ORIGINAL ARTICLE

The Physiological Society | american physiological society

Physiological Reports

Heightened levels of plasma growth differentiation factor 15 in men living with HIV

Neeti Agarwal[1] | **Claudia E. Ramirez Bustamante[1]** | **Huaizhu Wu[2]** |
Reina Armamento-Villareal[1,3] | **Jordan E. Lake[4]** | **Ashok Balasubramanyam[1]** |
Sean M. Hartig[1,5] ⬤

[1]Division of Diabetes, Endocrinology, and Metabolism, Baylor College of Medicine, Houston, Texas, USA

[2]Atherosclerosis and Lipoprotein Research, Baylor College of Medicine, Houston, Texas, USA

[3]Center for Translational Research on Inflammatory Diseases, Michael E DeBakey VA Medical Center, Houston, Texas, USA

[4]Division of Infectious Diseases, Department of Internal Medicine, McGovern Medical School, University of Texas Health Science Center, Houston, Texas, USA

[5]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, USA

Correspondence
Sean Hartig and Ashok Balasubramanyam, Division of Diabetes, Endocrinology, and Metabolism, Baylor College of Medicine, Houston, TX, USA.
Emails: hartig@bcm.edu and ashokb@bcm.edu

Abstract

Plasma biomarkers that reflect energy balance disorders in people living with HIV (PLWH) remain limited. Growth differentiation factor 15 (GDF15) abundance in plasma of mice and humans induces negative energy balance but also becomes highly elevated in obesity and other metabolic diseases. We sought to compare plasma GDF15 levels in PLWH and HIV-negative persons and mouse models expressing the HIV accessory protein Vpr (that recapitulate HIV-associated metabolic disorders) and determine their relationship to metabolic parameters. We measured liver *Gdf15* mRNA levels and plasma GDF15 levels in male Vpr mice and littermate controls. In parallel, we analyzed plasma GDF15 levels in 18 male PLWH on stable, long-term antiretroviral therapy and 13 HIV-negative men (6 healthy controls and 7 with metabolic syndrome). Plasma GDF15 levels were correlated with anthropometric and immune cell parameters in humans. Gene expression analysis of Vpr mouse liver demonstrated elevated *Gdf15* mRNA. Plasma GDF15 levels were also higher in Vpr mouse models. Levels of plasma GDF15 in PLWH were greater than in both HIV-negative groups and correlated positively with the CD4/CD8 T cell ratio in PLWH. Plasma GDF15 levels correlated positively with age in the HIV-negative subjects but not in PLWH. Since GDF15 levels predict fatty liver disease and energy balance disorders, further studies are warranted to determine the effect of GDF15 in mediating the metabolic disturbances that occur in Vpr mice and PLWH.

KEYWORDS
ART, energy balance, GDF15, HIV, metabolic syndrome

1 | INTRODUCTION

Persons living with HIV (PLWH) accumulate systemic metabolic alterations (Carr et al., 2000), the cardinal features of which include intra-abdominal fat accumulation, fatty liver disease, dyslipidemia, insulin resistance, and diabetes. The high prevalence of metabolic syndrome (MetSyn) in PLWH may relate to adipose tissue abnormalities and accelerated abdominal fat accumulation described among this group (Grant et al., 2016). Notably, compared to the general population, fatty liver disease in PLWH exhibits a more aggressive clinical course with a high frequency of non-alcoholic steatohepatitis (NASH) and fibrosis (Fourman et al., 2021; Vodkin et al., 2015). Treatment of these manifold defects using conventional lipid-lowering or insulin-sensitizing drugs and lifestyle interventions has been minimally effective (Carr, 2003). Despite the critical need, biomarkers of MetSyn in PLWH remain lacking and no therapies for HIV-associated fatty liver disease exist (Alkhouri et al., 2021).

Growth differentiation factor 15 (GDF15) is a circulating peptide that exerts weight-lowering effects when administered to rodents and non-human primates (Emmerson et al., 2017; Mullican et al., 2017). Intracellular stress drives the expression of GDF15 in the kidney, liver, skeletal muscle, and some immune cells. Other factors that elevate GDF15 include mitochondrial dysfunction (Coll et al., 2020; Hsiao et al., 2000; Keipert & Ost, 2021; Paralkar et al., 1998; Wang et al., 2021), the integrated stress response (Coll et al., 2020; Patel et al., 2019), and AMP-associated protein kinase (AMPK) activation (O'Neill et al., 2011). Consequently, GDF15 expression is upregulated in chronic inflammatory diseases (Moon et al., 2020), cardiovascular disease (Adela & Banerjee, 2015; Wang et al., 2021), non-alcoholic fatty liver disease (NAFLD) (Li et al., 2018) and diabetes (Adela & Banerjee, 2015; Hung et al., 2021; Wang et al., 2021).

Viral factors are central to the pathophysiology of metabolic dysfunction in PLWH (Dhurandhar, 2011; Lyons et al., 1982; Ramesh & Sanyal, 2004). We previously showed that the HIV accessory protein viral protein R (Vpr) circulates in the blood of PLWH even in the presence of viral-suppressive antiretroviral therapy (ART). We also demonstrated that forced expression of Vpr in mice recapitulates essential features of metabolic disorders in PLWH, including hyperlipidemia, dysregulated adipose tissue function, and NAFLD (Agarwal et al., 2013; Agarwal et al., 2017; Agarwal et al., 2021). Mitochondrial dysfunction caused by HIV-related factors and ART likely contribute to metabolic deterioration in PLWH (Sun et al., 2019), but the endocrine arms of these responses remain unclear. Along these lines, we explored GDF15 levels in the liver and serum of male Vpr mice and littermate controls. We also hypothesized GDF15 would be increased in the plasma of stable ART-treated PLWH relative to HIV-negative controls with and without MetSyn. Hence, we measured GDF15 levels in Vpr mice and PLWH and determined their relationships with immune reconstitution and available metabolic measures.

2 | MATERIALS AND METHODS

2.1 | Study design

We report data from male subjects who participated in studies of metabolic function and comorbidities as described below. In addition, we report data from 14 to 16 week-old male mice. Human protocols were approved by the Institutional Review Board of Baylor College of Medicine, the University of California, Los Angeles, and the University of Texas Health Science Center, Houston. Animal protocols were approved by the Baylor College of Medicine Institutional Review Board and Institutional Animal Care and Use Committee.

2.2 | Vpr transgenic (Vpr-Tg) mice

FVBN mice expressing phosphoenolpyruvate carboxykinase (*Pepck*) promoter-driven Vpr in the liver and adipose tissue under the control of a tetracycline-repressible (tTA) system were constructed at the NIH (Balasubramanyam et al., 2007).

2.3 | Synthetic Vpr

Synthetic Vpr (sVpr) was produced by solid-state peptide synthesis, purified, characterized by sequencing and mass spectrometry, and compared to viral Vpr by SDS-PAGE and immunoblotting (Henklein et al., 2000). Continuous delivery of sVpr or vehicle (sterile water) was achieved using Alzet pumps (Durect, Cupertino, CA). These were implanted subcutaneously in wild-type FVBN mice with a delivery rate of 0.25 μl/h to administer 5 μg of sVpr/24 h for 14 days (Agarwal et al., 2013; Agarwal et al., 2017; Agarwal et al., 2021; Balasubramanyam et al., 2007).

