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Follow-up of phenotype and metabolic changes in adult males living with HIV on combination antiretroviral therapy (CART) for a decade

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Combination Antiretroviral Therapy (CART) is the mainstay in the treatment of HIV infection. However, long-term CART causes fat redistribution, insulin resistance, and dyslipidaemia in patients living with HIV (PLWH). There is limited data from India that have studied these features exclusively in males. In this cross- sectional, observational study, male PLWH (*n* = 29; mean age: 45.5 years) treated on CART for a decade and above were recruited by purposive sampling method. Anthropometry and skinfold thickness were measured. Body composition analysis was done by DXA. Surrogate indices of insulin resistance (HOMA-IR, HOMA-Beta, and FG-IR) and insulin sensitivity (Mcauley index and QUICKI) were calculated. At end of the study period, Zidovudine-based ART accounted for 54.57% while Stavudine and Tenofovir based ART accounted for 28.37% and 17.05% respectively. Male PLWH on CART for more than a decade featured increased body weight, higher body fat percentage, total body fat mass higher abdominal adiposity and increased fat mass in upper limbs alongside, Furthemore, reduced beta cell function, impaired glucose tolerance, insulin resistance and lower testosterone levels were observed. The proportion of PLWH with diabetes, prediabetes and non-diabetic individuals were (20.6% *n* = 6), (34.5%; *n* = 10) and (44.8%: *n* = 13) respectively.

The Human Immuno-Deficiency Virus (HIV) infection is a significant global health challenge with socio-economic implications. According to the UNAIDS report of the year 2022, globally 38.4 million people were living with HIV (PLWH), 1.5 million people were newly infected with HIV and HIV related mortality was 650 000 in the year 2021. Notably, 28.7 million people accessed antiretroviral therapy in the same year[1]. A joint report from the Indian Council of medical research, the Ministry of health and family welfare and the National Aids Control Organisation (NACO) in India reported a total of 2.4 million PLWH, including 2.33 million adults, 1.05 million women, and 1.7 lakh young people aged between 15 and 24 years in the year 2021[2]. It is important to note that the prevalence of HIV infection and its associated mortality is higher in males than in females[3].

The Antiretroviral therapy (ART) in the treatment of HIV infection has improved life expectancy in PLWH. Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) inhibit viral replication but commonly induce mitochondrial toxicity[4]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have distinct effects against viral load but it may have neurologic and psychiatric side effects[5]. Integrase strand transfer inhibitors (INSTIs) are well-tolerated with neutral effects on cholesterol and triglycerides, making them preferred as first or second-line ART[6].

Fat redistribution syndromes including peripheral wasting, central adiposity and facial fat atrophy are common in PLWH on ART, with reported rates of upto 64% after one year of treatment[7]. In PLWH, the risk factors for lipodystrophy include the duration of ART, class of ART drugs used like NRTIs and protease inhibitors, low CD4 cell count, and high HIV-RNA levels prior to initiation of ART. In addition, the persistence of HIV load in tissue

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reservoirs can synergistically contribute to metabolic disorders in PWLH on ART[8].This includes lipoatrophy, lipohypertrophy[9], dyslipidaemia and disruptions in glucose metabolism, leading to increased cardiovascular risk[10] and accelerated senescence[11].

Worldwide, the prevalence of diabetes has increased exponentially over the last 25 years and nearly 1.31 billion people may develop diabetes by the year 2050[12]. Ethnically, the Indian population exhibits a phenotype of higher fat mass and visceral fat, along with lower muscle mass, and higher propensity for increased insulin resistance, hyperinsulinemia, and dyslipidaemia[13].

In patients living with HIV (PLWH) and on Combination antiretroviral therapy (CART), the burden of diabetes is a relative issue. A previous study conducted at our centre compared male patients with HIV infection (PLWH) receiving ART with ART-naïve HIV-infected individuals and BMI-matched HIV-negative, healthy controls. The study findings revealed that HIV-positive patients on CART had insulin resistance, impaired glucose tolerance, and dyslipidaemia along with a lipodystrophic habitus[14]. However, the long-term implications of CART on the phenotypic and metabolic profile in the same cohort had not been thoroughly investigated. Therefore, the aim of this study was to assess the long-term effects of CART on measures of body composition, surrogate indices of insulin resistance, insulin sensitivity, and lipid profile in male PLWH on CART.

Methods

This cross-sectional, observational study was approved by the Institutional Review Board (IRB) for Ethics in Research on Humans (IRB # 9405 dated 02 April 2015) at Christian Medical College Vellore, India. It was conducted to follow up male PLWH who were previously recruited from the department of infectious diseases of the institution between the years 2007–2008. The details of this study have been published earlier. Based on a previous study in HIV patients with lipodystrophy done at the same centre[14], the sample size of 31 subjects was determined with 80% power and a 5% significance level to detect an increase in fasting glucose from a baseline of approximately 102 units to 126 units using a paired student *t*-test at the 0.05 alpha level. Male PLWH aged between 30 and 60 years who were recruited in the previous study between the years 2007–2008[14] and on active CART till the end of study period were followed up in the year 2016 and included in the study by purposive sampling method. In the primary study, male PWLH with liver disease (serum alanine aminotransferase > 40 U/L) or renal failure (serum creatinine > 1.5 mg/dL) and those receiving anabolic steroids or glucocorticoids at the time of the study were excluded. Furthermore, PLWH diagnosed with diabetes prior to the start of the study and those with acquired immunodeficiency syndrome (AIDS)-defining illnesses within 3 months before screening were excluded[14].

The primary objective of the current study was to assess changes in body composition in a cohort of HIV-infected subjects including those initially diagnosed with lipodystrophy and living with Antiretroviral therapy (PLWH) for a decade and to compare their anthropometric parameters and body composition with age and BMI-matched, HIV negative heathy individuals as controls. The secondary objective was to evaluate the incidence and progression of metabolic abnormalities such as glucose intolerance and insulin resistance measured through surrogate indices namely the Homeostasis Model of Assessment of Insulin Resistance (HOMA-IR) and the Quantitative insulin-sensitivity check index (QUICKI) and the McAuley index. This assessment was done in HIV patients treated with combination antiretroviral therapy (CART) and compared with age and BMI-matched, healthy individuals as controls. We excluded patients with severe opportunistic infection and those with chronic hepatic or renal disease. Informed consent was obtained from all patients and confidentiality of study data was maintained at all stages of the study. The study protocol conformed to the guidelines of the Declaration of Helsinki 2013 and the ARRIVE guidelines.

Anthropometric measures namely the Body Mass Index (BMI) and the waist-to-hip ratio (WHR) were recorded. Weight was measured in kilograms using a digital weighing scale (Tulaman, HT 500 series, India) and height was measured in centimetres using a wall mounted stadiometer (Harpenden stadiometer, Holtain Limited, Dyfed, UK). Skinfold thickness at 4 sites (triceps, biceps, subscapular, and the supra-iliac region) were measured using a Harpenden calliper (Model # HSB-B1, Baty International, England). Biceps skinfold thickness was measured at the anterior surface of the biceps midway between the anterior axillary fold and the antecubital fossa. Triceps skinfold thickness was measured at the midpoint between the acromion and olecranon processes on the posterior side with the arm hanging by the side. Subscapular region skinfold thickness was measured below the inferior angle of the scapula. Waist circumference was measured with a non-elastic tape while participants stood in a relaxed state, and the measurement was taken midway between the iliac crest and the lowermost rib margin. Hip circumference was measured at the widest point of the gluteus maximus muscle. Waist circumference ≥ 90 cm was defined as abdominal obesity in males as per the National Institute for Health and care excellence (NICE) guidelines for South Asians[15].

Clinical assessment of lipodystrophy and body composition analysis

Clinical data of male PLWH on CART was obtained from the electronic databases of the institution. Clinical details such as duration of HIV, disease status, type and duration of antiretroviral therapy, the incidence of opportunistic infections, the presence and severity of lipodystrophy were obtained. The male PLWH available at the end of study period were compared to age and BMI matched controls for body composition profile, metabolic parameters, and surrogate indices of insulin resistance/sensitivity namely the HOMA-IR, the QUICKI and the McAuley index. We used the HIV outpatient study (HOPS) cohort severity scale to quantify lipodystrophy[16]. All participants underwent whole-body composition analysis on a Dual-energy X-ray Absorptiometry (DXA) scanner (Hologic DEXA Discovery QDR 4500). Bilateral sections and whole-body composition data including total body fat percentage, fat mass, lean mass, and truncal fat were obtained by analysing the regions of interest (ROI) using APEX software (Version 4.0.2). The coefficient of variation (CV) was 4%. The Hologic scanner has high precision and can detect small differences in lean mass and fat mass of 1–2% between two measurements

at 95% Confidence Interval[17]. With reference to our previous study in the same group[14], the indices of acquired lipodystrophy with cut-off values namely the Trunk fat/lower limb fat mass ratio (Cut off value > 2.28), the Trunk fat/total limb fat mass ratio (Cut off value > 1.68), the Triceps SFT (mm)/BMI ratio Cut-off value : ≤ 0.49, and the Abdominal skinfold thickness/triceps Cut-off value > 1.385) were calculated for PLWH on CART.

