

# Long-term body composition and metabolic changes in HIV-infected children switched from stavudine to tenofovir and from protease inhibitors to efavirenz

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**Abstract** This is an 8-year cohort study of 24 HIV-infected patients aged 5–17 years to assess body composition and metabolic changes after switching from lamivudine + stavudine (d4T) + protease inhibitors (PI) to lamivudine + tenofovir (TDF) + efavirenz (EFV). Body composition (dual-energy X-ray absorptiometry) and cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, glucose and insulin were measured annually. Linear mixed models and generalized linear mixed models were used to evaluate time changes of the outcome of interest. Body mass index increased linearly by 0.3 kg/m<sup>2</sup>/year ( $p < 0.001$ ); waist circumference increased non-linearly from 68 to 74 cm ( $p = 0.004$  for the linear term and  $p = 0.04$  for the quadratic term). Percent body fat, percent trunk fat and percent bone mineral content increased linearly by 0.6 %/year ( $p = 0.005$ ), 1.2 %/year ( $p < 0.001$ ) and 0.02 %/year ( $p = 0.04$ ), respectively. Percent arm fat remained stable ( $p = 0.5$ ), and percent

leg fat decreased linearly by 1.2 %/year ( $p < 0.001$ ). The probability of low HDL was 0.2 % at baseline and remained stable during the study. The probability of high triglycerides was 3 % at baseline and increased linearly to become 11 % at the 8th year of follow-up ( $p = ns$ ). The probability of high glucose was 1 % for the whole study duration. **Conclusions:** patients, after switching from d4T to TDF and from PI to EFV, show most of the changes in anthropometry and body composition associated with normal growth and no frankly pathological change in metabolic parameters.

**Keywords** Children · HIV infection · Body composition · Metabolic parameters

## Introduction

The degree to which body composition and metabolic abnormalities occur in HIV-infected paediatric patients is still a matter of debate. The lipodystrophy syndrome (LS), characterized by altered body fat distribution and metabolic disturbances, has been associated with antiretroviral (ARV) treatment in HIV-infected children [2, 7, 8, 32]. A recent cross-sectional study reported lower values of body mass index (BMI) and body fat in HIV-infected children as compared to control children [14]. Another cross-sectional study of HIV-infected children with extensive ARV treatment reported lower height, weight and limb fat as compared to controls [1]. The same study reported also a high frequency of lipid and glucose abnormalities among subjects treated with protease inhibitors (PI) [1]. A recent multicenter study showed that LS was present in nearly half of HIV-infected children with long-term ARV [8]. LS was associated with the use of stavudine (d4T) and ritonavir (RTV), white ethnicity and BMI

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[26]. Several adult studies reported the beneficial effect of switching from thymidine nucleoside to abacavir (ABC) or tenofovir (TDF) on peripheral lipoatrophy [5, 15, 18, 24]. In our study of HIV-infected lipoatrophic children, replacing d4T with TDF and PI with efavirenz (EFV) for 1.8 years was associated with a restoration of physiological fat accrual and better lipid profile [36, 37].

Perinatally HIV-infected patients have lower bone mass as compared to healthy peers [23]. Use of PIs is associated with lower bone mass [25, 33, 41], and the same is true for d4T, especially when used in association with RTV [41]. Some studies reported a decrease in bone mass after switching to a TDF-containing regimen [11, 30, 33], while other studies did not report any detrimental effect on skeletal health [6, 13, 38].

The aim of the present study was to describe the long-term (8-year) changes in growth, fat content and distribution, bone mass and metabolic parameters occurring in a series of 24 HIV-infected children who were switched from d4T to TDF and from PI to EFV.

## Materials and methods

### Study design

This is an 8-year cohort study of 24 HIV-infected Caucasian children and adolescents. At baseline, all enrolled patients were switched from d4T to TDF and from PI to EFV. We have previously reported data on these patients at 1.8 years after the switch [37]. The enrolment of patients began on April 2004, and it was completed by the end of November 2004. Anthropometry, body composition, immunological function and laboratory parameters were evaluated annually for eight consecutive years. Anthropometry and body composition measurements were always performed on the same day. The study was approved by the ethical committee of the Luigi Sacco Hospital, and informed consent was obtained from the legal guardians of the patients or the patients themselves if 18 years and older.