2.4 | Human plasma samples

De-identified samples from a completed, IRB-approved study of men with HIV at the University of California, Los Angeles, and the University of Texas Health Science Center Houston were used as convenience samples. All

participants provided written informed consent to use residual samples for future comorbidities research studies. General inclusion criteria included HIV-positive serostatus, male sex, age $\geq$ 50 years old, and HIV-1 RNA < 200 copies/ml on stable ART for $\geq$24 weeks (Netanya et al., 2018). These PLWH were non-diabetic and normoglycemic by fasting glucose measurements. Many were taking lipid-lowering therapy. De-identified samples of the HIV-negative healthy control subjects and HIV-negative subjects with MetSyn were obtained from Baylor College of Medicine IRB-approved studies. The healthy control subjects had no chronic illnesses, were not taking any medications, were normoglycemic, and met $\leq$2 of the criteria for MetSyn. The subjects with MetSyn were also normoglycemic and not taking any medications but met $\geq$3 non-glycemic criteria for MetSyn: waist circumference $\geq$ 102 cm, triglyceride levels $\geq$ 150 mg/dl, HDL-C < 40 mg/dl, and SBP $\geq$ 130 mm Hg or DBP $\geq$ 85 mm Hg. All plasma samples were stored at $-80°C$ until biomarker measurement. All PLWH were male, 67% White, 11% African, American, 11% Hispanic, and 11% Asian, aged 59 ± 1.73 years (mean $\pm$ SE), with a BMI of 27.1 ± 1.32 kg/m^2 (Table 1). All healthy control subjects were male, 67% White, 33% Hispanic, with a BMI of 24.55 ± 1.23 kg/m^2, aged 49 ± 7 years. All MetSyn subjects were male, 86% White, 14% African American, aged 61 ± 1.61 years (mean $\pm$ SE), with a BMI of 30.65 ± 0.87 kg/m^2.

2.5 | mRNA levels

Total RNA was extracted from livers of mice using the Trizol method (Invitrogen, Waltham, MA), transcribed using the RNA-to-cDNA kit (Applied Biosystems, Grand Island, NY), and PCR performed using the TaqMan® probe-based assay and Universal PCR Master Mix with an ABI 7000 Real-Time PCR System (Applied Biosystems, Grand Island, NY). mRNA expression levels of *Gdf15* were measured and normalized to *Pgk1* mRNA expression.

2.6 | GDF15 plasma analysis

GDF15 concentrations in plasma samples were measured by ELISA (Abcam, Milpitas, CA) following the manufacturer's protocols.

2.7 | Statistics

The primary endpoints were levels of GDF15 in men living with HIV compared to males with and without MetSyn, and in male Vpr-Tg and sVpr compared to wild-type littermates. Secondary endpoints included liver GDF15 mRNA expression and its correlation with clinical parameters such as age, CD4, and CD8 counts. We used convenience samples from male healthy, MetSyn, and HIV subjects available to us. Thus, we did not calculate a sample size for the human protocol. Sample sizes for animal studies were based on our previous data regarding the effects of Vpr on adipose tissue and the liver (Agarwal et al., 2013; Agarwal et al., 2017; Agarwal et al., 2021). Descriptive statistics of demographic characteristics are presented as median with interquartile range (IQR) and as mean and standard deviation when appropriate. Comparisons between groups were made using Mann-Whitney *U*. Correlation analyses were performed using Spearman's test. Linear regression

TABLE 1 Characteristics of participants at baseline

Characteristic	PLWH	HIV-negative healthy controls	HIV-negative metabolic syndrome patients	*p*-value
Number of subjects	18	6	7	
Age (years)	59 ± 1.73	49 ± 7	61 ± 1.61	HC vs. PLWH =0.27 MetSyn vs. PLWH =0.61
Male sex (number)	18	6	7	
Ethnicity				
White	12	4	5	
African American	2	0	2	
Asian	2	0	0	
Hispanic	2	2	0	
BMI (kg/m^2)	27.11 ± 1.32	24.55 ± 1.23	30.65 ± 0.87	HC vs. PLWH =0.81 MetSyn vs. PLWH =0.08

Note: All PLWH were normoglycemic on chronic antiretroviral therapy. All HIV-negative subjects were normoglycemic, on no medications. Non-glycemic MetSyn criteria included: Waist circumference $\geq$102 cm, triglyceride levels $\geq$150 mg/dl, HDL-C < 40 mg/dl, SBP $\geq$130 mm Hg, or DBP $\geq$85 mm Hg. MetSyn was defined by the presence of $\geq$3 of these criteria. HC = healthy control. Values represent mean $\pm$ SD.

analysis was performed relating GDF15 plasma levels to various demographic, anthropometric, and biochemical parameters in PLWH, control, and MetSyn groups. $p < 0.05$ was considered significant.

3 | RESULTS

3.1 | Vpr mice express elevated levels of Gdf15

Our retrospective analysis of RNA-Seq data (Agarwal et al., 2013; Agarwal et al., 2017) uncovered strong induction of *Gdf15* mRNA in the liver of Vpr-Tg mice. To further explore whether GDF15 is associated with metabolic disease in the Vpr mouse models, we measured *Gdf15* gene expression in the liver of male mice. Vpr-Tg mice showed 13-fold higher *Gdf15* mRNA expression in the liver than WT littermates ($p = 0.002$, Figure 1a) and 3-fold higher *Gdf15* mRNA expression in the liver of sVpr-treated mice compared to vehicle-treated mice ($p = 0.02$, Figure 1b). To determine the implications of increased *Gdf15* gene expression in the liver, we measured plasma GDF15 levels in the Vpr mice. As predicted from the fatty liver phenotype (Agarwal et al., 2017) and hepatic *Gdf15* expression changes, Vpr mice displayed elevated plasma levels of GDF15: 1.6-fold higher in Vpr-Tg mice compared to WT littermates ($p = 0.002$; Figure 1c) and approximately 3-fold higher in sVpr-treated mice compared to vehicle-treated mice ($p = 0.009$; Figure 1d).

3.2 | PLWH harbor elevated levels of circulating Gdf15

To establish whether plasma levels of GDF15 in PLWH differ from those of HIV-negative persons, we compared plasma GDF15 levels of the PLWH with those in plasma samples from both healthy control men and obese men with MetSyn (Table 1). Similar to observations in Vpr mice, plasma levels of GDF15 were 2-fold higher in the PLWH compared to controls ($p = 0.0007$) and 1.5-fold higher compared to MetSyn subjects ($p = 0.001$; Figure 2a). Median value of GDF15 in control was 174.1 pg/ml (IQR: 327.7–1156); in MetSyn subjects was 1002 pg/ml (IQR: 563–1174) and in PLWH was 1458 pg/ml (IQR: 1263–1667) (Table 2).

