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Effects of lifestyle modification and metformin on irisin and FGF21 among HIV-infected subjects with the metabolic syndrome

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Summary

Objective—Few studies have investigated irisin and FGF21 to elucidate the role of these hormones to regulate 'beiging' in HIV-infected patients.

Design—Fifty HIV-infected subjects with the metabolic syndrome were previously recruited and randomized to receive lifestyle modification (LSM) and/or metformin over 12 months. In the current study, we assessed FGF21 and irisin at baseline and after intervention. In addition, we assessed circulating FGF21 and irisin in relationship to brown adipose tissue (BAT) gene expression in dorsocervical subcutaneous fat biopsies from 13 HIV-infected subjects.

Results—At baseline, prior to intervention, HIV-infected subjects demonstrated increased log FGF21 (2·13 ± 0·06 *vs* 1·98 ± 0·05 pg/ml, $P = 0·05$) and log irisin (0·33 ± 0·02 *vs* 0·17 ± 0·04 μg/ml, $P = 0·003$) compared with healthy controls well matched based on waist circumference. After 12 months, HIV-infected subjects randomized to LSM demonstrated a relative reduction in FGF21 compared with those not randomized to LSM (−10 [−35,22] *vs* 40 [0,94] %change, $P = 0·01$). Changes in FGF21 were inversely associated with improved parameters of energy

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Declaration of interest

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Supporting Information

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homoeostasis, including increased REE (ρ = −0·34, *P* = 0·046) and max VO_2 (ρ = −0·38, *P* = 0·02), and reduced RQ (ρ = 0·40, *P* = 0·02) among all HIV-infected subjects. Increased UCP-1 (*r* = 0·75, *P* = 0·003), DIO2 (*r* = 0·58, *P* = 0·04) and CideA (*r* = 0·73, *P* = 0·01) gene expression in dorsocervical fat was significantly associated with FGF21 in HIV-infected subjects.

Conclusion—HIV-infected subjects with metabolic complications demonstrate increases in FGF21 in relationship to BAT gene expression. Relative reductions in FGF21 in those receiving long-term LSM relate to overall improvements in energy expenditure parameters. In contrast, irisin levels are elevated in HIV-infected subjects, but are not influenced by LSM nor associated with BAT gene expression.

Introduction

Brown adipose tissue (BAT) is a unique fat depot that can enhance metabolism through increased energy expenditure and may be protective of obesity-related complications. White adipose tissue (WAT) has the potential to transform into energetically favourable BAT-like depots, with adaptive mitochondrial biogenesis, in a process known as 'beiging.' Studies suggest that the 'beiging' process may be mediated by irisin and FGF21[1,2] through the activation of UCP-1.[3–5] FGF21 can be secreted from adipose tissue and is critical to the metabolic regulation of glucose and lipid homoeostasis.[6] Irisin is secreted from muscle with exercise and can act on adipose tissue to increase mitochondrial oxidation.[7] Lee *et al.* recently demonstrated that irisin and FGF21 may each play a distinct regulatory role in activating depot-specific BAT through shivering and nonshivering thermogenesis, respectively.[8] However, very limited human data are available as to the levels of these hormones and their association to energy parameters and BAT gene expression.

HIV-infected patients are prone to lipodystrophic changes, and these acquired fat redistributions contribute to mitochondrial dysfunction and other metabolic consequences. We have demonstrated unique regulation of BAT-like adipose tissue in the dorsocervical area among HIV-infected subjects, characterized by increased expression of type 2 deiodinase (DIO2).[9] We have also previously demonstrated that intervention with lifestyle modification (LSM) and metformin significantly improves fitness parameters in HIV-infected patients.[10] Exercise programmes and metformin both promote regulators associated with the 'beiging' process, including activation of the AMPK-SIRT1-PCG-1α pathway.[7,11] In that regard, this unique BAT-like phenotype in HIV-infected patients may be influenced by FGF21 and irisin in response to intervention from exercise or metformin.