### Study population

The recruitment of patients was performed at the Pediatric Clinic of the Luigi Sacco Hospital (Milan University, Milan, Italy). Inclusion criteria were: (1) vertical HIV infection; (2) treatment with HAART containing d4T, lamivudine and PI; (3) HIV RNA levels <50 cp/mL for at least 48 weeks before the study; (4) HIV RNA levels <50 cp/mL on two random determinations in the month prior to the study. Exclusion criteria were: (1) presence of AIDS-defining condition in the 48 weeks before the study and (2) previous treatment with non-nucleoside reverse transcriptase inhibitors (NNRTIs) or TDF.

### Anthropometry

Body weight and height were measured following international guidelines [17]. Waist circumference was measured at the midpoint between the last rib and the iliac crest. High waist circumference was defined as a waist  $\geq 90$ th percentile for age and gender using European–American growth charts [9, 40]. BMI was calculated as weight (in kilogram)/height (in metre)<sup>2</sup>. Standard deviation scores (SDS) of BMI were calculated using Italian growth charts [4]. Anthropometric measurements were performed by the same physician (SS) for the whole duration of the study. Pubertal status was evaluated by the same physician (SS) using Tanner’s criteria [35].

### Body composition

Total (without head) and appendicular (arms, legs and trunk) body composition was measured by the same physician (SM) using a dual-energy X-ray absorptiometry (DXA) scanner equipped with version 12 of paediatric software (Lunar Prodigy, GE-Lunar Radiation Corporation, WI, USA). The DXA scanner was calibrated daily following the manufacturer’s directions. The three-compartment DXA model separates body mass into lean tissue mass (LTM), fat mass (FM) and bone mineral content (BMC) [28]. The precision of the DXA scanner in children as measured in our laboratory is 1.5 % for BMC, 0.7 % for FM and 0.9 % for LTM. We calculated percent body fat (PBF) as FM/body mass, percent arm fat (PAF) as arm fat (both arms)/FM, percent leg fat (PLF) as leg fat (both legs)/FM, percent trunk fat (PTF) as trunk fat/FM and percent bone mass (PBM) as BMC/body mass.

### Immunological function

Total and percent CD4 T cells were measured in fresh blood samples using single-platform flow cytometry (Cytotron Absolute Cytometer, Ortho Cytometry, NJ, USA) and Immunocount II software (UK NEQAS, Watford, UK). CD4 T cells were identified using two combinations of directly labelled monoclonal antibodies (CD3/CD19/CD16 and CD3/CD4/CD8). HIV RNA was measured with the Quantiplex bDNA assay version 3.0 (Bayer Diagnostics, MA, USA) with a lower limit of detection of 50 cp/mL until 2009 and one of 37 cp/mL thereafter.

### Laboratory measurements

Fasting serum cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides and glucose were measured using standard laboratory methods. Low HDL cholesterol was defined as HDL

<40 mg/dL in subjects aged less than 16 years and as HDL <40 and <50 mg/dL, respectively, in males and females aged 16 years or more [40]. High triglycerides were defined as triglycerides  $\geq 150$  mg/dL [39]. High glucose was defined as fasting glucose  $\geq 100$  mg/dL [40]. Fasting serum insulin was measured using a chemoluminescence immunometric assay (Immunolite 2000, Medical Systems, Genoa, Italy). The homeostasis model assessment (HOMA) was used as surrogate marker of insulin resistance [39].