3.3 | Plasma GDF15 levels correlate with T cell subsets in PLWH

Chronic ART restores CD4$^+$ T cell (CD4) levels in PLWH. Especially in those with lipodystrophy or MetSyn, these changes are accompanied by increased levels of stress-associated hormones such as FGF21 (Domingo et al., 2010). We asked whether plasma GDF15 levels were also altered in relation to CD4 and CD8 T cell subsets. The PLWH showed a positive correlation of plasma GDF15 levels with CD4 count (Spearman's rho, $r_s = 0.62$, $p = 0.009$; Table 3), an inverse correlation of plasma GDF15 levels with CD8 count (Spearman's rho, $r_s = -0.56$, $p = 0.02$; Table 3) and a strong positive correlation with the CD4:CD8 ratio (Spearman's rho, $r_s = 0.71$, $p = 0.002$; Table 3; Figure 2b).

3.4 | Plasma GDF15 levels correlate with age in HIV-negative subjects but not in PLWH

We performed additional correlation analyses of GDF15 with available metabolic measurements and demographics. Both HIV-negative groups showed strong correlations of GDF15 levels with age (linear regression analysis $r^2 = 0.77$ and 0.80, respectively; $p = 0.0004$ and 0.007 respectively; Figure 2c) but this relationship was not apparent in the PLWH (linear regression analysis $r^2 = 0$; $p = 0.24$; Figure 2c).

4 | DISCUSSION

Plasma levels of GDF15, a member of the transforming growth factor-β superfamily, are increased in diabetes, cardiovascular disease, chronic inflammatory diseases, and NAFLD (Adela & Banerjee, 2015; Hsiao et al., 2000; Li et al., 2018; Moon et al., 2020). The purpose of this study was to understand how plasma GDF15 levels change in PLWH relative to HIV-negative persons with and without MetSyn. Higher levels of GDF15 in a spectrum of inflammatory and metabolic diseases formed the prediction that PLWH harbor elevated levels of GDF15 in plasma.

We noted differences in the degree of elevation of GDF15 levels between the two Vpr mouse models that likely occur due to differences in the nature of Vpr expression (Agarwal et al., 2013). In the Vpr-Tg model, transgenic expression of Vpr in *Pepck*-positive tissues (adipose tissue, liver, and kidney) occurs through most of the life of the animal. In contrast, in the sVpr model, the synthetic protein is infused in young adult mice for two weeks. Differences in tissue-specific gene and protein levels could occur due to the chronic versus short duration of the Vpr exposure strategies. Nonetheless, the energy balance phenotypes of both Vpr mouse models are very similar and recapitulate all critical aspects of HIV-associated fatty liver disease in PLWH.

GDNF family receptor alpha-like (GFRAL), a transmembrane receptor localized to the hindbrain (Emmerson

FIGURE 1 Increased mRNA levels of GDF15 in the liver and plasma of Vpr-Tg and sVpr-treated mice. (a) Gdf15 mRNA expression is increased in the liver of Vpr-Tg compared to WT mice ($p = 0.002$; $N = 8$ per group). (b) Gdf15 mRNA expression is increased in the liver of sVpr-treated compared to vehicle-treated mice ($p = 0.02$; $N = 8$ per group). (c) Increased GDF15 plasma levels in Vpr-Tg compared to WT mice ($p = 0.002$; $N = 6$ per group). (d) Increased GDF15 plasma levels in sVpr-treated compared to vehicle-treated mice ($p = 0.009$; $N = 5–6$ per group). Values are median with interquartile range. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$
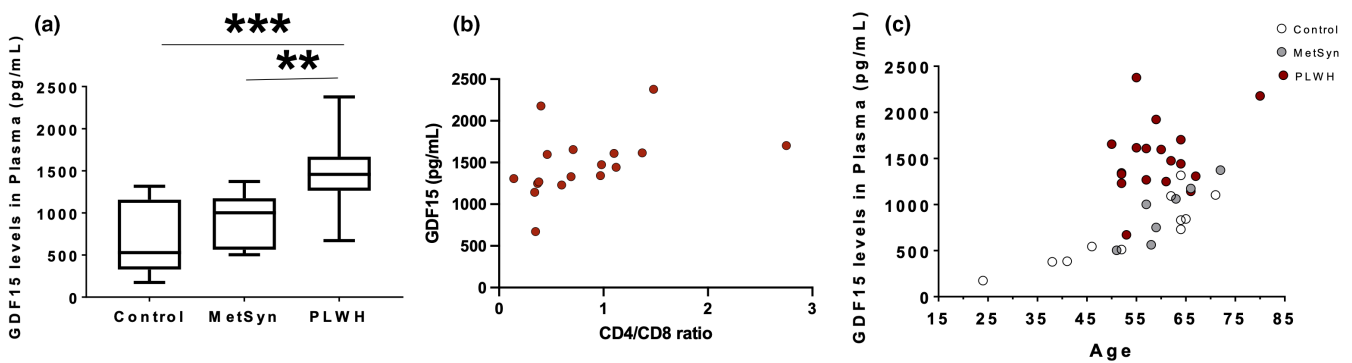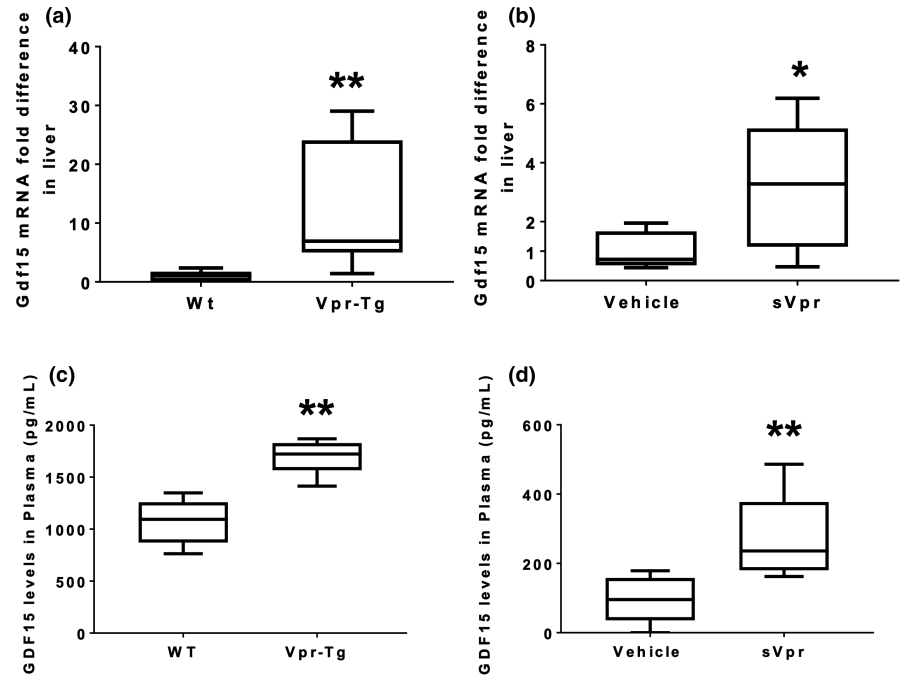