We took advantage of banked serum from two previous studies, one in which HIV-infected subjects with the metabolic syndrome were randomized to either LSM and/or metformin for 12 months and the other in which HIV-infected subjects with lipodystrophy underwent dorsocervical subcutaneous fat biopsies for evaluation of BAT-specific gene expression. We hypothesized that intervention with LSM and metformin would increase irisin and FGF21 levels, providing a potential mechanism for enhanced energy homoeostasis in HIV-infected subjects. Additionally, we hypothesized that expression of BAT-specific genes in dorsocervical fat would be relatively higher in HIV-infected patients compared with controls in association with increased irisin and FGF21. Indeed, this is the first study to our

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knowledge to assess irisin in HIV-infected subjects and the first study to assess both of these hormones with respect to the effects of metformin and exercise, two key strategies known to improve metabolic indices, in the HIV population. These studies provide novel insight into the role played by irisin and FGF21 to regulate energy homoeostasis and transformation of WAT towards 'beige' fat in HIV-infected subjects, a population predisposed to fat redistribution and metabolic complications.

Methods

Lifestyle and metformin interventional study

Subjects—Fifty HIV-infected subjects were prospectively recruited as previously described[10] for treatment with LSM and/or metformin. Eligibility requirements included documented HIV infection, age 18–65, stable antiretroviral therapy (ART) regimen ≥6 months and demonstration of National Cholesterol Education Program (NCEP) defined metabolic syndrome. Exclusion criteria are detailed elsewhere,[10] but main criteria were contraindication to physical activity, current use of diabetic medications, fasting blood sugar ≥126 mg/dl, alanine aminotransferase (ALT) ≥2·5 times upper limit of normal (ULN), creatinine ≥1·5 mg/dl, lactic acid ≥2 times ULN, allergy to metformin or pregnancy. Irisin and FGF21 were measured at baseline and after 12 months in the HIV-infected subjects randomized to LSM and/or metformin. In addition, irisin and FGF21 were measured in 50 non-HIV-infected control subjects well matched to HIV-infected subjects based on age, gender, race and iliac waist circumference (WC) for baseline comparisons. Assessments occurred in the resting state after an overnight fast. Informed written consent was obtained before study participation, and the protocol was approved by the Partners Human Research Committee.

Intervention—Eligible HIV-infected subjects were assigned to one of four groups in a randomized, placebo-controlled 2 × 2, 4 group factorial study conducted over 12 months: no LSM/placebo; LSM/placebo; no LSM/metformin; or LSM/metformin. According to randomization, groups received metformin or identical placebo 500 mg twice a day, with a dose increase to 850 mg twice a day after 3 months if lactic acid and creatinine remained in the normal range. Those randomized to LSM participated in three supervised exercise sessions per week complemented with once weekly dietary counselling session modelled after the LSM programme used in the diabetes prevention programme.[10]

Dorsocervical fat biopsy and gene expression study

Subjects—In a separate investigation, 13 male HIV-infected and 3 male non-HIV-infected control subjects were prospectively recruited as previously described[9] and underwent dorsocervical subcutaneous fat biopsies. Subjects were included if they were age 18–60 with a BMI 18–29·9 kg/m^2. Subjects were excluded if they had a history of diabetes or were on diabetic medications. In addition, HIV-infected subjects were required to demonstrate lipodystrophy upon examination and be on stable ART ≥12 months. One subject in the gene expression study had participated in the longitudinal study. This subject received LSM, but not metformin and finished his participation 3 years prior to the gene expression study.

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Informed written consent was obtained before study participation, and the protocol was approved by the Partners Human Research Committee.

Intervention—Dorsocervical subcutaneous adipose tissue was obtained through a 4-mm-diameter punch biopsy under local anaesthesia with 1% lidocaine. Samples were stored at −80 °C after being flash frozen in liquid nitrogen. RNA was extracted from tissue using an RNeasy minikit (QIAGEN, Valencia, CA, USA), and cDNA was prepared as previously described[9] to assess the following genes: TBP, UCP-1, DIO2, CideA, PGC-1α and PRDM-16. Quantitative RT-PCR assays were run in duplicates and quantified in the ABI Prism 7700 sequence detection system. Results for gene expression are reported as normalized to the housekeeping gene TBP and expressed as ratios in arbitrary units.

Metabolic evaluation

Metabolic and biochemical parameters—Fasting triglycerides, HDL, insulin and glucose, as well as creatinine, haemoglobin A1C (HbA1c), $CD4^+$ T-cell count and HIV viral load were measured.[10] REE was assessed by indirect calorimetry after the subject rested for 20 min (Delratrac, Yorba Linda, CA, USA).