#### Statistical analysis

Descriptive statistics of continuous variables are reported as means and standard deviations or confidence intervals (CI). Robust CIs were used to describe the continuous variables which had been estimated from the linear mixed model (LMM) used to evaluate time changes (see below). Categorical variables are reported as numbers or percentages. The changes in time of the continuous variables of interest (BMI, waist, PBF, PAF, PLF, PTF, PBM, total CD4, percent CD4, cholesterol, HDL, LDL, triglycerides, glucose, insulin and HOMA) were evaluated using a LMM employing: (1) the variable of interest (continuous) as response variable; (2) time (continuous, years), gender (discrete: 0=female; 1=male) and age at baseline (continuous, years) as predictors and; (3) the patient as random effect [10, 31]. The effect of baseline age and gender was controlled for because they are important predictors of body composition [3]. The changes in time of the dichotomous variables of interest (large waist, low HDL, high triglycerides, high glucose) [39] were evaluated using a generalized linear mixed model (GLMM) employing: (1) the variable of interest (dichotomous) as response variable, (2) time (continuous, years) as predictors, (3) a Bernoulli family with a log link and (4) the patient as random effect [10, 31]. Quadratic powers of age and time were evaluated to test the existence of non-linear relationships with continuous predictors. Robust 95 % CIs were always calculated. The LMM/GLMM is robust to missing data provided that missingness is random (MAR) [10].

## Results

### Baseline measurements

The baseline and final measurements of the 24 study patients are given in Table 1. These values were obtained from the LMM and are corrected for age at baseline and gender and missing data.

All the patients were Caucasians. They were aged from 5 to 17 years and were equally distributed between sexes. They had a good immune status as detected by high values of total and percent CD4 T cells.

### Changes in clinical status

Fifteen patients performed all yearly visits, four performed all visits with the exception of the last one and five lost one visit at the 3rd, 4th or 6th year. Because of this pattern of missingness and the lack of a clear association with the treatment, we assumed that data were MAR. All patients remained clinically stable, and no AIDS-defining events occurred during the 8-year follow-up.

### Changes in anthropometry and body composition

Figure 1 plots the changes in anthropometry and body composition that occurred during the 8-year follow-up. All the values are corrected for age at enrolment and sex using the LMM described under statistical analysis.

Mean (95 % CI) BMI was 18.9 (17.9 to 19.8) kg/m<sup>2</sup> at baseline and increased linearly by 0.3 (0.2 to 0.5) kg/m<sup>2</sup>/year ( $p < 0.001$ ). Waist circumference increased non-linearly from 68 (65 to 70) cm at baseline to 74 (67 to 80) cm at 8 years of follow-up ( $p = 0.004$  for linear term and  $p = 0.04$  for quadratic term).

PBF was 16.2 (13.4 to 19.0)% at baseline and increased linearly by 0.6 (0.2 to 1.0)%/year ( $p = 0.005$ ); PAF was 8.1 (7.5 to 8.6)% at baseline and remained stable during the study ( $p = 0.5$ ); PLF was 42.8 (39.6 to 45.8)% at baseline and decreased linearly by 1.1 (1.5 to 0.7)%/year ( $p < 0.001$ ); PTF was 49.1 (46.2 to 52.1)% at baseline and increased linearly by 1.2 (0.6 to 1.6)%/year ( $p < 0.001$ ); lastly, PBM was 4.1 (3.8 to 4.2)% at baseline and increased linearly by 0.02 (0.0006 to 0.05)%/year ( $p = 0.04$ ).

### Changes in immunological function

Figure 2 plots the immunological changes that occurred during the 8-year follow-up. (All values are corrected for age at enrolment and sex using the GLM described under statistical analysis). The mean number of CD4 cells at baseline was 846 (95 % CI 739 to 953) cells/mm<sup>3</sup> and remained stable during the 8-year follow-up ( $p = 0.3$ ). There was, however, a linear increase of the percentage of CD4 T cells of 0.5 (0.2 to 0.8)%/year from the baseline value of 36 (33 to 37)% ( $p < 0.001$ ). HIV RNA was undetectable in all subjects at all time points.