Biochemical and metabolic assessment

Venous blood samples were obtained from the participants following a 10-hour overnight fast for the biochemical assessment of fasting lipid profile, fasting insulin, glucose, serum calcium, phosphate, albumin, alkaline phosphatase levels, 25 OH vitamin D levels, blood haemoglobin, mean corpuscular volume (MCV), and CD4 count. Plasma glucose levels were measured using Glucose-hexokinase method. Serum total cholesterol, Low density lipoprotein cholesterol (LDL-C), High density lipoprotein cholesterol (HDL-C), and triglycerides were assessed by enzymatic colorimetric assay on an Olympus AU 2700 autoanalyzer (Olympus Optical Co., Ltd Japan). CD4 counts were estimated using flow cytometry (BD Biosciences fluorescence-activated cell sorter). Analytes including serum calcium (8.3–10.4 mg/dL), phosphorus (2.5–4.6 mg/dL), alkaline phosphatase (40–125 U/L), albumin (3.5–5.0 g/dL), serum creatinine (0.5–1.4 mg/dL), 25-hydroxyvitamin D [25(OH)D], and total testosterone (300-1,030 ng/dL) were measured using a fully automated and computerized microanalyzer. The normative range of each analyte is presented in parentheses. The intra-assay and inter-assay coefficients of variation ranged from 1 to 5%. To assess insulin resistance/sensitivity, fasting and 120 min post-prandial blood samples were collected for estimation of blood glucose as standard procedures[18]. Surrogate indices of insulin sensitivity/resistance were calculated using the following formula/equation:

1) HOMA–IR (Homeostasis Model of Assessment of Insulin Resistance) = (Fasting Insulin x Fasting glucose)/22.5[19].
2) HOMA-1% β (Homeostasis Model Assessment Beta) = (20 x Fasting Insulin)/(Fasting glucose − 3.5)[20].
3) QUICKI (Quantitative Insulin Sensitivity Check Index) = 1/[(log Fasting insulin) + (log fasting glucose)][21].
4) The McAuley index was calculated using the equation: Exponential of 2.63 − 0.28 x logarithm (fasting serum insulin (IU/ml)−0.31 x logarithm of (triglycerides (mmol/L)[22].

Statistical analysis

Data of continuous variables were checked for normal distribution. Paired samples *t*-test was applied to compare the means of anthropometric measures, body composition, biochemical parameters, and insulin resistance indices. Spearman correlation was applied to test for significance in association between CD4 count and independent variables. To test for the longitudinal effects of ART on indices of insulin resistance/sensitivity in male PLWH at the end of study period, the mixed model effect analysis was applied. Interaction terms were dropped from final models if they were not statistically significant at an alpha level of 0.05. Statistical Package for Social Sciences for Windows (SPSS Inc. version 21.0, Armonk, NY, USA) was used for analysis.

Results

In this study involving male PLWH on CART (mean age 45.5 ± 5.2 years at end of the study period), the mean duration of HIV infection was 11.6 ± 3.9 years and the mean duration of CART was 8.3 ± 2.4 years. The median age at diagnosis of HIV infection was 33 years. The treatment regimen comprised of a combination of two nucleoside HIV-1 reverse transcriptase inhibitor analogues (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI). None of the patients were on Protease inhibitors at any point of time. Out of 31 patients on CART recruited at baseline, fourteen patients were initially on a combination of Stavudine, Lamivudine and Nevirapine and then switched over to other ART drugs. Ten patients (26.6%) were on a combination of Zidovudine, Lamivudine, Nevirapine from baseline till the end of study. Two patients (6.6%) were initially on zidovudine, lamivudine and nevirapine and later changed to other ART drugs while one patient was on Zidovudine, Lamivudine and Efavirenz at end of study period. One patient was on Tenofovir, Disoproxil, and Lamivudine from baseline till the end of the study period while another patient was on Tenofovir, Lamivudine and Efavirenz at end of study period. Data on ART regimen was not available for two patients at the end of study period (Fig. 1**)**. Therefore, data of 29 patients was used for analysis.

At baseline, 55.1% ($n = 16/31$) of HIV patients did not feature lipodystrophy. At end of study period (median age: 45 years), 8 patients (27.5%) manifested lipodystrophy on face and 4 patients (13.8%) manifested lipodystrophy on the limbs. The degree of lipodystrophy worsened in 5 patients, while no worsening was observed in 9 patients. However, 6 patients (20.6%) did not show any indications of lipodystrophy.

Indices of acquired lipodystrophy

The surrogate indices of lipodystrophy were compared at baseline and at the end of the study period. The proportion of PWLH with Trunk fat/lower limb fat mass ratio > 2.28 was significantly higher at baseline. However, the mean values of Trunk fat/total limb fat mass ratio and abdominal skinfold/triceps skinfold thickness ratio did not differ significantly between baseline and at the end of study period as shown in Supplementary Table 1. Based on the duration of ART, the proportion of individuals with Trunk fat/lower limb fat mass ratio > 2.28 was significantly higher at baseline in PWLH on CART for more than ten years. However, the proportion of PLWH with Trunk fat/total limb fat mass ratio > 1.68 was higher at end of study period in those on CART for more than ten years. Furthermore, the proportion of male PWLH with Triceps skinfold thickness/BMI ratio ≤ 0.49 and Abdominal skinfold/Triceps skinfold thickness ratio > 1.38 were higher at end of study period in those on CART for more than 10 years as shown in Supplementary Table 2.

Amongst anthropometric measures, the mean values of waist and hip circumferences and waist-to-hip ratio were significantly higher at the end of end of study period, when compared to the baseline. However, no

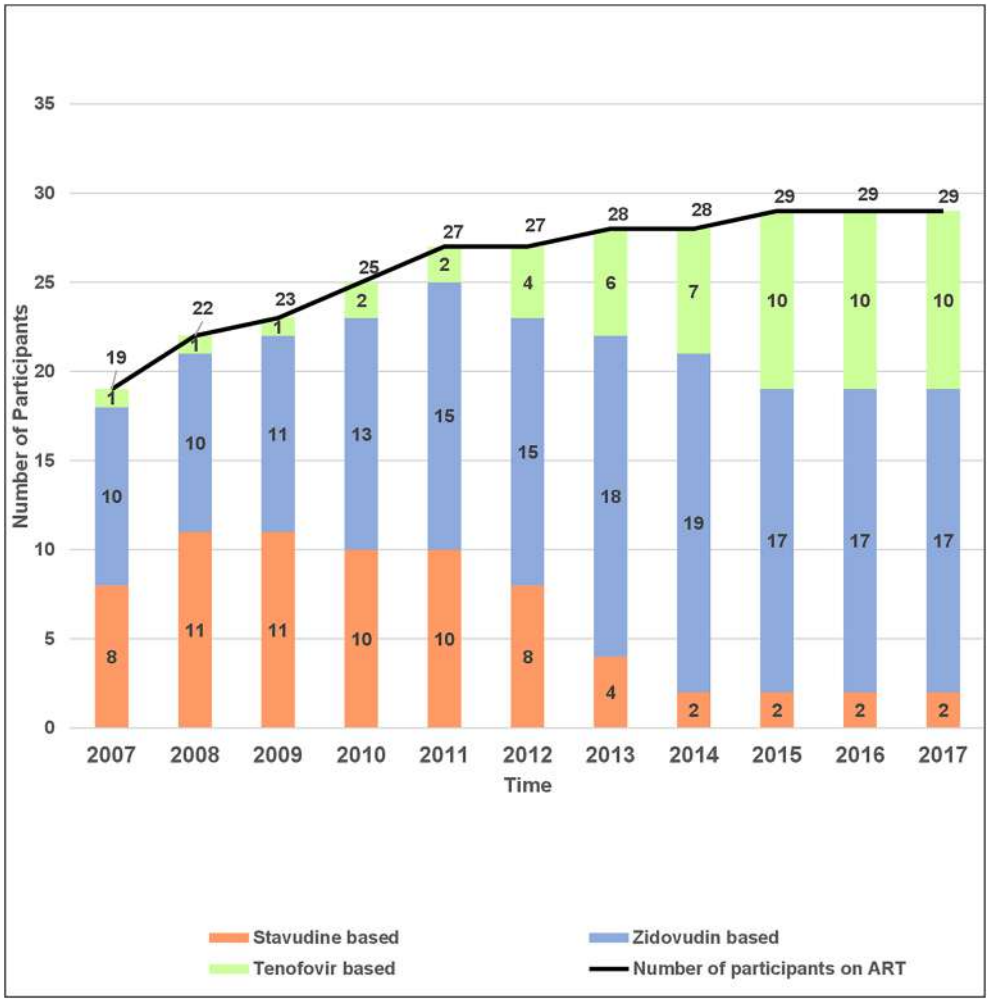


Fig. 1. Distribution of the anchor drug used in the Anti-Retroviral Therapy (ART) regimen in male PWLH for the study period.

