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CRISTIANE FONSECA DE ALMEIDA

LIPÍDEOS DIETÉTICOS, ÁCIDOS GRAXOS PLASMÁTICOS E SUA ASSOCIAÇÃO COM DOENÇA HEPÁTICA GORDUROSA NÃO ALCOÓLICA EM PESSOAS VIVENDO COM HIV/AIDS

Rio de Janeiro
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Tese apresentada ao Programa de Pós-Graduação *Stricto sensu* em Pesquisa Clínica em Doenças Infecciosas do Instituto Nacional de Infectologia Evandro Chagas/Fiocruz, como requisito ao título de Doutora em Ciências, sob a orientação do Dr.Hugo Perazzo Pedroso Barbosa e da Dr.ª Patrícia Dias de Brito

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Orientação: Dr.Hugo Perazzo Pedroso Barbosa

Dr.ª Patrícia Dias de Brito

Aprovado em:

BANCA EXAMINADORA

_____

Dr.ª Beatriz Gilda Jegerhorn Grinsztejn (Presidente)
INI/Fiocruz

_____

Dr.ª Cláudia Pinto Marques de Oliveira
USP

_____

Dr.ª Wilza Arantes Ferreira Peres
UFRJ

_____

Dr.ª Juliana Lauar Gonçalves
Centro Universitário UNA

_____

Dr.ª Paula Simplícia da Silva
INI/Fiocruz

_____

Dr.ª Célia Lopes da Costa (Revisor)
UERJ

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“Tudo vale a pena quando a alma não é pequena ...”.

Fernando Pessoa

De Almeida, CF. **Lipídeos dietéticos, ácidos graxos plasmáticos e sua associação com doença hepática gordurosa não alcoólica em pessoas vivendo com HIV/AIDS.** Rio de Janeiro, 2022. 83f. Tese [Doutorado em Pesquisa Clínica em Doenças Infecciosas] – Instituto nacional de Infectologia Evandro Chagas (INI), Fundação Oswaldo Cruz.

RESUMO

Introdução: A doença hepática gordurosa não-alcóolica (DHGNA) pode acometer até 40% das pessoas vivendo com HIV/AIDS (PVHA). Os lipídeos são peças chaves na patogênese e evolução desta doença. Porém, poucos estudos descreveram a relação dos lipídeos com DHGNA em PVHA. **Objetivos**: Avaliar ingestão de lipídeos e concentração plasmática de ácidos graxos (AG) e sua relação com a DHGNA e fibrose hepática. **Métodos**: Esta tese é composta por estudo transversal e estudo caso-controle utilizando dados da visita 1 (baseline) de indivíduos com mono-infecção pelo HIV incluídos no estudo de coorte PROSPEC-HIV (NCT02542020). Total de 721 participantes foram submetidos aos seguintes procedimentos: aferição de medidas antropométricas e avaliação de percentual de gordura corporal por bioimpedância elétrica; coleta de sangue para exames laboratoriais; avaliação clínica, avaliação nutricional, elastografia hepática transitória e avaliação do consumo alimentar por recordatório alimentar de 24h. Investigação sobre fibrose hepática e concentração plasmática de AG (por cromatografia a gás) foi realizada com sub-amostra de 142 participantes [71 indivíduos com fibrose hepática (caso) e 71 controles (sem fibrose)]. Modelos de regressão logística multivariada ajustados por fatores de confundimento foram realizadas nas diferentes análises. **Resultados**: Participantes com maior ingestão usual de gordura total apresentaram maior probabilidade de apresentar DHGNA em comparação aos de menor consumo [odds ratio ajustado (ORa): 1.91 (intervalo de confiança de 95% (IC95%) 1,06 -3,44)]. Participantes com moderada ingestão AG láurico [0,42 (IC95% 0,22-0,78)] tiveram menor probabilidade de apresentar DHGNA comparados com indivíduos com menor ingestão deste AG. Além disso, uma maior ingestão usual de AG miristoléico foi associada a menor probabilidade de apresentar DHGNA [0,56 (IC95% 0,32-0,99)]. Já os participantes com maior ingestão [ORa (IC95%)] de AG láurico [0,38 (0,18-0,80)], AG mirístico [0,38 (0,17-0,89)], AG palmitoléico [0,40 (0,19-0,82)] e AG oleico [0,35 (0,16-0,79)] demonstraram menor probabilidade de apresentar fibrose hepática quando comparados aos participantes com baixa ingestão desses FA. Por outro lado, o maior consumo de PUFA n-6 foi associado à presença de fibrose em comparação com os de menor ingestão [ORa=2,45 (IC95% 1,12-5,32)]. Em relação a concentração plasmática de AGs demonstramos que a maior concentração do AG palmítico está associada de forma independente com presença de fibrose hepática [ORa= 1,23 (IC95% 1,04-1,46); p=0.02]. **Conclusão**: Esses resultados enfatizam o importante papel dos lipídeos na patogênese de DHGNA e/ou fibrose hepática em PVHA. Reforçando assim a importância da avaliação e intervenção nutricional no cuidado a esta população.

Palavras chaves: 1. Consumo alimentar; 2. Lipídeos; 3. Perfil plasmático de lipídeos; 4. DHGNA; 5. Fibrose hepática; 6. HIV.

De Almeida, CF. **Relationship between dietary lipids and plasmatic fatty acids with nonalcoholic fatty liver disease in people with HIV/AIDS.** Rio de Janeiro, 2022. 83f. Tese [Doutorado em Pesquisa Clínica em Doenças Infecciosas] – Instituto nacional de Infectologia Evandro Chagas (INI), Fundação Oswaldo Cruz.

ABSTRACT

Background: Up to 40% of people living with HIV/AIDS (PLWHA) might have non-alcoholic fatty liver disease (NAFLD). Higher ingestion and plasmatic levels of lipids are cornerstones in the pathogenesis of this liver disease. However, data on the relationship of lipids and NAFLD remain scarce in PLWHA. **Objectives**: To evaluate the relationship of lipid intake and plasmatic concentration of fatty acids (FA) with NAFLD and liver fibrosis. **Methods**: This thesis is composed by a cross-sectional study and a case-control study using data from baseline visit of participants with HIV monoinfection included in the PROSPEC-HIV cohort study (NCT02542020). A total of 721 participants were submitted to the following procedures: measurement of anthropometric measurements and evaluation of body fat percentage by electrical bioimpedance; blood collection for laboratory tests; clinical evaluation, nutritional assessment, transient hepatic elastography and evaluation of food intake by 24-hour dietary recall. Plasmatic FA concentration (by gas chromatography) was conducted with a sub-sample of 142 participants [71 individuals with liver fibrosis (case) and 71 controls (without fibrosis)]. Multivariate logistic regression models adjusted for confounding factors were performed in the different analyses. **Results:** Participants with higher usual total fat intake had a higher odds for NAFLD compared to those with lower consumption [adjusted Odds ratio (aOR): 1.91 with a 95% confidence interval (95%CI) 1.06 - 3.44]. Participants with moderate intake of lauric FA [0.42 (0.22-0.78)] were less likely to have NAFLD compared to those with lower intake of this nutrient. Additionally, a higher usual intake of myistoleic FA was a significant protective factor for NAFLD [0.56 (0.32-0.99)]. On the other hand, participants with higher intake [aOR (95%CI)] of lauric FA [0.38 (95%CI 0.18-0.80)], Myristic FA [0.38 (0.17-0.89)], palmitoleic FA [0.40 (0.19-0.82)] and oleic FA [0.35 (0.16-0.79)] showed lower probability of presenting liver fibrosis when compared to participants with lower intake. Besides, the higher consumption of PUFA n-6 was associated with the presence of fibrosis compared to those with lower intake [ORa=2.45 (95%CI 1.12-5.32)]**.** Regarding plasma concentration of FA, we demonstrated that the higher concentration of palmitic FA is independently associated with liver fibrosis [ORa= 1.23 (95%CI 1.04-1.46); p=0.02]. **Conclusion**: These results emphasize the important role of lipids in the pathogenesis of NAFLD and/or liver fibrosis in PLWHA. Thus reinforcing the role of nutritional assessment and intervention in the health care of this population.

Key words: 1. Food intake; 2. Lipids; 3. Plasma lipid profile; 4. NAFLD; 5. Liver fibrosis; 6. HIV.

LISTA DE ABREVIATURAS E SIGLAS

AG	Ácidos graxos
ATP	Trifosfato de adenosina
AUDIT	*Alcohol Use Disorders Identification Test*
CAP	*Controlled Attenuation Parameter*
DMT	Dieta mediterrânea
DHGNA	Doença Hepática gordurosa não alcoolica
EHNA	Esteatohepatite não alcoólica
EHT	Elastografia hepática transitória
ERO	Espécie reativa de oxigênio
HAART	*Highly Active Antirretroviral Therapy*
HAS	Hipertensão arterial sistêmica
HIV	Vírus da imunodeficiência humana
IL-6	Interleucina 6
LSM	*Liver stiffness measurement*
MEC	Matriz extra celula
MUFA	*Mono-unsaturated fatty acid*
PCR	Proteina C reativa
PUFA	Ácidos graxos poliinsaturados
PUFA n-3	Ácidos graxos poliinsaturados do tipo n-3
PVHA	Pessoas vivendo com HIV/AIDS
R24H	Recordatório alimentar de 24h
RBP	Proteína ligado de retinol
SINAN	Sistema de notificação de agravos
SD1	Enzima desaturase-1
TARV	Terapia antiretroviral
TNF-α	Fator de necrose tumoral α
UNAIDS	Joint United Nations Programme on HIV/Aids

SUMÁRIO

1. INTRODUÇÃO

A infecção pelo vírus da imunodeficiência humana (HIV) é um problema de saúde pública que acomete milhões de pessoas mundialmente (UNAIDS, 2021). No Brasil, de 2007 até junho de 2021 foram notificados 381.793 casos de HIV, e em 2021 foram diagnosticados 32.701 novos casos de infecção pelo HIV (MINISTÉRIO DA SAÚDE, 2021). O uso da terapia antioretroviral (TARV) precoce reduziu a incidência de infecções oportunistas em pessoas vivendo com HIV/AIDS (PVHA), aumentou expectativa de vida e consequentemente a incidência de doenças não comunicáveis (CASTILHO et al., 2019).

A doença hepática gordurosa não-alcóolica (DHGNA) caracteriza-se pelo acúmulo anormal de gordura no parênquima hepático na ausência da ingestão abusiva de álcool. Sua apresentação clínica pode variar desde a esteatose hepática simples até esteatohepatite não alcóolica (EHNA) que pode progredir para fibrose avançada (cirrose) e suas complicações, como o carcinoma hepatocelular (RINELLA, 2015). A presença da DHGNA em PVHA vem sendo associada com a obesidade e o ganho de peso. Este fato reforça a necessidade de medidas de prevenção e tratamento destas morbidades, dentre as quais destacamos o cuidado nutricional (KOETHE et al., 2016). Vários estudos têm reportado uma alta prevalência de DHGNA e/ ou fibrose hepática em PVHA (AEPFELBACHER et al., 2019; PEMBROKE et al., 2017a; VUILLE-LESSARD et al., 2016). A patogênese da DHGNA está fortemente associada com disfunção metabólica e sua progressão para formas graves baseia-se na presença de resistência insulínica, estresse oxidativo e ativação de cascata inflamatória (SHARP; SCHULTZ; COPPELL, 2018).

A ingestão energética excessiva com consequente acúmulo de lipídeo nos hepatócitos corrobora para o estresse oxidativo, injúria no retículo endoplasmático e apoptose celular resultando em lipotoxicidade que induz a resposta inflamatória e a fibrinogênese (MACHADO; CORTEZ-PINTO, 2014). Estudos confirmaram o efeito dos hábitos alimentares no desenvolvimento de DHGNA. Indivíduos com DHGNA apresentaram consumo excessivo de carboidratos simples, gordura saturada e baixa ingestão de gordura monoinsaturada e fibras (DE CASTRO; CALDER, 2017; FEROLLA et al., 2013).

A alimentação tem um papel central na regulação da inflamação crônica (CAVICCHIA et al., 2009). A dieta do tipo ocidental tem sido associada com altos níveis de citocinas inflamatórias (PHILLIPS et al., 2018) e, consequentemente, com o desenvolvimento da DHGNA (CANTERO et al., 2018). Por outro lado, a dieta tipo mediterrânea (DMT)

associa-se com baixas concentrações plasmáticas de citocinas inflamatórias (CAVICCHIA et al., 2009), além de contribuir para a redução da esteatose hepática e melhorar a resistência insulínica (RYAN et al., 2013).

Dentre os fatores dietéticos, a gordura tem sido estudada como um dos principais nutrientes envolvidos no desenvolvimento e progressão da DHGNA. Pacientes diagnosticados com DHGNA frequentemente apresentam concentração plasmática aumentada de ácidos graxos (AG) (MACIEJEWSKA et al., 2018)**.** Os AG podem ser obtidos na dieta ou produzidos pelo próprio organismo, em um processo chamado lipogênese que é regulado por fatores nutricionais (dieta), hormonais, enzimáticos e genéticos (POLACOW; JUNIOR; H, 2007)**.** Além disso, a avaliação de subtipos de AG dietéticos pode ser relevante devido as suas funções e efeitos metabólicos distintos (VERGANI, 2019).

Portanto, mais relevante do que a quantidade de gordura total, parece ser o tipo de gordura ingerida na dieta. A composição dos AG dietéticos influencia o metabolismo hepático, e está diretamente ligada à composição lipídica do fígado (JURADO-RUIZ et al., 2017). Por este motivo, a composição de AG plasmáticos (GAMBINO et al., 2016), nos eritrócitos e no tecido hepático têm sido utilizadas como biomarcadores de ingestão de gorduras (ANDERSEN; SOLVOLL; DREVON, 1996)**,** e também refletem o metabolismo e a produção endógena de AG.

1.1. REVISAO DE LITERATURA

1.1.1. Epidemiologia e morbidades em PVHA

A infecção pelo HIV ainda é considerada um problema de saúde pública acometendo 37,7 milhões [30,2 milhões - 45,1 milhões] de pessoas mundialmente e 1,5 milhões [1 milhões-2 milhões] de pessoas foram recentemente infectadas por HIV em 2021 (UNAIDS, 2021). No Brasil, em 2020 foram diagnosticados 32.701 novos casos de infecção pelo HIV, com uma taxa de detecção de 14,1/100.000 habitantes. De 2007 até junho de 2021, foram notificados no Sinan (Sistema de informações de agravos de notificação) 381.793 casos de infecção pelo HIV no Brasil, sendo 165.247 (43,3%) na região Sudeste, 75.618 (19,8%) na região Nordeste, 75.165 (19,7%) na região Sul, 36.218 (9,5%) na região Norte e 29.545 (7,7%) na região Centro Oeste. Desde o ano de 2012 os dados demonstram que houve um

decréscimo na taxa de detecção da AIDS no Brasil. Essa redução tem sido mais significativa desde a recomendação de "tratamento para todos", implementado em 2013. Cabe ressaltar que parte da redução recente desta taxa pode estar relacionada à subnotificação de casos, em virtude da pandemia de covid-19 (MINISTÉRIO DA SAÚDE, 2021).

Desde o início da TARV a taxa de mortalidade global em PVHA vem sendo reduzida, especialmente guiada pela acentuada redução da mortalidade associada à síndrome da imunodeficiência adquirida (SIDA) (GRINSZTEJN et al., 2013). De 2014 para 2018 houve uma redução de 22,8% na taxa de mortalidade – possivelmente, em consequência da recomendação do "tratamento universal" e da ampliação do diagnóstico precoce da infecção pelo HIV (MINISTÉRIO DA SAÚDE, 2021). Desta forma, com o melhor controle da infecção pelo HIV, também observa-se uma redução na ocorrência de infecções oportunistas (BRASIL, 2017), assim como, uma mudança no perfil da doença, com maior incidência de doenças crônicas e conseguente aumento da expectativa de vida das PVHA (GUIMARÃES et al., 2017).

Estas doenças crônicas podem ser o resultado da combinação de diversos fatores como: infecção pelo HIV, presença prévia de morbidades, toxicidade relacionada à TARV e ativação inflamatória persistente. O estado inflamatório crônico predispõe a um perfil aterogênico e parece estar relacionado ao aparecimento de alterações metabólicas como dislipidemias, resistência insulínica, síndrome metabólica e esteatose hepática (MIRANI et al., 2015).