Body composition assessment—Abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) areas were assessed by MRI at the level of the L4 pedicle.[12,13] Dual-energy X-ray absorptiometry (Hologic A, Waltham, MA, USA) was used to assess fat and lean mass body composition.

Measurement of irisin and FGF21—Serum samples were obtained from previously unthawed blood aliquoted into 2-mL Sarstedt microtubes and stored at −80 °C. Data on irisin and FGF21 were not previously analysed in either study. FGF21 was measured by ELISA (R&D Systems, intra-assay variability 3·4%) and irisin by competitive ELISA (Adipogen, intra-assay variability 6·9%).

Biostatistical analysis

Irisin and FGF21 levels were log-transformed to establish normality of distribution in the lifestyle and metformin study. Comparisons between HIV-infected and control subjects were made using Student's *t*-test or Wilcoxon rank-sum test for normally and non-normally distributed continuous data, respectively, and x^2 test for categorical variables. Data are presented as mean ± standard error of the mean (SEM) or median [inter-quartile range (IQR)], depending on normality of distribution. Irisin and FGF21 at baseline and change over 12 months were compared among the four groups; overall significance was determined by the Kruskal–Wallis test. Subsequently, a factorial analysis was performed using the Wilcoxon rank-sum test for comparison of irisin and FGF21 at baseline and change over 12 months between groups with no LSM vs LSM (regardless of placebo or metformin assignment) and placebo vs metformin (regardless of LSM assignment) after determining there was no interaction between LSM and metformin interventions for either irisin or FGF21. Data from interim time points were not available for analysis. We performed a sensitivity analysis with the last observation carried forward (LOCF) using baseline values for subjects who did not complete the study. Relationship of metabolic variables to the

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change in irisin and FGF21 over 12 months was assessed using Spearman's correlation coefficient.

With 50 evaluable patients, the study was powered at 80% to detect a 0·81 SD change in FGF21 and irisin at $P < 0·05$ for each comparison. After correction for those lost to follow-up ($n = 14$), the study was powered at 80% to assess a 0·96 SD change in FGF21 and irisin at $P < 0·05$ for each comparison.

Irisin and FGF21 levels were evaluated in relationship to BAT gene expression among HIV-infected subjects and non-infected subjects using linear regression. To further assess the impact of HIV status and FGF21 on expression of specific BAT genes, multivariate regression modelling was performed including both HIV-infected subjects and controls, assessing for individual effects of HIV status and FGF21, as well as the interaction between these terms, as the independent variables in models for each gene. Statistical significance was defined as $P \leq 0·05$ for both studies. Analysis was performed using JMP for SAS (Carey, NC, USA).

Results

Lifestyle and metformin interventional study

Demographic and clinical characteristics—Controls and HIV-infected subjects were well matched with respect to age, race, gender and WC. HIV-infected subjects had a long duration since HIV diagnosis of 14 years with a mean $CD4^+$ T-cell count of 576 cell/μl. Despite good immunologic control, the HIV population demonstrated significantly more metabolic abnormalities, including decreased HDL cholesterol and increased respiratory quotient (RQ), VAT, triglycerides, fasting glucose and HOMA-IR (Table 1). Log FGF21 (2·13 ± 0·06 *vs* 1·98 ± 0·05 pg/ml, $P = 0·05$) and log irisin (0·33 ± 0·02 *vs* 0·17 ± 0·04, μg/ml, $P = 0·003$) levels were increased among HIV-infected subjects compared with controls at baseline (Table 1). At baseline, neither viral load ($P = 0·32$ and $P = 0·28$), $CD4^+$ T-cell count ($P = 0·24$ and $P = 0·59$) and current ART use ($P = 0·67$ and $P = 0·31$) nor duration of ART use ($P = 0·29$ and $P = 0·69$) was related to FGF21 or irisin, respectively, in the HIV cohort.

Effect of LSM and metformin on FGF21 and irisin in HIV-infected subjects—HIV-infected subjects randomized to LSM demonstrated a relative reduction in FGF21 after 12 months compared with those not randomized to LSM (−10 [−35,22] *vs* 40 [0,94] % change, $P = 0·01$) (Table 2). In the sensitivity analysis using LOCF to account for noncompleters ($n = 14$), the per cent change in FGF21 after 12 months remained significantly different between the lifestyle modification group compared with those who underwent no lifestyle intervention (0 [−26,2] *vs* 14 [0,48] % change, $P = 0·01$). In contrast, changes in irisin were not significantly different in those subjects randomized to LSM ($P = 0·66$), and this result was confirmed in the LOCF analysis ($P = 0·95$). Per cent changes in irisin and FGF21 did not differ by randomization status with respect to metformin vs placebo (Table 3), and findings were similar in the sensitivity analysis using LOCF. Per cent changes in the four group analysis for irisin and FGF21 are detailed in Table 4. With respect

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to safety reporting, there was no significant difference in the per cent change in lactic acid levels (n = 36) in those assigned to metformin vs. placebo (16 ± 9 *vs* 9 ± 10%, P = 0·61).