significant change was noted for the mean value of BMI. The mean values of skinfold thickness of triceps showed a statistically significant decrease ($p < 0.01$), whereas the mean value supra-iliac skinfold thickness showed a statistically significant increase ($p < 0.01$) at the end of the study period. There was a trend towards an increase in abdominal and subscapular skinfold thickness, but did not reach statistical significance. Amongst the measures of body composition assessed on DXA, the mean values of total body fat mass ($p < 0.05$), lean mass ($p < 0.01$), total body weight ($p < 0.01$), and total fat mass in upper limbs ($p < 0.01$) were significantly higher at end of study period, when compared to baseline values. Specifically, there was an incremental trend in truncal fat mass at end of study period, though not statistically significant. It may be noted that the mean body fat percentage, truncal fat percentage and fat mass in legs did not differ significantly when compared to baseline, as shown in Table 1.

The male PLWH on CART manifested metabolic derangements. As for the surrogate indices in PLWH, the mean value of the McAuley index was significantly lower at end of study period. However, the mean values of HOMA-IR and QUICKI did not differ significantly from baseline till the end of study period. Furthermore, the mean values of fasting and post prandial blood glucose levels were significantly higher whereas the mean value of serum phosphorus and testosterone were significantly lower at end of study period when compared to baseline values. However, the mean value of blood haemoglobin, MCV at end of study period did not differ significantly when compared to the mean values of the same at baseline. Notably, the mean value of CD4 count was significantly higher at end of study period when compared to baseline in PLWH on CART as shown in Table 2.

Out of 29 patients followed-up at the end of the study period, the proportion of male PLWH with diabetes, prediabetes and non-diabetic individuals were ($n = 6$; 20.6%), ($n = 10$; 34.5%) and ($n = 13$; 44.8%) respectively. At baseline, 3 patients were diagnosed of diabetes (10.3%) and 6 patients (20.6%) were diagnosed of prediabetes, while 20 patients (64.5%) were non-diabetic as shown in Fig. 2.

Variables	Male PLWH on CART at baseline ($n=31$)	Male PLWH on CART at the end of study period ($n=29$)	p-value
Body Mass Index (kg/m^2)	23.1 ± 3.1	23.3 ± 3.7	0.57
Waist circumference (cms)	83.5 ± 9	89.6 ± 11.5	< 0.01
Hip circumference (cms)	89.6 ± 6.3	91.8 ± 7.4	< 0.05
Waist Hip ratio	0.92 ± 0.05	0.97 ± 0.07	< 0.01
Mid-arm circumference (cms)	26.5 ± 2.6	25.8 ± 2.2	0.10
Skinfold thickness			
Triceps (mms)	10.1 ± 3.6	7.0 ± 3.7	< 0.01
Subscapular (mms)	20.2 ± 11.2	21.4 ± 12.5	0.33
Abdomen (mms)	18.8 ± 7.4	20.4 ± 5	0.50
Suprailiac (cms)	1.0 ± 0.5	1.7 ± 0.3	< 0.01
Triceps skinfold/BMI ratio	0.42 ± 0.12	0.29 ± 0.16	< 0.05
Abdomen skinfold/triceps ratio	2.0 ± 0.6	3.3 ± 2.8	< 0.05
Body composition on DXA			
Total body weight (kgs)	60.9 ± 9.8	64.6 ± 11.7	< 0.01
Lean mass (kgs)	45. 6 ± 5.9	47.8 ± 7.0	< 0.01
Body Fat (%)	21.2 ± 6.3	22.4 ± 4.7	0.17
Total fat mass (kgs)	13.2 ± 5.6	14.9 ± 5.2	< 0.05
Fat mass in trunk (kgs)	7.4 ± 3.7	8.1 ± 3.5	0.07
Fat mass in limbs (gms)	646.8 ± 321.4	853.4 ± 353.5	< 0.01
Fat (%) trunk	23.9 ± 7.7	24.3 ± 5.9	0.67
Fat mass in upper limbs (kgs)	1.2 ± 0.6	1.7 ± 0.7	< 0.01
Fat mass in lower limbs (kgs)	3.7 ± 1.5	3.9 ± 1.4	0.46
Trunk fat/lower limb fat mass ratio	2.13 ± 0.7	2.18 ± 0.11	0.77

Table 1. Anthropometric and body composition profile in all PLWH patients on CART at baseline and at end of study period. PLWH: Patients living with HIV. Values are presented as Mean ± SD. p value < 0.05; Statistically significant.

Comparison of cases with controls

The male PLWH on CART (cases) were compared with age and BMI matched, healthy HIV negative males (controls). The mean body weight of cases, mid-arm circumference and skinfold thickness of triceps were significantly lower in cases when compared to controls. In contrast, an increase in the mean values of suprailiac skinfold thickness and waist circumference was noted in cases indicating truncal adiposity. However, no significant differences were observed for waist-to-hip ratio and waist and hip circumferences when compared with controls. Body composition analysis on DXA demonstrated significantly lower mean values for fat mass and fat percentage in lower limbs in cases. Notably, no significant differences were observed for fat mass and fat percentage in the arms and truncal region when compared to controls. The mean values of fasting glucose and fasting insulin did not differ significantly between cases and controls. Notably, the mean value of the McAuley index, serum testosterone, total cholesterol and HDL-cholesterol were significantly lower in cases, when compared to controls. However, no significant differences were observed for surrogate indices namely the HOMA-IR and the QUICKI as shown in Table 3.

Comparisons based on duration of CART

We categorised the participants of the study based on the duration of CART as those with less than, and as those with more than 10 years of CART. In patients on CART for less than 10 years, the mean values of waist to hip ratio, and total lean mass were significantly higher when compared to baseline. The mean values of waist circumference and truncal fat mass were 6.4 cm, 0.4 kg higher respectively at the end of study period. Amongst skinfold measurements, the mean values of subscapular skinfold thickness and suprailiac skinfold thickness were higher at the end of the study period. Furthermore, increase in fat mass in arms, and decrease in fat mass in the legs was also observed. Concomitantly, an increase in the mean value of truncal fat mass to total limb fat mass ratio and a decrease in total limb fat mass to total fat mass ratio was observed as shown in Table 4.

As for the biochemical parameters in male PLWH on CART for less than 10 years, the mean values of post prandial blood glucose, LDL cholesterol, total cholesterol and serum calcium were significantly higher than baseline mean values. The mean value of HDL cholesterol was higher at the end of the study period, though statistically not significant. Notably, participants in this group had elevated fasting blood glucose levels, insulin level, triglycerides alongside lower value of QUICKI and McAuley Index (surrogate measure of insulin sensitivity), indicative of insulin resistance.

In response to CART, the mean value of CD4 count was significantly higher at end of study period when compared to baseline. However, no changes were observed in the mean values of blood haemoglobin and serum creatinine. Notably, the mean values of serum phosphorus and testosterone were significantly lower when compared to baseline value as shown in Table 5.