1.1.2 Doença hepática gordurosa não-alcóolica (DHGNA)

As doenças hepáticas ainda representam causas importantes de morbi-mortalidade em PVHA, especialmente associadas com coinfecções por hepatites virais. Porém, novas drogas dispensadas pelo Sistema Único de Saúde apresentam alta eficácia no controle da replicação do vírus da hepatite B (BOYD et al., 2017) e na erradicação do vírus da hepatite C (PERAZZO et al., 2017). Em contrapartida, estudos sugerem aumento da prevalência de doenças hepáticas não-comunicáveis (CASTILHO et al., 2019).

A DHGNA caracteriza-se pelo acúmulo de lipídeo no citoplasma dos hepatócitos em pessoas sem consumo abusivo de bebidas alcóolicas ou outras doenças hepáticas crônicas, sendo referida como manifestação hepática da síndrome metabólica (YOUNOSSI et al., 2016). A apresentação clínica da DHGNA pode variar desde a esteatose hepática simples até a

sua forma grave conhecida como esteatohepatite não alcóolica (EHNA) que pode evoluir para fibrose hepática e progredir para cirrose e carcinoma hepatocelular. Estudos vêm descrevendo associação da DHGNA com aumento da mortalidade relacionada a doença cardiovascular (YOUNOSSI et al., 2016). Esteatose hepática caracteriza-se pelo acúmulo de gordura em mais de 5% dos hepatócitos, enquanto na EHNA há presença de injúria inflamatória e degenerativa nos hepatócitos.

A presença de progressão da fibrose é a principal causa de doença hepática descompensada (ACHARYA et al., 2021) e está associada com aumento da mortalidade. Esta caracteriza-se pela deposição excessiva de proteínas da matriz extracelular (MEC), especialmente alfa colágeno tipo 1 e está associada a grandes alterações na quantidade e composição da MEC. A fibrose hepática é um processo dinâmico; geralmente é secundário a lesões hepáticas e inflamação, progride em diferentes taxas e também é influenciado por fatores ambientais e genéticos. A progressão da fibrose acarreta alteração da arquitetura hepática provocando alteração da função e danos fisiopatológicos do fígado. Cirrose representa o estágio mais avançado de fibrose hepática (ISMAIL; PINZANI, 2009).

Estima-se que a DHGNA esteja presente em até 30% da população geral e o aumento de sua prevalência acontece de forma paralela à epidemia de obesidade (RINELLA, 2015). Indivíduos com obesidade e diabetes do tipo 2 são considerados grupos de alto risco para DHGNA devido a sua íntima associação com fatores metabólicos, principalmente resistência insulínica. Entretanto a prevalência da DHGNA parece ser maior em pacientes infectados pelo HIV quando comparados à população geral (ANGULO, 2007). Estudos sugerem que a fibrose e a esteatose hepática podem estar presentes em até 40% das PVHA (NÚÑEZ-TORRES et al., 2017; PEMBROKE et al., 2017b). Recentemente Perazzo, et al (2018) demonstraram que a prevalência de fibrose e esteatose hepática em PVHA monoinfectados em uso de TARV é de 6% e 35%, respectivamente (PERAZZO et al., 2018b). Além disso, EHNA e fibrose avançada podem chegar a 55% e 11%, respectivamente, nos pacientes infectados pelo HIV com elevação das enzimas hepáticas (MORSE et al., 2015). Assim como na população geral, a incidência de DHGNA/EHNA ou esteatose/fibrose hepática em PVHA pode estar associada com a epidemia de obesidade/sobrepeso (KOETHE et al., 2016), mas também com fatores específicos associados à TARV, como lipodistrofia, insulino-resistência, toxicidade mitocondrial e alteração glicêmica/lipídica (SHLAY et al., 2007).

A patogênese da DHGNA está fortemente conectada com desordens metabólicas e com o contexto inflamatório da obesidade (SHARP; SCHULTZ; COPPELL, 2018), ademais sua progressão para formas graves baseia-se na presença de resistência insulínica, estresse

oxidativo e ativação de cascata inflamatória, conhecida como teoria do "*double or multiple-hits*" (DAY; JAMES, 1998). A resistência insulínica provoca aumento da liberação de ácidos graxos não esterificados (ácidos graxos livres) pelo tecido adiposo (lipólise) que são incorporados como triglicerídeos intra-hepáticos resultando na esteatose hepática e representando a primeira injúria ou o "*first-hit*". A partir deste momento, a presença de estresse oxidativo e ativação de cascata inflamatória associados com complexa interação entre hepatócitos, tecido adiposo, liberação de citocinas inflamatórias, ativação de células estreladas e células de Kupffer ("*multiple-hits*") resultam em apoptose/necrose e progressão para EHNA (PERAZZO; POYNARD; DUFOUR, 2014).

1.1.3 Consumo alimentar e DHGNA

A modificação do estilo de vida é a primeira opção para o tratamento da DHGNA, sendo a perda de peso corporal o objetivo mais importante para a maioria das pessoas com esta doença (RINELLA, 2015). Este fato foi demonstrado em um estudo onde pacientes com DHGNA apresentaram grau de melhora histológica hepática proporcional a quantidade de peso corporal perdido (WONG et al., 2013).

A dieta tem um reconhecido impacto no desenvolvimento das doenças metabólicas, em parte pela modulação do perfil inflamatório (YANG et al., 2020), dentre elas a DHGNA (CHAN et al., 2015). Desta forma, dietas podem ser consideradas saudáveis ou não, de acordo com seu padrão, composição de nutrientes e com a presença de nutrientes específicos.

De acordo com o *Guideline* da Sociedade Européia Nutrição Clínica e Metabolismo, indivíduos sobrepeso/obesos com DHGNA devem perder 7 a 10% de peso corporal com objetivo de melhorar esteatose hepática e bioquímica hepática, e uma perda de peso maior que 10% pode melhorar a fibrose. Uma dieta hipocalórica é recomendada para atingir a perda de peso corporal. Além disso a dieta mediterrânea também é recomendada para reduzir esteatose e melhorar a sensibilidade insulínica (BISCHOFF et al., 2020).

A dieta ocidental; que é rica em carne vermelha, produtos ricos em gordura saturada, farinhas refinadas, carboidratos simples, *fast food* e bebidas açucaradas; tem sido associada com altos níveis de proteína c-reativa (PCR) e interleucina 6 (IL-6) (IP et al., 2019; PHILLIPS et al., 2018). Além disso uma maior ingestão de proteína de origem animal e de carnes processadas está positivamente associada a marcadores de DHGNA (RIETMAN et al., 2018), fibrose hepática (SOLEIMANI et al., 2019) e resistência insulínica (ZELBER-SAGI et

al., 2018). Por outro lado, a dieta tipo mediterrânea (DMT), rica em grãos integrais, peixes, frutas e vegetais verdes, com moderada ingestão de azeite de oliva e baixa ingestão de carne vermelha, tem sido associada com baixos níveis de inflamação. A DMT apresenta alta proporção de AG monoinsaturados (MUFA) em relação aos AG saturados e 30-40 % de gordura total (TRICHOPOULOU et al., 2014).

Outro ponto interessante é que nutrientes específicos como: AG graxos n-3, MUFA, fibras, vitamina E, vitamina C, betacaroteno e magnésio também têm sido consistentemente associados a menores níveis de inflamação (CAVICCHIA et al., 2009).

Dentre os fatores dietéticos que podem ser relacionados ao desenvolvimento da DHGNA, destacamos os lipídeos devido as suas funções metabólicas distintas. Os AG e a ingestão total de gordura são relevantes no desenvolvimento da DHGNA e na progressão para a fibrose hepática, pois afetam a taxa de síntese hepática de triacilglicerol. Em contra ponto, alguns tipos de AG podem proporcionar benefícios à saúde através de alterações na composição do AG tecidual ou da indução de vias de sinalização celular (JURADO-RUIZ et al., 2017).

Os AG poliinsaturados do tipo n-3 (PUFA n-3) podem ter ação anti-inflamatória e antioxidante pela redução da liponeogênese e aumento da β-oxidação hepática (JUMP et al., 2016), e por isso, são considerados como potencial estratégia na prevenção e/ou tratamento da DHGNA (CUI et al., 2021). Estudos experimentais descreveram ação antioxidante e maior sensibilidade insulínica nos grupos que receberam alimentação suplementada com PUFA n-3 (VALENZUELA et al., 2016). Em PVHA, a suplementação diária de PUFA n-3 associada com prática de atividade física associou-se com redução da concentração plasmática de triglicerídeos (WOHL et al., 2005). Já em um ensaio clínico duplo cego, randomizado placebo controlado a suplementação de PUFA n-3 associou-se significativamente com redução da concentração plasmática de enzimas hepáticas e redução da fibrose (CANSANÇÃO et al., 2020). A literatura também descreve um papel protetor dos MUFAs, como o oleico, que parece reduzir a lipogênese hepática pela redução da ativação das proteínas ligadoras do retinol (RBP), além de promover a redução da concentração plasmática de citocinas inflamatória (DE CASTRO; CALDER, 2017).

Por outro lado, a maior ingestão de gordura saturada pode induzir resistência insulínica e diabetes (LEAMY; EGNATCHIK; YOUNG, 2013). Em um ensaio clínico que randomizou indivíduos com peso corporal normal para o consumo de 750 kcal extras de gordura saturada ou de PUFA, foi observado que o peso corporal aumentou em 1,6 kg em

ambos os grupos, mas o percentual de gordura hepática foi significantemente maior no grupo que ingeriu gordura saturada (ROSQVIST et al., 2014).

O índice glicêmico da dieta e a ingestão de subtipos de carboidratos também têm sido associados à DHGNA (VALTUEÑA et al., 2006). Esta associação pode ser observada com a ingestão de carboidratos simples, como a frutose, que estimula a lipogênese promovendo o depósito de gordura no fígado. Além disso em um estudo com animais foi demonstrado que o maior consumo de frutose presente em alimentos industrializados elevou a translocação bacteriana e aumentou a concentração do fator de necrose tumoral α (TNF-α) (BERGHEIM et al., 2008).

Indivíduos com DHGNA apresentam ingestão aumentada de carboidratos simples, gordura saturada e baixa ingestão de micronutrientes (DE CASTRO; CALDER, 2017). Um estudo caso-controle utilizando recordatório alimentar de 24h (R24h) demonstrou que a ingestão de açúcar foi maior em pacientes com DHGNA do que nos indivíduos saudáveis (ZOLFAGHARI et al., 2016). Já em um estudo brasileiro demonstrou que a maior parte dos pacientes excedeu a recomendação de consumo de energia e de gordura saturada e 76% ingeriram menos fibras alimentares que o recomendado (FEROLLA et al., 2013).

1.1.4 Metabolismo Lipídico e DHGNA

A gordura dietética tem papel relevante na patogênese da DHGNA, entretanto a modulação do metabolismo lipídico hepático pelos AG é complexa. A composição dos AG dietéticos influencia o metabolismo hepático (JUÁREZ-HERNÁNDEZ et al., 2016). A composição de AG plamático, nos eritrócitos e no tecido tem sido utilizada como instrumento na avaliação do perfil de ingestão de gorduras e do metabolismo lipídico hepático dos indivíduos. O acúmulo de gordura hepática induz inflamação em células hepáticas, que é o importante mediador da fibrogênese (GAMBINO et al., 2016).

Sabe-se que dietas hiperglicídicas estimulam consideravelmente a lipogênese *de novo*, e esta quando aumentada é reconhecida como um importante contribuinte para o desenvolvimento da DHGNA, assim como para outras situações que envolvem a resistência insulínica (POLACOW; JUNIOR; H, 2007). Esta lipogênese ocorre quando há produção excessiva ou desnecessária de trifosfato de adenosina (ATP). Desta forma uma série de reações enzimáticas são iniciadas pela presença de acetil-coA no citosol, ocorrendo assim a síntese “de novo” de AG (VERLENGIA, ROZANGELA; LIMA, THAIS MARTINS, 2002).

O produto final principal deste processo é o palmitato livre, que é precursor dos AG saturados de cadeia longa e por isso pode ser um bom marcador da lipogênese hepática (LEE et al., 2015).

Estresse oxidativo tem sido sugerido como um importante fator responsável pela evolução da doença hepática pela sua contribuição no processo de progressão de fibrose hepática. As espécies reativas de oxigênio (ERRO) derivados do AG araquidônico, estimulam a proliferação de células estrelares hepáticas responsáveis pelo processo fibrótico. Além disso, o desequilíbrio dentre os AG n-6/n-3 pode estar envolvido no estress oxidativo em pacientes cirróticos, pois aumenta a formação de isoprastanos (BASILI et al., 2014).

Os AG saturados são considerados mais tóxicos do que suas contrapartes insaturadas, resultando em uma cascata lipotóxica progressiva. Eles aumentam a saturação dos fosfolipídios da membrana, promovem o estresse oxidativo e afetam o metabolismo mitocondrial (LEAMY; EGNATCHIK; YOUNG, 2013).

O AG palmitoléico endógeno pode ser usado como biomarcador de lipogênese, e este pode estar aumentado no plasma pela produção endógena através do processo de dessaturação do ácido palmítico que é mediado pela enzima desaturase-1 (SD1) (LEE et al., 2015). Park e colaboradores encontraram correlação entre concentrações plasmáticas de AG palmítico, palmitoléico e paramêtros metabólicos e inflamatórios em pacientes com DHGNA (PARK et al., 2010). Este fato por ser explicado pelo seu maior potencial citotóxico destes AG para as células hepáticas (PARK et al., 2010).

Em um estudo com pacientes com DHGNA avaliados por elastrografia hepática foi demonstrado que indivíduos com fibrose hepática avançada apresentaram maior percentual dos AG palmítico, esteárico e oléico nos eritrócitos, sendo o palmítico considerado um preditor independente para fibrose avançada (CANSANÇÃO et al., 2018a). Já em outro estudo comparando indivíduos com e sem DHGNA, a gordura saturada total e o AG PUFA n-6 plasmáticos estavam positivamente relacionados ao DHGNA, enquanto que o AG docosaehexanóico, que é um PUFA n-3, foi negativamente correlacionado com o risco de DHGNA (ZHENG et al., 2012a).

Um estudo com amostra limitada de indivíduos não-infectados pelo HIV descreveu que os metabólitos dos AG do tipo PUFA podem ser utilizados como biomarcadores para EHNA, apresentando maiores níveis de PUFA quando comparados a controles (LOOMBA et al., 2015).

1.2 JUSTIFICATIVA

Nas últimas décadas observa-se redução da mortalidade associada às infecções oportunistas com concomitante aumento da incidência de doenças crônicas e da expectativa de vida da PVHA. A DHGNA vem apresentando relevância científica e social, por ter sua prevalência global aumentada de forma concomitante à epidemia de obesidade. Estudos vêm sugerindo maior prevalência de DHGNA em PVHA em comparação com a população geral. Isto pode ser explicado pela alta prevalência de resistência insulínica, desregulação glicêmica e lipídica, além do uso prolongado de antiretrovirais.

A avaliação do consumo alimentar é fundamental para o melhor entendimento da patogênese e tratamento da DHGNA, assim como na prevenção da progressão para sua forma grave que pode acarretar no desenvolvimento da fibrose hepática. A ingestão de alguns tipos de AG com ação anti-inflamatória ou metabólica contribui para redução do acúmulo de gordura no parênquima hepático e injúria hepatocelular. As recomendações nutricionais estão focadas na perda de peso corporal, e na redução da ingestão de lipídeos totais com ênfase na gordura saturada. Entretanto demais tipos de ácidos graxos apresentam funcões bioativas, dependendo de suas características moleculares, que podem conferir efeitos protetores ou de risco para DHGNA.

A concentração plasmática de AG específicos parece correlacionar-se com o acúmulo de lipídeos hepáticos e com a progressão da doença. Desta forma, a determinação da concentração plasmática de AG pode auxiliar na melhor compreensão das alterações metabólicas e também na detecção de formas graves relacionadas com DHGNA, podendo representar potenciais biomarcadores na DHGNA. Portanto, avaliação da composição dietética e concentração plasmática de AG podem auxiliar na elaboração de estratégias para diagnóstico precoce das formas graves, prevenção e tratamento da DHGNA.

Alguns estudos avaliaram a correlação da dieta e concentrações plasmáticas de AG com diversos espectros da DHGNA na população geral. Porém, dados sobre a relação entre fatores dietéticos/concentrações plasmáticas de AG em PVHA são escassos. Portanto, esperamos que os resultados inéditos desta tese contribuam para maior entendimento das alterações metabólicas, melhor abordagem nutricional destes pacientes, assim como para elaboração de estratégias nutricionais e clínicas para prevenção e tratamento da DHGNA em PVHA.

