Journal of Antimicrobial Chemotherapy Advance Access published February 5, 2013

J Antimicrob Chemother
doi:10.1093/jac/dks540

Journal of Antimicrobial Chemotherapy

Switching to lopinavir/ritonavir with or without abacavir/lamivudine in lipoatrophic patients treated with zidovudine/abacavir/lamivudine

J. I. Bernardino[1]*, F. Pulido[2], E. Martinez[3], J. Arrizabalaga[4], P. Domingo[5], J. Portilla[6], A. Ocampo[7], J. Muñoz[8], R. Torres[9] and J. R. Arribas[1] on behalf of the GESIDA-6008-KRETA Study Group†

[1]Hospital Universitario La Paz, IdiPAZ, Madrid, Spain; [2]Hospital Universitario 12 de Octubre i+12, Madrid, Spain; [3]Hospital Clínic, Institut d'Investigacions Biomediques August Pi i Sunyer, University of Barcelona, Barcelona, Spain; [4]Hospital Donostia, San Sebastián, Spain; [5]Hospital de la Santa Creu i San Pau, University of Barcelona, Barcelona, Spain; [6]Hospital General de Alicante, Alicante, Spain; [7]Complexo Hospitalario Universitario de Vigo, Pontevedra, Spain; [8]Hospital de Basurto, Bilbao, Spain; [9]Hospital Severo Ochoa, Leganes, Madrid, Spain

*Corresponding author. Tel: +34 91 727 70 99; Fax: +34 91 729 00 33; E-mail: jibernardino.hulp@salud.madrid.org
†Members of the GESIDA-6008-KRETA Study Group are listed in the Acknowledgements section.

Received 7 September 2012; returned 20 October 2012; revised 10 December 2012; accepted 19 December 2012

Background: Discontinuation of thymidine nucleoside reverse transcriptase inhibitors (tNRTIs) is the only proven strategy for improving lipoatrophy. It is unclear whether switching to NRTI-sparing or to non-thymidine NRTI-containing therapy has differential effects on body fat recovery.

Methods: This was a 96 week, open-label, randomized study in suppressed patients with moderate/severe lipoatrophy and no prior virological failure while receiving a protease inhibitor and who had their triple NRTI regimen (zidovudine/lamivudine/abacavir) switched to lopinavir/ritonavir plus abacavir/lamivudine for a 1 month run-in period and then randomized to lopinavir/ritonavir plus abacavir/lamivudine versus lopinavir/ritonavir monotherapy. The KRETA trial is registered with ClinicalTrials.gov (number NCT00865007).

Results: Of 95 patients included, 88 were randomized to lopinavir/ritonavir plus abacavir/lamivudine ($n=44$) or lopinavir/ritonavir monotherapy ($n=44$). Median (IQR) baseline limb fat was 2.5 (1.6–3.7) kg in the lopinavir/ritonavir plus abacavir/lamivudine group and 2.5 (2.0–5.4) kg in the lopinavir/ritonavir monotherapy group. Six patients in the triple therapy group and 13 in the monotherapy group had discontinued study drugs by week 96. Although there were limb fat gains in each group at weeks 48/96 (+324/+358 g in lopinavir/ritonavir plus abacavir/lamivudine, $P=0.09/0.07$, versus +215/+416 g in the lopinavir/ritonavir monotherapy group, $P=0.28/0.16$), differences between groups were not significant [difference +109 g (95% CI −442, +660)/−57 g (95% CI −740, +625)].

Conclusions: In lipoatrophic patients treated with zidovudine/lamivudine/abacavir, switching to lopinavir/ritonavir monotherapy had no additional benefit in limb fat recovery relative to switching to lopinavir/ritonavir with abacavir/lamivudine. These data suggest that non-thymidine nucleosides such as abacavir/lamivudine are not an obstacle to limb fat recovery.

Keywords: HIV, lipoatrophy, thymidine nucleoside analogues, NRTI-sparing regimen, lopinavir/ritonavir monotherapy

Introduction

Combination antiretroviral therapy (cART) has improved life expectancy in HIV-infected patients, but has as a counterpart a number of metabolic disturbances, such as insulin resistance, dyslipidaemia and body fat abnormalities.[1,2] Body composition abnormalities are associated with a worse cardiovascular risk profile and depression, and as a consequence impact negatively on adherence and quality of life.[3–6]

HIV-associated lipodystrophy is still present even though the probability of developing lipoatrophy has decreased substantially in developed countries as the pattern of cART prescription has significantly changed. Prevalence of lipoatrophy varies from 50% in the early cohort studies to 25% in recent cohorts.[7–10] Thymidine nucleoside analogue reverse transcriptase inhibitors (tNRTIs) are clearly involved in the development of lipodystrophy, especially in combination with first-generation protease inhibitors (PIs).[11–13] Clinical trials in antiretroviral-naive HIV-infected patients have clearly shown that regimens that include non-tNRTIs such as abacavir or tenofovir are associated with a lower incidence of lipoatrophy.[14–17]

Replacement of stavudine or zidovudine with abacavir or tenofovir has led to modest limb fat gains and a more favourable lipid profile.[18–21] Clinical trials of tNRTI-sparing regimens, mainly a combination of PIs and a non-nucleoside reverse transcriptase inhibitor (NNRTI), have shown similar increases in limb fat but with unacceptable hyperlipidaemia.[17,22–24]

Although tenofovir, abacavir and lamivudine have a risk of lipoatrophy much lower than tNRTIs, they are NRTIs and share mitochondrial dysfunction as the common pathway for toxicity. For this reason, it could be hypothesized that complete removal of NRTIs might induce greater limb fat gains than switching to non-tNRTIs. In fact the Abbott-613 trial[25] suggested a beneficial role of PI monotherapy for limb fat recovery.