FIGURE 2 Correlates of GDF15 plasma levels in PLWH. (a) Increased GDF15 plasma levels in PLWH ($N = 18$) compared to HIV-negative healthy controls ($p = 0.0007$; $N = 6$) and HIV-negative MetSyn patients ($p = 0.0014$; $N = 7$). (b) GDF15 levels correlated positively with CD4/CD8 ratio in PLWH (Spearman correlation coefficient $r_s = 0.71$, $p = 0.002$). (c) GDF15 levels correlated positively with age in the healthy controls ($R^2 = 0.77$, $p = 0.0004$) and persons with MetSyn ($R^2 = 0.80$, $p = 0.007$) but not in PLWH ($R^2 = 0.09$, $p = 0.2351$)

TABLE 2 GDF15 levels in the study populations

	PLWH	HIV-negative healthy controls	HIV-negative metabolic syndrome patients
Number of subjects	18	6	7
GDF15 level (pg/ml)	1507 ± 391.6 (1458, 1263–1667)	671.4 ± 441.9 (528, 327.9–1156)	918.4 ± 322.7 y

Note: Values represent mean $\pm$ SD. Numbers in parenthesis represent mean and interquartile range (median, lower quartile–upper quartile). p values are based on Mann Whitney U test. Units of measurement of GDF15 levels are pg/ml.

et al., 2017; Mullican et al., 2017), binds GDF15 and mediates its satiety effects in rodents. Additional studies demonstrated that GDF15 critically mediates the appetite and weight loss effects of metformin (Day et al., 2019). GDF15 also reduces physical activity in rodents (Macia et al., 2012; Wang et al., 2013). Our finding of elevated levels of GDF15 protein in the plasma and mRNA expression in the liver of Vpr mice adds to a complex picture of the endocrine effects of GDF15. We recently showed that Vpr disrupts energy balance and thermogenic regulation of subcutaneous adipose tissue in mice (Agarwal et al., 2021). Vpr-Tg mice cannot defend body temperature when exposed to cold and display lower energy expenditure at room temperature (Agarwal et al., 2021). Mullican et al.

TABLE 3 Spearman correlation of GDF15 plasma levels with clinical parameters in PLWH

	r_s-value	p-value
Age (years)	0.08597	0.73
Ethnicity	−0.02464	0.92
Body weight (kg)	−0.07327	0.77
Body mass index (BMI, kg/m^2)	−0.03199	0.90
Glucose (mg/dl)	0.3348	0.19
Glycated hemoglobin (HbA1c, %)	−0.4454	0.23
Total cholesterol (mg/dL)	−0.2134	0.41
High density lipoprotein (HDL-C, mg/dl)	−0.08621	0.41
Low density lipoprotein (LDL, mg/dl)	−0.1887	0.47
Triglycerides (TG, mg/dl)	−0.2465	0.34
Aspartate aminotransferase (AST, mg/dl)	−0.04712	0.86
Alanine aminotransferase (ALT, mg/dl)	−0.00491	0.99
White blood cell count (WBC, ×10^9/L)	0.4794	0.06
CD4 count (cells/mm^3)	0.6242	0.009
CD8 count (cells/mm^3)	−0.5632	0.020
CD4:CD8 ratio	0.7083	0.002

reported that the GDF15 effect on body mass is due to reduced food intake (increased satiety) rather than increased energy expenditure (Johnen et al., 2007; Mullican et al., 2017; Yang et al., 2017). While we have not rigorously investigated satiety in our mouse models, we have not observed significant differences in food intake or activity between the Vpr mice and their respective controls (Agarwal et al., 2021). Hence alternative mechanisms probably underlie energy balance changes occurring in parallel with elevated GDF15 in these mice. In our study, we used male mice, which show greater sensitivity to genetic and dietary interventions that cause fatty liver disease (Clegg & Mauvais-Jarvis, 2018) and most faithfully model the human samples available to us. Additional studies are underway to test whether GDF15 levels in Vpr mice and PLWH are associated with fatty liver and resistance to the central nervous effects of GDF15 in male and female mice, similar to other hormone resistance paradigms in PLWH (Bourgi et al., 2018).

There is emerging evidence that GDF15 accumulates in the plasma of older adults (Alcazar et al., 2021; Kim et al., 2022; Yamaguchi et al., 2021). Linear regression analysis showed that GDF15 levels in the plasma of healthy controls and MetSyn HIV-negative subjects correlate positively with age. There was no such association in the PLWH. Although the age nominally varied amongst the subjects by ten years, our data suggest that circulating GDF15 levels in PLWH are not dependent on age. PLWH develops age-related comorbidities more frequently and at earlier ages than individuals without HIV (Rodriguez-Penney et al., 2013; Schouten et al., 2014). Thus, higher GDF15 levels in PLWH occurring

independently of age may mirror the confluence of older PLWH with the prevalence of advanced age-related metabolic comorbidities, such as NAFLD, and significantly raises the disease burden in PLWH.

GDF15 evolved as a signal of cellular stress and represents a biomarker of disorders of mitochondrial function. The lack of a relationship of GDF15 levels with age in the PLWH could partially reflect the phenomenon of accelerated aging in PLWH on ART, with premature development of age-related diseases. It has been demonstrated that ART accelerates mitochondrial aging through the clonal expansion of mtDNA mutations and thereby induces mitochondrial dysfunction (Payne et al., 2011). Furthermore, ART induces hallmarks of aging, including persistent low-grade inflammation and senescence in various cell types, even after relatively short exposures (Caron et al., 2008). We additionally saw a positive correlation between GDF15 plasma concentration and circulating CD4 count and CD4:CD8 ratio, which could reflect the need for immune reconstitution on ART to mount sufficient stress responses. Furthermore, insight will be gained from the measurement of GDF15 levels in PLWH after contemporary ART and in the context of accelerated age-related disorders such as sarcopenia and NAFLD.

GDF15 secretion in rodents derives from energetic stress on peripheral tissues, including the liver (Lockhart et al., 2020). Outside of GFRAL requirements for GDF15 effects on energy balance, the downstream signaling mechanisms of GDF15 and the regulation of its tissue expression have not been well defined. Furthermore, whether GDF15 serves a causal role in metabolic diseases or simply reflects energetic stress remains controversial. Key intracellular proteins governing GDF15 expression include transcription factors of the integrated stress pathway such as ATF4 (Patel et al., 2019), whose regulation becomes altered in human and rodent NAFLD (Puri et al., 2008; Seo et al., 2009). Our studies of Vpr mice allow testing of possible mechanisms supporting GDF15 secretion and action. Plausible mechanisms based on known stimuli of GDF15 expression (Hsiao et al., 2000) in the liver include Vpr-induced mitochondrial dysfunction (Agarwal et al., 2021) and overall hepatocellular stress induced by multiple mechanisms leading to NAFLD (Agarwal et al., 2017) in the Vpr mice. It is also possible that an unidentified transcriptional pathway linked to Vpr′s promiscuous ability to co-regulate host genes related to lipid/sterol metabolism or oxidative stress (Agarwal et al., 2017, 2021) or to inhibit PPARγ (Agarwal et al., 2013) could be responsible. In addition to MetSyn, plasma GDF15 may also be a biomarker of pulmonary hypertension and heart failure risk in PLWH with low-level viremia (Elvstam et al., 2019; Scherzer et al., 2018; Secemsky et al., 2015). Whether GDF15 mediates

maladaptive or adaptive responses to organ stress in the liver or elsewhere remains an open question.

