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Carbohydrate, lipid, bone and inflammatory markers in HIV-positive adolescents on antiretroviral therapy and hormonal contraception

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Abstract

Background: Little is known about the cumulative effect of HIV antiretroviral therapy (ART) and hormonal contraception (HC) on metabolism and inflammation in HIV-positive women.

Methods: We conducted a cross-sectional assessment of markers for carbohydrate, lipid, bone metabolism, inflammation and coagulation in HIV-positive adolescents on ART and HC (*n*=37) versus on ART only (*n*=51) in Thailand. The Wilcoxon rank-sum test was used to assess differences between groups.

Results: The median age was 19.5 years. Most adolescents (95%) were perinatally infected. All were on ART for a median of 9 years. HC used was progestin only (*n*=21); combined oral contraceptive (COC) tablets (*n*=6) for the whole study period or alternating between progestin only and COC (*n*=10). Prevalence of any metabolic abnormalities was 99%. Four biomarkers were significantly higher with HC *vs* no HC: insulin (10.3 *vs* 6.2 µU/mL, *P*=0.002), insulin resistance (1.89 *vs* 1.19 mass units, *P*=0.005), 25-OH vitamin D (33.2 *vs* 20.2 ng/mL, *P*<0.0001) and C-terminal telopeptide (690 *vs* 530 ng/L, *P*=0.011). Triglycerides and D-dimer were significantly lower with HC (103 *vs* 139 mg/dL, *P*=0.014 and 140 *vs* 155 ng/mL, *P*=0.003, respectively). There was no relationship between the type of HC or ART and the above differences.

Conclusion: Perinatally infected HIV-positive adolescents on ART in this pilot study had a high prevalence of metabolic abnormalities. Bone turnover markers and insulin resistance were significantly higher with HC. Research on the cumulative effect of HIV, ART and HC on metabolism and inflammation in adolescents with HIV is important in order to devise strategies for preventing and mitigating long-term comorbidities.

Introduction

Antiretroviral therapy (ART) allows HIV-positive adolescents, infected perinatally or behaviourally, to lead healthy lives [1]. Approximately half of the individuals are girls. Many are becoming sexually active and encouraged to use an effective contraceptive method, including hormonal contraception (HC) in addition to condoms to prevent unintended pregnancy [2,3]. However, there are many questions related to the use of HC in HIV-positive women and one major issue is related to undesirable metabolic changes. HC use in the general population has been associated, to different degrees, with unfavourable changes in carbohydrate metabolism [4–7], lipid profile [7–9], bone turnover markers reflecting bone health [10–11] and markers for inflammation and coagulation [5,6]. Changes vary depending on the HC type and dosage, the metabolic marker assessed, and confounding factors including body weight.

These metabolic markers can be affected by HIV infection and/or ART. Many studies have reported a high prevalence of insulin resistance (IR) and lipid abnormalities in perinatally or behaviourally HIV-infected adolescents [12,13]. ART significantly influences these changes [14–16], thus contributing to the lifetime risk of cardiovascular disease. Markers for inflammation and coagulation, including C-reactive protein (CRP), interleukin-6 (IL-6) and D-dimer are elevated with HIV infection and ART [17,18], which can further contribute to increased severity of comorbidities. HIV infection alone has also been associated with an increase in bone turnover markers and a decrease in bone mineral density in adults and children [19–22].

Little is known about the cumulative effect of HIV, ART and HC on metabolism and inflammation in women, and no studies have been performed in adolescents [23,24]. Womack *et al*. found that in a cohort of HIV-positive and HIV-negative women, progestin-only and combined HC impact metabolic outcomes differently [23]. Progestin-only HC was associated with lower high density lipoprotein (HDL) and greater insulin resistance in HIV-infected and uninfected women. Bekinska *et al*. highlighted the importance of studies addressing the loss of bone mineral density in HIV-positive women using progestin-only HC [24]. This study reports on the levels of metabolic and inflammatory markers in HIV-positive female adolescents on ART and 48 weeks of HC use and compares results with a control group of HIV-positive adolescents on ART only.