In exploratory analyses, we constructed multivariate models using dichotomous variables for LSM and metformin, individually, as well as the two-way interactions between these two variables to see their effect on the changes in FGF21 and irisin. The interactions between LSM and metformin were not significantly associated either with changes in FGF21 (P = 0·87) or irisin (P = 0·62), in each respective model. Lifestyle modification remained significantly and independently associated with the change in FGF21 in this model controlling for any interaction between LSM and metformin (β estimate = −35·190, P = 0·04).

Relationship of changes in FGF21 and irisin to changes in metabolic parameters in the interventional study—Changes in FGF21 were inversely associated with improvements in metabolic indices, including increased REE (ρ = −0·34, P = 0·046) and max VO_2 (ρ = −0·38, P = 0·02), and reduced RQ (ρ = 0·40, P = 0·02) among all subjects, but there was no association between changes in irisin and energy homoeostasis parameters. Neither changes in FGF21 nor irisin were associated with changes in lipid or glucose parameters over the duration of the study.

Dorsocervical fat biopsies and gene expression study

Demographic and clinical characteristics—HIV-infected subjects with lipodystrophy and control subjects had similar BMI measurements. Detailed demographic and clinical characteristics between HIV-infected subjects and healthy controls can be found in Supplementary Table 1.

Relationship of gene expression in dorsocervical fat biopsies to FGF21 and irisin in HIV-infected subjects—In HIV-infected subjects with lipodystrophy, gene expression of UCP-1 (r = 0·75, P = 0·003), DIO2 (r = 0·58, P = 0·04) and CideA (r = 0·73, P = 0·01) was significantly associated with serum FGF21. Gene expression of PGC-1α (r = 0·60, P = 0·12) also tended to be related to FGF21 among the HIV-infected subjects, but this relationship did not reach statistical significance. PRDM16 (r = 0·007, P = 0·99) expression did not correlate significantly with FGF21 in the HIV population. In addition, there were no significant relationships between irisin and gene expression in dorsocervical fat depots in the HIV-infected subjects. In multivariate modelling for UCP-1 and CideA, there was an interaction between HIV status and FGF21 levels, such that gene expression of UCP-1 (P = 0·03) and CideA (P = 0·04) increased more among HIV-infected subjects with higher FGF21 levels.

Discussion

This is the first study to our knowledge investigating irisin in any HIV cohort, and the first to assess changes in irisin and FGF21 in HIV-infected subjects with the metabolic syndrome in response to LSM or metformin. In our study, FGF21 levels were elevated at baseline in HIV-infected subjects compared with well-matched controls. Animal models demonstrate that increased FGF21 is associated with improved metabolic parameters, including lower

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body weight and increased O_2 consumption and energy expenditure.[14] A limited number of human studies, in contrast, demonstrate that increased FGF21 independently predicts progression to metabolic syndrome[15] and is associated with adverse body composition, such as increased waist to hip ratio.[16] We demonstrate similar findings in the basal state in our HIV subjects, a metabolically challenged population with increased waist to hip ratio, when compared to controls.

Our data extend these prior findings in a carefully performed long-term 1-year interventional study of LSM. We demonstrate no increase and relative reductions over time in FGF21 among those randomized to LSM *vs* not. The relative reductions in FGF21 were associated with improved fitness and increased energy expenditure parameters over 1 year. These data in our longer term study with HIV-infected subjects differ from data reported in other short-term exercise studies in non-HIV-infected healthy subjects, which demonstrate increased FGF21.[17,18] In comparison with LSM, we saw no effects of metformin on FGF21. These data contrast with Zhang *et al.*, who reported a decrease in FGF21 in diabetic subjects receiving metformin for a short-term 1-week course.[19] Our data, from a long-term study, do not support the hypothesis that LSM increases FGF21 as a mechanism to increase REE in HIV-infected subjects.

