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Key relationships between non-alcoholic fatty liver disease, insulin resistance, diabetes mellitus, frailty, integrase-strand transferase inhibitor-based antiretroviral therapy and weight gain in people living with HIV

PhD thesis

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1. Abstract (English version)

In the past three decades, advances in antiretroviral therapies (ART) changed the clinical scenario of people living with HIV (PLWH). The wasting syndrome was a characteristic of the pre-ART era, while in nowadays, we are witnessing to increasing incidence and prevalence of cardiometabolic conditions, such as weight gain associated with use of ART, non-alcoholic fatty liver disease (NAFLD), insulin resistance (IR) and diabetes. Moreover, this clinical scenario puts PLWH at higher risk to experience prematurely geriatric syndromes and frailty.

However, many questions still remain unanswered. How much NAFLD, as a multisystemic state, could determine frailty in PLWH? Can complex endocrine pathways involved in the pathogenesis of NAFLD inform specific and personalized interventions in PLWH? What is the role of IR and what of diabetes in the natural history of NAFLD and NAFLD with fibrosis in PLWH? Does weight gain associated with, in particular, use of integrase inhibitors (INSTI) depend on pre-existing cardiometabolic conditions prior to switch to INSTI? Are INSTI still a metabolic friendly option in PLWH? What is the weight gain definition that is associated with clinically relevant outcomes?

This thesis, based on three major studies performed in a large observational cohort of PLWH, tries to address these questions. In particular, the contribution of NAFLD with and without fibrosis to frailty, as a measure of biological age, was investigated. The dynamic interplay among endocrine disorders and NAFLD or NAFLD with fibrosis, using Bayesian networks, was also explored. Weight and body mass index (BMI) changes were depicted in a large cohort and in the subsets of PLWH without diabetes and IR at the time to switch to INSTI. Finally, we attempted to identify the cut-off of weight or BMI increase associated with IR incidence in PLWH switching to INSTI.

In detail, in paper I, in the study that comprised 707 PLWH, predictors of frailty were: liver steatosis (OR=2.1, 1.3-3.5), fibrosis (OR=2, 1-3.7), NAFLD with fibrosis (OR=9.2, 5.2-16.8), diabetes (OR=1.7, 1-2.7) and multimorbidity (OR=2.5, 1.5-4). NAFLD with fibrosis was associated with frailty 3-fold more than multimorbidity, indicating that NAFLD with fibrosis as a multisystemic construct. In paper II, in the study including 1434 PLWH, IR alone (OR=2.36, 95%CI:1.37-4.14, p=0.002) was more strongly associated with NAFLD than diabetes alone (OR=1.41, 95%CI:1.05-1.90, p=0.022). DAG model for NAFLD with fibrosis

showed a direct association with IR-diabetes only. Diabetes alone (OR=2.76, 95%CI:1.77-4.24, p<0.001) was associated with NAFLD with fibrosis, while IR alone was not. In paper III, that comprised 2437 PLWH, switching to INSTI based regimens (vs. INSTI-naive) was associated with a lower risk of IR (HR=0.71, $CI_{95\%}$: 0.52, 0.98, p=0.025). A 1% weight increase reduced the total protective effect of INSTI by 21.1% over one year of follow-up, allowing us to identify a 5% weight increase as a clinically meaningful weight gain definition.

Overall, obtained results underline the importance of cardiometabolic conditions and its implications in PLWH. NAFLD with fibrosis exceeded multimorbidity in the prediction of frailty and it is mostly driven by diabetes, while NAFLD alone is more strongly associated with IR. A data-driven weight-gain definition in relation to IR as a clinically meaningful endpoint was identified. Switching to INSTI in PLWH without pre-existing metabolic abnormalities offsets negative effects of weight gain on IR incidence, confirming INSTI regimens as a metabolically satisfactory option in PLWH.

2. Abstract (versione italiana)

Negli ultimi tre decenni, i progressi nelle terapie antiretrovirali (ART) hanno cambiato lo scenario clinico delle persone che convivono con l'HIV (PLWH). La sindrome “wasting” era una caratteristica dell'era pre-ART, mentre attualmente stiamo assistendo ad una crescente incidenza e prevalenza di condizioni cardiometaboliche, come aumento di peso associato all'uso di ART, steatosi epatica non alcolica (NAFLD), insulina resistenza (IR) e diabete. Inoltre, questo scenario clinico espone la PLWH a un rischio maggiore di manifestare sindromi geriatriche prematuramente e fragilità.

Tuttavia, molte domande rimangono ancora senza risposta. Quanto NAFLD, come stato multisistemico, potrebbe determinare la fragilità in PLWH? Le complesse vie endocrine coinvolte nella patogenesi della NAFLD possono informare interventi specifici e personalizzati nella PLWH? Qual è il ruolo della IR e del diabete nella storia naturale della NAFLD e della NAFLD con fibrosi nella PLWH? L'aumento di peso associato, in particolare, all'uso di inibitori dell'integrasi (INSTI), dipende da condizioni cardiometaboliche preesistenti prima del passaggio a INSTI? INSTI è ancora un'opzione metabolicamente favorevole in PLWH? Qual è la definizione di aumento di peso associata agli outcome clinicamente rilevanti?

Questa tesi, basata su tre studi principali eseguiti in un'ampia coorte osservativa di PLWH, cerca di rispondere a queste domande. In particolare, è stato studiato il contributo della NAFLD con e senza fibrosi alla fragilità, come misura dell'età biologica. È stata anche esplorata l'interazione dinamica tra disturbi endocrini e NAFLD o NAFLD con fibrosi, utilizzando reti bayesiane. Le variazioni del peso e dell'indice di massa corporea (BMI) sono state rappresentate in un'ampia coorte e nei sottogruppi di PLWH senza diabete e IR al momento del passaggio a INSTI. Infine, abbiamo tentato di identificare il cut-off dell'aumento di peso o BMI associato all'incidenza di IR nel passaggio da PLWH a INSTI.

In dettaglio, nel paper I, nello studio che comprendeva 707 PLWH, i predittori di fragilità erano: steatosi epatica (OR=2.1, 95%CI 1.3-3.5), fibrosi (OR=2, 95%CI 1-3.7), NAFLD con fibrosi (OR=9.2, 95%CI 5,2-16,8), diabete (OR=1,7, 1-2,7) e multimorbidità (OR=2,5, 1,5-4). La NAFLD con fibrosi è stata associata a fragilità 3 volte più della multimorbidità, indicando che la NAFLD con fibrosi è un costrutto multisistemico. Nel paper II, nello studio che includeva 1434 PLWH, l'IR (OR=2,36, 95%CI:1,37-4,14, p=0,002) era più fortemente associata alla NAFLD rispetto al diabete (OR=1,41, 95%CI:1,05- 1,90, p=0,022). Il modello DAG per NAFLD con fibrosi ha mostrato un'associazione diretta solo con il diabete-IR. Il solo diabete (OR=2,76, 95%CI:1,77-4,24, p<0,001) era associato a NAFLD con fibrosi, mentre la sola IR non lo era. Nel paper III, che comprendeva 2437 PLWH, il passaggio a regimi basati su INSTI (rispetto a INSTI-naive) era associato a un minor rischio di IR (HR=0,71, CI95%: 0,52, 0,98, p=0,025). Un aumento di peso dell'1% ha ridotto l'effetto protettivo totale di INSTI del 21,1% in un anno di follow-up, consentendoci di identificare un aumento di peso del 5% come definizione clinicamente significativa di aumento di peso.

Nel complesso, i risultati ottenuti sottolineano l'importanza delle condizioni cardiometaboliche e le sue implicazioni nel PLWH. La NAFLD con fibrosi ha superato la multimorbilità nella previsione della fragilità, ed è principalmente guidata dal diabete, mentre la NAFLD da sola è più fortemente associata all'IR. È stata identificata una definizione di aumento di peso basata sui dati in relazione alla IR come endpoint clinicamente significativo. Il passaggio a INSTI in PLWH senza anomalie metaboliche preesistenti compensa gli effetti negativi dell'aumento di peso sull'incidenza di IR, confermando i regimi INSTI come un'opzione metabolicamente soddisfacente in PLWH.

3. Aims

3.1. Hypothesis

The hypotheses of the studies reported in the PhD thesis were the following:

- NAFLD with or without fibrosis could be a significant determinants of frailty in PLWH, in the context of a multisystemic nature of both these conditions;
- The complex endocrine pathways explored in observational real-life cohorts of PLWH may depict specific patterns involved in NAFLD pathogenesis and indicate specific personalized interventions in the management of NAFLD;
- Weight gain observed in PLWH switching to INSTI may depend on pre-existing cardiometabolic conditions;
- Switching to INSTI-based regimens may be overall metabolically favorable.

3.2. Objectives

The overall objectives of the PhD thesis were the following:

- to investigate the correlation between liver steatosis and significant fibrosis alone and in association (NAFLD with fibrosis) and frailty, as a measure of biological age, in PLWH (paper I);
- to explore, using Bayesian networks, the dynamic interplay among endocrine disorders and NAFLD or NAFLD with fibrosis, in relatively large and well-characterized cohort of PLWH (paper II);
- to explore weight and BMI changes in a large cohort and in the subsets of PLWH without diabetes and insulin resistance (IR) at the time of switch to INSTI in the attempt to identify the cut-off of weight or BMI increase associated with incidence of IR in PLWH switching to INSTI (paper III).

4. Paper I

4.1. Brief study outline

NAFLD should not be considered as a liver condition only, but rather as a multisystemic state involving other organs [1]. Indeed, NAFLD has been linked to increased risk of diabetes, cardiovascular disease, and chronic kidney disease in the general population. In this scenario, NAFLD is not only seen as a risk factor, but can play an important role in the complex pathogenesis of co-morbidities. Accumulation of co-morbidities is described by multimorbidity, but this construct fails to describe the clinical complexity of aging, that can rather be conceptualized through the construct of frailty, which may depict vulnerable individuals at higher risk to adverse health outcomes [2].

In this study we aimed to investigate the contribution of liver steatosis and significant fibrosis alone and in association (NAFLD with fibrosis) to frailty as a measure of biological age in people living with HIV (PLWH). In a cross-sectional study of consecutive patients attending Modena HIV Metabolic Clinic in 2018-2019, PLWH with hazardous alcohol intake and viral hepatitis co-infection were excluded. Liver steatosis was diagnosed by controlled attenuation parameter (CAP), while liver fibrosis was diagnosed by liver stiffness measurement (LSM). NAFLD was defined as presence of liver steatosis (CAP≥248 dB/m), while significant liver fibrosis or cirrhosis (stage ≥F2) as LSM≥7.1 kPa. Frailty was assessed using a 36-Item frailty index (FI). Logistic regression was used to explore predictors of frailty using steatosis and fibrosis as covariates.

4.2. Key findings

This study, that included 707 PLWH (mean age 53.5 years, 76.2% males), explored in detail liver steatosis and fibrosis in relation to chronological age and frailty as a proxy of biological age. NAFLD with fibrosis was present in 10.2%; 18.9% and 3.9% of patients were classified as frail and most-frail, respectively. The highest peaks for liver steatosis, fibrosis and NAFLD are observed in PLWH at the age of 51-55 (43.4%, 21.4% and 15.1% respectively) and at the age 65+, which is defined as geriatric age (46.2%, 25.6% and 15.4% respectively). In PLWH with NAFLD with fibrosis, frailty was identified in 69%.

Univariate analysis demonstrated that neurocognitive impairment (OR=5.1, 1.6-15), vitamin D insufficiency (OR=1.94, 1.2-3.2), obesity (OR=8.1, 4.4-14.6), diabetes (OR=3.2, 1.9-5.6) and osteoporosis (OR=0.37, 0.16-0.76) were significantly associated with NAFLD with fibrosis. Predictors of FI included: steatosis (OR=2.1, 1.3-3.5), fibrosis (OR=2, 1-3.7), NAFLD with fibrosis (OR=9.2, 5.2-16.8), diabetes (OR=1.7, 1-2.7) and multimorbidity (OR=2.5, 1.5-4). Liver steatosis and NAFLD with fibrosis were associated with frailty. NAFLD with fibrosis exceeded multimorbidity in the prediction of frailty, suggesting the former as an indicator of metabolic age in PLWH. NAFLD with fibrosis would reflect better biological age (frailty) of the individual, consistent with our initial hypothesis and the main finding. NAFLD with fibrosis and frailty showed almost a linear association. In our multivariate analysis, NAFLD with fibrosis was associated with frailty, 3-fold more than multimorbidity, suggesting that NAFLD with fibrosis as a multisystemic construct [1] exceeds the construct of multimorbidity, defined as a simple sum of single co-morbidities. Given this strong association with frailty as a measure of biological age, we may suggest NAFLD with fibrosis as an indicator to assess the metabolic age of an individual.

5. Paper II

5.1. Brief study outline

Metabolic syndrome and obesity are the cornerstones of liver fibrosis in PLWH [3]. Recent data suggest that PLWH with NAFLD are at higher risk of incident diabetes and dyslipidemia [4]. In the general population, NAFLD is also related to many endocrine alterations, such as hypothyroidism [5], hypogonadism [6], insulin resistance (IR) [7], diabetes [8], vitamin D insufficiency [9] and insulin growth factor-1 (IGF-1) axis disorders [10], while these associations remain mainly unexplored in PLWH. The complex endocrine pathways are rarely studied together in observational real-life cohorts which may depict specific patterns involved in NAFLD pathogenesis and indicate specific personalized interventions in the management of NAFLD. Therefore, our objective was to explore, using Bayesian networks, the dynamic interplay among endocrine disorders and NAFLD or NAFLD with fibrosis, in relatively large and well-characterized cohort of PLWH.

This was a cross-sectional study of PLWH attending Modena HIV Metabolic Clinic, Italy in the period June 2018-January 2020. NAFLD with fibrosis was defined as the contemporary presence of NAFLD and significant liver fibrosis (liver stiffness measurement ≥7.1 kPa).

Learning Bayesian Networks were applied to identify the conditional probabilities and relationship network among the different predictors (endocrine disorders, age and sex) and the outcomes through a Directed Acyclic Graph (DAG). Each DAG model was accompanied by a multivariable logistic regression that quantified the effect of identified direct relationships between the predictors and NAFLD or NAFLD with fibrosis.

5.2. Key findings

We enrolled 1434 PLWH (75.5% males), mean age of 54.2 years. NAFLD was diagnosed in 39.3%, while NAFLD with fibrosis in 8.2% of PLWH. DAG model for NAFLD identified direct associations with age, insulin resistance (IR) and vitamin D insufficiency. IR alone (OR=2.36, 95%CI:1.37-4.14, p=0.002) was more strongly associated with NAFLD than diabetes alone (OR=1.41, 95%CI:1.05-1.90, p=0.022). DAG model for NAFLD with fibrosis showed a direct association with IR-diabetes only. Diabetes alone (OR=2.76, 95%CI:1.77-4.24, p<0.001) was associated with NAFLD with fibrosis, while IR alone was not.

This study was conducted in a relatively large sample size and well-characterized cohort of PLWH. The addition of DAG models to merely traditional multivariable models gave the possibility to emulate the complex endocrine pathways and feedback mechanisms involved in the natural history of NAFLD in PLWH. The exploration of these pathways could inform the current clinical care and management of NAFLD in PLWH in the absence of specific therapeutic interventions. In consideration of contemporary assessment of multiple disorders, we were able to quantify the weight of each examined variable in the complex endocrine pathways. We show that IR, diabetes and vitamin D insufficiency are of paramount importance in PLWH, while none of the other endocrine disorders were related to NAFLD and NAFLD with fibrosis in PLWH. Although some of these results were partially expected, they are once more emphasizing the need to focus therapeutic and lifestyles interventions such as vitamin D supplementation and physical activity while we are waiting for more promising and effective pharmacological agents for NASH [11]. To conclude, DAG models revealed that IR is more strongly associated with NAFLD than diabetes, while NAFLD with fibrosis is mainly driven by diabetes rather than IR. This suggests a significant role of diabetes on fibrosis progression in PLWH. Vitamin D supplementation might be considered in PLWH with NAFLD.

6. Paper III

6.1. Brief study outline

Weight gain is a common phenomenon affecting PLWH starting or switching ART [12–15]. Weight gain implies generalized, abdominal and ectopic fat increase that is commonly associated with insulin resistance (IR), assessed with HOMA which predicts diabetes, metabolic syndrome and cardiovascular disease. It is multifactorial in nature and it is difficult to disentangle its drivers including genetic factors, environment, lifestyles, drugs, and the complex immuno-metabolic alterations that are often present in PLWH undergoing INSTI with or without tenofovir alfenamide (TAF). A weight gain definition which considers a meaningful weight or BMI change associated with clinically relevant outcomes, including cardiometabolic conditions, is missing. There is a conflicting data if INSTI are associated with a higher risk of IR [16,17]. Therefore, we explored weight and BMI changes in a large cohort and in the subsets of individuals without diabetes and IR at the time of switch to INSTI in the attempt to identify the cut-off of weight or BMI increase associated with incidence of IR in PLWH switching to INSTI.

This was a longitudinal matched-cohort study including PLWH attending MHMC, divided into two groups: INSTI-naive and INSTI-switchers (INSTI-s) matched for similar observation time since entrance in the cohort (T0-T1). Time zero (T0) represents the date of the first visit at the MHMC in both groups. In INSTI-s, time 1 (T1) is the visit date of switching to INSTI. In INSTI-n, T1 was chosen as the closest visit with a similar observation time between T0 and T1 as in INSTI-s (within a 1-month tolerance). A total of 54826 weight assessments were analyzed comprising 46377 observations in 1412 PLWH INSTI-n and 8449 observations in 1025 PLWH INSTI-s. When PLWH with diabetes and IR at T1 were excluded, the number of observations was 36063 and 5261, respectively. A maximum of 5 observations in INSTI-n were selected, to match one observation in INSTI-s to maintain the proportion of the observations in the two groups in the cohort. Time to diabetes or IR event was defined as the time from T1 to diabetes or IR diagnosis or to the last observation for censored subjects (T2). The effect of switching to INSTI on percentage of weight and BMI change was tested through a linear mixed model. Trajectories of weight and BMI change were compared through piece-wise regression identifying three time periods: (i) prior to switch to INSTI and matched observation of INSTI-n (pre INSTI-s/n), (ii) at the time of switch to INSTI and matched observation of INSTI-n up to two years (early INSTI-s/n), and (iii) after two years

from switching to INSTI and matched observation of INSTI-n (late INSTI-s/n).A mediation analysis was performed to explore the mediation effect of weight and BMI change in the causal path between the switch to INSTI and IR incidence.

6.2. Key findings

In the entire cohort, weight and BMI were similar between the two groups at T1. PLWH switching to INSTI-s displayed a significantly higher prevalence of cardiometabolic conditions including diabetes (14.4% vs. 6.9%, p<0.001), metabolic syndrome (37.2% vs. 18.9%, p<0.001), cardiovascular disease (5.8% vs. 2.2% p<0.001) at T1. In the entire cohort, based on 54826 weight assessments, trends for weight (β 0.386, $CI_{95\%}$: 0.216, 0.555, p<0.001) and BMI (β 0.142, $CI_{95\%}$: 0.080, 0.203, p<0.001) increase were significantly higher in INSTI-s (vs. INSTI-n) in the first two years after switching, but no differences were observed between the two groups in the late period. This latter result is encouraging and could suggest that in the long term PLWH undergoing INSTI have the same trend in weight gain as INSTI-n. We confirmed an early weight gain in the first two years after switch to INSTI. Contrary to randomized clinical trials, we were able to project weight change in the late period, beyond 96 weeks. We also observed a subset of PLWH undergoing INSTI and TAF in which no plateau of weight gain was observed at the follow-up, similarly to what hypothesized in the ADVANCE trial [14,18].

Our main finding, based on a large sample size in a real-life cohort, shows that switching to INSTI (vs. INSTI-n) in PLWH was associated with a lower risk of IR (HR=0.71, $CI_{95\%}$: 0.52, 0.98, p=0.025). However, a 1% weight increase reduced the total protective effect of INSTI by 21.1% over one year of follow-up, allowing us to identify a 5% weight increase as a clinically meaningful weight gain definition. These data imply that switching to INSTI in PLWH without IR at T1 is not associated with higher risk of metabolic alterations when compared to INSTI-n, confirming INSTI regimens as a metabolically satisfactory option in PLWH. We highlight that switch to INSTI is more frequently offered to PLWH with pre-existing cardiometabolic conditions. This strategy clusters PLWH at higher risk to develop weight gain and adverse health outcomes. Conversely, switching to INSTI in PLWH without metabolic abnormalities offsets negative effects of weight gain on IR incidence. This study contributd to a data-driven weight-gain definition in relation to IR as a clinically meaningful endpoint to be used in clinical and research settings.

7. Discussion

In the last 30 years, all the advances in the antiretroviral therapy were followed by the significant cardiometabolic impact in PLWH. In the era of contemporary ART and “silver tsunami” [19] that is affecting PLWH, this thesis tries to contribute to the better understanding of interplay between aging, depicted by frailty, and cardiometabolic conditions, depicted by insulin resistance, diabetes, NAFLD and weight gain. These findings underline once more a crucial role of cardiometabolic conditions that may determine significantly frailty trajectories. The obtained results from paper I imply that NAFLD with fibrosis was associated with frailty 3-fold more than multimorbidity. Thus, NAFLD with fibrosis as a multisystemic construct [1] exceeds the construct of multimorbidity, defined as a simple sum of single co-morbidities. Moreover, dedicated lifestyles’ interventions on NAFLD could considerably reduce the burden of frailty in PLWH.

In the era of successful treatment for chronic hepatitis C virus infection and optimal control of hepatitis B virus infection, NAFLD has risen as the most prevalent chronic liver condition in PLWH [20]. NAFLD as a multisystemic construct is also related to diverse endocrine alterations in the general population, such as vitamin D deficiency, IR or diabetes, while PLWH with NAFLD are at higher risk of incident diabetes [4]. This thesis tried to fill the knowledge gap by exploring more profoundly the interplay among IR, diabetes and vitamin D insufficiency and NAFLD in PLWH. Results from paper II showed that IR alone is more strongly associated with NAFLD than diabetes, while NAFLD with fibrosis was mainly driven by diabetes rather than IR. DAG models also revealed that age and vitamin D insufficiency had a direct impact on NAFLD, but not on NAFLD with fibrosis. These results provide a novel insight into dynamics of IR, diabetes and NAFLD and NAFLD with fibrosis in PLWH.
An important relationship between NAFLD and IR emerged from our findings and some previous reports, but both conditions are still not used as end-points in PLWH, although EACS guidelines have dedicated the whole chapter on prevention, diagnosis and treatment of NAFLD in PLWH. Our findings could reinforce further efforts to use NAFLD and IR as significant cardiometabolic outcomes in randomized clinical trials of PLWH.

On the other hand, weight gain has been identified as an important issue in the current HIV care and research. Despite being addressed in all recent clinical trials regarding novel antiretroviral agent and INSTI in particular [14,18], it is still poorly characterized and its cardiometabolic impact remains still elusive. Results from paper III offered a definition of

weight gain that is associated with an clinically meaningful outcome, such as IR. Firstly, weight trajectories were analyzed in the entire population of PLWH, then in the subsets of PLWH without diabetes and IR at the time of switch to INSTI. This approach was used in order to depict weight trajectories in clinically very heterogenous PLWH, i.e. PLWH with already present metabolic alterations (entire population) vs. PLWH that could be considered metabolically “healthy”. Trends of weight and BMI increase were observed in all PLWH regardless of ART regimens, but also confirmed greater weight gain among PLWH switching to INSTI in the first two years after the switch. However, weight gain varied across the entire population and two subsets, showing that PLWH at highest risk of weight gain were those with already present metabolic alterations at the time of switch to INSTI. Further analysis indicated that switching to INSTI (vs. INSTI-n) in PLWH was associated with a lower risk of IR and that 1% weight increase reduced the total protective effect of INSTI by 21.1% over one year of follow-up, thus identifying a 5% weight increase as a clinically meaningful weight gain definition, that could be used in clinical trials.

In the light of presented results, future research should focus on the relationship between ART classes and NAFLD, weight gain and NAFLD. In order to capture these link, longitudinal data is needed with at least annual assessments for NAFLD. Furthermore, with the aging epidemic affecting PLWH, longitudinal studies on cardiometabolic predictors of frailty transitions are necessary. Such research would allow to detect areas of intervention and prevention that could compress the frailty to an older age. Another interesting topic involves the relationship between sarcopenia, sarcopenic obesity, frailty and cardiometabolic conditions.

In conclusion, the obtained results underline the importance of cardiometabolic conditions and its implications in PLWH. NAFLD with fibrosis exceeded multimorbidity in the prediction of frailty and it is mostly driven by diabetes, while NAFLD alone is more strongly associated with IR. A data-driven weight-gain definition in relation to IR as a clinically meaningful endpoint was identified. Switching to INSTI in PLWH without pre-existing metabolic abnormalities offsets negative effects of weight gain on IR incidence, confirming INSTI regimens as a metabolically satisfactory option in PLWH.

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9. PAPER I

Liver steatosis and non-alcoholic fatty liver disease with fibrosis are predictors of frailty in people living with HIV

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ABSTRACT

Objective

The aim was to investigate the contribution of liver steatosis and significant fibrosis alone and in association (NAFLD with fibrosis) to frailty as a measure of biological age in people living with HIV (PLWH).

Design

This was a cross-sectional study of consecutive patients attending Modena HIV Metabolic Clinic in 2018-2019.

Methods

Patients with hazardous alcohol intake and viral hepatitis co-infection were excluded. Liver steatosis was diagnosed by controlled attenuation parameter (CAP), while liver fibrosis was diagnosed by liver stiffness measurement (LSM). NAFLD was defined as presence of liver steatosis (CAP≥248), while significant liver fibrosis or cirrhosis (stage ≥F2) as LSM≥7.1 kPa. Frailty was assessed using a 36-Item frailty index (FI). Logistic regression was used to explore predictors of frailty using steatosis and fibrosis as covariates.

