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Background. Perinatal treatment with lopinavir boosted by ritonavir (LPV/r) is associated with steroidogenic abnormalities. Long-term effects in infants have not been studied.

Methods. Adrenal-hormone profiles were compared at weeks 6 and 26 between human immunodeficiency virus (HIV)-1–exposed but uninfected infants randomly assigned at 7 days of life to prophylaxis with LPV/r or lamivudine (3TC) to prevent transmission during breastfeeding. LPV/r in vitro effect on steroidogenesis was assessed in H295R cells.

Results. At week 6, 159 frozen plasma samples from Burkina Faso and South Africa were assessed (LPV/r group: n = 92; 3TC group: n = 67) and at week 26, 95 samples from Burkina Faso (LPV/r group: n = 47; 3TC group: n = 48). At week 6, LPV/r-treated infants had a higher median dehydroepiandrosterone (DHEA) level than infants from the 3TC arm: 3.91 versus 1.48 ng/mL ($P < .001$). Higher DHEA levels (>5 ng/mL) at week 6 were associated with higher 17-OH-pregnenolone (7.78 vs 3.71 ng/mL, $P = .0004$) and lower testosterone (0.05 vs 1.34 ng/mL, $P = .009$) levels in LPV/r-exposed children. There was a significant correlation between the DHEA and LPV/r AUC levels ($\rho = 0.40$, $P = .019$) and C_{trough} ($\rho = 0.40$, $P = .017$). At week 26, DHEA levels remained higher in the LPV/r arm: 0.45 versus 0.13 ng/mL ($P = .002$). Lopinavir, but not ritonavir, inhibited CYP17A1 and CYP21A2 activity in H295R cells.

Conclusions. Lopinavir was associated with dose-dependent adrenal dysfunction in infants. The impact of long-term exposure and potential clinical consequences require evaluation.

Keywords. HIV infant prophylaxis; lopinavir; adrenal function impairment; CYP21 and CYP17 inhibition.

The overall safety of antiretrovirals (ARVs) in children living with human immunodeficiency virus (HIV) is close to, or better than, that observed in adults [1–8], in whom aging and certain cofactors may aggravate the impact of treatment [9–11]. This is not necessarily the case for newborns and infants, for whom the immaturity of certain enzymatic processes is likely to modify the metabolism of drugs and their tolerance [12–15]. This applies to indirect exposure to maternal treatment both in utero and through breastfeeding and direct exposure, such as postnatal prophylaxis given to the newborn, as well as treatment of infants with HIV, for whom it is now recommended to initiate treatment as early as possible [12, 16–18]. The evaluation of the tolerance to drugs initiated in the perinatal period is not straightforward, as ARV treatment is given as a combination of several molecules of different classes [19] and the identification and statistical interpretation of the incidence of rare clinical events require large trials or cohorts. A complementary approach consists of studying the "biological signature" induced by a molecule with respect to its molecular structure and potential biological interference of normal processes. Such a signature does not necessarily predict the occurrence of overt clinical toxicity but raises attention to its potential risk.

We previously identified abnormalities of steroidogenesis in newborns exposed to pre- and postnatal ritonavir-boosted lopinavir (LPV/r) [20]. LPV/r-induced adrenal disturbances have also been observed in adolescents [21] and pregnant women treated with LPV/r [22].

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The large randomized Agence Nationale de Recherches sur le SIDA et des hépatites virales (ANRS) 12174 trial [23] compared 2 extended, single-drug postnatal prophylaxis regimens consisting of either LPV/r or lamivudine (3TC) in HIV-1–exposed, breastfed infants without HIV. It offered a unique opportunity to retrospectively evaluate the adrenal hormonal profile, without interference of either HIV infection or other drugs used in combination. In addition, we separately assessed the effect of both lopinavir and ritonavir on 3 adrenal enzymes in a cultured adrenal-cell model of steroidogenesis.

METHODS

Study Population

The ANRS 12174 trial is described in detail elsewhere [23]. Briefly, it was a multisite, randomized, double-blind, controlled trial conducted in 1273 mother–infant pairs in Burkina Faso, South Africa, Uganda, and Zambia between December 2009 and February 2013 (clinical trials.gov NCT00640263). The trial enrolled HIV-exposed newborns without HIV of asymptomatic mothers who were not eligible for antiretroviral therapy, according to World Health Organization recommendations at the time of the trial (ie, CD4 blood count >350 cells/μL). The mothers did not receive any ARVs during the trial but could have received ARVs during pregnancy. Newborns received 7 days of nevirapine from birth to inclusion at day 7 of life, according to national guidelines at that time. Participants with a negative HIV-1 DNA polymerase chain reaction at day 7 were randomly assigned at a 1:1 ratio to LPV/r or 3TC. The regimen was maintained until the end of breastfeeding plus 1 week for a maximum duration of 50 weeks. The randomization was stratified by site.

The present secondary outcome evaluation (Supplementary Figure 1) was performed on frozen samples collected in 2 of the 4 sites: Ouagadougou University Teaching Hospital, Burkina Faso, and the East London Hospital Complex, South Africa. Children with at least 1 frozen sample available at week 6 and/or week 26 were included in this substudy. All of the children included were still receiving the assigned treatment at week 26. Samples from South Africa at week 26 were not available, especially because only 48.8% of the South African children were still exclusively breastfed at week 22 [24]. Plasma levels of lopinavir and 3TC available at week 6 in South Africa for 29 infants were also analyzed [23].