What are the potential mechanisms by which LSM may contribute to relative reductions in FGF21? A potential answer to this question was suggested by Lee *et al.* in a recent publication demonstrating the capacity of FGF21 to increase BAT expression in a depot-specific fashion. Specifically, FGF21 was shown to increase UCP-1 and local heat production by subcutaneous neck adipocytes in association with increased BAT expression.[8] In this regard, we postulate that FGF21 levels may increase among subjects with the metabolic syndrome in a compensatory fashion to promote ‘beiging’ and thermogenesis of adipose tissue in specific ectopic neck depots. With exercise, other stimuli to BAT-mediated thermogenesis occur, leading to a reduction in signalling for FGF21. Lee *et al.* postulate that specific myokines, such as irisin, are released in a process analogous to shivering, to activate BAT and dissipate heat in an FGF21 primed milieu, pointing out the differential regulation of these hormones during exercise.[8] Our data are consistent, in part, with the hypothesis of Lee *et al.* suggesting reduced FGF21 with exercise, although we do not show a simultaneous increase in irisin. Moreover, Lee *et al.* demonstrated reduced FGF21 in the context of short-term exercise, whereas our data are obtained after long-term exercise.

Our findings relating FGF21 to BAT genes in ectopic adipose tissue provide some further evidence that basal increases of these hormones in subjects with metabolic derangements may be adaptive or compensatory to regulate specific genes in the brown fat lineage. Alternatively, resistance to FGF21 may occur in metabolic syndrome, akin to leptin resistance in obesity, but the positive association we show between increased FGF21 and BAT gene expression argues against this. Our prior studies have revealed increased DIO2 and a unique intermediate phenotype of fat in the dorsocervical area in HIV-infected subjects, which is not typical brown fat and does not image on PET.[9] We now show that expression of BAT genes, including UCP-1, DIO2 and CideA, in these cervical depots is increased in relationship to FGF21 among HIV-infected subjects with lipodystrophy. *A prior* study demonstrated increased UCP-1 expression through mRNA extraction from

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dorsocervical fat depots in HIV-infected subjects, [20] but UCP-1 expression in these HIV-infected subjects was not assessed in relationship to FGF21 or irisin as in our current study. In addition, biopsies from dorsocervical fat in HIV-infected subjects revealed abundant mitochondria-rich cytoplasm and a 'dual' appearance of WAT and BAT.[21,22] Taken together, these data suggest that dorsocervical fat may represent an intermediate phenotype, unique from classical BAT, with preferential 'beiging' in a compensatory fashion among HIV-infected subjects with metabolic dysregulation.

In contrast to studies of FGF21, irisin has been shown to have a similar role in animal and human metabolism. Increased levels of irisin in animal models have been associated with favourable effects on metabolic parameters. Bostrom *et al.* demonstrated that irisin was associated with improved energy expenditure and glucose tolerance in mice on a high-fat diet.[7] To complement these animal studies, irisin was negatively associated with BMI and percentage fat mass and positively associated with insulin sensitivity in obese non-HIV-infected subjects.[23] In contrast, our data demonstrate, for the first time, increased irisin levels in metabolically challenged HIV-infected patients. However, the role of irisin in response to exercise in human biology is less clear. Some studies have demonstrated that short-term exercise training in healthy subjects reduced irisin levels or did not have a beneficial effect.[24–26] Lee *et al.*, however, demonstrated that irisin levels increase in healthy subjects after short-term endurance exercise in association with shivering and that irisin also increases expression of brown and 'beige' fat gene expression in human cervical adipocytes.[8] Endurance rather than intensity of exercise may be more influential on irisin secretion.[8] In contrast, we did not see an association of irisin with BAT gene expression nor an increase in irisin after a successful long-term exercise programme that increased VO_2 and REE, but we assessed irisin levels separate in time from acute exercise training sessions. The role of irisin in the regulation of changes in metabolic parameters with long-term lifestyle modification thus remains unclear.