2.OBJETIVOS

2.1 OBJETIVO GERAL:

- Avaliar o consumo alimentar de lipídeos, concentração plasmática de ácidos graxos e sua relação com espectros clínicos da DHGNA em PVHA.

2.2 OBJETIVOS ESPECÍFICOS:

- Objetivo I: Analisar a associação entre o consumo alimentar de lipídeos, DHGNA e fibrose hepática;

- Objetivo II: Investigar a associação entre a concentração plasmática de ácidos graxos e fibrose hepática.

3. DESENVOLVIMENTO

3.1 ARTIGO 1

Os resultados obtidos nesta tese serão apresentados em formato de artigos. O primeiro artigo responde ao objetivo I da tese (analisar a associação o consumo alimentar de lipídeos DHGNA e fibrose hepática), e foi publicado na revista *Nutrients* (Nutrients. 2021 Sep 29;13(10):3462. doi: 10.3390/nu13103462) "*Relationship between Dietary Fatty Acid Intake with Nonalcoholic Fatty Liver Disease and Liver Fibrosis in People with HIV"*.

nutrients

MDPI

Article

Relationship between Dietary Fatty Acid Intake with Nonalcoholic Fatty Liver Disease and Liver Fibrosis in People with HIV

Cristiane Fonseca de Almeida [1,2], Paula Simplicio da Silva [1,2], Claudia Santos de Aguiar Cardoso [1,2], Nathalia Gorni Moreira [2], Julliana Cormack Antunes [1,2], Michelle Morata de Andrade [3], Julio Silva [3], Marina Campos Araujo [4], Wilza Arantes Ferreira Peres [5], Pedro Emmanuel Alvarenga Americano do Brasil [1,6], Ronaldo Ismerio Moreira [7], Sandra W. Cardoso [7], Valdilea G. Veloso [7], Beatriz Grinsztejn [7], Patricia Dias de Brito [1,2] and Hugo Perazzo [7,*]

1 Grupo de Pesquisa Clínica em Nutrição e Doenças Infecciosas (GPClin_Nut), Instituto Nacional de Infectologia Evandro Chagas—Fundação Oswaldo Cruz (FIOCRUZ), 21040-360 Rio de Janeiro, Brazil; cristiane.almeida@ini.fiocruz.br (C.F.d.A.); paula.simplicio@ini.fiocruz.br (P.S.d.S.); claudia.cardoso@ini.fiocruz.br (C.S.d.A.C.); julliana.cormack@ini.fiocruz.br (J.C.A.); pedro.brasil@ini.fiocruz.br (P.E.A.A.d.B.); patricia.brito@ini.fiocruz.br (P.D.d.B.)

2 Serviço de Nutrição (SENUT), Instituto Nacional de Infectologia Evandro Chagas—Fundação Oswaldo Cruz (FIOCRUZ), 21040-360 Rio de Janeiro, Brazil; nathalia_gorni@hotmail.com

3 Plataforma de Pesquisa Clínica, Instituto Nacional de Infectologia Evandro Chagas—Fundação Oswaldo Cruz (FIOCRUZ), 21040-360 Rio de Janeiro, Brazil; Michelle.morata@ini.fiocruz.br (M.M.d.A.); julio.lima@ini.fiocruz.br (J.S.)

4 Escola Nacional de Saúde Pública—Fundação Oswaldo Cruz (FIOCRUZ), 21031-210 Rio de Janeiro, Brazil; mcamposaraujo@gmail.com

5 Instituto de Nutrição Josué de Castro, Universidade Federal do Rio de Janeiro (UFRJ), 21941-902 Rio de Janeiro, Brazil; wilza@nutricao.ufrj.br

6 Laboratório de Pesquisa em Imunização e Vigilância em Saúde, Instituto Nacional de Infectologia Evandro Chagas—Fundação Oswaldo Cruz (FIOCRUZ), 21040-360 Rio de Janeiro, Brazil

7 Laboratório de Pesquisa Clínica em DST/AIDS (LAPCLIN-AIDS), Instituto Nacional de Infectologia Evandro Chagas—Fundação Oswaldo Cruz (FIOCRUZ), 21040-360 Rio de Janeiro, Brazil; ronaldo.ismerio@ini.fiocruz.br (R.I.M.); sandra.wagner@ini.fiocruz.br (S.W.C.); valdilea.veloso@ini.fiocruz.br (V.G.V.); beatriz.grinsztejn@gmail.com (B.G.)

* Correspondence: hugo.perazzo@ini.fiocruz.br; Tel.: +55-21-3865-9128

Abstract: We aimed to evaluate the relationship between food intake of lipids with nonalcoholic fatty liver disease (NAFLD) and/or liver fibrosis in people living with HIV/AIDS (PLWHA). In this cross-sectional study, transient elastography was used to detect the presence of NAFLD and/or liver fibrosis. The dietary intake of fats and fatty acids (FA) were assessed by two 24 h dietary recalls (24-HDR) (n = 451). Multivariate logistic regression models were performed. Participants with higher intake of total fat were associated with higher odds for NAFLD compared to those with lower consumption [adjusted odds ratio (aOR) = 1.91 (95% confidence interval (95% CI) 1.06–3.44)]. Furthermore, participants with intermediate intake of n6-PUFA (n6-poly-unsaturated FA) and lauric FA had lower odds for NAFLD, respectively aOR = 0.54 (95% CI 0.3–0.98) and aOR = 0.42 (95% CI 0.22–0.78). Additionally, a higher intake of myristoleic FA (fourth quartile) was a significant protective factor for NAFLD [aOR = 0.56 (95% CI 0.32–0.99)]. Participants with higher intake of lauric FA [0.38 (95% CI 0.18–0.80)], myristic FA [0.38 (0.17–0.89)], palmitoleic FA [0.40 (0.19–0.82)] and oleic FA [0.35 (0.16–0.79)] had positively less odds of having liver fibrosis. On the other hand, higher intake of n-6 PUFA was significantly associated with fibrosis [aOR = 2.45 (95% CI 1.12–5.32)]. Dietary assessment of total fat and FA should be incorporated into HIV care as a tool for preventing NAFLD and fibrosis in PLWHA.

Keywords: dietary fats; liver fibrosis; NAFLD; HIV infection

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1. Introduction

Globally, 38 million people have been living with the human immunodeficiency virus (HIV) [1]. The use of early combined antiretroviral therapy (c-ART) has been decreasing the incidence of opportunistic diseases and increased the life expectancy in people living with HIV/AIDS (PLWHA) [2]. In contrast, the prevalence of non-communicable diseases has been dramatically increasing in PLWHA in the last decade [3]. Non-alcoholic fatty liver disease (NAFLD) is characterized by abnormal accumulation of fat in the liver in the absence of abusive alcohol intake. Clinical presentation of NAFLD can range from simple steatosis to nonalcoholic steatohepatitis (NASH) that can progress to cirrhosis and its complications, such as hepatocellular carcinoma. The presence of advanced liver fibrosis is the main predictor of mortality in individuals with NAFLD [4]. Several studies have been reporting the burden of NAFLD and/or liver fibrosis in PLWHA [5–7].

Dietary habits seem to play an important role in the pathogenesis of NAFLD. The Western diet has been associated with high levels of inflammatory cytokines [8] and a higher prevalence of NAFLD in the general population [9]. On the other hand, the Mediterranean diet can reduce fatty liver and improve insulin resistance status [10]. However, the influence of specific nutrients has not been fully elucidated [11]. Among dietary factors, total fat intake and analysis of the specific subtype of fatty acid (FA) intake might be relevant due their functional and metabolic distinct effects [12]. This might be reinforced because the dietary FA composition impacts liver metabolism, leading to triglyceride accumulation in the liver tissue [13]. However, this relationship has not been completely studied, especially in PLWHA. Studies conducted in Brazil showed that patients with NAFLD had high energy and lipid consumption [14]. Additionally, studies have demonstrated that PLWHA presented more likely an unhealthy food intake pattern [15]. However, the relationship between dietary fat intake and their subtype of fatty acid with NAFLD and liver fibrosis has not been studied in PLWHA. Therefore, the aim of this study was to evaluate the relationship between dietary fatty acid intake and NAFLD and/or the presence of liver fibrosis in HIV mono-infected individuals.

2. Materials and Methods

2.1. Study Design and Participants

This cross-sectional study analyzed data collected at the baseline visit from the longitudinal PROSPEC-HIV study (NCT02542020) that has been conducted at Evandro Chagas National Institute of Infectious Diseases (INI/FIOCRUZ, Rio de Janeiro, Brazil) [16]. All participants with HIV infection enrolled in the PROSPEC-HIV study from June 2015 to January 2019 were eligible for this analysis. Participants with viral hepatitis co-infection defined by positive HCV-antibody or positive HBsAg; excessive alcohol consumption defined by the Alcohol Use Disorders Identification Test (AUDIT) score $\geq$8 [17]; use of lipids supplements or missing laboratory/inconsistent data on dietary assessment were excluded. This study was approved by the Ethical Committee from INI/FIOCRUZ (IRB 32889514.4.0000.5262). All participants signed an informed consent upon enrollment in the PROSPEC-HIV study.

2.2. Clinical Assessment and HIV Infection History

Clinical records collected at baseline visit of PROSPEC HIV study included age, sex at birth, self-reported skin-color [18], years of study and presence of co-morbidities. Dyslipidemia, hypertension, type-2 diabetes and metabolic syndrome were defined according to the International Diabetes Federation [19]. Anthropometric measures, such as weight, height and waist circumference were measured by trained research assistants. Participants were considered as lean, overweight and obese if body mass index (BMI) < 25 Kg/m^2, BMI = 25 to 29.99 Kg/m^2 and BMI $\geq$ 30 Kg/m^2, respectively [20]. A bioelectrical impedance analyzer (Biodynamics® 450, Sao Paulo, Brazil) with 4-electrode (hand-feet) and frequency of 50 kHz was used to assess body fat percentage. All bioelectrical impedances were performed by a single operator in fasted participants in supine

position [21]. The following data were available at the INI/FIOCRUZ HIV clinical cohort: (i) date of first positive HIV antibody test; (ii) date of initiation of any antiretroviral drug; (iii) dates of start and end of combined antiretroviral therapy (c-ART) and (iv) $CD4^+$ T-lymphocyte count and HIV viral load from the closely day of clinical visit.

2.3. Laboratory Tests and Transient Elastography

Blood tests were performed after an overnight fasting and analyzed in a centralized laboratory using an analyzer Dimension-RxL-Max (Siemens Healthcare Diagnostic, Hoffman Estates, IL, USA). Liver tests, such as alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) were measured using an enzymatic assay. Glucose was measured using the hexokinase method; total and high-density lipoprotein (HDL) cholesterol and triglycerides were determined using enzymatic methods. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation [16]. Insulin was determined using chemiluminescent immunoassay (CLIA) and the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated by the formula: [fasting insulin (mIU/L) × fasting glucose (mg/dL)]/405 [22].

Transient elastography (TE) by FibroScan (EchoSens, Paris, France) was performed by a single experienced (>2000 examinations) operator (HP) to detect the presence of NAFLD and/or liver fibrosis following a previously described validated procedure [16]. The results defined as a median of 10 valid measures and expressed in kPa were considered as reliable for analysis if the following criteria had been met: (1) at least 10 valid measurements; (2) an interquartile range (IQR) lower than 30% of the median of liver stiffness measurement (LSM) for fibrosis or Controlled Attenuation Parameter (CAP) for steatosis; and (3) a success rate of more than 60%. The results of XL probe were considered in participants with unreliable TE exams with the M probe. NAFLD was defined by CAP $\geq$ 248 dB/m [23]. Presence of significant liver fibrosis (METAVIR stage F $\geq$ 2) was defined by LSM $\geq$ 7.1 kPa or $\geq$6.2 kPa with M or XL probe, respectively [24].

2.4. Dietary Data

The dietary intake of macronutrients, fat subtypes and FA were assessed by the 24 h dietary recall (24-HDR) method. Briefly, a nutritionist investigator requested the participants to self-report all foods and beverages consumed through the last 24 h. These reports must include details of food preparation and type of oil or fat used, as well as amount of food consumed in household measurements [25]. The 24-HDR was applied using the Automated Multiple-Pass method to structure the interview and to increase the accuracy of the report, minimizing any memory bias [26]. In addition, the 24-HDR was performed in two non-consecutive days: a face-to-face interview during the clinical visit and a remote interview by telephone a few days later. Data of each food and/or beverage item reported by the participant were converted to milligrams/grams and/or milliliters/liters, and these data were entered into a nutritional analysis software (Diet Win Professional Plus 3.0® package software) that uses the Brazilian nutrient database, known as TACO ("Tabela Brasileira de Composição de Alimentos"). The implausibility in self-report intake was verified when individual report less than three foods items. In addition, the 24-HDR that had extreme values (outliers) of caloric intake were reviewed by boxplot graphs to evaluate possible inconsistent data.

The statistical modeling technique Multiple Source Method (MSM) was used to estimate the usual intake of nutrients of participants and to correct the intrapersonal dietary variability (https://msm.dife.de, accessed on 7 July 2019). The use of this approach to correct this variability avoids the need of multiple dietary interviews to estimate individual dietary intake [27]. Nutrients were adjusted by energy density method (the ratio between usual nutrient intake and total usual energy intake) expressed as a percentage to evaluate the relative contribution of these nutrients to the diet [28]. Fiber intake was calculated per 1000 kcal using the following formula: total fiber (g) × 1000 kcal/total energy intake.

2.5. Statistical Analysis

Categorical variables were reported as absolute (n) and relative frequency (%) and continuous variables as median and interquartile range (IQR). We used Chi-square and Mann—Whitney tests to compare proportions and medians, respectively. All nutrients were analyzed in proportion of energy intake (E%). Direct Acyclic Graphs (DAGs) were created with assumptions on the relationship among co-variables and outcomes (NAFLD or fibrosis) using the DAGitty as a browser-based environment (http://www.dagitty.net/, accessed on 6 December 2019) (Figure 1). DAG, as illustrated in Figure 1, is a theoretical model described through a graph that permits qualitative and visual assessment of confounding factors. These DAGs supported our decision about the most parsimonious models for NAFLD and fibrosis to avoid collinearity and confounding. Logistic multivariate models considered occurrence of NAFLD and liver fibrosis as outcomes, each nutrient alone (in quartiles) as independent variables (assuming quartile 1, lowest consumption as the reference), and age, sex and duration of c-ART as confounders as well as usual energy intake (kcal) to minimize the underreporting of the food intake method. Statistical analyses were performed using R version 3.6.3 and considering p-values < 0.05 as statistically significant.

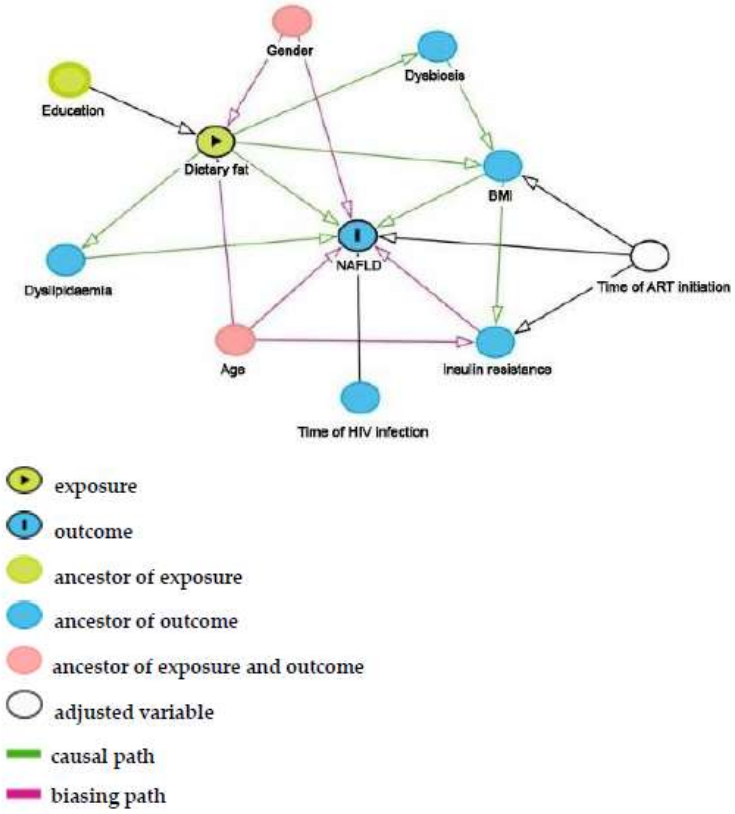

Figure 1. Direct Acyclic Graph for association of dietary fats and fatty acids with liver fibrosis and NAFLD in HIV patients, Rio de Janeiro, Brazil.

