# Departament de Medicina

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Títol: *A 48-week study of fat molecular alterations in HIV naïve patients starting tenofovir/emtricitabine with lopinavir/ritonavir or efavirenz: Greater deleterious effects of efavirenz.*

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## Abstract

***Background***: Conflicting reports on the effects of efavirenz (EFV) and lopinavir/ritonavir (LPV/r) on subcutaneous adipose tissue (SAT) have been described.

***Objective***: To assess the 48-week molecular and clinical effects of LPV/r and EFV, both combined with tenofovir/emtricitabine (TDF/FTC), on SAT of HIV-infected, antiretroviral-naïve patients.

***Methods***: Forty-four adult HIV-infected were started LPV/r or EFV combined with TDF/FTC. Fasting metabolic tests, HIV RNA, CD4 cell count, and whole body and segmental fat measured by dual X-ray absorptiometry scans were obtained at study entry and at week 48. Mitochondrial DNA (mtDNA) and transcripts for mitochondrially-related genes, and genes involved in inflammation, adipocyte differentiation and metabolism were assessed in paired SAT biopsies.

***Results***: Total, HDL, LDL, VLDL cholesterol and triglycerides increased significantly in the LPV/r and total and LDL cholesterol in the EFV group. Whole body fat and limb fat mass increased significantly in the LPV/r but not in the EFV group. MtDNA and cytochrome oxidase subunit II (COII) did not change and cytpchrome b increased significantly in EFV patients. Tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and monocyte chemotactic protein-1 (MCP-1) gene expression did not change in the LPV/r group, but significantly increased in the EFV group. Interleukin 18 (IL-18) decreased in LPV/r group while it increased in EFV group.

***Conclusion***: Starting antiretroviral therapy with a LPV/r or EFV-based regime was associated with a significant increase in limb fat mass in the LPV/r group. EFV exposure was associated with an increased expression of genes encoding for inflammatory cytokines in SAT.


Keywords: lopinavir/ritonavir, efavirenz, tenofovir/emtricitabine, HALS, subcutaneous fat, adipocyte differentiation, mitochondrial DNA, PPAR-$\gamma$, cytochrome b, TNF-$\alpha$, GPR78, MCP-1, interleukin-18.

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## Introduction

Antiretroviral drug toxicity is still casting a shadow over the doubtless efficacy of highly active antiretroviral therapy (HAART), although its overall prevalence seems to have decreased in recent times [1]. Among antiretroviral drug-associated toxicity, that causing body fat redistribution, the so-called HIV/HAART-associated lipodystrophy syndrome (HALS), is one of the most feared by both patients and caregivers [2]. HALS is feared because of the devastating consequences in terms of self-image and psychological health, and because there is no intervention to successfully reverse this complication [2]. Therefore, much effort should be made to prevent its appearance. The best preventive measures which can be offered to patients to prevent HALS include early start of HAART, and above all starting with drugs with a benign fat profile [2].

Antiretroviral drugs with a known deleterious profile on adipocyte biology and function are the thymidine analogues and to a lesser extent didanosine, whereas non-nucleoside reverse transcriptase inhibitors (NNRTI) have usually been considered as benign drugs with respect to their effects on adipose depots [3]. First-generation protease inhibitors (PI) such as nelfinavir and indinavir also exhibited significant fat toxicity and their negative effects were synergistic with those of nucleoside reverse transcriptase inhibitors (NRTI) [4]. On the other hand, lopinavir/ritonavir (LPV/r) and atazanavir have been linked in some studies mostly to increased trunk fat or even to visceral adipose tissue hypertrophy [5-7]. The ACTG 5142 clinical trial which compared head-to-head LPV/r and efavirenz (EFV) combined with different NRTI backbones changed the perception of the neutral effects of EFV on fat. In that trial, EFV-based regimes were found associated with a higher risk of lipoatrophy, defined as a limb fat loss > 20% from the baseline, across all NRTI groups compared with LPV/r [8].

This intriguing finding prompted us to perform a molecular and clinical study to assess the effects of LPV/r and EFV, both combined with tenofovir/emtricitabine (TDF/FTC) on gene markers of adipocyte biology and function in SAT, in HIV-infected, antiretroviral naïve patients, who started their first HAART regime. Our working hypothesis was that molecular markers of adipocyte biology and function would improve in both arms.

## Patients and Methods

### Subjects

Patients were selected from the same HIV-1 infection clinic cohort, with the baseline visit between July 2008 and January 2010, at the *Hospital de la Santa Creu i Sant Pau*, which serves a population of 1708 HIV-1-infected patients on active follow-up. Inclusion criteria were having an established diagnosis of HIV-1 infection, antiretroviral-naïve who started antiretroviral therapy with TDF/FTC combined fixed dose formulation plus LPV/r or with TDF/FTC/EFV compact pill. The primary endpoint of the study was the change in limb fat mass measured by dual energy X-ray absorptiometry (DEXA) scan from baseline to 48 weeks. Secondary endpoints were changes in molecular markers of adipocyte biology and function at SAT level.

At the time of study entry no patient used any other drug known to influence glucose or lipid metabolism or fat distribution, such as anabolic hormones or systemic corticosteroids, recombinant human growth hormone, or appetite stimulants. Additional exclusion criteria included: fasting glucose > 6.1 mmol/l, daily alcohol intake ≥ 40 g/daily, hypothyroidism, serum creatinine > 150 mmol/l and alanine aminotransferase o aspartate aminotransferase > 5 x upper limit of normal, anemia, > 10% loss in body weight in the preceding 6 months, and any active AIDS-defining disease. The patients were instructed

not to modify diet, exercise or other habits during the study. The diagnosis of AIDS was based on the 1993 revised case definition of the Centers for Disease Control and Prevention CDC [9].

All patients provided written informed consent before enrollment. The study was approved by the Ethics Committee of the *Hospital de la Santa Creu i Sant Pau* and complied with the provisions of the Good Clinical Practice guidelines and the Declaration of Helsinki.

Baseline and follow-up measurements and treatments were done according to standard clinical practice at the HIV infection clinic. At study entry each patient had HIV infection history and demographic data recorded and anthropometric, blood pressure, viro-immunological, and metabolic parameters measured. These measurements were repeated every 3 months. DEXA scans and abdominal SAT biopsies were performed at baseline and at week 48. Gene expression in SAT was assessed with baseline and 48-week samples.