Variables	Male PWLH at baseline; ($n=31$)	Male PWLH at the end of study period; ($n=29$)	p-value
Fasting Glucose (mg/dL)	97.1 ± 14.8	111.5 ± 44.4	< 0.05
Post prandial Glucose(mg/dL)	130.9 ± 41	163.9 ± 90.3	< 0.05
Fasting insulin (µU/L)	13.5 ± 9.2	11.2 ± 6.9	0.15
Total cholesterol (mg/dl)	188.4 ± 53	199.2 ± 43.5	0.177
Triglycerides (mg/dl)	207.9 ± 116.8	213.8 ± 99.6	0.79
HDL cholesterol (mg/dl)	38.3 ± 8.6	41.6 ± 12.0	0.16
LDL cholesterol (mg/dl)	113.5 ± 39.6	120.4 ± 28.6	0.24
HOMA-IR	3.5 ± 2.5	3.2 ± 2.5	0.45
QUICKI	0.3 ± 0.04	0.3 ± 0.04	0.60
HOMA Beta	149 ± 102	113.2 ± 104	0.05
McAuley index	9.4 ± 1.4	5.2 ± 0.7	< 0.01
Albumin (gm/dL)	4.6 ± 0.3	4.7 ± 0.3	0.06
Calcium (mg/dL)	9.0 ± 0.4	9.2 ± 0.3	< 0.05
Phosphorus (mg/dL)	3.8 ± 0.6	3.4 ± 0.5	< 0.05
Vitamin D (ng/ml)	19.3 ± 8.8	20.0 ± 13.7	0.64
Alkaline Phosphatase (U/L)	84.8 ± 21	98.6 ± 30.0	0.04
Testosterone (ng/dL)	490 ± 204.3	344 ± 54	< 0.001
Parathyroid hormone (pg/ml)	52.9 ± 25	53.3 ± 25	0.94
Blood haemoglobin (g/dL)	14.0 ± 2.0	14.0 ± 1.1	0.92
Mean Corpuscular Volume (fL)	97.8 ± 15.9	105.8 ± 13.4	0.12
CD4 count (cell/mm^3)	510.9 ± 243.7	810.6 ± 290.5	< 0.001
Serum creatinine (mg/dL)	0.94 ± 0.1	0.93 ± 0.1	0.95

Table 2. Biochemical profile in male PLWH on CART at baseline and at end of study period. PLWH: Patients living with HIV. Values are presented as Mean ± SD. p value < 0.05; Statistically significant.

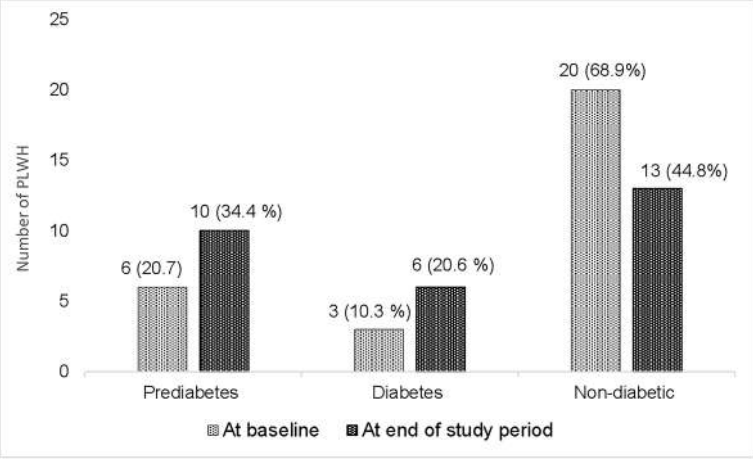


Fig. 2. Proportion of male PWLH with prediabetes/diabetes at baseline and at the end of study period.

In male PLWH on CART for more than 10 years, the mean body weight had increased by 3.8 kg (6.5%) at the end of study period. The mean values of waist circumference, waist to hip ratio and supra-iliac skinfold thickness were significantly higher, whereas the mean value of triceps skinfold thickness was significantly lower at end of study period, when compared to baseline. Amongst the measures of body composition, the mean values of total fat mass, truncal fat mass, fat mass in the right and the left arm and fat mass in the left leg were significantly higher at end of study period, when compared to baseline. Notably, no statistically significant changes were observed in mean values of BMI, mid arm circumference, subscapular skinfold and abdominal skinfold thickness as shown in Table 6.

Amongst biochemical variables, the mean values of fasting and post prandial blood glucose were higher at the end of study period in male PLWH on CART for more than 10 years. Notably, the mean values of the HOMA-IR Beta (surrogate index of beta cell function) and the McAuley index (surrogate index of insulin sensitivity) were significantly lower at end of study period when compared to baseline value in this group of male PLWH.

Variables	Male PLWH on CART (Cases; $n=29$)	Healthy, HIV negative males (Controls, $n=31$)	*P* value
Age (years)	45.77 ± 4.7	45.2 ± 6.2	0.66
Total body weight (kgs)	64.3 ± 11.3	71.1 ± 13.8	< 0.05
Body Mass Index (kg/m^2)	23.31 ± 3.7	24.84 ± 4.1	0.10
Waist circumference (cms)	89.6 ± 11.5	93.4 ± 12.1	0.18
Hip circumference (cms)	91.7 ± 7.4	94.3 ± 9.4	0.20
Waist Hip ratio	0.97 ± 0.07	0.98 ± 0.06	0.42
Mid-arm circumference (cms)	25.8 ± 2.1	31.6 ± 3.0	< 0.01
Skinfold thickness			
Biceps (mms)	5.6 ± 2.8	7.2 ± 4.2	0.07
Triceps (mms)	7.0 ± 3.6	14.7 ± 7.2	< 0.01
Subscapular (mms)	21.3 ± 21.5	18.3 ± 7.1	0.33
Suprailiac (cms)	1.6 ± 1.3	0.99 ± 0.5	< 0.01
Body composition on DXA			
Fat left arm (grams)	853.4 ± 353.5	963.7 ± 407.8	0.23
Fat % left arm	21.8 ± 5.8	22.2 ± 6.3	0.36
Fat right arm (grams)	905.45 ± 387.5	1006.97 ± 409.1	0.29
Fat % right arm	21.2 ± 5.3	21.9 ± 6.2	0.36
Fat left leg (kgs)	1.9 ± 0.6	3.0 ± 1.2	< 0.01
Fat % left leg	19.4 ± 5	24.6 ± 6.6	< 0.01
Fat right leg (kgs)	1.9 ± 0.71	3.1 ± 1.2	< 0.01
Fat % right leg	18.9 ± 5.08	25.0 ± 6.95	< 0.01
Fat trunk (kgs)	8.1 ± 3.4	8.0 ± 3.5	0.85
Fat % trunk	24.3 ± 6	22.3 ± 6.3	0.19
Biochemical variables			
Fasting glucose (mg/dL)	111.4 ± 44	100.4 ± 10.3	0.73
Fasting plasma insulin (µU/ml)	11.22 ± 6.8	11.7 ± 8	0.77
Total cholesterol (mg/dL)	198 ± 41.7	173.4 ± 34.8	< 0.05
HDL- Cholesterol (mg/dL)	41.7 ± 11.8	36.0 ± 4.8	< 0.05
LDL- Cholesterol (mg/dL)	119.7 ± 27.8	114.5 ± 28.8	0.47
Triglycerides (mg/dL)	217 ± 101.4	173.8 ± 110.1	0.11
Testosterone (ng/dl)	343.3 ± 53.1	548.4 ± 182.5	< 0.01
Surrogate indices			
HOMA-IR	3.2 ± 2.5	3.7 ± 3.6	0.50
QUICKI	0.34 ± 0.0	0.34 ± 0.0	0.97
McAuley index	5.2 ± 0.7	11.7 ± 1.7	< 0.01

Table 3. Comparison of anthropometric and body composition profile in cases and controls. PLWH: Patients living with HIV. Values are presented as Mean ± SD. p value < 0.05; Statistically significant.

Furthermore, the mean value of serum testosterone was significantly lower whereas the mean values of CD4 count and blood corpuscular volume were significantly higher at the end of study period, when compared to baseline. The mean values of fasting insulin, total cholesterol, triglycerides and LDL- cholesterol were lower whereas the mean values of vitamin D and alkaline phosphatase were higher at the end of study period, though statistically not significant as shown in Table 7.

The mixed model affect analysis showed that in male PLWH on ART for more than a decade, the index of insulin resistance HOMA-IR and the index of beta cell function – HOMA beta changed significantly at the end of study period. Specifically, the beta coefficient of HOMA-IR was higher than that of HOMA-beta showing the increase in insulin resistance and significant decrease in beta cell function. The unit change in QUICKI index was comparatively lower when compared to the unit change in McAuley index as shown in Table 8.