The study protocol was approved by the National Ethics Committee for Health Research in Burkina Faso, the Medicines Control Council in South Africa, and the Regional Committee for Medical and Health Ethics in Norway. All mothers signed a written informed consent.

Hormonal Assays

Plasma steroid concentrations were measured at the Steroid Research and Mass Spectrometry Unit, Center of Child and Adolescent Medicine, Justus Liebig University, Germany, by gas chromatography–mass spectrometry [25, 26], as previously described (see Supplementary Methods). The samples were transported frozen and maintained at −20°C before dosage.

In Vitro Studies of the Effect of the Drugs on Steroidogenesis

The impact of lopinavir and ritonavir on adrenal steroidogenesis was tested using a well-established steroid-producing adrenocortical cell line, NCI-H295R cells (American Type Culture Collection CRL2128). Steroidogenesis was studied using radiolabeled substrates, for which the metabolism by the cells was revealed by thin-layer chromatography [27]. This approach allowed testing of the activities of 3β-hydroxysteroid dehydrogenase (HSD3B2), cytochrome P450 (CYP)17A1, and CYP21A2, and their potential inhibition separately (see Supplementary Methods Appendix). Various concentrations of the drugs were tested: from low concentrations of 0 to 8 μg/mL (0–13 μM) to a high concentration of 20 μg/mL (31 μM) for lopinavir and from 0 to 0.5 μg/mL (0–0.7 μM) for ritonavir. These concentrations were chosen based on the fact that the LPV/r combination inhibits CYP3A at 1.1 μM (half maximal inhibitory concentration [IC_{50}]) in vivo and ritonavir alone at 0.14 μM (IC_{50}) in liver microsomes [28] and that the median LPV trough concentrations at weeks 6 and 26 in this cohort were 2.8 and 5.6 mg/L, respectively [29].

Statistical Analysis

The data are presented as medians with interquartile ranges (IQRs) for continuous variables and as frequencies with percentages for categorical variables. The Pearson's chi-square test or the Fisher's exact test, when appropriate, was used for comparisons of categorical variables and the Wilcoxon Mann–Whitney test for continuous variables. For comparisons of hormone levels, the continuous variable was replaced by a categorical variable if more than 25% of values of the hormone level were under the limit of quantification (LoQ), without evidence of poor chromatography quality. This categorical variable took on the following values: less than the LoQ and LoQ or greater. Comparisons between treatment groups were corrected for multiple testing by the Benjamini-Hochberg method of the false discovery rate family methods.

Results for plasma DHEA levels were compared with normal values based on age and sex [30]. A multivariable logistic regression model was used to evaluate the association between high DHEA plasma levels (greater than or equal to the upper limit of normal [ULN]) and treatment group adjusted for sex and site. The interaction between treatment group and the other factors was evaluated using likelihood ratio tests.

The nonparametric correlation test of Spearman (ρ) was used for the correlation analysis between hormone and drug levels. Enzyme activities were compared using Student's *t* test. The data were analyzed using SAS version 9.4 (SAS Institute, Inc).

Table 1. Characteristics of the Patients and Their Mothers at Inclusion at Day 7 After Birth

	All (n = 161)	LPV/r (n = 93)	3TC (n = 68)	*P*
South Africa, n (%)	63 (39)	43 (46)	20 (29)	.031
Girls, n (%)	64 (40)	39 (42)	25 (37)	.51
Infant birth weight, kg	3.0 [2.7–3.3]	3.0 [2.7–3.3]	3.0 [2.6–3.3]	.69
Maternal age, years	27.9 [24.1–32.8]	27.5 [25.0–33.0]	28.0 [23.0–32.5]	.40
Parity, n	2 [2–3]	2 [2–3]	2 [2–4]	.32
Gestational age, weeks	27.9 [24.1–32.8]	27.5 [25.0–33.0]	28.0 [24.1–32.8]	.40
Predelivery CD4 count, cells/μL	519 [426–647]	536 [421–648]	503 [434; 647]	.79
Undetectable maternal plasma HIV-1 RNA viral load, n (%)	78 (48)	40 (43)	38 (56)	.11
Maternal plasma HIV-1 RNA,[a] log copies/mL	3.25 [2.92–3.67]	3.44 [2.99–3.87]	3.08 [2.77–3.51]	.026

Data are median [IQR] unless otherwise indicated. Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; LPV/r, lopinavir boosted by ritonavir; 3TC, lamivudine.
[a]Detectable viral loads (n = 53 in the LPV/r group and n = 40 in the 3TC group).

RESULTS

Among the 161 infants included in the substudy, 93 (58%) were in the LPV/r arm (54 boys and 39 girls) and 68 (42%) were in the 3TC arm (43 boys and 25 girls). The maternal and infant characteristics of the 2 treatment groups were comparable (Table 1). Among the 159 frozen samples available at week 6, 92 (58%) were from the LPV/r arm and 67 (42%) from the 3TC arm. At week 26, 95 frozen samples were available: 47 infants (49%) treated by LPV/r (27 boys and 20 girls) and 48 (51%) by 3TC (32 boys and 16 girls).

Adrenal Hormone Levels

At week 6 (Figure 1A; Table 2, part A), the median level of DHEA was 2.6-fold higher among infants receiving LPV/r than among those receiving 3TC (3.91 ng/mL [IQR, 2.11–8.48 ng/mL] vs 1.48 ng/mL [IQR, 0.46–3.40 ng/mL]; $P < .001$).