**Body composition measurements**

Subjects were weighed on calibrated scales after removing shoes, outdoor clothing, and other heavy items. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters. Waist circumference was measured to the nearest millimeter using anatomical landmarks as defined for the Third National Health and Nutrition Evaluation Survey [10].

Whole body DEXA scans (Hologic QDR-4500A Hologic, Inc, 590 Lincoln St, Waltham, MA 02154, USA) were conducted by a single operator on all the patients. The operator was blinded to antiretroviral treatment. The percentage of fat at the arms, legs and central abdomen (calculated from the mass of fat versus lean and bone mass), as well as total lean body mass in kilograms, was recorded. To assess fat symmetry distribution, the following fat ratios were analyzed: trunk/limb fat ratio by dividing trunk fat by appendicular fat [11], fat mass ratio by dividing the percentage of trunk fat by the percentage of lower limb fat [12], fat mass index by dividing whole body fat by squared height [13], and leg fat percentage normalized to BMI by dividing the percentage of leg fat mass by BMI [14].

**Definition of lipoatrophy, lipohypertrophy, and metabolic syndrome**

Lipoatrophy was defined as a decrease in limb fat > 20% with respect to baseline, whereas lipohypertrophy was defined as an increase in trunk fat > 20% with respect to baseline value [8, 15]. The metabolic syndrome was defined according to the U.S. National Cholesterol Education Program (NCEP) Adult Treatment Panel III Guidelines [16] and modified as recommended in the latest American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement [17].

**Biochemistry laboratory measurements**

All laboratory investigations were performed after a 12 h overnight fast and at least 5 minutes after seating prior to phlebotomy to avoid hemoconcentration, as previously described [16]. Insulin resistance was estimated by the homeostasis model assessment method (HOMA-R) as the product of the fasting concentrations of plasma insulin (μunits/ml) and plasma glucose (mmol/l) divided by 22.5 [18].

**Fat tissue samples**

Fat tissue samples were obtained from SAT abdominal depot through a small surgical biopsy performed by an 8 mm punch under local anesthesia with mepivacaine, at

baseline and at week 48. One half of the SAT obtained was immediately frozen in liquid nitrogen and stored at -80ºC until RNA extraction.

### Genes profiled in subcutaneous fat

After homogenization in RLT buffer (Qiagen, Hilden, Germany), DNA was isolated using standard phenol/chloroform procedures and RNA was isolated using a column-affinity based methodology that included on-column DNA digestion (RNeasy; Qiagen). One microgram of RNA was transcribed into cDNA using MultiScribe reverse transcriptase and random-hexamer primers (TaqMan Reverse Transcription Reagents; Applied Biosystems, Foster City, California, CA, USA). For quantitative mRNA expression analysis, TaqMan reverse transcriptase (RT)-polymerase chain reaction (PCR) was performed on the ABI PRISM 7700HT sequence detection system (Applied Biosystems). The TaqMan RT-PCR reactions were performed in a final volume of 25 µl using TaqMan Universal PCR Master Mix, No AmpErase UNG reagent and primer pair probes specific for transcripts encoding the master regulators of adipogenesis peroxisome proliferator-activated receptor-γ (PPAR-γ) (Hs00234592_m1) and CCAAT-enhancer binding protein-α (C/EBPα) (Hs00269972_s1), the marker genes of terminal adipogenic differentiation glucose transporter-4 (GLUT4)(Hs00168966_m1), and fatty acid bind protein-4 (aP2/FABP4) (Hs00609791_m1), the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α Hs00174128_m1), interleukin 18 ( IL-18)(Hs99999040_m1), and monocyte chemotactic protein-1 (MCP-1)( Hs00234140_m1**)** and the marker gene for endoplasmic reticulum stress 78 kDa glucose-regulated protein (GPR78). The transcript levels for the mtDNA-encoded genes cytochrome b (Cyt b) (Hs02596867_s1**)** and cytochrome oxidase subunit II (COII)( Hs02596865_g1) were determined using the same Taqman RT-PCR reactions method. The assessment of mtDNA levels was performed using cyt-b probe (MT-CYB) and referred to nuclear DNA, as determined by the amplification of the intronless gene CEBPα (CEBPA) [19]. Controls with no RNA, primers, or RT were included in each set of experiments. Each sample was run in duplicate, and the mean value of the duplicate was used to calculate the mRNA levels for the genes of interest. Values were normalized to that of the reference control (18S ribosomal RNA) using the comparative 2-ΔCT method, following the manufacturer's instructions. Parallel calculations performed using the reference gene PPIA (Hs99999904_m1) yielded essentially the same results.

### Statistical analyses

Data are expressed as frequencies and percentages or median with interquartile range (IQR, percentile 25 – percentile 75). HIV-1 RNA copies/ml were analysed after a log10 transformation. We used the Fisher's exact test to compare categorical variables and the Mann-Whitney test and the Wilcoxon test for independent and dependent continuous data. All analyses were performed with the Statistical Package for Social Sciences version 17.0 (SPSS, Chicago, IL) and the level of significance for all tests was set at the two-sided 0.05 level.

## Results

### Population studied

The study group was made up of 44 patients, 23 treated with TDF/FTC plus LPV/r and 21 with TDF/FTC/EFV. Demographics of patients at baseline are shown in table 1, where it is apparent that both groups were well balanced. The mean age of the patients

was 39.2 ± 10.5 years (median [IQR]: 36.5 [31.5-45.5] years). There were 36 men (81.8%) and 8 women (18.2%).

**Viro-immunological status**

Both groups were well balanced too with respect to virological and immunological parameters at baseline, including the percentage of patients with less than 200 CD4 cells and those with viral load ≥5 $\log_{10}$ copies/ml (Table 1). Seventy percent of patients had undetectable viral load at week 48, and among those who had it detectable the median viral load was 42 (IQR: 25-51) copies/ml. The median increase in CD4 cell count was > 200 cells/$mm^3$, and the decrease in viral load was >3.5 $\log_{10}$ copies/ml in both groups (Table 1).