### Changes in laboratory measurements

Figure 2 plots the changes in laboratory parameters that occurred during the 8-year follow-up. (All values are corrected for age at enrolment and sex using the GLM described under statistical analysis). Cholesterol was 158

**Table 1** Measurements of the children at the enrolment into the study

	Baseline	8-year follow-up	<i>p</i> value <sup>a</sup>
<i>N</i>	24	20	NA
Age (years) (50th, 25th, 75th percentiles)	12 (9, 16)	20 (17, 25)	NA
Pubertal stage (Tanner)			NA
Prepubertal (%)	33.3	0.0	
Early pubertal (%)	66.7	0.0	
Late pubertal (%)	0.0	100.0	
CDC clinical stage			NA
Class A+N (%)	37.5	?	
Class B+C (%)	62.5	?	
BMI (kg/m <sup>2</sup> ) <sup>b</sup>	18.9 (17.9 to 19.8)	21.0 (19.8 to 22.3)	<0.001
BMI (SDS) <sup>b,c</sup>	-0.2 (-0.5 to 0.2)	0.6 (0.1 to 1.1)	<0.001
Waist circumference (cm) <sup>b</sup>	68 (65 to 70)	74 (67 to 80)	0.004 (lt) 0.04 (qt)
Percent body fat (%) <sup>b</sup>	16.2 (13.4 to 19.0)	20.6 (17.5 to 23.7)	0.005
Percent arm fat (%) <sup>b</sup>	8.1 (7.5 to 8.6)	7.8 (6.9 to 8.6)	0.5
Percent leg fat (%) <sup>b</sup>	42.8 (39.6 to 45.8)	35.0 (31.7 to 38.2)	<0.001
Percent trunk fat (%) <sup>b</sup>	49.1 (46.2 to 52.1)	57.2 (53.7 to 60.7)	<0.001
Percent bone mineral content (%) <sup>b</sup>	4.1 (3.8 to 4.2)	4.2 (4.0 to 4.4)	0.04
CD4 T cells <sup>b</sup>	846 (739 to 953)	792 (683 to 901)	0.3
Percent CD4 T cells <sup>b</sup>	36 (33 to 37)	39 (37 to 41)	<0.001
Cholesterol (mg/dL) <sup>b</sup>	158 (152 to 163)	171 (163 to 179)	<0.001
HDL cholesterol (mg/dL) <sup>b</sup>	60 (55 to 64)	56 (51 to 60)	<i>p</i> =0.08 (lt) <i>p</i> =0.04 (qt)
LDL-cholesterol (mg/dL) <sup>b</sup>	87 (79 to 95)	99 (90 to 109)	<i>p</i> =0.01 <i>p</i> =0.01
Triglycerides (mg/dL) <sup>b</sup>	69 (62 to 75)	97 (80 to 115)	<0.001
Glucose (mg/dL) <sup>b</sup>	87 (84 to 89)	82 (78 to 84)	<i>p</i> <0.001 (lt) <i>p</i> =0.03 (qt)
Insulin(μU/mL) <sup>b</sup>	8 (6 to 11)	6 (0.8 to 1.6)	0.02
HOMA <sup>b</sup>	1.8 (0.3)	1.2 (0.2)	0.009
Large waist circumference (%) <sup>b</sup>	1.0	2.0	0.01 0.04
Low HDL cholesterol (%) <sup>b</sup>	2.0	2.0	0.9
High triglycerides (%) <sup>b</sup>	3.0	11.0	0.2
High glucose (%) <sup>b</sup>	1.0	1.0	0.2

NA not available, *lt* linear term of time, *qt* quadratic term of time

<sup>a</sup>The *p* value corresponds to the effect of time in the linear or generalized linear mixed models employed for analysis (see text and figures for details)