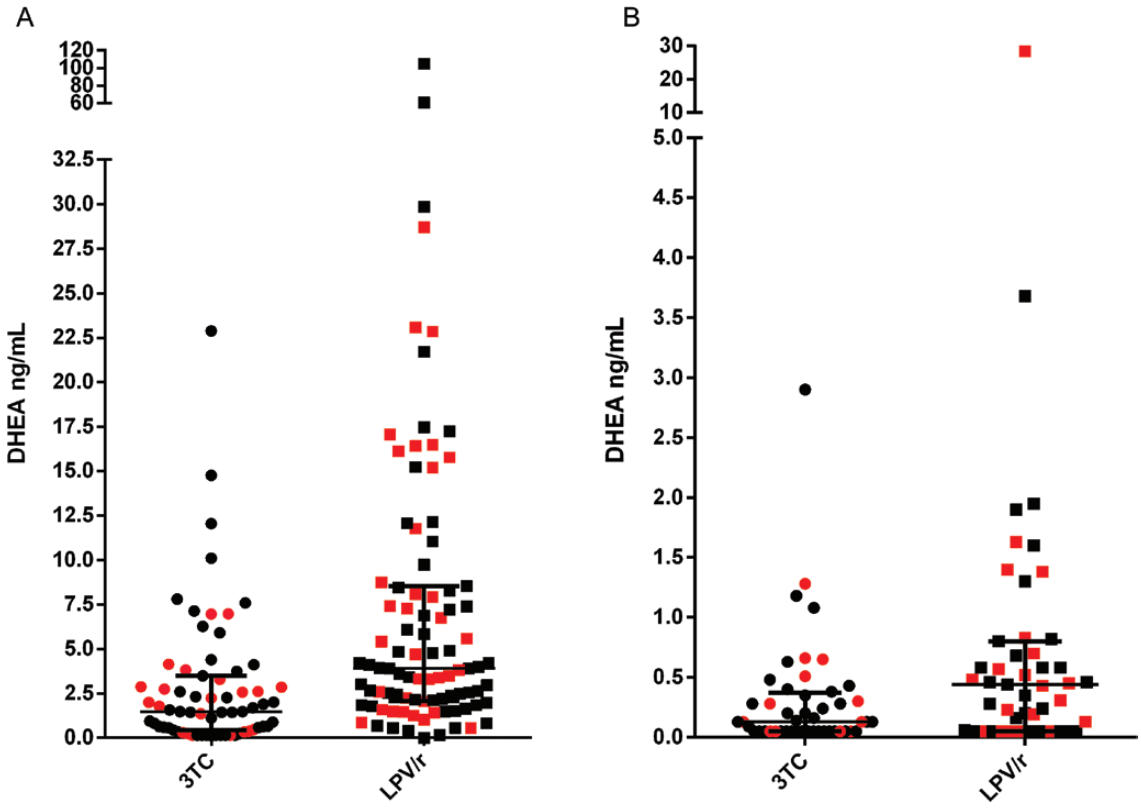

Figure 1. Distribution of plasma DHEA levels for 3TC- and LPV/r-treated patients at weeks 6 (*A*) and 26 (*B*). Red dots: girls; black dots: boys. Normal DHEA values at week 6: between 0.59 and 4.15 ng/mL for boys and 0.44 and 1.63 ng/mL for girls. Normal DHEA values at week 26: 0.19–2.11 ng/mL for boys and 0.56–2.70 ng/mL for girls. Abbreviations: DHEA, dehydroepiandrosterone; LPV/r, lopinavir boosted by ritonavir; 3TC, lamivudine.

Table 2. Plasma Hormone Levels at Weeks 6 and 26

	A. Burkina Faso and South Africa—Week 6 (Both Sexes)			B. Burkina Faso—Week 26 (Both Sexes)		
	LPV/r (n = 92)	3TC (n = 67)	P[a]	LPV/r (n = 47)	3TC (n = 48)	P[a]
DHEA levels, ng/mL	3.91 [2.09–8.50]	1.48 [0.44–3.50]	<.001	…	…	…
<0.3 ng/mL	2 (2)	12 (18)	.004	19 (40)	35 (73)	.005
≥ULN	51 (55)	23 (34)	.029	2	1	NA
≥3× ULN	25 (27%)	4 (6)	.004	1	0	NA
Other hormone levels						
DHT <0.3 ng/mL	48 (52)	38 (57)	.73	45 (96)	48 (100)	.30
T <0.3 ng/mL	39 (42)	26 (39)	.76	40 (85)	43 (90)	.71
4A <0.3 ng/mL	79 (86)	64 (96)	.13	47 (100)	48 (100)	NA
17-OHP <0.3 ng/mL	35 (38)	21 (31)	.59	37 (79)	40 (83)	.71
17-OHPRE, ng/mL	5.85 [2.68–9.91]	5.10 [2.79–10.68]	.99	1.25 [0.54–3.11]	1.16 [0.48–1.87]	.74
AD <0.15 ng/mL	76 (83)	60 (90)	.44	41 (87)	46 (96)	.30
S <0.3 ng/mL	32 (35)	20 (30)	.71	22 (47)	22 (46)	.92
B <3 ng/mL	55 (60)	45 (67)	.59	32 (68)	38 (79)	.37
Prog <1.5 ng/mL	91 (99)	66 (99)	.88	45 (96)	48 (100)	.30
F,[b] µg/dL	…	…	…	9.42 [5.76–12.98]	7.05 [4.38–10.18]	.10
F <4 µg/dL[b]	32 (35)	31 (46)	.33	…	…	…

Data are median [IQR] or n (%). Abbreviations: AD, androstenediol; B, 11-OH-corticosterone; DHEA, dehydroepiandrosterone; DHT, dihydro-testosterone; F, cortisol; IQR, interquartile range; LoQ, limit of quantification; LPV/r, lopinavir boosted by ritonavir; NA, not applicable; Prog, progesterone; S, 11-deoxycortisol; T, testosterone; ULN, upper limit of normal; 3TC, lamivudine; 4A, 4-androstenedione; 17-OHP, 17-OH-progesterone; 17-OHPRE, 17-OH-pregnenolone.