In this study, the median age of male PLWH at the end of study period was 45 years. The median percentage of male PWLH with diabetes (20.6%; $n=6$) and prediabetes (34.4%; $n=10$) were higher at the end of the study period, when compared to the incidence of diabetes (10.3%; $n=6$) and prediabetes (20.6%; $n=6$) at baseline.

Spearman correlation analysis

At the end of study period, a significant negative correlation was noted between the nadir CD4 count at the end of study period and QUICKI (*rho* −0.43; $p<0.05$). In contrast, significant positive correlation was observed between nadir CD4 count and anthropometric measures namely BMI (*rho*: 0.55, $p<0.05$), waist circumference (*rho*: −0.43; $p<0.05$) and mid-arm circumference (*rho*: 0.46, $p<0.05$). Amongst measures of body composition,

Variables	Baseline profile of male PLWH on CART for less than 10 years ($n=11$)	Profile of male PLWH on CART for less than 10 years at the end of study period ($n=11$)	p-value
Body Mass Index (kg/m^2)	22.6 ± 3.9	23.1 ± 5.2	0.60
Waist circumference (cms)	82.8 ± 12.4	88.4 ± 16.0	0.06
Hip circumference (cms)	87.5 ± 8.5	90.4 ± 10.7	0.22
Waist to Hip ratio	0.93 ± 0.06	0.97 ± 0.09	< 0.05
Mid-arm circumference (cms)	26.3 ± 3.5	25.4 ± 2.5	0.15
Skinfold thickness			
Triceps (mms)	10.4 ± 1.3*	9.1 ± 1.8*	0.51
Subscapular (mms)	17.7 ± 3.8*	20.8 ± 4.1*	0.22
Abdomen (cms)	1.9 ± 0.2*	2.2 ± 0.5	0.61
Suprailiac (cms)	1.1 ± 0.2*	1.8 ± 0.4*	0.15
Abdomen/triceps ratio	1.97 ± 0.20*	3.26 ± 0.99*	0.56
Triceps/BMI ratio	0.44 ± 0.03*	0.38 ± 0.06*	0.42
Body composition on DXA			
Total body weight (kgs)	58.1 ± 13.6	61.9 ± 15.4	0.05
Lean mass (kgs)	42.0 ± 6.7	45.4 ± 9.6	< 0.05
Total body Fat (%)	23.0 ± 7.4	22.6 ± 4.6	0.80
Total fat mass (kgs)	58.1 ± 13.6	62.9 ± 7.2	0.09
Fat trunk (kgs.)	7.3 ± 4.8	7.7 ± 4.2	0.60
Fat (%) trunk	24 ± 9.1	23.8 ± 6.4	0.98
Fat mass (left arm) (gms)	719.3 ± 132.0*	799.± 105 *	0.30
Fat mass (right arm) (gms)	726.4 ± 146.5*	885.2 ± 134.2	0.07
Fat mass (left leg) (kgs)	2.3 ± 0.9	2.0 ± 0.9	0.23
Fat mass (right leg) (kgs)	2.2 ± 0.9	2.0 ± 0.6	0.07
Trunk fat mass/total limb fat mass ratio	1.15 ± 0.07	1.29 ± 0.11	0.34
Total limb fat/total fat mass ratio	0.43 ± 0.03	0.40 ± 0.05	0.18

Table 4. Anthropometric and body composition profile in male PLWH on CART less than 10 years at baseline and at the end of study period. PLWH: Patients living with HIV. Values are presented as Mean ± SD or Standard Error of Mean (SEM) indicated by *. p value < 0.05; Statistically significant.

significant positive correlation was observed between the nadir CD4 count at the end of study period and total fat mass (*rho*: 0.39; $p < 0.05$), fat in trunk region (*rho*: 0.39, $p < 0.05$) and fat mass in right arm (*rho*: 0.39; $p < 0.05$).

Discussion

In this study, male PWLH were on a combination of non-nucleoside reverse transcriptase inhibitors (NNRTIs) and two nucleoside analogues (NRTI) which were preferred treatment ART modalities for their unique antiviral activity, high specificity, and low toxicity[23]. In this study male PLWH on CART for more than 10 years featured abdominal obesity as demonstrated by higher mean values of waist circumference, waist-to-hip ratio and supra-iliac skinfold thickness at the end of study period. Furthermore, increased truncal and peripheral adiposity in arms and legs was also observed in this group. As for lipid markers, the mean values of LDL cholesterol and total cholesterol were significantly higher than the baseline mean values. This can be attributed to the cumulative exposure to NNRTIs and NRTI for a long duration probably causing hypercholesterolaemia as evidenced in a study on 1664 individuals with HIV including Caucasians, Black Africans and other ethnic groups[24]. An observational cohort study from Poland, evaluated the changes in lipid profile of 70 PLWH (males $n = 58$ females; $n = 12$) before implementation of ART and after 1 year of continuous ART. At the end of the first year of ART, significant increases in total cholesterol, LDL cholesterol, HDL cholesterol, and non-HDL cholesterol levels were observed when compared to the baseline lipid profile[25].However, this study did not evaluate for changes in measures of body composition or insulin resistance.

A meta-analysis of observational and interventional studies with comparable ART-treated and ART-naïve populations from sub-Saharan Africa demonstrated ART associated elevated total cholesterol, triglycerides, LDL-cholesterol, and lower HDL cholesterol cumulatively leading to increased cardiovascular risk. However, no significant association was observed between ART and risk of hypertension and diabetes in PWLH from the Sub-Saharan region[26].

Altered fat repartition, diagnosed as lipodystrophy, has been related to first-generation nucleoside-reverse-transcriptase-inhibitors (NRTIs) (Stavudine and Zidovudine) and some protease inhibitors[8].Fat deficiency in lipodystrophy leads to a combination of metabolic disturbances including insulin resistance, hypertriglyceridemia, and ectopic fat accumulation[27] eventually resulting in increased risk of cardiovascular diseases in HIV patients on CART. Globally, this burden is anticipated to increase, given the rapid scale-up of ART regimens and increasing lifespan of HIV-infected patients on long-term ART therapy[28].

Variables	Baseline biochemical profile of male PLWH on CART for less than 10 ($n=11$)	Biochemical Profile of male PWLH on CART for less than 10 years at end of study period ($n=11$)	p-value
Fasting Glucose (mg/dL)	95.1 ± 15.3	104.3 ± 33.8	0.27
Post prandial Glucose(mg/dL)	118.6 ± 48	146.6 ± 63.4	< 0.05
Fasting insulin (µU/L)	8.7 ± 2.1*	9.5 ± 2.1*	0.60
Total cholesterol (mg/dL)	144.5 ± 27.2	191.3 ± 30.1	< 0.05
Triglycerides (mg/dL)	188.1 ± 112	207.9 ± 80.1	0.55
HDL cholesterol (mg/dL)	30.5 ± 4.6	39.9 ± 14.9	0.07
LDL cholesterol (mg/dL)	89.2 ± 27.5	116.5 ± 16.5	< 0.05
QUICKI	0.36 ± 0.01	0.35 ± 0.01	0.20
HOMA -IR Beta	1.7 ± 0.3	1.5 ± 0.2	0.16
McAuley index	8.8 ± 1.0	5.0 ± 0.6	< 0.01
Albumin (gm/dL)	4.3 ± 0.4	4.6 ± 0.3	0.15
Calcium (mg/dL)	8.8 ± 0.4	9.2 ± 0.2	< 0.05
Phosphorus (mg/dL)	4.2 ± 0.6	3.2 ± 0.4	< 0.01
Vitamin D (ng/ml)	26.4 ± 7.3	25.5 ± 19.9	0.88
Alkaline Phosphatase (U/L)	82.1 ± 21.5	104.2 ± 38.6	0.14
Testosterone (ng/dL)	490.6 ± 138.1	339.7 ± 57.3	< 0.05
Parathyroid hormone (pg/ml)	41.0 ± 4.3*	52.2 ± 9.5*	0.29
Blood haemoglobin (g/dL)	14.3 ± 1.6	14.3 ± 1.0	0.85
Mean Corpuscular Volume (fL)	105 ± 10	107 ± 15	0.70
CD4 count (cell/mm^3)	253.2 ± 57.0*	758 ± 88.1*	< 0.01
Serum creatinine (mg/dL)	0.95 ± 0.11	0.95 ± 0.1	1.0

Table 5. Biochemical profile in male PLWH on CART less than 10 years at baseline and at end of study period. PLWH: Patients living with HIV. Values are presented as Mean ± SD or Standard Error of Mean (SEM) indicated by *. p value < 0.05; Statistically significant.