Methods

We conducted a pilot, cross-sectional comparison of adolescents who had been using HC for at least 48 weeks versus those not on HC, for biomarkers of carbohydrate, lipid, bone metabolism, inflammation and coagulation. The study enrolled female adolescents with HIV at five sites in Thailand – HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT), Bangkok; Faculty of Medicine, Khon Kaen University, Khon Kaen;

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Inclusion criteria for both groups were: age between 12 and 24 years, perinatally or behaviourally HIV-infected and on stable ART. The HC group had to be on HC for at least 48 weeks. The control group, not on HC, were required not to have taken HC for the past 24 weeks. The following hormonal contraceptions were accepted in the HC group: combined oral contraceptive (COC) tablets, depot medroxyprogesterone acetate (DMPA) injection every 12 weeks or hormonal implant. For the COC tablets, Marvelon 28 (0.150 mg desogestrel/0.030 mg ethinylestradiol) was used (Organon, Oss, the Netherlands) in a standard dose of one pill per day. The hormonal implant, Implanon, containing 68 mg etonogestrel (Organon Oss, the Netherlands) was used. Exclusion criteria included being pregnant at the time of enrolment, having an active opportunistic infection and mental or other physical conditions that could limit the informed participation in the study.

No formal sample size calculation was performed due to the lack of preliminary data. Thirty-seven participants were enrolled in the group with HC and 51 in the group without HC. Eleven control subjects elected to start HC after their visit and contributed data to both groups. Participation was voluntary, all study-related procedures were free of charge and all participants and caregivers (if participants were younger than 18 years of age) gave written informed consent. The study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University in Bangkok, and the respective IRBs at each site.

The following assay profiles were performed on fasting bloods from each participant: carbohydrate metabolism – fasting blood sugar (FBS), glycated haemoglobin (HbA1c) and insulin; lipid metabolism – total cholesterol, triglycerides (TG) and HDL; bone metabolism – 25-OH vitamin D, C-terminal telopeptide (β-CTx) and N-terminal propeptide (P1NP); markers for immune inflammation and coagulation – high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and D-dimer. Collected blood was centrifuged and stored at –80°C. Laboratory tests were performed at the College of American Pathologists certified HIV-NAT laboratory or at Bangkok RIA Laboratory in Bangkok, Thailand using standard methods. Laboratory tests for HbA1c were done locally with fresh blood at the study site. The values of homeostatic model assessment of insulin resistance (HOMA-IR) were calculated by a formula. The upper limit of normal (ULN) for each biological marker was as follows: FBS >105 mg/dL; HbA1c >5.5%; insulin >25 mcU/mL; HOMA-IR >3 mass units; total cholesterol >200 mg/dL; TG>150 mg/dL; HDL<40 mg/dL; hs-CRP >3 mg/L; IL-6 >5 pg/mL; D-dimer >250 ng/mL. 25-OH vitamin D<30 ng/mL was considered below the lower limit of normal. Biomarker levels below the lower limit of detection were read as equal to the lower limit of detection. Reference ranges for β-CTx and P1NP are presently being established; for this assessment the upper 95th percentile reference limit from the most recent data for Asian women below 24 years of age: β-CTx >1024 ng/L; P1NP >87 ng/mL [25,26] were used as ULN values. Demographic and medical data of study participants were collected from medical records within 6 months of enrolment.

We used STATA/IC version 11.2 for Windows (Statacorp LP, TX, USA) for statistical analysis. Characteristics at the time of the metabolic parameter assessment were summarised by calculating the median and interquartile range (IQR) for quantitative data, and number and percentage for categorical data. The percentage of participants with biomarker levels above the ULN was calculated for each biomarker by HC group. Additionally, for each participant, we calculated the prevalence of metabolic groups (carbohydrate, lipid, bone metabolism and inflammatory markers) with at least one marker above the ULN, or below the norm for 25-OH vitamin D.

The significance of the difference of measured biomarkers in the two groups was assessed using the Wilcoxon rank-sum test. For biomarkers, which showed significant difference in the group with HC in comparison to the group without HC, a dichotomised endpoint was used (e.g. proportion of participants with biomarkers levels >ULN) logistic regression was then applied to test whether the differences were influenced by factors under study.

We performed a sensitivity analysis for the subgroup of 11 participants who contributed data to both groups with and without HC, applying a Wilcoxon paired sample test. All hypothesis tests were two-sided with 5% significance level.