**Metabolic changes over time**

Metabolic, anthropometric and fat parameters, including fat distribution symmetry indexes, were well balanced without statistically significant differences between groups at baseline (Table 2). Eight patients (18.2%) had metabolic syndrome at baseline, a percentage that did not increase significantly during the study period (11 patients, 25%). Over the 48 weeks of study, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and triglycerides increased significantly in patients exposed to LPV/r, whereas in the EFV arm only total cholesterol and LDL cholesterol had significant increases (Table 3). Despite these changes, there were not significant differences in total cholesterol/HDL ratio between groups either at baseline or at week 48. With respect to glucose homeostasis, there were not significant changes over time in fasting glucose, fasting insulin and HOMA, except for fasting glucose in the EFV group (Table 3). There were not statistically significant differences in metabolic parameters between groups at week 48, except for a higher triglycerides in the LPV/r group and higher fasting glucose in the EFV group (Table 3). No patient was taking lipid-lowering drugs at baseline, whereas 5 (12.7%) in the LPV/r and one (4.7%) in the EFV arm were taking them at week 48 (P = 0.2304).

**Body composition changes over time**

Whole body fat, lower limb fat and limb fat mass increased significantly from baseline in the LPV/r group, whereas a non-significant increase was observed in the same parameters in the EFV one (Table 4). The mean increase in limb fat mass at week 48 was 994 ± 732 grams in the LPV/r and 1181 ± 628 grams in the EFV group, respectively (P = 0.9832). Two (9.5%) patients in the LPV/r and 2 (8.7%) in the EFV group had a limb fat loss ≥ 20% with respect of baseline at week 48 (P = 1.0). Percentages of limb fat loss > 10% of baseline at week 48 were 21.7% and 14.3%, respectively (P = 0.7010). Two patients (8.7%) in the LPV/r and none in the EFV group had an increase in trunk fat mass ≥ 20% from baseline (P = 0. 5101). Fat distribution symmetry indexes did not change significantly over time (Table 4). At week 48 there were no significant differences between both groups in terms of fat parameters and fat distribution symmetry indexes. Bone mineral content (BMC) and bone mineral density (BMD) significantly decreased over the study period, and at week 48 BMC was significantly lower in the LPV/r with respect to EFV group (Table 4).

**Mitochondrial DNA and gene expression in SAT over time**

Paired fat biopsies were available for 18 patients (78.3%) in the LPV/r and 17 patients (80.9%) in the EFV group. There were not statistically significant differences with the whole group between patients who had paired biopsies in each arm in terms of viro-immunological, metabolic and fat parameters at baseline.

*Mitochondrial DNA and mitochondrially-related genes*

Baseline mtDNA abundance and COII and Cyt b gene expression levels in LPV/r and EFV groups were comparable (P = 0.8819, P = 0.1372, and P = 0.1979, respectively). The relative abundance of mtDNA did not increase significantly, and COII did not change significantly in either group (Figure 1). Cyt b mRNA significantly increased in the EFV but not in the LPV/r group (Figure 1). At week 48, the relative abundance of mtDNA and of COII and Cyt b transcripts were not different between groups (P = 0.3871, P = 0.8880, and P = 0.8430, respectively).

*Adipocyte differentiation and lipid metabolism*

Overall, transcript levels for adipogenesis-related genes at baseline were not significantly different, with the exception of GLUT4 that was higher in the LPV/r group (P = 0.0014). The expression of PPAR-$\gamma$ and C/EBP$\alpha$, the two major master regulators of adipogenic differentiation, did not change significantly during the study (Figure2). In addition, consistently with these observations, the expression of marker genes of terminal differentiation of adipose cells, such as GLUT4 and aP2/FABP, did not change significantly (Figure2). Between groups comparison at week 48 did not show differences for any of those transcripts (P = 0.8950, P = 0.2601, and P = 0.4678, respectively).

*Genes involved in inflammation*

MCP-1, IL-18, GPR78, and TNF-$\alpha$ transcript levels at baseline were comparable between groups (P = 0.6677, P = 0.1372, P = 0. 9736, and P = 0.9473, respectively). MCP-1 and TNF-$\alpha$ transcripts did not change significantly over 48 weeks in the LPV/r group, whereas they showed a significant increase in the EFV group (Figure 3). GPR78 gene expression, an indicator of the endoplasmic reticulum stress process closely related to inflammation, did not change significantly in both groups (Figure 3). IL-18 transcript level significantly decreased in the LPV/r but increased significantly in the EFV group (Figure 3). Between groups comparison at week 48 did not show any difference for MCP-1, GPR78 and TNF-$\alpha$ transcript levels (P = 0.2219, P = 0.5743, and 0.7917, respectively), but there were significant differences for IL-18 gene expression (P = 0.0050).

## Discussion

This study shows that LPV/r and EFV both combined with TDF/FTC for 48 weeks, are associated with different changes in molecular markers of adipocyte biology and function. However, none of them was associated with relevant changes in the abundance of mtDNA. LPV/r had a neutral effect on the markers studied, whereas EFV was associated with an increase in Cyt b and in markers of inflammation. From a clinical point of view, LPV/r was associated with significant increases in subcutaneous fat without concomitant changes in the fat distribution symmetry indexes, whereas EFV was not associated with increases in subcutaneous fat or with changes in fat distribution symmetry indexes. From a metabolic point of view, LPV/r was associated with significant increases in total cholesterol, triglycerides, HDL, LDL and VLDL cholesterol, whereas in the EFV arm total cholesterol, LDL and VLDL cholesterol also increased significantly. However, total cholesterol/HDL ratio did not change significantly in either arm.

A number of clinical trials have examined the effect of LPV/r and EFV on fat depots; in two of them [8, 15], EFV was associated with a higher incidence of lipoatrophy defined as > 20% limb fat loss from baseline value. However, in these two studies the NRTI backbone in the EFV arm contained zidovudine (AZT) [15] or was composed of stavudine and AZT in 66% of the patients in the second one [8], which could have had a substantial

contribution to fat loss. Notwithstanding that, whichever NRTI backbone was used, EFV was associated with higher degrees of lipoatrophy than LPV/r, and this was true even when TDF/FTC were the NRTI used [8]. These results are at odds with fat sub-studies in other clinical trials which show increases in subcutaneous fat in antiretroviral-naïve patients who start an EFV-based regime in combination with TDF/FTC [7,20]. Moreover, no differences were detected in these studies regarding the percentage of patients who experienced fat loss either with the 10% or the 20% threshold [7, 20]. Therefore, EFV from a clinical point of view seems safe in terms of subcutaneous adipose tissue toxicity. In our study, both groups showed an increase in subcutaneous fat, although this increase only reached statistical significance for patients in the LPV/r arm. In addition, fat distribution symmetry indexes remained unchanged throughout the study which suggests that the fat gained was symmetrically distributed. As expected, bone mineral content and density significantly decreased during the study, and this decrease was more pronounced in the LPV/r arm. It is well known that bone mineralization parameters decrease when HAART is started, and that this decrease is greater if TDF is part of the HAART regime and even greater when TDF is combined with a boosted PI [21, 22].