3. Results

3.1. Study Characteristics

A total of 727 participants with HIV infection were included in the PROSPEC-HIV study from June 2015 to January 2019. For this analysis, participants were excluded due to viral hepatitis coinfection (n = 95), abusive alcohol intake (n = 123), use of lipid supplement

(n = 6), 24-HDR with missing (n = 4) or inconsistent dietary data (n = 4) or missing data of serum insulin (n = 44). The flowchart of the study population is depicted in Figure 2.

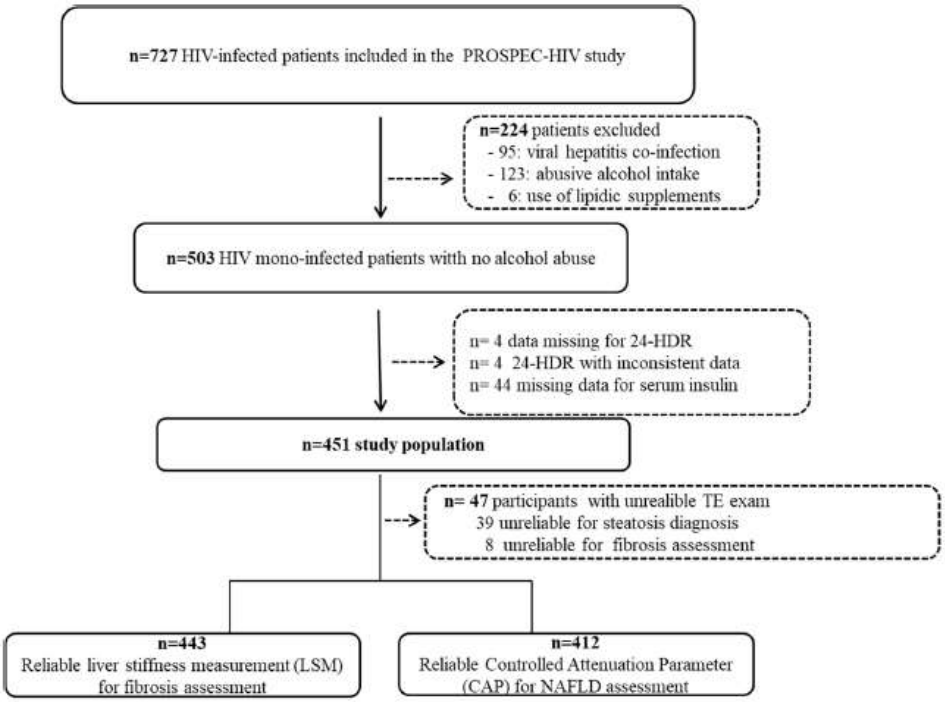

Figure 2. Flow chart of patient recruitment, Rio de Janeiro, Brazil.

A total of 451 participants [60.3 female, median age of 45 (IQR, 36–53) years, 33.9% with metabolic syndrome, median BMI = 25 (IQR, 23–29) Kg/m^2, 96.7% under c-ART during a median time of 7 (IQR, 4–14) years] were included in this analysis. Table 1 describes clinical and demographic characteristics of the participants. CAP and LSM values were unreliable with M and XL probes in 9% (n = 39) and 2% (n = 8) of participants, respectively. Therefore, the association of lipid dietary intake with NAFLD and significant fibrosis was assessed in 412 and 443 HIV mono-infected participants, respectively. The prevalence of NAFLD and significant fibrosis were 37% (95% CI, 32–41) [n = 152] and 16% (95% CI, 12–20) [n = 72], respectively. Supplementary Tables S1 and S2 describe the socio-demographic and clinical characteristics of participants with NAFLD and liver fibrosis.

Table 1. Clinical and demographic characteristics of included participants with HIV mono-infection in INI/FIOCRUZ. Rio de Janeiro, Brazil.

Variables	All (n = 451)
Social and demographic	
Female sex [a]	272 (60.3)
Age, years [b]	45 (36–53)
Self-reported skin color [a]	
White	214 (47.5)
Brown	139 (30.8)
Black	94 (20.8)
Others	4 (0.9)
Education [a] < 8 years of study	209 (46.4)

Table 1. *Cont.*

Variables	All (n = 451)
Comorbidities	
Diabetes mellitus [a]	46 (10.2)
Hypertension [a]	100 (22.2)
Dyslipidemia [a]	78 (17.3)
Metabolic syndrome [a]	150 (33.9)
Biochemistry	
ALT, IU/L [b]	29 (23–43)
AST, IU/L [b]	25 (20–33)
Alkaline phosphatase, IU/L [b]	89 (70–111)
GGT, IU/L [b]	45 (32–70)
Total cholesterol, mg/dL [b]	185 (158–219)
LDL—cholesterol, mg/dL [b]	112 (90–138)
HDL—cholesterol, mg/dL [b]	43 (35–54)
Triglycerides, mg/dL [b]	124 (84–171)
Fasting glucose, mg/dL [b]	93 (88–100)
Insulin, um/L	11 (8–16)
HOMA-IR	3 (2–4)
Nutritional Status	
Body mass index, (kg/m^2) [b]	25 (23–29)
Lean [<25 Kg/m^2] [a]	207 (45.9)
Overweight [25–29.99 Kg/m^2] [a]	153 (33.9)
Obesity [≥30 Kg/m^2] [a]	91 (20.2)
Body fat, (%), by bioimpedance [b]	30 (24–35)
Waist circumference, (cm) [b]	87 (79–95)
HIV history and characteristics	
Duration of HIV infection, years [b]	10 (5–17)
CD4+ T-lymphocyte count (cells/m^3) [b]	665 (421–881)
Detectable HIV RNA viral load (>40 cópias/mm^3) [a]	74 (16.1)
Current c-ART use [a]	436 (96.7)
Duration of c-ART, years [b]	7 (4–14)

Data expressed as n (%) [a] or median (IQR) [b]. ALT, alanine transaminase; ART, antiretroviral therapy; AST, aspartate transaminase; BMI, body mass index; GGT, gamma-glutamyltransferase, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; waist circumference.

3.2. Relationship between Dietary Intake and NAFLD or Liver Fibrosis

The Table 2 summarizes the association between quartiles of usual intake of nutrients with NAFLD in HIV participants. Considering the multivariate models, higher usual intake of total carbohydrates (highest quartile) was associated with lower odds for NAFLD [aOR = 0.44 (95% CI 0.24–0.8); p = 0.01] when compared to the lower intake range (reference quartile). Furthermore, participants with intermediate intake of fiber (third quartile), n6-PUFA (n6-poly-unsaturated FA) (second quartile), lauric FA (third quartile) had significantly lower odds for NAFLD when compared to the reference quartile, respectively aOR = 0.51 (95% CI 0.27–0.96); p = 0.04; aOR = 0.54 (95% CI 0.3–0.98); p = 0.04, and aOR = 0.42 (95% CI 0.22–0.78); p = 0.01. Additionally, a higher intake of myristoleic FA (fourth quartile) was a significant protective factor for NAFLD [aOR = 0.56 (95% CI 0.32–0.99), p = 0.05]. In contrast, participants with higher (fourth quartile) usual intake of total fat had higher odds for NAFLD compared to those with lower consumption [aOR = 1.91 (95% CI 1.06–3.44), p = 0.03].

Table 2. Logistic multivariate model considering dietary intake and presence of nonalcoholic fatty liver disease (NAFLD) [CAP ≥ 248 dB/m] in participants with HIV mono-infection (n = 412)—INI/FIOCRUZ, Rio de Janeiro, Brazil.

	NAFLD	
Variables	**Univariate Model**	**Multivariate Models**
	OR (95% CI)	**aOR (95% CI)**
Energy; kcal		
Q1 < 1587.76	Reference	Reference
Q2 (1587.76–1952.72)	0.80 (0.46–1.39)	1.00 (0.50–2.00)
Q3 (1952.72–2299.45)	0.73 (0.42–1.28)	1.07 (0.43–2.68)
Q4 > 2299.45	0.68 (0.39–1.21)	1.43 (0.36–5.69)
Carbohydrate; % kcal		
Q1 < 49.07	Reference	Reference
Q2 (49.07–53.16)	0.62 (0.35–1.07)	0.56 (0.31–1.01)
Q3 (53.16–56.79)	0.56 (0.32–0.99)	0.56 (0.31–1.01)
Q4 > 56.79	0.48 (0.27–0.85)	0.44 (0.24–0.80)
Protein; % kcal		
Q1 < 14.49	Reference	Reference
Q2 (14.49–16.12)	1.19 (0.67–2.12)	0.99 (0.54–1.82)
Q3 (16.12–17.93)	1.37 (0.77–2.45)	1.18 (0.64–2.18)
Q4 > 17.93	1.71 (0.97–3.04)	1.41 (0.75–2.65)
Total fat; % kcal		
Q1 < 28.56	Reference	Reference
Q2 (28.56–31)	0.95 (0.53–1.68)	0.97 (0.53–1.76)
Q3 (31–34.38)	0.70 (0.39–1.26)	0.65 (0.35–1.21)
Q4 > 34.38	1.81 (1.04–3.17)	1.91 (1.06–3.44)
Fiber, density; g/1000 kcal		
Q1 < 8.47	Reference	Reference
Q2 (8.47–10.13)	0.87 (0.49–1.52)	0.71 (0.39–1.29)
Q3 (10.13–11.80)	0.68 (0.38–1.21)	0.51 (0.27–0.96)
Q4 > 11.80	1.13 (0.65–1.98)	0.88 (0.48–1.60)
Saturate fat; % kcal		
Q1 < 8.36	Reference	Reference
Q2 (8.36–9.59)	0.91 (0.51–1.61)	0.89 (0.49–1.62)
Q3 (9.59–10.77)	0.80 (0.45–1.41)	0.85 (0.46–1.55)
Q4 > 10.77	1.45 (0.83–2.54)	1.62 (0.9–2.92)
PUFA fat; % kcal		
Q1 < 5.87	Reference	Reference
Q2 (5.87–7.23)	0.63 (0.36–1.11)	0.57 (0.31–1.02)
Q3 (7.23–8.28)	0.68 (0.38–1.19)	0.60 (0.33–1.08)
Q4 > 8.28	0.77 (0.44–1.35)	0.70 (0.39–1.26)
MUFA fat; % kcal		
Q1 < 7.47	Reference	Reference
Q2 (7.47–8.38)	1.04 (0.59–1.83)	0.78 (0.43–1.43)
Q3 (8.38–9.48)	0.74 (0.42–1.34)	0.58 (0.31–1.07)
Q4 > 9.48	1.32 (0.75–2.31)	0.93 (0.49–1.76)
Trans FA; % kcal		
Q1 < 0.28	Reference	Reference
Q2 (0.28–0.37)	0.77 (0.44–1.37)	0.80 (0.44–1.46)
Q3 (0.37–0.46)	1.34 (0.77–2.35)	1.28 (0.71–2.31)
Q4 > 0.46	0.81 (0.46–1.43)	0.68 (0.37–1.25)
Cholesterol; % kcal		
Q1 < 0.1	Reference	Reference
Q2 (0.1–0.12)	1.33 (0.80–2.23)	1.25 (0.73–2.15)
Q3 (0.12–0.14)	0.99 (0.55–1.79)	0.84 (0.44–1.6)
Q4 > 0.14	1.14 (0.65–1.98)	0.96 (0.51–1.79)

Table 2. *Cont.*

	NAFLD	
Variables	**Univariate Model**	**Multivariate Models**
	OR (95% CI)	**aOR (95% CI)**
n-6 PUFA; % kcal		
Q1 < 3.47	Reference	Reference
Q2 (3.47–4.34)	0.57 (0.32–1.01)	0.54 (0.30–0.98)
Q3 (4.34–5.28)	0.73 (0.41–1.27)	0.66 (0.37–1.20)
Q4 > 5.28	0.64 (0.36–1.13)	0.59 (0.32–1.08)
n-3 PUFA; % kcal		
Q1 < 0.41	Reference	Reference
Q2 (0.41–0.53)	0.75 (0.42–1.31)	0.71 (0.40–1.29)
Q3 (0.53–0.67)	0.72 (0.41–1.28)	0.65 (0.36–1.19)
Q4 > 0.67	0.89 (0.51–1.56)	0.76 (0.42–1.38)
Lauric FA (12:00); % kcal		
Q1 < 0.08	Reference	Reference
Q2 (0.08–0.13)	0.69 (0.40–1.19)	0.80 (0.45–1.40)
Q3 (0.13–0.18)	0.42 (0.23–0.77)	0.42 (0.22–0.78)
Q4 > 0.18	0.74 (0.44–1.27)	0.75 (0.43–1.32)
Myristic FA (14:00); % kcal		
Q1 < 2.36	Reference	Reference
Q2 (2.36–3.27)	1.23 (0.70–2.16)	1.25 (0.69–2.24)
Q3 (3.27–5.14)	1.00 (0.56–1.79)	1.09 (0.59–2.00)
Q4 > 5.14	1.34 (0.76–2.36)	1.26 (0.70–2.29)
Palmitic FA (16:00); % kcal		
Q1 < 2.58	Reference	Reference
Q2 (2.58–3.09)	1.23 (0.70–2.15)	1.30 (0.73–2.33)
Q3 (3.09–3.63)	1.30 (0.74–2.27)	1.20 (0.67–2.15)
Q4 > 3.63	0.89 (0.50–1.58)	0.82 (0.45–1.50)
Stearic FA (18:00); % kcal		
Q1 < 1.06	Reference	Reference
Q2 (1.06–1.3)	1.13 (0.65–1.99)	1.28 (0.72–2.30)
Q3 (1.3–1.57)	1.27 (0.73–2.22)	1.19 (0.66–2.14)
Q4 > 1.57	0.87 (0.49–1.55)	0.81 (0.44–1.49)
Arachidic FA (20:00); % kcal		
Q1 < 0.035	Reference	Reference
Q2 (0.035–0.04)	0.89 (0.53–1.51)	0.76 (0.44–1.33)
Q3 (0.04–0.05)	1.06 (0.61–1.83)	0.87 (0.49–1.55)
Q4 > 0.05	1.37 (0.66–2.83)	1.02 (0.47–2.21)
Myristoleic FA (14:1); % kcal		
Q1 < 0.02	Reference	Reference
Q2 (0.02–0.03)	0.78 (0.46–1.31)	0.72 (0.42–1.25)
Q3 (0.03–0.04)	0.53 (0.29–0.99)	0.63 (0.33–1.21)
Q4 > 0.04	0.70 (0.41–1.19)	0.56 (0.32–0.99)
Palmitoleic FA (16:1); % kcal		
Q1 < 0.17	Reference	Reference
Q2 (0.17–0.21)	0.80 (0.45–1.43)	0.80 (0.44–1.45)
Q3 (0.21–0.26)	1.27 (0.75–2.16)	1.06 (0.61–1.84)
Q4 > 0.26	1.08 (0.61–1.91)	0.87 (0.47–1.60)
Oleic FA (18:1); % kcal		
Q1 < 4.29	Reference	Reference
Q2 (4.29–5.09)	0.97 (0.55–1.71)	0.97 (0.54–1.75)
Q3 (5.09–5.94)	0.86 (0.49–1.52)	0.71 (0.39–1.29)
Q4 > 5.94	1.08 (0.61–1.89)	0.87 (0.47–1.58)
Linoleic FA (18:2-n6); % kcal		
Q1 < 3.45	Reference	Reference
Q2 (3.45–4.33)	0.61 (0.35–1.08)	0.59 (0.33–1.08)
Q3 (4.33–5.26)	0.75 (0.43–1.32)	0.70 (0.39–1.26)
Q4 > 5.26	0.66 (0.38–1.17)	0.63 (0.34–1.14)

Table 2. *Cont.*

	NAFLD	
Variables	**Univariate Model**	**Multivariate Models**
	OR (95% CI)	**aOR (95% CI)**
Linolenic FA (18:3-n3); % kcal		
Q1 < 0.4	Reference	Reference
Q2 (0.4–0.51)	0.71 (0.40–1.26)	0.68 (0.38–1.24)
Q3 (0.51–0.63)	0.77 (0.44–1.34)	0.67 (0.37–1.21)
Q4 > 0.63	0.88 (0.50–1.55)	0.77 (0.43–1.40)
n6/n3 PUFA ratio; g		
Q1 < 7.45	Reference	Reference
Q2 (7.45–8.21)	0.61 (0.35–1.07)	0.59 (0.33–1.06)
Q3 (8.21–9.18)	0.66 (0.37–1.16)	0.69 (0.38–1.25)
Q4 > 9.18	0.84 (0.48–1.46)	0.96 (0.54–1.71)

Multivariate models adjusted by usual energy intake, age, gender and duration of c-ART. ART, antiretroviral therapy; CAP, controlled attenuation parameter; E%, energy percent; FA, fatty acid; g, gram; kcal, kilocalories; MUFA, mono-unsaturated FA; NAFLD, nonalcoholic fatty liver disease; PUFA, poly-unsaturated FA; Q, quartile.