The GESIDA-6008-KRETA clinical trial investigated the potential additional benefits on limb fat recovery of a completely NRTI-sparing regimen of lopinavir/ritonavir monotherapy as an alternative to a tNRTI-switching strategy in HIV-infected patients with moderate to severe lipoatrophy.

Patients and methods

Study design

This Phase IV open-label, multicentre, randomized, 96 week trial compared the effect on limb fat recovery of lopinavir/ritonavir plus abacavir/lamivudine versus lopinavir/ritonavir monotherapy in patients virologically suppressed while receiving co-formulated zidovudine/lamivudine/abacavir. The study was performed in 10 Spanish HIV treatment centres. This clinical trial was approved by the Regional Ethics Committee for Clinical Research of the Community of Madrid, the local ethics committees for clinical research at each site and the Spanish Agency for Medicine and Healthcare Products. All patients provided written informed consent prior to study entry and the study was conducted according to International Good Clinical Practice guidelines. The study was sponsored by Grupo de Estudio de SIDA (GESIDA) of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) and funded by Abbott Laboratories. The KRETA trial is registered with ClinicalTrials.gov (number NCT00865007).

The primary endpoint was the absolute change in limb fat mass measured by dual X-ray absorptiometry (DEXA) scan from baseline to 48 weeks. Secondary endpoints were absolute change in limb fat at 96 weeks, percentage change in limb fat mass at 48/96 weeks and changes in fasting cholesterol [total, high-density lipoprotein (HDL) cholesterol and calculated low-density lipoprotein (LDL) cholesterol] and triglycerides at 48/96 weeks. Additional endpoints were incidence of adverse events, virological rebounds (two consecutive HIV-1 RNA measurements >400 copies/mL) and change in CD4 cell count between baseline and 48/96 weeks.

Eligible patients were HIV-1-infected patients aged ≥18 years, treated with co-formulated zidovudine/lamivudine/abacavir for >24 weeks and with no prior virological failure while receiving a PI-based regimen. Individuals with clinical moderate to severe lipoatrophy at one or more facial/body sites according to the Lipodystrophy Severity Grading Scale (LSGS) definition[8] and with a viral load <50 copies/mL for at least the previous 6 months were invited to participate. Exclusion criteria were pregnancy, serum hepatitis B surface antigen positivity, active opportunistic disease or wasting syndrome, need for treatment with agents known to have potential major interactions with lopinavir/ritonavir, or treatment with antineoplastic or antidiabetic drugs, anabolic steroids or growth hormone in the last 16 weeks.

Eligible subjects had zidovudine/lamivudine/abacavir switched to lopinavir/ritonavir plus abacavir/lamivudine for a 1 month run-in period. The run-in period was designed to guarantee tolerance to lopinavir/ritonavir before exposing patients to monotherapy. After this run-in period, patients tolerating the new regimen were randomized in a 1:1 ratio to stop (monotherapy group) or to continue abacavir/lamivudine co-formulation (triple therapy group). Randomization was centralized and computer generated (Clin Stat v.08.05.96. Department of Public Health Sciences, St George's Hospital Medical School). Randomization was stratified by nadir CD4 cell count (below or above 100 cells/mm^3), duration of exposure to zidovudine (less or more than 3 years) and DEXA centre.

Data collection

Patients were assessed at baseline, week 4, week 12 and every 12 weeks thereafter until week 96. At study visits clinical data were collected and blood samples were drawn for laboratory tests after an overnight fast. Laboratory analysis included CD4 cell count, measurement of plasma HIV-1 RNA, full blood count, plasma chemistry profiles and a fasting lipid panel. All laboratory determinations were performed locally at each site except for plasma HIV-1 RNA at baseline and 24, 48, 72 and 96 weeks and whenever viral rebound (>50 copies/mL) occurred. These determinations were performed centrally at the Laboratory of Molecular Microbiology at the Hospital 12 de Octubre in Madrid.

Total body DEXA (limb fat, trunk fat, total body fat and lean mass) scans were obtained at baseline, week 48 and week 96 within 14 days of the scheduled visit. Fat mass ratio was selected as a marker of body fat distribution. This is the ratio between the percentage of trunk fat and the percentage of limb fat as previously described.[26] Imaging was performed at five radiology sites. Standardized scanning protocols based on each manufacturer's specifications were used across all sites. Follow-up scans were performed with the same equipment and by the same technician for each patient. For DEXA scans, patients were positioned straight on the table, with all body parts in the scan field, palms down and separated from the thighs and legs rotated inward 25°. Two sites used Hologic (Hologic Inc., Bedford, MA, USA), three used Lunar (GE Healthcare Lunar, Madison, WI, USA) and one used Norland (Fisher Biomedical Inc., Venice, FL, USA) scans.

Virological analysis

Every 6 months, central HIV-1 plasma viral load was determined at a central laboratory using automatic RNA extraction and amplification using Cobas® TaqMan® HIV-1 v2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA). Tests with detectable plasma HIV-1 RNA (>50 copies/mL) were repeated 2 weeks apart for confirmation, and then monthly until viral load became undetectable again or until viral load increased to >400 copies/mL in two consecutive samples. Viral genotyping was performed in all samples with viral load >400 copies/mL. For the present analysis, lopinavir/ritonavir- and NRTI-associated resistance mutations were defined according to the list published by the International AIDS Society-USA in 2008.[27]

Therapeutic failure was defined in the triple group as two consecutive measurements of HIV RNA >400 copies/mL separated by at least 2 weeks. Patients randomized to the monotherapy group who fulfilled this definition were not considered failures if at the time of failure there was no evidence of lopinavir/ritonavir genotypic resistance, were re-induced with abacavir/lamivudine and were suppressed to <50 copies/mL. Conversely, failure to reach HIV RNA <50 copies/mL 16 weeks after re-induction was considered as therapeutic failure in the monotherapy group.