LPV/r has been associated with a range of disturbances in adipocyte biology and function in *in vitro* studies, including impairment of adipocyte differentiation [23, 24] and up-regulation of inflammatory-related genes [23]. Moreover, LPV/r has been associated with adverse effects on mitochondrial function measured by an increased reactive oxygen species (ROS) production and has also adversely affected insulin sensitivity [24]. However, impairment of insulin sensitivity by LPV/r seems very dependent on the length of exposure [25]. These findings are at odds with our *ex vivo* findings of a neutral action of TDF/FTC plus LPV/r on SAT expression of genes related to adipocyte differentiation, inflammation and mitochondrial function measured by mtDNA content and expression of COII and Cyt b genes. EFV has been linked in *in vitro* studies to impairment of adipocyte differentiation, decreased lipogenesis, promotion of inflammation [23, 26, 27], and mitochondrial toxicity [28]. Moreover, in the hepatocyte model, EFV has been associated with profound changes in mitochondrial bioenergetics reducing cell respiration by inhibition of complex I of the mitochondrial respiratory chain (MRC), reducing intracellular ATP levels and increasing ROS production [29]. In an *ex vivo* study, a decrease in Cyt b and increase in uncoupling protein-2 (UCP-2) gene expression was found, suggesting a shift to anaerobic metabolism within SAT [30]. We did not find any sign of mitochondrial toxicity among patients exposed to EFV measured by mtDNA content whereas Cyt b gene expression even increased significantly during the study. This discrepancy may be explained by the fact that in case of toxicity mitochondrial mass may increase [31], but also because in one study [30] half of the patients were exposed to AZT and when Cyt b expression was analyzed by AZT or TDF exposure, there were significant differences between both arms.

The present study did not reveal relevant changes in adipogenic genes caused by LPV/r- and EFV-based HAART for 48 weeks. The main difference in terms of gene expression at SAT level between LPV/r- and EFV-based HAART in this study was that the latter was associated with up-regulation of the genes encoding for inflammatory cytokines (i.e. TNF-$\alpha$, IL-18 and MCP-1). This finding is in line with those from *in vitro* adipocyte models where EFV is associated with increases in TNF-$\alpha$ and MCP-1 transcript levels [23, 27]. In these models, LPV/r was also associated with such an increase [23, 27], but to a lesser extent. However, this was not reproduced in our *ex vivo* samples for LPV/r-treated patients. On the other hand, protease inhibitors, including lopinavir, have been reported to cause endoplasmic reticulum stress in multiple tissues and cells including adipocytes [32, 33], and this effect is known to be coupled to a pro-inflammatory induction [34]. The present data on unaltered GPR78, a marker of endoplasmic reticulum stress, indicate that this process does not appear to be involved in the preferential pro-inflammatory induction observed in the LPV/r-exposed SAT. The establishment of an inflammatory environment at

SAT and systemic level is thought to play an important part in the development of HALS [35]. Whether the increase in SAT inflammatory markers in the EFV arm can be linked to the absence of significant improvement in SAT parameters in the EFV arm, may only be a matter of speculation at present.

This study has limitations; first, this is a non-randomized study and therefore no causal relationship must be established. Notwithstanding that, both groups were comparable with regard to clinical, virologic, fat, and even SAT molecular levels at baseline. Second, the limited number of patients and samples may have prevented us from finding relevant differences both in molecular and clinical parameters. Third, it has not explored the mitochondrial bioenergetics in samples from the patients, and this has been described both *in vitro* and *ex vivo* as a possible mechanism of SAT toxicity, especially by EFV [36]. Fourth, it has not explored the expression of genes involved in glucocorticoid generation which may play a role in adipocyte differentiation [30].

## Conclusion

In summary, after 48 weeks of therapy, TDF/FTC plus LPV/r significantly increased limb fat and lipid parameters, whereas TDF/FTC/EFV did not significantly increase limb fat but increased total and LDL cholesterol. The expression of genes involved in adipocyte differentiation and mitochondria-related genes did not change significantly in SAT from LPV/r-exposed patients, whereas Cyt b, and especially inflammation-related genes were significantly up-regulated in SAT from TDF/FTC/EFV-exposed patients.

## Tables and figures

**Table 1. Demographics, baseline and viro-immunological characteristics of the population studied**

| Characteristic | LPV/r (n = 23) | EFV (n = 21) | P value |
|---|---|---|---|
| **Age, yrs** | 37.0 (30.2-45.0) | 36.0 (32.0-46.2) | 0.8049 |
| **Males, n (%)** | 18 (78.3) | 18 (85.7) | 0.8034 |
| **Risk factor for HIV infection** | | | |
| **MsM, n (%)** | 12 (52.2) | 14 (66.7) | 0.6179 |
| **Heterosexual, n (%)** | 8 34.8() | 5 (23.8) | |
| **IVDU, n (%)** | 3 (13.0) | 2 (9.5) | |
| **Duration of HIV infection, months** | 9.0 (2.0-25.7) | 10.0 (2.7-34.7) | 0.4651 |
| **AIDS, n (%)** | 7 (30.4) | 6 (28.6) | 0.8923 |
| **Smokers, n (%)** | 18 (78.3) | 15 (71.4) | 0.8617 |
| **Alcohol consumption, g/d** | 20.0 (0.0-36.0) | 18.0 (0.0-26.0) | 0.4948 |
| **HBV co-infection, n (%)** | 1 (4.3) | 2 (9.5) | 0.4936 |
| **HCV co-infection, n (%)** | 3 (13.0) | 1 (4.8) | 0.6086 |
| **Baseline CD4 cell count, cells/mm$^3$** | 195 (45-318) | 199 (108-323) | 0.6982 |
| **Baseline CD4 count, %** | 15 (4.0-18.7) | 17 (10.5-21.2) | 0.3229 |
| **Nadir CD4 cell count < 200 cells/mm$^3$, n (%)** | 11 (47.8) | 11 (52.4) | 0.7627 |
| **Nadir CD4 cell count < 100 cells/mm$^3$, n (%)** | 7 (30.4) | 5 (23.8) | 0.7403 |
| **CD4 increase through 48 weeks, cells/mm$^3$** | 234 (109-437) | 264 (167-520) | 0.5106 |
| **Baseline CD8 cell count, cells/mm$^3$** | 690 (355-1113) | 760 (437-1017) | 0.8509 |
| **Baseline HIV-1 RNA, $\log_{10}$ copies/ml** | 5.10 (4.60-5.65) | 5.47 (4.98-5.65) | 0.2850 |
| **Baseline HIV-1 RNA > 5 $\log_{10}$ copies/ml , n (%)** | 13 (56.5) | 15 (71.4) | 0.3596 |
| **Undetectable HIV-1 RNA at week 48, n (%)** | 16 (71.4) | 15 (69.6) | 1.0 |
| **Decrease HIV-1 RNA over 48 weeks, $\log_{10}$ copies/ml** | -3.67 (-4.35- [-3.29]) | - 4.05 (-4.8-[-3.41]) | 0.3911 |