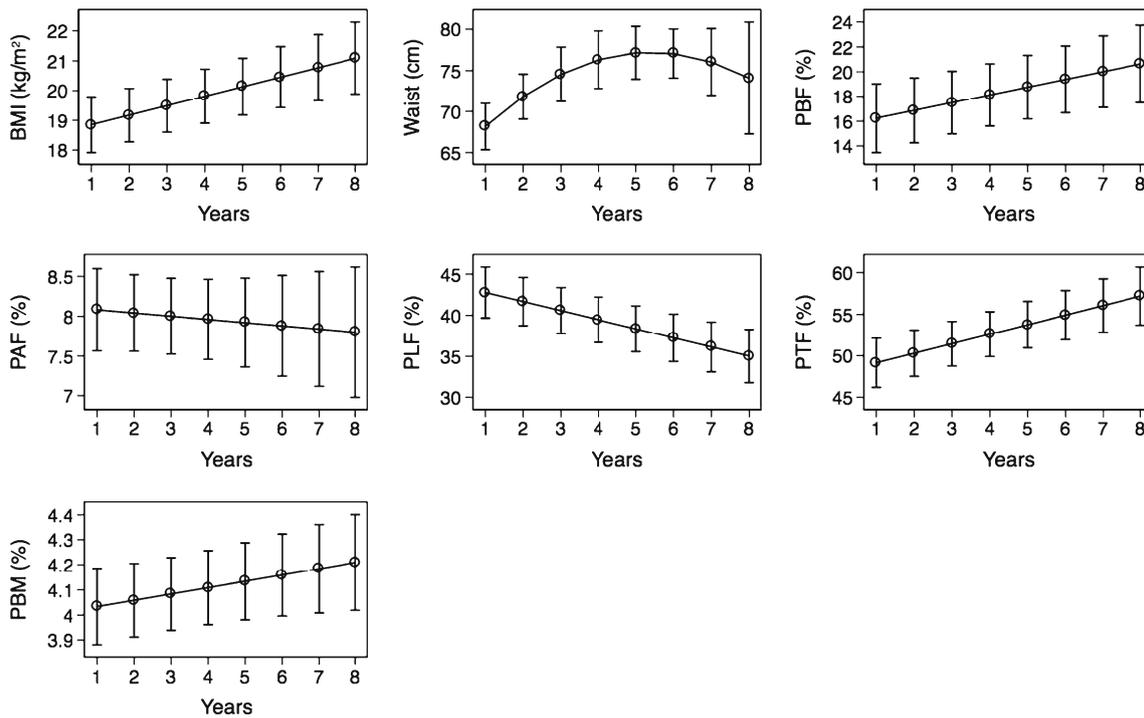
<sup>b</sup>Values are means and confidence intervals for continuous variables and percentages for dichotomous variables and were estimated from linear or generalized linear mixed models

<sup>c</sup>Available only for subjects aged ≤20 years

(95 % CI 152 to 163)mg/dL at baseline and increased linearly by 2 (0.8 to 3.1) mg/dL/year (*p*<0.001). HDL decreased non-linearly from 60 (55 to 64)mg/dL at baseline to 56 (51 to 60)mg/dL at 8 years (*p*=0.08 for the linear term and *p*=0.04 for the quadratic term). LDL increased non-linearly from 87 (79 to 95)mg/dL at baseline to 99 (90 to 109)mg/dL at 8 years of follow-up (*p*=0.01 for the linear term and *p*=0.01 for the quadratic term). Triglycerides were 69 (62 to 65)mg/dL at baseline and increased linearly by 4 (2 to 6) mg/dL/year (*p*<0.001). Glucose decreased non-linearly from 87 (84 to 89)mg/dL at baseline to 82 (78 to 84)mg/dL at 8 years (*p*<0.001 for the linear term and *p*=0.003 for the

quadratic term). Insulin was 8 (6 to 11)μU/mL at baseline and decreased linearly by 0.3 (0.6 to -0.4)μU/L/year (*p*=0.02). HOMA was 1.8 (1.1 to 2.4)units at baseline and decreased linearly by 0.08 (0.14 to -0.02)units/year (*p*=0.009).