[a]Wilcoxon Mann–Whitney test or Pearson chi-square test corrected for multiple testing by Benjamini-Hochberg method.

[b]Data available for 91 children at week 26 (n = 45 in the LPV/r group and n = 46 in the 3TC group).

Moreover, 55% (n = 51) of children in the LPV/r group versus 34% (n = 23) in the 3TC group had DHEA levels at the ULN or higher ($P = .029$). Very high DHEA levels (≥3× ULN) were also more frequent in the LPV/r group (27%, n = 25) than in the 3TC group (6%, n = 4) ($P = .004$). Conversely, the proportion of infants with unquantifiable DHEA levels (under the LoQ) was lower in the LPV/r group than in the 3TC group (2% vs 18%, $P = .004$). High DHEA levels were independently associated with LPV/r group, girls, and South Africa site (Supplementary Table 1). No interaction between treatment group and sex or study site was identified (Supplementary Table 2).

The levels of dihydro-testosterone (DHT), testosterone, 4-androstenedione, 17-OH-progesterone (17-OHP), 17-OH-pregnenolone (17-OHPRE), androstenediol, 11-deoxycortisol, 11-OH-corticosterone, and cortisol were all within the normal range (data not shown). The proportion of children with unquantifiable values for these hormones was similar between the 2 arms at week 6 and week 26 (Table 2).

Among children receiving LPV/r at week 6 (Table 3), those with high plasma DHEA levels (≥3× ULN) had higher 17-OHPRE levels (8.99 ng/mL [IQR, 6.14–11.00 ng/mL] vs 4.19 ng/mL [IQR, 2.16–8.85 ng/mL]; $P = .004$) than children with plasma DHEA levels less than 3 times the ULN. The proportion of children with undetectable testosterone levels and undetectable DHT levels was higher in high DHEA group: 76% (n = 19) versus 30% (n = 20) ($P < .001$) and 76% (n = 19) versus 43% (n = 29) ($P = .010$), respectively.

A comparison of the hormonal levels depending on the DHEA level was not possible at week 26, as there was only 1 infant with a DHEA level 3 or more times the ULN (from the LPV/r-arm).

At 26 weeks (Figure 1B and Table 2, part B), the proportion of children with plasma DHEA levels under the LoQ remained lower in the LPV/r group than in the 3TC group (40% [n = 19] vs 73% [n = 35]; $P = .005$). Cortisol levels were also higher but within normal ranges among LPV/r-treated children (9.42 µg/

Table 3. Plasma Hormone Levels in the High-DHEA-Level and Low-DHEA-Level Patient Groups in Burkina Faso and South Africa in LPV/r-Treated Patients—Week 6 (Both Sexes)

	High-DHEA Group (DHEA ≥3× ULN) (n = 25)	Low-DHEA Group (DHEA <3× ULN) (n = 67)	P[a]
DHT <0.3 ng/mL	19 (76)	29 (43)	.010
T <0.3 ng/mL	19 (76)	20 (30)	<.001
4A <0.3 ng/mL	16 (64)	63 (94)	.001
17-OHP <0.3 ng/mL	12 (48)	23 (34)	.26
17-OHPRE, ng/mL	8.99 [6.14–11.00]	4.19 [2.16–8.85]	.010
AD <0.15 ng/mL	20 (80)	56 (84)	.69
S <0.3 ng/mL	3 (12)	29 (43)	.010
B <3 ng/mL	12 (48)	43 (64)	.20
Prog <1.5 ng/mL	24 (96)	67 (100)	.14
F <4 ng/mL	4 (16)	28 (42)	.035

Data are median [IQR] or n (%). Abbreviations: AD, androstenediol; B, 11-OH-corticosterone; DHEA, dehydroepiandrosterone; DHT, dihydro-testosterone; F, cortisol; IQR, interquartile range; LPV/r, lopinavir boosted by ritonavir; Prog, progesterone; S, 11-deoxycortisol; T, testosterone; ULN, upper limit of normal; 4A, 4-androstenedione; 17-OHP, 17-OH-progesterone; 17-OHPRE, 17-OH-pregnenolone.

[a]Wilcoxon Mann–Whitney test or Pearson chi-square test corrected for multiple testing by Benjamini-Hochberg method.

dL [IQR, 5.76–12.98 µg/dL] vs 7.05 µg/dL [IQR, 4.38–10.18 µg/dL]; $P = .10$).

Between week 6 and week 26, the DHEA levels of almost all patients in both groups decreased to normal values for their age. Three children had DHEA levels greater than the ULN range at week 26, 2 in the LPV/r group and 1 in the 3TC group (Table 2, part B, and Figure 2).

Drug Plasma Levels and Hormonal Disturbances

Among the 29 infants with available lopinavir and 3TC plasma levels at week 6 (Figure 3), there was a significant positive correlation between plasma DHEA levels and the LPV/r area under the curve (AUC) level ($\rho = 0.40$, $P = .019$) and residual concentration, C_{trough} ($\rho = 0.40$, $P = .017$). There was no correlation between DHEA and 3TC levels. There was also no correlation with the levels of any other steroids.