Statistical analysis

Changes in quantitative measures from baseline to weeks 48/96 within each treatment group were tested for significance using paired *t*-tests; comparisons between treatment groups were performed using unpaired *t*-tests. Where changes were non-normally distributed, values were analysed using non-parametric tests (Mann–Whitney *U*-tests). All qualitative

variables were analysed using χ^2 tests or Fisher's exact tests, as appropriate. Additional analyses adjusted for baseline-imbalanced characteristics were performed.

Analyses were performed on an intention-to-treat (ITT) basis, with all patients being analysed in the groups to which they were randomized and with a baseline DEXA scan performed. Due to technical problems a DEXA scan was not obtained in one patient randomized to the triple therapy group and therefore this patient was excluded from the ITT analysis. A per protocol (PP) analysis was also done, including all patients randomized and with a baseline and 48-week DEXA scan performed. Missing values were imputed using a last observation carried forward approach for the ITT analysis but not for the PP analysis.

Sensitivity analyses were planned to include patients that had withdrawn from the study between 24 and 48 weeks with a DEXA scan performed in the discontinuation visit for the primary endpoint. For the secondary endpoint of percentage change in limb fat mass, an analysis replacing missing data for the 48 week DEXA scan with the worst and the best limb fat percentage was done.

To ensure a power of 80% to detect a 500 g difference in mean limb fat mass between the two groups at 48 weeks (with SD 850 g) at the 5% level of significance, 46 subjects per group were required. Assuming a 10% dropout rate, we aimed to include 50 patients per group.

Results

Demographics and subject disposition

Between November 2008 and September 2009, 95 patients were recruited and started the 1 month run-in period with abacavir/lamivudine plus lopinavir/ritonavir. Eighty-eight patients were finally randomized (44 per group). Thirty-one patients in the monotherapy group and 38 in the triple therapy group reached 96 weeks (Figure 1). Patients were well matched for baseline characteristics (Table 1) except for a higher proportion of men having sex with men in the triple group (24%) relative to the monotherapy group (6%), and longer exposure to zidovudine in the triple therapy group. Median time of exposure to zidovudine in the triple therapy group was 8.6 years compared with 6.6 years in the monotherapy group ($P=0.001$).

Changes in regional fat mass

Patients were balanced at baseline for total fat, trunk fat, limb fat and fat mass ratio (Table 1). Over 48 weeks, mean (SD) limb fat gain in the monotherapy group was 215 (1161) g by week 48 and 324 (1205) g in the triple therapy group (Figure 2). The mean difference in limb fat recovery between the monotherapy group and the triple therapy group at week 48 was −109 g (95% CI −660, 441; $P=0.694$) (Table 2), and this difference was not further modified after adjustment for baseline imbalanced variables (duration of HIV infection, total cholesterol, time on tNRTIs and time on zidovudine). Mean changes in total fat, trunk fat and fat mass ratio did not vary significantly between treatment groups. Mean (SD) limb fat gain by week 96 was 415.8 (1670) g in the monotherapy group and 358.5 (1207) g in the triple therapy group. The mean difference in limb fat