Lipodystrophy can manifest as two distinct phenotypes: fat accumulation (lipo-hypertrophy) or fat loss (lipoatrophy). Lipo-hypertrophy occurs in the truncal areas and manifests as abdominal obesity, mammary hypertrophy, accumulation of fat on the neck, or lipomas. In contrast, lipoatrophy occurs on the face, gluteal regions, arms, and legs. In some patients, both these manifestations may coexist[29]. The clinical lipoatrophy syndrome in CART-treated patients emanates from specific adipocyte and stromal vascular cell damage[30] and differs significantly from the "wasting syndrome" The wasting syndrome primarily arises from a profound caloric deficit causing loss of muscle mass instead of fat[23,29].

Globally, the age-standardised total prevalence of diabetes was higher in males (6.5%) when compared to females (5.8%) between the years 1990 to 2021[12]. Amongst Asian Indians males aged between 45 years to 49 years, the prevalence of diabetes in India increased from 8.8% in the year 1990 to 11.1% in the year 2016[31].

A previous cohort study from the same institution had demonstrated a high prevalence of cardiometabolic risk markers in urban and rural cohorts of the general population. Specifically, in individuals aged between 29 and 42 years, the prevalence of overweight/obesity increased from 17 to 51%; that of type 2 diabetes from 3 to 16%; that of hypertension from 2 to 20% and that of hypertriglyceridemia from 16 to 30%[32].

A previous study in HIV patients on ART for 12 months ($n=30$) and ART naive HIV patients ($n=30$), reported the incidence of diabetes in HIV patients on ART as 20%[33]. In comparison to the former study[33], the incidence of diabetes in PLWH aged above 45 years had increased from 10.3% at baseline to 20.6% at the endpoint as shown in this study. Notably, islet cell autoimmunity or beta cell destruction has not been evidenced in HIV patients[34].

Insulin resistance in PLWH on CART has been studied in other populations. A cross-sectional study by Freitas and colleagues from Portugal, compared 217 clinically stable HIV-infected male and female patients receiving cART for 3 years against 74 BMI matched controls. It was noted that HIV-infected patients had significantly higher waist/hip ratio, fasting glucose, triglyceride, insulin and HOMA-IR indicative of dysglycemia[35]. A study by Bigolonia and colleagues in PLWH ($n=39$) on CART for three years demonstrated a consistent increase in insulin resistance and a concomitant decrease in insulin secretion. All patients had impaired fasting glucose at baseline, which worsened during the course of ART, irrespective of the type of ART regimen[36].

In a multiethnic study involving 327 HIV-infected and 3240 HIV-uninfected subjects aged between 35 and 70 years, reported that nucleoside and non-nucleoside reverse transcriptase inhibitor therapy were associated with a higher risk of diabetes in PLWH. HIV infection by itself was not the risk factor for diabetes, but the cumulative effect of increasing age, BMI and continued CART, leading to the differential level of immune activation and inflammatory response in HIV infected when compared to uninfected persons[37].Weight gain is common in PLWH on ART and may be attributed to a reduction in inflammation-related catabolism[38], the composition of ART regimen and demographic factors[39]. In this study, the mean values of total body mass, waist circumference, waist-to-hip ratio, truncal fat mass, and fat in upper limbs and total fat mass were significantly

Variables	Baseline profile of male PLWH on CART for more than 10 years($n=18$)	Profile of male PLWH on CART for more than 10 years, at the end of study period ($n=18$)	p-value
Body Mass Index (kg/m^2)	23.6 ± 2.5	23.5 ± 3.2	0.77
Waist circumference (cms)	84.5 ± 6.1	91.1 ± 9	< 0.01
Hip circumference (cms)	90.7 ± 5.0	92.2 ± 4.9	0.07
Waist Hip ratio	0.93 ± 0.02	0.97 ± 0.06	< 0.01
Mid-arm circumference (cms)	26.8 ± 1.8	26.2 ± 1.9	0.11
Skinfold thickness			
Triceps (cms)	0.9 ± 0.3	0.6 ± 0.1	< 0.01
Subscapular (cms)	2.1 ± 1.1	2.3 ± 1.2	0.45
Abdomen (cms)	1.8 ± 0.6	1.9 ± 1.3	0.70
Suprailiac (cms)	1.1 ± 0.5	1.8 ± 1.3	< 0.05
Abdominal/triceps ratio	2.02 ± 0.16	3.31 ± 0.61	< 0.05
Triceps/BMI ratio	0.40 ± 0.12	0.24 ± 0.24	0.27
Body composition measures			
Total body weight (kgs)	62.9 ± 7.2	65.8 ± 9.5	0.05
Lean mass (kgs)	47.7 ± 4.6	49.2 ± 5.2	0.13
Total body Fat (%)	20.5 ± 5.6	22.2 ± 4.9	0.06
Total fat mass (kgs)	13.1 ± 4.4	15.1 ± 4.8	< 0.05
Fat trunk (kgs.)	7.8 ± 2.9	8.5 ± 3.1	< 0.01
Fat (%) trunk	24.1 ± 7.1	24.9 ± 5.8	0.42
Fat mass (left arm) (gms)	620 ± 58.8*	888.7 ± 86.3*	< 0.01
Fat mass (right arm) (gms)	570.5 ± 47.9*	917.7 ± 47.9*	< 0.01
Fat mass (left leg) (kgs)	1.6 ± 0.6	1.8 ± 0.5	< 0.05
Fat mass (right leg) (kgs)	1.5 ± 0.6	1.7 ± 0.5	0.07
Trunk fat/limb fat mass ratio	2.5 ± 0.6	1.57 ± 0.2	< 0.01
Total limb fat/total fat mass ratio	0.33 ± 0.04	0.36 ± 0.03	0.20

Table 6. Anthropometry and body composition profile in male PLWH on CART more than 10 years at baseline and at end of study period. PLWH: Patients living with HIV. Values are presented as Mean ± SD or Standard Error of Mean (SEM) indicated by *. p value < 0.05; Statistically significant.

higher when compared to baseline values. This phenotype predisposes to insulin resistance which is evident with the mean value of the Mcauley index (a surrogate index of insulin sensitivity) being significantly lower at end of study period. A large study of 22,972 male PLWH on ART including NNRTI, PI and INSTI reported 10 times higher odds for weight gain in individuals aged above 33 years. It was reported that after three years of ART, 32% of normal-BMI PLWH initiated on an INSTI-based regimen had become overweight, compared to 29% of those on a PI-based regimen and 25% on a NNRTI-based regimen[40]. A pooled analysis of eight randomised controlled trials comprising of more than 5000 treatment-naive PLWH initiated on ART demonstrated weight gain was significant in PWLH on newer ART regimens. Specifically, integrase strand transfer inhibitor based ART regimen was associated with more weight gain when compared to protease inhibitors or nonnucleoside reverse transcriptase inhibitors (NNRTIs) based ART regimen[41].

Lipoatrophy, characterized by fat loss is often associated with higher cumulative exposure to NRTIs, particularly Stavudine[28]. In this study, the male PLWH were on Zidovudine, Lamivudine, Nevirapine and Stavudine as the first-line ART as practised in most low and middle income countries[42]. However, we observed no significant changes for BMI in PWLH on ART for less than or more than ten years, whereas the mean waist circumference had significantly increased in PLWH on ART for more than 10 years. This was concomitant with significant increases in truncal fat mass, fat mass in right and left legs, total fat mass and a significant decrease in insulin sensitivity in the same group. On pooled analysis, a significant increase in fat mass, truncal fat mass, fat mass in upper limbs, waist and hip circumferences and waist-to-hip ratio were observed at end of study period, when compared to baseline values. However, significantly lower mid-arm circumference, triceps skinfold thickness and fat percentage in lower limbs were observed in PLWH on ART when compared with healthy individuals. This could be due to loss of muscle mass in the arms and the cumulative effect of fat loss in the legs due to prolonged CART regimen in male PLWH.