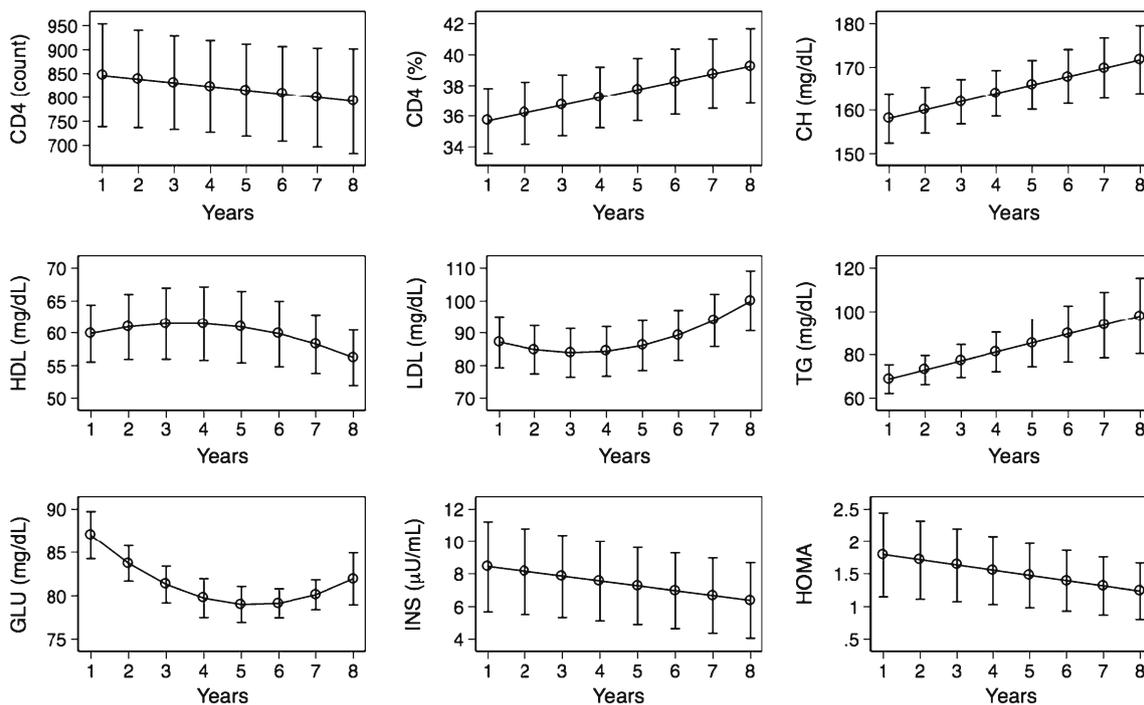
As estimated from the GLMM, the probability of a large WC was 1 % at baseline, reached a maximum of 11 % at 5 years and virtually returned to the baseline level (2 %) at the 8th year (*p*=0.01 for the linear term and *p*=0.04 for the quadratic term). The probability of low HDL was 2 % at baseline and remained stable during the study (*p*=0.9). The probability of high triglycerides was 3 % at baseline and increased linearly to become 11 % at the 8th year of follow-



**Fig 1** Changes in anthropometry and body composition during the study (mixed linear regression model). *BMI* body mass index, *PBF* percent body fat, *PAF* percent arm fat, *PLF* percent leg fat, *PTF* percent trunk fat, *PBM* percent bone mineral mass

up even if the change was not statistically significant owing to the low number of subjects relative to events ( $p=0.2$ ).

The probability of high glucose equal to 1 % for the whole study duration ( $p=0.2$ ).



**Fig 2** Changes in immunological and laboratory parameters during the study (mixed linear regression model). *CD4* CD4 T cells, *CH* total cholesterol, *HDL* HDL cholesterol, *LDL* LDL cholesterol, *TG*

triglycerides, *GLU* glucose, *INS* insulin, *HOMA* homeostasis model assessment of insulin resistance

## Discussion

We reported the 8-year changes in anthropometry, body fat distribution, bone mass and metabolic parameters, which occurred in HIV-infected children after switching from d4T to TDF and from PI to EFV.