The association between usual intake of nutrients in quartiles with occurrence of liver fibrosis in HIV mono-infected participants is summarized in Table 3. After adjustment for confounding factors, the usual intake of protein had only a statistical non-significant trend [aOR = 2.13 (95% CI 0.96–4.70); $p = 0.06$] to be associated with liver fibrosis. In multivariate models, participants with moderate usual intake of lauric FA [second quartile; aOR = 0.38 (0.18–0.80); $p = 0.01$], myristic FA [third quartile; aOR = 0.38 (0.17–0.89), $p = 0.03$], palmitoleic FA [third quartile; aOR = 0.40 (0.19–0.82); $p = 0.01$] and oleic FA [third quartile; aOR = 0.35 (0.16–0.79); $p = 0.79$] had lower risk of presence of liver fibrosis compared to those with low usual intake (lowest quartile) of these FAs. On the other hand, intermediate usual intake of n-6 PUFA (third quartile) was significantly associated with the presence of liver fibrosis compared to low intake [aOR = 2.45 (95% CI 1.12–5.32); $p = 0.02$].

Table 3. Logistic multivariate model considering dietary intake and presence of liver fibrosis (stage F ≥ 2) [LSM ≥ 7.1 kPa or ≥ 6.2 kPa with M or XL probe] in participants with HIV mono-infection ($n = 443$)—INI/FIOCRUZ. Rio de Janeiro, Brazil.

	Fibrosis	
Variables	**Univariate Model**	**Multivariate Models**
	OR [95%IC]	**aOR [95%IC]**
Energy; kcal		
Q1 < 1587.76	Reference	Reference
Q2 (1587.76–1952.72)	0.97 (0.49–1.89)	0.97 (0.43–2.20)
Q3 (1952.72–2299.45)	0.82 (0.41–1.64)	0.80 (0.26–2.46)
Q4 > 2299.45	0.50 (0.23–1.08)	0.48 (0.08–2.79)
Carbohydrate; % kcal		
Q1 < 49.07	Reference	Reference
Q2 (49.07–53.16)	1.33 (0.65–2.70)	1.37 (0.67–2.82)
Q3 (53.16–56.79)	1.25 (0.61–2.59)	1.33 (0.64–2.76)
Q4 > 56.79	0.99 (0.47–2.09)	0.97 (0.46–2.07)
Protein; % kcal		
Q1 < 14.49	Reference	Reference
Q2 (14.49–16.12)	1.94 (0.91–4.18)	1.75 (0.81–3.82)
Q3 (16.12–17.93)	1.11 (0.48–2.54)	0.97 (0.41–2.28)
Q4 > 17.93	2.61 (1.24–5.49)	2.13 (0.96–4.70)

Table 3. *Cont.*

	Fibrosis	
Variables	**Univariate Model**	**Multivariate Models**
	OR [95%IC]	**aOR [95%IC]**
Total fat; % kcal		
Q1 < 28.56	Reference	Reference
Q2 (28.56–31)	1.45 (0.75–2.80)	1.55 (0.79–3.05)
Q3 (31–34.38)	0.69 (0.33–1.46)	0.70 (0.33–1.49)
Q4 > 34.38	0.64 (0.30–1.37)	0.64 (0.30–1.39)
Fiber; g/1000 kcal		
Q1 < 8.47	Reference	Reference
Q2 (8.47–10.13)	0.77 (0.37–1.61)	0.65 (0.30–1.38)
Q3 (10.13–11.80)	0.83 (0.40–1.72)	0.69 (0.33–1.46)
Q4 > 11.80	1.21 (0.61–2.39)	0.95 (0.47–1.95)
Saturated fat; % kcal		
Q1 < 8.36	Reference	Reference
Q2 (8.36–9.59)	1.03 (0.52–2.02)	1.04 (0.53–2.08)
Q3 (9.59–10.77)	0.88 (0.44–1.77)	0.96 (0.47–1.96)
Q4 > 10.77	0.60 (0.28–1.28)	0.63 (0.29–1.36)
PUFA fat; % kcal		
Q1 < 5.87	Reference	Reference
Q2 (5.87–7.23)	1.67 (0.84–3.33)	1.61 (0.80–3.25)
Q3 (7.23–8.28)	0.94 (0.44–2.00)	0.89 (0.41–1.91)
Q4 > 8.28	1.03 (0.49–2.19)	0.96 (0.45–2.06)
MUFA fat; % kcal		
Q1 < 7.47	Reference	Reference
Q2 (7.47–8.38)	0.68 (0.33–1.41)	0.54 (0.26–1.14)
Q3 (8.38–9.48)	0.85 (0.42–1.70)	0.67 (0.33–1.38)
Q4 > 9.48	0.84 (0.42–1.68)	0.54 (0.25–1.17)
Trans FA; % kcal		
Q1 < 0.28	Reference	Reference
Q2 (0.28–0.37)	0.87 (0.40–1.90)	0.89 (0.41–1.96)
Q3 (0.37–0.46)	1.37 (0.65–2.88)	1.24 (0.58–2.65)
Q4 > 0.46	1.90 (0.94–3.84)	1.68 (0.82–3.45)
Cholesterol; % kcal		
Q1 < 0.1	Reference	Reference
Q2 (0.1–0.12)	1.20 (0.63–2.32)	1.10 (0.57–2.15)
Q3 (0.12–0.14)	1.05 (0.49–2.24)	0.87 (0.40–1.92)
Q4 > 0.14	1.29 (0.65–2.57)	1.03 (0.49–2.18)
n-6 PUFA; % kcal		
Q1 < 3.47	Reference	Reference
Q2 (3.47–4.34)	2.10 (0.96–4.59)	2.10 (0.95–4.63)
Q3 (4.34–5.28)	2.57 (1.19–5.54)	2.45 (1.12–5.32)
Q4 > 5.28	1.53 (0.68–3.47)	1.40 (0.61–3.21)
n-3 PUFA; % kcal		
Q1 < 0.41	Reference	Reference
Q2 (0.41–0.53)	0.66 (0.31–1.42)	0.64 (0.30–1.39)
Q3 (0.53–0.66)	1.34 (0.67–2.66)	1.27 (0.63–2.54)
Q4 > 0.655	1.08 (0.53–2.19)	0.94 (0.46–1.93)
Lauric FA (12:00); % kcal		
Q1 < 0.08	Reference	Reference
Q2 (0.08–0.13)	0.34 (0.16–0.72)	0.38 (0.18–0.80)
Q3 (0.13–0.18)	0.47 (0.23–0.98)	0.49 (0.24–1.02)
Q4 > 0.18	0.60 (0.31–1.16)	0.63 (0.32–1.22)
Myristic FA (14:00); % kcal		
Q1 < 2.36	Reference	Reference
Q2 (2.36–3.27)	0.97 (0.50–1.87)	0.98 (0.50–1.91)
Q3 (3.27–5.135)	0.36 (0.16–0.83)	0.38 (0.17–0.89)
Q4 > 5.135	0.85 (0.43–1.69)	0.80 (0.40–1.60)

Table 3. *Cont.*

	Fibrosis	
Variables	**Univariate Model**	**Multivariate Models**
	OR [95%IC]	**aOR [95%IC]**
Palmitic FA (16:00); % kcal		
Q1 < 2.58	Reference	Reference
Q2 (2.58–3.09)	0.71 (0.36–1.41)	0.73 (0.36–1.46)
Q3 (3.09–3.625)	0.66 (0.32–1.34)	0.62 (0.30–1.28)
Q4 > 3.625	0.71 (0.36–1.41)	0.64 (0.32–1.30)
Stearic FA (18:00); % kcal		
Q1 < 1.06	Reference	Reference
Q2 (1.06–1.3)	0.76 (0.38–1.51)	0.82 (0.41–1.64)
Q3 (1.3–1.57)	0.50 (0.23–1.07)	0.48 (0.22–1.04)
Q4 > 1.57	0.95 (0.48–1.87)	0.91 (0.46–1.80)
Arachidic FA (20:00); % kcal		
Q1 < 0.035	Reference	Reference
Q2 (0.035–0.04)	0.93 (0.48–1.81)	0.81 (0.41–1.6)
Q3 (0.04–0.05)	1.00 (0.50–1.98)	0.83 (0.41–1.69)
Q4 > 0.05	1.29 (0.53–3.14)	0.98 (0.39–2.46)
Myristoleic FA (14:1); % kcal		
Q1 < 0.02	Reference	Reference
Q2 (0.02–0.03)	0.65 (0.34–1.27)	0.63 (0.32–1.24)
Q3 (0.03–0.04)	0.51 (0.23–1.14)	0.59 (0.26–1.34)
Q4 > 0.04	0.71 (0.37–1.38)	0.65 (0.33–1.27)
Palmitoleic FA (16:1); % kcal		
Q1 < 0.17	Reference	Reference
Q2 (0.17–0.21)	0.78 (0.40–1.52)	0.80 (0.40–1.58)
Q3 (0.21–0.26)	0.46 (0.23–0.95)	0.40 (0.19–0.82)
Q4 > 0.26	0.68 (0.34–1.35)	0.52 (0.25–1.09)
Oleic FA (18:1); % kcal		
Q1 < 4.29	Reference	Reference
Q2 (4.29–5.09)	0.74 (0.37–1.46)	0.71 (0.36–1.43)
Q3 (5.09–5.94)	0.40 (0.18–0.88)	0.35 (0.16–0.79)
Q4 > 5.94	0.89 (0.46–1.73)	0.70 (0.35–1.41)
Linoleic FA (18:2-n6); % kcal		
Q1 < 3.45	Reference	Reference
Q2 (3.45–4.33)	1.61 (0.75–3.44)	1.63 (0.75–3.51)
Q3 (4.33–5.23)	2.17 (1.04–4.53)	2.08 (0.99–4.38)
Q4 > 5.23	1.30 (0.59–2.84)	1.19 (0.54–2.64)
Linolenic FA (18:3-n3); % kcal		
Q1 < 0.4	Reference	Reference
Q2 (0.4–0.51)	0.68 (0.31–1.46)	0.67 (0.31–1.45)
Q3 (0.51–0.63)	1.32 (0.67–2.61)	1.25 (0.63–2.49)
Q4 > 0.63	1.04 (0.51–2.14)	0.92 (0.44–1.91)
n6/n3 PUFA ratio, g		
Q1 < 7.445	Reference	Reference
Q2 (7.445–8.21)	1.69 (0.82–3.51)	1.69 (0.81–3.52)
Q3 (8.21–9.18)	1.57 (0.75–3.30)	1.62 (0.77–3.44)
Q4 > 9.18	1.20 (0.56–2.60)	1.27 (0.58–2.78)

Multivariate models adjusted by usual energy intake, age, gender and duration of c-ART. ART, antiretroviral therapy; E%, energy percent; kcal, kilocalories; LSM, liver stiffness measurement; MUFA, mono-unsaturated FA; NAFLD, nonalcoholic fatty liver disease; PUFA, poly-unsaturated FA; Q, quartile.

4. Discussion

This study highlighted the association of dietary fat intake with the presence of NAFLD and/or fibrosis in PLWHA. To the best of our knowledge, this is the one of the first studies that has demonstrated the role of FA intake, and that high ingestion of total fat can increase the odds of NAFLD in PLWHA, independently of energy intake, age, sex and

duration of c-ART. We demonstrated that participants with high usual intake of total fat had 91% more odds of having NAFLD.

A high-fat diet can be a trigger for liver fatty infiltration [29], might cause dysbiosis [30] and increase intestinal permeability leading to accumulation of triglycerides in hepatocytes contributing to NAFLD [31]. Our findings were aligned with a Korean study that showed a higher odds for NAFLD, determined by ultrasonography, in individuals with higher fat intake, quantified by food frequency questionnaire (FFQ) [32]. Similarly, high levels of Fatty Liver Index, a serological biomarker for detection of steatosis, were associated with a higher intake of total fat in a Dutch population [33]. In addition, a Brazilian cross-sectional study that assessed dietary intake in a limited sample of 96 participants with NAFLD using 24-HDR reported that most individuals consumed a higher total fat amount than recommended [14].

In contrast with previous publications, our study did not report association between total saturated fat intake and NAFLD. Instead, we described that moderate consumption of lauric FA was significantly associated with a lower odd of NAFLD and liver fibrosis in PLWHA. Lauric is a saturated medium-chain FA (MCFA) which is directly transported to the liver, where it is rapidly metabolized by β oxidation and also provokes a thermogenic response [34]. An experimental study reported that mice fed with lauric FA diet had lower obesity-related metabolic disorders and lower levels of plasma markers of liver function (alanine and aspartate aminotransferases) than mice fed with palmitic FA [35]. The present study also reported that participants with HIV who had moderated their consumption of myristic FA had less likely odds of having liver fibrosis compared to those with low intake. This might be explained by the fact that myristic, a saturated FA found in coconut and milk products, seems to be more rapidly metabolized (both β-oxidation and elongation) in hepatocytes [36].

The relationship between a high intake of monounsaturated fatty acid (MUFA) and improvement on lipid profile has been extensively described in previous studies that reported the benefits of the Mediterranean diet [10,37,38]. The present study reinforces this concept since we demonstrated that a moderate consumption of myristoleic FA, an MUFA, was a protective nutrient for NAFLD associated with lower odds of NAFLD. Additionally, moderate consumption of palmitoleic and oleic MUFAs were associated with a reduction of at least 60% in the odds for developing liver fibrosis. Several studies demonstrated that a diet rich in oleic acid can improve plasma lipid profile, inflammatory cytokines (INF-, IL-6), insulin sensitivity and macrophage infiltration, reducing histological features of NAFLD and liver fat [13,39,40]. Besides, previous studies reported that palmitoleic FA could impact glucose metabolism improving and/or preventing insulin resistance and type-2 diabetes [41].

Few studies have investigated the associations of n-6 PUFA intake with NAFLD. In our study, participants with a moderate consumption of n-6 PUFA had less likely NAFLD. This result is in agreement with a cross-sectional study that investigated the association of n-6 PUFA intake with NAFLD in adults using data from the National Health and Nutrition Examination Survey (NHANES). Those authors also used 24-HDR and demonstrated that n-6 PUFA intake was inversely associated with NAFLD [42]. In contrast, we reported that moderate consumption of n-6 PUFA increased the risk of liver fibrosis, probably related a pro-inflammatory activity [43]. These results were aligned with a study by Cortez-Pinto et al. which demonstrated that patients with biopsy-proven NASH had a significantly higher intake of n-6 PUFA and higher n6/n3 ratio, determined by FFQ, compared to controls [44].

We demonstrated that the moderate consumption of some fatty acids was associated with lower odds of NAFLD or liver fibrosis, but this was not observed in higher quartile. We suppose that the effect of dose—response might not be adequate for association of fatty acids with NAFLD or liver fibrosis because the moderate intake has a beneficial effect over excessive consumption.

We reported that a higher usual intake of total carbohydrate was associated with lower odds for NAFLD. Although the literature has shown that high intakes of dietary sugars have been associated with increased risk for NAFLD, there is no consensus about the effects of total carbohydrates on this liver disease [11]. Studies assessing nutrient intake and dietary patterns have showed that a high consumption of monosaccharides and disaccharides (fructose and sucrose) was positively associated with NAFLD [45,46]. We were unable to analyze the different subtypes of carbohydrates ingested, but we can suggest that our result reflects a high intake of polysaccharides, originating from beans and cereals, which are very common in Brazilian eating habits and are also sources of dietary fiber [47].