This study has limitations. Control subjects were not randomized to undergo intervention with LSM or metformin, so the longitudinal effects on irisin and FGF21 could not be evaluated in this group. Nonetheless, baseline data from a well-matched non-HIV control group do provide insight into critical physiologic differences in FGF21 and irisin between HIV and non- HIV-infected groups. Moreover, systemic FGF21 and irisin were evaluated, which may not be reflective of autocrine or paracrine activity at the level of adipose tissue or muscle. The interventional study experienced a dropout rate consistent with other LSM studies, but results were confirmed in a conservative LOCF analysis. Future studies should be designed to confirm the effect of LSM on FGF21 in a larger cohort, enabling further detailed assessments of statistical significance. We demonstrate that BAT gene expression from dorsocervical biopsies in HIV-infected patients is associated with FGF21 at baseline, but the longitudinal effects of exercise on specific genes associated with the 'beiging' process, and the relationship of FGF21 and irisin to expression patterns in these genes should also be investigated.

In conclusion, prior studies in an HIV population have not investigated the effects of exercise on irisin and FGF21 nor related these hormones to BAT gene expression in ectopic dorsocervical fat. We have now demonstrated in HIV-infected subjects, a population prone

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to metabolic complications, that basal FGF21 levels are increased when compared with a healthy cohort and that FGF21 levels are relatively reduced with LSM. The effects of increased FGF21 may be to promote increased expression of BAT genes in a unique ectopic adipose depot, as suggested by our gene expression studies. Indeed, our data extend a number of the findings of Lee *et al.* in a human model characterized by frequent metabolic perturbations. These studies advance our understanding of the physiological role of FGF21 in human metabolism and highlight novel relationships between FGF21 and BAT genes in HIV-infected patients. Further studies are needed to understand the relationship of FGF21, lifestyle modification and 'beiging' of adipose tissue in this population.

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Table 1

Baseline demographic and clinical characteristics of controls and HIV-infected subjects

	Controls (n = 50)	HIV-infected (n = 50)	P value*
Demographics			
Age (years)	47 ± 1	47 ± 1	0·99
Race (%)			
Caucasian	68	66	0·83
Gender (%)			
Male	76	76	1·00
Current lipid lowering therapy use (n)	7	10	0·42
HIV parameters			
$CD4^+$ T-cell count (cells/μl)	N/A	576 ± 41	–
Log HIV RNA viral load (copies/ml)	N/A	1·98 ± 0·09	–
Duration HIV (years)	N/A	14 ± 1	–
Duration ART use (years)	N/A	6 ± 1	–
Current PI use (%)	N/A	56	–
Current NRTI use (%)	N/A	86	–
Current NNRTI use (%)	N/A	22	–
Energy parameters			
Resting energy expenditure (kcal/d)	1944 [1613,2169]	1939 [1694,2237]	0·93
VO_2 max (ml/kg/min)	N/A	22·8 ± 0·6	–
Respiratory quotient	0·80 ± 0·01	0·84 ± 0·01	0·05
Metabolic parameters			
Waist circumference (cm)	103 ± 2	105 ± 2	0·32
Waist to hip ratio	0·95 ± 0·01	0·99 ± 0·01	0·001
BMI(kg/m^2)	29·2 [26·4,31·8]	28·9 [26·9,32·3]	1·00
VAT area (cm^2)	142·6 [73·9,183·5]	181·6 [113·6,258·0]	0·006
SAT area (cm^2)	290·2 [225·0,396·2]	249·8 [144·1,354·8]	0·056
Total fat mass (%)	28 ± 1	28 ± 1	0·67
Total lean body mass (%)	70 ± 1	72 ± 1	0·23
SBP (mmHg)	120 ± 2	121 ± 2	0·83
DBP (mmHg)	77 ± 1	78 ± 1	0·76
Triglycerides (mg/dl)	91 [70,124]	165 [123,235]	<0·0001
HDL Cholesterol (mg/dl)	46 [39,53]	34 [30,45]	<0·0001
Fasting glucose (mg/dl)	89 [83,93]	94 [87,102]	0·005
Haemoglobin A1c (%)	5·5 [5·2,5·7]	5·5 [5·0,5·8]	0·90
HOMA-IR	0·78 [0·49,1·52]	1·56 [0·65,2·53]	0·02
Creatinine (mg/dl)	1·02 ± 0·03	1·01 ± 0·03	0·84
Biochemical Parameters			
Log FGF21 (pg/ml)	1·98 ± 0·05	2·13 ± 0·06	0·05
(FGF21 (pg/ml))	(89·6 [48·1,176·2])	(107·5 [79·4,182·8])	
Log irisin (μg/ml)	0·17 ± 0·04	0·33 ± 0·02	0·003

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	Controls (*n* = 50)	HIV-infected (*n* = 50)	*P* value*
(Irisin (μg/ml))	(1·9 [0·8,3·0])	(2·0 [1·7,2·6])	

ART, antiretroviral therapy; PI, protease inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NNRTI, non-nucleoside reverse-transcriptase inhibitor; VO_2 max, maximal oxygen consumption; N/A, not available; BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homoeostatic model assessment of insulin resistance.