**Values are expressed as medians (95%CI) unless otherwise specified. TDF = tenofovir, FTC = emtricitabine, LPV/r = lopinavir/ritonavir, EFV = efavirenz, MsM = men who have sex with men, IVDU = intravenous drug users, HBV = Hepatitis B virus, HCV = Hepatitis C virus, AIDS = acquired immune deficiency syndrome**

**Table 2. Baseline anthropometric, metabolic and fat parameters**

| Parameter | LPV/r (n = 23) | EFV (n = 21) | P value |
|---|---|---|---|
| **Weight, kg** | 68.5 (60.0-74.9) | 69.0 (62.4-74.3) | 0.8971 |
| **Height, cm** | 173.0 (168.2-176.7) | 172.0 (166.5-180.0) | 0.9906 |
| **BMI, kg/m$^2$** | 22.6 (20.4-24.6) | 22.8 (20.9-23.9) | 0.9612 |
| **Waist circumference, cm** | 81.5 (77.0-87.0) | 81.5 (76.0-85.0) | 0.6735 |
| **WHR** | 0.86 (0.83-0.92) | 0.85 (0.81-0.89) | 0.4049 |
| **Systolic BP, mm Hg** | 110.0 (110.0-120.0) | 120.0 (110.0-127.7) | 0.1452 |
| **Diastolic BP, mm Hg** | 70.0 (60.0-80.0) | 75.0 (70.0-80.0) | 0.1385 |
| **Total cholesterol, mmol/l** | 4.04 (3.33-4.59) | 3.86 (3.69-4.56) | 0.8972 |
| **Triglycerides, mmol/l** | 1.45 (0.92-2.03) | 1.14 (1.01-1.40) | 0.3180 |
| **HDL cholesterol, mmol/l** | 0.87 (0.79-0.97) | 1.02 (0.85-1.11) | 0.0900 |
| **Total cholesterol/HDL ratio** | 4.33 (3.50-5.49) | 4.09 (3.46-4.61) | 0.2621 |
| **LDL cholesterol, mmol/l** | 2.29 (1.82-2.67) | 1.42 (2.09-2.92) | 0.3215 |
| **VLDL cholesterol, mmol/l** | 0.67 (0.40-0.88) | 0.54 (0.47-0.65) | 0.4416 |
| **Fasting glucose, mmol/l** | 4.80 (4.52-5.17) | 4.80 (4.60-5.05) | 0.7333 |
| **Fasting insulin, pmol/l** | 28.0 (11.42-97.25) | 15.2 (3.3-35.0) | 0.1912 |
| **HOMA-IR** | 1.77 (0.53-3.60) | 0.73 (0.65-1.40) | 0.5338 |
| **Whole body fat, g** | 12270 (9435-17392) | 15743 (9294-17613) | 0.4558 |
| **Trunk fat, g** | 6052 (4429-8340) | 6632 (4539-9532) | 0.4771 |
| **Left leg fat, g** | 1932 (1315-3234) | 2814 (1771-3483) | 0.3229 |
| **Lower limb fat, g** | 3743 (2706-6425) | 5591 (3537-6864) | 0.3229 |
| **Limb fat mass, g** | 5056 (3472-8152) | 7077 (4284-9050) | 0.3062 |
| **Trunk/limb fat ratio** | 1.13 (0.99-1.26) | 1.05 (0.83-1.18) | 0.2596 |
| **Fat mass ratio** | 1.49 (1.29-1.75) | 1.34 (1.07-1.59) | 0.2641 |
| **Leg fat percentage normalized to BMI** | 0.79 (0.73-0.96) | 1.14 (0.85-1.31) | 0.1037 |
| **Fat mass index, kg/m$^2$** | 4.30 (3.05-5.92) | 5.28 (3.96-6.69) | 0.3402 |
| **Metabolic syndrome, n (%)** | 6 (26.1) | 2 (9.5) | 0.3023 |
| **BMC total, g** | 2241 (2034-2448) | 2325 (2228-2616) | 0.1145 |
| **BMD, g/cm$^2$** | 1.09 (1.05-1.11) | 1.12 (1.07-1.16) | 0.1068 |

**Values are expressed as medians (95%CI) unless otherwise specified. LPV/r = lopinavir ritonavir, EFV = efavirenz, BMI = body mass index, WHR = waist to hip ratio, HDL = high density lipoprotein, LDL = low density lipoprotein, VLDL = very low density lipoprotein, HOMA-IR = homeostasis model assessment method , BMC = bone mineral content, BMD = bone mineral density**