Another limitation of our human study is the lack of biological and ethnic diversity. Accumulating evidence demonstrates that the prevalence and severity of NAFLD are affected by sex and age (Vilar-Gomez et al., 2018). In HIV-negative adults, NAFLD prevalence is higher in men than in pre-menopausal women (or aged ≤ 50–60 years), while NAFLD tends to become more common in women after menopause or after age 50 (Long et al., 2018). All plasma samples in the study were male, which does not allow testing of female influences on GDF15 levels in PLWH. Estrogen inhibits HIV transcription (Das et al., 2018) and may engender the differences in MetSyn prevalence between men and women living with HIV. Nonetheless, there remains little information regarding the mechanisms that contribute to the higher prevalence and severity of NAFLD among PLWH and the role of gender, race, and ethnicity on these risk factors (Lim & Bernstein, 2018; Soti et al., 2018). Unfortunately, most of the available data on NAFLD in PLWH is disproportionately from studies of male subjects (Maurice et al., 2017). Considering that women and minorities living with HIV have a greater prevalence of obesity and are at greater risk to experience poorer outcomes and increased weight gain from HIV treatment (Erlandson et al., 2016; Soti et al., 2018), there is a clear need for biomarker studies of NAFLD in these communities of PLWH.

Ethnic differences in GDF15 have been reported in acute coronary syndrome, with median GDF15 concentrations in the range of 1244–1835 ng/dl in Europeans (Zhang et al., 2016) and 650 ng/dl in Asians (Lin et al., 2014). Conversely, in a multiethnic cohort of middle-aged Dallas County adult residents, 53% of African American subjects had GDF15 levels equal to or greater than 1800 ng/dl, whereas only 17% of White subjects had GDF15 levels in that range (Rohatgi et al., 2012). Although our study population was predominantly White and had the same number of African American subjects in the PLWH and MetSyn groups, larger studies in PLWH and controls would be needed to assess the role of ethnicity in GDF15 expression in PLWH. In addition, the specific role of ART on GDF15 secretion was not evaluated in this cohort of PLWH because the current standard of care is to treat all PLWH with ART.

GDF15 levels are elevated in NAFLD in proportion to disease severity (Koo et al., 2018). Specific HIV-related risk factors (e.g., effects of persistent viral factors such as Vpr, immune activation, and antiretroviral drugs) may result in an altered course of liver disease and sustained GDF15 secretion. Development and progression of steatosis and the risk for progression towards NASH are more pronounced in patients receiving ART (Bischoff et al., 2021). To our knowledge, this is the first study that measured circulating GDF15 in PLWH and orthogonal mouse models. Our data suggest that GDF15 levels are elevated in PLWH on suppressive ART and that Vpr could be a specific, causative HIV-associated factor that either directly modulates its expression in the liver or provokes its expression and secretion as a result of persistent hepatocellular stress from HIV-related risk factors.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
A.B., J.E.L., and S.M.H. conceptualized the study. N.A, A.B., C.E.R-B., and S.M.H. designed experiments. J.E.L, H.W., and R.V. provided clinical specimens. N.A. performed ELISA and qPCR studies. N.A, A.B., C.E.R-B., and S.M.H wrote the manuscript with editorial input from all authors. All authors provided interpretations of the data. All authors have approved the submitted manuscript.

ETHICS STATEMENT
This study was approved by the IRB at UCLA and UTHSC-Houston and was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their participation in the study.

ORCID
Sean M. Hartig https://orcid.org/0000-0002-2695-2072

REFERENCES

Adela, R., & Banerjee, S. K. (2015). GDF-15 as a target and biomarker for diabetes and cardiovascular diseases: A translational prospective. *Journal of Diabetes Research*, *2015*, 490842. https://doi.org/10.1155/2015/490842

Agarwal, N., Iyer, D., Gabbi, C., Saha, P., Patel, S. G., Mo, Q., Chang, B., Goswami, B., Schubert, U., Kopp, J. B., Lewis, D. E., & Balasubramanyam, A. (2017). HIV-1 viral protein R (Vpr) induces fatty liver in mice via LXRα and PPARα dysregulation: Implications for HIV-specific pathogenesis of NAFLD. *Scientific Reports*, *7*(1), 13362. https://doi.org/10.1038/s41598-017-13835-w

Agarwal, N., Iyer, D., Patel, S. G., Sekhar, R. V., Phillips, T. M., Schubert, U., Oplt, T., Buras, E. D., Samson, S. L., Couturier, J., Lewis, D. E., Rodriguez-Barradas, M. C., Jahoor, F., Kino, T., Kopp, J. B., & Balasubramanyam, A. (2013). HIV-1 Vpr induces adipose dysfunction in vivo through reciprocal effects on PPAR/GR co-regulation. *Science Translational Medicine*, *5*(213), 213ra164. https://doi.org/10.1126/scitranslmed.3007148

Agarwal, N., Iyer, D., Saha, P., Cox, A. R., Xia, Y., Utay, N. S., Somasundaram, A., Schubert, U., Lake, J. E., Hartig, S. M., & Balasubramanyam, A. (2021). HIV-1 viral protein R couples metabolic inflexibility with white adipose tissue thermogenesis. *Diabetes*, *70*(9), 2014–2025. https://doi.org/10.2337/db20-0888

Alcazar, J., Frandsen, U., Prokhorova, T., Kamper, R. S., Haddock, B., Aagaard, P., & Suetta, C. (2021). Changes in systemic GDF15 across the adult lifespan and their impact on maximal muscle power: The Copenhagen Sarcopenia Study. *Journal of Cachexia, Sarcopenia and Muscle*, *12*(6), 1418–1427. https://doi.org/10.1002/jcsm.12823

Alkhouri, N., Tincopa, M., Loomba, R., & Harrison, S. A. (2021). What does the future hold for patients with nonalcoholic steatohepatitis: Diagnostic strategies and treatment options in 2021 and beyond? *Hepatology Communications*, *5*(11), 1810–1823. https://doi.org/10.1002/hep4.1814

Balasubramanyam, A., Mersmann, H., Jahoor, F., Phillips, T. M., Sekhar, R. V., Schubert, U., Brar, B., Iyer, D., Smith, E. O., Takahashi, H., Lu, H., Anderson, P., Kino, T., Henklein, P., & Kopp, J. B. (2007). Effects of transgenic expression of HIV-1 Vpr on lipid and energy metabolism in mice. *American Journal of Physiology. Endocrinology and Metabolism*, *292*(1), E40–E48. https://doi.org/10.1152/ajpendo.00163.2006

Bischoff, J., Gu, W., Schwarze-Zander, C., Boesecke, C., Wasmuth, J. C., van Bremen, K., Dold, L., Rockstroh, J. K., & Trebicka, J. (2021). Stratifying the risk of NAFLD in patients with HIV under combination antiretroviral therapy (cART). *EClinicalMedicine*, *40*, 101116. https://doi.org/10.1016/j.eclinm.2021.101116