In Vitro Impact of Ritonavir and Lopinavir on Steroidogenesis

We then tested the effect of ritonavir and lopinavir separately on steroidogenesis in human adrenal H295R cells. Specifically, we assessed the potential inhibition of 3 enzymes involved in the production of DHEA, 17-OHPRE, and 17-OHP: HSD3B2 (converting pregnenolone to DHEA), CYP17A1 (converting pregnenolone to progesterone), and CYP21A2 (converting 17-OHP to 11-deoxycortisol). Lopinavir moderately inhibited CYP17A1 activity at the high concentration of 20 µg/mL (31 µM) (Figure 4A), whereas it significantly inhibited 21-hydroxylase (CYP21A2) activity at lower doses: 8 µg/mL (13 µM) (Figure 4B). In contrast, lopinavir had no effect on 3β-hydroxysteroid dehydrogenase (3βHSD2; HSD3B2) activity (Figure 4C). The effects were specific to lopinavir, as we found no such effects for ritonavir (Figure 4).

DISCUSSION

In this prospective, double-blind, randomized trial, we confirmed that LPV/r initiated at 7 days among HIV-1–exposed infants without HIV provokes early, dose-dependent disruption of the steroid hormone profile of some of these infants. The comparator was 3TC, a nucleoside analog without any known adrenal toxicity after 25 years of use, including in newborns. DHEA plasma levels at 6 and 26 weeks of treatment were elevated in approximately one-third of infants treated with LPV/r. DHEA levels physiologically decrease between the first and sixth months of life in infants. After 6 months, the adrenal glands can be considered to be mature [31]. This

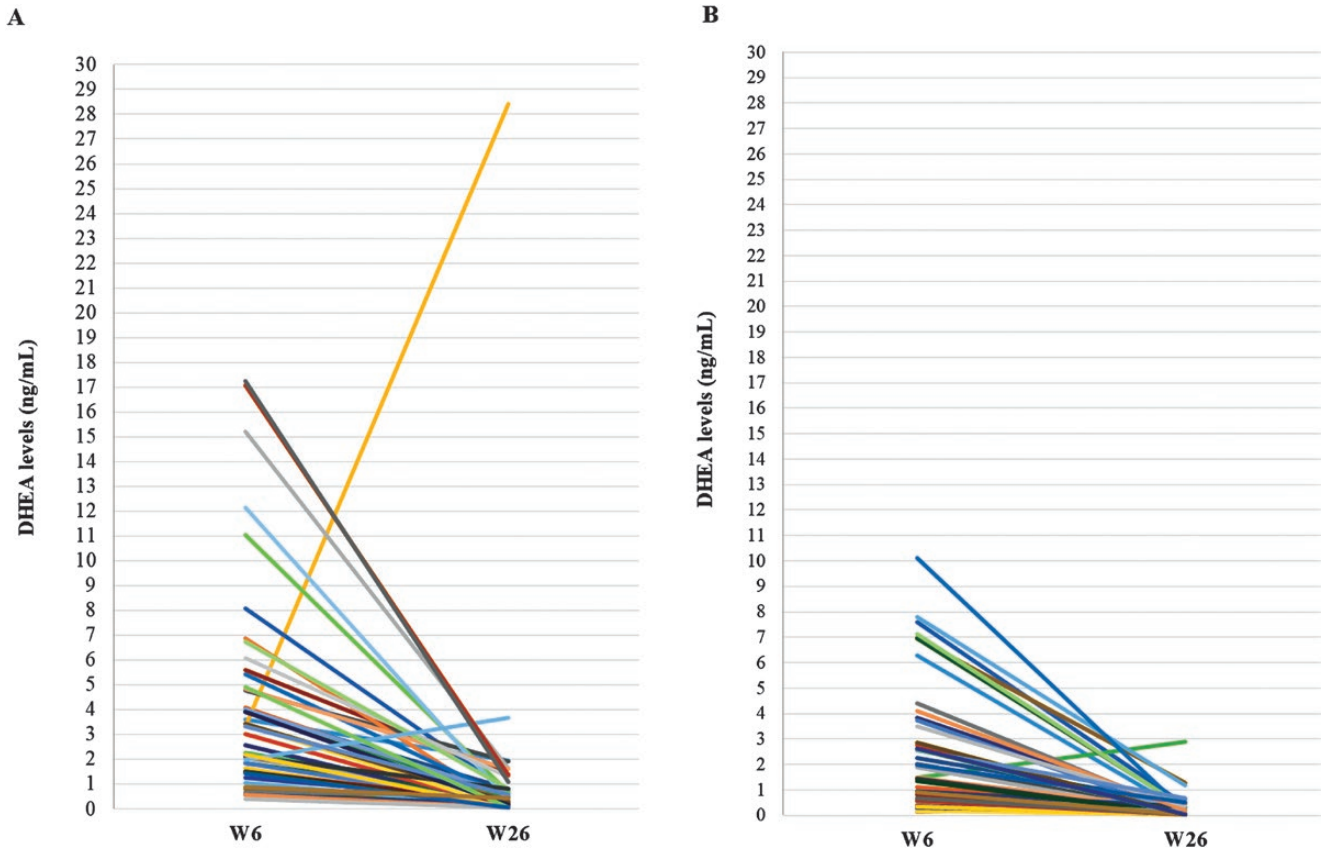

Figure 2. Evolution of DHEA levels for each patient between weeks 6 and 26 according to treatment group: LPV/r (*A*) and 3TC (*B*). Abbreviations: DHEA, dehydroepiandrosterone; LPV/r, lopinavir boosted by ritonavir; W, week; 3TC, lamivudine.