Another piece of evidence presented in this study revealed that a moderate consumption of dietary fiber was associated with lower odds for NAFLD, which remains in agreement with previous publications [46,48]. The benefits of dietary fiber have been extensively validated in overall metabolic health due to improvement of insulin sensitivity. Additionally, dietary fiber can prevent/control obesity through its effects on satiety, reducing the frequency of eating and the portion of food [49]. The fermentation of fiber, due to the interaction with gut microbes, can provide short-chain FA, key microbial metabolites that promote a protective and nourishing role for colonocytes, ensuring the preservation of the intestinal barrier and consequently protecting liver function [48].

The major limitations of our study are the cross-sectional study design and the lack of liver biopsy as the reference for the presence of NAFLD and/or liver fibrosis. Our study design does not allow us to conjecture any conclusions about the causality between dietary intake and incidence of NAFLD and/or fibrosis. In the present study, the presence of liver steatosis, for the definition of NAFLD, and the presence of fibrosis were defined using an extensive validated non-invasive method, such as transient electrography [50,51]. The same threshold of CAP measurement ($\geq$248 dB/m) was used independent of the probe because LSM would be 1.5 to 2.0 kPa lower by the XL probe compared to M probe [52], but CAP seems to be similar in both probes [53]. A potential criticism would be the lack of physical activity assessment using validated questionnaires. We acknowledge that when informing the participant about the presence of liver steatosis and/or fibrosis during the clinical visit, it could affect in the second 24-HDR, due to significant changes in dietary habits. However, this source of bias was mitigated since we did not notice any important difference in food energy intake between first and second 24-HDRs. We are aware that 24-HDR is a self-reporting instrument for dietary intake assessment that might lead to underreporting, and to minimize this bias, we used the Automated Multiple-Pass method. Nevertheless, this is a practical and validated method that is considered the least biased to examine association between diet and disease and has been widely used in epidemiological and dietary monitoring studies [25]. Finally, we assume that two 24-HDRs might be insufficient to evaluate usual fat intake. However, we adjusted nutrients for total energy intake to minimize misreporting [53], as well as the potential variability on dietary intake using a well-established statistical method, such as MSM, to estimate usual intake [28]. The 24-HDR is a validated method that has been recommended as the least biased of the self-reporting instruments when compared to the other instruments such as FFQ and food record [25,54]. Alternative measurements which would be easier to implement in clinical practice are dietary screeners, which allow the assessment of aspects of the diet, such as specifics nutrients, rather than the total diet [55]. The lack of biochemical analysis of fatty acids is also a limitation of our study. Additionally, our study design hinders the evaluation of whether food intake can lead to higher prevalence of NAFLD in PLWHA compared to controls, since the PROSPEC-HIV was not set to include uninfected individuals. Studies comparing prevalence of NAFLD or food intake of PLWHA compared to controls (uninfected individuals) remain lacking in Brazil. However, the diet quality seems to be lower in PLWHA, and this population presents high prevalence of inappropriate food intake, despite the fact that PLWHA have undergone the same culture and influences as the general population [56,57]. The last point to highlight is the statistical

methodological choices. We used the "Multivariate Nutrient Density Method" [58] due to the need to adjust the total energy consumption methodology (as mentioned earlier). This choice of statistical model highlighted the nutrients of interest (lipids) and avoided the discussion of diet, i.e., the influence of other nutrient intake on the correlation of lipid nutrients and their results. Thus, this choice can be considered a limitation of the study.

The main strengths of this study remain the dietary intake evaluation in a well-characterized large sample of people with HIV mono-infection, the quality methodology of data analysis and the use of DAG, supporting the choice of confounding variables. Clinical assessment, TE exams, bioelectrical impedances and blood samples were performed on the same day in the PROSPEC study. Additionally, all TE exams were performed for a single experimented operator in fasting patients, and blood analyses were performed in a centralized laboratory.

5. Conclusions

In conclusion, the current study showed that a higher usual intake of total fat increased the risk of NAFLD. Additionally, consumption of specific FAs was associated with lower and/or higher odds for presence of liver diseases in HIV mono-infected participants. These results reinforced the role of diet in the pathogenesis of NAFLD and/or liver fibrosis in PLWHA. Dietary assessment of total fat and FA could be incorporated into HIV care, and this strategy should be used as a tool for preventing NAFLD and fibrosis in PLWHA. Additionally, dietary supplementation of specific fatty acids, such as myristoleic FA, could be important in nutritional care of PLWHA.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/nu13103462/s1, Table S1: Clinical and demographic characteristics of participants with HIV mono-infection in INI/FIOCRUZ and nonalcoholic fatty liver disease (NAFLD) [CAP $\geq$ 248 dB/m; n = 152, prevalence = 37%], Rio de Janeiro, Brazil, Table S2: Clinical and demographic characteristics of participants with HIV mono-infection in INI/FIOCRUZ and significant liver fibrosis (stage F $\geq$ 2) [LSM $\geq$ 7.1 kPa or $\geq$6.2 kPa with M or XL probe; n = 72, prevalence = 16%], Rio de Janeiro, Brazil.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethical Committee from INI/FIOCRUZ (IRB 32889514.4.0000.5262) on 16 July 2016. All participants signed an informed consent form upon enrollment in the PROSPEC-HIV study.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

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Abbreviations

ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; AUDIT, Alcohol Use Disorders Identification Test; BMI, body mass index; CAP, controlled attenuation parameter; CI, confidence interval; E%, energy percent; FA, fatty acid; FFQ, frequency food questionnaire; GGT, gamma-glutamyltransferase; 24-HDR, 24 h dietary recall; HDL, high-density cholesterol; IQR, interquartile range; LDL, low-density cholesterol; LSM, liver stiffness measurement; MUFA, mono-unsaturated FA; nonalcoholic NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OR, odds ratio; PUFA, poly-unsaturated FA; Q, quartile; ULN, upper limit of normal; WHO, World Health Organization.

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3.2 ARTIGO 2

Este artigo: "*Plasmatic Fatty acid composition and its relationship with liver fibrosis in people with HIV: a case-control study"* responde ao objetivo II (investigar a associação entre a concentração plasmática de ácidos graxos e fibrose hepática). Este será submetido a revista científica. As revistas alvos de escolha são: 1) *Nutrition Research* e, 2) *Metabolism: Clinical and Experimental.*

Plasmatic Fatty acid composition and its relationship with liver fibrosis in people with HIV: a case-control study

Cristiane Fonseca de Almeida[1,2], Wilza Arantes Ferreira Peres[5], Paula Simplício da Silva[1,2], Claudia Santos de Aguiar Cardoso[1,2], Michelle Morata de Andrade[3], Julio Castro-Alves[3], Marcel de Souza Borges Quintana[3], Marina Campos Araujo[4], Karla Yasmin Dias Fraga[5,] Julliana Antunes Cormack [1,2], Ronaldo Ismerio Moreira[7], Sandra W Cardoso[7], Valdilea G. Veloso[7], Beatriz Grinsztejn[7], Patricia Dias de Brito[1,2], Hugo Perazzo[7]

[1]Grupo de Pesquisa Clínica em Nutrição e Doenças Infecciosas (GPClin_Nut); Instituto Nacional de Infectologia Evandro Chagas - Fundação Oswaldo Cruz (FIOCRUZ); 21040-360; Rio de Janeiro; Brazil.

[2]Serviço de Nutrição (SENUT); Instituto Nacional de Infectologia Evandro Chagas - Fundação Oswaldo Cruz (FIOCRUZ); 21040-360; Rio de Janeiro; Brazil

[3]Plataforma de Pesquisa Clínica; Instituto Nacional de Infectologia Evandro Chagas - Fundação Oswaldo Cruz (FIOCRUZ); 21040-360; Rio de Janeiro; Brazil

[4]Escola Nacional de Saúde Pública - Fundação Oswaldo Cruz (FIOCRUZ); 21031-210; Rio de Janeiro; Brazil

[5] Instituto de Nutrição Josué de Castro - Universidade Federal do Rio de Janeiro (UFRJ); 21941-902; Rio de Janeiro; Brazil.

[1,6] Laboratório de Pesquisa em Imunização e Vigilância em Saúde Clínica; Instituto Nacional de Infectologia Evandro Chagas - Fundação Oswaldo Cruz (FIOCRUZ); 21040-360; Rio de Janeiro; Brazil

[7]Laboratório de Pesquisa Clínica em DST/AIDS (LAPCLIN-AIDS); Instituto Nacional de Infectologia Evandro Chagas - Fundação Oswaldo Cruz (FIOCRUZ); 21040-360; Rio de Janeiro; Brazil.

Keywords

Fattys acid composition; Liver fibrosis; NAFLD; HIV infection.

Corresponding author

Hugo Perazzo; MD; PHD

Laboratório de Pesquisa Clínica em DST/AIDS (LAPCLIN-AIDS)

Instituto Nacional de Infectologia Evandro Chagas- Fundação Oswaldo Cruz

Av. Brasil, 4365 - CEP 21040-360 - Rio de Janeiro - Brazil

E-mail: hugo.perazzo@ini.fiocruz.br
Tel: +55 21 3865-9128 / Fax +55 21 2564-4933

ABSTRACT

Background: The relationship between plasmatic fatty acid (FA) composition and liver fibrosis remains scarce in people living with HIV/AIDS (PLWHA). We aimed to evaluate the association of plasmatic FAs and liver fibrosis in HIV mono-infected individuals. **Methods:** This case-control study included PLWHA with liver fibrosis (cases) and randomly selected subjects without fibrosis (controls) from the PROSPEC-HIV study (NCT02542020). Participants with viral hepatitis, abusive alcohol consumption and lipids supplements use were excluded. Liver fibrosis was defined using transient elastography (TE) by liver stiffness measurement (LSM) ≥ 7.1 kPa or ≥ 6.2 kPa with M or XL probe, respectively. All HIV mono-infected participants with liver fibrosis identified at the baseline PROSPEC-HIV visit were included. Controls (1:1) were randomly selected among those HIV mono-infected participants without liver fibrosis. Plasmatic FA profile, dietary lipid intake, anthropometric measures, and blood samples were assessed. Plasmatic fatty acid was analyzed using gas chromatography and intake of fats lipids were assessed by two 24-hour dietary recall (24-HDR). Multivariate logistic regression models adjusted by age, sex at birth and duration of antiretroviral therapy (ART) were performed. **Results:** A total of 142 participants (71 cases and 71 controls) [62% female, median age=46 (IQR, 37-53) years, 14.8% with diabetes, median CD4 count=655 cells/mm3, 96.5% under ART] were included. Higher percentages of plasmatic palmitc acid (16:0) and saturated fatty acids (SFA) were observed in participants with liver fibrosis (cases) compared to those without (controls). Presence of higher percentage of plasmatic palmitc acid (16:0) was associated with an increased odds for liver fibrosis [aOR=1.23 (95%CI 1.04-1.46); p=0.02] in multivariate models.

Conclusion: This study demonstrated the potential role of the plasmatic FA composition in the pathogenesis of liver fibrosis in PLWHA.

Keywords: Fatty acid composition; Liver fibrosis; NAFLD; HIV infection

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most frequent causes of chronic liver disease worldwide due to the strong relationship of this condition with metabolic features (YOUNOSSI et al., 2018). NAFLD can progress to advanced liver fibrosis, especially driven by presence of non-alcoholic steatohepatitis (NASH) that is characterized by lobular necro-inflammatory activity (ULLAH et al., 2019). The hypothesis of "multiple hits" has been proposed in the pathogenesis of NAFLD/NASH and progression to fibrosis/cirrhosis. Fat infiltration of hepatocytes (steatosis) is often referred as the "first hit" that might be followed by proinflammatory cytokine cascade activation, oxidative stress and apoptosis leading to liver fibrosis/cirrhosis ("multiple hits") (HEYENS et al., 2021).

Excessive accumulation of lipids in the liver has adverse effects on cell functions and it is termed lipotoxicity (PARK et al., 2010). Plasmatic composition of fatty acid (FA) has been used as a marker of altered liver metabolism because reflects endogenous FA metabolism, and is related to the dietary lipids. Plasmatic FA are the major mediator of liver steatosis. Increased levels of plasma FA, in addition of insulin resistance, are a crucial link between obesity (VERGANI, 2019). Recent studies demonstrated conflicting results about this topic. In a study with patients with NAFLD was demonstrated that individuals with advanced liver fibrosis had a higher percentage of palmitic, stearic and oleic FA in erythrocytes. Palmitic FA was considered an independent predictor for advanced fibrosis (CANSANÇÃO et al., 2018b). In another study comparing individuals with and without NAFLD showed that in plasma, total saturated fat and PUFA n-6 FA were positively related to NAFLD, while docosaehexanoic FA (PUFA n-3) was negatively correlated with the risk of NAFLD (ZHENG et al., 2012b).

Moreover, several studies have reported the burden of NAFLD and/or liver fibrosis in people living with HIV/AIDS (PLWHA) (AEPFELBACHER et al., 2019; PEMBROKE et al., 2017a; VUILLE-LESSARD et al., 2016). Many risk factors for NAFLD in PLWHA are similar to those in the general population, including obesity, insulin resistance, type-2 diabetes and dyslipidemia. However, additional mechanisms are hypothesized to contribute to a higher prevalence of NAFLD in PLWHA, such as higher visceral adipose tissue, long-term use of combined antiretroviral therapy (c-ART), inflammatory status and gut dysbiosis (YANAVICH et al., 2022; PERAZZO et al., 2018a; SOTI et al., 2018). Despite the extensive description of prevalence and risk factors for NAFLD and fibrosis, data on the relationship between those liver diseases and levels of plasmatic FA remains scarce in PLWHA.

Therefore, this study aimed to evaluate the relationship between of levels of plasmatic fatty acids and liver fibrosis in HIV mono-infected individuals.

METHODS

Study design and population

This case-control study used baseline data from the PROSPEC-HIV cohort study (NCT02542020) that has been conducted at Evandro Chagas National Institute of Infectious Diseases (INI/FIOCRUZ, Rio de Janeiro, Brazil) (PERAZZO et al., 2018a). PROSPEC-HIV study included adults with HIV infection from June 2015 to January 2019. For this analysis, we excluded participants with viral hepatitis co-infection defined by positive HCV-antibody or positive HBsAg; excessive alcohol consumption defined by the Alcohol Use Disorders Identification Test (AUDIT) score ≥8 (SAUNDERS et al., 1993); use of lipids supplements or missing laboratory/inconsistent data on dietary assessment. A sample size of 126 participants (63 cases and 63 controls) was achieved considering averages of the plasma concentration of fatty acids, a significance level of 5% (α=0.05) and power of 80% (β=0.80). Presence of liver fibrosis (METAVIR stage ≥ F2) was defined by liver stiffness measurement (LSM) ≥ 7.1 kPa or ≥ 6.2 kPa with probe M or XL, respectively. All participants with liver fibrosis enrolled in the PROSPEC-HIV study from June/2015 to January/2019 were included as cases (presence of fibrosis) in this analysis. Then, the control group (1:1) was randomly selected from participants without fibrosis from the same PROSPEC-HIV study population. This study was approved by the Ethical Committee from INI/FIOCRUZ (IRB 32889514.4.0000.5262). All participants signed an informed consent upon enrollment in the PROSPEC-HIV study.

Clinical assessment and HIV infection history

Age, sex at birth, self-report skin-color (TRAVASSOS et al., 2011), years of study and presence of co-morbidities were recorded as clinical data. Dyslipidemia, hypertension, type-2 diabetes and metabolic syndrome were defined according to the International Diabetes Federation (ALBERTI; ZIMMET; SHAW, 2006). Anthropometric measures, such as body mass index (BMI) and waist circumference, were measured by a trained operator. Body fat percentage was measured using a bioelectrical impedance analyzer (Biodynamics® 450, Sao Paulo, Brazil). The procedures performed are described elsewhere (DE ALMEIDA et al., 2021). Data regarding HIV infection history were available at the INI/FIOCRUZ HIV cohort: (i) date of first positive HIV antibody test; (ii) date of initiation of any antiretroviral drug; (iii)

dates of start and end of combined antiretroviral therapy (c-ART) and (iv) CD4 $^{+}$ T-lymphocyte count and HIV viral load from the closest day of the study visit.

Laboratory tests and transient elastography

Blood tests were performed in a centralized laboratory using an analyzer Dimension-RxL-Max (Siemens Healthcare Diagnostic, Illinois, USA). Enzymatic assay was used to measure liver tests, such as alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT). Glucose was measured using the hexokinase method; total and high-density lipoprotein (HDL)-cholesterol and triglycerides were determined using enzymatic methods. Insulin was determined using chemiluminescent immunoassay (CLIA) and the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated using the following formula: [fasting insulin (mIU/L)*fasting glucose (mg/dL)] / 405 (BOYKO; JENSEN, 2007).

