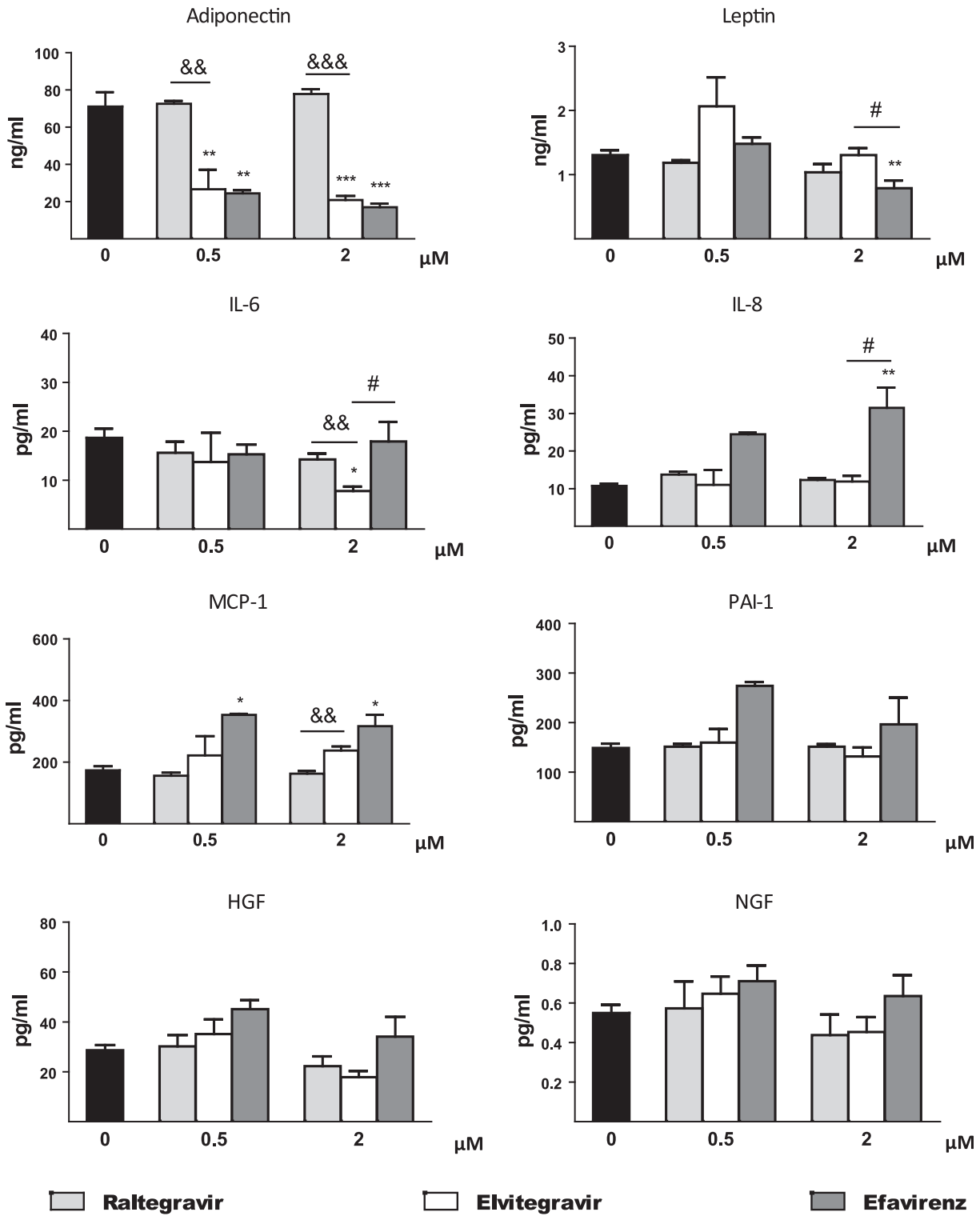

Fig. 3. Effects of elvitegravir on the release of adipokines and cytokines by SGBS human adipocytes in culture. SGBS human preadipocytes were differentiated in culture in the presence of the indicated concentrations of drugs. Values represent concentrations of adipokines and cytokines in the cell culture medium, presented as means ± SEMs from 4 to 5 independent experiments. *P < 0.05, **P < 0.01, and ***P < 0.001 for each drug treatment vs. control; #P < 0.05 for elvitegravir vs. efavirenz treatment at the same concentration.; $^{\&}$P < 0.05, $^{\&\&}$P < 0.01, $^{\&\&\&}$P < 0.001 for elvitegravir vs. raltegravir treatment at the same concentration.