Table 1. Characteristics of study participants at the time of metabolic parameter assessment

Variable	With HC, *n*=37 Median (IQR)/*n* (%)	Without HC, *n*=51 Median (IQR)/*n* (%)	Difference, *P*-value
Age (years)	20 (18–21)	19 (17–21)	0.41
Method of HIV acquisition			0.743
Perinatally	35 (95)	49 (96)	
Behaviourally	2 (5)	2 (4)	
Weight(kg)	46 (40–54)	48 (42–55)	0.348
Height (cm)	153 (151–155)	156 (153–160)	0.007
BMI	20.1 (17.6–21.9)	19.6 (17.8–21.8)	0.866
ART duration (years)	9 (7–10)	9 (5–12)	
Type of ART			
PI regimen	12 (32)	31 (61)	0.0221
TDF regimen	9 (24)	20 (45)	0.0874
CD4 cell counts (cells/mm3)	640 (359–832)	590 (384–846)	0.568
HIV-RNA (copies/mL)			0.835
<40	16 (70)	35 (68)	
40–999	1 (4)	8 (16)	
≥1000	6 (26)	8 (16)	
	(2901–283,891)	(2283–488,037)	
Type of HC			
Progestin only (DMPA or implant)	21 (57)	–	
COC tablet only	6 (16)	–	
Mixed*	10 (27)	–	
Ever on HC	37 (100)	10 (20%)	

HC: hormonal contraception; IQR: interquartile range; BMI: body mass index; ART: antiretroviral therapy; PI: protease inhibitor; TDF: tenofovir DF; DMPA: depot medroxyprogesterone acetate; COC: combined oral contraceptive.
* Mixed: alternated between progestin-only method or COC pill during the 48-week study period.

Results

Between December 2013 and August 2015 we enrolled 37 adolescents with HC and 51 in a control group without HC. Eleven participants had both pre- and post-HC visits and contributed data to both groups. Characteristics at the time of metabolic parameter assessment are presented in Table 1. The median age in the group with HC was 20 years, and 19 years in the group without HC. Over 95% of the participants in each group were perinatally infected. The median ART duration for both groups was 9 years; 74% in the group with HC and 84% in the group without HC had HIV-RNA <1000 copies/mL. Twenty-one of 37 (57%) participants used a progestin-only method (DMPA or implant) for the whole study period, six (16%) used COC tablets for the whole study period and 10 (27%) alternated between progestin-only or combined methods during the study period.

The overall prevalence of any metabolic abnormalities was 99%, with 97% in the HC group and 100% in group without HC. In the HC group 86% showed changes in at least two groups of measured biomarkers (carbohydrates, lipids, bone and inflammatory markers) versus 94% in the group without HC. The prevalence of abnormalities did not differ between groups.

The median and interquartile (IQR) biomarker levels for each of the HC groups are presented in Table 2. There was significant difference between the two groups in six of the biomarkers. Insulin (P=0.002), HOMA-IR (P=0.005), 25-OH vitamin D (P<0.0001) and β-CTx (P=0.011) were higher with HC. Levels of these biomarkers above the ULN, and below the norm for 25-OH vitamin D, were not significantly influenced by the type of HC (progestin only, combined or changing between the two options over the 48-week study period) and the type of ART (tenofovir versus other nucleoside reverse transcriptase inhibitors as a backbone regimen; protease inhibitor(PI) versus non-nucleoside reverse transcriptase inhibitors-based regimens). Triglycerides (P=0.014) and D-dimer (P=0.003) were significantly lower in the HC group.

Table 2. Biomarker levels in HIV-positive adolescents on ART with and without HC, and proportion of patients with biomarkers above the upper limit of normal