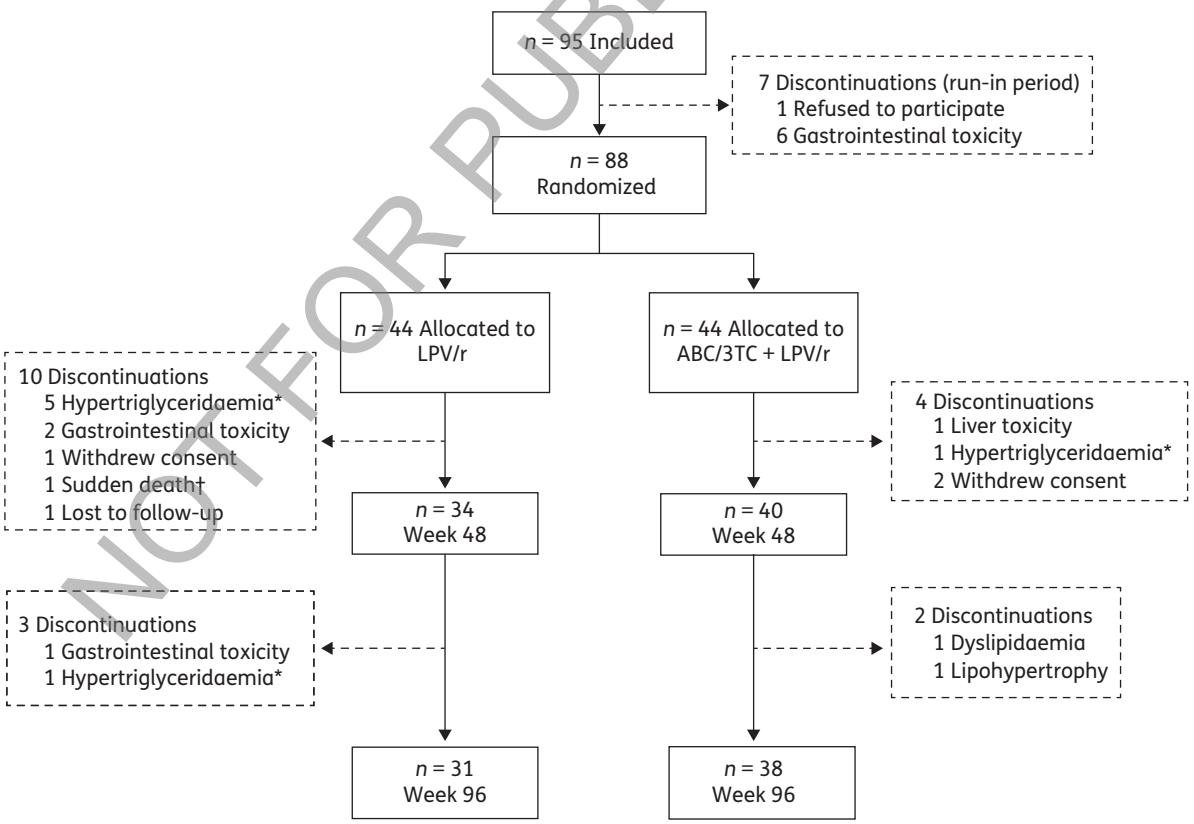

Figure 1. Flow of patients through the trial from baseline to 96 weeks. ABC, abacavir; LPV/r, lopinavir/ritonavir; 3TC, lamivudine. *Discontinuations due to hypertriglyceridaemia were decided by investigators (all grade 4 despite treatment with fibrates). †Considered not related to study drug by the investigator.

Table 1. Baseline characteristics; randomized patients, $n=88$

	Treatment group			
	monotherapy, $n=44$	triple therapy, $n=44$	total, $n=88$	*P* value
Age (years)	44.7 (41.5–52)	45 (42.1–50.8)	44.8 (41.8–51.1)	NS
Male sex	26 (59.1)	33 (75)	59 (67)	NS
Mode of HIV transmission				
men who have sex with men	8 (18.2)	17 (38.6)	25 (28.4)	0.033
heterosexual	22 (50)	14 (31.8)	36 (40.9)	0.083
injection drug user	11 (25)	15 (34)	26 (29.5)	NS
other	3 (6.8)	2 (4.5)	5 (5.7)	NS
Duration of HIV infection (years)	11 (8.7–13.7)	11.8 (10.5–16.7)	11.4 (9.3–15)	0.09
CDC category AIDS	26 (59.1)	20 (45.5)	46 (52.3)	NS
Body mass index (kg/m^2)	24 (20.9–25.8)	23.3 (21–25)	23.5 (21–25)	NS
Waist circumference (cm)	83.5 (75.9–91.3)	86 (80–91.5)	85 (78–91.5)	NS
Hip circumference (cm)	91 (87–95.5)	89.5 (86–96)	90 (86–96)	NS
HCV+ antibody	16 (36.4)	22 (50)	38 (43.2)	NS
Nadir CD4+ (cells/mm^3)	189 (40–298)	233 (128–285)	222 (104–291)	NS
Total CD4+ (cells/mm^3)	675 (512–885)	766 (543–1002)	697 (524–946)	NS
Total body fat (kg)	12.9 (8.9–16.5)	9.8 (7.4–15.4)	11.6 (8–15.7)	NS
Trunk fat (kg)	8.5 (5.6–11.1)	8.2 (5.1–10.8)	8.4 (5.4–10.8)	NS
Limb fat (kg)	2.5 (1.9–5.3)	2.5 (1.6–3.6)	2.5 (1.7–4.2)	NS
Fat mass ratio	1.9 (1.4–2.9)	2.1 (1.6–3.1)	2.0 (1.5–3.1)	NS
Total cholesterol (mg/dL)	247.5 (195–268)	214.2 (179.5–263)	224 (187–268)	0.075
HDL cholesterol (mg/dL)	43.8 (38–58.5)	40 (36–49.5)	41.5 (37–53)	NS
LDL cholesterol (mg/dL)	136 (98–167)	112 (86–142)	120 (94–160)	NS
Triglycerides (mg/dL)	270 (156–398)	246 (134–342)	254 (151–378)	NS
Total/HDL cholesterol ratio	5.3 (4.1–6.7)	5.2 (3.8–6.3)	5.2 (3.9–6.5)	NS
Time on zidovudine (years)	6.7 (5.2–7.9)	8.5 (6.4–11.3)	7.4 (5.4–9.2)	0.003
Time on stavudine (years)	2.9 (2.1–4)	3.7 (1.6–5)	3.1 (1.9–4)	NS
Time on thymidine analogues (years)	7.9 (5.5–10.3)	10.5 (7.7–11.7)	9.4 (6.3–11)	0.006
Patients included by DEXA centre				
Hologic	24 (54.5)	24 (54.5)		NS
Lunar	10 (22.7)	10 (22.7)		NS
Norland	10 (22.7)	10 (22.7)		NS

HCV, hepatitis C virus; NS, not significant.
Values are expressed as the median (IQR) for continuous variables and *n* (%) for categorical variables.

between the monotherapy and triple therapy groups by week 96 was 57.35 g (95% CI −625, 739.7; $P=0.867$) (Table 2). There were no statistically significant differences in total fat, trunk fat and fat mass ratio between treatment groups. Intra-group analysis showed that mean absolute increases in limb fat were not statistically significant either in the monotherapy group or in the triple therapy group at both 48 and 96 weeks.

From baseline to 48 weeks limb fat increased by 24.7% and 10% in the triple and monotherapy groups, respectively. At 96 weeks limb fat percentage had increased by 30% and 15.7% in the triple and monotherapy groups, respectively. Although the intra-group increase in limb fat percentage was statistically significant in the triple group at both 48 and 96 weeks, differences between treatment groups were not (Table 2).

Due to the great variability in limb fat change in the study population, we also evaluated if there was any difference in the percentage of patients gaining or losing limb fat at different cut-offs (<10%, 10%–20% or >30%). There were no differences between groups in the distribution of limb fat percentage change at 48 and 96 weeks (data not shown).

Due to the higher than expected discontinuation rate, two different sensitivity analyses were performed. Patients who discontinued between weeks 24 and 48 and had a DEXA scan at the time of discontinuation were included in the primary endpoint analysis ($n=78$). There was no statistically significant difference between treatment groups (triple therapy arm minus monotherapy arm) regarding absolute change in limb fat mass at 48 weeks (mean difference 34 g; 95% CI −493, 561). For the secondary analysis of relative change in limb fat mass (fat loss or gain <10%, 10%–20% or >30%), missing values were substituted either for the best or the worst relative percentage change. Again, there was no statistically significant difference between treatment groups (data not shown).