these drugs on systemic metabolism, and to some signs of healthier effects of elvitegravir on lipid profile relative to those of efavirenz (Sax et al., 2012). However, it is noteworthy that raltegravir, another HIV integrase inhibitor, did not show any deleterious effects on adipogenesis, adipocyte-related gene expression or pro-inflammatory cytokines in human adipocytes, consistently with previous reports in rodent adipocyte cell models (Pérez-Matute et al., 2011; Minami et al., 2011).

The fact that the current study used an in vitro approach is an obvious limit with respect to the relevance of our findings for the treatment of patients. Elvitegravir plasma concentrations in patients administered clinical doses of the compound reach peak levels in the 5 μM range and show a Cmin (minimum plasma concentration) of approximately 1 μM (Ramanathan et al., 2011). This indicates that the concentration range at which elvitegravir was found here to exert most of its deleterious effects in vitro may be reached in vivo. However, elvitegravir is highly protein bound, with a plasma-free fraction of approximately 1% (Ramanathan et al., 2011). In the present human adipocyte cell culture studies the drugs are added to serum-free adipocyte culture medium, -an inherent condition of this in vitro cell model-; thus, dose for dose, the effective concentration of elvitegravir in cell culture may be higher than the actual free elvitegravir concentration in blood, where serum proteins may bind substantial amounts of the drug. A similar consideration may be placed for efavirenz, which protein binding capacity is also as high as that for elvitegravir. On the other hand, in contrast with efavirenz, for which information on differential tissue distribution in patients is available (Dupin et al., 2002), to our knowledge there are no available data relating to the potential differential accumulation of elvitegravir (or raltegravir) in adipose tissue and blood.

In summary, this study suggests that elvitegravir, at moderate concentrations, would not likely exert profound deleterious effects on adipose tissue development or on the endocrine function of adipose tissue (adipokine and cytokine release) in treated patients, but totally neutral effects cannot be anticipated. Moreover, on the basis of this comparative study, the deleterious effects of elvitegravir are likely to be similar to those of efavirenz, but somewhat less intense, especially for the induction of pro-inflammatory signals. However, elvitegravir shows deleterious effects on human adipocytes that do not occur for raltegravir. It should be taken into account that the overall alterations in lipid metabolism in HIV-1-infected patients are thought to be caused not by a single drug but by the complex interactions of a given drug with other drug components of antiretroviral treatment regimens as well as underlying HIV-1-infection-related alterations. Thus, further studies assessing lipid abnormalities in patients treated with elvitegravir as a component of a drug cocktail will be necessary to confirm a metabolic-friendly effect of this integrase inhibitor and/or identify potential lipid alterations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.antiviral.2016.05.013.