Results

We analyzed 707 PLWH (mean age 53.5 years, 76.2% males, median CD4 700 µL, 98.7% with undetectable HIV RNA). NAFLD with fibrosis was present in 10.2%; 18.9% and 3.9% of patients were classified as frail and most-frail, respectively. Univariate analysis demonstrated that neurocognitive impairment (OR=5.1, 1.6-15), vitamin D insufficiency (OR=1.94, 1.2-3.2), obesity (OR=8.1, 4.4-14.6), diabetes (OR=3.2, 1.9-5.6), metabolic syndrome (OR=2.41, 1.47–3.95) and osteoporosis (OR=0.37, 0.16-0.76) were significantly associated with NAFLD with fibrosis. Predictors of FI included: steatosis (OR=2.1, 1.3-3.5), fibrosis (OR=2, 1-3.7), NAFLD with fibrosis (OR=9.2, 5.2-16.8), diabetes (OR=1.7, 1-2.7) and multimorbidity (OR=2.5, 1.5-4).

Conclusion

Liver steatosis and NAFLD with fibrosis were associated with frailty. NAFLD with fibrosis exceeded multimorbidity in the prediction of frailty, suggesting the former as an indicator of metabolic age in PLWH.

Background

Non-alcoholic fatty liver disease (NAFLD) has become an emerging condition in general aging population and the most common cause of chronic liver disease [1]. The predictions show that NAFLD prevalence will increase by 21%, from 83.1 million in 2015 to 101 million in 2030 in the United States [2]. NAFLD is defined as fat accumulation in >5% hepatocytes not attributable to consumption of alcohol and may be associated to fibrosis. Both steatosis and fibrosis could be diagnosed by non-invasive diagnostic tools, such as transient elastography (TE) with controlled attenuation parameter (CAP). On the other side, the diagnosis of non-alcoholic steatohepatitis (NASH) is based on liver biopsy that combines liver steatosis with hepatocyte ballooning and lobular inflammation, that potentially leads to progressive liver fibrosis and hepatic failure [1].

Recent studies confirm the increasing burden of NAFLD in people living with HIV (PLWH), as viral hepatitis prevalence and associated mortality decline [3]. Moreover, liver disease is a leading cause of non-AIDS related deaths in PLWH [4].

Nevertheless, NAFLD should not be considered as a liver condition only, but rather as a multisystemic state involving other organs [5]. Indeed, NAFLD has been linked to increased risk of type 2 diabetes mellitus, cardiovascular disease, and chronic kidney disease in the general population. In this scenario, NAFLD is not only seen as a risk factor, but can play an important role in the complex pathogenesis of non-infectious co-morbidities (NICMs) [5]. This relationship remains unexplored in PLWH, despite metabolic syndrome and its components are frequent and consistently reported as risk factors for NAFLD in this population [6].

Accumulation of NICMs described by multimorbidity, is a typical feature in people reaching geriatric age, nevertheless this construct fails to describe the clinical complexity of aging. For this reason, geriatric medicine has conceptualized the construct of frailty to identify individuals more vulnerable to adverse health outcomes and to provide suitable clinical interventions.

So far, it has been shown that frailty represents a more accurate measure of an individual's biological age that may replace the traditional metric of chronological age, which does not necessarily correspond [7]. Frailty is defined as a condition characterized by the reduction

of homeostatic reserves exposing the individual to a greater risk of negative outcomes such as multimorbidity, falls, disability, nursing home placement and death [8].

In the clinical setting, how to measure frailty is still a matter of debate. At the organ level, sarcopenia assessment has been proposed as an equivalent of frailty, while for a more profound and holistic approach, frailty phenotype and frailty index had been used [9]. In fact, while sarcopenia is an important component of frailty, it may be seen as a one-dimensional approach, while the aging in an individual is a complex process where multiple factors play a role. The association of NAFLD and sarcopenia has been investigated both in HIV and the general population [10–12]. Our group has recently reported that sarcopenia assessed by grip strength increased the risk of NAFLD [13], while the data regarding association between NAFLD and frailty remains scarce (Supplementary figure 1).

We hypothesized that NAFLD could be a significant determinant of frailty, in the context of a multisystemic nature of both these conditions. Therefore, the objective of the study was to investigate the correlation between liver steatosis and significant fibrosis alone and in association (NAFLD with fibrosis) and frailty, as a measure of biological age, in PLWH.

Material and methods

Study design

This was a cross-sectional study that included consecutive PLWH attending Modena HIV Metabolic Clinic (MHMC) from June 2018 to May 2019. MHMC is a tertiary level referral center established in 2004 where PLWH are screened for NICMs and immuno-metabolic disorders including NAFLD, geriatric syndromes and frailty to better describe their aging trajectory.

Inclusion and exclusion criteria

We included ART-experienced PLWH who were evaluated for liver steatosis and fibrosis by transient elastography at MHMC. Patients with hepatitis B (HBV), hepatitis C (HCV) co-infection and hazardous alcohol intake were excluded from the study. HBV and HCV co-infection diagnosis were based on serology, while the alcohol intake was evaluated through

an AUDIT questionnaire that collects self-reported information, using as cut-offs 30g/day for men and 20g/day for women [14].

Assessment of steatosis and fibrosis

Liver stiffness measurement (LSM) and associated CAP were evaluated using TE with M probe. Liver steatosis was diagnosed by CAP as follows: S0 (no steatosis; CAP<248 dB/m), S1 (mild steatosis; 248≥ CAP <268 dB/m), S2 (moderate steatosis; 269≥ CAP<280 dB/m), S3 (severe steatosis; CAP≥ 280 dB/m) [15]. All measurements >248 dB/m were considered as NAFLD. Liver fibrosis was diagnosed by LSM as follows: stage F0-F1 (mild fibrosis, LSM<7.1 kPa), F2-F3 (significant fibrosis, 7.1≥LSM<13 kPa), F4 (cirrhosis, LSM≥13 kPa) [16]. NAFLD with fibrosis was defined as the contemporary presence of liver steatosis (CAP≥248) and significant liver fibrosis or cirrhosis (stage ≥F2). In the unavailability of liver biopsy, we considered NAFLD with fibrosis as a proxy of NASH .

Covariates

Demographic, anthropometric, HIV-related and immune-metabolic variables were collected on the same day of the visit at MHMC. Co-morbidities were defined using the European AIDS Clinical Society (EACS) guidelines [17]. Hypertension was defined as two consecutive measurements of blood pressure >140/90 mmHg. T2DM was defined as fasting serum glucose levels >126 mg/dL or HbA1C >6.5%. Diagnoses of hypertension and T2DM were also identified based on the current use of antihypertensive or antidiabetic drugs. Obesity was diagnosed as body mass index (BMI) > 30 kg/m^2. Dyslipidemia was defined as elevated total or low density lipoprotein (LDL) cholesterol or low high density lipoprotein (HDL) cholesterol above laboratory limits. Chronic kidney disease (CKD) as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m2 calculated using the CKD-Epi equation. Laboratory values for the diagnosis of CKD and dyslipidemia were confirmed in two consecutive measurements. Metabolic syndrome was defined using MetS ATPIII classification [18], including three or more of the following five criteria: waist circumference over 102 cm (men) or 88 cm (women), blood pressure over 130/85 mmHg, fasting triglyceride level over 150 mg/dl, fasting HDL cholesterol level less than 40 mg/dl (men) or 50 mg/dl (women), and fasting blood glucose over 100 mg/dl [18]. Cardiovascular disease included myocardial infarction, coronary artery disease, peripheral vascular disease, stroke, and angina pectoris, as well as coronary artery bypass grafting and angioplasty, based on

diagnoses recorded in patient files. Chronic obstructive pulmonary disease was defined as post-bronchodilator FEV1/FVC <0.70 with spirometry. Vitamin D insufficiency was defined as < 20 ng/mL (< 50 nmol/L). Osteoporosis in postmenopausal women and men aged ≥50 years was defined as a bone mass index (BMD) T-score ≤-2.5 and in premenopausal women and men aged <50 years as a BMD Z-score ≤-2 and fragility fracture. The presence of a cancer was considered if a diagnosis was reported in the patient's medical history. Depression was evaluated using the CES-D questionnaire. Neurocognitive impairment was evaluated using CogState battery that comprises six tasks, one for each cognitive domain [19]. Multi-morbidity was defined as ≥2 comorbidities in the same individual.

Frailty assessment

Frailty was determined using 36-Item frailty index (FI) generated by a standardized comprehensive geriatric assessment, previously validated at MHMC and constructed from health variables collected at the same study visit (Supplementary table 1). Each variable included in the FI was coded with a value of 1 when a deficit was present, and 0 when it was absent. Missing values were removed from both the numerator and the denominator of the FI [20]. The FI for each patient visit was calculated as the ratio between the number of deficits present and the total number of deficits assessed. Missing values were removed from both the numerator and denominator of the FI (FI = $\frac{\sum Deficit}{(36-\sum Missingvalues)}$). Each FI was computed when a minimum of 80% of valid data for the health variables was available. We categorized PLWH according to FI score as fit (<0.25), frail (0.25-0.4), most frail (>0.4) [21]. Of note, NAFLD, selected as a covariate of interest for this study, was excluded from the computation of the FI.

Statistical analysis

Results were expressed as mean and standard deviation (± SD), or median and interquartile range (IQR) for continuous variables based on the normality of distribution, and as frequencies and percentages for categorical variables. Student's t-test and ANOVA were applied to identify statistical difference for the continuous variables with normal distribution, while the Mann-Whitney and Walls-Kruskal test was used for those without normal distribution. The $\chi 2$ test was performed to assess the frequency of the categorical variables. Logistic regression models were built to explore the contribution of liver steatosis and significant fibrosis alone and in association (NAFLD with fibrosis) to frailty along with age,

gender, diabetes mellitus and multi-morbidity. Multivariate regression models included covariates with a p-value<0.05 in univariable analysis or covariates that were determined *a priori* to be clinically important, based on previous literature.

Subgroups of PLWH with liver steatosis, significant fibrosis and NAFLD with fibrosis were mutually exclusive. The missing data was removed from denominator and was not considered in the calculations. The numbers between square brackets [N°] refer to number of people in which the given variable was available. The significance of the tests was set to 0.05. The statistical program R, v. 3.6.0 in GNU Linux environment was used to analyze the data.

This study was approved by the University of Modena and Reggio Emilia ethics committee according to the Helsinki declaration.

Results

We analyzed 707 PLWH. Mean age was 53.5 (±8.2) years, 76.2% were males, mean BMI was 24.6 (±4.2), 18.3% had T2DM, median CD4 was 700 µL (IQR=540-889), HIV RNA viral load was undetectable in 98.7% of cases. Prevalence of NAFLD was 39.7%, while NAFLD with fibrosis was present in 10.2%, frail and most-frail in 18.9% and 3.9%, respectively. Study population characteristics according to frailty index categories is provided in Supplementary table 2. Detailed description of liver steatosis and fibrosis is provided in Supplementary table 3.

Supplementary figure 2 depicts trends of prevalence of steatosis, fibrosis and NAFLD with fibrosis across different chronological age categories (<45, 46-50, 51-55, 56-60, 61-65, >65 years). The highest peaks for all three conditions are observed in PLWH at the age of 51-55 (43.4%, 21.4% and 15.1% respectively) and at the age 65+, which is defined as geriatric age (46.2%, 25.6% and 15.4% respectively).

With regards to a biological age, assessed by FI, prevalence of frailty increased with higher levels of liver steatosis and fibrosis, and NAFLD with fibrosis (Figure 1). Frailty was present in 41.2% and 52.3% in PLWH with mild/moderate and severe liver steatosis respectively (Figure 1A, p<0.001). A similar pattern is observed for liver fibrosis, i.e. FI scores >0.25 were found in 54.8% and 70% PLWH with significant fibrosis and cirrhosis, respectively (Figure

1B, p<0.001). In PLWH with NAFLD with fibrosis, frailty was identified in 69% (Figure 1C, p<0.001).

NAFLD with fibrosis group showed a higher prevalence of obesity (36% vs. 6%) (p<0.001) (Table 1). With regards to HIV variables, PLWH with NAFLD with fibrosis presented a longer mean HIV duration and lower CD4 nadir. The use of NNRTI was observed in 24% of PLWH with more than two times higher use in people without NAFLD with fibrosis. This observation was accompanied by higher HDL cholesterol levels, while LDL and total cholesterol levels were lower (Table 1).

Univariate analysis demonstrated that neurocognitive impairment (OR=5.08, 1.61-14.96), vitamin D insufficiency (OR=1.94, 1.18-3.24), obesity (OR=8.06, 4.44-14.55), diabetes mellitus (OR=3.24, 1.91-5.59), metabolic syndrome (OR=2.41, 1.47–3.95) and osteoporosis (OR=0.37, 0.16-0.76) were associated with NAFLD with fibrosis (Table 2).

To better explore this association, a multivariate logistic model was built. Independent positive predictors for FI were steatosis (OR=2.12, 1.3-3.45) and fibrosis (OR=1.95, 1.03-3.66) alone and in association – NAFLD with fibrosis (OR=9.19, 5.17-16.79), diabetes mellitus (OR=1.65, 1-2.74) and multimorbidity (OR=2.46, 1.53-4.01) (Figure 2).

Discussion

The relationship between liver steatosis and significant fibrosis alone and in association (NAFLD with fibrosis) and frailty is not a widely investigated topic in PLWH and general population.

This study explored in detail liver steatosis and fibrosis in relation to chronological age and frailty as a proxy of biological age. With regards to chronological age, steatosis and NAFLD with fibrosis follow the same trend of prevalence across age groups in our study. The curves have an initial increase until the age of 50, followed by a plateau that occurs between 50-65. The prevalence of NAFLD across age remains controversial. Most studies showed that NAFLD prevalence increases with advancing age [22]. However, the cross-sectional nature of the study and a small subset of patients older than 65 years do not permit us to make strong conclusions about prevalence of liver steatosis and NAFLD with fibrosis in geriatric PLWH.

It remains unclear if NAFLD with fibrosis is regulated by age or is a regulator of biological age itself. According to the second scenario, NAFLD with fibrosis would reflect better biological age (frailty) of the individual, consistent with our initial hypothesis and the main finding. NAFLD with fibrosis and frailty showed almost a linear association. In our multivariate analysis, NAFLD with fibrosis was associated with frailty, 3-fold more than multimorbidity, suggesting that NAFLD with fibrosis as a multisystemic construct [5] exceeds the construct of multimorbidity, defined as a simple sum of single NICMs. Given this strong association with frailty as a measure of biological age, we may suggest NAFLD with fibrosis as an indicator to assess the metabolic age of an individual. From the present study emerges lack of association between chronological age and frailty index, both in univariate and multivariate analysis. This is not consistent with previous studies from our group and to what was shown in general population [21]. Such finding could be justified by different of cut-offs to identify frail individuals or choice not to use FI as a continuous variable, as in the previously quoted study [21].

Our multivariate analysis showed that male gender is protective against frailty, which is in line with observations in general population [23]. In HIV setting were identified different variables associated with frailty in males (VACS index, C-reactive protein and falls) and females (CRP, AIDS, and menopause) [24]. These observations imply that choice of variables that entered in the frailty index used in our study might have played a role in the gender difference. However, further studies are needed to investigate patterns of frailty with the regards to gender.

As expected, obesity, BMI, waist circumference, higher aminotransferase, GT and triglycerides and lower HDL levels were strongly associated with NAFLD with fibrosis suggesting that PLWH share common metabolic patterns occurring in the general population [25]. Similar findings were obtained in a large cohort study that comprised PLWH with and without metabolic syndrome in which obesity and insulin resistance were key factors associated with liver fibrosis independently of the HIV duration and exposure to ART [26]. However, the pathogenesis of NAFLD in PLWH has some additional features [27], expressed by viro-immunological parameters, depicted by low nadir and longer HIV duration or other factors that may contribute to immune activation in PLWH. This suggests that also an “immunological scar” that might be depicted by higher levels of sCD163 is associated with metabolic harm and hepatic inflammation, captured by NAFLD with fibrosis [26].

We arbitrary chose a definition of NAFLD with fibrosis based on the European Association for the Study of the Liver (EASL) definitions of liver steatosis and fibrosis singularly evaluated with TE [16]. This combined definition was used in order to offer a proxy measurement of NASH. Liver fibrosis, as the most important histological determinant of NASH, is associated with long-term negative outcomes in the general population and PLWH [28,29].

We found that not only NAFLD with fibrosis, but also liver steatosis was associated with frailty, implying that health care interventions that are proven to be effective, such as weight loss and other lifestyle changes [30,31], should be reinforced and promoted in order to decrease the burden of frailty, as well as the burden of NAFLD with fibrosis in PLWH.

In patients in which lifestyle changes are not sufficient to treat NAFLD/NASH, some other therapeutic options are considered, such as pharmacologic treatment or liver transplantation [32,33]. In the last few years, an increased number of clinical trials for treatment of NAFLD/NASH are noted [34,35], recognizing this condition as one of the major challenges in contemporary and future health care. We observed that 69% of PLWH with NAFLD with fibrosis were frail in our study. The only validated test to prove NASH is liver biopsy [1,16]. NASH diagnosis is rarely made because of the unavailability and invasiveness of liver biopsy, implying that the selection of the affected patients for pharmacologic treatment will also be an issue. Frailty assessment could serve as an additional criterion in decision making, as it is more feasible in the routine clinical practice.

This study has a number of limitations. Some of these are intrinsic to the cross-sectional nature of the study, which cannot reveal a causative association between NAFLD with fibrosis and frailty. The absence of a control group does not allow us to show whether demographic, anthropometric, HIV variables and comorbidities associated both with NAFLD with fibrosis and frailty represent a different pattern of PLWH when compared to the general population.

In conclusion, liver steatosis and fibrosis alone and in association were associated with frailty. NAFLD with fibrosis exceeded multimorbidity in the prediction of frailty, suggesting the former as an indicator of metabolic age in PLWH.

Conflict of interest

GS has acted as speaker for Merck, BMS, Gilead, Abbvie, ViiV, served as an advisory board member for Merck, BMS and Novartis has received research funding from Merck and Echosens. GG received research grant and speaker honorarium from Gilead, ViiV, MERCK and Jansen. GG attended advisory boards of Gilead, ViiV and MERCK. Other authors reported no conflict of interest.

Authors contributions

JM, FS, AM, GS and GG conceptualized and designed the study. JM, VM and GG wrote the manuscript. JM, GS and GG did the supervision of the final version of the manuscript. All the authors contributed to discussion and revision of the manuscript.

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Academic presentations

The results of this study were presented at 17th EACS (6-9 November 2019, Basel, Switzerland) as an oral presentation and at 21st International Workshop on Co-Morbidities and Adverse Drug Reactions in HIV (5-6 November 2019, Basel, Switzerland) as a poster.

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Table 1. Demographic, anthropometric HIV and clinical characteristics in PLWH according to the presence of NAFLD with fibrosis.

Variable	No NAFLD with fibrosis	NAFLD with fibrosis	p
N	635 (89.8%)	72 (10.2%)	
Sex, male (%)	479 (75.4%)	60 (83.3%)	0.20
Age, years, mean (±SD), [N°]	53.2 (8.1) [635]	55.8 (8.2) [72]	0.02
BMI, kg/m^2, mean (±SD), [N°]	24.2 (4) [633]	28.7 (4.2) [72]	<0.001
Waist circumference, cm, mean (±SD), [N°]	90.8 (34.4) [595]	104.9 (11.2) [67]	<0.001
Obesity (%)	38 (6%)	26 (36.1%)	<0.001
Mild/moderate physical activity (%)	292 (46%)	18 (25%)	0.001
HIV duration, months, median (IQR), [N°]	275 (165.5-338) [615]	322 (229-372) [71]	0.007
Nadir CD4, median, c/µL, median (IQR) [N°]	224 (100-328.8) [606]	180 (67-287) [68]	0.03
Current CD4, median, c/µL, median (IQR) [N°]	700 (542.3-884.8) [622]	689 (436-891) [69]	0.39
Current CD8, median, c/µL, median (IQR) [N°]	778.5 (581.8-1038) [620]	823 (550-1054) [69]	0.92
CD4/CD8 ratio, mean (±SD), [N°]	1 (0.5) [620]	0.98 (0.5) [69]	0.65
Undetectable viral load (%)	627 (98.7%)	71 (98.6%)	0.99
Type 2 diabetes (%)	99 (15.6%)	30 (41.7%)	<0.001
Polypharmacy (%)	81 (12.8%)	17 (23.6%)	0.02
Current exposure to NNRTI (%)	160 (28.1%)	9 (13.6%)	0.02
FIB-4. mean (±SD), [N°]	1.5 (0.9) [285]	1.6 (0.6) [37]	0.02
AST, U/l, mean (±SD), [N°]	24.1 (12.5) [605]	30.2 (14.9) [65]	<0.001
γGT, U/L, mean (±SD), [N°]	29 (28.9) [600]	48.1 (36) [65]	<0.001
Total cholesterol, mg/dl, mean (±SD), [N°]	180.9 (35.6) [603]	166.9 (41.9) [65]	0.003
LDL cholesterol, mg/dl, mean (±SD), [N°]	117.8 (32.7) [600]	106.1 (38.7) [64]	0.004
HDL cholesterol, mg/dl, mean (±SD), [N°]	51.6 (15) [600]	43.8 (10.6) [65]	<0.001
Triglycerides, mg/dl, mean (±SD), [N°]	133.5 (90) [603]	169.8 (108.2) [64]	0.002
Metabolic syndrome (%)	176 (27.7%)	49 (68.1%)	<0.001
Grip strength, kg, mean, (±SD), [N°]	36 (9.6) [573]	37.3 (8.3) [68]	0.46
4m walk speed test, seconds, mean, (±SD) [N°]	3.6 (0.6) [588]	3.8 (0.6) [66]	0.005
30s chair-stand test, mean (±SD) [N°]	8.1 (2.8) [603]	8.6 (2.8) [67]	0.12

Abbreviations: AST - aspartate aminotransferase; BMI – body mass index; HDL – high density lipoprotein; HIV – human immunodeficiency virus; IQR – interquartile range; LDL – low density lipoprotein; [N°] – number of people in which the given variable was available; NAFLD – non-alcoholic fatty liver disease; NNRTI – non-nucleoside reverse transcriptase inhibitors; SD – standard deviation

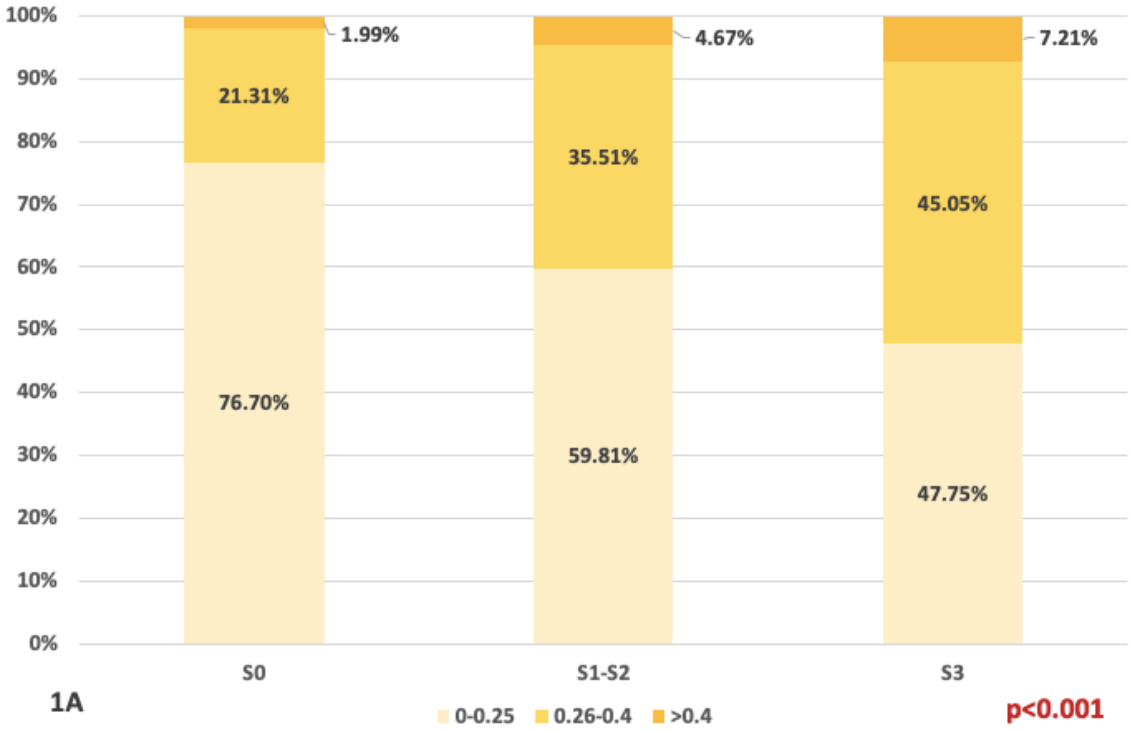
Table 2. Univariate analysis demonstrates that neurocognitive impairment, vitamin D insufficiency, obesity, T2DM and osteoporosis are associated with NAFLD with fibrosis.