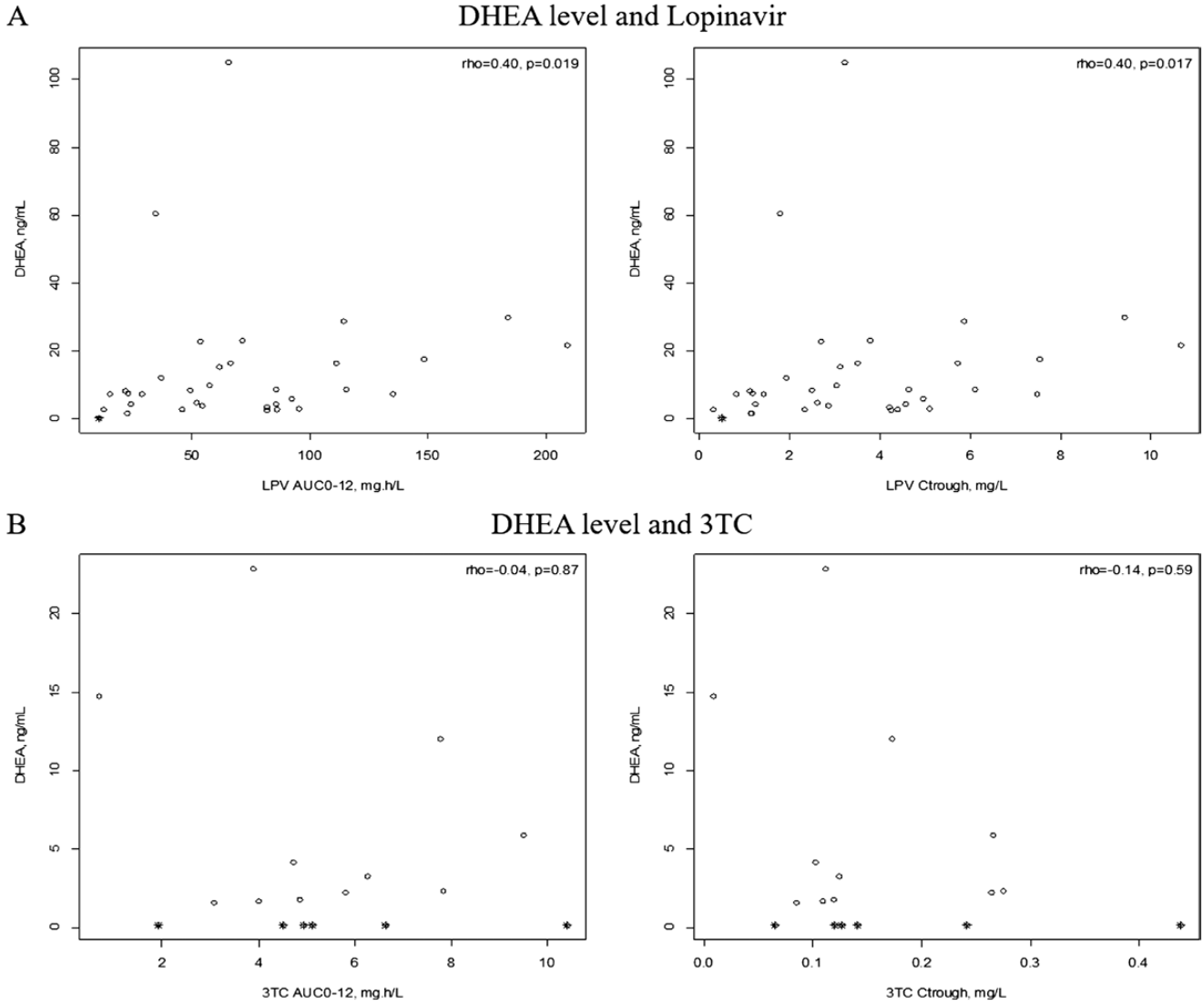

Figure 3. Correlations between plasma lopinavir AUC or C_{trough} and plasma DHEA levels (*A*) and between plasma 3TC AUC or residual concentration (C_{trough}) and plasma DHEA levels (*B*). Asterisks indicate undetectable DHEA plasma levels. Nonparametrical correlation test of Spearman (ρ), $^*P \leq .05$. Abbreviations: AUC, area under the curve; C_{trough}, residual concentration; DHEA, dehydroepiandrosterone; 3TC, lamivudine.

certainly explains why the plasma DHEA levels decreased between weeks 6 and 26, even though they remained higher in the LPV/r-treated group.

The strength of this study is that it assessed children without HIV, randomly allocated to a single drug, and the results are not distorted by infection with HIV itself. As suggested by the difference in drug effect between South Africa and Burkina Faso, the cytochrome gene polymorphisms could play a role in the variability of the drug's effects. The cytochrome P450 polymorphism can be related to the genetic background, which could limit the generalizability of the findings.

In addition, we may have missed other possible adrenal-hormone disturbances due to insufficient sample size. For example, the median cortisol level was higher in the LPV/r-treated group at week 6 than in the 3TC-treated group, but the difference did not reach significance. Thus, our data mainly focused on DHEA levels.