References

Alessi, M.C., Poggi, M., Juhan-Vague, I., 2007. Plasminogen activator inhibitor-1, adipose tissue and insulin resistance. Curr. Opin. Lipidol. 18, 240–245.
Bell, L.N., Cai, L., Johnstone, B.H., Traktuev, D.O., March, K.L., Considine, R.V., 2008. A central role for hepatocyte growth factor in adipose tissue angiogenesis. Am. J. Physiol. Endocrinol. Metab. 294, E336–E344.
Blanco, J.L., Whitlock, F., Milinkovic, A., Moyle, G., 2015. HIV integrase inhibitors: a new era in the treatment of HIV. Expert. Opin. Pharmacother. 16, 1313–1324.
Bruun, J.M., Pedersen, S.B., Richelsen, B., 2000. Interleukin-8 production in human adipose tissue. Inhibitory effects of anti-diabetic compounds, the thiazolidinedione ciglitazone and the biguanide metformin. Horm. Metab. Res. 32, 537–541.
Bruun, J.M., Pedersen, S.B., Richelsen, B., 2001. Regulation of interleukin 8 production and gene expression in human adipose tissue in vitro. J. Clin. Endocrinol. Metab. 86, 1267–1273.
Bulló, M., Peeraully, M.R., Trayhurn, P., Folch, J., Salas-Salvadó, J., 2007. Circulating nerve growth factor levels in relation to obesity and the metabolic syndrome in women. Eur. J. Endocrinol. 157, 303–310.
Cohen, C., Elion, R., Ruane, P., Shamblaw, D., DeJesus, E., Rashbaum, B., Chuck, S.L., Yale, K., Liu, H.C., Warren, D.R., Ramanathan, S., Kearney, B.P., 2011. Randomized, phase 2 evaluation of two single-tablet regimens elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate versus efavirenz/emtricitabine/tenofovir disoproxil fumarate for the initial treatment of HIV infection. AIDS 25, F7–F12.
Díaz-Delfín, J., del Mar Gutiérrez, M., Gallego-Escuredo, J.M., Domingo, J.C., Gracia Mateo, M., Villarroya, F., Domingo, P., Giralt, M., 2011. Effects of nevirapine and efavirenz on human adipocyte differentiation, gene expression, and release of adipokines and cytokines. Antivir. Res. 91, 112–121.
Díaz-Delfín, J., Domingo, P., Mateo, M.G., Gutierrez Mdel, M., Domingo, J.C., Giralt, M., Villarroya, F., 2012. Effects of rilpivirine on human adipocyte differentiation, gene expression, and release of adipokines and cytokines. Antimicrob. Agents Chemother. 56, 3369–3444.
Dupin, N., Buffet, M., Marcelin, A.G., Lamotte, C., Gorin, I., Ait-Arkoub, Z., Tréluyer, J.M., Bui, P., Calvez, V., Peytavin, G., 2002. HIV and antiretroviral drug distribution in plasma and fat tissue of HIV-infected patients with lipodystrophy. AIDS 16, 2419–2424.
El Hadri, K., Glorian, M., Monsempes, C., Dieudonné, M.N., Pecquery, R., Giudicelli, Y., Andreani, M., Dugail, I., Fève, B., 2004. In vitro suppression of the lipogenic pathway by the nonnucleoside reverse transcriptase inhibitor efavirenz in 3T3 and human preadipocytes or adipocytes. J. Biol. Chem. 279, 15130–15141.
Gallego-Escuredo, J.M., Del Mar Gutierrez, M., Diaz-Delfin, J., Domingo, J.C., Mateo, M.G., Domingo, P., Giralt, M., Villarroya, F., 2010. Differential effects of efavirenz and lopinavir/ritonavir on human adipocyte differentiation, gene expression and release of adipokines and pro-inflammatory cytokines. Curr. HIV Res. 8, 545–598.
Gazzard, B.G., et al., 2008. British HIV Association guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy. HIV Med. 9, 563–608.
Giralt, M., Domingo, P., Villarroya, F., 2011. Adipose tissue biology and HIV-infection. Best. Pract. Res. Clin. Endocrinol. Metab. 25, 487–499.
Hammer, S.M., et al., 2008. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-U.S.A. panel. JAMA 300, 555–570.