Bourgi, K., Wanjalla, C., & Koethe, J. R. (2018). Inflammation and metabolic complications in HIV. *Current HIV/AIDS Reports*, *15*(5), 371–381. https://doi.org/10.1007/s11904-018-0411-2

Caron, M., Auclair, M., Vissian, A., Vigouroux, C., & Capeau, J. (2008). Contribution of mitochondrial dysfunction and oxidative stress to cellular premature senescence induced by antiretroviral thymidine analogues. *Antiviral Therapy*, *13*(1), 27–38. https://doi.org/10.1177/135965350801300103

Carr, A. (2003). HIV lipodystrophy: Risk factors, pathogenesis, diagnosis and management. *AIDS*, *17*(Suppl 1), S141–S148. https://doi.org/10.1097/00002030-200304001-00017

Carr, A., Miller, J., Law, M., & Cooper, D. A. (2000). A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: Contribution to protease inhibitor-related lipodystrophy syndrome. *AIDS*, *14*(3), F25–F32. https://doi.org/10.1097/00002030-200002180-00001

Clegg, D. J., & Mauvais-Jarvis, F. (2018). An integrated view of sex differences in metabolic physiology and disease. *Molecular Metabolism*, *15*, 1–2. https://doi.org/10.1016/j.molmet.2018.06.011

Coll, A. P., Chen, M., Taskar, P., Rimmington, D., Patel, S., Tadross, J. A., Cimino, I., Yang, M., Welsh, P., Virtue, S., Goldspink, D. A., Miedzybrodzka, E. L., Konopka, A. R., Esponda, R. R., Huang, J. T., Tung, Y. C. L., Rodriguez-Cuenca, S., Tomaz, R. A.,

Harding, H. P., ... O'Rahilly, S. (2020). GDF15 mediates the effects of metformin on body weight and energy balance. *Nature*, *578*(7795), 444–448. https://doi.org/10.1038/s41586-019-1911-y

Das, B., Dobrowolski, C., Luttge, B., Valadkhan, S., Chomont, N., Johnston, R., Bacchetti, P., Hoh, R., Gandhi, M., Deeks, S. G., Scully, E., & Karn, J. (2018). Estrogen receptor-1 is a key regulator of HIV-1 latency that imparts gender-specific restrictions on the latent reservoir. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(33), E7795–E7804. https://doi.org/10.1073/pnas.1803468115

Day, E. A., Ford, R. J., Smith, B. K., Mohammadi-Shemirani, P., Morrow, M. R., Gutgesell, R. M., Lu, R., Raphenya, A. R., Kabiri, M., McArthur, A. G., McInnes, N., Hess, S., Pare, G., Gerstein, H. C., & Steinberg, G. R. (2019). Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss. *Nature Metabolism*, *1*(12), 1202–1208. https://doi.org/10.1038/s42255-019-0146-4

Dhurandhar, N. V. (2011). A framework for identification of infections that contribute to human obesity. *The Lancet Infectious Diseases*, *11*(12), 963–969. https://doi.org/10.1016/S1473-3099(11)70274-2

Domingo, P., Gallego-Escuredo, J. M., Domingo, J. C., Gutiérrez, M. d. M., Mateo, M. G., Fernández, I., Vidal, F., Giralt, M., & Villarroya, F. (2010). Serum FGF21 levels are elevated in association with lipodystrophy, insulin resistance and biomarkers of liver injury in HIV-1-infected patients. *AIDS*, *24*(17), 2629–2637. https://doi.org/10.1097/QAD.0b013e3283400088

Elvstam, O., Medstrand, P., Jansson, M., Isberg, P. E., Gisslen, M., & Bjorkman, P. (2019). Is low-level HIV-1 viraemia associated with elevated levels of markers of immune activation, coagulation and cardiovascular disease? *HIV Medicine*, *20*(9), 571–580.

Emmerson, P. J., Wang, F., Du, Y., Liu, Q., Pickard, R. T., Gonciarz, M. D., Coskun, T., Hamang, M. J., Sindelar, D. K., Ballman, K. K., Foltz, L. A., Muppidi, A., Alsina-Fernandez, J., Barnard, G. C., Tang, J. X., Liu, X., Mao, X., Siegel, R., Sloan, J. H., ... Wu, X. (2017). The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. *Nature Medicine*, *23*(10), 1215–1219. https://doi.org/10.1038/nm.4393

Erlandson, K. M., Zhang, L., Lake, J. E., Schrack, J., Althoff, K., Sharma, A., Tien, P. C., Margolick, J. B., Jacobson, L. P., & Brown, T. T. (2016). Changes in weight and weight distribution across the lifespan among HIV-infected and -uninfected men and women. *Medicine (Baltimore)*, *95*(46), e5399. https://doi.org/10.1097/MD.0000000000005399

Fourman, L. T., Stanley, T. L., Zheng, I., Pan, C. S., Feldpausch, M. N., Purdy, J., Aepfelbacher, J., Buckless, C., Tsao, A., Corey, K. E., Chung, R. T., Torriani, M., Kleiner, D. E., Hadigan, C. M., & Grinspoon, S. K. (2021). Clinical predictors of liver fibrosis presence and progression in human immunodeficiency virus-associated nonalcoholic fatty liver disease. *Clinical Infectious Diseases*, *72*(12), 2087–2094. https://doi.org/10.1093/cid/ciaa382

Grant, P. M., Kitch, D., McComsey, G. A., Collier, A. C., Bartali, B., Koletar, S. L., Erlandson, K. M., Lake, J. E., Yin, M. T., Melbourne, K., Ha, B., & Brown, T. T. (2016). Long-term body composition changes in antiretroviral-treated HIV-infected individuals. *AIDS*, *30*(18), 2805–2813. https://doi.org/10.1097/QAD.0000000000001248

Henklein, P., Bruns, K., Sherman, M. P., Tessmer, U., Licha, K., Kopp, J., de Noronha, C. M., Greene, W. C., Wray, V., & Schubert, U.

(2000). Functional and structural characterization of synthetic HIV-1 Vpr that transduces cells, localizes to the nucleus, and induces G2 cell cycle arrest. *Journal of Biological Chemistry*, *275*(41), 32016–32026. https://doi.org/10.1074/jbc.M004044200

Hsiao, E. C., Koniaris, L. G., Zimmers-Koniaris, T., Sebald, S. M., Huynh, T. V., & Lee, S. J. (2000). Characterization of growth-differentiation factor 15, a transforming growth factor beta superfamily member induced following liver injury. *Molecular and Cellular Biology*, *20*(10), 3742–3751.

Hung, H. C., Wu, H. T., Lin, C. H., Chou, H. W., Ou, H. Y., & Chang, C. J. (2021). Associations between GDF15 levels and pre-diabetes in non-obese subjects. *Journal of Investigative Medicine*, *70*(1), 79–84. https://doi.org/10.1136/jim-2021-001805

Johnen, H., Lin, S., Kuffner, T., Brown, D. A., Tsai, V. W., Bauskin, A. R., Wu, L., Pankhurst, G., Jiang, L., Junankar, S., Hunter, M., Fairlie, W. D., Lee, N. J., Enriquez, R. F., Baldock, P. A., Corey, E., Apple, F. S., Murakami, M. M., Lin, E. J., ... Breit, S. N. (2007). Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. *Nature Medicine*, *13*(11), 1333–1340.