These data underline the potential effect of LPV/r on the adrenal glands of infants, especially at an early stage (6 weeks after the treatment starts). There were no overt clinical signs of adrenal dysfunction in any members of the cohort from which this substudy was derived, up to 1 year of age [23]. However, subtle signs of adrenal dysfunction were not reported as local clinicians were not trained to detect them specifically. LPV/r did have a deleterious effect on weight gain among the 1273 participants of the ANRS 12174 trial [25, 31, 32]. Here, high DHEA levels were associated with increased 17-OHPRE levels, which could favor the occurrence of hyperandrogenism [33, 34], usually associated with accelerated growth. The hormonal disturbances reported here cannot therefore explain the lower growth

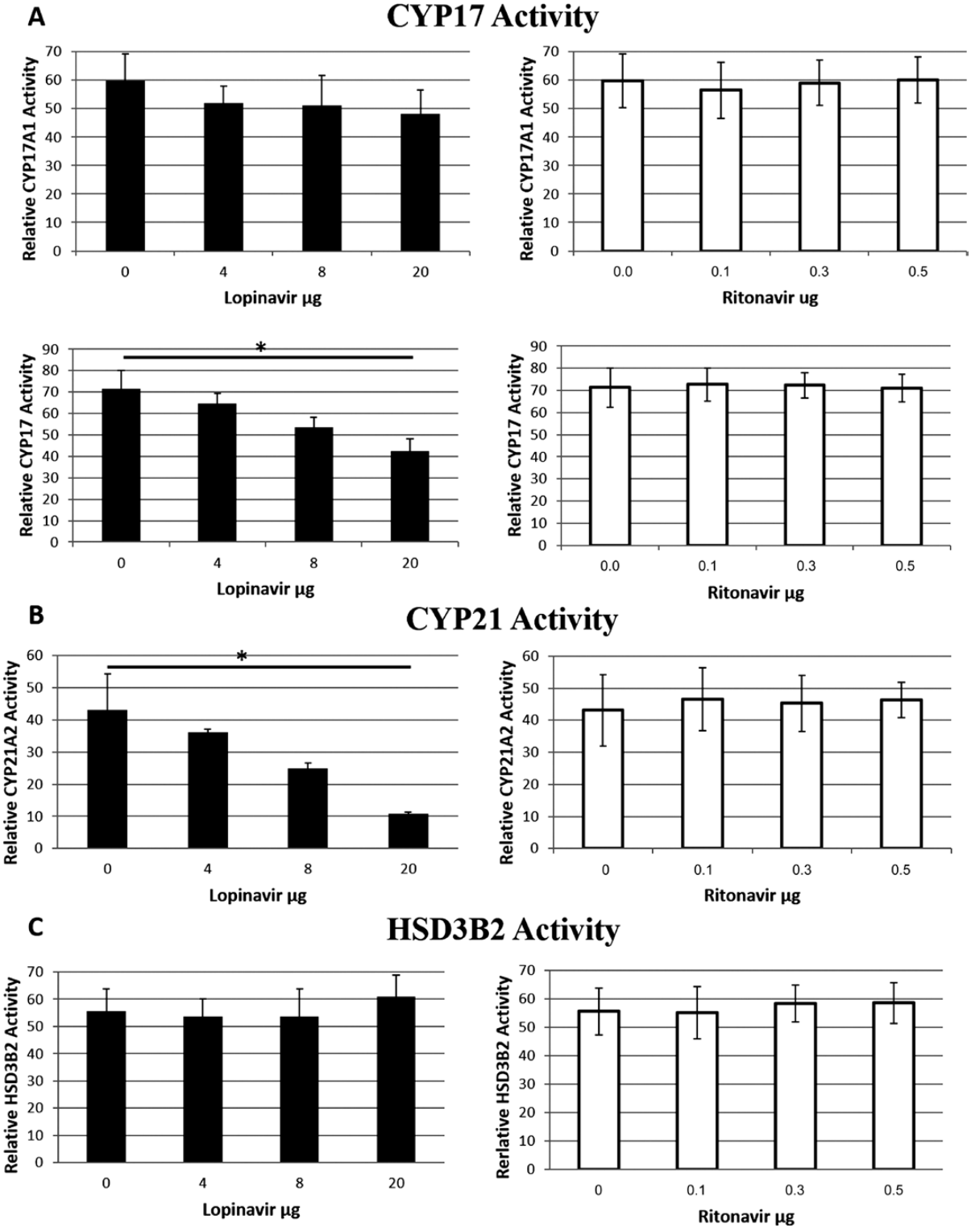

Figure 4. Lopinavir, but not ritonavir, inhibits the steroidogenic enzymes CYP21A2 and CYP17A1. NCI cells were treated with various concentrations of lopinavir and ritonavir as shown. Overall steroidogenesis of NCI cells was assessed using the 3H radiolabeled substrate pregnenolone. *A*, CYP17A activity was tested by examining the conversion of the 3H-radiolabeled substrate pregnenolone to 17-OHP + 17OHPRE + 11-deoxycortisol. In addition, the conversion of 14C-radiolabeled progesterone to 17-OHP + 11-deoxycortisol was also assessed. Lopinavir inhibited CYP17 activity in a dose-dependent manner. Ritonavir showed no effect. *B*, CYP21A2 activity was assessed by the conversion of the 3H-radiolabeled substrate 17-OHP to the product 11-deoxycortisol. Lopinavir inhibited CYP21 activity in a dose-dependent manner. Ritonavir showed no effect. *C*, HSD3B2 activity was tested by the conversion of 3H-radiolabeled DHEA to androstenedione. Neither lopinavir nor ritonavir showed any effect on HSD3B2 activity. The graphs present the relative conversion ratios (product/substrate) by the specific enzymes as shown on thin-layer chromatograms. $^*P \leq .05$. Abbreviations: DHEA, dehydroepiandrosterone; HSD3B2, 3β-hydroxysteroid dehydrogenase; NCI, National Cancer Institute human tumor cell line; 17-OHP, 17-OH-progesterone; 17-OHPRE, 17-OH-pregnenolone.

of children in the LPV/r arm found in this previous study in the same trial [32]. However, such possible hyperandrogenism may have hidden or compensated a potential growth deficiency in some of the patients.