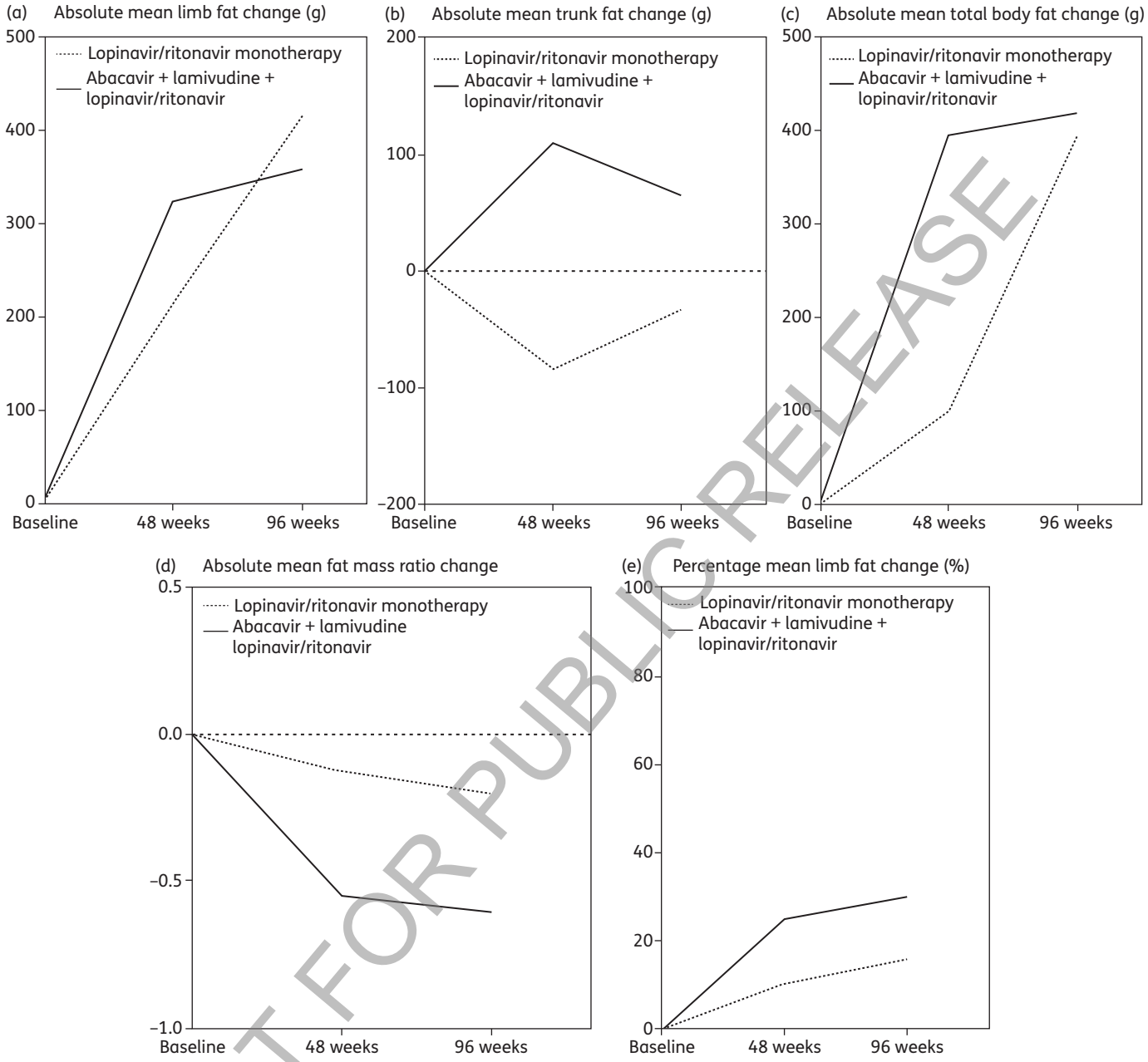

Figure 2. Absolute mean changes in (a) limb fat, (b) trunk fat, (c) total body fat and (d) fat mass ratio and (e) percentage change in limb fat mass.

Lipids and other laboratory markers

Substantial increases were observed in total cholesterol, LDL cholesterol, triglycerides and total cholesterol/HDL cholesterol ratio during the 4 week run-in period (Table 3). After randomization there was a statistically significant increase in HDL cholesterol in the monotherapy group at both 48 weeks [5 mg/dL (95% CI 1.7, 8.1); $P=0.03$] and 96 weeks [6.6 mg/dL (95% CI 2.8, 10.2); $P=0.01$]. Total cholesterol/HDL cholesterol ratio was also reduced in the monotherapy group at 48 weeks [−0.53 (95% CI −0.85, −0.21); $P=0.002$] and 96 weeks [−0.6 (95% CI −1.02, −0.17); $P=0.007$]. There was also a statistically significant reduction in triglycerides in the monotherapy group at week 48 [−74 mg/dL (95% CI −119.5, −28.6); $P=0.002$], but this difference was not significant at 96 weeks. Excluding the run-in period, there were no statistically significant intra-group changes in the triple therapy group comparing 48/96 week data and data at the time of randomization.