Presence of liver fibrosis was defined by liver stiffness measurement (LSM) using transient elastography (TE) by FibroScan (EchoSens, Paris, France) performed by a single experienced (>2,000 examinations) operator (HP), as previously described (PERAZZO et al., 2018a). The LSM result was defined as a median of 10 valid measures and expressed in kPa. TE exams were considered as reliable for analysis if the following criteria had been met: (1) at least 10 valid measurements; (2) an interquartile range (IQR) lower than 30% of the median of LSM, and (3) a success rate of more than 60%. Presence of liver fibrosis (METAVIR stage F≥2) was defined by LSM ≥ 7.1 kPa or ≥ 6.2 kPa with M or XL probe, respectively (WONG et al., 2012).

Dietary data

The dietary intake of lipids and FA were evaluated by the 24-hour dietary recall (24-HDR) method in two non-consecutive days. The 24-HDR was applied using the Automated Multiple-Pass method to structure the interview and to increase the accuracy of the report (MOSHFEGH et al., 2008). Data of each food and/or beverage item reported by the participant were entered into a nutritional analysis software (Diet Win Professional Plus 3.0®package software), as previously described (DE ALMEIDA et al., 2021). Additionally, Multiple Source Method (MSM) was used to estimate the nutrients usual intake of participants and to correct the intrapersonal dietary variability (https://msm.dife.de). Nutrients were adjusted by energy density method expressed as a percentage (FREEDMAN et al., 2011).

Plasmatic fatty acid composition assessment

FA contents were analyzed by gas chromatography, using Agilent Technologies 7890A CG System equipment, equipped with flame ionization detector, coupled to the EZChrom Elite CDS program (Agilent Technologies, Inc., C.A., U.S.A). Plasma samples were submitted to lipid extraction, saponification and direct alkaline methylation by the method proposed by American Oil Chemist' Society AOCS 2b-11(AMERICAN OIL CHEMISTS' SOCIETY (AOCS), 2017).

Then, the samples were injected into the gas chromatograph equipment with a flame ionization detector. The methyl esters were separated into a fused silica capillary column SP-2560 of polysiloxane bis-cyanopropyl (Sulpeco Inc., PA, USA), with a length of 100m x 0.25μm x 0.20μm of internal diameter whose temperature programming was established at 100ºC with an increase rate of 3ºC/minute, reaching the temperature of 140ºC. The temperatures of the injector and detector were 250ºC. Gas flows used: (Linde Gases, RJ, BR) were 0.75 mL/min for drag gas (hydrogen); 25 mL/min for auxiliary gas (nitrogen); 30 mL/min and 400 mL/min for hydrogen and synthetic air of flame, respectively. FA methylated were identified based on the comparison with the relative retention time of the pattern peaks (Nu-Chek Prep. Inc., mixture of methyl esters 463) and the quantification of each FA was performed using the internal standard C13:0 (Sigma-Aldrich) as a reference. The assay generated data on 36 fatty acids and the fatty acid composition was expressed as percentage of the total fatty acids analyzed in plasma.

Statistical Analysis

PLWHA with liver fibrosis were considered as cases and controls (PLWHA without liver fibrosis) were randomly selected from the PROSPEC-HIV cohort. Categorical variables were reported as absolute (n) and relative frequency (%) and continuous variables as median and interquartile range (IQR). We used Chi-square and Mann-Whitney tests to compare proportions and medians, respectively. Plasmatic FA were analyzed in proportion (%) of total FA and all nutrients in energy intake (E%). Direct Acyclic Graphs (DAGs) were created with assumptions on the relationship among co-variables and outcome to support our decision about the most parsimonious models for liver to avoid collinearity and select confounders (DE ALMEIDA et al., 2021). Logistic multivariate regression models considered plasmatic fatty acids as independent variables and presence/absence of liver fibrosis as outcome; and multivariate models were adjusted for age, sex, duration of c-ART. Statistical analyses were performed using R version 3.6.3 and considering p-values < 0.05 as statistically significant.

RESULTS

Initially, there were 727 eligible participants with HIV infection in the PROSPEC-HIV study from June 2015 to January 2019. Participants were excluded due to viral hepatitis coinfection (n=95), abusive alcohol intake (n=123), use of lipid supplement (n=6), unreliable for liver fibrosis assessment (n=8) or missing data of analysis laboratory (n=6). Therefore, 489 participants were eligible for this case-control analysis. A total of 71 participants (14.5%) among those eligible had liver fibrosis. Thus, we analyzed data from 142 participants, 71 controls and 71 cases (Figure 1). Overall, 62% were female, median (IQR) age was 46 (37-53) years and median BMI was 26.7 (23.3-30.1) Kg/m^2; 14.8% had type-2 diabetes, 28.2% had hypertension and lipodystrophy was present in 17.6% of participants. Participants had a median (IQR) HIV infection duration of 8.5 (4.5-14.8) years, median CD4+ t-lymphocyte count of 665 (477-861) $cells/m^3$ and 96.5% were under c-ART (Table 1). Participants with liver fibrosis were significantly older [median age; 47 (40 -56) vs 43 (IQR, 33-52) years; p =0.009], and had higher levels of ALT, AST and GGT compared to controls. Additionally, participants with liver fibrosis had higher BMI [29 (23.7-32.7) vs 25.9 (23-28.3) Kg/m2; p=0.005] and higher proportion of metabolic features, such as type-2 diabetes (22.5% vs 7%, p=0.009) and hypertension (40.8% vs 15.5%, p<0.001) when compared with controls. On the other hand, there was no differences in HIV characteristics between cases and controls (Table 1).

Assessment of dietary intake

Table 2 describes the dietary intake by cases and controls. PLWHA with fibrosis (cases) had higher ingestion of protein compared to those without fibrosis (controls) [Median (IQR) 17.1% (14.8-18.9) vs 16% (13.9-17.1); p=0.041]. There were no difference on fats and fatty acids intake between cases and controls participants.

Plasmatic fatty acid

Table 3 reports the median plasmatic levels of fatty acids according to presence or absence of liver fibrosis. Participants with liver fibrosis had higher levels of palmitc acid (16:0) compared to those without fibrosis [23 %(IQR, 20.8-24.8) vs 21.8% (20.8-23.1); p=0.006]. Similarly, saturated FA (SFA) were higher in cases compared to controls [31.7% (30-33.9) vs 30.8% (20.9-32.2); p=0.041]. On the other hand, there was no significant difference in plasmatic levels of others FA between cases and controls.

A higher percentage of plasmatic palmitc acid (16:0) was associated with presence of liver fibrosis [OR=1.26 (1.06-1.49), p=0.010] and this association has remained after adjustment for confounding factors [aOR=1.23 (95%CI 1.04-1.46); p=0.02]. Furthermore, an increase in the higher percentage of total plasmatic SFA was significant associated with liver fibrosis on univariate analysis [OR=1.15 (95%CI 1-1.32); p=0.04], but this association disappeared after adjustment (Table 4).

DISCUSSION

This study is characterized by the plasmatic levels of FA in PLWHA with and without liver fibrosis. In addition, the present study highlighted the association of higher percentages of saturated FA, specifically the association of palmitic acid with liver fibrosis. To the best of our knowledge, this is the one of the first studies that evaluated plasmatic FA according to presence/absence of liver fibrosis in PLWHA.

Increased circulating free FAs could be a major cause of hepatic lipotoxicity that might lead to hepatocytes injury (HLIWA et al., 2021). In present study, we showed that a higher percentage of plasmatic saturated FA was associated with presence of liver fibrosis. This result is aligned with the study from Shim et al. that evaluated plasmatic levels of FA in uninfected people with fatty liver disease detected by abdominal ultrasound (SHIM; CHOI; PARK, 2017). This might be explained by cellular apoptosis caused by toxicity related to SFA accumulation in hepatocytes (HLIWA et al., 2021). Moreover, an in vitro study demonstrated that SFAs have induce proinflammatory cytokines and insulin resistance (GOLDBERG; GINSBERG, 2006).

Palmitic acid (C16:00), a saturated FA mostly intrinsically produced, has an important role in the pathogenesis of liver injury. Palmitic acid is the first fatty acid synthesized and this fatty is precursor to longer fatty acids. This FA may be elongated to stearic acid (C18:00) or to longer fatty acids. Both FA C16:00 and C18:00 may be desaturated to form palmitolate (C16:1) and oleate (C18:1), respectively (MATO et al., 2019; SOFTIC; COHEN; KAHN, 2016). Unsaturated FA such C18:01 are preferentially utilized on triglyceride synthesis, thereby protects against palmitate-induced lipotoxicity (LISTENBERGER et al., 2003). Therefore, palmitic acid might be considered a potential candidate biomarker for de novo lipogenesis (KAWANO; COHEN, 2013). Additionally, palmitic FA remains a cornerstone for inflammasome activation, leads to hepatic stellate cells activation and production of IL-1α and IL-1β in Kupffer cells (DUAN; LIU; WU, 2017; MIURA et al., 2013; JOSHI-BARVE et al., 2007). In the present study, we demonstrated that higher levels of palmitic FA in plasm

was independently associated with the presence of liver fibrosis. This result is aligned with previous studies that included uninfected individuals. Cansanção et al. demonstrated that a higher percentage of palmitic FA in red blood cells was associated with liver disease progression using TE as the reference in Brazilian individuals with NAFLD. However, those authors also reported that the proportion of stearic acid, oleic acid and total MUFA was also significantly increased in participants with advanced liver fibrosis (LSM ≥ 7.9 kPa) (CANSANÇÃO et al., 2018b). In a study with NAFLD-biopsy proven using FA composition in plasma cholesteryl esters, was demonstrated that those subjects with NASH had significantly higher levels of palmitic acid, palmitoleic acid, oleic acid and γ- linoleic compared to those without. Furthermore, higher palmitic acid levels were associated with a higher NAFLD activity score (NAS) (PARK et al., 2010).

Despite several studies reporting the relationship between palmitic acid and liver fibrosis in uninfected individuals (PURI et al., 2009; SHIM; CHOI; PARK, 2017), data regarding this relation in PLWHA remains scarce. A limited sample size cross-sectional study compared fatty acid composition in erythrocytes between 20 HIV-infected men with NAFLD, 21 uninfected men with NAFLD and 7 healthy controls (uninfected HIV without NAFLD). This study demonstrated that higher proportion of palmitic FA in participants HIV/NAFLD compared to controls (M ARENDT et al., 2011). However, differently of our findings, those authors showed a significant lower plasmatic proportion of n-3 PUFA in participants HIV/NAFLD compared to controls. This fact might be explained because this fatty acid is associated with an inflammatory and immunological process present in the pathogenesis of HIV infection (DE ALMEIDA et al., 2021; BOWMAN et al., 2019).

In present study, we did not observe a significant association between lipids ingestion and presence of liver fibrosis. We are aware that only 15% of the circulating FA are derived from diet in people with NAFLD (VERGANI, 2019).Therefore, our hypothesis remains on the fact that the profile of plasm FA, probably, was influenced by endogenous metabolism (*de novo* lipogenesis) (LEE et al., 2015). NAFLD subjects often show an increase in *de novo* lipogenesis, and is a major pathway in the pathogenesis of NAFLD and NASH. This may be an adaptive mechanism for the generation of metabolic signals that direct lipids toward beneficial pathways to improve energy balance even in the setting of excess FA accumulation, a concept known as lipoexpediency (MATO et al., 2019).

On the other hand, we reported that median usual intake of protein was higher in HIV individuals with liver fibrosis compared to controls. Soleimani et al demonstrated that higher intake of red meat was associated with liver fibrosis in uninfected individuals (SOLEIMANI

et al., 2019). This relationship can be explained by the fact that high protein intake might be associated with insulin resistance and oxidative stress (DEHGHANSERESHT et al., 2020). The iron content of red meats can generate free radicals, that might cause cellular and tissue damage (MEHTA; FARNAUD; SHARP, 2019). Moreover, a higher protein diet might potentially lead to gut microbiome dysbiosis and pro-inflammatory status. A high-protein diet affects the gut microbiome, because it favors a potentially pathogenic and proinflammatory microbiota profile which increases ammonia, phenols and hydrogen sulfide concentrations that induce mucosal inflammation. Indeed, the dysregulation of gut endothelial barrier function that allows for the translocation of bacterial components and leads to hepatic inflammation (MOKHTARI; GIBSON; HEKMATDOOST, 2017).

The major limitations of our study were the absence of a HIV-uninfected control group and the lack of liver biopsy as the reference for the presence of the liver fibrosis. We used data from the PROSPEC-HIV cohort study, which included PLWHA. Moreover, we defined presence of the liver fibrosis using an extensive validated non-invasive tool, as transient elastography by Fibroscan. Another limitation is the unbalance between cases and controls regarding BMI. However, it reflects the clinical characteristics of the population. The present analysis included all participants identified as having liver fibrosis at baseline visit of the PROSPEC-HIV cohort (n=71) and controls (1:1) were randomly selected among those participants without liver fibrosis. Furthermore, our final sample size (n=142) was higher than that calculated (n=126). The mains strengths of this study remain the fact that we evaluated the relationship of plasmatic FA composition in a well-characterized sample of people with HIV mono-infection and the quality of fatty acid composition determination. Additionally, all TE exams were performed for a single experimented operator and all blood analyses were performed in a centralized laboratory.

CONCLUSION

In conclusion, this study demonstrated the role of the saturated FA, especially palmitic FA, associated with liver fibrosis. These results reinforce the importance of the quality of the lipids in pathogenesis of liver fibrosis in this population. Therefore, those findings emphasize the importance of nutrition in prevention and treatment of liver fibrosis in PLWHA.

Abbreviations

ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; AUDIT, Alcohol Use Disorders Identification Test; BMI, body mass index; CI, confidence interval; E%, energy per cent; FA, Fatty acid; GGT, gamma-glutamyltransferase; 24-HDR, 24-hour dietary recall; HDL, high-density cholesterol; IQR, interquartile range; LDL, low-density cholesterol; LSM, liver stiffness measurement; LSM, liver stiffness measurement; MUFA, mono-unsaturated FA; NAFLD, non-alcoholic fatty liver disease; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OR, odds ratio; PLWHA, people living hiv aids; PUFA, poly-unsaturated FA; Q, quartile; ULN, upper limit of normal, SFA, saturated FA; TE, transient elastography and WHO, World Health Organization.

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Table 1. Characteristics of participants with HIV mono-infection by presence/absence of liver fibrosis (METAVIR stage F≥2) [LSM ≥ 7.1 kPa or ≥ 6.2 kPa with M or XL probe].