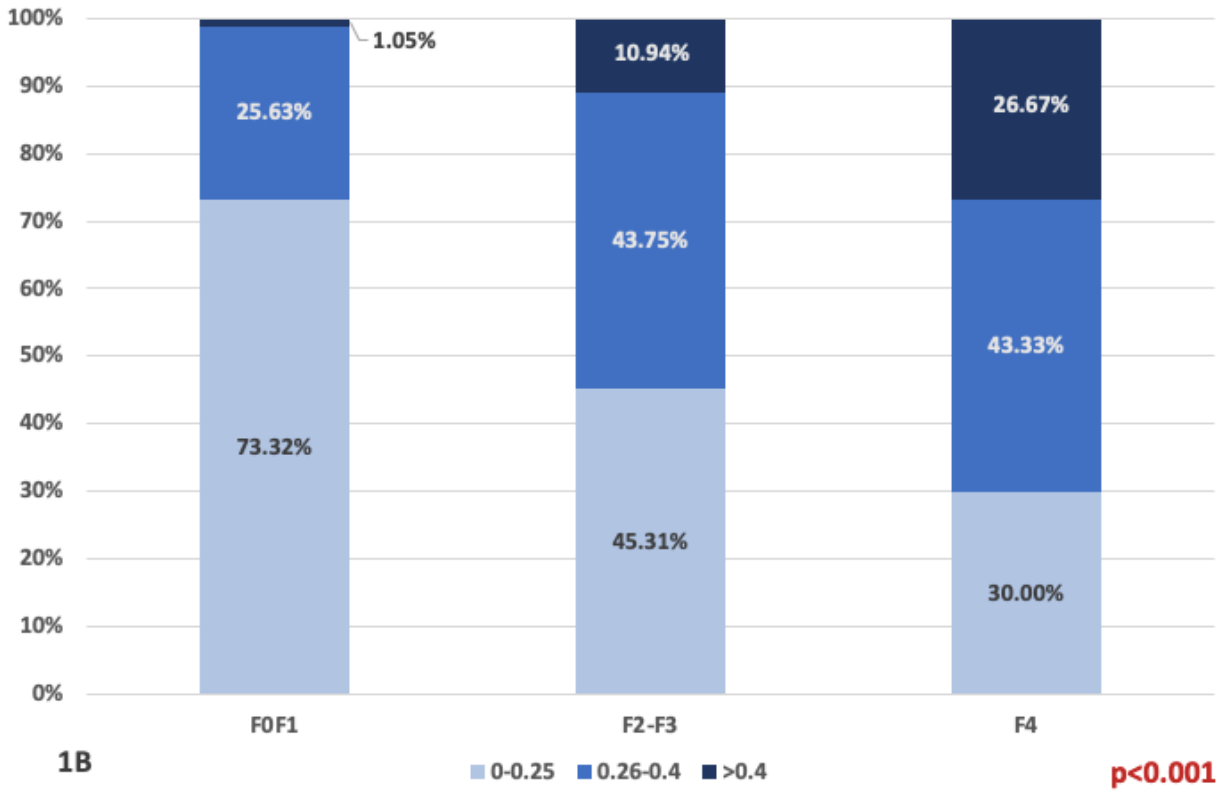
	NAFLD with fibrosis		
	OR	**IQR**	**p**
Depression	1.78	0.93-3.31	0.07
NC impairment	5.08	1.61-14.96	<0.01
Vitamin D insufficiency	1.94	1.18-3.24	0.01
Obesity	8.06	4.44-14.55	<0.01
Hypertension	1.59	0.95-2.7	0.08
Type 2 diabetes	3.28	1.91-5.59	<0.01
CVD	0.85	0.25-2.29	0.78
CKD	0.71	0.35-1.35	0.32
COPD	1.32	0.38-3.57	0.62
Osteoporosis	0.37	0.16-0.76	0.01
Dyslipidemia	0.92	0.47-1.92	0.8
Cancer	1.36	0.70-2.50	0.34
Metabolic syndrome	2.41	1.47-3.95	<0.01

Abbreviations: CKD – chronic kidney disease; COPD – chronic obstructive pulmonary disease; CVD – cardiovascular disease; IQR – interquartile range; NAFLD – non-alcoholic fatty liver disease; NC – neurocognitive, OR – odds ratio.

Figures

Figure 1 shows the association between frailty index divided into three groups (fit (<0.25), frail (0.25-0.4), most frail (>0.4)) and liver steatosis (1A), liver fibrosis (1B) and NAFLD with fibrosis (1C). **Abbreviations:** F0-F1 – mild fibrosis; F2-F3 – significant fibrosis; F4 – cirrhosis; S0 – without steatosis; S1-S2 – mild-moderate steatosis; S3 – severe steatosis.

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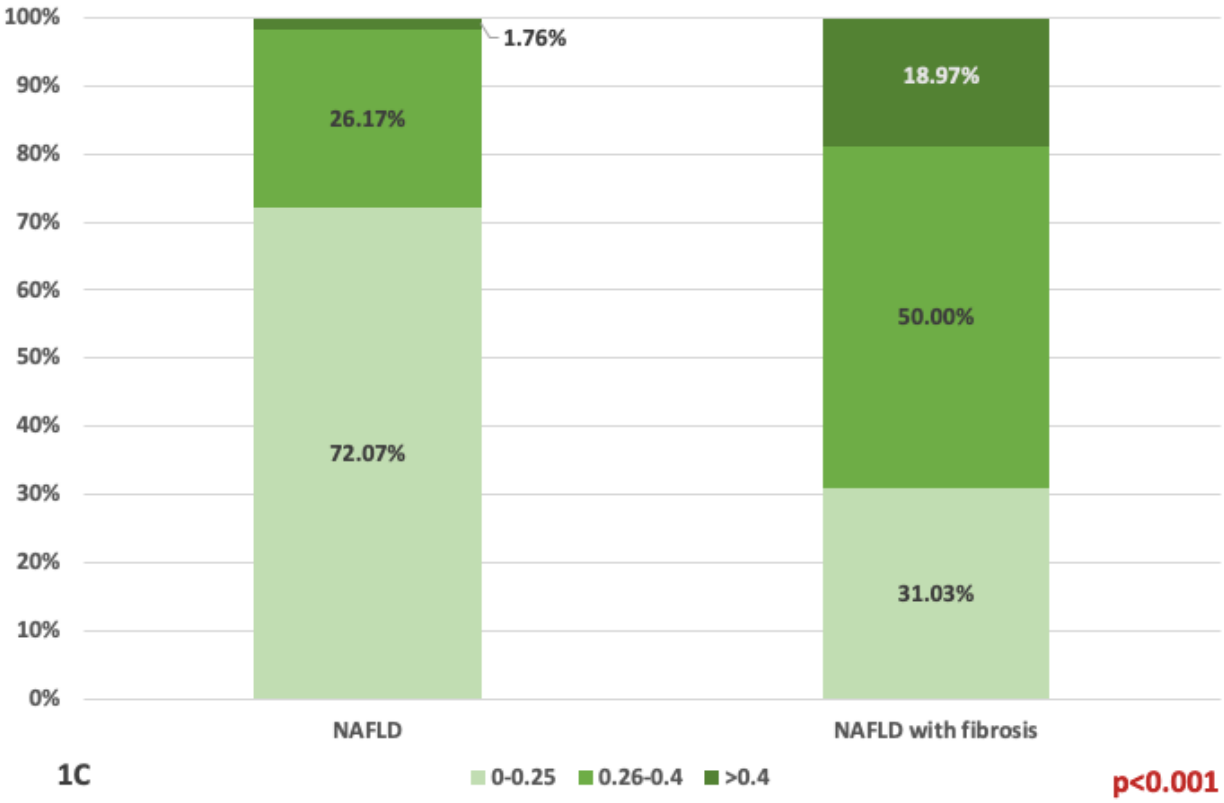
F0F1
F2-F3
F4
0-0.25
0.26-0.4
>0.4

1B

p<0.001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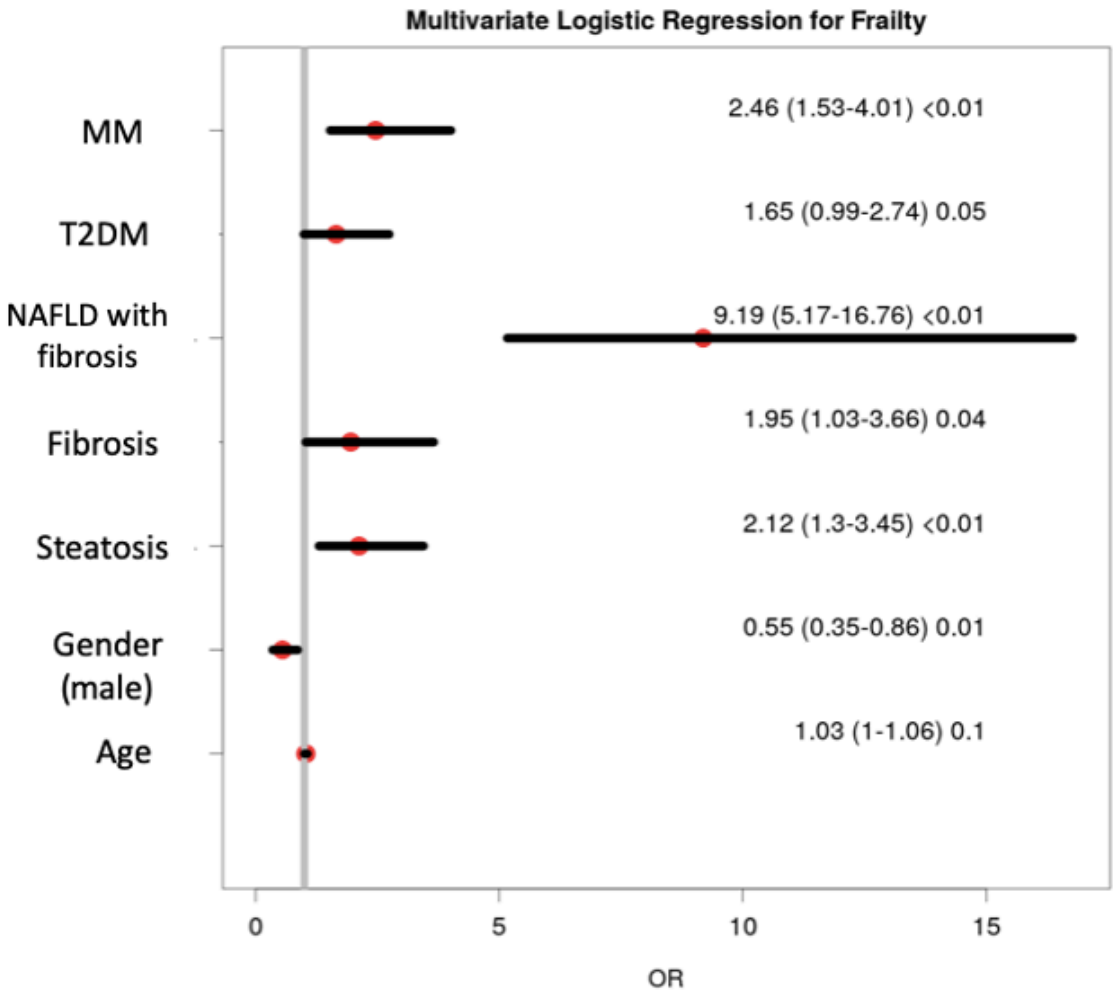
50.00%
31.03%
NAFLD
NAFLD with fibrosis
0-0.25
0.26-0.4
>0.4

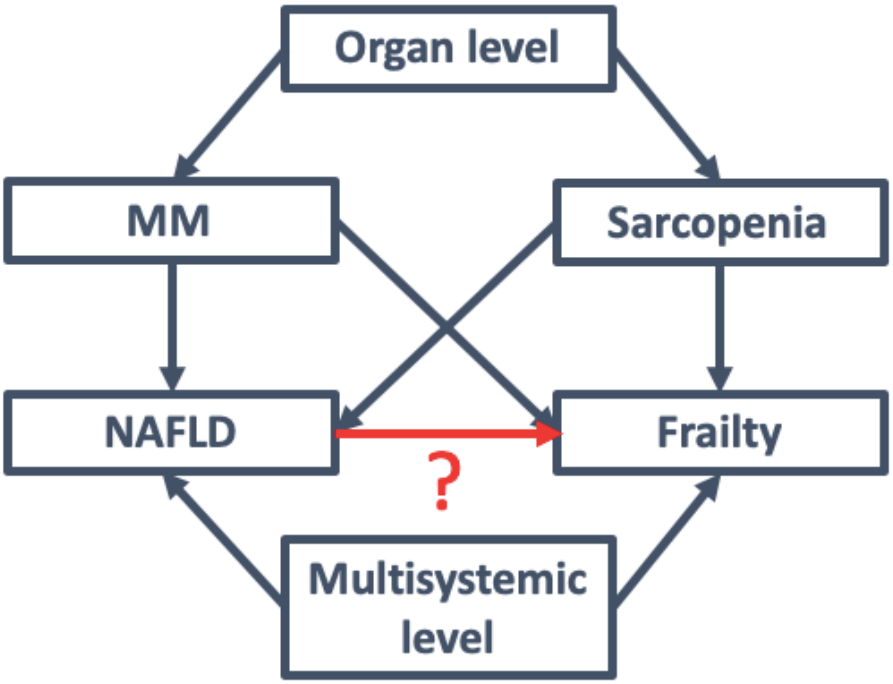
1C

p<0.001

Figure 2 depicts a multivariate logistic model that identified independent predictors for FI were liver steatosis and fibrosis alone and in association (NAFLD with fibrosis), diabetes and multi-morbidity. **Abbreviations:** MM – multimorbidity; T2DM – type 2 diabetes mellitus.



Supplementary figure 1 shows the interplay between organ level (depicted by multimorbidity and sarcopenia) and multisystemic level (depicted by NAFLD and frailty).



Supplementary table 1. Variables of 36-item frailty index

	Variable	Cut-offs and used criteria	Available data of total 707 patients
1	Lipoatrophy	Multicenter AIDS Cohort Study (MACS) criteria	707
2	Lipohypertrophy	MACS criteria	707
3	Menopause or male hypogonadism	If female: FSH > 30 IU/l and LH < 30 IU/l and/or absence of menstruation >1 year If male: testosterone < 300 ng/dl	674
4	High or low BMI	<18 or >25 kg/m^2	705
5	High waist circumference	If female: >88 cm If male: >102 cm	662
6	High visceral adipose tissue	VAT > 130 cm^2 or VAT/TAT ratio >0.5	340
7	Sarcopenia or pre-sarcopenia	Fat-free mass index < 1 SD	688
8	Insulin resistance	Homeostasis Model Assessment – Insulin Resistance > 2	299
9	High total cholesterol	>200 mg/dl	637
10	High low-density lipoprotein	>100 mg/dl	634
11	Low high-density lipoprotein	<40 mg/dl	632
12	High triglycerides	>150 mg/dl	635
13	High homocysteine	If female: >10 mmol/l If male: >15 mmol/l	646
14	Abnormal white blood cell counts	<4000 cells/ml	637
15	Anemia	If female: <10 g/dl If male: <12 g/dl	637
16	Hepatitis C coinfection	Positive	614
17	Hepatitis B coinfection	Hepatitis B antigen positive	641
18	Vitamin D insufficiency	<30 ng/ml	681
19	Polypharmacy	>5 drug classes (excluding antiretroviral therapy)	707
20	Abnormal parathyroid hormone	>60 pg/ml	682
21	Elevated D-dimer	>Sample mean (358)	617
22	Elevated C-reactive protein	>0.7 mg/l	630
23	Sedentary lifestyle	< 3 h/week physical activity	668

24	Atherosclerosis	Coronary artery calcium score > 100 or intima media thickness > 0.85 mm	478
25	Hyponatremia	<125 mmol/l	628
26	Proteinuria or albuminuria	>5 mg/mmol	128
27	Elevated aspartate transaminase	>31 U/l	638
28	Elevated alanine transaminase	>31 U/l	640
29	Abnormal alkaline phosphatase	<38 or >126 U/l	633
30	Elevated g-glutamyl transferase	>55 U/l	633
31	Low platelets	<150 billion/l	640
32	Abnormal potassium	<3.5 or >5.3 mEq/l	630
33	Abnormal phosphorus	<2.5 or >5.1 mg/dl	128
34	Abnormal thyroid-stimulating hormone	<0.27 or >4.2 mIU/l	406
35	Elevated total bilirubin	>1 mg/dl	627
36	Unemployment	Self-report	705

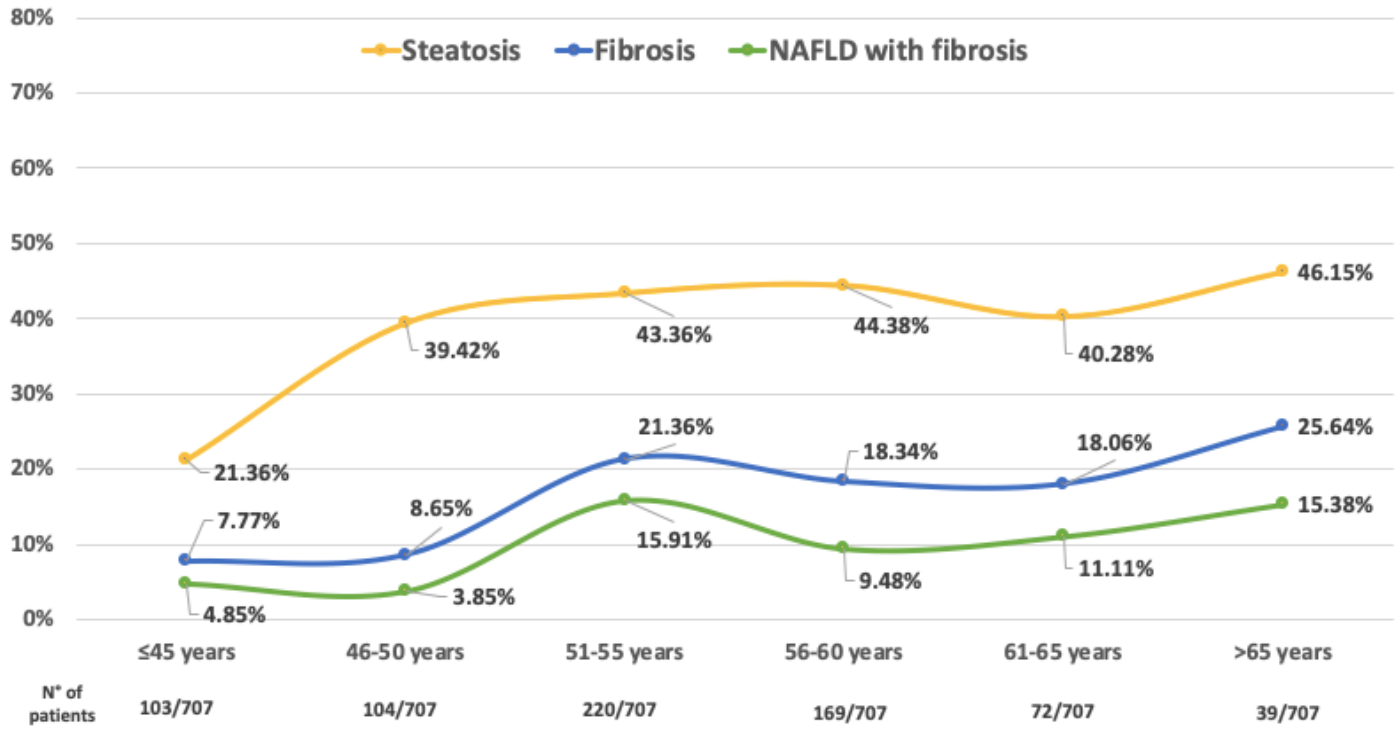
Supplementary table 2 describes study population according to categories of frailty index.

Variable	Fit (0 - 0.25)	Frail (0.26 - 0.4)	Most frail (>0.4)	p
N	531 (77.2%)	130 (18.9%)	27 (3.9%)	
Demographic and Anthropometrics				
Sex, male (%)	414 (78%)	88 (67.7%)	19 (70.4%)	0.04
Age, years, mean (±SD), [N°]	53.2 (8.1) [531]	54 (8.8) [130]	56 (7.6) [27]	0.16
HIV Parameters				
HIV duration, months, median (IQR), [N°]	274 (157-338.8) [518]	291.5 (220.3-347) [124]	320 (216-347) [25]	0.06
Nadir CD4, median, c/µL, median (IQR) [N°]	220 (100-322) [509]	215 (105-326) [121]	250 (48-329) [25]	0.99
Current CD4, median, c/µL, median (IQR) [N°]	698.5 (535.3-880) [520]	673 (556-932) [125]	829 (602.5-1052.5) [27]	0.27
Current CD8, median, c/µL, median (IQR) [N°]	775 (575.5-1037.3) [518]	836 (595-1008) [125]	854 (722-1275) [27]	0.12
CD4/CD8 ratio, mean (±SD), [N°]	1.01 (0.5) [518]	0.98 (0.5) [125]	0.95 (0.6) [27]	0.86
Undetectable HIV RNA viral load (%)	526 (99.1%)	128 (98.5%)	25 (92.6%)	0.02
NICM				
Type 2 diabetes (%)	94 (17.7%)	24 (18.5%)	9 (33.3%)	0.12
Multimorbidity (%)	277 (52.2%)	79 (60.8%)	22 (81.5%)	0.004
Hypertension (%)	224 (42.2%)	61 (46.9%)	14 (51.9%)	0.41
CVD (%)	21 (4%)	10 (7.7%)	4 (14.8%)	0.01
CKD (%)	93 (17.5%)	34 (26.2%)	8 (29.6%)	0.03
COPD (%)	18 (3.4%)	6 (4.6%)	3 (11.1%)	0.12
Osteoporosis (%)	97 (18.3%)	35 (26.9%)	10 (37%)	0.009

Abbreviations: CKD – chronic kidney disease; COPD – chronic obstructive pulmonary disease; CVD – cardiovascular disease HIV – human immunodeficiency virus; IQR –

interquartile range; [N°] – number of people in which the given variable was available; NAFLD – non-alcoholic fatty liver disease; SD – standard deviation.

Supplementary figure 2 depicts prevalence of steatosis, fibrosis and evolutive NAFLD across age groups.



Supplementary table 3 shows prevalence of NAFLD, levels of steatosis and fibrosis and NAFLD with fibrosis.

	Prevalence (%) N=707
NAFLD - CAP>248 dB/m	281 (39.7%)
Levels of steatosis	
- S1 (mild steatosis; 248≥ CAP<268 dB/m)	95 (13.4%)
- S2 (moderate steatosis; 269≥ CAP<280 dB/m)	38 (5.4%)
- S3 (severe steatosis; CAP≥ 280 dB/m)	148 (20.9%)
Fibrosis	
- F2F3 (significant fibrosis; 7.1≥LSM<13 kPa)	82 (11.6%)
- F4 (cirrhosis; LSM≥13 kPa)	36 (5.1%)
NAFLD with fibrosis - NAFLD + significant fibrosis/cirrhosis	72 (10.2%)

Abbreviations: LSM – liver stiffness measurement; NAFLD – non-alcoholic fatty liver disease

PAPER II

Endocrine pathways of non-alcoholic fatty liver disease in people living with HIV

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Word count: 3106; **Abstract:** 248; **Number of tables:** 3; **Number of figures:** 2; **References:** 38; **Supplementary material:** 4 tables, 5 figures**.**

Keywords: NAFLD; insulin resistance; diabetes; vitamin D insufficiency; HIV; PLWH

Key points:

- Insulin resistance (IR) is more strongly associated with NAFLD than diabetes in people living with HIV (PLWH).

- NAFLD with fibrosis is mainly driven by diabetes and not IR.

- Vitamin D insufficiency is associated with NAFLD in PLWH

Submitted to Clinical Infectious Diseases

ABSTRACT

Background

Non-alcoholic fatty liver disease (NAFLD) has multisystemic nature that involves different immuno-metabolic-endocrine pathways. The objective was to explore, using Bayesian networks, the dynamic interplay among endocrine disorders and NAFLD or NAFLD with fibrosis in people living with HIV (PLWH).

Methods

This was a cross-sectional study of PLWH attending Modena HIV Metabolic Clinic, Italy in the period June 2018-January 2020. NAFLD was assessed by transient elastography as controlled attenuation parameter ≥248 dB/m. NAFLD with fibrosis was defined as the contemporary presence of NAFLD and significant liver fibrosis (liver stiffness measurement ≥7.1 kPa). Bayesian networks were applied to identify the relationship among the endocrine disorders and the outcomes through a Directed Acyclic Graph (DAG).

Results

We enrolled 1434 PLWH (75.5% males), mean age of 54.2 years. NAFLD was diagnosed in 39.3%, while NAFLD with fibrosis in 8.2% of PLWH. DAG model for NAFLD identified direct associations with age, insulin resistance (IR) and vitamin D insufficiency. IR alone (OR=2.36, 95%CI:1.37-4.14, p=0.002) was more strongly associated with NAFLD than diabetes alone (OR=1.41, 95%CI:1.05-1.90, p=0.022). DAG model for NAFLD with fibrosis showed a direct association with IR-diabetes only. Diabetes alone (OR=2.76, 95%CI:1.77-4.24, p<0.001) was associated with NAFLD with fibrosis, while IR alone was not.

Conclusion

DAG models revealed that IR is more strongly associated with NAFLD than diabetes, while NAFLD with fibrosis is mainly driven by diabetes rather than IR. This suggests a significant role of diabetes on fibrosis progression in PLWH. Vitamin D supplementation might be considered in PLWH with NAFLD

Background

In the era of successful management of chronic viral hepatitis, non-alcoholic fatty liver disease (NAFLD) has prevailed as the most frequent cause of chronic liver disease in the general population [1] and may progress to non-alcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma. Indeed, NASH represents the evolving indication for liver transplantation in Europe, increasing from 1.2% in 2002 to 8.4% in 2016 [2].

The burden of NAFLD in people living with HIV (PLWH) follows similar trends of general population, with an estimated overall prevalence of 35% [3,4]. However, pathogenesis of NAFLD in PLWH has some additional features that may be attributed to virus itself and to antiretroviral therapy [5,6]. In particular, PLWH, even when adequately virally suppressed, have higher levels of monocyte activation markers sCD14 and sCD163, activated CD4+ and CD8+ T cells and regulatory T-cells [7–9]. For instance, higher sCD163 levels are associated with greater hepatic inflammation [8,10].

Taking into consideration the multifactorial nature of NAFLD, it is often described as a multisystemic state associated with many cardiometabolic conditions [11]. Similarly to general population, metabolic syndrome and obesity are the cornerstones of liver fibrosis in PLWH [10]. Recent data suggest that PLWH with NAFLD are at higher risk of incident diabetes and dyslipidemia [12]. Furthermore, NAFLD with fibrosis, as a multisystemic state, exceeds the construct of multimorbidity, defined as sum of single co-morbidities in the prediction of frailty [13].

In the general population, NAFLD is related to many endocrine alterations, such as hypothyroidism [14], hypogonadism [15], insulin resistance [16], diabetes [17], vitamin D insufficiency [18] and insulin growth factor-1 (IGF-1) axis disorders [19], while these associations remain mainly unexplored in PLWH. The complex endocrine pathways are rarely studied together in observational real-life cohorts which may depict specific patterns involved in NAFLD pathogenesis and indicate specific personalized interventions in the management of NAFLD.

Therefore, our objective was to explore, using Bayesian networks, the dynamic interplay among endocrine disorders and NAFLD or NAFLD with fibrosis, in relatively large and well-characterized cohort of PLWH.

Methods

Study design

This was a cross-sectional study that included consecutive PLWH attending Modena HIV Metabolic Clinic (MHMC) from June 2018 to January 2020. MHMC is a tertiary level referral center established in 2004 where PLWH are screened for co-morbidities, immuno-metabolic disorders including NAFLD and endocrine disorders including insulin resistance, vitamin D deficiency, hypo- and hyperthyroidism, hypogonadism.