In this study, the treatment regimen comprised of a combination of two nucleoside analogues (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI). The mean serum testosterone level at end of study period was significantly lower (29%) when compared to the baseline values. In PLWH on ART for more than 10 years, the mean decrease in testosterone levels was 31.4%, while in PLWH on ART for less than 10 years, the mean decrease in testosterone levels was 30%. The mean value of serum testosterone in PWLH on ART for a duration less than or greater than 10 years was higher than 300 ng/dl when compared to the former. However, in the current study hypogonadism (serum testosterone < 300 ng/dl) was noted only in 4 (13.7%) patients. A

Variables	Baseline profile of male PLWH on CART for more than 10 years ($n=18$)	Profile of male PWLH on CART for more than 10 years at the end of study period ($n=18$)	p-value
Fasting Glucose (mg/dL)	98.7 ± 15.7	117.8 ± 53.6	0.08
Post prandial Glucose(mg/dL)	142.4 ± 33.2	184.8 ± 107.3	0.08
Fasting insulin (μU/L)	17.1 ± 9.1	13.3 ± 6.4	0.13
Total cholesterol (mg/dL)	218.2 ± 44	203.3 ± 49	0.09
Triglycerides (mg/dL)	253.7 ± 32.6*	230.9 ± 27.5*	0.54
HDL cholesterol (mg/dL)	30.5 ± 4.6	39.9 ± 15	0.44
LDL cholesterol (mg/dL)	129.6 ± 38.4	121.8 ± 34.1	0.24
QUICKI	0.31 ± 0.02	0.31 ± 0.02	0.99
HOMA-IR Beta	3.4 ± 0.3*	3.1 ± 0.4*	< 0.05
McAuley index	10.4 ± 1.3	5.4 ± 0.7	< 0.01
Albumin (gm/dL)	4.7 ± 0.09	4.8 ± 0.3	0.40
Calcium (mg/dL)	8.8 ± 0.45	9.2 ± 0.2	0.59
Phosphorus (mg/dL)	3.57 ± 0.5	3.51 ± 0.5	0.63
Vitamin D (ng/ml)	15.1 ± 5.6	16.6 ± 8.0	0.44
Alkaline Phosphatase (U/L)	86.9 ± 22.5	92.0 ± 23.7	0.32
Testosterone (ng/dL)	500.4 ± 60.7*	343.3 ± 13.2*	< 0.05
Parathyroid hormone (pg/ml)	62.3 ± 26.7	54 ± 23	0.17
Blood haemoglobin (g/dL)	13.6 ± 2.5	13.5 ± 1.1	0.96
Mean Corpuscular Volume (fL)	86.4 ± 15.8	102.9 ± 12.0	< 0.05
CD4 count (cell/mm^3)	509.4 ± 75.7*	802.7 ± 91.6*	< 0.01
Serum creatinine (mg/dL)	0.93 ± 0.1	0.93 ± 0.1	1.00

Table 7. Biochemical profile in male PLWH on CART more than 10 years at baseline and at end of study period. PLWH: Patients living with HIV. Values are presented as Mean ± SD or Standard Error of Mean (SEM), SEM is indicated by *. p value < 0.05; Statistically significant.

Group 1	β coefficient (SE)	95% CI	*P* value
HOMA-IR	2.2 (0.8)	(0.49, 3.9)	< 0.05
QUICKI	−0.024 (0.01)	(−0.04, 0.00)	< 0.05
HOMA BETA	1.9 (0.62)	(0.64, 3.2)	< 0.01
MCAULEY index	0.75 (0.44)	(−1.6, 0.17)	1.06 (NS)

Table 8. Mixed model effect in PLWH on ART for more than a decade. p value < 0.05; Statistically significant. The unit change in indices namely HOMA-IR, HOMA beta QUICKI, and the McAuley index are presented in Figs. 3**(a)**, **(b)**, **(c) and (d).**

recent meta-analysis has reported that the prevalence rate of acquired hypogonadism in male PWLH was 26%[43]. A study on Indian MLWH ($n=225$; aged between 18 and 70 years) and on highly active ART for a duration of 8 years reported a prevalence rate of 39% of hypogonadism (serum testosterone < 300 ng/dl)[44]. The SWISS cohort study in 139 Caucasian male PLWH treated on zidovudine/lamivudine-based CART for two years reported secondary hypogonadism even at low or normal luteinising hormone levels[45]. The interpretation of the results of testosterone assays is more difficult in men with HIV due to several confounding factors such as increasing age, lower baseline CD4 count and lower Vitamin D levels in PLWH on ART[46].

To sum up, in this cross-sectional, observational study, male PLWH on CART for more than a decade featured increased body weight, higher body fat percentage, total body fat mass, higher abdominal adiposity as shown by higher truncal fat percentage, waist circumference and triceps skinfold. In addition, increased fat mass in upper limbs alongside, reduced beta cell function, impaired glucose tolerance, insulin resistance and lower testosterone levels were also observed when compared to baseline.

Considering the alteration in body habitus and onset of insulin resistance and lipodystrophy that may occur in PLWH on CART, early diagnosis of the same set of patients with appropriate substitution of ART including new generation ART drugs may be advocated. Furthermore, early detection of insulin resistance and prediabetes in such patients are likely to help in ART regimen adjustments to minimize the metabolic adverse effects. The management strategies for diabetes and dyslipidaemia in PLWH will differ when compared to the general population and thereby requires a cautious choice of pharmacotherapy. PLWH and pre-existing diabetes require clinical counselling about the possible alterations in metabolic function, and the chances of drug interactions between oral antidiabetic drugs and ART. PLWH who are detected to have diabetes at onset of therapy or later, may benefit from insulin as it is safe and effective[47]. The results of this cross-sectional study are intended to

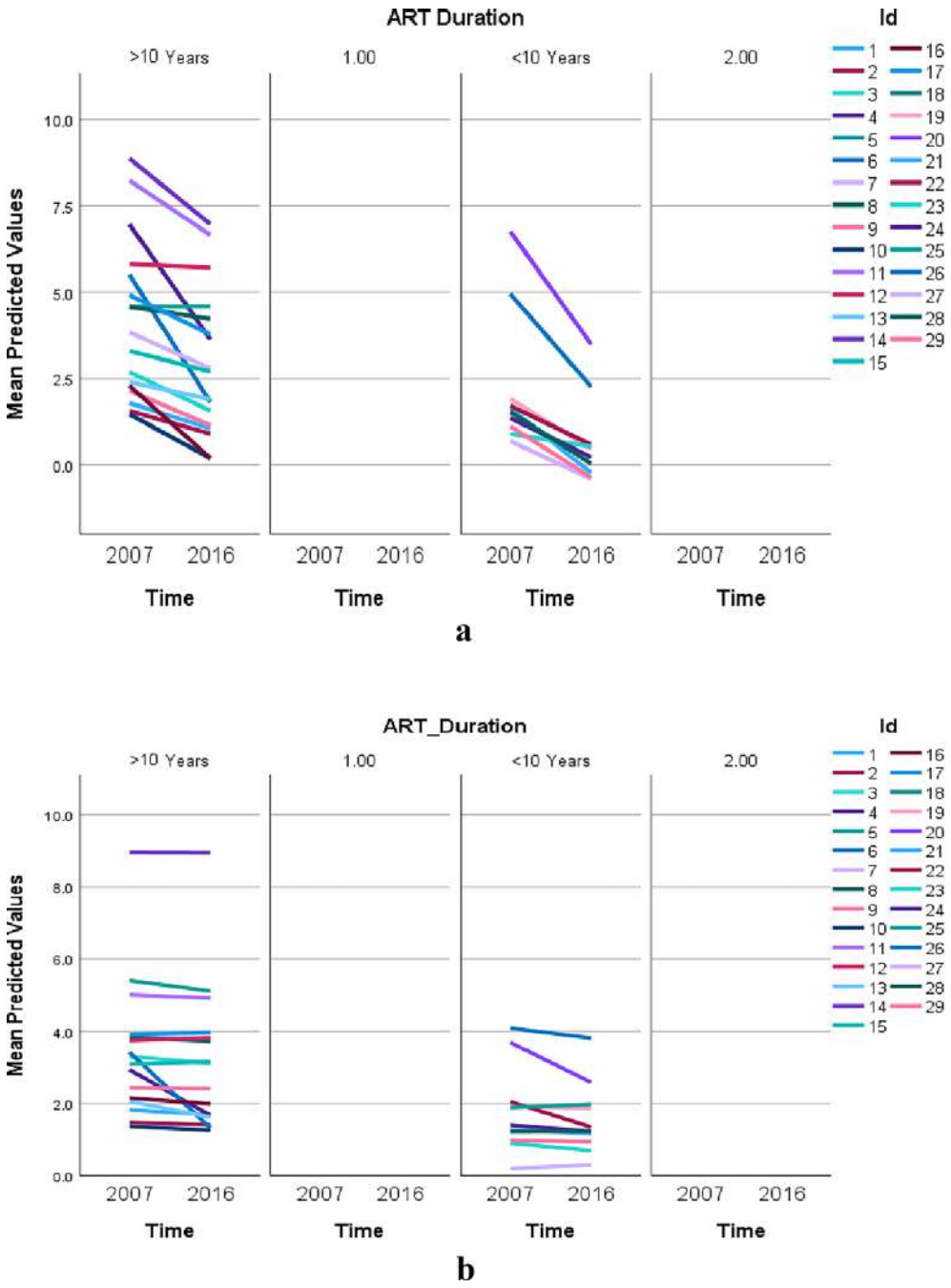
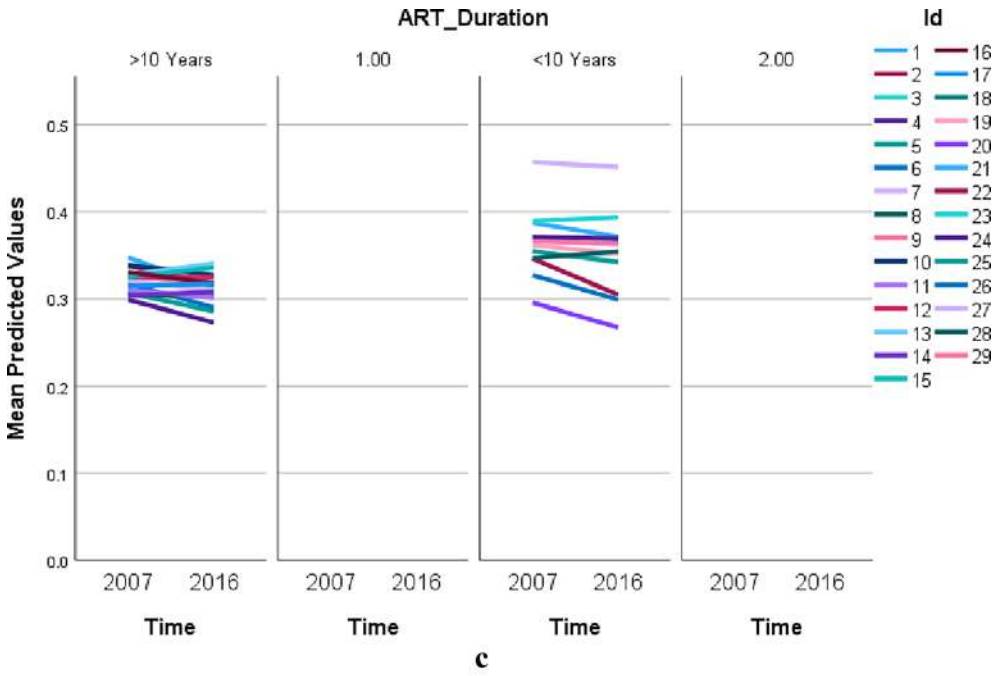