The excipient of LPV/r contains propylene glycol (Kaletra [LPV/r]). Very few studies have assessed the effect of this molecule on steroidogenesis and did not show any inhibition [35]. It is therefore unlikely that propylene glycol is responsible for the hormonal disruption found in this study.

According to the ANRS 12174 initial trial [36] and other substudies about breastfeeding [24] and pharmacokinetics on drugs [29], we can attest that the mean drug adherence was high and slightly better in the 3TC group (93%; SD, 15%) than in the LPV/r group (90%; SD, 14%) ($P < .0001$), which emphasizes the hormonal effects found in the LPV/r.

One of several pathophysiological mechanisms could explain the hormonal profile we observed among the children on LPV/r (ie, a large increase in DHEA levels associated with increased 17-OHP and 17-OHPRE plasma levels). First, inhibition of 21-hydroxylase (CYP21A2, a cytochrome P450 enzyme) would lead to the accumulation of 17-OHP and, upstream, 17-OHPRE and DHEA (Figure 5). Patients with congenital adrenal hyperplasia due to *CYP21A2* mutations have classically elevated plasma 17-OHP and DHEA levels. Inhibition of HSD3B2 [37] would typically lead to the accumulation of 17-OHPRE and DHEA (as seen in this study), but HSD3B2 is not a cytochrome P450 enzyme, the main suspected target of all HIV-protease inhibitors [38, 39]. Third, inhibition of 17,20 lyase (CYP17A1) could result in an increase in 17-OHPRE and pregnenolone levels [40, 41] but cannot explain the elevation of DHEA and would, on the contrary, decrease its level. Finally, the combined partial enzymatic disruption of the 2 cytochrome P450 enzymes (CYP17A1 and CYP21A2) could explain the specific atypical hormonal profile observed here. We excluded a defect in 5-α-reductase activity as testosterone levels and the testosterone to DHT ratio were not increased. A 17ßHSD3/5 defect was also excluded, as androstenedione levels were not increased.

The in vitro enzymatic study on CYP17, CYP21, and HSD3B2 enzymatic activity supports these hypotheses: lopinavir, but remarkably not ritonavir, induces both inhibition of CYP21 and CYP17, but not HSD3B2.

Mc Donald et al [22] and Balogun et al [42] showed that LPV/r exposure in pregnant women living with HIV was associated with increased estradiol levels, higher DHEAS (DHEA-sulfate) levels in cord blood of women exposed to protease inhibitor–based ART and a direct correlation between cord blood DHEAS and maternal estradiol levels, in accordance with our data: this increase may be due to higher availability of DHEA, which is the placental precursor of estradiol.

Finally, such adrenal disruption linked to the partial inhibition of the cytochrome P450 enzymes should be considered when evaluating the early and prolonged treatment of children with HIV with LPV/r, which may last several years, and certainly when concomitant drugs metabolized through cytochrome P450 are given.

Conclusions

Lopinavir, one of the major anti-HIV drugs commonly used in infants and children, is associated with dose-dependent adrenal dysfunction in infants. Adrenal enzyme disruption is plausible through the combined partial inhibition of cytochrome P450 CYP17 and CYP21. Treatment of children beyond 26 weeks, who have a fully mature adrenal gland, and the potential clinical consequences of this biological signature merit further investigation, as well as the comparative effects of the other HIV protease or integrase inhibitors.

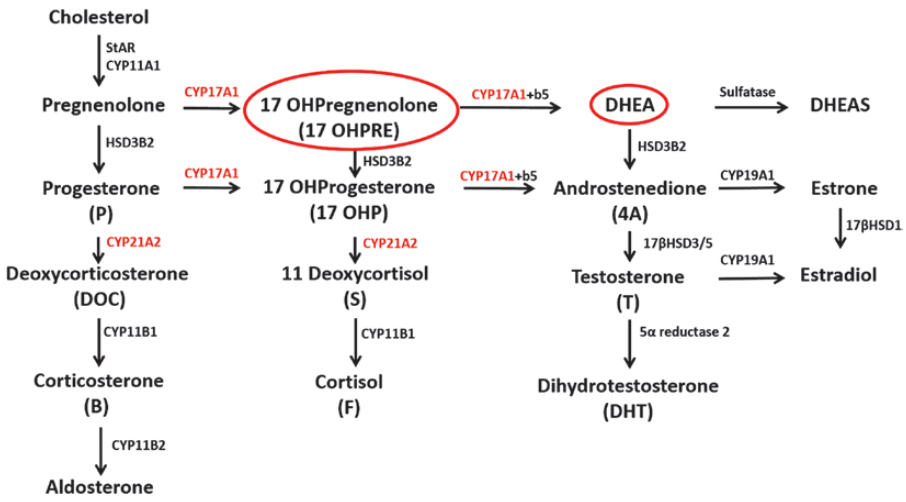

Figure 5. Schematic of steroidogenesis in human postnatal adrenals and impact of lopinavir. Partially inhibited enzymes by lopinavir shown in red. Abbreviation: DHEAS, dehydroepiandrosterone sulfate.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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