**Table 3. Change in anthropometric and metabolic parameters over 48 weeks in patients treated with TDF/FTC plus LPV/r or EFV**

| | LPV/r (n = 23) | | | EFV (n =21) | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Week 48 | Within group P value | Baseline | Week 48 | Within group P value | Between groups P value wk. 48 |
| **Weight, kg** | 68.5 (60.0-74.9) | 71.80 (66.0-77.0) | **0.0537** | 69.0 (62.4-74.3) | 74.1 (65.1-79.5) | **0.0079** | **0.4884** |
| **BMI, kg/m$^2$** | 22.59 (20.37-24.65) | 23.5 (22.04-25.22) | **0.0653** | 22.78 (20.99-23.96) | 24.85 (23.26-26.39) | **0.0122** | **0.2678** |
| **Waist circumference, cm** | 81.5 (77.0-87.0) | 84.5 (81.0-92.0) | **0.1066** | 81.5 (76.0-85.0) | 86.5 (80.5-91.0) | **0.1316** | **0.8491** |
| **Systolic BP, mm Hg** | 110 (110-120) | 120 (110-120) | **0.2036** | 120 (110-127) | 120 (120.128) | **0.6836** | **0.3670** |
| **Diastolic BP, mm Hg** | 70 (60-70) | 70 (65-75) | **0.6365** | 75 (70-80) | 75 (70-80) | **0.9005** | **0.1362** |
| **Total cholesterol, mmol/l** | 4.04 (3.33-4.59) | 5.22 (4.28-6.21) | **0.0001** | 3.86 (3.69-4.56) | 4.72 (4.20-5.22) | **0.0011** | **0.1296** |
| **HDL cholesterol, mmol/l** | 0.87 (0.79-1.29) | 1.04 (0.90-1.35) | **0.0089** | 1.02 (0.85-1.11) | 1.14 (0.89-1.35) | **0.0504** | **0.7338** |
| **Total cholesterol/HDL ratio** | 4.33 (3.50-5.49) | 4.81 (3.83-5.98) | **0.5323** | 4.09 (3.46-4.61) | 3.96 (3.31-5.05) | **0.4344** | **0.2467** |
| **LDL cholesterol, mmol/l** | 2.29 (1.82-2.67) | 3.16 (2.46-3.97) | **0.0025** | 2.42 (2.09-2.92) | 2.70 (2.38-3.03) | **0.0451** | **0.1778** |
| **VLDL cholesterol, mmol/l** | 0.67 (0.40-0.88) | 0.93 (0.67-1.13) | **0.0226** | 0.54 (0.47-0.65) | 0.60 (0.46-0.74) | **0.4690** | **0.0110** |
| **Triglycerides, mmol/l** | 1.45 (0.92-2.03) | 2.14 (1.46-2.74) | **0.0180** | 1.14 (1.01-1.40) | 1.28 (1.01-1.59) | **0.2360** | **0.0108** |
| **Fasting glucose, mmol/l** | 4.80 (4.52-5.17) | 5.00 (4.80-5.20) | **0.2120** | 4.80 (4.60-5.05) | 5.20 (5.07-5.42) | **0.0031** | **0.0399** |
| **Fasting insulin, pmol/l** | 28.0 (13.7-97.2) | 7.15 (2.10-17.8) | **0.2060** | 17.5 (13.0-35.0) | 8.45 (2.60-15.35) | **0.4175** | **0.8169** |
| **HOMA-IR** | 1.77 (0.53-3.60) | 0.55 (0.45-1.61) | **0.8520** | 0.73 (0.65-1.40) | 1.15 (0.51-1.95) | **0.1230** | **0.4337** |

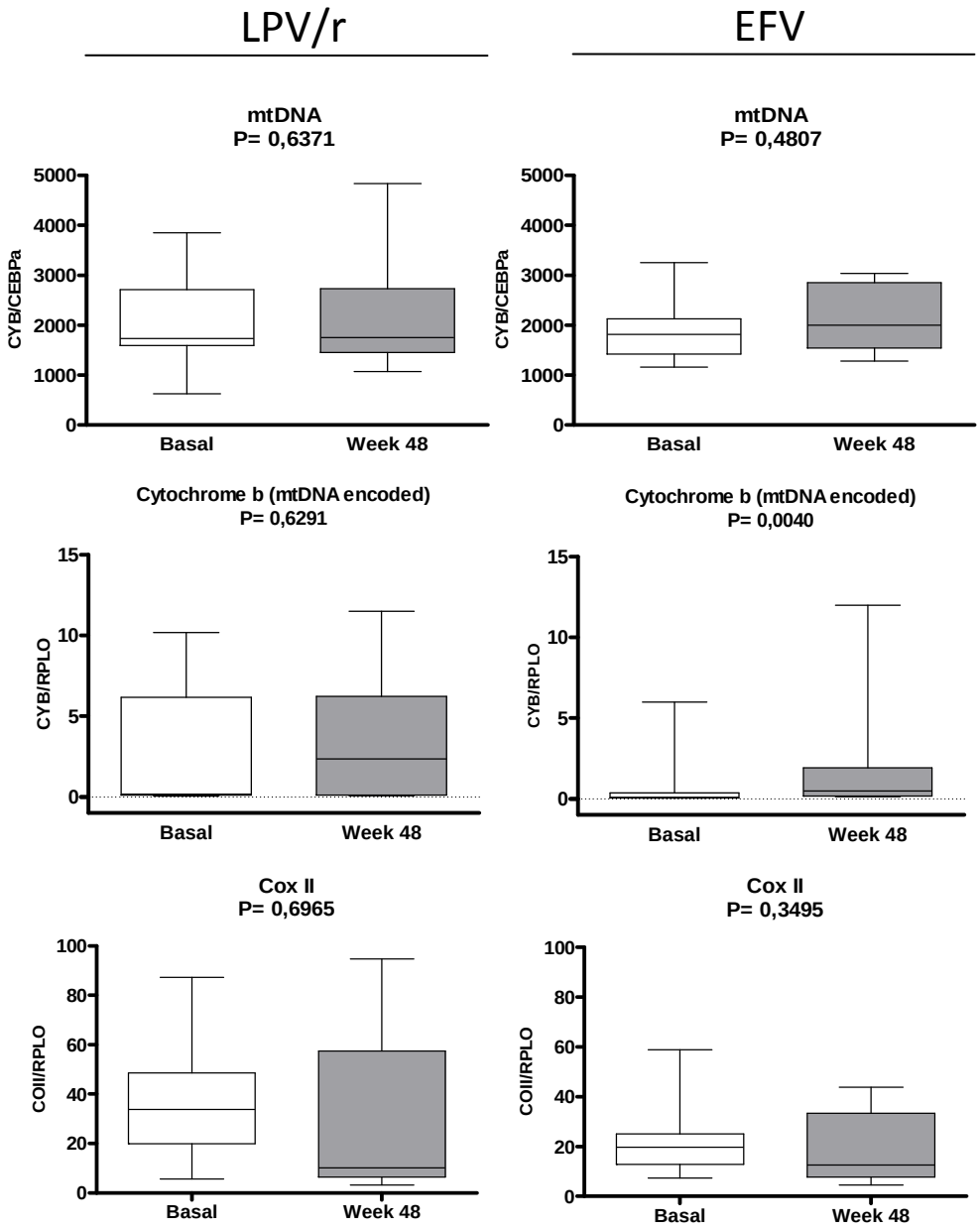
**All parameters expressed as median (interquartile range) unless otherwise specified. BMI = body mass index, BP = blood pressure, HDL = high density lipoprotein, LDL = low density lipoprotein, VLDL = very low density lipoprotein, mmol = milimoles, pmol = picomoles, HOMA = homeostasis model assessment method**