Keipert, S., & Ost, M. (2021). Stress-induced FGF21 and GDF15 in obesity and obesity resistance. *Trends in Endocrinology and Metabolism*, *32*(11), 904–915. https://doi.org/10.1016/j.tem.2021.08.008

Kim, M., Walston, J. D., & Won, C. W. (2022). Associations between elevated growth differentiation factor-15 and sarcopenia among community-dwelling older adults. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *77*(4), 770–780. https://doi.org/10.1093/gerona/glab201

Koo, B. K., Um, S. H., Seo, D. S., Joo, S. K., Bae, J. M., Park, J. H., Chang, M. S., Kim, J. H., Lee, J., Jeong, W. I., & Kim, W. (2018). Growth differentiation factor 15 predicts advanced fibrosis in biopsy-proven non-alcoholic fatty liver disease. *Liver International: Official Journal of the International Association for the Study of the Liver*, *38*(4), 695–705. https://doi.org/10.1111/liv.13587

Li, D., Zhang, H., & Zhong, Y. (2018). Hepatic GDF15 is regulated by CHOP of the unfolded protein response and alleviates NAFLD progression in obese mice. *Biochemical and Biophysical Research Communications*, *498*(3), 388–394. https://doi.org/10.1016/j.bbrc.2017.08.096

Lim, H. W., & Bernstein, D. E. (2018). Risk factors for the development of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, including genetics. *Clinics in Liver Disease*, *22*(1), 39–57. https://doi.org/10.1016/j.cld.2017.08.008

Lin, J. F., Wu, S., Hsu, S. Y., Yeh, K. H., Chou, H. H., Cheng, S. T., Wu, T. Y., Hsu, W. T., Yang, C. C., & Ko, Y. L. (2014). Growth-differentiation factor-15 and major cardiac events. *American Journal of the Medical Sciences*, *347*(4), 305–311. https://doi.org/10.1097/MAJ.0b013e318291cd4e

Lockhart, S. M., Saudek, V., & O'Rahilly, S. (2020). GDF15: A hormone conveying somatic distress to the brain. *Endocrine Reviews*, *41*(4), bnaa007. https://doi.org/10.1210/endrev/bnaa007

Long, M. T., Pedley, A., Massaro, J. M., Hoffmann, U., Ma, J., Loomba, R., Chung, R. T., & Benjamin, E. J. (2018). A simple clinical model predicts incident hepatic steatosis in a community-based cohort: The Framingham Heart Study. *Liver International: Official Journal of the International Association for the Study of the Liver*, *38*(8), 1495–1503. https://doi.org/10.1111/liv.13709

Lyons, M. J., Faust, I. M., Hemmes, R. B., Buskirk, D. R., Hirsch, J., & Zabriskie, J. B. (1982). A virally induced obesity syndrome in mice. *Science*, *216*(4541), 82–85. https://doi.org/10.1126/science.7038878

Macia, L., Tsai, V. W., Nguyen, A. D., Johnen, H., Kuffner, T., Shi, Y. C., Lin, S., Herzog, H., Brown, D. A., Breit, S. N., & Sainsbury, A. (2012). Macrophage inhibitory cytokine 1 (MIC-1/GDF15) decreases food intake, body weight and improves glucose tolerance in mice on normal & obesogenic diets. *PLoS One*, *7*(4), e34868.

Maurice, J. B., Patel, A., Scott, A. J., Patel, K., Thursz, M., & Lemoine, M. (2017). Prevalence and risk factors of non-alcoholic fatty liver disease in HIV-monoinfection. *AIDS*, *31*(11), 1621–1632. https://doi.org/10.1097/QAD.0000000000001504

Moon, J. S., Goeminne, L. J. E., Kim, J. T., Tian, J. W., Kim, S. H., Nga, H. T., Kang, S. G., Kang, B. E., Byun, J. S., Lee, Y. S., Jeon, J. H., Shong, M., Auwerx, J., Ryu, D., & Yi, H. S. (2020). Growth differentiation factor 15 protects against the aging-mediated systemic inflammatory response in humans and mice. *Aging Cell*, *19*(8), e13195. https://doi.org/10.1111/acel.13195

Mullican, S. E., Lin-Schmidt, X., Chin, C. N., Chavez, J. A., Furman, J. L., Armstrong, A. A., Beck, S. C., South, V. J., Dinh, T. Q., Cash-Mason, T. D., Cavanaugh, C. R., Nelson, S., Huang, C., Hunter, M. J., & Rangwala, S. M. (2017). GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and non-human primates. *Nature Medicine*, *23*(10), 1150–1157. https://doi.org/10.1038/nm.4392

Netanya, S., Utay, D. W. K., Yeh, E., Fichtenbaum, C. J., Lederman, M. M., Estes, J. D., Deleage, C., Magyar, C., Nelson, S. D., Klingman, K. L., Bastow, B., Luque, A. E., McComsey, G. A., Douek, D. C., Currier, J. S., & Lake, J. E. (2018). A5317 AIDS clinical trials group team. Telmisartan therapy does not improve lymph node or adipose tissue fibrosis more than continued antiretroviral therapy alone. *The Journal of Infectious Diseases*, *217*(11), 1770–1781. https://doi.org/10.1093/infdis/jiy064

O'Neill, H. M., Maarbjerg, S. J., Crane, J. D., Jeppesen, J., Jorgensen, S. B., Schertzer, J. D., Shyroka, O., Kiens, B., van Denderen, B. J., Tarnopolsky, M. A., Kemp, B. E., Richter, E. A., & Steinberg, G. R. (2011). AMP-activated protein kinase (AMPK) beta1beta2 muscle null mice reveal an essential role for AMPK in maintaining mitochondrial content and glucose uptake during exercise. *Proceedings of the National Academy Sciences of the United States of America*, *108*(38), 16092–16097.

Paralkar, V. M., Vail, A. L., Grasser, W. A., Brown, T. A., Xu, H., Vukicevic, S., Ke, H. Z., Qi, H., Owen, T. A., & Thompson, D. D. (1998). Cloning and characterization of a novel member of the transforming growth factor-beta/bone morphogenetic protein family. *Journal of Biological Chemistry*, *273*(22), 13760–13767.

Patel, S., Alvarez-Guaita, A., Melvin, A., Rimmington, D., Dattilo, A., Miedzybrodzka, E. L., Cimino, I., Maurin, A. C., Roberts, G. P., Meek, C. L., Virtue, S., Sparks, L. M., Parsons, S. A., Redman, L. M., Bray, G. A., Liou, A. P., Woods, R. M., Parry, S. A., Jeppesen, P. B., ... O'Rahilly, S. (2019). GDF15 provides an endocrine signal of nutritional stress in mice and humans. *Cell Metabolism*, *29*(3), 707–718.e708. https://doi.org/10.1016/j.cmet.2018.12.016

Payne, B. A., Wilson, I. J., Hateley, C. A., Horvath, R., Santibanez-Koref, M., Samuels, D. C., Price, D. A., & Chinnery, P. F. (2011). Mitochondrial aging is accelerated by antiretroviral therapy through the clonal expansion of mtDNA mutations. *Nature Genetics*, *43*(8), 806–810. https://doi.org/10.1038/ng.863

Puri, P., Mirshahi, F., Cheung, O., Natarajan, R., Maher, J. W., Kellum, J. M., & Sanyal, A. J. (2008). Activation and dysregulation of the unfolded protein response in non-alcoholic fatty liver disease. *Gastroenterology*, *134*(2), 568–576. https://doi.org/10.1053/j.gastro.2007.10.039

Ramesh, S., & Sanyal, A. J. (2004). Hepatitis C and non-alcoholic fatty liver disease. *Seminars in Liver Disease*, *24*(4), 399–413.