Variable	With HC, *n*=37 Median (IQR)/*n* (%)	Without HC, *n*=51 Median (IQR)/*n* (%)	Difference, *P*-value
FBS (mg/dL)	79 (74–87)	79 (76–85)	0.987
FBS >105 mg/dL	2 (5)	3 (6)	
HbA1c (%)	4.9 (4.7–5.2)	5 (4.6–5.3)	0.163
HbA1c >5.5%	1 (3)	3 (6)	
Insulin (μU/mL)	10.3 (6.3–17.4)	6.2 (3.7–10.5)	0.002
Insulin >25 μU/mL	5 (14)	2 (4)	
HOMA-IR (mass units)	1.89 (1.22–3.69)	1.19 (0.7–2.09)	0.005
HOMA-IR >3	11 (30)	9 (18)	
Total cholesterol (mg/dL)	183 (160–193)	179 (143–211)	0.866
Total cholesterol >200 mg/dL	6 (16)	18 (35)	
TG (mg/dL)	103 (75–144)	139 (93–200)	0.0137
TG >150 mg/dL	9 (24)	21 (41)	
HDL cholesterol (mg/dL)	43 (34–60)	42 (34–53)	0.551
HDL cholesterol <40 mg/dL	15 (41)	21 (41)	
25-OH vitamin D (ng/mL)	33.2 (27.9–36.1)	20.2 (16.3–25.8)	<0.0001
25-OH vitamin D >30 ng/mL*	23 (62)	6 (12)	
P1NP (ng/mL)	116 (94–156)	114 (71–157)	0.651
P1NP >87 ng/mL	28 (76)	35 (69)	
β-CTx (ng/L)	690 (480–850)	530 (340–700)	0.011
β-CTx >1024 ng/L	5 (14)	2 (4)	
Il-6 (pg/mL)	6.3 (4.1–15.1)	5.08 (1.65–18.07)	0.115
Il-6 >5 pg/mL	23 (62)	26 (51%)	
D-dimer (ng/mL)	140	155 (140–212)	0.003
D-dimer >250 ng/mL	3 (8)	10 (20)	
Hs-CRP (mg/L)	1.7 (0.4–3.51)	0.81 (0.38–1.85)	0.264
Hs-CRP >3 mg/L	11 (30)	10 (20)	

HC: hormonal contraception; ART: antiretroviral therapy; FBS: fasting blood sugar; HOMA-IR: insulin resistance; TG: triglycerides; HDL: high density lipoproteins; P1NP: N-terminal propeptide; β-CTx: C-terminal telopeptide; Il-6: interleukin-6; Hs-CRP; high sensitivity C-reactive protein.
* 25-OH vitamin D<30 ng/mL was considered below the lower limit of normal.

The sub-analysis of data from the 11 participants who contributed to both groups showed similar patterns before and after HC as in the cross-sectional analysis of no HC versus HC (Table 3). Significant differences were reached for two biomarkers only: 25-OH vitamin D increased by 11 ng/mL (P=0.0033); β-CTx increased by 260 ng/L (P=0.0262). Although not statistically significant, there was a tendency for increased insulin (P=0.0505), HOMA-IR (P=0.0912) and P1NP (P=0.0505) with HC use.

Discussion

In this study we have found a high prevalence of metabolic abnormalities among Thai HIV-positive female adolescents on ART. Over 90% of participants had at least two biomarkers from different metabolic pathways outside the limit of normal. In the group that had received at least 48 weeks of HC, levels of insulin and HOMA-IR, as well as of β-CTx and 25-OH vitamin D, were significantly higher compared to the group without HC, in spite of a twice-lower PI use. TG and D-dimer showed significantly lower levels in the group with HC versus the group without HC.

The association between HIV infection, ART and increased HOMA-IR has been demonstrated in many studies [12,14–16]. Evidence that HC influences carbohydrate metabolism in the general population is less strong [27]. However, some authors have reported that HC significantly increases HOMA-IR [4–7]. We also observed a tendency for increased insulin and HOMA-IR with HC in the prospective sub-analysis of the 11 HIV-positive adolescents who had biomarkers available before and after starting HC. In contrast to the earlier report from Womack *et al*. [23], we did not find a relationship between insulin and HOMA-IR level abnormalities and the type of HC used. In summary, our data suggest that HC can contribute towards the development of insulin resistance in HIV-positive adolescents on ART.