Regarding differences between treatment groups, at week 48 there was a statistically significant reduction in triglycerides [mean difference −61 mg/dL (95% CI −121.5, 0.57); $P=0.048$] and in the total cholesterol/HDL cholesterol ratio [mean difference −0.81 (95% CI −1.38, 0.23); $P=0.006$] in favour of the monotherapy group. Differences in lipids between treatment groups were

Table 2. Change in body composition and difference between treatment arms

	48 weeks				96 weeks			
	mean [median (IQR)] change				mean [median (IQR)] change			
Measurement	monotherapy	triple therapy	mean (95% CI) difference between arms[a]	*P* value	monotherapy	triple therapy	mean (95% CI) difference between arms[a]	*P* value
Total fat (g)	98.5 [80 (−1823, 1624)]	395 [−309 (−1593, 1538)]	−296.12 (−2032, 1440)	0.73	394 [33 (−2500, 2559)]	419 [−246 (−2489, 1668)]	−25 (−1961, 1911)	0.98
Total fat (%)	9.7 [0.6 (−16, 13)]	10.2 [−2.5 (−12, 16)]	−0.46 (−26.8, 25.8)	0.97	11.9 [0.5 (−17, 19)]	12 [−2 (−15, 16)]	−0.18 (−30.5, 30.1)	0.99
Limb fat (g)	215 [84 (−308, 596)]	324.2 [190 (−407, 816)]	−109.27 (−660.32, 441.7)	0.69	415.8 [−39 (−446, 622)]	358.5 [139 (−415, 883)]	57.35 (−625, 739.7)	0.86
Limb fat (%)[b]	10 [2.5 (−11, 19.5)]	24.7 [5.8 (−14, 33.5)]	−14.76 (−40.2, 10.67)	0.25	15.7 [−0.8 (−14, 21)]	30 [5.7 (−12, 41)]	−14.4 (−47, 18.21)	0.38
Trunk fat (g)	−84 [−29 (−1553, 1160)]	110 [−8 (−1470, 1062)]	−194 (−1425.2, 1037)	0.75	−33.4 [242 (−1904, 1681)]	64.5 [23 (−1632, 851)]	−97.9 (−1379.6, 1183.8)	0.88
Trunk fat (%)	13.7 [−0.2 (−16.5, 13)]	9 [−0.1 (−14, 16)]	4.7 (−29.7, 39.14)	0.785	13.6 [1.5 (−20, 18)]	9 [0.5 (−17, 16)]	4.58 (−31.6, 40.7)	0.80
Fat mass ratio[c]	−0.1 [0 (−0.3, 0.1)]	−0.5 [−0.1 (−0.5, 0.1)]	−0.42 (−1.12, 0.28)	0.46	−0.2 [−0.1 (−0.5, 0)]	−0.6 [−0.1 (−0.5, 0)]	−0.4 (−1.04, 0.23)	0.20

[a]Mean value of lopinavir/ritonavir minus abacavir plus lamivudine plus lopinavir/ritonavir.
[b]Statistically significant intra-group difference in limb fat (%) in the triple therapy arm at 48 and 96 weeks: 48 weeks, 24.7 (95% CI 4.1, 45.4); $P=0.02$; and 96 weeks, 30.1 (95% CI 6.4, 53.7); $P=0.014$.
[c]Statistically significant intra-group difference in fat mass ratio in the monotherapy arm at 96 weeks: −0.2 (95% CI −0.37, −0.02); $P=0.026$.

Table 3. Differences in lipid profile during the trial

		Mean (SD) change from baseline[a] to 48 weeks				Mean (SD) change from baseline[a] to 96 weeks			
	Mean (SD) change during run-in period, *n*=87	monotherapy, *n*=44	triple therapy, *n*=43	Mean (95% CI) difference between groups[b]	*P* value	monotherapy arm, *n*=44	triple therapy, *n*=43	Mean (95% CI) difference between groups[b]	*P* value
Total cholesterol (mg/dL)	44 (38)	−7 (36)	10.2 (46)	−17.1 (−34.7, 049)	0.057	−8.7 (38.8)	4.7 (44)	−13.37 (−31, 4.3)	0.136
HDL cholesterol (mg/dL)[c]	−1.9 (9.4)	5 (10.5)	−0.6 (19.2)	5.6 (−1.02, 12.28)	0.096	6.6 (12.1)	2.7 (23.2)	3.8 (−4.1, 11.8)	0.342
LDL cholesterol (mg/dL)	19.4 (31.6)	0.3 (30.4)	16.6 (41)	−16.3 (−32.7, 0.11)	0.052	−3.4 (28.6)	8.2 (44)	−11.6 (−28.6, 5.4)	0.178
Triglycerides (mg/dL)[d]	151.6 (202)	−74 (149)	−13 (129)	−61 (−121.5, 0.57)	0.048	−45.6 (261)	−27.6 (172.7)	−18 (−114.2, 78.1)	0.711
Total/HDL cholesterol ratio[c]	1 (1.1)	−0.5 (1.1)	0.3 (1.5)	−0.81 (−1.38, 0.23)	0.006	−0.6 (1.4)	0.0 (2)	−0.62 (−1.37, 0.13)	0.104

[a]Baseline (end of run-in period).
[b]Mean value of lopinavir/ritonavir minus abacavir plus lamivudine plus lopinavir/ritonavir.
[c]Statistically significant intra-group difference in HDL cholesterol and total/HDL cholesterol in the monotherapy arm at 48 and 96 weeks. HDL cholesterol: 48 weeks, 5 (95% CI 1.7, 8.1); $P=0.03$; and 96 weeks, 6.5 (95% CI 2.8, 10.2); $P=0.01$. Total/HDL cholesterol: 48 weeks, −0.53 (95% CI −0.85, −0.21); $P=0.002$; and 96 weeks, −0.6 (95% CI −1.02, −0.17); $P=0.007$.
[d]Statistically significant intra-group difference in triglycerides in the monotherapy arm at 48 weeks: −74 (95% CI −119.5, −28.6); $P=0.002$.

not statistically significant at 96 weeks. Lipid-lowering therapy with either statins or fibrates was started during the trial in 11 patients (25%) in the monotherapy group and in 13 patients (29.5%) in the triple therapy group.