*Normally distributed data reported as mean ± standard error of the mean or percentages; P value by Student's *t*-test or x^2 test, respectively. Non-normally distributed data reported are reported as median [inter-quartile range]; *P* value by Wilcoxon rank-sum test.

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Table 2

Effect of lifestyle modification on FGF21 and irisin in HIV-infected subjects

	No lifestyle modification (*n* = 24)	Lifestyle modification (*n* = 26)	*P* value*
FGF21 (pg/ml)			
Baseline	98·9 [83·2,232·5]	117·4 [60·5,178·7]	0·99
12 months†	171·3 [89·6,630·2]	108·0 [80·5,216·5]	
% change at 12 months	40 [0,94]	−10 [−35,22]	0·01
Irisin (μg/ml)			
Baseline	2·02 [1·69,2·70]	2·13 [1·66,2·59]	0·78
12 months†	2·24 [1·62,3·02]	1·96 [1·74,2·53]	
% change at 12 months	2 [−19,13]	1 [−15,21]	0−66

*Non-normally distributed data are reported as median [inter-quartile range]; *P* value by Wilcoxon rank-sum test.

†Nineteen subjects randomized to no lifestyle modification and 17 subjects randomized to lifestyle modification completed the interventional study at 12 months.

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Table 3

Effect of metformin on FGF21 and irisin in HIV-infected subjects

	Placebo (*n* = 22)	Metformin (*n* = 28)	*P* value*
FGF21 (pg/ml)			
Baseline	112·3 [72·3,219·1]	103·1 [80·5,199·1]	0·79
12 months†	145·4 [71·7,538·7]	140·1 [89·6,258·5]	
% change at 12 months	15 [−32,46]	8 [−21,42]	0−78
Irisin (μg/ml)			
Baseline	2·50 [1·96,2·94]	1·94 [1·52,2·32]	0·007
12 months†	2·43 [1·65,2·90]	1·92 [1·57,2·29]	
% change at 12 months	13 [−12,20]	−2 [−19,11]	0·28

*Non-normally distributed data are reported as median [inter-quartile range]; *P* value by Wilcoxon rank-sum test.

†Seventeen subjects randomized to no metformin and 19 subjects randomized to metformin completed the interventional study at 12 months.

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Table 4

Effect of lifestyle modification, metformin or both on FGF21 and irisin in HIV-infected subjects

	No lifestyle modification-placebo (*n* = 11)	Lifestyle modification-placebo (*n* = 11)	No lifestyle modification-metformin (*n* = 13)	Lifestyle modification- metformin (*n* = 15)	*P* Value*
FGF21 (pg/ml)					
Baseline	109·9 [86·6, 171·5]	114·7 [50·7, 362·0]	96·6 [81·3,405·2]	120·2 [63·1,169·2]	0·99
12 months†	171·3 [62·9,532·9]	130·4 [80·3,672·8]	169·9 [111·2,634·6]	92·2 [67·6,165·6]	
% change at 12 months	43 [4,124]	−30 [−39,25]	29 [−21,57]	−10 [−24,16]	0·08
Irisin (μg/ml)					
Baseline	2·09 [1·71,2·73]	2·59 [2·01,3·13]	1·91 [1·44,2·39]	1·96 [1·55,2·36]	0·04
12 months†	2·37 [1·60,3·15]	2·53 [1·97,2·92]	1·98 [1·64,3·13]	1·92 [1·56,2·08]	
% change at 12 months	9 [−12,17]	16 [−13,22]	−4 [−19,12]	−2 [−22,13]	0·66

*Non-normally distributed data are reported as median [interquartile range]; Overall *P* value by Kruskal–Wallis test.

†Nine subjects randomized to no lifestyle modification-placebo, eight subjects randomized to lifestyle modification-placebo, 10 subjects randomized to no lifestyle modification-metformin, and nine subjects randomized to lifestyle modification-metformin completed the interventional study at 12 months.