Haubrich, R.H., Riddler, S.A., DiRienzo, A.G., Komarow, L., Powderly, W.G., Klingman, K., Garren, K.W., Butcher, D.L., Rooney, J.F., Haas, D.W., Mellors, J.W., Havlir, D.V., AIDS Clinical Trials Group (ACTG) A5142 Study Team, 2009. Metabolic outcomes in a randomized trial of nucleoside, nonnucleoside and protease inhibitor-sparing regimens for initial HIV treatment. AIDS 23, 1109–1118.
Lihn, A.S., Richelsen, B., Pedersen, S.B., Haugaard, S.B., Rathje, G.S., Madsbad, S., Andersen, O., 2003. Increased expression of TNF-alpha, IL-6, and IL-8 in HALS: implications for reduced adiponectin expression and plasma levels. Am. J. Physiol. Endocrinol. Metab. 285, E1072–E1152.
Minami, R., Yamamoto, M., Takahama, S., Ando, H., Miyamura, T., Suematsu, E., 2011. Comparison of the influence of four classes of HIV antiretrovirals on adipogenic differentiation: the minimal effect of raltegravir and atazanavir. J. Infect. Chemother. 17, 183–188.
Pérez-Matute, P., Pérez-Martínez, L., Blanco, J.R., Oteo, J.A., 2011. Neutral actions of Raltegravir on adipogenesis, glucose metabolism and lipolysis in 3T3-L1adipocytes. Curr. HIV Res. 9, 174–179.
Pérez-Molina, J.A., Domingo, P., Martínez, E., Moreno, S., 2008. The role of efavirenz compared with protease inhibitors in the body fat changes associated with highly active antiretroviral therapy. J. Antimicrob. Chemother. 62, 234–279.
Ramanathan, S., Mathias, A.A., German, P., Kearney, B.P., 2011. Clinical pharmacokinetic and pharmacodynamic profile of the HIV integrase inhibitor elvitegravir. Clin. Pharmacokinet. 50, 229–273.
Riddler, S.A., Haubrich, R., DiRienzo, A.G., Peeples, L., Powderly, W.G., Klingman, K.L., Garren, K.W., George, T., Rooney, J.F., Brizz, B., Lalloo, U.G., Murphy, R.L., Swindells, S., Havlir, D., Mellors, J.W., AIDS Clinical Trials Group Study A5142 Team, 2008. Class-sparing regimens for initial treatment of HIV-1 infection. N. Engl. J. Med. 358, 2095–2106.
Rockstroh, J.K., DeJesus, E., Henry, K., Molina, J.M., Gathe, J., Ramanathan, S., Wei, X., Plummer, A., Abram, M., Cheng, A.K., Fordyce, M.W., Szwarcberg, J., GS-236-0103 Study Team, 2013. A randomized, double-blind comparison of coformulated elvitegravir/cobicistat/emtricitabine/tenofovir DF vs ritonavir-boosted atazanavir plus coformulated emtricitabine and tenofovir DF for initial treatment of HIV-1 infection: analysis of week 96 results. J. Acquir. Immune. Defic. Syndr. 62, 483–489.
Sax, P.E., DeJesus, E., Mills, A., Zolopa, A., Cohen, C., Wohl, D., Gallant, J.E., Liu, H.C., Zhong, L., Yale, K., White, K., Kearney, B.P., Szwarcberg, J., Quirk, E., Cheng, A.K., GS-US-236-0102 study team, 2012. Co-formulated elvitegravir, cobicistat, emtricitabine, and tenofovir versus co-formulated efavirenz, emtricitabine, and tenofovir for initial treatment of HIV-1 infection: a randomised, double-blind,

phase 3 trial, analysis of results after 48 weeks. Lancet 379, 2439–2487.
Schlüter, A., Yubero, P., Iglesias, R., Giralt, M., Villarroya, F., 2002. The chlorophyll-derived metabolite phytanic acid induces white adipocyte differentiation. Int. J. Obes. Relat. Metab. Disord. 26, 277–1280.
Stray, K.M., Bam, R.A., Birkus, G., Haom, J., Lepistm, E.I., Yant, S.R., Ray, A.S., Cihlar, T., 2013. Evaluation of the effect of cobicistat on the in vitro renal transport and cytotoxicity potential of tenofovir. Antimicrob. Agents. Chemother. 57, 4982–4991.
Wabitsch, M., Brenner, R.E., Melzner, I., Braun, M., Möller, P., Heinze, E., Debatin, K.M., Hauner, H., 2001. Characterization of a human preadipocyte cell strain with high capacity for adipose differentiation. Int. J. Obes. Relat. Metab. Disord. 25, 8–15.