**Table 4. Change in fat parameters over 48 weeks in patients treated with TDF/FTC plus LPV/r or EFV**

| | LPV/r (n = 23) | | | EFV (n = 21) | | | |
|---|---|---|---|---|---|---|---|
| **Parameter** | **Baseline** | **Week 48** | **Within group P value** | **Baseline** | **Week 48** | **Within group P value** | **Between groups P value (wk. 48)** |
| **Whole body fat, g** | 12270 (9435-17392) | 15152 (10428-22207) | **0.0424** | 15743 (10417-17923) | 16887 (13643-22605) | **0.0370** | **0.6353** |
| **Trunk fat, g** | 6052 (4429-8340) | 7040 (5271-10863) | **0.1225** | 6686 (4158-7698) | 7187 (5161-12401) | **0.1793** | **0.8744** |
| **Left leg fat, g** | 1932 (1315-3234) | 2628 (1614-3363) | **0.0577** | 2814 (1771-3483) | 3309 (2277-3718) | **0.1680** | **0.2059** |
| **Lower limb fat, g** | 3743 (2706-6425) | 5298 (3216-6957) | **0.0329** | 5591 (3537-6864) | 6858 (4561-7451) | **0.0937** | **0.2357** |
| **Limb fat mass, g** | 5056 (3472-8152) | 6884 (4133-9144) | **0.0293** | 7077 (4584-8839) | 8682 (5881-9478) | **0.0809** | **0.2684** |
| **Trunk/limb fat ratio** | 1.13 (0.99-1.26) | 1.16 (0.99-1.32) | **0.5486** | 1.05 (0.83-1.18) | 0.97 (0.83-1.31) | **0.6741** | **0.3230** |
| **Fat mass ratio** | 1.49 (1.29-1.75) | 1.51 (1.29-1.75) | **0.5073** | 1.34 (1.07-1.59) | 1.28 (1.05-1.73) | **0.6352** | **0.2357** |
| **% leg fat/BMI** | 0.79 (0.73-0.96) | 1.03 (0.65-1.23) | **0.2956** | 1.14 (0.85-1.31) | 1.12 (0.93-1.37) | **0.0630** | **0.1498** |
| **Fat mass index, kg/m$^2$** | 4.30 (3.05-5.92) | 4.83 (3.55-7.34) | **0.2439** | 5.28 (3.96-6.69) | 5.51 (4.20-8.37) | **0.0216** | **0.1701** |
| **Metabolic syndrome, n (%)** | 6 (26.1) | 8 (34.8) | **0.7486** | 2 (9.5) | 3 (14.3) | **1.0** | **0.2225** |
| **BMC total, g** | 2241 (2034-2448) | 1781 (1670-1878) | **< 0.0001** | 2325 (2228-2616) | 1924 (1783-1989) | **< 0.0001** | **0.0081** |
| **BMD, g/cm$^2$** | 1.09 (1.05-1.11) | 1.05 (1.01-1.09) | **< 0.0001** | 1.12 (1.07-1.16) | 1.12 (1.09-1.16) | **< 0.0001** | **0.0753** |

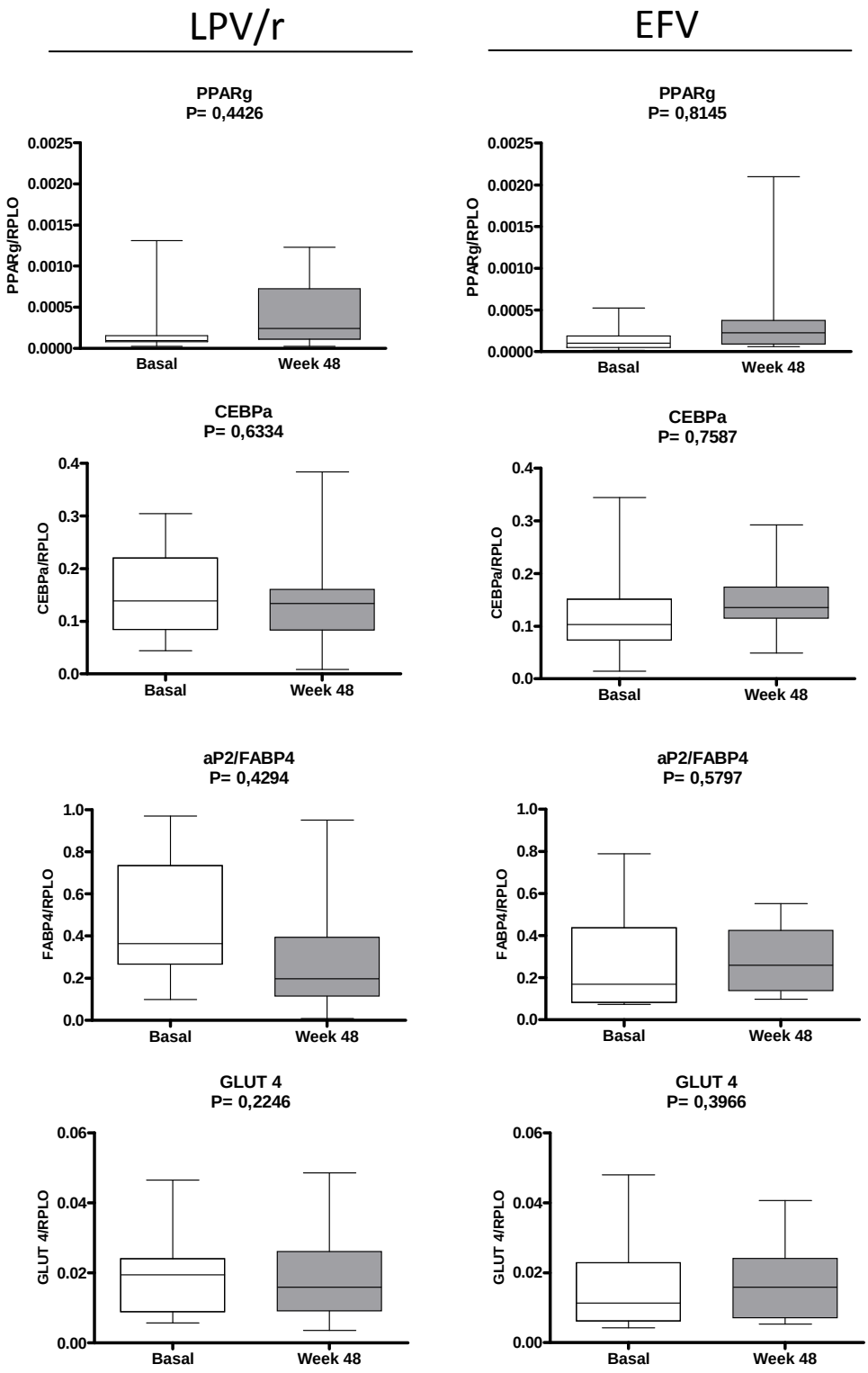
**All parameters expressed as median (interquartile range) unless otherwise specified. BMI = body mass index, BMC = bone mineral content, BMD = bone mineral density**