Inclusion and exclusion criteria

We included ART-experienced PLWH who were evaluated for NAFLD and fibrosis by transient elastography at MHMC. Patients with hepatitis B (HBV), hepatitis C (HCV) co-infection and hazardous alcohol intake were excluded from the study.

Covariates

Demographic, anthropometric, HIV-related and immune-metabolic variables were collected on the same day of the visit at MHMC. Co-morbidities were defined using the European AIDS Clinical Society (EACS) guidelines [20]. Diabetes was defined as fasting serum glucose levels >126 mg/dL or HbA1C >6.5%. Diagnosis of diabetes was also based on the current use of antidiabetic drugs. Vitamin D insufficiency was defined as <20 ng/mL. Hypothyroidism was defined as TSH >4.2 μIU/ml or TSH <0.35 μIU/ml. Male hypogonadism was defined as <3.0 ng/ml or LH ≥8.9 mIU/ml. Female hypogonadism was defined as estradiol <15 ng/ml, LH > 10 ng/ml and FSH >25 ng/ml or as estradiol <15n g/ml and LH <1 ng/ml and FSH <1 ng/ml. Menopause was defined as the amenorrhea for at least one year. Insulin resistance was defined by Homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: HOMA-IR = [fasting glucose (mg/dL) x fasting insulin (mU/ml)]/405 [21]. IR was defined as HOMA-IR score ≥ 2. IGF-1 deficiency was defined as IGF-1 <67 ng/ml.

Outcome measures

Outcomes of the study were NAFLD and NAFLD with fibrosis. Liver stiffness measurement (LSM) and associated controlled attenuation parameter (CAP) were evaluated using TE with M probe. All CAP measurements ≥248 dB/m were considered as NAFLD. Liver fibrosis was diagnosed by LSM as follows: stage F0-F1 (mild fibrosis, LSM<7.1 kPa), F2-F3 (significant

fibrosis, 7.1≥LSM<13 kPa), F4 (cirrhosis, LSM≥13 kPa) [22]. NAFLD with fibrosis was defined as the contemporary presence of liver steatosis (CAP≥248) and significant liver fibrosis or cirrhosis (stage ≥F2) [13].

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for normally distributed continuous variables, as median and interquartile range (IQR) for non-normally distributed continuous variables, and as frequencies and percentages for categorical variables. Student's t-test and ANOVA were performed to identify statistical difference for the normally distributed continuous variables, while Mann-Whitney and Kruskal-Wallis tests were used for not normally distributed continuous variables. The $\chi 2$ (chi-squared) test was applied for categorical variables.

Logistic regressions were conducted to identify independent predictors for NAFLD and NAFLD with fibrosis, using as covariates age, gender, HIV-related variables and endocrine disorders. In order to depict effect of HIV duration, but to avoid the collinearity with age, the univariate linear regression was used to calculate "the residual" between age and HIV duration (in figure called "residual HIV months").

Learning Bayesian Networks were applied to identify the conditional probabilities and relationship network among the different predictors (endocrine disorders, age and sex) and the outcomes through a Directed Acyclic Graph (DAG). Each node represents a variable, and the arc between nodes describes a direct influence of the parent node (the node from which the arrow start) on the child node (the node on which the arrow points to) [23]. A set of restraints were applied. In particular, only arrows that ultimately point to NAFLD were allowed, while sex and age could also affect the endocrine axes and NAFLD, but not to be affected by any other variable. Several DAG models for both outcomes were tested in order to better understand the interactions between endocrine disorders and the outcomes. DAGs that illustrated the best causal pattern between predictors and two outcomes, were represented as the main material of this paper, while the rest was included in the Supplementary material. Each DAG model was accompanied by a multivariable logistic regression that quantified the effect of identified direct relationships between the predictors and NAFLD or NAFLD with fibrosis. Finally, the probabilities of the outcomes were calculated based on the presence/absence of each predictor that showed direct association with the outcome in the DAG model.

All statistical tests were two-sided and assumed a significance level of 5%. The statistical program R (v. 3.6.0) and Phyton were used to analyze and clean the data.

This study was approved by the University of Modena and Reggio Emilia ethics committee according to the Helsinki declaration.

Results

We enrolled 1434 PLWH (75.5% males). Mean age was 54.2 (±9.1) years, mean BMI 24.2 (Q1,Q3: 22.2-26.4) kg/m^2, current median CD4 cell count 702/μL (Q1,Q3: 541.5-900.5). HIV RNA viral load was undetectable in 98.3% of cases. NAFLD was diagnosed in 563 patients (39.3%), while NAFLD with fibrosis in 116 (8.2%) (Table 1).

As expected, PLWH with NAFLD had higher waist circumference, greater visceral adipose tissue and subcutaneous tissue area. Additionally, longer HIV duration and higher current CD4 cell count were related to NAFLD, while exposure to various antiretroviral regimens was not different between the groups (Table 1).

With regards to endocrine alterations, PLWH with NAFLD had a higher prevalence of IR (56.7% vs. 29.2%, p<0.001), vitamin D insufficiency (43.7% vs. 32.8%, p<0.001) and diabetes (22.0% vs. 14.6%, p<0.001). Other endocrine disorders were not correlated to NAFLD. PLWH with NAFLD exhibited significantly higher levels of hemoglobin A1C, total and LDL cholesterol, triglycerides and lower levels of HDL cholesterol and IGF-1 (Table 1). The characteristics of PLWH according to the presence or absence of NAFLD with fibrosis are provided in the Supplementary table 1.

To better depict the complex interactions between endocrine disorders and NAFLD, several Bayesian networks were developed. The first DAG model for NAFLD identified direct associations with age, IR and vitamin D insufficiency, but not with diabetes. IR was related to NAFLD both directly and indirectly, through vitamin D insufficiency (Supplementary figure 1). These direct associations were explored in multivariable logistic regression in which age >50 years (OR=1.91, 95%CI: 1.50 – 2.45, p<0.001), IR (OR=2.30, 95%CI: 1.44 – 3.71, p=0.001) and vitamin D insufficiency (OR=1.64, 95%CI: 1.32 – 2.05, p<0.001) were associated with higher risk of NAFLD (Supplementary table 2).

The second DAG model for NAFLD, in which PLWH with IR were excluded, showed direct associations with age, diabetes and vitamin D insufficiency (Supplementary figure 2). In the logistic regression, diabetes (OR=1.43, 95%CI: 1.08 – 1.90, p=0.012) was associated with NAFLD, while age >50 years and vitamin D insufficiency exhibited similar odds ratio as in the previous model (Supplementary table 3).

The third DAG model for NAFLD, in which PLWH with IR and diabetes were combined in one variable (IR + diabetes), identified direct associations with age, IR + diabetes, and vitamin D insufficiency (Figure 1). In the logistic regression model, IR + diabetes was split into three mutually exclusive categories: (i) diabetes alone, (ii) IR alone, (iii) IR + diabetes, while “no IR + no diabetes” was used as a reference. IR alone (OR=2.36, 95%CI: 1.37 - 4.14, p=0.002) was more strongly associated with NAFLD than diabetes alone (OR=1.41, 95%CI: 1.05 - 1.90, p=0.022), while IR + diabetes had an additive effect in predicting NAFLD (OR=2.63, 95%CI: 1.12 - 6.64, p=0.031). Vitamin D insufficiency displayed a similar association with NAFLD as in the previous two DAG models (Table 2). Furthermore, in PLWH with age >50 years, vitamin D insufficiency, and both IR and diabetes, the probability of presenting NAFLD was 83.5%. Conversely, if none of the previous was present, the probability of NAFLD decreased to 25.4% (data presented within the text only).

Two Bayesian networks were also developed for NAFLD with fibrosis. The first DAG model for NAFLD with fibrosis identified direct associations with diabetes, IR and hypogonadism. IR was related to NAFLD with fibrosis both directly and indirectly, through diabetes**.** Vitamin D was neither directly nor indirectly linked to NAFLD with fibrosis (Supplementary figure 3). In the logistic regression, both IR (OR=2.26, 95%CI: 1.16 – 4.12, p=0.011) and diabetes (OR=3.18, 95%CI: 2.11 – 4.74, p<0.001) were associated with higher risk of NAFLD with fibrosis (Supplementary table 4). The second DAG model for NAFLD with fibrosis was created in similar fashion as Figure 1, identifying IR + diabetes and hypogonadism in direct relation to this outcome (Figure 2). In the logistic regression model, using the same mutually exclusive categories as reported in Table 2, diabetes alone (OR=2.76, 95%CI: 1.77 – 4.24, p<0.001) and IR + diabetes (OR=11.81, 95%CI: 4.87 – 27.86, p<0.001) were associated with NAFLD with fibrosis, while IR alone and hypogonadism were not (Table 3). Additionally, if PLWH had IR, diabetes and hypogonadism, the probability of having NAFLD with fibrosis was 35.5%. Conversely, if none of these conditions were present, the probability of NAFLD with fibrosis decreased to 7% (data presented within the text only).

To better explore the impact of hypogonadism on NALFD with fibrosis, a logistic regression, independent from DAG model, was conducted. However, hypogonadism, along with HIV variables, was not associated with this outcome (Supplementary figure 4). In the similar model to the previous one, vitamin D insufficiency was related to NAFLD with fibrosis (OR=2.02, 95%CI: 1.33 – 3.06, p<0.001) (Supplementary figure 5).

Discussion

The originality of this paper stands in the contemporary assessment of multiple endocrine pathways that are shown to be involved in NAFLD pathogenesis. We used Bayesian networks to explore the dynamic interplay among endocrine disorders, and NAFLD or NALFD with fibrosis in PLWH. Our main finding suggest that IR alone is more strongly associated with NAFLD (OR=2.36, 95%CI: 1.37 - 4.14, p=0.002) than diabetes (OR=1.41, 95%CI: 1.05 - 1.90, p=0.022). On the other side, NAFLD with fibrosis was mainly driven by diabetes rather than IR. Moreover, all DAG models showed a direct impact of age and vitamin D insufficiency on NAFLD, but not on NAFLD with fibrosis.

The relationship between NAFLD and age has not been clarified completely. According to a meta-analysis, NAFLD prevalence increases with age [24], while the other studies report that prevalence of NAFLD increases with age up to a certain point, then stabilizes with a plateau in the last decades of life [25]. Recently, in the HIV setting, our group showed that prevalence of both NAFLD and NAFLD with fibrosis increases with age and it was highly associated with frailty, a measure of biological age [13]. In the current study, we confirm an association between age and NAFLD. However, we observed the lack of direct association between age and NAFLD with fibrosis. This finding could be justified by the fact that NAFLD-related conditions such as obesity, diabetes or IR, may have a more prominent role than age itself [26] in the context of the complex endocrine pathways. Additionally, this finding may be also explained by the survival bias, as older people are more susceptible to negative outcomes associated with NAFLD, such as liver cirrhosis, hepatocellular carcinoma and death [27].

The pathogenetic link between NAFLD and vitamin D insufficiency is a widely investigated topic in the general population, but poorly described in the PLWH. Many studies showed that low levels of vitamin D are associated with higher risk of NAFLD and advanced liver

disease [28,29]. Our results confirm the direct association between vitamin D insufficiency and NAFLD, but no direct link with NAFLD with fibrosis. However, a multivariable regression, independently of DAG model, showed that PLWH with vitamin D insufficiency are at increased risk for NAFLD with fibrosis, suggesting that endocrine axes other than vitamin D may have a more significant role in the advanced stages of NAFLD. These consistent results suggest that PLWH with NAFLD may benefit from vitamin D supplementation, that is already indicated in the EACS guidelines for other diseases [30]. Nevertheless, adherence to vitamin D supplementation might be challenging in PLWH. The studies in the general population are still inconclusive, but however encouraging, regarding the effect of this therapeutic intervention in the improvement of NAFLD [31–33].

IR and diabetes have been extensively studied in the PLWH, but rarely together in relation to NAFLD or NAFLD with fibrosis. In all our DAG models, IR was highly associated with NAFLD, while the impact of diabetes depended on how IR variable was treated statistically. In particular, when IR and diabetes were analyzed in the same DAG model, but as separate and not mutually exclusive variables (i.e. PLWH with IR and diabetes were attributed both to IR and diabetes node), diabetes was not directly linked to NAFLD. When PLWH with IR were excluded from the analysis, the relationship between diabetes and NAFLD appeared. These data indicated that IR and diabetes are “competing” for the same spot, in which IR showed greater “affinity” for NAFLD. This hypothesis was confirmed in the final DAG model in which IR and diabetes were combined in one variable, but in the subsequent logistic regression model were considered as mutually exclusive categories, in which was showed that IR was more strongly associated with NAFLD than diabetes.

These findings suggest the importance of physical activity, which is the first line treatment for both IR and NAFLD [34,35]. In HIV setting, the MACS Cohort reported that higher physical activity was associated with lower prevalence of IR in PLWH [36], while the impact of lifestyles’ intervention on NAFLD has not been described yet. However, Erlandson et al. demonstrated that greater intensity of physical exercise is needed in older PLWH to obtain similar metabolic improvements to the general population [37]. Physical activity should be promoted in PLWH in a pro-active manner, along with vitamin D supplementation, in order to prevent negative health outcomes associated with both NAFLD and IR, such as incident diabetes, NASH, hepatocellular carcinoma, liver transplantation and cardiovascular disease [10,12]. We underline that the probability of NAFLD was 83.5% if the patient was older than

50 years, suffered from IR or diabetes and had vitamin D insufficiency, which justifies the proposed interventions.

Both presented Bayesian networks showed that diabetes had a strong association with NAFLD with fibrosis, while IR was less potent in this prediction. When IR and diabetes alone were analyzed in the same model, diabetes “beaten” IR in the “battle” for NAFLD with fibrosis spot. Furthermore, absence of diabetes, IR and hypogonadism, reduced the probability of NAFLD with fibrosis to 7% in PLWH. These results could imply that diabetes have an important effect on NAFLD progression in PLWH, depicted by NAFLD with fibrosis. This is in line with the recent reports that showed that liver fibrosis was two-time higher in PLWH with diabetes [12].

This study suffers from several limitations. Some are intrinsic to the cross-sectional nature of the study that cannot identify the causal relationship between the variables. The absence of a control group does not allow us to show whether HIV status along with hormonal and endocrine variables is associated with NAFLD and NAFLD with fibrosis. The impact of single antiretroviral agents was not explored. Further studies should focus more on vitamin D, IR and diabetes, HIV specific features and weight gain in the prediction of NAFLD. We did not assess lean NAFLD in this study, which has been shown to be peculiar in PLWH [38]. Additionally, our DAG models relied partially on knowledge- based approaches, therefore, they are not entirely data-driven, which might potentially mislead our conclusions in the absence of clear pathogenetic mechanisms behind the observed relationships.

Some strengths should also be recognized. This study was conducted in a relatively large sample size and well-characterized cohort of PLWH. The addition of DAG models to merely traditional multivariable models gave the possibility to emulate the complex endocrine pathways and feedback mechanisms involved in the natural history of NAFLD in PLWH. The exploration of these pathways could inform the current clinical care and management of NAFLD in PLWH in the absence of specific therapeutic interventions.

Moreover, in consideration of contemporary assessment of multiple disorders, we were able to quantify the weight of each examined variable in the complex endocrine pathways. We show that IR, diabetes and vitamin D insufficiency are of paramount importance in PLWH, while none of the other endocrine disorders were related to NAFLD and NAFLD with fibrosis.

Although some of these results were partially expected, they are once more emphasizing the need to focus therapeutic and lifestyles interventions such as vitamin D supplementation and physical activity while we are waiting for more promising and effective pharmacological agents for NASH [6].

In conclusion, we described a dynamic interplay among various endocrine disorders and NAFLD in PLWH. DAG models revealed that IR is more strongly associated with NAFLD than diabetes, while NAFLD with fibrosis is mainly driven by diabetes rather than IR. We may argue that diabetes has potential effect on fibrosis progression in PLWH with NAFLD. Vitamin D supplementation might be considered in PLWH with NAFLD.

Conflict of interest

GG and CM received research grant and speaker honorarium from Gilead, ViiV, MERCK and Jansen. GG and CM attended advisory boards of Gilead, ViiV and MERCK. JM, LG, SR, SC, DF, SB, AC, GF, VI, CD, VR and GS reported no conflict of interest.

Authors' contributions

JM, GG and GS conceptualized and designed the manuscript. JM, LG and GG wrote and revised the manuscript. SR, SC, DF and SB did the statistical analysis. JM, GG, GS, CM, SR, SC did the supervision of the final version of the manuscript. All the authors contributed to discussion and revised the manuscript.

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Table 1. Demographic, anthropometric, relevant HIV, endocrine variables and comorbidities according to the presence or absence of NAFLD.

	NAFLD (N=563, 39.3%)	**No NAFLD (N=871, 60.7%)**	**p**
Demographic and anthropometric variables			
Age, mean (± SD) [N°]	55.6 (8.7) [563]	53.2 (9.2) [871]	**<0.001**
Males, N (%)	434 (77.1%)	649 (74.5%)	0.296
BMI, kg/m^2, median (IRQ) [N°]	26.1 (24.2 - 28.7) [527]	23.2 (21.5 - 25.1) [815]	**<0.001**
Waist circumference, cm, median (IRQ) [N°]	97.0 (90.0 - 104.0) [514]	86.0 (81.0 - 93.0) [794]	**<0.001**
SAT, cm^3, median (IRQ) [N°]	204.5 (152.0 - 283.8) [76]	165.0 (105.0 - 234.0) [89]	**0.006**
VAT, cm^3, median (IRQ) [N°]	214.0 (152.0 - 300.0) [77]	126.0 (92.0 - 186.0) [89]	**<0.001**
HIV related variables			
HIV duration, months, median (IRQ) [N°]	285.0 (180.0 - 339.5) [535]	260.0 (152.75 - 337.0) [812]	**0.010**
Nadir CD4, c/µL, median (IQR) [N°]	35.1 (29.42 - 40.8) [514]	35.3 (29.6 - 41.48) [802]	0.367
Current CD4, c/µL, median (IRQ) [N°]	725.5 (574.5 - 928.25) [516]	682.0 (522.5 - 879.0) [803]	**0.004**
CD4/CD8 ratio, median (IRQ) [N°]	0.95 (0.68-1.3) [504]	0.95 (0.69 – 1.29) [784]	0.594
Current exposure to NRTIs, N (%)	427 (75.8%)	624 (71.6%)	0.090
Current exposure to NNRTIs, N (%)	180 (32.0%)	290 (33.3%)	0.643
Current exposure to PIs, N (%)	148 (26.3%)	232 (26.6%)	0.933
Current exposure to INSTIs, N (%)	304 (54.0%)	425 (48.8%)	0.061
Endocrine disorders			
Hypothyroidism, N (%)	126 (22.4%)	182 (20.9%)	0.570
Male hypogonadism, N (%)	38 (6.8%)	53 (6.1%)	0.817
Female hypogonadism, N (%)	129 (22.9%)	222 (25.5%)	1.000
Menopause, N (%)	27 (4.8%)	53 (6.1%)	0.867
Insulin resistance, N (%)	59 (56.7%)	40 (29.2%)	**<0.001**
Vitamin D insufficiency, N (%)	246 (43.7%)	286 (32.8%)	**<0.001**
IGF deficiency, N (%)	12 (2.1%)	11 (1.3%)	0.288
Diabetes, N (%)	124 (22.0%)	127 (14.6%)	**<0.001**
Hormone-related variables			
Hemoglobin A1C, mmol/mol, median (IRQ) [N°]	35.0 (32.0 - 38.0) [370]	33.0 (30.75 - 36.0) [568]	**<0.001**
Glucose, ml/dl, median (IRQ) [N°]	94.0 (86.0 - 105.0) [435]	89.0 (82.0 - 96.0) [661]	**<0.001**
Insulin, µIU/ml, median (IRQ) [N°]	9.0 (6.6 - 13.5) [108]	6.9 (4.6 - 9.8) [158]	**0.001**
HOMA Index, median (IRQ) [N°]	2.2 (1.4 - 3.3) [104]	1.3 (0.9 - 2.1) [137]	**<0.001**
Total cholesterol, mg/dl, median (IRQ) [N°]	184.0 (158.0 - 209.0) [434]	178.0 (153.0 - 201.0) [661]	**0.015**
LDL cholesterol, median (IRQ) [N°]	118.0 (95.5 - 139.5) [431]	114.0 (91.0 - 135.5) [647]	**0.036**
HDL cholesterol, ml/dl, median (IRQ) [N°]	46.0 (39.0 - 54.5) [431]	52.0 (43.0 - 63.0) [652]	**<0.001**
Triglycerides, ml/dl, median (IRQ) [N°]	131.0 (95.0 - 193.25) [432]	100.0 (74.0 - 142.0) [657]	**<0.001**
TSH, µIU/ml, median (IRQ) [N°]	1.9 (1.3 - 2.5) [153]	1.9 (1.3 - 2.7) [248]	0.551
T4, pg/ml, median (IRQ) [N°]	12.3 (10.9 - 13.7) [112]	12.2 (11.0 - 13.4) [163]	0.593
Estradiol, pg/ml, median (IRQ) [N°]	23.5 (17.0 - 34.0) [150]	22.5 (11.0 - 30.0) [248]	0.232
FSH, mIU/ml, median (IRQ) [N°]	8.7 (5.2 – 29.0) [151]	7.8 (4.7 - 32.0) [247]	0.383

LH, mIU/ml, median (IRQ) [N°]	7.9 (5.7 - 16.7) [150]	8.6 (5.1 - 21.6) [246]	0.387
Testosterone, ng/ml, median (IRQ) [N°]	4.5 (3.3 - 5.7) [106]	5.4 (4.1 - 6.4) [159]	0.002
Prolactin, ng/ml, median (IRQ) [N°]	10.4 (7.4 - 13.9) [150]	10.0 (7.7 - 14.2) [247]	0.437
PTH, pg/ml, median (IRQ) [N°]	42.0 (31.9 - 52.9) [430]	36.8 (28.5 - 50.4) [654]	0.120
IGF1, ng/ml, median (IRQ) [N°]	152.7 (124.9 - 185.5) [425]	170.2 (133.7 - 202.7) [657]	**<0.001**

Abbreviations: BMI – body mass index; FSH – Follicle-stimulating hormone; HDL – high density lipoprotein; HIV – human immunodeficiency virus; HOMA - Homeostatic Model Assessment for insulin resistance; IGF1 - Insulin-like growth factor 1; INSTI – integrase strand transfer inhibitors; IQR – interquartile range; LDL – low density lipoprotein; LH - luteinizing hormone; [N°] – number of people in which the given variable was available; NNRTI – non-nucleoside reverse transcriptase inhibitors; NRTI – nucleoside reverse transcriptase inhibitors; PI – protease inhibitors; PTH - parathyroid hormone; SAT – subcutaneous adipose tissue; TSH – thyroid stimulating hormone; VAT – visceral adipose tissue

Table 2. Multivariable logistic regression model for NAFLD in which IR and diabetes are used as a combined variable and “No IR + no diabetes” as a reference.

Predictors	NAFLD		
	Odds ratio	95% CI	p
Age >50 vs. ≤ 50 years	1.81	1.41 – 2.33	**<0.001**
Diabetes alone vs. Normal	1.41	1.05 – 1.90	**0.022**
IR alone vs. Normal	2.36	1.37 – 4.14	**0.002**
IR + diabetes vs. Normal	2.63	1.12 – 6.64	**0.031**
Vitamin D insufficiency	1.64	1.31 – 2.05	**<0.001**

Figure 1. DAG model for NAFLD identified direct associations with age, IR and diabetes combined in one variable and vitamin D insufficiency. **Abbreviations:** HYPOGON – hypogonadism; HYPOTHYR – hypothyroidism; IGF DEF – insulin growth factor deficiency**;** IR + DM - insulin resistance + diabetes; VIT D - vitamin D insufficiency.