HIV infection itself and use of ART has also been associated with an increase in bone turnover markers and a decrease in bone mineral density in adults and children [19–22]. The use of HC, especially progestin-only products, had been associated with poorer bone health. In 2004, the US Food and Drugs Administration (FDA)

Table 3. Biomarker levels in 11 participants on ART, who participated in both with and without HC (longitudinal sensitivity analysis)

Variable	First time point (without HC), *n*=11 Median (IQR)	Second time point (with HC), *n*=11 Median (IQR)	*P*-value
FBS (mg/dL)	76 (68–78)	78 (73–87)	0.0912
HbA1c (%)	5.1 (4.5–5.5)	4.9 (4.6–5.2)	0.593
Insulin (μU/mL)	4.2 (3.4–10.5)	10.3 (8.2–15.3)	0.0505
HOMA-IR (mass units)	0.8 (0.7–1.9)	2.0 (1.5–3.1)	0.0912
Total cholesterol (mg/dL)	180 (153–209)	181 (138–193)	0.859
TG (mg/dL)	119 (90–167)	100 (75–138)	0.328
HDL cholesterol (mg/dL)	42 (28–50)	49 (34–67)	0.062
25-OH vitamin D (ng/mL)	19 (15–26)	30 (23–36)	0.0033
P1NP (ng/mL)	100 (65–142)	117 (111–239)	0.0505
β-CTx (ng/L)	480 (330–750)	740 (480–1310)	0.0262
Il-6 (pg/mL)	18 (3–28.4)	19.4 (6.2–58.3)	0.248
D-dimer (ng/mL)	172 (140–217)	140 (140–214)	0.271
Hs-CRP (mg/L)	1.2 (0.8–10.1)	1.98 (0.3–5.1)	0.594

HC: hormonal contraception; ART: antiretroviral therapy; FBS: fasting blood sugar; HOMA-IR: insulin resistance; TG: triglycerides; HDL: high density lipoproteins; P1NP: N-terminal propeptide; β-CTx: C-terminal telopeptide; Il-6: interleukin-6; Hs-CRP: high sensitivity C-reactive protein.

added a black box warning to the package insert of DMPA stating that women who use DMPA contraceptive injection may have significant bone mineral density loss. The warning also stated that it was unknown whether its use during adolescence or early adulthood, a critical period of bone growth, would reduce peak bone mass and increase the risk of osteoporotic fracture later in life. Later on the message was softened [11].

However, little is known about the interaction between HIV, ART, HC and bone metabolism [24]. The present study has found higher levels of two bone turnover markers in HIV-positive adolescents on ART and at least 48 weeks of HC. β-CTx levels were significantly higher in the group with HC in comparison to the group without HC, though the HC group was about 1 year older than the other group; this significant difference was observed as well in the prospective sub-analysis of the of 11 HIV-positive adolescents. Levels of P1NP were also higher in the group with HC compared to the group without HC; however, the difference was not statistically significant. The association became stronger in the longitudinal sub-analysis of the 11 HIV-positive adolescents with pre- and post-HC data. Bone turnover markers, among which are β-CTx for bone resorption and P1NP for bone formation, are gaining in importance for monitoring responses to osteoporosis treatments thath inhibit bone turnover. The extent to which these markers can be used as predictors for fracture risk is unclear [28] and reference ranges are currently being established [25,26]. Corroborating other reports, 25-OH vitamin D levels were significantly higher with HC [29]. This is likely to be due to the steroidal nature of 25-OH vitamin D and its common origin with sex steroid hormones from cholesterol. There are conflicting reports regarding the relationship between 25-OH vitamin D levels and bone turnover markers [30,31].

The TG and D-dimer levels were significantly different between groups in our study. TG were lower in the group with HC, which could possibly derive from the lower use of PIs in this group. D-dimer, in contrast to other studies, was lower in the HC group [32]. The duration of ART, which could influence D-dimer levels, was comparable between the two groups [33].

The main limitation of this pilot study is its small sample size and the cross-sectional nature of the study. Nevertheless, it does contribute data that are lacking in the adolescent Asian population. We have described here the high prevalence of metabolic abnormalities in Thai HIV-positive adolescents on ART, and shown that differences of bone turnover markers and insulin resistance could occur with HC in this population. The interaction between HIV, ART and HC may have long-term deleterious effects. As HC is being increasingly prescribed for adolescents with the potential for many years of use during their reproductive years [4,5,] there is an urgent need for additional research to determine the nature and extent of metabolic abnormalities associated with HC use in the setting of HIV and ART, in order to devise strategies to prevent and mitigate long-term comorbidities. In addition, the overall high prevalence of metabolic abnormalities in young HIV-positive women requires special attention and comprehensive care [34].

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Conflict of interests

The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above. The authors have no conflict of interest. The study does not have a clinical trial registration number.

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