Figure 1. Relative abundance of mitochondrial DNA and cytochrome oxidase b transcript levels in abdominal SAT samples from patients exposed to LPV/r and EFV, both combined with TDF/FTC.



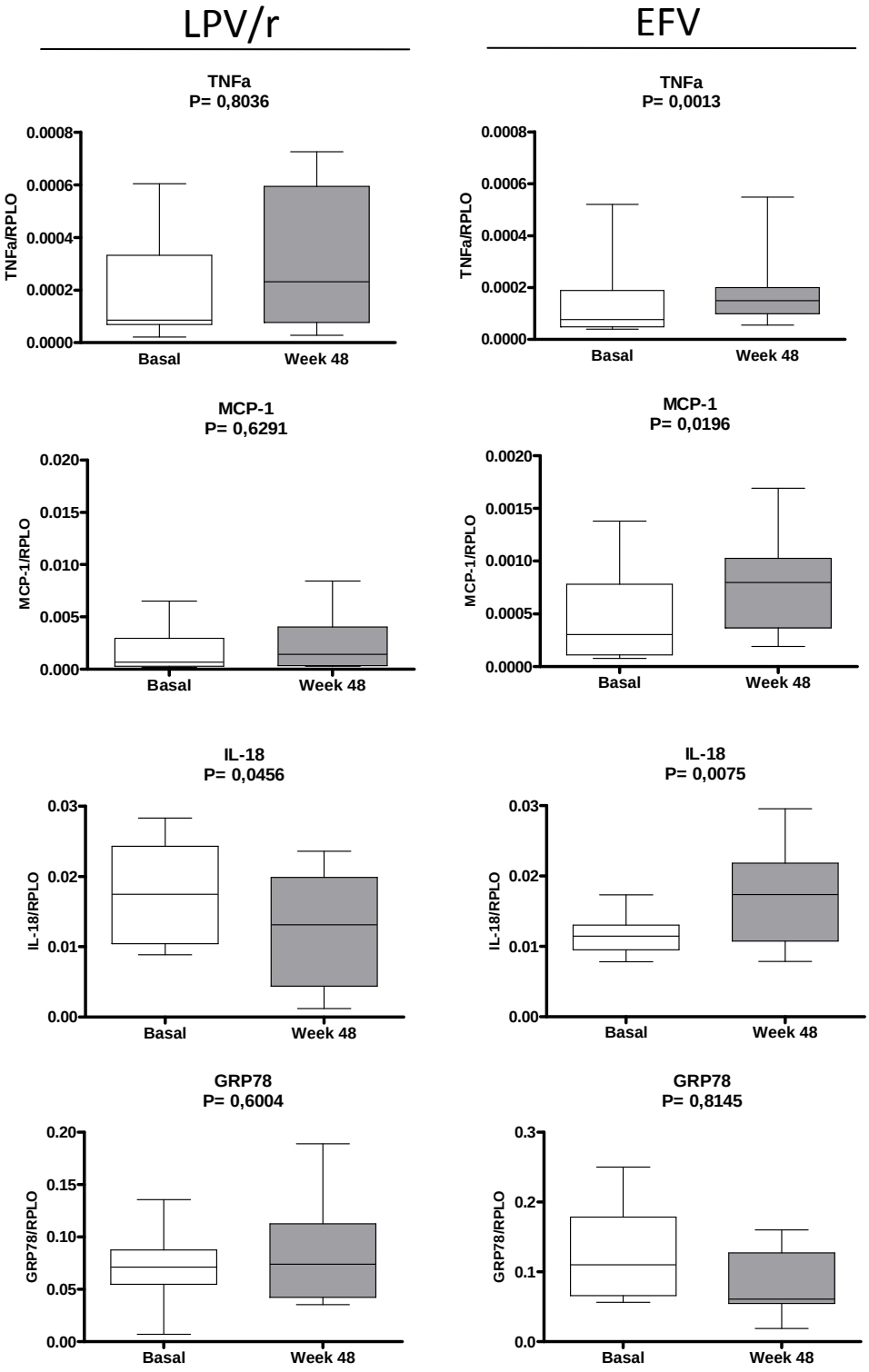

LPV/r = lopinavir/ritonavir, EFV = efavirenz, mtDNA = mitochondrial DNA, Cox II = cytochrome oxidase subunit II

Figure 2. PPAR-γ and LPL transcript levels in abdominal SAT samples from patients exposed to LPV/r and EFV, both combined with TDF/FTC.




LPV/r = lopinavir/ritonavir, EFV = efavirenz, PPAR-γ = peroxisome proliferator-activated receptor gamma, LPL = lipoprotein lipase, CEBPa = CCAAT-enhancer binding protein-α, aP2/FABP4 = fatty acid bind protein-4, GLUT 4 = glucose transporter-4.

Figure 3. TNF-α, IL-18, and MCP-1 transcript levels in abdominal SAT samples from patients exposed to LPV/r and EFV, both combined with TDF/FTC.




LPV/r = lopinavir/ritonavir, EFV = efavirenz, TNF-α = tumor necrosis factor alpha, MCP-1 = monocyte chemotactic protein 1, IL-18 = interleukin 18, GRP78 = 78 kDa glucose-regulated protein.

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