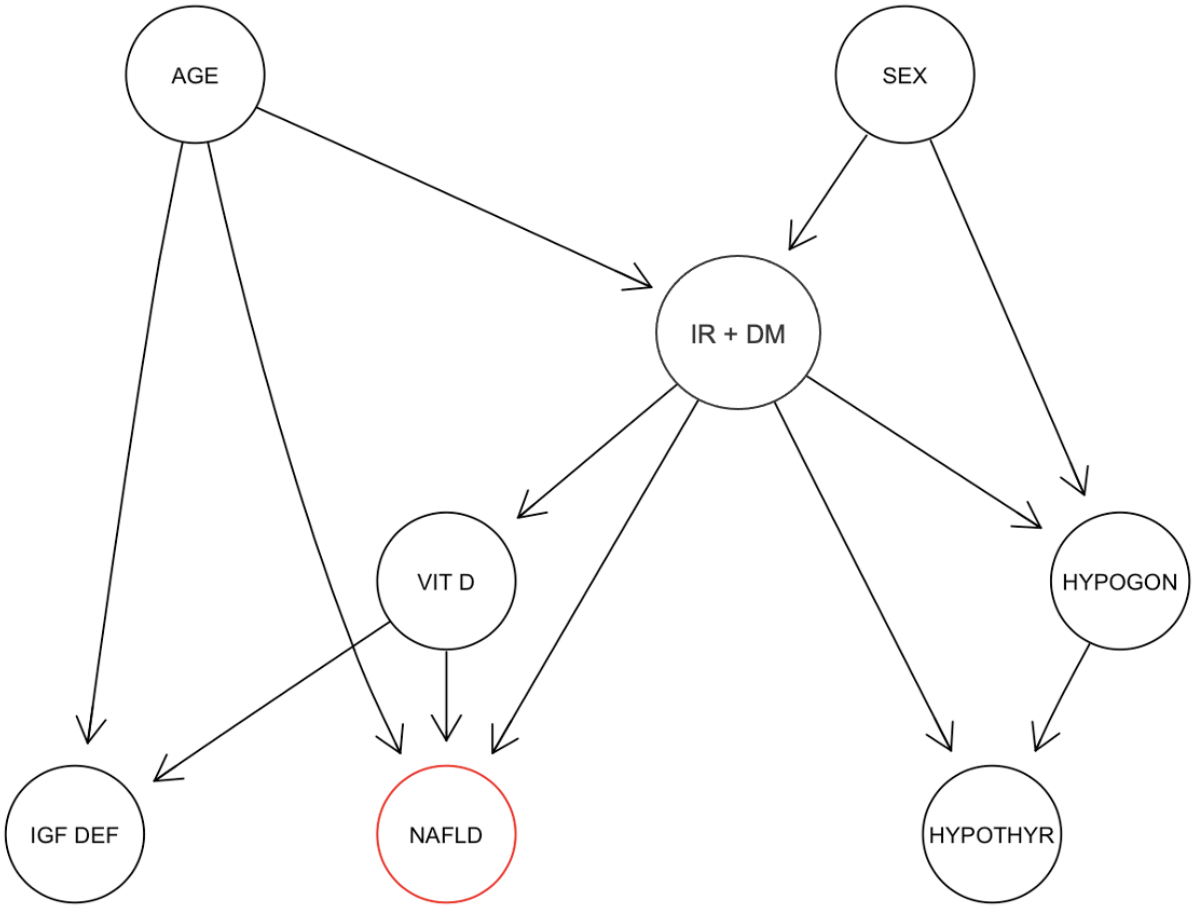
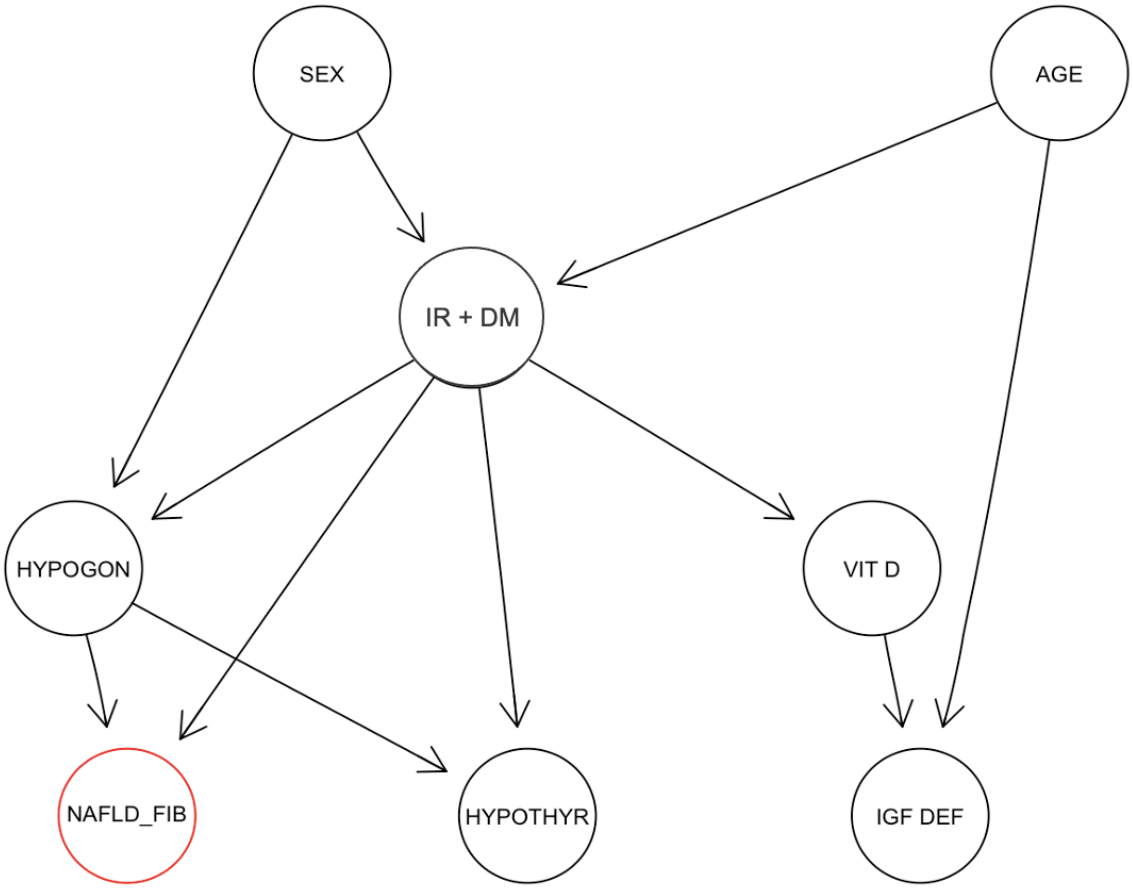


Table 3. Multivariable logistic regression model for NAFLD with fibrosis in which IR and diabetes are used as a combined variable and “No IR + no diabetes” as a reference.

Predictors	NAFLD with fibrosis		
	Odds ratio	95% CI	p
No IR + diabetes vs. Normal	2.76	1.77 – 4.24	**<0.001**
IR + no diabetes vs. Normal	1.18	0.35 – 3.00	0.756
IR + diabetes vs. Normal	11.81	4.87 – 27.86	**<0.001**
Hypogonadism	1.07	0.70 – 1.66	0.760

Figure 2. DAG model for NAFLD with fibrosis in which IR and diabetes are used as a combined variable, identified direct association with IR-diabetes only. **Abbreviations:** HYPOGON – hypogonadism; HYPOTHYR – hypothyroidism; IGF DEF – insulin growth factor deficiency**;** IR + DM - insulin resistance + diabetes; VIT D - vitamin D insufficiency.



Supplementary table 1. Demographic, anthropometric, relevant HIV, endocrine variables and comorbidities according to the presence of NAFLD without fibrosis or NAFLD with fibrosis.

	NAFLD with fibrosis N=117	No NAFLD with fibrosis N=1317	p
Demographic and anthropometric variables			
Age, mean (± SD) [N°]	56.4 (9.6) [117]	54.0 (9.0) [1317]	0.006
Male, N° (%)	95 (81.2%)	988 (75.0%)	0.168
BMI, kg/m^2, median (IRQ) [N°]	29.2 (25.9 - 32.5) [105]	24 (21.9 - 26.1) [1237]	<0.001
Waist circumference, cm, median (IRQ) [N°]	105.5 (99.0 - 112.8) [102]	89.0 (83.0 - 97.0) [1206]	<0.001
SAT, cm^3, median (IRQ) [N°]	213.0 (199.0 - 302.0) [13]	180.0 (115.0 - 237.3) [152]	0.491
VAT, cm^3, median (IRQ) [N°]	294.0 (237.0 - 320.0) [13]	152.0 (101.0 - 221.0) [153]	0.002
HIV related variables			
HIV duration in months, median (IRQ) [N°]	288.0 (178.3 - 375.0) [110]	269.0 (159.0 - 336.0) [1237]	0.019
Nadir CD4, c/µL, median (IQR) [N°]	35.0 (27.9 - 41.0) [106]	35.3 (29.7 - 41.2) [1210]	0.249
Current CD4, c/µL, median (IRQ) [N°]	664.5 (567.3 - 930.0) [106]	703.0 (541.0 - 897.0) [1213]	0.975
CD4/CD8 ratio, median (IRQ) [N°]	0.9 (0.6 - 1.3) [103]	1.0 (0.7 - 1.3) [1185]	0.393
Current exposure to NRTIs, N° (%)	84 (71.8%)	967 (73.4%)	0.785
Current exposure to NNRTIs, N° (%)	34 (29.1%)	436 (33.1%)	0.429
Current exposure to PIs, N° (%)	36 (30.8%)	344 (26.1%)	0.326
Current exposure to INSTIs, N° (%)	57 (48.7%)	672 (51.0%)	0.703
Endocrine alterations			
Hypothyroidism, N° (%)	25 (21.4%)	283 (21.5%)	0.999
Male hypogonadism, N° (%)	12 (10.3%)	79 (6.0%)	0.173
Female hypogonadism, N° (%)	22 (18.8%)	329 (25.0%)	1.000
Hypotestosteronemia, N° (%)	9 (7.7%)	65 (4.9%)	0.304
Menopause, N° (%)	4 (3.4%)	76 (5.8%)	0.838
Insulin resistance, N° (%)	17 (89.5%)	82 (36.9%)	<0.001
Vitamin D insufficiency, N° (%)	55 (47.0%)	477 (36.2%)	0.008
IGF deficiency, N° (%)	4 (3.4%)	19 (1.4%)	0.213
Diabetes, N° (%)	45 (38.5%)	206 (15.6%)	<0.001
Hormone-related variables			
Hemoglobin A1C, mmol/mol, median (IRQ) [N°]	37.0 (33.0 - 42.5) [67]	34.0 (31.0 - 37.0) [871]	<0.001
Glucose, ml/dl, median (IRQ) [N°]	99.5 (86.75 - 114.75) [80]	90.0 (83.0 - 99.0) [1016]	<0.001
Insulin, µIU/ml, median (IRQ) [N°]	11.8 (9.0 - 20.1) [19]	7.3 (5.25 - 11.15) [247]	0.001
HOMA Index, median (IRQ) [N°]	3.64 (2.4 - 5.87) [19]	1.61 (1.05 - 2.39) [222]	<0.001
Total cholesterol, mg/dl, median (IRQ) [N°]	170.0 (145.25 - 196.0) [80]	182.0 (155.0 - 204.0) [1015]	0.101
LDL cholesterol, median (IRQ) [N°]	112.0 (91.0 - 131.0) [79]	116.0 (93.0 - 138.0) [999]	0.300
HDL cholesterol, ml/dl, median (IRQ) [N°]	42.0 (37.0 - 50.5) [79]	50.0 (42.0 - 60.25) [1004]	<0.001

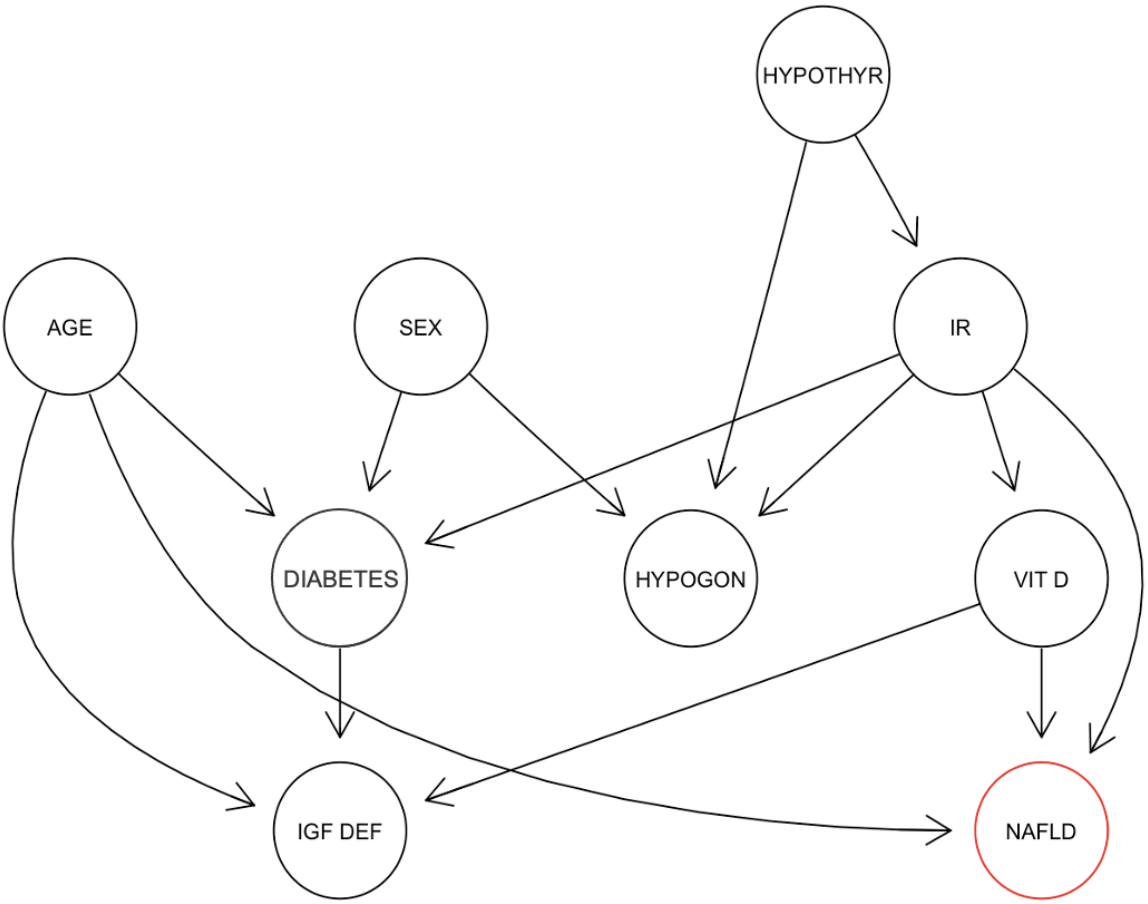
Triglycerides, ml/dl, median (IRQ) [N°]	153.0 (113.25 - 214.25) [80]	111.0 (81.0 - 154.0) [1009]	<0.001
TSH, µIU/ml, median (IRQ) [N°]	1.77 (1.2 - 2.44) [30]	1.9 (1.3 - 2.57) [371]	0.793
T4, pg/ml, median (IRQ) [N°]	12.7 (10.88 - 13.5) [22]	12.2 (10.9 - 13.5) [253]	0.788
Estradiol, pg/ml, median (IRQ)	24.0 (22.0 - 40.0) [29]	23.0 (13.0 - 32.0) [369]	0.851
FSH, mIU/ml, median (IRQ) [N°]	9.0 (7.0 - 13.8) [29]	8.3 (4.9 - 31.8) [369]	0.228
LH, mIU/ml, median (IRQ) [N°]	8.2 (7.0 - 14.6) [29]	8.2 (5.4 - 19.8) [367]	0.345
Testosterone, ng/ml, median (IRQ) [N°]	4.6 (3.2 - 6.45) [22]	5.1 (3.9 - 6.0) [243]	0.652
Prolactin, ng/ml, median (IRQ) [N°]	10.4 (8.8 - 15.1) [29]	10.05 (7.57 - 14.0) [368]	0.139
IGF1, ng/ml, median (IRQ) [N°]	139.1 (104.5 - 162.55) [79]	167.1 (131.45 - 196.3) [1003]	<0.001
IGFBP3, ng/ml, median (IRQ) [N°]	2888.0 (2175.0 - 3404.0) [65]	3042.0 (2641.0 - 3586.0) [739]	0.005

Abbreviations: BMI – body mass index; FSH – Follicle-stimulating hormone; HDL – high density lipoprotein; HIV – human immunodeficiency virus; HOMA - Homeostatic Model Assessment for insulin resistance; IGF1 - Insulin-like growth factor 1; INSTI – integrase strand transfer inhibitors; IQR – interquartile range; LDL – low density lipoprotein; LH - luteinizing hormone; [N°] – number of people in which the given variable was available; NNRTI – non-nucleoside reverse transcriptase inhibitors; NRTI – nucleoside reverse transcriptase inhibitors; PI – protease inhibitors; PTH - parathyroid hormone; SAT – subcutaneous adipose tissue; TSH – thyroid stimulating hormone; VAT – visceral adipose tissue

Supplementary table 2. Multivariable logistic regression model for NAFLD in which PLWH with both diabetes and IR are included.

Predictors	NAFLD		
	Odds ratio	95% CI	p
Age >50 vs. ≤ 50 years	1.91	1.50 – 2.45	**<0.001**
Insulin resistance	2.30	1.44 – 3.71	**0.001**
Vitamin D insufficiency	1.64	1.32 – 2.05	**<0.001**

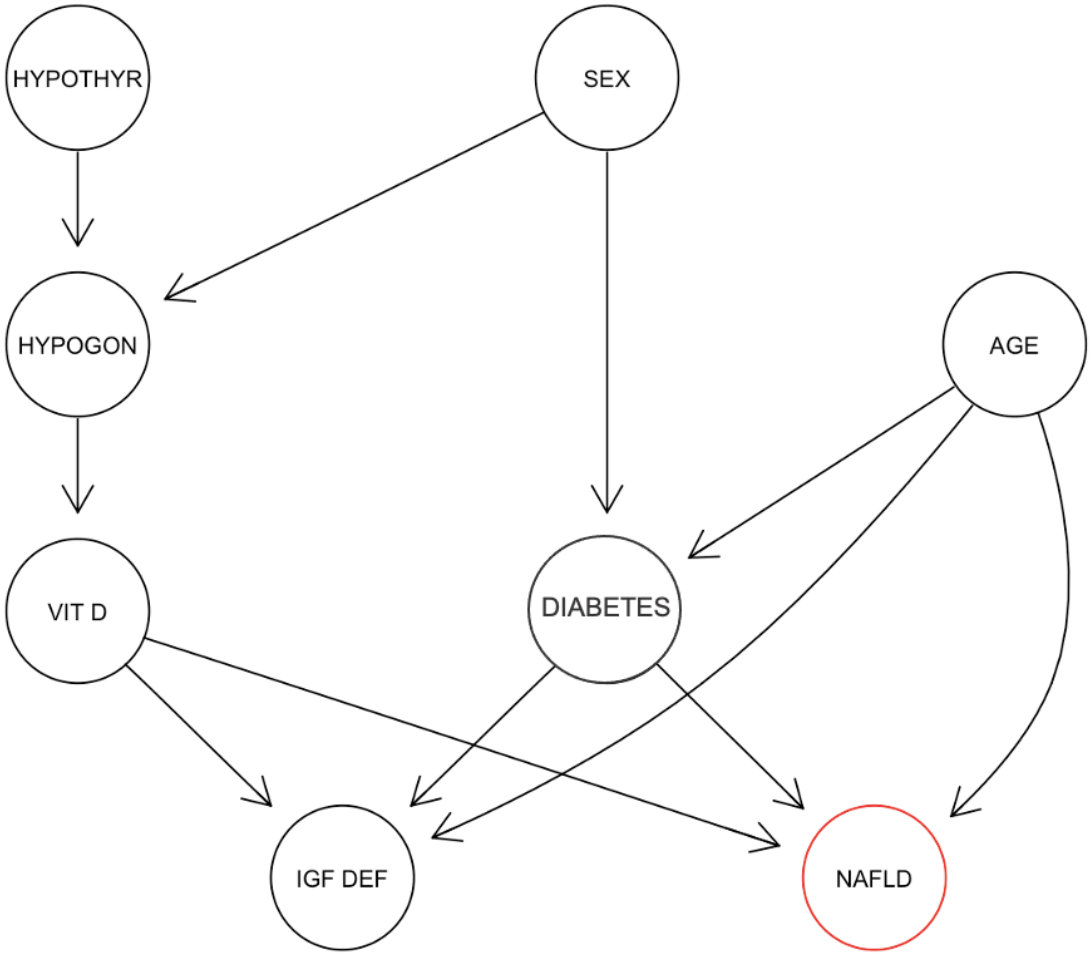
Supplementary figure 1. DAG model for NAFLD identified direct associations with age, IR and vitamin D insufficiency, but not with diabetes. IR was related to NAFLD both directly and indirectly, through vitamin D insufficiency**. Abbreviations:** HYPOGON – hypogonadism; HYPOTHYR – hypothyroidism; IGF DEF – insulin growth factor deficiency**;** IR - insulin resistance; VIT D - vitamin D insufficiency.



Supplementary table 3. Multivariable logistic regression model in which PLWH with IR are excluded and PLWH with diabetes were used in the prediction of the NAFLD.

Predictors	NAFLD		
	Odds ratio	**95% CI**	**p**
Age >50 vs. ≤ 50 years	1.77	1.38 – 2.28	**<0.001**
Diabetes	1.43	1.08 – 1.90	**0.012**
Vitamin D insufficiency	1.68	1.35 – 2.10	**<0.001**

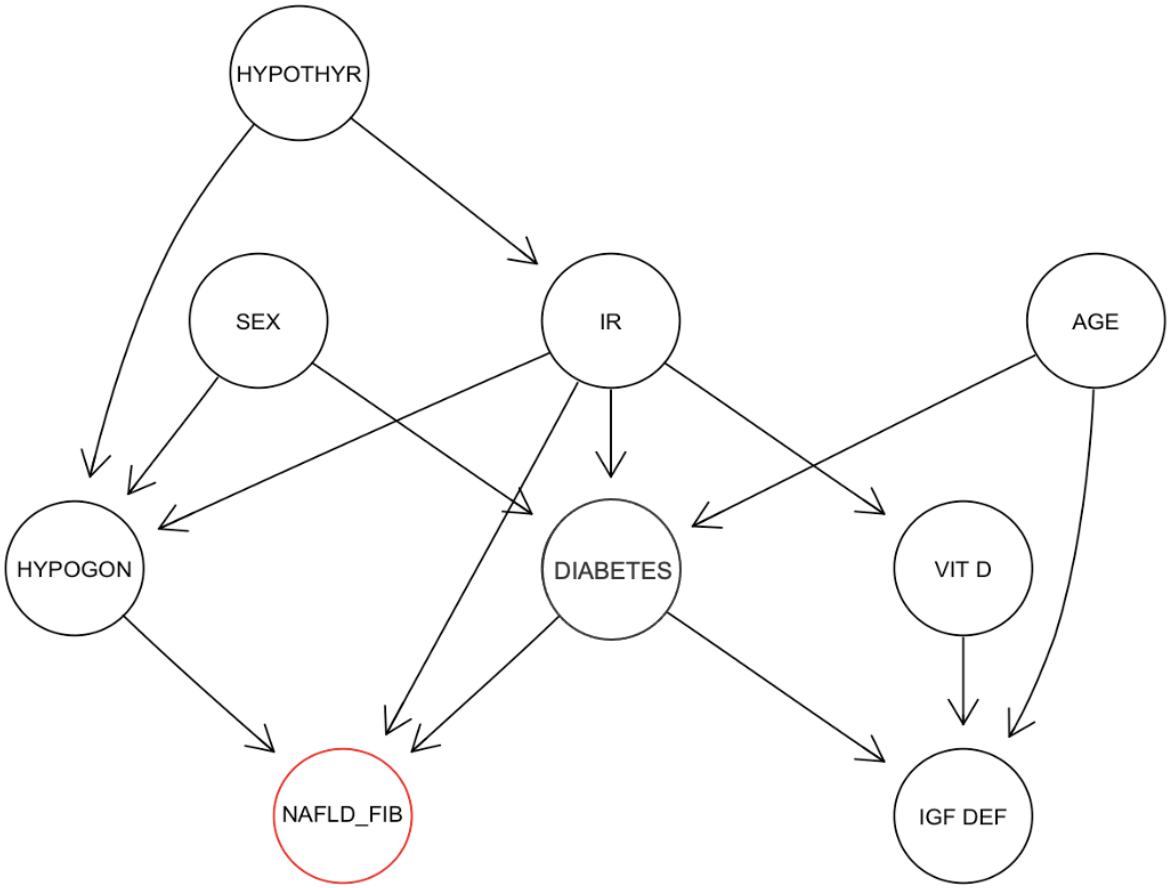
Supplementary figure 2. DAG model for NAFLD identified direct associations with age, diabetes and vitamin D insufficiency. PLWH with IR (and without diabetes) were excluded. **Abbreviations:** HYPOGON – hypogonadism; HYPOTHYR – hypothyroidism; IGF DEF – insulin growth factor deficiency; VIT D - vitamin D insufficiency.



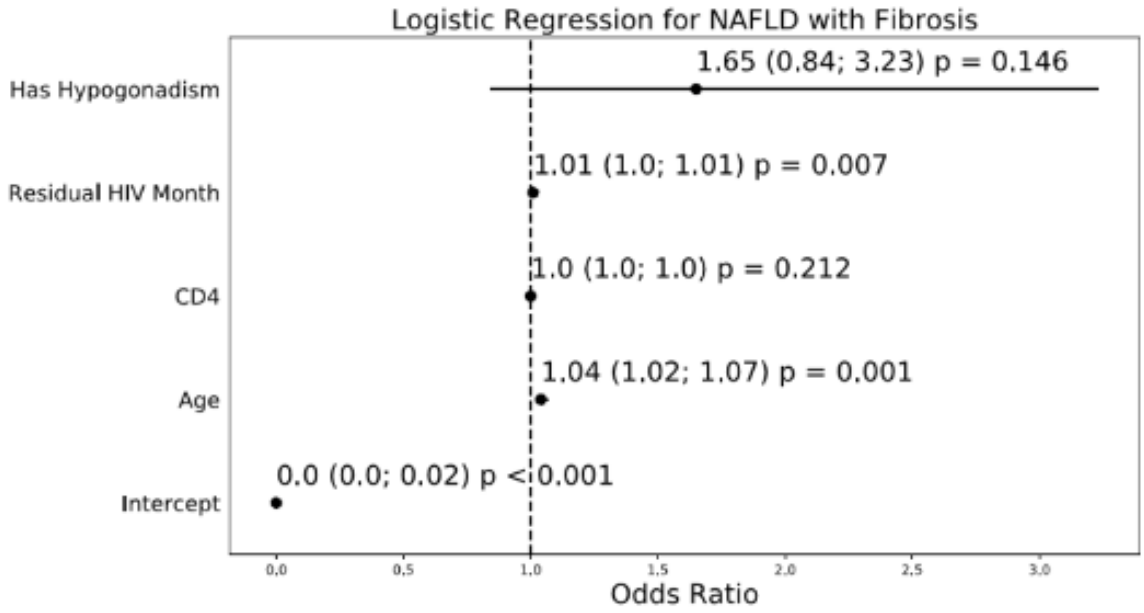
Supplementary table 4. Multivariable logistic regression model for NAFLD with fibrosis in which PLWH with both diabetes and IR are included.