	All (n=142)	**Controls (n=71) [absence of liver fibrosis]**	**Cases (n=71) [presence of liver fibrosis]**	**P value**
Social and demographic				
Female gender[a]	88 (62)	47 (66.2)	41 (57.7)	0.387
Age, years[b]	46 (37-53)	43 (33-52)	47 (40 -56)	**0.009**
Skin color[a]				
White	63 (44.4)	31 (43.7)	32 (45.1)	0.694
Brown	45 (31.7)	25 (35.2)	20 (28.2)	
Black	33 (23.2)	15 (21.1)	18 (25.4)	
Others	1 (0.7)	0 (0)	1 (1.4)	
Education [a] <8 years of study	63 (44.7)	28 (39.4)	35 (50)	0.275
Biochemistry				
ALT, IU/L[b]	31 (23-44)	27 (23-40)	35 (24.5-54)	**0.014**
AST, IU/L[b]	25 (20-32)	23 (19.5-28.5)	27 (21.5-37.5)	< **0.001**
Alkaline phosphatase, IU/L[b]	91 (75-109)	91 (78-102.5)	90 (71.2-111)	0.833

GGT, IU/L[b]	45 (32.2-84)	40 (31-62.5)	53 (35.5-114.5)	**0.003**
Total cholesterol, mg/dL[b]	182 (157-209.8)	185 (162-213)	176 (153.5-203.5)	0.119
LDL - cholesterol, mg/dL[b]	112 (87.5-132)	115 (91.5-138)	103 (80-125.5)	0.103
HDL - cholesterol, mg/dL[b]	43 (37-54)	44 (38.5-57.5)	42.5 (35-52.8)	0.163
Triglycerides, mg/dL[b]	123.5 (84-171)	110 (87.5-138.5)	140 (80-184.5)	0.074
Fasting glucose, mg/dL[b]	94 (89-102)	92 (88-98)	95 (89.5-103)	0.085
Insulin, um/L	11.1 (7.8-15.9)	10 (8.1-13)	12.4 (7.5-19.7)	0.127
HOMA-IR	2.5 (1.7-3.8)	2.5 (1.9-3.3)	3.1 (1.6-4.6)	0.149
Nutritional Status				
BMI(kg/m^2) [b]	26.7 (23.3-30.1)	25.9 (23-28.3)	29 (23.7-32.7)	**0.005**
BMI categories				**< 0.001**
Lean [< 25 Kg/m^2][a]	55 (38.7)	31 (43.7)	24 (33.8)	
Overweight [25 – 29,99 Kg/m^2] [a]	50 (35.2)	32 (45.1)	18 (25.4)	
Obesity [≥ 30 Kg/m^2][a]	37 (26.1)	8 (11.3)	29 (40.8)	
Body fat percentage[b]	31.1 (24.5-36)	30.4 (25.2-34.6)	31.6 (23.7-37.6)	0.483
Waist circumference (cm)[b]	87 (79-98)	84 (78.8 - 90)	91 (82.5-104.5)	**< 0.001**
Comorbidities				
Diabetes mellitus[a]	21 (14.8)	5 (7)	16 (22.5)	**0.009**
Hypertension[a]	40 (28.2)	11 (15.5)	29 (40.8)	**< 0.001**
Dyslipidaemia[a]	25 (17.6)	9 (12.7)	16 (22.5)	0.123
Metabolic Syndrome[a]	54 (38.6)	22 (31.4)	32 (45.7)	0.083
Lipodystrofy[a]	25 (17.6)	7 (9.9)	18 (25.4)	**0.015**
HIV history				
Duration of hiv, years[b]	8.5 (4.5-14.8)	7.4 (4.9-13.4)	10.5 (4.4-15.6)	0.260
CD4 count (cells/m^3)[b]	655 (477-861)	670 (547-860)	651 (417-843)	0.200
CD4 count (<200cells/m3)[a]	4 (2.8)	0 (0)	4 (5.7)	0.058
Detectable HIV viral load (>40copies/m^3)[a]	25 (17.7)	9 (12.7)	16 (22.9)	0.173
Nadir CD4 count <100 cells/m^3[b]	48 (33.8)	20 (28.2)	28 (39.4)	0.214
Current ART use[a]	137 (96.5)	69 (97.2)	68 (95.8)	1
Duration of ART, years[b]	6.7 (3-14.3)	5.7 (3.1-10.3)	10.1 (3.1-15.1)	0.136

Data expressed as (a) absolute (%) or (b) median [IQR]. Controls were randomly selected (1:1) among participants with liver fibrosis from the PROSPEC-HIV cohort. ALT, alanine aminotransferase; ART, antiretroviral therapy; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein;

Table 2. Dietary intake by presence/absence of liver fibrosis (METAVIR stage F≥2) [LSM ≥ 7.1 kPa or ≥ 6.2 kPa with M or XL probe] in participants with HIV mono-infection

	All (n=142)	**Controls (n=71) [absence of liver fibrosis]**	**Cases (n=71) [presence of liver fibrosis]**	**P value**
Dietary intake (% E)				
Energy (kcal)	1896.4 (1541.7-2192.8)	1930.7 (1549.8-2248.2)	1874.4 (1533.2-2177.5)	0.541
Carbohydrate	53.1 (49.2-56.7)	53.2 (48.6-56.7)	53.1 (49.9-56.5)	0.581
Protein	16.4 (14.6-18.2)	16 (13.9-17.1)	17.1 (14.8-18.9)	**0.041**
Fibre	10.3 (8.5-11.9)	10.1 (8.6-11.9)	10.5 (8.4-12)	0.357
Saturate fat	9.5 (8.3-10.5)	9.6 (8.5-10.5)	9.2 (8.1-10.3)	0.358
PUFA fat	7.3 (6.2-8.5)	7.5 (5.8-8.6)	7.1 (6.3-8.4)	0.721
MUFA fat	8.3 (7.4-9.5)	8.3 (7.5-9.6)	8.4 (7.3-9.3)	0,840
Trans fatty acid	0.4 (0.3-0.5)	0.4 (0.3-0.4)	0.4 (0.3-0.5)	0.099
Cholesterol	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.701
n-6 PUFA	4.5 (3.6-5.5)	4.4 (3.5-5.6)	4.5 (3.6-5.3)	0.878
n-3 PUFA	0.6 (0.4-0.7)	0.6 (0.4-0.7)	0.6 (0.4-0.7)	0.898
Lauric fatty acid (12:00)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.304
Myristic fatty acic (14:00)	2.9 (2-4.9)	3 (2.1-4.8)	2.9 (2-5.1)	0.987
Palmitc fatty acid (16:00)	3 (2.5-3.4)	3 (2.5-3.4)	2.9 (2.5-3.5)	0.738
Stearic fatty acid (18:00)	1.2 (1-1.5)	1.2 (1-1.4)	1.3 (1-1.6)	0.465
Palmitoleic fatty acid (16:1)	0.2 (0.2-0.2)	0.2 (0.2-0.3)	0.2 (0.2-0.2)	0.496
Oleic fatty acid (18:1)	5 (4.2-5.9)	5 (4.2-5.8)	4.8 (4.1-6.1)	0.872
Linoleic fatty acid (18:2-n6)	4.5 (3.6-5.4)	4.4 (3.5-5.6)	4.5 (3.6-5.2)	0.862
Linolenic fatty acid (18:3-n3)	0.6 (0.4-0.7)	0.5 (0.4-0.7)	0.6 (0.4-0.7)	0.886
n6/n3 ratio	8.3 (7.5-9.3)	8.3 (7.5-9.5)	8.3 (7.5-9.2)	0.490

Data expressed as median [IQR]. Controls were randomly selected (1:1) among participants with liver fibrosis from the PROSPEC-HIV cohort. E%, energy percent; FA, fatty acid; FA, fatty acid; LSM,

liver stiffness measurement; MUFA, mono-unsaturated FA; PUFA, poly-unsaturated FA and SFA, saturated FA.

Table 3. Plasmatic fatty acid composition in participants with HIV mono-infection of cases and controls considering presence/absence of liver fibrosis (METAVIR stage F≥2) [LSM ≥ 7.1 kPa or ≥ 6.2 kPa with M or XL probe].

	All (n=142)	**Controls (n=71) [absence of liver fibrosis]**	**Cases (n=71) [presence of liver fibrosis]**	**P value**
FA (% of total fatty acids)				
C14:0 (miristic acid)	1 (0.9-1.4)	1 (0.9-1.3)	1 (0.8-1.4)	0.637
C16:0 (palmitic acid)	22.2 (20.8-23.8)	21.8 (20.8-23.1)	23 (20.8-24.8)	**0.006**
C18:0 (stearic acid)	6.4 (5.6-7.1)	6.5 (5.7-7.1)	6.2 (5.6-7.1)	0.794
C20:0 (arachidic acid)	0.6 (0.5-0.8)	0.6 (0.5-0.8)	0.6 (0.4-0.8)	0.136
Σ SFA	31.1 (29.4-33.1)	30.8 (29.4-32.2)	31.7 (30-33.9)	**0.041**
C14:1 9c (miristoleic acid)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.1 (0.1-0.1)	0,230
C16:1 9c n-7 (palmitoleic acid)	1.9 (1.5-2.7)	1.9 (1.5-2.6)	2 (1.4-2.7)	0,98
C18:1 9c n-9 (oleic acid)	17.5 (15.6-19)	17.6 (15.6-19.1)	17.3 (15.6-18.9)	0.838
Σ MUFA	23.2 (21.5-25.3)	23.8 (21.6-25)	22.9 (21.4-25.6)	0.757
C18:2 n-6 (linoleic acid)	31.2 (27.1-34.3)	31.1 (27.3-34.1)	31.5 (26.5-34.6)	0.94
C18:3 n-6 (γ -linoleic acid)	0.1 (0-0.1)	0.1 (0-0.1)	0.1 (0-0.1)	0.231
C18:3 n-3 (α-linoleic acid)	0.9 (0.7-1)	0.8 (0.7-1)	0.9 (0.7-1)	0.98
C20:4 5c n-6 (arachidonic acid)	8.1 (6.7-9.6)	8.4 (7.1-9.8)	7.6 (6.4-9.3)	0.066
C20:5 n-3 (eicosapentaenoic acid)	0.5 (0.4-0.6)	0.5 (0.4-0.7)	0.5 (0.4-0.6)	0.329
C22:6 n-3 (docosahexaenoic acid)	1.3 (1-1.6)	1.3 (1-1.6)	1.3 (1.1-1.6)	0.639
Σ PUFA	46 (41.3-48.7)	46.4 (42.3-48.1)	45.6 (40.8-49.5)	0.23
Σ Trans	0.9 (0.8-1.1)	1 (0.8-1.1)	0.9 (0.8-1)	0.139
n-6	40.1 (35.8-43.1)	40.3 (36.7-42.7)	39.5 (35.3-44.2)	0.283
n-3	3.1 (2.7-3.6)	3 (2.7-3.5)	3.1 (2.8-3.7)	0.555
(n-6):(n3)	12.9 (10.8-14.7)	13.1 (10.8-14.9)	12.8 (10.6-14.4)	0.315

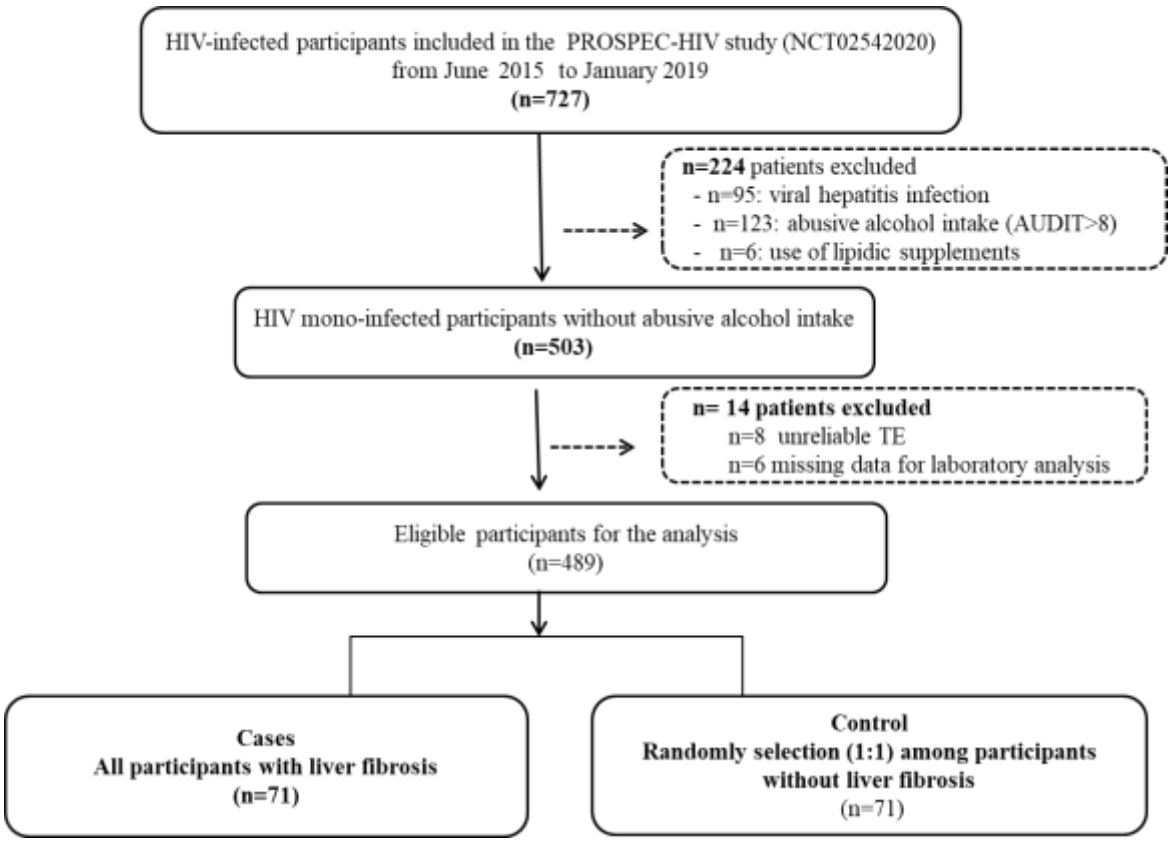
Data expressed as median [IQR]. Controls were randomly selected (1:1) among participants with liver fibrosis from the PROSPEC-HIV cohort. FA, fatty acid; FA, fatty acid; LSM, liver stiffness measurement; MUFA, mono-unsaturated FA; PUFA, poly-unsaturated FA and SFA, saturated FA

Table 4. Logistic regression considering plasmatic fatty acid and presence (cases; n=71) / absence (controls; n=71) of liver fibrosis (METAVIR stage F≥2) [LSM ≥ 7.1 kPa or ≥ 6.2 kPa with M or XL probe] in participants with HIV mono-infection (N= 142, controls= 71 and cases=71)- INI/FIOCRUZ. Rio de Janeiro, Brazil.

	OR [95%IC]	**P value**	**aOR [95%IC]**	**P value**
FA (% of total fatty acids)				
C14:0 (miristic acid)	1.01 (0.47-2.18)	0.98	0.78 (0.34-1.78)	0.55
C16:0 (palmitic acid)	1.26 (1.06-1.49)	**0.01**	1.23 (1.04-1.46)	**0.02**
C18:0 (stearic acid)	0.96 (0.71-1.3)	0.79	0.98 (0.72-1.34)	0,90
C20:0 (arachidic acid)	0.48 (0.16-1.45)	0.19	0.37 (0.11-1.19)	0,10
Σ SFA	1.15 (1-1.32)	**0.04**	1.13 (0.98-1.3)	0.09
C14:1 9c (miristoleic acid)	0.03 (0-21.14)	0,30	0.02 (0-17.8)	0.26
C16:1 9c (palmitoleic acid)	1.08 (0.72-1.63)	0.72	0.95 (0.61-1.48)	0.82
C18:1 9c (oleic acid)	1.01 (0.9-1.14)	0.84	0.93 (0.82-1.07)	0.32
Σ MUFA	1.04 (0.98-1.11)	0.23	1.01 (0.94-1.08)	0.77
C18:2 n-6 (linoleic acid)	1 (0.93-1.07)	0.94	1.03 (0.96-1.11)	0,40
C18:3 n-3 (α-linoleic acid)	0.72 (0.18-2.91)	0.65	0.85 (0.19-3.71)	0.83
C20:4 5c (arachidonic acid)	0.87 (0.75-1)	0.05	0.88 (0.76-1.03)	0.11
C20:5 n-3 (eicosapentaenoic acid)	1.57 (0.44-5.65)	0.49	1.02 (0.26-4.03)	0.98
C22:6 n-3 (docosahexaenoic acid)	1.2 (0.71-2.05)	0.49	1.09 (0.63-1.91)	0.75
Σ PUFA	2.5 (0.03-230.37)	0.69	0.86 (0.01-100.52)	0.95
Σ Trans	0.25 (0.04-1.45)	0.12	0.21 (0.03-1.35)	0,10
n6	0.97 (0.91-1.03)	0.28	1 (0.93-1.07)	0.93
n3	1.11 (0.76-1.62)	0.61	1.02 (0.68-1.53)	0.92
(n-6):(n3)	0.94 (0.85-1.03)	0.19	0.97 (0.88-1.06)	0.48

*Multivariate logistic regression models adjusted by age, gender and duration of c-ART. ART, antiretroviral therapy. ; FA, fatty acid; LSM, liver stiffness measures; MUFA, mono-unsaturated FA; PUFA, poly-unsaturated FA and SFA, saturated FA.

Figure 1. Flow-chart of the study



4. CONCLUSÃO

Esses resultados reforçaram a importância dos lipídeos na patogênese da DHGNA e/ou fibrose hepática em PVHA. Foi demonstrado que uma maior ingestão de gordura total aumentou o risco de DHGNA, e AG específicos foram associados a menores e/ou maiores chances de presença de doenças hepáticas em PVHA. Demonstrando assim que não só a quantidade de lipídeo dietético deve ser monitorada, mas também a qualidade dos diferentes tipos de lipídeos ingeridos. Além disso, foi demonstrado uma maior concentração plasmática de AG saturados, especialmente o AG palmítico em indivíduos com fibrose hepática. Podendo este este fato estar associado com o aumento da neolipogenese, via metabólica muito importante na DHGNA. Sendo assim, estes resultados reforçam o papel de destaque da intervenção nutricional como ferramenta estratégica na atenção a saúde desta população.

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