The lipoatrophic-inducing effect of d4T has been reported by both adult and paediatric studies. d4T-associated lipoatrophy improves after replacement of d4T by either ABC or zidovudine (ZDV) [21, 29]. In lipoatrophic HIV-infected adults with extensive ARV therapy, switching from a nucleoside reverse transcriptase inhibitor (NRTI, d4T or ZDV) to ABC improved peripheral lipoatrophy [5]. However, limb fat mass had increased only by about 11 % at 6 months, and it was suggested that at this rate, it might take five or more years for limb fat mass to return to normal [5]. A partial remission of lipoatrophy was also reported in HIV-infected adults after switching from a thymidine analogue to ABC [18]. HIV-infected children treated with d4T have lower fat in the limbs and higher fat in the trunk [14], and both d4T and RTV have been recently reported to be risk factors for paediatric LS [26]. We previously reported that lipoatrophic children have a restoration of physiological fat accrual 1.8 years after switching from d4T to TDF and from PI to EFV [37]. In the present study, we showed no frankly pathological change in total and appendicular body fatness 8 years after a switch from d4T to TDF and from PI to EFV. It is possible that the good control of viral replication observed during the study and a generally greater ability of HIV-infected children to recover as compared to adults may partly explain our findings, which are based on the longest available follow-up.

The use of TDF has been associated with a marked reduction of bone mineral density in HIV-infected adults, especially during the first months of treatment [12, 34]. Paediatric data are, however, conflicting. Some studies report in fact a marked reduction of bone mass following treatment with TDF [11, 30, 33]. Other studies have, however, shown that TDF has no detrimental effect on skeletal health [6, 13, 38]. In the present study, the use of TDF was not associated with pathological bone mass changes. We observed that PBM increased linearly of 0.02 (0.01)%/year, but the changes were below the error value of the technique for bone mineral measurements. The finding further underlines that switching to TDF does not harm bone mineral composition, and this may be partially dependent upon the good immune recovery shown by our patients for the whole study duration.

Switching from PI to NNRTI is an effective strategy for controlling hyperlipidemia [19, 27]. A robust and sustained reduction in LDL cholesterol and triglycerides has been reported in HIV-infected adults after the substitution of d4T with TDF [16]. Even if both d4T dose reduction and

switching to TDF were associated to decrease in plasma lipids, significant changes were apparent only among the patients who had switched from d4T to TDF [22]. The replacement of PI with EFV was associated with a decrease of LDL cholesterol and triglycerides [20]. Our experience confirms that switching from d4T to TDF and from PIs with EFV is associated with an improvement in lipid profile [36]. In the present study, the metabolic abnormalities were very few and of doubtful clinical relevance. Waist circumference was highest at the mid of the study, but the number of children with high waist was similar at baseline and 8-year visits (1 vs 2%). Low HDL and high glucose were very uncommon (<1 %) for the whole study duration. Although there was a trend for increasing triglycerides, the probability of high triglycerides was only 11 % at 8 years of follow-up.

Although this study is the longest performed to date, it is not without limitations. First, our cohort was small ( $n=24$ ), as is to be expected from a single-centre study. However, the power to model the continuous outcome–time relationships was substantial owing to the large number of time points. Moreover, very few patients missed a follow-up visit, and none was lost to follow-up. Nevertheless, due to the small number of subjects included in the study, some metabolic effects may have not been detected. Second, the lack of a control group, dictated by ethical reasons as DXA exposes to ionizing radiation, impeded us to contrast the changes in body composition of our patients to those of control children. The lack of a control group prevented us to correctly evaluate the modest percentage leg fat decrease observed during the study period.

In conclusion, replacing PI with EFV and d4T with TDF appears to be a successful treatment option in HIV-infected paediatric patients. Such strategy allows for continued virological suppression and maintenance of immune recovery and is not associated with frankly pathological abnormalities of body composition and metabolism.

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