Predictors	NAFLD		
	Odds ratio	95% CI	p
Hypogonadism	1.06	0.70 – 1.64	0.793
Insulin resistance	2.26	1.16 – 4.12	**0.011**
Diabetes	3.18	2.11 – 4.74	**<0.001**

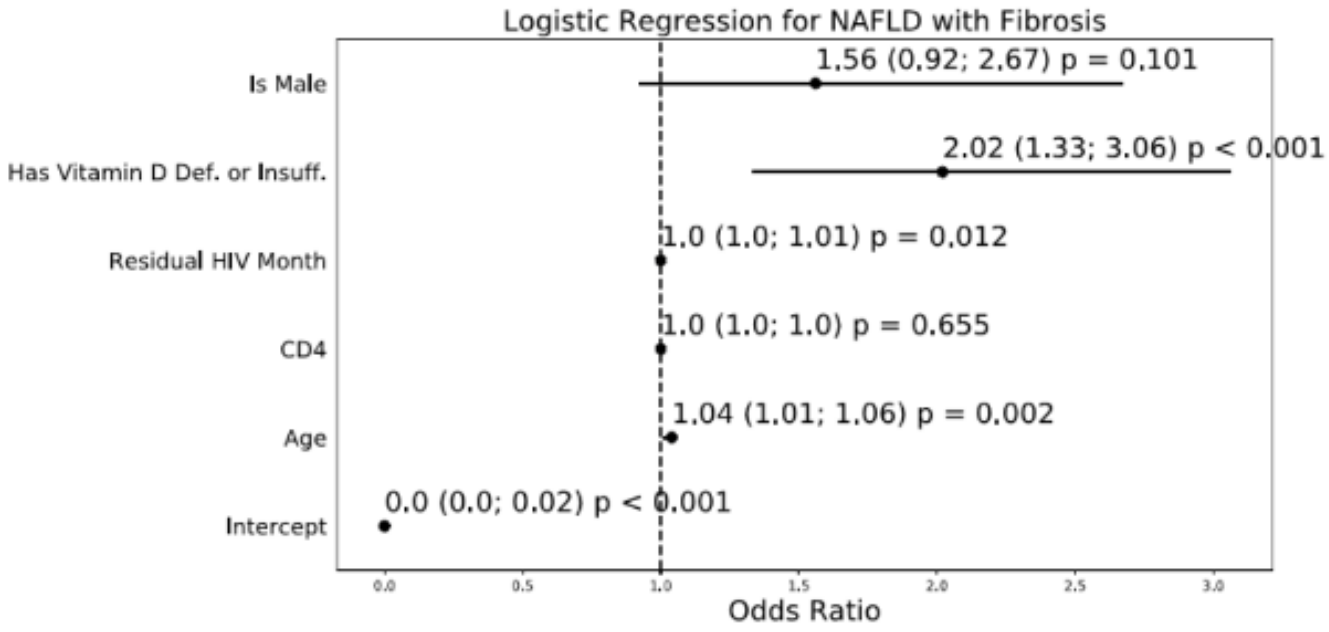
Supplementary figure 3. DAG model for NAFLD with fibrosis identified direct associations with diabetes, IR and hypogonadism. IR was related to NAFLD with fibrosis both directly and indirectly, through diabetes**. Abbreviations:** HYPOGON – hypogonadism; HYPOTHYR – hypothyroidism; IGF DEF – insulin growth factor deficiency**;** IR - insulin resistance; VIT D - vitamin D insufficiency.



Supplementary figure 4. Multivariable logistic regression model in which hypogonadism, along with HIV variables, was considered in the prediction of NAFLD with fibrosis.



Supplementary figure 5. Multivariable logistic regression model in which vitamin D insufficiency, along with HIV variables, was considered in the prediction of NAFLD with fibrosis.



PAPER III

Switching to INSTI offsets negative effects of weight gain on incidence of insulin resistance in people living with HIV

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Research in context

Evidence before this study

Weight gain is a common phenomenon affecting PLWH starting or switching ART and may induce obesity in the tail of distribution. It is multifactorial in nature and it is difficult to disentangle its drivers including genetic factors, environment, lifestyles, drugs, and the complex immuno-metabolic alterations that are often present in PLWH undergoing integrase inhibitors (INSTI) with or without tenofovir alfenamide (TAF).

A weight gain definition which considers a meaningful weight or BMI change associated with clinically relevant outcomes, including cardiometabolic conditions, is missing. Weight gain implies generalized, abdominal and ectopic fat increase that is commonly associated with insulin resistance (IR), assessed with HOMA which predicts diabetes, metabolic syndrome and cardiovascular disease.

Literature research was undertaken through PubMed, Embase, Cochrane Review, ISI Web of Science, and SCOPUS up to 8 March 2021. We also reviewed presentations and abstracts from 2021 Conference on Retroviruses and opportunistic infections. This search revealed that weight gain is a hot topic and its implications in the context of contemporary ART strategies are still uncertain.

Added value of this study

We analyzed 2437 PLWH, respectively 1025 INSTI-switchers (INSTI-s) and 1412 INSTI-naives (INSTI-n). These groups were matched for similar observational time since entrance in the cohort. Among these patients, data granularity comprised 1823 and 634 PLWH who did not have diabetes or IR at the time of switch.

In the entire cohort, based on 54826 weight assessments, trends for weight (β 0.386, $CI_{95\%}$: 0.216, 0.555, $p<0.001$) and BMI (β 0.142, $CI_{95\%}$: 0.080, 0.203, $p<0.001$) increase were significantly higher in INSTI-s (vs. INSTI-n) in the first two years after switching, but no differences were observed between the two groups in the subsequent period.

Use of INSTI (HR=0.71, $CI_{95\%}$: 0.52, 0.98, p=0.025) had a protective effect on IR incidence. A weight increase by 1% reduced the total protective effect of INSTI by 21.1% over one year

of follow-up, allowing us to identify a 5% weight increase as a clinically meaningful weight gain definition.

Implications of all the available evidence

The results of this real-life cohort clarify the existing conflicting results on the contribution of INSTI to weight gain focusing the attention on diabetes and IR. We contribute to a data-driven weight-gain definition in relation to IR as a clinically meaningful endpoint to be used in clinical and research settings.

We highlight that switch to INSTI is more frequently offered to PLWH with pre-existing cardiometabolic conditions. This strategy clusters PLWH at higher risk to develop weight gain and adverse health outcomes. On the contrary, switching to INSTI in PLWH without metabolic abnormalities offsets negative effects of weight gain on IR incidence.

ABSTRACT

Background

Weight gain is a matter of concern in PLWH switching to integrase inhibitors (INSTI). The objective was to explore weight and BMI changes in a large cohort and in the subsets of individuals without diabetes and insulin resistance (IR) at the time of switch to INSTI, in the attempt to identify the cut-off of weight or BMI increase associated with IR incidence in PLWH switching to INSTI.

Methods

This was a longitudinal matched-cohort study including PLWH attending Modena HIV Metabolic Clinic (MHMC), Italy, divided into two groups: INSTI-naive and INSTI-switchers (INSTI-s) matched for similar observation time since entrance in the cohort. The effect of switching to INSTI on percentage of weight and BMI change was tested through a linear mixed model. A mediation analysis was performed to explore the mediation effect of weight and BMI change in the causal path between the switch to INSTI and IR incidence.

Findings

We analyzed 2437 PLWH, respectively 1025 INSTI-s and 1412 INSTI-n, in a total of 54826 weight assessments. At the time of switch, median age was 45 years, 70.1% were males, median BMI was 23.3 kg/m^2. In the subset of 634 PLWH without IR, use of INSTI (HR=0.71, $CI_{95\%}$: 0.52, 0.98, p=0.025) had a protective effect on IR incidence. A weight increase by 1% reduced the total protective effect of INSTI by 21.1% over one year of follow-up, allowing us to identify a 5% weight increase as a clinically meaningful weight gain definition.

Interpretation

This study contributes to a data-driven weight-gain definition in relation to IR as a clinically meaningful endpoint. Switching to INSTI in PLWH without pre-existing metabolic abnormalities offsets negative effects of weight gain on IR incidence, confirming INSTI regimens as a metabolically satisfactory option in PLWH.

Funding

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Background

In the past 30 years, advances in antiretroviral therapies (ART), which drove the impressive change in HIV natural history, were paralleled with different anthropometric phenotypes in people living with HIV (PLWH). In detail, wasting syndrome was described in pre-ART, lipoatrophy in the early-ART, lipohypertrophy in the late-ART era [1]. Contemporary ART, mainly based on integrase strand transfer inhibitors (INSTI), has been associated with weight gain, both in naïve and experienced PLWH, generating the fear of an obesity epidemic affecting this population [1–4]. Recently, use of tenofovir alfenamide (TAF), independently of INSTI use, has also been linked to weight gain [5].

However, weight gain related to INSTI use is still poorly characterized. We are still struggling to disentangle multifactorial drivers of weight gain, including genetic, environmental, lifestyles, impact of drugs, and the complex immuno-metabolic alterations often present in PLWH starting or switching to INSTI. Furthermore, we are lacking a weight gain definition which considers a meaningful weight or BMI change associated with clinically relevant outcomes, including cardiometabolic conditions. Current literature suggests evaluating either a continuous measure of weight increase or a categorical arbitrary cut-off of 5% increase in weight or rather a 7% in BMI. The former cut-off is derived, as opposed to the magnitude of weight change recommended in lifestyle interventions as initial treatment of cardiometabolic conditions in the general population [6]. The latter has been used to describe toxicities of antipsychotic drugs [7].

Weight gain implies generalized, abdominal and ectopic fat increase that is commonly associated with glucose homeostasis derangement, depicted by insulin resistance (IR) [8]. IR is defined as a decreased ability of insulin to induce glucose uptake in insulin sensitive tissues. IR is far more than a precursor of development of type 2 diabetes, it is associated with metabolic syndrome and also predicts future CVD in adults without HIV [9]. In HIV setting, the Multicenter AIDS Cohort Study (MACS) and Women's Interagency Health Study (WIHS) also identified association between IR and subclinical cardiovascular disease and cognitive impairment [10,11]. Risk of IR induced by ART is mediated by a complex and tightly regulated network of cellular signals that lead to significant changes in adipocyte function. In particular, protease inhibitors (PIs) are shown to inhibit adipocyte differentiation [12–14] and alter expression of adipogenic factors, like SREBP-1 and ZMPSTE24 [15]. On the contrary, raltegravir does not appear to influence adipocyte differentiation [16].

Nevertheless, in adipocytes derived from obese individuals, INSTI are still associated with oxidative stress and mitochondrial dysfunction [17].

Given the discordant data regarding risk for IR and weight gain in PLWH undergoing INSTI-based regimens, the objective of this study was to explore weight and BMI changes in a large cohort and in the subsets of individuals without diabetes and insulin resistance (IR) at the time of switch to INSTI in the attempt to identify the cut-off of weight or BMI increase associated with incidence of IR in PLWH switching to INSTI.

Methods

Study design

This was an observational longitudinal matched-cohort study that included ART-experienced PLWH attending Modena HIV Metabolic Clinic (MHMC) from January 2004 to December 2019. MHMC is a tertiary level referral center established in 2004 where PLWH are screened for co-morbidities, immuno-metabolic disorders, geriatric syndromes and frailty, as previously described. PLWH undergo at each visit a total body composition analysis including accurate measure of body weight with Dual-energy X-ray absorptiometry (DEXA).

Inclusion and exclusion criteria

Consecutive ART-experienced PLWH, INSTI-naïve at baseline, aged ≥ 18 years attending MHMC were included in the initial analysis.

Matching criteria

PLWH who were INSTI-naïve (INSTI-n) at the first visit at the MHMC (baseline – T0) were included. During follow-up, PLWH were divided in two groups: INSTI-n vs. INSTI-switchers (INSTI-s) that comprised raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG) based regimens. Bictegravir (BIC) was not included because of the more recent availability in the clinical setting with limited follow-up data.
A matching was performed to select study population, based on similar observation time since entrance in the MHMC cohort (T1-T0). Time zero (T0) represents the date of the first

visit at the MHMC in both groups. In INSTI-s, time 1 (T1) is the visit date of switching to INSTI. In INSTI-n, T1 was chosen as the closest visit with a similar observation time between T0 and T1 as in INSTI-s (within a 1-month tolerance) (Supplementary figure 1).

A total of 54826 weight assessments were analyzed comprising 46377 observations in 1412 PLWH INSTI-n and 8449 observations in 1025 PLWH INSTI-s. When PLWH with diabetes and IR at T1 were excluded, the number of observations was 36063 and 5261, respectively. A maximum of 5 observations in INSTI-n were selected, to match one observation in INSTI-s to maintain the proportion of the observations in the two groups in the cohort.

Time to diabetes or IR event was defined as the time from T1 to diabetes or IR diagnosis or to the last observation for censored subjects (T2) (Supplementary figure 1).

Covariates

Demographic, anthropometric, HIV-related and immune-metabolic variables were collected on the same day of the visit at MHMC, including fasting blood glucose and insulin. Co-morbidities were defined using the European AIDS Clinical Society (EACS) guidelines [18]. Diabetes was defined as fasting serum glucose levels >126 mg/dL or HbA1C >6.5%. Diagnosis of diabetes was also made based on the current use of antidiabetic drugs. Obesity was diagnosed as body mass index (BMI) > 30 kg/m^2. Metabolic syndrome was defined using MetS ATPIII classification [19], including three or more of the following five criteria: waist circumference over 102 cm (men) or 88 cm (women), blood pressure over 130/85 mmHg, fasting triglyceride level over 150 mg/dl, fasting HDL cholesterol level less than 40 mg/dl (men) or 50 mg/dl (women), and fasting blood glucose over 100 mg/dl [19]. Cardiovascular disease (CVD) was defined as the presence of major cardiovascular events including myocardial infarction, coronary artery disease, peripheral vascular disease, stroke, and angina pectoris, as well as coronary artery bypass grafting and angioplasty, based on diagnoses recorded in patient files, regardless of the ASCVD score.

Outcomes

The outcome of the study was insulin resistance (IR), evaluated by Homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: HOMA-IR =

[fasting glucose (mg/dL) x fasting insulin (mU/ml)]/405 [20]. IR was defined as HOMA-IR score ≥ 2.

Statistical analysis

Data were summarized using median and upper (Q3) and lower quartile (Q1) for continuous variables and counts and percentages for categorical variables. Comparisons among groups at T0, T1 and T2 were performed using Kruskal-Wallis for continuous variables, and $\chi 2$ test for categorical variables.

The effect of switching to INSTI on percentage of weight and BMI changes was tested through multilevel models fitted using linear mixed model (LMM) [21], considering the matching and the longitudinal design of the study (i) in the whole population (1412 INSTI-n and 1025 INSTI-s), (ii) in the subset of PLWH without diabetes at T1 (1140 INSTI-n and 683 INSTI-s), and (iii) in the subset of PLWH without IR at T1 and available HOMA-IR index (465 INSTI-n and 169 INSTI-s). Trajectories of weight and BMI change were compared through piece-wise regression identifying three time periods: (i) prior to switch to INSTI and matched observation of INSTI-n (pre INSTI-s/n), (ii) at the time of switch to INSTI and matched observation of INSTI-n up to two years (early INSTI-s/n), and (iii) after two years from switching to INSTI and matched observation of INSTI-n (late INSTI-s/n). The same estimations were preformed also for INSTI-s and INSTI-n with or without TAF in the total population.

In the subset of 1823 and 634 PLWH who did not have diabetes and IR at T1, respectively, a Cox proportional hazard model with time dependent covariates (percentage weight or BMI change) was applied to test the direct effect of switching to INSTI on diabetes and IR. The effect on the outcome was expressed as hazard ratio (HR) with 95% confidence intervals ($CI_{95\%}$). Key confounders were sex, age, and HOMA-IR at T1.

A mediation analysis [22] was performed to explore the mediation effect of weight and BMI change in the causal path between the switch to INSTI and the time to IR event, assuming a constant increase of weight and BMI per year. Mediation models were adjusted for age, sex, baseline weight or BMI and HOMA-IR.

In order to identify cut-off of clinically meaningful weight gain in INSTI-s, the probability of IR after one year of follow-up was estimated at varying percentage of weight or BMI change compared to INSTI-n, assuming no variation of weight or BMI in this last group.

The missing data was reported in the tables. All statistical tests were two-sided and assumed a significance level of 5%. The statistical program R (v. 4.0.2) was used to analyze the data.

This study was approved by the University of Modena and Reggio Emilia ethics committee according to the Helsinki declaration.

Results

Characteristics of the total population

We analyzed 2437 PLWH, respectively 1025 INSTI-s and 1412 INSTI-n. At T0, median age was 45 (Q1, Q3: 41.0, 50.0) years, 70.1% were males, median BMI was 23.1 (Q1, Q3: 21.1, 25.5), 83 (5.6%) patients were obese, median CD4 was 552.0 cell/μL (Q1, Q3: 392.0, 740.0) and HIV RNA viral load was undetectable in 85.5% of cases (Table 1).

Weight and BMI were similar between the two groups at T1. PLWH switching to INSTI-s displayed a significantly higher prevalence of cardiometabolic conditions including diabetes (14.4% vs. 6.9%, p<0.001), metabolic syndrome (37.2% vs. 18.9%, p<0.001), cardiovascular disease (5.8% vs. 2.2% p<0.001) at T1 (Table 2A).

After a median follow-up (T0 to T2) of 6.7 (Q1, Q3: 3.0, 11.0) years, median weight change was 2.9% (Q1, Q3: -1.7, 8.5) in INSTI-s and 1.6% (Q1, Q3: -2.2, 5.7) in INSTI-n (p<0.001), while median BMI changed by 2.8% (Q1, Q3 :-1.9, 8.7) in INSTI-s and 1.7% (Q1, Q3: -2.4, 5.9) in INSTI-n (p<0.001). No differences were observed between the INSTI-s vs. INSTI-n in the incidence of diabetes (7.7% vs. 8.2%) and cardiovascular disease (2.0% vs. 2.6%), while the incidence of metabolic syndrome was higher in INSTI-n (19.3% vs. 8.8%, p<0.001) (Table 2A).

Characteristics of the subset of PLWH without diabetes at T1

In the subset of 1823 PLWH without diabetes at T1 (683 INSTI-s and 1140 INSTI-n), prevalence of metabolic syndrome was higher in PLWH switching to INSTI (34.3% vs. 27.9%, p=0.004) (Table 2B).

After a median follow-up (from T1 to T2) of 2.7 (Q1, Q3: 1.4-4.6) years, INSTI-s experienced higher median weight change (1.7%, Q1, Q3: -1.5, 5.6 vs. 1.1%, Q1, Q3: -1.9, 4.3, p<0.003) and median BMI change (1.4%, Q1, Q3: -1.5, 5.5 vs. 0.8%, Q1, Q3: -2.4, 4.3, p=0.023). No differences were observed between the INSTI-s vs. INSTI-n in the incidence of diabetes (9.7% vs. 10.4%) and metabolic syndrome (9.8% vs. 9.1%), while cardiovascular disease (2.2% vs. 0.9%, p=0.019) was higher in INSTI-s (Table 2B).

None of the drugs within the INSTI class were associated with higher incidence of diabetes (Supplementary table 2). The other anthropometric and HIV characteristics of the subset of PLWH without diabetes at T1 are shown in Supplementary Tables 2 and 3.

Characteristics of the subset of PLWH without IR at T1

In order to explore the interplay between INSTI-s and weight with IR, the analysis was restricted to the subset of PLWH without IR at T1 and in which HOMA-IR was available at least at T1 and T2. The sample size reduced to 634 patients, 465 (73.3%) INSTI-n and 169 (26.7%) INSTI-s. No differences were observed between the groups in the prevalence of metabolic syndrome and cardiovascular disease at T1 (Table 2C).

After a median follow-up (from T1 to T2) of 2.0 (Q1, Q3: 1.1, 3.4) years, weight and BMI changes were not different between INSTI-s and INSTI-n (weight: 1.7%, Q1, Q3: -1.7, 5.2 vs. 1.2%, Q1, Q3: -1.8, 4.3, p=0.299; BMI: 1.6%, Q1, Q3: -1.5-5.5 vs. 1.1%, Q1, Q3: -1.7, 4.7, p=0.396). The incidence of diabetes (2.4% vs. 1.5%), metabolic syndrome (9.5% vs. 7.5%) and cardiovascular disease (1.2% vs. 1.3%) was similar in the two groups. On the contrary, PLWH undergoing INSTI-n regimens had higher incidence of IR in comparison to INSTI-s (42.3% vs. 25.4%, p<0.001) (Table 2C).

PLWH who developed IR at T2 were less frequently undergoing 2DR regimens (Supplementary table 4). The other anthropometric and HIV characteristics, including physical activity and metabolic parameters, of the subset of PLWH without IR at T1 are shown in Supplementary tables 4 and 5.

Trends of weight and BMI change in INSTI-s and INSTI-n

Table 3 depicts estimations derived from LMM of weight and BMI trends over time for INSTI-s and INSTI-n in the entire cohort (Table 3A), in the subset of PLWH without diabetes at T1 (Table 3B) and without IR at T1 (Table 3C). The comparisons comprise three time periods: (i) prior to switch to INSTI and matched observation of INSTI-n (pre INSTI-s/n), (ii) at the time of switch to INSTI up to two years of follow-up and matched observation of INSTI-n (early INSTI-s/n), and (iii) after two years from switching to INSTI and matched observation of INSTI-n (late INSTI-s/n). These data are also represented in Figure 1. The models were fitted for weight and BMI including: total population (Figure 1A and 1D), subset of PLWH without diabetes at T1 (Figure 1B and 1E), and subset of PLWH without IR at T1 (Figure 1C and 1F).

In the entire cohort, based on 54826 weight assessments, trends for weight (β 0.386, $CI_{95\%}$: 0.216, 0.555, p<0.001) and BMI (β 0.142, $CI_{95\%}$: 0.080, 0.203, p<0.001) increase were significantly higher in early-INSTI-s (vs. early INSTI-n), but no difference in trends for weight and BMI increase were observed between the two groups in the late period after the switch. Moreover, the line slopes of weight and BMI changes in late period of the switch were similar to the trends before the switch for both INSTI-s (β 0.036, $CI_{95\%}$: 0.001, 0.072, p=0.053 for weight and -0.012, $CI_{95\%}$: -0.044, 0.020, p=0.761 for BMI) and INSTI-n (β 0.001, $CI_{95\%}$: -0.086, 0.089, p=1.0 for weight and 0.006, $CI_{95\%}$: -0.007, 0.019, p=0.576 for BMI) (Table 3A, Figure 1A and 1D). Additionally, trends for weight (β 0.209, $CI_{95\%}$: 0.023, 0.396, p=0.028), but not BMI (β 0.058, $CI_{95\%}$: -0.009, 0.126, p=0.090), showed a significant increase among INSTI-s with TAF (vs. INSTI-s without TAF) (Supplementary table 1, Supplementary figure 2).

In the subset of PLWH without diabetes at T1, based on 36063 weight assessments, trends for weight (Figure 1B) and BMI (Figure 1E) change were similar to the previous model for the whole cohort both for INSTI-s and INSTI-n, with the exception of trends for BMI change in the late period after the switch that were lower for INSTI-s (β -0.032, $CI_{95\%}$: -0.059, -0.004, p=0.024) (Table 3B).

In the subset of PLWH without IR at T1, based on 5261 weight assessments, only trends for weight increase (β 0.633, $CI_{95\%}$: 0.079, 1.186, p=0.025) were significantly higher in early-INSTI-s (vs. early INSTI-n). No difference in trends for weight and BMI increase were observed between the two groups in the late period after the switch (Table 3C, Figure 1C and 1F).

The association between switching to INSTI and diabetes/IR

The incidence rate of IR was 14.6 cases for 100 patient years. The effect of switching to INSTI on diabetes and IR was tested through a Cox proportional hazard model with the percentage weight or BMI change as time dependent covariate (Table 4). In the model 4A, use of INSTI (HR=1.39, $CI_{95\%}$: 1.08, 1.78, p=0.009) was associated with a higher risk of diabetes. However, after adjusting for HOMA-IR at T1 (model 4B), use of INSTI was no longer associated with diabetes (HR=1.19, $CI_{95\%}$: 0.91, 1.56, p=0.201). Conversely, use of INSTI (HR=0.71, $CI_{95\%}$: 0.52, 0.98, p=0.025) had a protective effect when IR was considered as an outcome (Table 4C).

The mediation effect of weight and BMI change and switch to INSTI on incidence of IR

The estimated mediation effect of weight and BMI change in the causal path between switching to INSTI and IR shows a reduction of the total protective INSTI effect of 21.1% and 19.4% due to the increase of 1% of weight or BMI in one year of follow-up, respectively (Figure 2A and B). Through the fitted Cox regression model, a 5% increase of baseline weight and 12% of baseline BMI were estimated among INSTI-s which offsets the reduction of IR onset risk induced by switching to INSTI (Supplementary figure 3A and 3B).

Discussion

Our main finding, based on a large sample size in a real-life cohort, shows that switching to INSTI (vs. INSTI-n) in PLWH was associated with a lower risk of IR (HR=0.71, $CI_{95\%}$: 0.52, 0.98, p=0.025). However, a 1% weight increase reduced the total protective effect of INSTI by 21.1% over one year of follow-up, allowing us to identify a 5% weight increase as a clinically meaningful weight gain definition. These data imply that switching to INSTI in PLWH without IR at T1 is not associated with higher risk of metabolic alterations when compared to INSTI-n, confirming INSTI regimens as a metabolically satisfactory option in

PLWH. We highlight that switch to INSTI is more frequently offered to PLWH with pre-existing cardiometabolic conditions. This strategy clusters PLWH at higher risk to develop weight gain and adverse health outcomes. Conversely, switching to INSTI in PLWH without metabolic abnormalities offsets negative effects of weight gain on IR incidence.

In 54826 weight assessments, this study analyzed the relationship between INSTI switch and weight or BMI change in a cohort of 2437 ART-experienced PLWH over a median of 6.7 years of follow-up. Our data suggest that trends of weight and BMI increase were observed in all PLWH regardless of ART regimens, but also confirmed greater weight gain among PLWH switching to INSTI in the first two years after the switch. Interestingly, in the late period after the switch, the estimated trends of weight and BMI change in both INSTI-s and INSTI-n were similar. This latter result is encouraging and could suggest that in the long term PLWH undergoing INSTI have the same trend in weight gain as INSTI-n
How can we interpret these real-life data in the context of the results of randomized clinical trials (RCT), for instance the ADVANCE study? First of all, RCT included a non-metabolically selected population similar to our parenteral dataset (including PLWH with diabetes and IR at the time of switch). We confirmed an early weight gain in the first two years after switch to INSTI. Contrary to RCT, we were able to project weight change in the late period, beyond 96 weeks. Lastly, we also observed a subset of PLWH undergoing INSTI and TAF in which, apparently, no plateau of weight gain was present in the follow-up, similarly to what hypothesized in the ADVANCE trial [3,23].

It should be emphasized that switching to INSTI was not driven by virological failure, but by longer HIV duration and lower nadir CD4, possibly harboring a higher prevalence of cardiometabolic conditions such as metabolic syndrome, diabetes and cardiovascular disease, as depicted at the time of switch (T1). Furthermore, visceral adipose tissue area was greater in INSTI-s at T1, although the observed value might not be clinically significant, lower as it is the cut-off currently used to define visceral obesity [24,25]. Nevertheless, a less favorable profile of INSTI-s at T1 was not associated with higher incidence of cardiometabolic conditions at follow-up, regardless of greater weight and BMI increase. This is in line with recent data from the REPRIEVE study, which showed that INSTI based regimens were associated with higher BMI, higher risk of obesity and waist circumference, but not with increased fasting glucose, LDL cholesterol, hypertension or metabolic syndrome [26].