Virological and safety results

At week 48 the proportion of patients with viral load <50 copies/mL in the PP population was 88.2% in the monotherapy group and 97.5% in the triple therapy group. At 96 weeks there were three virological failures (confirmed HIV RNA >400 copies/mL), two in the monotherapy group and one in the triple therapy group. A single major PI mutation (82A) was detected after virological failure in one patient in the monotherapy group. Four patients in the monotherapy group were successfully re-induced with nucleosides. No significant changes were observed in CD4 count during the trial.

Thirty-four patients in the monotherapy group and 40 in the triple therapy group completed 48 weeks of follow-up, and 31 in the monotherapy and 38 in the triple therapy group completed the 96 week visit. Most of the discontinuations occurred before 24 weeks and were due to drug-related adverse events (Figure 1). Among those who discontinued, the median time to discontinuation was 32 weeks in the monotherapy group compared with 22 weeks in the triple therapy group. Adverse events were statistically significantly more frequent in the monotherapy group [42 (95%) versus 35 (79.5%); $P=0.024$]. Discontinuations due to adverse events were more frequent in the monotherapy group [11 (25%) versus 4 (9%); $P=0.047$], although there were no differences in either serious adverse events [8 (18.2%) versus 5 (11.4%); $P=0.367$] or drug-related adverse events [30 (68.2%) versus 30 (68.2%); $P=1$].

Discussion

In our study, switching to either lopinavir/ritonavir plus abacavir/lamivudine or lopinavir/ritonavir monotherapy led to similar small limb fat gains after 2 years of follow-up in patients with severe lipoatrophy. Nevertheless, we did not find any statistically significant difference in absolute limb fat gain between the two strategies.

Our main result differs from those of other clinical trials, which had demonstrated a partial but significant recovery of lipoatrophy after tNRTIs were switched to either abacavir or tenofovir.[19–21] Limb fat recovery in those trials after 2 years of follow-up has been estimated as 400–1000 g.[19,28]

Other NRTI-sparing strategies have obtained different results. In ACTG 5125, 62 patients were randomized to two nonthymidine analogues with efavirenz or to lopinavir/ritonavir plus efavirenz.[22] After a median of 102 weeks, the NRTI-sparing group gained a median of 782 g of limb fat. Another study in lipoatrophic HIV-infected patients showed that thigh fat volume increased by 12% and 30% at 48 and 96 weeks, respectively, after switching to a regimen with an NNRTI plus a PI.[24]

The role of PI monotherapy in limb fat recovery has also been evaluated both in antiretroviral-naive patients and as a switching strategy in patients with suppressed viral replication. A substudy of the MONARK trial showed that, in antiretroviral-naive HIV-infected patients, median limb fat loss was lower in the lopinavir/ritonavir monotherapy group than in the triple combination group of lopinavir/ritonavir plus zidovudine/lamivudine.[29] In the MONOI trial, which explored darunavir/ritonavir monotherapy in patients with viral suppression, there was a mean increase of 340 g of limb fat in the darunavir/ritonavir monotherapy group contrasting with a decrease of 20 g in the triple therapy group at 48 weeks. The difference was statistically significant at 48 weeks but not at week 96.[30] In another simplification study, the KALESOLO trial, switching to lopinavir/ritonavir monotherapy was associated with a median increase of 160 g at 48 weeks.[31]

The limb fat increase found in the GESIDA-6008-KRETA trial is lower than previously reported increases, although it is consistent with more recent trials, such as MONOI, in which the monotherapy group obtained a median limb fat gain of 330 g at 96 weeks. Percentage increases in limb fat in those studies in which this variable was studied were ~30% at 96 weeks.[19,24] Relative limb fat gain in the KRETA study was higher than that in the MONOI trial at 96 weeks in monotherapy groups (15.7% versus 8.4%). Changes in visceral fat could explain the difference between absolute and relative limb fat gain, although this point cannot be confirmed because visceral fat CT scans were not performed in our study. Another possible explanation for the diversity of limb fat gain obtained in previous boosted PI monotherapy studies could be the different use of tNRTI in the control arm. This varied from 100% in the MONARK and Abbott-613 trials to 38% in the KALESOLO trial and 32.6% in the MONOI trial.

One intriguing fact is the more rapid gain of limb fat in the triple therapy group at 48 weeks followed by a tapering off at 96 weeks. This could be just a consequence of inter-subject variability amplified by other factors, such as sample size and discontinuation rate.

A number of factors might help to explain our results. Patients included in the GESIDA-6008-KRETA study had severe lipoatrophy at baseline (median limb fat was only 2.5 kg) and the longest prior exposure to tNRTI (median 9.4 years) of published studies. As an example, in the RAVE study median baseline limb fat was 3000 g and in the SWEET study median exposure to zidovudine was 3 years.[20,21] In the SWEET trial, the lowest limb fat recovery was reported in patients with ≥3 years of treatment with zidovudine, suggesting that long-term use of zidovudine may lead to a quasi-irreversible loss of limb fat. Our results confirm this point.

It is important to point out that zidovudine was included in the control regimen in those studies reporting a higher limb fat recovery in patients treated with PI monotherapy.

Switching to either lopinavir/ritonavir with abacavir/lamivudine or lopinavir/ritonavir monotherapy did not result in a benign lipid profile in this population with severe lipoatrophy. During the 1 month run-in period total cholesterol and triglycerides increased by 44 and 152 mg/dL, respectively. In a previous tNRTI substitution study there was an improvement in lipids that was greater with tenofovir than with abacavir.[20] The less favourable profile in our study could be related to long-term use of tNRTI, severe lipoatrophy and accumulated mitochondrial toxicity. After randomization, the lopinavir/ritonavir monotherapy group had a more favourable lipid profile, especially in total/HDL cholesterol ratio at 48 weeks, but this difference was no longer significant at 96 weeks.