Rodriguez-Penney, A. T., Iudicello, J. E., Riggs, P. K., Doyle, K., Ellis, R. J., Letendre, S. L., Grant, I., & Woods, S. P. (2013). Group HIVNComorbiditiesiies in persons infected with HIV: increased burden with older age and negative effects on health-related quality of life. *AIDS Patient Care STDS*, *27*(1), 5–16.

Rohatgi, A., Patel, P., Das, S. R., Ayers, C. R., Khera, A., Martinez-Rumayor, A., Berry, J. D., McGuire, D. K., & de Lemos, J. A. (2012). Association of growth differentiation factor-15 with coronary atherosclerosis and mortality in a young, multiethnic population: Observations from the Dallas Heart Study. *Clinical Chemistry*, *58*(1), 172–182. https://doi.org/10.1373/clinchem.2011.171926

Scherzer, R., Shah, S. J., Secemsky, E., Butler, J., Grunfeld, C., Shlipak, M. G., & Hsue, P. Y. (2018). Association of biomarker clusters with cardiac phenotypes and mortality in patients with HIV infection. *Circulation: Heart Failure*, *11*(4), e004312. https://doi.org/10.1161/CIRCHEARTFAILURE.117.004312

Schouten, J., Wit, F. W., Stolte, I. G., Kootstra, N. A., van der Valk, M., Geerlings, S. E., Prins, M., Reiss, P., Reiss, P., Wit, F. W. N. M., van der Valk, M., Schouten, J., Kooij, K. W., van Zoest, R. A., Elsenga, B. C., Prins, M., Stolte, I. G., Martens, M., Moll, S., … Group AGCS. (2014). Cross-sectional comparison of the prevalence of age-associated comorbidities and their risk factors between HIV-infected and uninfected individuals: The AGEhIV cohort study. *Clinical Infectious Diseases*, *59*(12), 1787–1797. https://doi.org/10.1093/cid/ciu701

Secemsky, E. A., Scherzer, R., Nitta, E., Wu, A. H., Lange, D. C., Deeks, S. G., Martin, J. N., Snider, J., Ganz, P., & Hsue, P. Y. (2015). Novel biomarkers of cardiac stress, cardiovascular dysfunction, and outcomes in HIV-infected individuals. *JACC: Heart Failure*, *3*(8), 591–599. https://doi.org/10.1016/j.jchf.2015.03.007

Seo, J., Fortuno, E. S. 3rd, Suh, J. M., Stenesen, D., Tang, W., Parks, E. J., Adams, C. M., Townes, T., & Graff, J. M. (2009). Atf4 regulates obesity, glucose homeostasis, and energy expenditure. *Diabetes*, *58*(11), 2565–2573. https://doi.org/10.2337/db09-0335

Soti, S., Corey, K. E., Lake, J. E., & Erlandson, K. M. (2018). NAFLD and HIV: Do sex, race, and ethnicity explain HIV-related risk? *Current HIV/AIDS Reports*, *15*(3), 212–222. https://doi.org/10.1007/s11904-018-0392-1

Sun, J., Longchamps, R. J., Piggott, D. A., Castellani, C. A., Sumpter, J. A., Brown, T. T., Mehta, S. H., Arking, D. E., & Kirk, G. D. (2019). Association between HIV infection and mitochondrial DNA copy number in peripheral blood: A population-based, prospective cohort study. *The Journal of Infectious Diseases*, *219*(8), 1285–1293. https://doi.org/10.1093/infdis/jiy658

Vilar-Gomez, E., Calzadilla-Bertot, L., Wai-Sun Wong, V., Castellanos, M., Aller-de la Fuente, R., Metwally, M., Eslam, M., Gonzalez-Fabian, L., Alvarez-Quinones Sanz, M., Conde-Martin, A. F., De Boer, B., McLeod, D., Hung Chan, A. W., Chalasani, N., George, J., Adams, L. A., & Romero-Gomez, M. (2018). Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease: A multi-national cohort study. *Gastroenterology*, *155*(2), 443–457.e417. https://doi.org/10.1053/j.gastro.2018.04.034

Vodkin, I., Valasek, M. A., Bettencourt, R., Cachay, E., & Loomba, R. (2015). Clinical, biochemical and histological differences between HIV-associated NAFLD and primary NAFLD: A case-control study. *Alimentary Pharmacology & Therapeutics*, *41*(4), 368–378. https://doi.org/10.1111/apt.13052

Wang, D., Day, E. A., Townsend, L. K., Djordjevic, D., Jorgensen, S. B., & Steinberg, G. R. (2021). GDF15: Emerging biology and therapeutic applications for obesity and cardiometabolic disease. *Nature Reviews Endocrinology*, *17*(10), 592–607. https://doi.org/10.1038/s41574-021-00529-7

Wang, X. M., Li, J., Yan, M. X., Liu, L., Jia, D. S., Geng, Q., Lin, H. C., He, X. H., Li, J. J., & Yao, M. (2013). Integrative analyses identify osteopontin, LAMB3 and ITGB1 as critical pro-metastatic genes for lung cancer. *PLoS One*, *8*(2), e55714. https://doi.org/10.1371/journal.pone.0055714

Yamaguchi, Y., Zampino, M., Tanaka, T., Bandinelli, S., Osawa, Y., Ferrucci, L., & Semba, R. D. (2021). Elevated plasma growth and differentiation factor 15 predicts incident anemia in older adults aged 60 years and older. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *76*(7), 1192–1197. https://doi.org/10.1093/gerona/glaa324

Yang, L., Chang, C. C., Sun, Z., Madsen, D., Zhu, H., Padkjaer, S. B., Wu, X., Huang, T., Hultman, K., Paulsen, S. J., Wang, J., Bugge, A., Frantzen, J. B., Norgaard, P., Jeppesen, J. F., Yang, Z., Secher, A., Chen, H., Li, X., … Jorgensen, S. B. (2017). GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. *Nature Medicine*, *23*(10), 1158–1166. https://doi.org/10.1038/nm.4394

Zhang, S., Dai, D., Wang, X., Zhu, H., Jin, H., Zhao, R., Jiang, L., Lu, Q., Yi, F., Wan, X., & Cui, H. (2016). Growth differentiation factor-15 predicts the prognoses of patients with acute coronary syndrome: A meta-analysis. *BMC Cardiovascular Disorders*, *16*, 82. https://doi.org/10.1186/s12872-016-0250-2