A pooled analysis of randomized clinical trials in ART-naive PLWH showed that weight gain is a multifactorial phenomenon, associated with baseline demographic and HIV characteristics such as lower CD4 cell count, higher HIV RNA viral load, female sex and black race [27]. However, this analysis reported 2-year follow up data that may not be sufficient to capture longer-term metabolic consequences [27]. Additionally, PLWH included in these trials were rarely stratified by preexisting cardiometabolic abnormalities and monitored across time. Likewise, the median age of PLWH enrolled was <40 years [27], which does not fully reflect current scenario of the “silver tsunami” affecting PLWH [28] that are more prone to develop or already have cardiometabolic conditions. Moreover, in experienced individuals with previous exposure to ART, drivers of weight gain may be the result of more complex interplay between various ART strategies, age, and preexisting cardiometabolic abnormalities.

Therefore, in order to better depict the impact of preexisting metabolic abnormalities, we restricted the analysis to 1823 PLWH without diabetes at T1. The drivers of switching to INSTI were similar to those of the entire population and had similar patterns in weight and BMI trends. The link between INSTI and diabetes has not been clearly established [29]. Nevertheless, some data from randomized clinical trials indicate that INSTI, when used with TAF, may have long-term risk of diabetes [23,29] and cases of new-onset hyperglycaemia after initiation of dolutegravir have been described [30]. In this subgroup of our cohort, initial analyses showed that use of INSTI was associated with diabetes incidence, but after adjustment for HOMA at T1, this relationship disappeared, suggesting that diabetes onset is rather justified by preexisting metabolic alterations.

To better explore this subclinical metabolic signal associated with IR, we restricted the analyses to 634 PLWH with no IR at T1. IR as an exclusion criterion at the time of switch identified a population with low burden of cardiometabolic conditions.

However, the relationship between INSTI and IR remains controversial. DTG and RAL can induce insulin resistance in human and simian adipose tissue [17], but observational studies and randomized clinical trials did not observe the negative impact of switch to INSTI on IR [31–33]. In detail, Gorwood et al described that DTG and, to a lesser extent, RAL were associated with greater extracellular matrix production and lipid accumulation in adipose stem cells and/or adipocytes that led to IR [17]. On the other side, Calza et al. showed that the switch from a PI/r to INSTI reduced both HOMA index and serum leptin levels, thus

improving insulin sensitivity [31], while Dirajlal et al. did not observe any differences in HOMA index in PLWH undergoing RAL versus PIs [33].

We also described clinical characteristics of PLWH developing IR over a two-year follow up, in whom a slight weight increase was observed (2.1% vs. 0.6%). These individuals had higher HOMA, dyslipidemia and lower physical activity at T1, suggesting the need for dedicated counseling on lifestyle interventions in order to address modifiable risk factors. The relationship between HOMA and physical activity is well established in the general population [34] and in PLWH. In the MACS Cohort, which included 1281 patients (581 PLWH), it was shown that higher physical activity was associated with lower prevalence of IR in PLWH, while cumulative and current exposure to ART had no effect on HOMA values [35].

Some limitations are intrinsic to the observational nature of the study. As previously described, this analysis required careful selection of the study population and appropriate matching. INSTI-s and INSTI-n were matched for observational time in the cohort and adjusted for age, sex and baseline BMI. The criterion, although not related to an HIV specific variable, was used to homogenize similar clinical care, and ART exposure and availability in the two groups. The time difference in the observation period between arms is intrinsic to the study design given that data were censored at the occurrence of IR, in this group only. Lastly, we did not explore the impact of single drugs within the INSTI class and TAF in relation to IR.

The original approach of this study was twofold. Firstly, we were able to provide data granularity in a large sample of PLWH, progressively selecting three subsets of individuals, with clinical or subclinical glucose metabolism impairment, which allowed to depict the impact of INSTI-s on weight or BMI change according to baseline characteristics. Moreover, body weight was accurately assessed with total body DEXA, which provides a standardized reliable measure [36]. Secondly, we contemporary explored competing phenomena associated with IR, respectively INSTI-s and weight gain, through the mediation analyses.

After more than a half decade of discussion regarding short-term weight gain after starting [3,4,23] or switching ART [37], we are still missing a clinically meaningful weight gain definition. This issue was widely discussed in the ADVANCE study in the context of cultural challenges related to weight gain and its prevention and treatment. Authors wondered which

weight gain threshold should be used when switching-out from ongoing DTG based regimens [23]. An on-going ACTG study is addressing reversibility of weight gain through switching from INSTI to doravirine [38], a new non-nucleoside reverse transcriptase inhibitor. Hopefully, results from this trial would enlighten future therapeutic strategies in PLWH who experience weight gain.

In the meanwhile, as we are waiting for these results, our study could significantly fill this knowledge gap, by suggesting the use of IR as a relevant clinical outcome in observational and randomized trials. Moreover, this real-life cohort clarifies the existing conflicting results on the impact of INSTI on weight gain, focusing the attention on diabetes and IR.

In conclusion, we contribute to a data-driven weight-gain definition in relation to IR as a clinically meaningful endpoint to be used in clinical and research settings. In particular, in PLWH without IR at the time of switch, we identified a value of a 5% weight and/or a 12% BMI increase from the time of switch to be associated to negative health outcomes.

Conflict of interest

GG and CM received research grant and speaker honorarium from Gilead, ViiV, MERCK and Jansen. GG and CM attended advisory boards of Gilead, ViiV and MERCK. JM, SR, DF, SB, MM, FC, GD, GC and SC reported no conflict of interest.

Authors' contributions

GG, JM, SR and SC conceptualized and designed the manuscript. GG and JM wrote and revised the manuscript. SR, SC, DF and SB did the statistical analysis. GG, JM, SR, SC did the supervision of the final version of the manuscript. All the authors contributed to discussion and revised the manuscript.

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Table 1. Demographic, HIV and metabolic characteristics in INSTI-s and INSTI-n at baseline and follow-up.

	INSTI naive (N=1412)	**INSTI switchers (N=1025)**	**Total (N=2437)**	**p**
Demographic and anthropometric characteristics at baseline (T0)				
Male sex, N (%)	963 (68.2%)	746 (72.8%)	1709 (70.1%)	**0.015**
Age, years, median (Q1, Q3)	44.0 (40.0, 49.0)	46.0 (42.0, 51.0)	45.0 (41.0, 50.0)	**<0.001**
Waist circumference, cm, median (Q1, Q3) [N°]	85.0 (79.0, 92.0) [1395]	86 (80.0, 94.0) [1022]	85.5 (80.0, 93.0) [2398]	**0.017**
VAT, cm^2, median (Q1, Q3), [N°]	107.0 (71.0, 156.2) [912]	119.0 (75.0, 176.0) [635]	111.0 (73.0, 165.0) [1547]	**0.006**
Obesity, N (%)	83 (5.9%)	54 (5.3%)	137 (5.6%)	0.519
Physical activity, N (%) [N°] No Mild Intense	[1390] 888 (63.9%) 350 (25.2%) 152 (10.9%)	[1013] 622 (61.4%) 283 (27.9%) 108 (10.7%)	[2403] 1510 (62.8%) 633 (26.3%) 260 (10.8%)	0.316
HIV variables at baseline (T0)				
HIV duration, months, median (Q1, Q3), [N°]	161.0 (92.0, 225.0) [1381]	185.0 (114.0, 246.0) [1001]	168.0 (100.0, 233.0) [2382]	**<0.001**
Nadir CD4, c/μL, median (Q1, Q3), [N°]	200.0 (88.0, 300.0) [1375]	180.0 (76.0, 296.0) [991]	196.0 (80.0, 300.0) [2382]	**0.019**
Current CD4, c/μL**, median (Q1, Q3),** [N°]	565.0 (405.0, 753.0) [1344]	536.0 (375.0, 718.0) [969]	552.0 (392.0, 740.0) [2313]	**<0.001**
CD4/CD8 ratio, median (Q1, Q3), [N°]	0.8 (0.5, 1.1) [482]	0.7 (0.5, 1.0) [471]	0.7 (0.5, 1.1) [953]	**0.014**
HIV RNA viral load, median (Q1, Q3), [N°]	1086 (86.4%) [1241]	708 (84.2%) [841]	1794 (85.5%) [2089]	0.159
Metabolic variables at baseline (T0)				
HOMA index, median (Q1, Q3), [N°]	2.4 (1.4, 4.0) [1201]	2.4 (1.4, 4.0) [851]	2.4 (1.4, 4.0) [2052]	0.593
Total cholesterol, mg/dl, median (Q1, Q3), [N°]	190.0 (160.0, 222.0) [1281]	190.0 (161.0, 219.0) [925]	190.0 (160.0, 221.0) [2206]	0.916
LDL cholesterol, mg/dl, median (Q1, Q3), [N°]	114.0 (90.0, 142.0) [1259]	116.0 (92.0, 139.0) [905]	115.0 (91.0, 141.0) [2164]	0.612
HDL cholesterol, mg/dl, median (Q1, Q3), [N°]	45.0 (37.0, 55.0) [1267]	43.0 (35.0, 53.0) [910]	44.0 (36.0, 54.0) [2177]	**0.020**
Triglycerides, mg/dl, median (Q1, Q3), [N°]	138.0 (94.0, 210.0) [1280]	144 (99.0, 218.0) [921]	132.0 (91.0, 200.0) [2201]	0.094
ART at baseline (T0)				
Current exposure to PI, N (%)	695 (49.2%)	574 (56.0%)	1269 (52.1%)	**<0.001**
Current exposure to NRTI, N (%)	1294 (91.6%)	920 (89.8%)	2214 (90.8%)	**<0.001**
Current exposure to NNRTI, N (%)	661 (46.8%)	331 (32.3%)	992 (40.7%)	**<0.001**
Follow-up duration, years, median (Q1, Q3)	5.3 (2.1, 9.4)	8.5 (4.4, 12.3)	6.7 (3.0, 11.0)	**<0.001**
ART at follow-up (T2)				
Exposure to TDF, N (%)	570 (40.4%)	102 (10.0%)	672 (27.6%)	**<0.001**
Exposure to TAF, N (%)	191 (13.5%)	164 (16.0%)	355 (14.6%)	0.088
2 DR therapy, N (%)	103 (7.7%)	344 (33.6%)	447 (18.9%)	**<0.001**
3 DR therapy, N (%)	905 (67.3%)	559 (54.5%)	1464 (61.8%)	**<0.001**

Abbreviations: 2 DR – two-drug regimen; 3 DR – three drug regimen; ART – antiretroviral therapy; HDL – high density lipoprotein; HIV – human immunodeficiency virus; HOMA - Homeostatic Model Assessment for insulin

resistance; INSTI – integrase strand transfer inhibitors; Q1, Q3 – interquartile range; LDL – low density lipoprotein; [N°] – number of people in which the given variable was available; NNRTI – non-nucleoside reverse transcriptase inhibitors; NRTI – nucleoside reverse transcriptase inhibitors; PI – protease inhibitors; TAF – tenofovir alafenamide; TDF - tenofovir disoproxil fumarate; VAT – visceral adipose tissue

Table 2. Anthropometrics and cardiometabolic conditions at baseline and follow-up in INSTI-s and INSTI-n.

2A	**Time of the switch (T1)**			**Follow-up (T2)**		
Total population (N=2437)	**INSTI-n** N=1412	**INSTI-s** N=1025	**p**	**INSTI-n** N=1412	**INSTI-s** N=1025	**p**
Anthropometrics						
Weight, kg, median (Q1, Q3)	67.0 (59.0, 75.0)	68.0 (59.6, 76.0)	0.243	68.0 (60.0, 77.0)	70.0 (61.5, 78.5)	**0.002**
Weight change, %, median (Q1, Q3)				1.6 (-2.2, 5.7)	2.9 (-1.7, 8.5)	**<0.001**
Weight gain >5%, N (%)				400 (28.3%)	408 (39.8%)	**<0.001**
BMI, kg/m^2, median (Q1, Q3)	23.1 (21.1, 25.3)	23.3 (21.2, 25.7)	0.089	23.5 (21.5, 25.9)	24.0 (21.9, 26.4)	**<0.001**
BMI change, %, median (Q1, Q3)				1.7 (-2.4, 5.9)	2.8 (-1.9, 8.7)	**<0.001**
BMI gain >7%, N (%)				303 (21.5%)	328 (32.0%)	**<0.001**
Cardiometabolic conditions*						
MetS, N (%)	267 (18.9%)	381 (37.2%)	**<0.001**	272 (19.3%)	90 (8.8%)	**<0.001**
Diabetes, N (%)	97 (6.9%)	148 (14.4%)	**<0.001**	115 (8.2%)	79 (7.7%)	0.690
CVD, N (%)	31 (2.2%)	59 (5.8%)	**<0.001**	37 (2.6%)	20 (2.0%)	0.279
IR, N (%)	626 (52.6%)	421 (52.7%)	0.970	436 (50.6%)	174 (52.6%)	0.551

2B						
Subset of PLWH without diabetes at T1 (N=1823)	**INSTI-n** N=1140	**INSTI-s** N=683	**p**	**INSTI-n** N=1140	**INSTI-s** N=683	**p**
Anthropometrics						
Weight, kg, median (Q1, Q3)	67.3 (59.0, 75.5)	68.0 (59.3, 75.2)	0.669	68.0 (60.0, 76.5)	70.0 (62.7, 79.9)	**0.012**
Weight change, %, median (Q1, Q3)				1.1 (-1.9, 4.3)	1.7 (-1.5, 5.6)	**0.003**
Weight gain >5%, N (%)				244 (21.4%)	190 (27.8%)	**0.002**
BMI, kg/m^2, median (Q1, Q3)	23.3 (21.1, 25.5)	23.4 (21.2, 25.8)	0.239	23.5 (21.4, 25.9)	24.4 (22.3, 27.3)	**0.001**
BMI change, %, median (Q1, Q3)				0.8 (-2.4, 4.3)	1.4 (-1.5, 5.5)	**0.023**
BMI gain >7%, N (%)				172 (15.1%)	123 (18.0%)	0.101
Cardiometabolic conditions*						
MetS, N (%)	318 (27.9%)	234 (34.3%)	**0.004**	104 (9.1%)	67 (9.8%)	0.626
Diabetes, N (%)	0	0		118 (10.4%)	66 (9.7%)	0.637
CVD, N (%)	36 (3.2%)	31 (4.5%)	0.129	10 (0.9%)	15 (2.2%)	**0.019**
IR, N (%)	549 (48.2%)	333 (48.8%)	0.805	163 (18.0%)	106 (20.8%)	0.205

2C						
Subset of PLWH without IR at T1 (N=634)	**INSTI-n** N=465	**INSTI-s** N=169	**p**	**INSTI-n** N=465	**INSTI-s** N=169	**p**
Anthropometrics						
Weight, kg, median (Q1, Q3)	64.5 (56.7, 72.0)	65.0 (57.5, 73.5)	0.300	68.0 (60.0, 77.0)	69.0 (61.0, 76.9)	0.269
Weight change, %, median (Q1, Q3)				1.2 (-1.8, 4.3)	1.7 (-1.7, 5.2)	0.299
Weight gain >5%, N (%)				92 (19.8%)	43 (25.4%)	0.124
BMI, kg/m^2, median (Q1, Q3)	22.2 (20.4, 24.1)	22.7 (20.4, 25.0)	0.201	23.5 (21.4, 25.8)	23.8 (21.6, 26.2)	0.078
BMI change, %, median (Q1, Q3)				1.1 (-1.7, 4.7)	1.6 (-1.5, 5.5)	0.396
BMI gain >7%, N (%)				57 (12.3%)	32 (18.9%)	**0.032**
Cardiometabolic conditions*						
MetS, N (%)	54 (11.6%)	17 (10.1%)	0.583	35 (7.5%)	16 (9.5%)	0.427
Diabetes, N (%)	8 (1.7%)	5 (3.0%)	0.331	7 (1.5%)	4 (2.4%)	0.463
CVD, N (%)	8 (1.7%)	7 (4.1%)	0.076	6 (1.3%)	2 (1.2%)	0.915
IR, N (%)	0	0		197 (42.3%)	43 (25.4%)	**<0.001**

Table 3. Estimations of weight and BMI trends over time for INSTI-s and INSTI-n in total population, subset of PLWH without diabetes at T1 and without IR at T1. The comparisons derived from three time periods: (i) prior to switch to INSTI and matched observation of INSTI-n (pre INSTI-s/n), (ii) at the time of switch to INSTI up to two years of follow-up and matched observation of INSTI-n (time early INSTI-s/n), and (iii) after two years from switching to INSTI and matched observation of INSTI-n (late INSTI-s/n).

3A

Total population (N=2437)	**Weight (see Figure 1A)**		**BMI (see Figure 1D)**	
	Estimate (*CI*$_{95\%}$)	**p**	**Estimate (*CI*$_{95\%}$)**	**p**
Time pre INSTI-s vs. pre INSTI-n	0.041 (0.004, 0.078)	0.029	0.011 (-0.003, 0.024)	0.115
Time early INSTI-s vs. early INSTI-n	0.386 (0.216, 0.555)	<0.001	0.142 (0.080, 0.203)	<0.001
Time late INSTI-s vs. late INSTI-n	0.007 (-0.062, 0.075)	0.851	-0.007 (-0.032, 0.017)	0.557
Time early INSTI-s vs. pre INSTI-s	0.506 (0.325, 0.687)	<0.001	0.179 (0.113, 0.245)	<0.001
Time late INSTI-s vs. early INSTI-s	-0.505 (-0.709, -0.301)	<0.001	-0.191 (-0.265, -0.117)	<0.001
Time early INSTI-s vs. late INSTI-n	0.511 (0.340, 0.682)	<0.001	0.183 (0.121, 0.245)	<0.001
Time late INSTI-s vs. pre INSTI-s	0.036 (0.001, 0.072)	0.053	-0.012 (-0.044, 0.020)	0.761
Time late INSTI-n vs. pre INSTI-n	0.001 (-0.086, 0.089)	1.000	0.006 (-0.007, 0.019)	0.576

3B

Subset of PLWH without diabetes at T1 (N=1823)	**Weight (see Figure 1B)**		**BMI (see Figure 1E)**	
	Estimate (*CI*$_{95\%}$)	**p**	**Estimate (*CI*$_{95\%}$)**	**p**
Time pre INSTI-s vs. pre INSTI-n	0.021 (-0.026, 0.068)	0.379	0.007 (-0.010, 0.024)	0.417
Time early INSTI-s vs. early INSTI-n	0.521 (0.324, 0.718)	<0.001	0.174 (0.102, 0.245)	<0.001
Time late INSTI-s vs. late INSTI-n	-0.068 (-0.144, 0.008)	0.078	-0.032 (-0.059, -0.004)	0.024
Time early INSTI-s vs. pre INSTI-s	0.467 (0.254, 0.679)	<0.001	0.163 (0.086, 0.240)	<0.001
Time late INSTI-s vs. early INSTI-s	-0.523 (-0.759, -0.288)	<0.001	-0.198 (-0.283, -0.112)	<0.001
Time early INSTI-s vs. late INSTI-n	0.455 (0.257, 0.654)	<0.001	0.166 (0.094, 0.238)	<0.001
Time pre INSTI-s vs. late INSTI-s	-0.057 (-0.157, 0.043)	0.447	-0.035 (-0.072, 0.001)	0.062
Time pre INSTI-n vs. late INSTI-n	0.032 (-0.009, 0.073)	0.172	0.004 (-0.011, 0.019)	0.922

3C

Subset of PLWH without IR at T1 (N=634)	**Weight (see Figure 1C)**		**BMI (see Figure 1F)**	
	Estimate (*CI*$_{95\%}$)	**p**	**Estimate (*CI*$_{95\%}$)**	**p**
Time pre INSTI-s vs. pre INSTI-n	0.235 (0.069, 0.401)	0.006	0.033 (-0.050, 0.117)	0.433
Time early INSTI-s vs. early INSTI-n	0.633 (0.079, 1.186)	0.025	0.230 (-0.053, 0.513)	0.112
Time late INSTI-s vs. late INSTI-n	-0.092 (-0.422, 0.239)	0.586	-0.071 (-0.240, 0.097)	0.408
Time early INSTI-s vs. pre INSTI-s	0.399 (-0.243, 1.042)	0.263	0.399 (-0.243, 1.042)	0.491
Time late INSTI-s vs. early INSTI-s	-0.799 (-1.581, -0.017)	0.045	-0.268 (-0.669, 0.133)	0.220
Time early INSTI-s vs. late INSTI-n	0.707 (0.122, 1.293)	0.016	0.197 (-0.103 0.496)	0.230
Time pre INSTI-s vs. late INSTI-s	-0.400 (-0.838, 0.038)	0.088	-0.128 (-0.352, 0.096)	0.440
Time pre INSTI-n vs. late INSTI-n	-0.073 (-0.218, 0.072)	0.552	-0.023 (-0.097, 0.051)	0.842

Table 4. Hazard ratios from a multiple Cox regression model for diabetes and IR.

Diabetes mellitus in subset of PLWH without diabetes at T1 (panel A)				**Diabetes mellitus in subset of PLWH without diabetes at T1 (panel B)**			
Predictors	**HR**	**$CI_{95\%}$**	**p**	*Predictors*	**HR**	**$CI_{95\%}$**	**p**
Use of INSTI	1.39	1.08 – 1.78	**0.009**	Use of INSTI	1.19	0.91 – 1.56	0.201
Male sex	1.40	1.10 – 1.79	**0.005**	Male sex	0.98	0.76 – 1.26	0.856
Age	1.06	1.05 – 1.07	**<0.001**	Age	1.06	1.05 – 1.08	**<0.001**
BMI change	1.02	1.00 – 1.03	**0.014**	Weight change	1.02	1.00 – 1.04	**0.017**
				HOMA at T1	1.17	1.14 – 1.19	**<0.001**

Insulin resistance in subset of PLWH without IR at T1 (panel C)			
Predictors	**HR**	**$CI_{95\%}$**	**p**
Use of INSTI	0.71	0.52 – 0.98	**0.025**
Male sex	1.44	1.14 – 1.83	**0.006**
Age	1.01	1.00 – 1.03	0.072
Weight change	1.07	1.05 – 1.09	**<0.001**
HOMA at T1	3.73	2.87 – 4.83	**<0.001**

Abbreviations: HR –hazard ratio; BMI – body mass index; CI: confidence interval; HOMA - Homeostatic Model Assessment for insulin resistance; INSTI – integrase strand transfer inhibitors; IR – insulin resistance; T1 – time of switch to INSTI; T2 - IR or diabetes diagnosis or last observation available if no IR or diabetes (follow-up).

Figure 1. Weight and BMI trends over time for INSTI-s and INSTI-n in total population (A, D), subset of PLWH without diabetes at T1 (B, E) and without IR at T1 (C, F).