Our study has several limitations. First, DEXA scans were not centrally read and three different systems were used. This limitation could be partially compensated by the fact that each patient

acted as its own control and by DEXA centre stratification. Although randomization was also stratified by exposure to zidovudine, some baseline characteristics, such as median time on tNRTIs, were misbalanced, probably due to the limited sample size. Another limitation is the unexpectedly high discontinuation rate, which compromised the study's power to detect differences in limb fat recovery. We had calculated a 10% drop-out rate but the discontinuation rate was doubled in the monotherapy group.

In conclusion, the GESIDA-6008-KRETA study provides evidence that in severe lipoatrophic patients treated with zidovudine/abacavir/lamivudine, with a long history of tNRTI use, switching to lopinavir/ritonavir monotherapy had no additional benefit in limb fat recovery relative to switching to abacavir/lamivudine plus lopinavir/ritonavir. Our data suggest that non-thymidine nucleosides such as abacavir plus lamivudine are not an obstacle for limb fat recovery.

Acknowledgements

This study would not have been possible without the collaboration of all the patients, medical and nursing staff, and data managers who have taken part in the project. Special thanks to Herminia Esteban from the SEIMC-GESIDA Foundation on behalf of the coordinating centre staff.

Members of the GESIDA-6008-KRETA Study Group

José I. Bernardino, José R. Arribas, Juan González-García, Jose M. Peña, Mª Luisa Montes, Juan Miguel Castro and Rocío Campos (Hospital Universitario La Paz, Madrid), Federico Pulido, Rafael Rubio, Jara Llenas, Silvana Fiorante, Mariano Matarranz and Natalia Ortiz (Hospital Universitario 12 de Octubre, Madrid), Rafael Torres, Juan José Jusdado, Miguel Cervero and José Luis Agud (Hospital Severo Ochoa, Leganes), Antonio Rivero, Julian de la Torre-Cisneros, Jose M. Kindelán, Rafael Jurado, Milagros García-Lázaro and Angela Camacho (Hospital Provincial, Córdoba), Esteban Martinez, Laura Zamora, Mariano Larrousse, Montserrat Loncá and Jose Maria Gatell (Hospital Clinic, Institut d'Investigacions Biomediques August Pi i Sunyer, Barcelona), Pere Domingo, Mª Antonia Sambeat, Maria del Mar Gutierrez and Gracia Maria Mateo (Hospital de la Santa Creu i San Pau, Barcelona), Josefa Muñoz, Oscar Ferrero, Sofía Ibarra, Zurine Zubero, Juan Miguel Santamaría, Nekane Juaristi and Josu Baraiaetxaburu (Hospital de Basurto, Bilbao), Julio Arrizabalaga, Miguel Angel Goneaga, Francisco Rodriguez-Arrondo, Miguel A. Von Wichmann, Jose A. Iribarren, M. J. Bustinduy, Maialen Ibarguren and Ioana Riaño (Hospital Donostia, San Sebastian), Antonio Ocampo, Alfredo Rodriguez Da Silva, Maite Perez and Celia Miralles (Complexo Hospitalario Universitario de Vigo) and Joaquín Portilla and Livia Giner (Hospital General de Alicante, Alicante).

Funding

This work was supported by an unrestricted grant from Abbott Laboratories. The funding source had no role in the study design, data collection, analysis and interpretation of the data, preparation of the manuscript or the decision to submit the manuscript for publication.

Transparency declarations

J. I. B. has received research funding, consultancy fees or lecture sponsorships from Abbott, Gilead Sciences, Bristol-Myers-Squibb, ViiV Healthcare and Janssen. F. P. has received advisory fees, speaker fees and grant support from ViiV Healthcare, Janssen, Abbott, Bristol-Myers Squibb, Gilead and Merck Sharp & Dohme. E. M. has received research funding, consultancy fees or lecture sponsorships from or served on advisory boards for Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Ferrer Group, Gilead Sciences, GlaxoSmithKline, Merck Sharp & Dohme, Theratechnologies, Tibotec and ViiV Healthcare. J. A. has received advisory fees, speaker fees and grant support from Bristol-Myers Squibb, Gilead, Roche and GlaxoSmithKline. P. D. has received advisory fees, speaker fees and grant support from ViiV Healthcare, Janssen, Abbott, Bristol-Myers Squibb, Gilead, Merck Sharp & Dohme, Boehringer Ingelheim, Ferrer International and Theratechnologies. J. P. has received advisory fees, speaker fees and grant support from ViiV Healthcare, Janssen, Abbott, Bristol-Myers Squibb, Gilead, Merck Sharp & Dohme and Roche. A. O. has received consultancy fees or lecture sponsorship from or served on advisory boards for Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Merck Sharp & Dohme, Janssen and ViiV Healthcare. J. M. has received advisory fees, speaker fees and grant support from ViiV Healthcare, Janssen, Abbott, Bristol-Myers Squibb, Gilead, Merck Sharp & Dohme and Boehringer-Ingelheim. J. R. A. has received advisory fees, speaker fees and grant support from ViiV Healthcare, Tibotec, Janssen, Abbott, Bristol-Myers Squibb, Gilead and Merck Sharp & Dohme. R. T. has none to declare.

Author contributions

J. I. B., F. P., E. M. and J. R. A. contributed equally to the study design, data analysis, data interpretation and writing of the report. J. I. B., F. P., E. M., J. A., P. D., J. P., A. O., J. M., R. T. and J. R. A. contributed to the collection of data representing the most active centres. All authors reviewed and approved the final version of the article. J. I. B. had full access to all the data and had the final responsibility for the decision to submit this report for publication.

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