Fig. 3. **(a)**: Mixed model analysis showing differences in HOMA-IR between groups. **(b)** : Mixed model analysis showing differences in HOMA beta between groups. **(c)**: Mixed model analysis showing differences in QUICKI between groups. **(d)** : Mixed model analysis showing differences in McAuley index between groups.

provide the impetus for prospective, randomised controlled studies of the interactions among drug and host factors in the development of fat distribution abnormalities in PLWH.

The merits of the study include detailed body composition assessment, biochemical analysis, and assessment of insulin resistance in a demographically homogenous cohort of male PWLH on CART who were followed-up after 10 years at a single centre. This excludes the effects of gender on the phenotype and body composition, unlike other studies which include male and female patients with HIV. Secondly, this study provides data on the longitudinal phenotype changes in male PWLH who were on a combination of two nucleoside analogues (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) ART, unlike others studies which reported data on CART inclusive of protease inhibitors.

The limitations of the study are acknowledged. This study included a relatively small sample size of Asian Indian males with HIV at baseline and the same cohort was followed up after 10 years. Therefore, hence the results are applicable only to Asian Indian males. Subsequent studies should include female PWLH to explore



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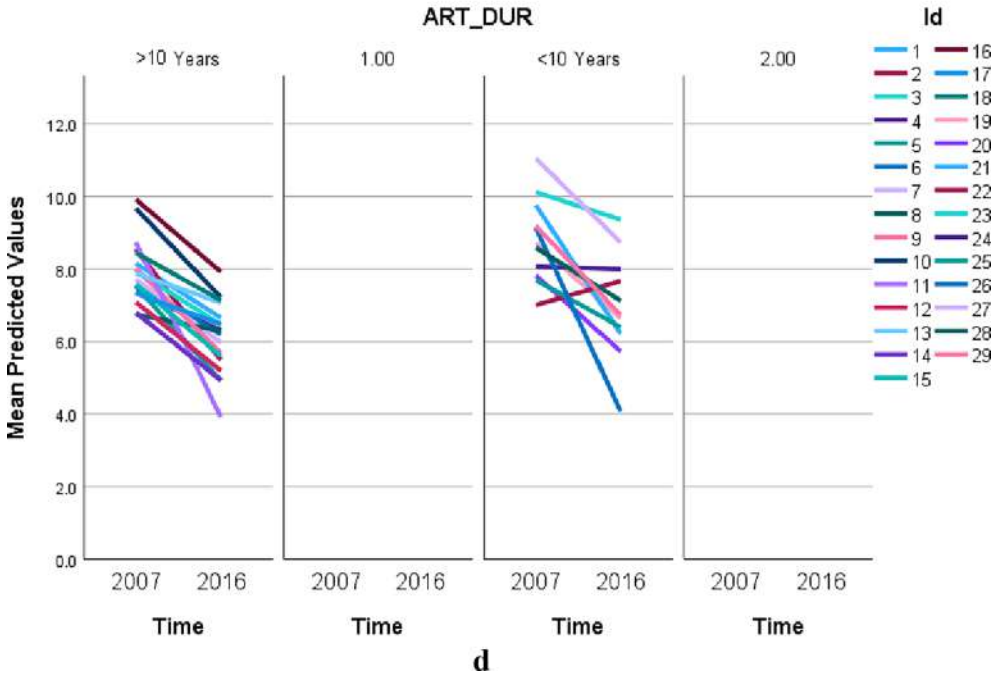

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Fig. 3. (continued)

potential gender specific differences in phenotypic and metabolic outcome due to CART. The healthy, non-HIV males (controls) recruited at baseline were not followed-up till the end of study, therefore longitudinal data of the control group was not available for comparisons. As quantitative data of diet and physical activity was not obtained at baseline and the end of the study, therefore the confounding effects of these factors, if any could not be studied. Furthermore, this is an observational study and therefore the cause and effect of metabolic changes in PLWH on ART cannot be definitively ascertained.

It is important to mention that the HIV viral load testing facility was not available at the institution during the study period and therefore it was not analysed for all PWLH. As of the year 2017, most of the study patients were part of the National AIDS Control Programme sponsored by the Government of India, and antiretroviral therapies were provided free of cost along with CD4 testing. In cognizance of the same, the government of India declared HIV viral load testing a standard diagnostic criterion for all PWLH in the year 2017. This was

in accordance to the National "*Test and Treat*" policy launched on April 28, 2017. The National AIDS Control Organization (NACO) in India has formulated policies to adhere to the global targets of ending HIV in India, and to reduce the new infections annually and the mortality rate by 80% by the year 2025–2026. It is envisaged to achieve this by promoting universal access to viral load testing facilities to people in need of ART as mentioned in the report of the National Aids Control Organisation (NACO) for the year 2021. Subsequently, in the year 2024, there were 64 public-sector viral testing laboratories operating under the National AIDS Control Program in India[48]. As of today, HIV viral load testing is a standard protocol at the study centre and is applied in all studies in PLWH.

Data availability

The datasets generated in the current study are available from the corresponding author on reasonable request from the readers.

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Author contributions

NT, PR and AH conceptualized the study. SS collected data and conducted the study. GR analysed and interpreted the data. SA drafted, edited, and revised the manuscript. DB and FKJ contributed to discussion. PK and NT reviewed the manuscript and contributed to discussion. NT is the guarantor for the study. All authors read the manuscript and consented for publication.

Declarations

Conflict of interest

The authors declare no conflict of interest in this study.

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Additional information

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