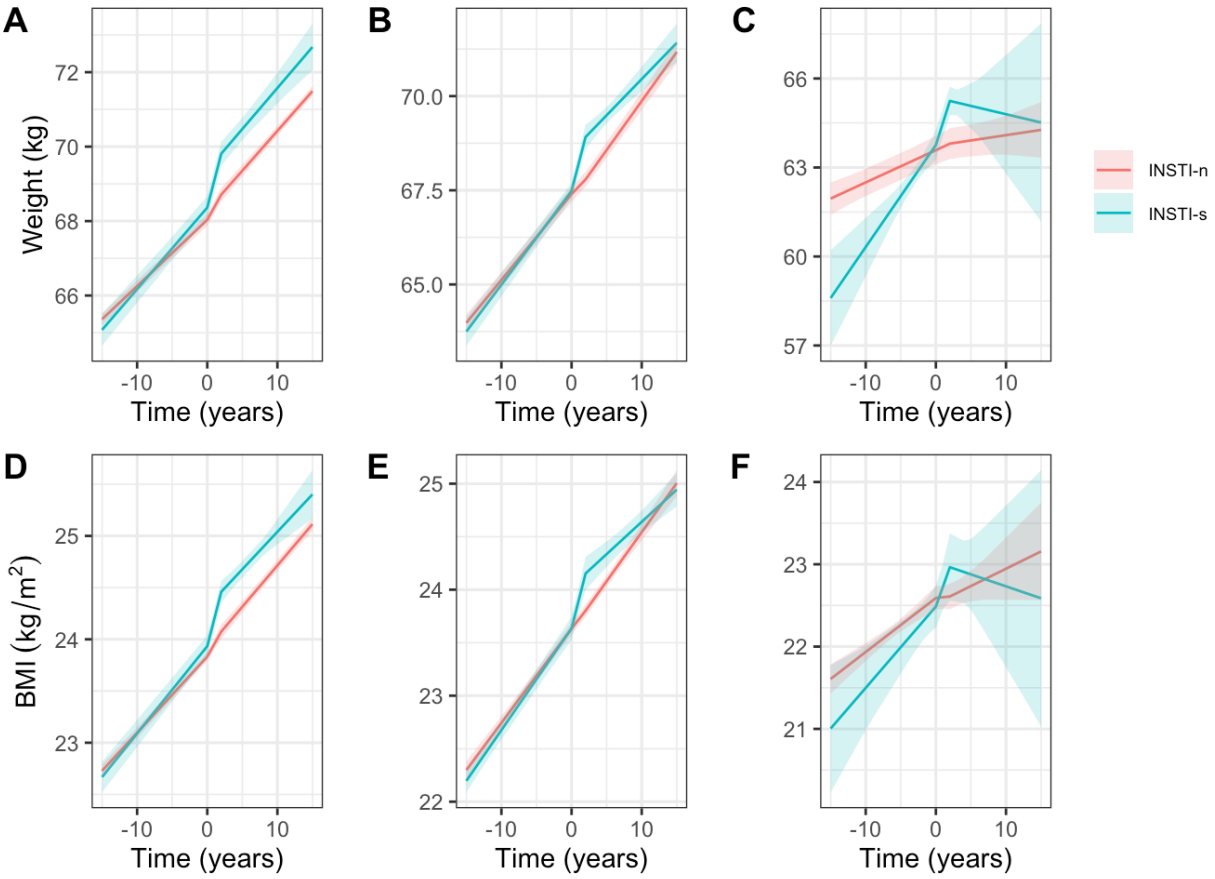
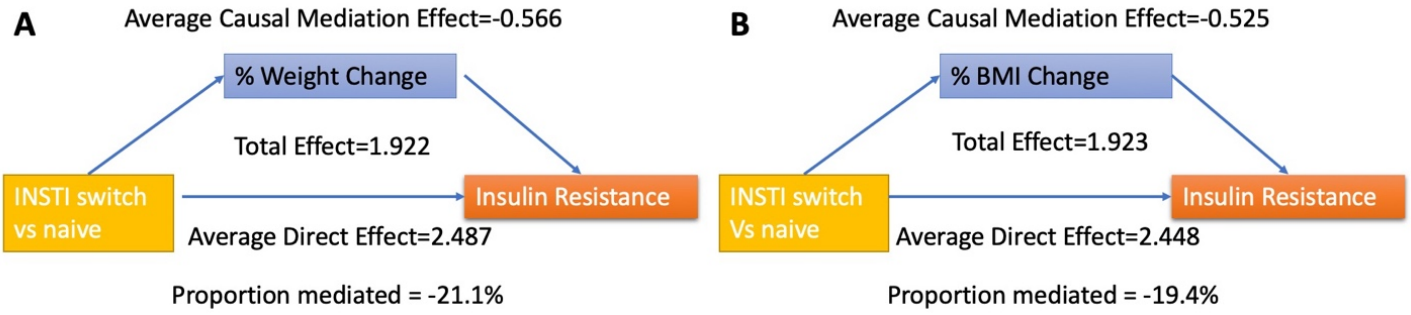
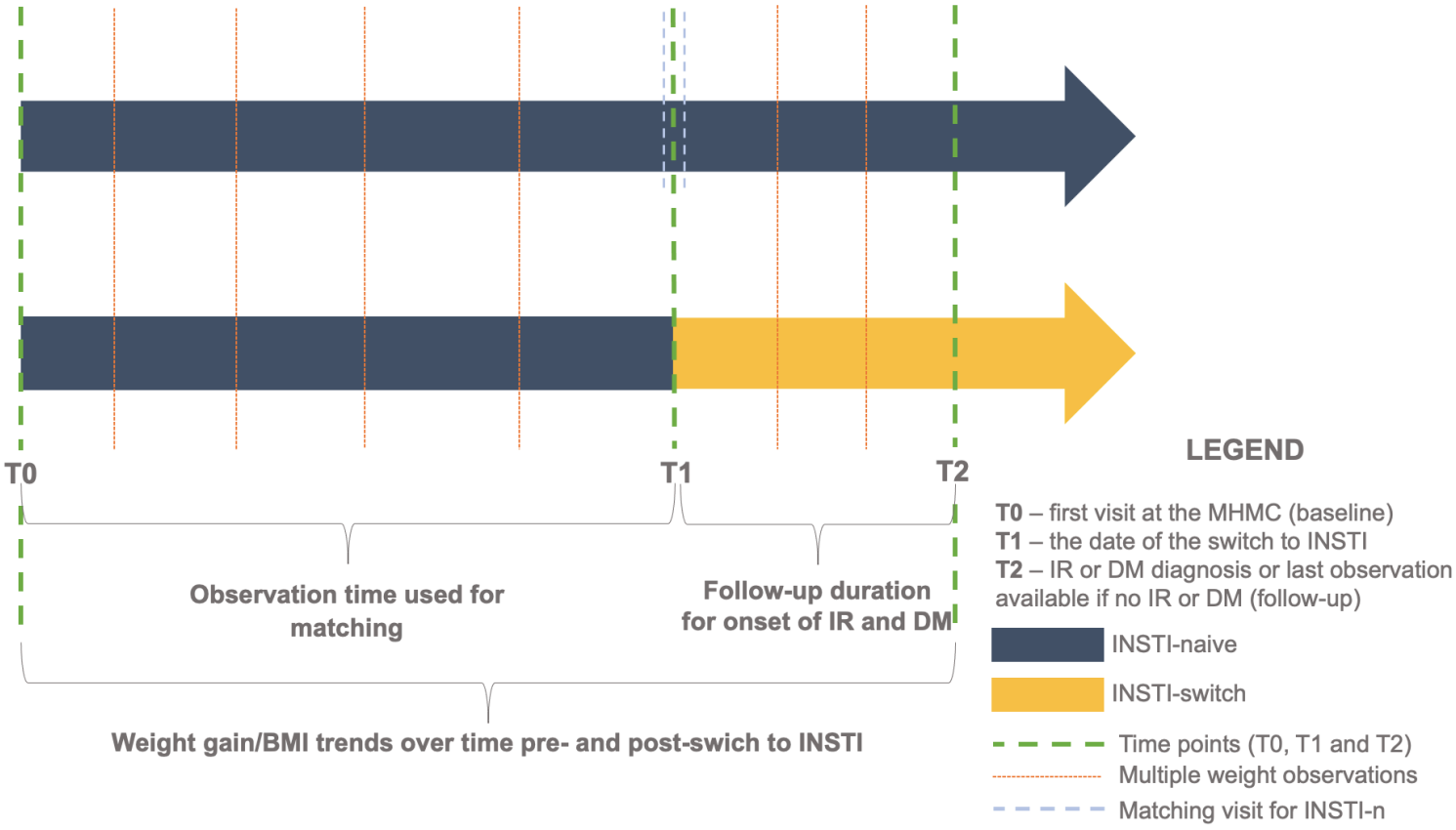


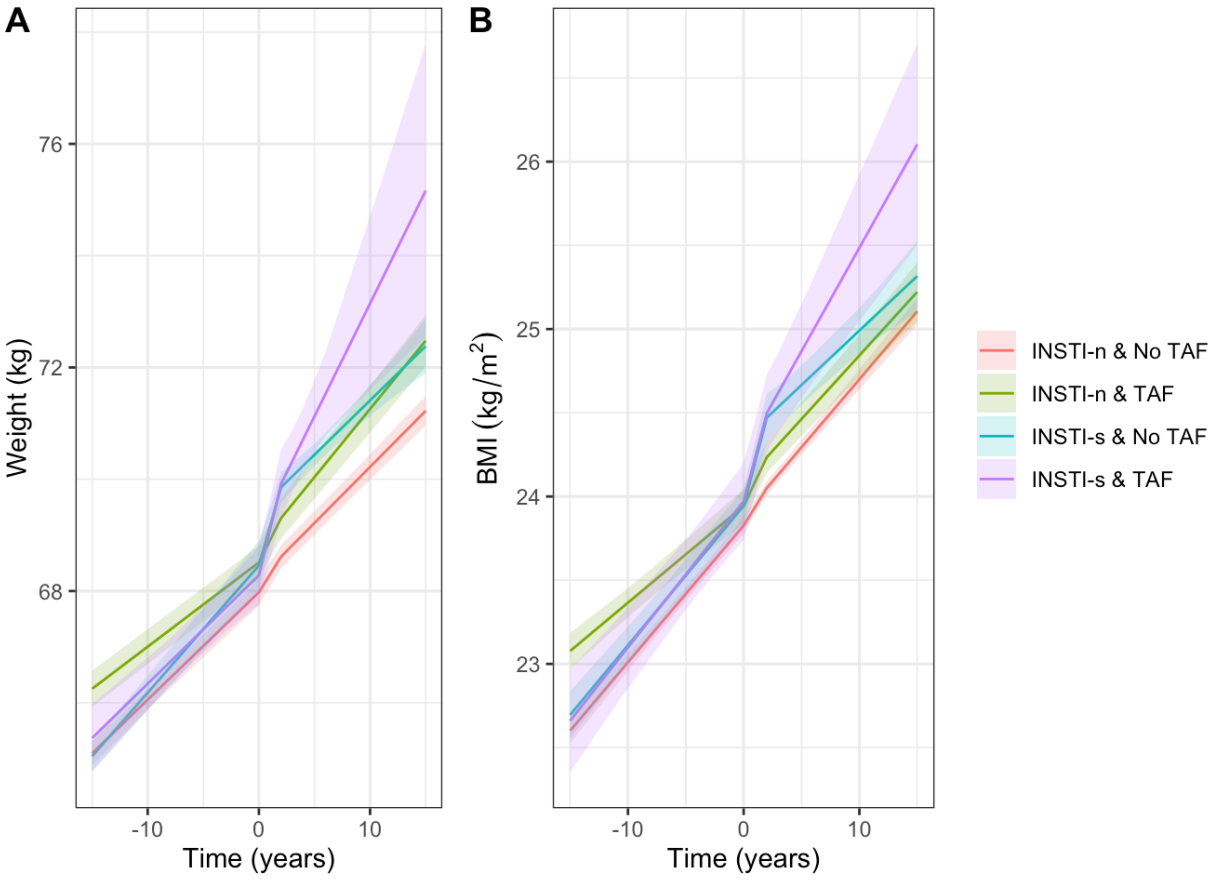
Figure 2. The mediation effect of weight change (A) and BMI (B) in the causal path between the switch to INSTI and the incidence of IR. Both models were adjusted for age and sex and HOMA at baseline.



Supplementary figure 1 depicts study design.



Supplementary figure 2. Weight (A) and BMI (B) trends of total population over time for INSTI-s and INSTI-n undergoing ART regimens with and without TAF.



Supplementary table 1. Estimations of weight and BMI trends over time for INSTI-s and INSTI-n with or without TAF in total population.

	Weight		**BMI**	
	Estimate ($CI_{95\%}$)	p	Estimate ($CI_{95\%}$)	p
Time post INSTI-s & TAF vs post INSTI-s & No TAF	0.209 (0.023, 0.396)	**0.028**	0.058 (-0.009, 0.126)	0.090
Time post INSTI-s & TAF vs INSTI-s & TAF	-0.414 (-0.945, 0.117)	0.156	-0.139 (-0.332, 0.053)	0.201
Time post INSTI-s & No TAF vs INSTI-s & No TAF	-0.506 (-0.746, -0.266)	**<0.001**	-0.198 (-0.285, -0.111)	**<0.001**

Supplementary table 2. Demographic, HIV and metabolic characteristics at baseline and follow-up in subset of PLWH without diabetes at T1 according to presence of diabetes at follow-up.

	No diabetes (N=1639)	**Diabetes (N=184)**	**Total (N=1823)**	**p**
Male sex, N (%)	1103 (67.3%)	139 (75.5%)	1242 (68.1%)	0.023
Age, years, median (Q1, Q3)	48.0 (43.0, 53.0)	50.0 (46.0, 54.2)	48.0 (43.0, 53.0)	<0.001
BMI, kg/m^2, median (Q1, Q3)	23.3 (21.1, 25.5)	24.1 (22.0, 26.7)	23.4 (21.2, 25.6)	0.002
Weight, kg, median (Q1, Q3)	67.1 (59.0, 75.0)	69.7 (61.6, 78.2)	67.5 (59.0, 75.5)	0.008
Waist circumference, cm, median (Q1, Q3) [Missing values]	86.0 (80.0, 92.0) [27]	88.0 (82.0, 95.0) [0]	86.0 (80.0, 93.0) [27]	<0.001
VAT, cm^2, median (Q1, Q3) [Missing values]	111.0 (70.5, 159.5) [836]	149.5 (91.2, 206.2) [64]	114.0 (74.0, 166.5) [900]	<0.001
HIV duration, years, median (Q1, Q3) [Missing values]	217.0 (137.0, 280.5) [24]	207.0 (148.0, 267.5) [1]	217.0 (140.0, 279.0) [25]	0.930
Nadir CD4, c/µL, median (Q1, Q3) [Missing values]	199.0 (83.5, 300.0) [36]	178.5 (84.0, 276.5) [2]	197.0 (83.0, 300.0) [38]	0.218
Current CD4, c/µL, median (Q1, Q3) [Missing values]	622.0 (456.0, 836.0) [68]	563.0 (427.0, 799.0) [11]	620.0 (450.0, 835.0) [79]	0.110
HOMA, median (Q1, Q3)	1.9 (1.1, 2.9)	3.6 (1.9, 5.8)	1.9 (1.2, 3.2)	<0.001
Current exposure to PI, N (%)	753 (45.9%)	95 (51.6%)	848 (46.5%)	0.142
Current exposure to NRTI, N (%)	1351 (82.4%)	145 (78.8%)	1496 (82.1%)	0.224
Current exposure to NNRTI, N (%)	598 (36.5%)	71 (38.6%)	669 (36.7%)	0.575
Follow-up duration, years, median (Q1, Q3)	2.8 (1.4, 4.8)	2.3 (1.1, 4.2)	2.7 (1.3, 4.6)	0.041
ART at follow-up				
Exposure to TDF, N (%)	471 (28.7%)	67 (36.4%)	538 (29.5%)	0.030
Exposure to TAF, N (%)	241 (14.7%)	8 (4.3%)	249 (13.7%)	<0.001
Exposure to RAL, N (%)	292 (17.8%)	41 (22.3%)	333 (18.3%)	0.137
Exposure to DTG, N (%)	279 (17.0%)	22 (12.0%)	301 (16.5%)	0.079

Exposure to EVG, N (%)	46 (2.8%)	3 (1.6%)	49 (2.7%)	0.350
2 DR therapy, N (%)	277 (17.3%)	35 (19.3%)	312 (17.5%)	0.499
3 DR therapy, N (%)	1013 (63.4%)	95 (52.5%)	1108 (62.2%)	0.004

Abbreviations: BMI – body mass index; DTG – dolutegravir; EVG – elvitegravir; HOMA - Homeostatic Model Assessment for insulin resistance; INSTI – integrase strand transfer inhibitors; Q1, Q3 – lower and upper quartile; NNRTI – non-nucleoside reverse transcriptase inhibitors; NRTI – nucleoside reverse transcriptase inhibitors; RAL – raltegravir; PI – protease inhibitors; VAT – visceral adipose tissue

Supplementary table 3. Demographic, HIV and metabolic characteristics in INSTI-s and INSTI-n at baseline and follow-up in subset of PLWH without diabetes at T1.

	INSTI-n (N=1140)	**INSTI-s (N=683)**	**Total (N=1823)**	**p**
Male sex, N (%)	770 (67.5%)	472 (69.1%)	1242 (68.1%)	0.488
Age, years, median (Q1, Q3)	47.0 (42.0, 52.0)	50.0 (46.0, 55.0)	48.0 (43.0, 53.0)	<0.001
Waist circumference, cm, median (Q1, Q3) [Missing values]	86.0 (80.0, 92.0) [11]	87.0 (81.0, 93.5) [16]	86.0 (80.0, 93.0) [27]	0.004
VAT, cm^2, median (Q1, Q3) [Missing values]	109.0 (69.0, 158.5) [529]	124.0 (82.0, 179.2) [371]	114.0 (74.0, 166.5) [900]	0.001
HIV duration, years, median (Q1, Q3) [Missing values]	201.0 (129.2, 261.8) [14]	244.5 (165.8, 305.0) [11]	217.0 (140.0, 279.0) [25]	<0.001
Nadir CD4, c/μL, median (Q1, Q3) [Missing values]	200.0 (92.8, 300.0) [20]	170.0 (70.0, 291.0) [18]	197.0 (83.0, 300.0) [38]	0.002
Current CD4, c/μL, median (Q1, Q3) [Missing values]	621.0 (464.0, 835.0) [51]	617.0 (431.5, 832.0) [28]	620.0 (450.0, 835.0) [79]	0.393
CD4/CD8 ratio, median (Q1, Q3) [Missing values]	0.8 (0.6, 1.1) [424]	0.8 (0.5, 1.1) [115]	0.8 (0.6, 1.1) [539]	<0.001
HIV RNA viral load, median (Q1, Q3) [Missing values]	777 (93.5%) [309]	374 (98.7%) [304]	1151 (95.1%) [613]	<0.001
HOMA, median (Q1, Q3)	1.9 (1.2, 3.3)	2.0 (1.2, 3.2)	1.9 (1.2, 3.2)	0.498
Current exposure to PI, N (%)	585 (51.3%)	263 (38.5%)	848 (46.5%)	<0.001
Current exposure to NRTI, N (%)	1058 (92.8%)	438 (64.1%)	1496 (82.1%)	<0.001
Current exposure to NNRTI, N (%)	557 (48.9%)	112 (16.4%)	669 (36.7%)	<0.001
Follow-up duration, years, median (Q1, Q3)	2.3 (1.2, 4.2)	3.1 (1.9, 5.5)	2.7 (1.3, 4.6)	<0.001
ART at follow-up				
Exposure to TDF, N (%)	470 (41.2%)	68 (10.0%)	538 (29.5%)	<0.001
Exposure to TAF, N (%)	158 (13.9%)	91 (13.3%)	249 (13.7%)	0.747
2 DR therapy, N (%)	85 (7.7%)	227 (33.2%)	312 (17.5%)	<0.001
3 DR therapy, N (%)	743 (67.7%)	365 (53.4%)	1108 (62.2%)	<0.001

Abbreviations: 2 DR – two-drug regimen; 3 DR – three drug regimen; ART – antiretroviral therapy; HIV – human immunodeficiency virus; HOMA - Homeostatic Model Assessment for insulin resistance; INSTI – integrase strand transfer inhibitors; Q1, Q3 – lower and upper quartile; NNRTI – non-nucleoside reverse transcriptase inhibitors; NRTI – nucleoside reverse transcriptase inhibitors; PI – protease inhibitors; TAF – tenofovir alafenamide; TDF - tenofovir disoproxil fumarate; VAT – visceral adipose tissue

Supplementary table 4. Demographic, HIV and metabolic characteristics at baseline and follow-up in subset of PLWH without IR at T1 according to presence of IR at follow-up.

	No IR (N=394)	IR (N=240)	Total (N=634)	p
Male sex, N (%)	263 (66.8%)	170 (70.8%)	433 (68.3%)	0.284
Age, years, median (Q1, Q3)	45.0 (40.0, 50.0)	46.0 (41.0, 51.0)	45.0 (41.0, 50.0)	0.126
BMI, kg/m^2, median (Q1, Q3)	22.4 (20.5, 24.1)	22.4 (20.4, 24.6)	22.4 (20.4, 24.3)	0.621
Weight, kg, median (Q1, Q3)	65.0 (57.1, 71.7)	64.5 (56.9, 74.0)	64.9 (57.0, 72.3)	0.721
Waist circumference, cm, median (Q1, Q3) [Missing values]	82.0 (77.0, 88.0) [4]	83.0 (78.0, 89.0) [1]	82.0 (77.0, 88.0) [5]	0.084
VAT, cm^2, median (Q1, Q3) [Missing values]	84.0 (54.5, 113.5) [207]	96.5 (60.5, 131.5) [90]	88.0 (58.0, 123.0) [297]	0.014
Physical activity, N (%) No Mild Intense	156 (39.7%) 175 (44.5%) 62 (15.8%)	133 (55.6%) 82 (34.3%) 24 (10.0%)	289 (45.7%) 257 (40.7%) 86 (13.6%)	<0.001
HIV duration, years, median (Q1, Q3) [Missing values]	158.5 (79.0, 235.5) [4]	167.0 (89.0, 236.0) [3]	160.0 (80.5, 236.0) [7]	0.548
Nadir CD4, c/µL, median (Q1, Q3) [Missing values]	242.0 (136.0, 343.0) [13]	201.0 (80.0, 300.0) [2]	221.0 (109.5, 320.0) [15]	0.005
Current CD4, c/µL, median (Q1, Q3) [Missing values]	641.0 (480.0, 821.0) [17]	536.0 (392.0, 716.8) [14]	600.0 (431.0, 786.5) [31]	<0.001
HOMA, median (Q1, Q3)	1.1 (0.8, 1.3)	1.4 (1.0, 1.7)	1.1 (0.8, 1.5)	<0.001
Total cholesterol, mg/dl, median (Q1, Q3)	190.5 (167.2, 218.0)	196.0 (162.0, 222.5)	193.0 (166.0, 219.0)	0.809
LDL cholesterol, mg/dl, median (Q1, Q3)	116.0 (95.0, 139.0)	120.0 (93.0, 142.0)	117.0 (94.5, 141.0)	0.416
HDL cholesterol, mg/dl, median (Q1, Q3)	50.0 (43.0, 62.0)	47.5 (39.0, 57.0)	49.0 (41.0, 61.0)	<0.001
Triglycerides, mg/dl, median (Q1, Q3)	105.0 (76.0, 150.0)	121.0 (85.5, 173.2)	110.0 (79.8, 159.0)	0.002
Current exposure to PI, N (%)	170 (43.1%)	117 (48.8%)	287 (45.3%)	0.169
Current exposure to NRTI, N (%)	328 (83.2%)	215 (89.6%)	543 (85.6%)	0.027
Current exposure to NNRTI, N (%)	173 (43.9%)	99 (41.2%)	272 (42.9%)	0.512
Follow-up duration, years, median (Q1, Q3)	2.2 (1.1, 4.1)	1.5 (1.0, 2.5)	2.0 (1.1, 3.4)	<0.001

HOMA at follow-up, median (Q1, Q3)	1.0 (0.8, 1.4)	2.5 (2.2, 3.1)	1.5 (0.9, 2.3)	<0.001
Weight change, %, median (Q1, Q3)	0.6 (-2.2, 4.2)	2.1 (-0.4, 5.7)	1.4 (-1.8, 4.5)	<0.001
BMI change, %, median (Q1, Q3)	0.6 (-2.1, 4.5)	2.4 (-0.6, 5.7)	1.2 (-1.6, 4.8)	<0.001
ART at follow-up				
Exposure to TDF, N (%)	142 (36.0%)	117 (48.8%)	259 (40.9%)	0.002
Exposure to TAF, N (%)	32 (8.1%)	7 (2.9%)	39 (6.2%)	0.008
Exposure to RAL, N (%)	79 (20.1%)	28 (11.7%)	107 (16.9%)	0.006
Exposure to DTG, N (%)	33 (8.4%)	13 (5.4%)	46 (7.3%)	0.164
Exposure to EVG, N (%)	14 (3.6%)	2 (0.8%)	16 (2.5%)	0.034
2 DR therapy, N (%)	58 (15.3%)	17 (7.4%)	75 (12.3%)	0.004
3 DR therapy, N (%)	251 (66.1%)	151 (65.7%)	402 (65.9%)	0.919

Abbreviations: 2 DR – two-drug regimen; 3 DR – three drug regimen; ART – antiretroviral therapy; DTG – dolutegravir; EVG – elvitegravir; HIV – human immunodeficiency virus; HOMA - Homeostatic Model Assessment for insulin resistance; INSTI – integrase strand transfer inhibitors; Q1, Q3 – lower and upper quartile; LDL – low density lipoprotein; [N°] – number of people in which the given variable was available; NNRTI – non-nucleoside reverse transcriptase inhibitors; NRTI – nucleoside reverse transcriptase inhibitors; PI – protease inhibitors; RAL – raltegravir; TAF – tenofovir alafenamide; TDF - tenofovir disoproxil fumarate; VAT – visceral adipose tissue

Supplementary table 5. Demographic, HIV and metabolic characteristics in INSTI-s and INSTI-n at baseline and follow-up in subset of PLWH without IR at T1.

	INSTI-n (N=465)	**INSTI-s (N=169)**	**Total (N=634)**	**p**
Male sex, N (%)	322 (69.2%)	111 (65.7%)	433 (68.3%)	0.393
Age, years, median (Q1, Q3)	45.0 (39.0, 50.0)	47.0 (43.0, 52.0)	45.0 (41.0, 50.0)	<0.001
Waist circumference, cm, median (Q1, Q3) [Missing values]	82.0 (77.0, 88.0) [2]	84.0 (78.2, 90.0) [3]	82.0 (77.0, 88.0) [5]	0.011
VAT, cm^2, median (Q1, Q3) [Missing values]	89.0 (58.0, 125.5) [210]	86.5 (58.5, 116.5) [87]	88.0 (58.0, 123.0) [297]	0.541
HIV duration, years, median (Q1, Q3) [Missing values]	154.0 (76.8, 228.0) [5]	189.0 (95.5, 268.5) [2]	160.0 (80.5, 236.0) [7]	0.002
Nadir CD4, c/μL, median (Q1, Q3) [Missing values]	224.0 (122.5, 320.0) [10]	219.0 (91.5, 325.5) [5]	221.0 (109.5, 320.0) [15]	0.667
Current CD4, c/μL, median (Q1, Q3) [Missing values]	600.0 (434.0, 785.0) [24]	608.5 (423.8, 803.2) [7]	600.0 (431.0, 786.5) [31]	0.891
HOMA, median (Q1, Q3)	1.1 (0.9, 1.5)	1.1 (0.8, 1.5)	1.1 (0.8, 1.5)	0.759
Current exposure to PI, N (%)	222 (47.7%)	65 (38.5%)	287 (45.3%)	0.038
Current exposure to NRTI, N (%)	437 (94.0%)	106 (62.7%)	543 (85.6%)	<0.001
Current exposure to NNRTI, N (%)	244 (52.5%)	28 (16.6%)	272 (42.9%)	<0.001
Follow-up duration, years, median (Q1, Q3)	2.0 (1.1, 3.3)	2.1 (1.1, 3.5)	2.0 (1.1, 3.4)	0.141
HOMA at follow-up, median (Q1, Q3)	1.6 (0.9, 2.4)	1.2 (0.9, 2.0)	1.5 (0.9, 2.3)	0.007
ART at follow-up				
Exposure to TDF, N (%)	235 (50.5%)	24 (14.2%)	259 (40.9%)	<0.001
Exposure to TAF, N (%)	25 (5.4%)	14 (8.3%)	39 (6.2%)	0.178
2 DR therapy, N (%)	28 (6.3%)	47 (27.8%)	75 (12.3%)	<0.001
3 DR therapy, N (%)	309 (70.1%)	93 (55.0%)	402 (65.9%)	<0.001

Abbreviations: 2 DR – two-drug regimen; 3 DR – three drug regimen; ART – antiretroviral therapy; HIV – human immunodeficiency virus; HOMA - Homeostatic Model Assessment for insulin resistance; INSTI – integrase strand transfer inhibitors; Q1, Q3 – lower and upper quartile; NNRTI – non-nucleoside reverse transcriptase inhibitors; NRTI – nucleoside reverse transcriptase inhibitors; PI – protease inhibitors; TAF – tenofovir alafenamide; TDF - tenofovir disoproxil fumarate; VAT – visceral adipose tissue

Supplementary figure 3. The probability of IR in INSTI-s after one year of follow-up at varying percentage of weight (panel A) or BMI (panel B) change compared to INSTI-n, assuming no variation of weight or BMI in the latter group.