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FACULDADE DE MEDICINA

"Fatty liver disease: Relevance of nutritional determinants and nutritional care in the general population and in HIV patients"

Sara Raquel Osório Policarpo

Orientadores:

Prof.ª Doutora Helena Maria Ramos Marques Coelho Cortez-Pinto

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Tese especialmente elaborada para obtenção do grau de Doutor em Ciências e Tecnologias da Saúde, Ramo de Nutrição

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A impressão desta tese foi aprovada pelo Conselho Científico da Faculdade de Medicina de Lisboa em reunião de 18 de julho de 2023.

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‘Be curious. And however difficult life may seem there is always something you can do and succeed at. It matters that you just don´t give up.’

Stephen Hawking

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O caminho percorrido nestes quatro anos e meio foi duro, cheio de percalços e com bastantes desvios do trilho original. Não teria sido possível chegar até aqui sem o imenso contributo de algumas pessoas.

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PREAMBLE

I came across my two fields of interest in clinical practice, infectious diseases and hepatology, quite accidentally.

Since my first internship, during my bachelor's degree, I have dedicated myself to the field of clinical nutrition. In Hospital Santa Cruz, I had the opportunity to be the sole responsible for nutritional support in the unit of renal care to patients with Human Immunodeficiency Virus (HIV), and since then I have dedicated my clinical practice to this group of patients, having conducted my bachelor (2009) and master´s degree dissertations (2014) on this topic.

After the master´s degree, I was privileged to be invited as a faculty member in Laboratório de Nutrição, Faculdade de Medicina da Universidade de Lisboa, coordinated by Professor Helena Cortez-Pinto.

When developing a project for nutritional care of patients co-infected with HIV and Hepatitis C virus, my interest in the field of hepatology grew and I was fortunate to be invited, by Professor Helena Cortez-Pinto, as a member of a multidisciplinary consultation dedicated to fatty liver disease to be initiated in the outpatient hepatology clinic (2016). This allowed me to substantially increase my knowledge in this field and be aware of some gaps in the nutritional management of these patients, observing major problems that could generate research questions.

Coupling my research interest, and my areas of expertise in clinical practice, conducting a PhD in the field of hepatology and HIV was a bonus, so I initiated my PhD work at the end of 2018, eager to contribute to the scientific community and patients' quality of nutritional care.

The main aim of the initial project was to characterize the prevalence of non-alcoholic fatty liver disease in HIV patients, determine risk factors associated (related and not related with HIV infection) and conduct a randomized dietary intervention trial, to evaluate if the dietary management established for NAFLD patients suited HIV patients and led to the same improvements in clinical outcomes.

Conducting a PhD while working full time as a clinical dietitian and teaching was especially challenging, but field work was always exciting for me, a way of interacting with patients and improving my clinical practice.

Unfortunately, in 2020, mid-way through my intervention trial, Covid-19 happened and made it impossible to pursue the goals that had initially been established.

After much consideration and anticipating that Covid-19 restrictions in clinical practice were to be maintained, the initial project was redesigned, and the dietary intervention trial and screening of new patients was terminated. That obliged me to rethink the project and come up with new innovative ways of addressing relevant questions, including the comparison of the HIV cohort with a nationwide group and the topic of metabolic fatty liver disease (MAFLD), a new nomenclature recently proposed.

As such, apart from other publications and communications that I have authored within this period (as listed in my CV), the following list of original publications are part of this thesis:

Study 1: Policarpo S, Machado MV, Barreira D, Cortez-Pinto H. NAFLD Nutritional Management: Results from a Multidisciplinary Approach GE Port J Gastroenterol. 2021 Dec 1;29(6):401-408. doi: 10.1159/000519932. eCollection 2022 Nov (Quartile 3).

Study 2: Policarpo S, Machado MV, Medeiros FC, Carvalhana S, Leitão J, Cortez-Pinto H. Dietary intake as a risk factor for NAFLD in HIV patients: a comparison with a nationwide cohort (submitted to JAIDS – Quartile 1)

Study 3: Policarpo S. Machado MV, Cortez-Pinto H. Telemedicine as a tool for dietary intervention in NAFLD-HIV patients during the COVID-19 lockdown: A randomized controlled trial Clin Nutr ESPEN. 2021 Jun; 43:329-334. Doi: 10.1016 (Quartile 2)

Study 4: Policarpo S, Carvalhana S, Craciun A, Crespo RR, Cortez-Pinto H. Do MAFLD Patients with Harmful Alcohol Consumption Have a Different Dietary Intake? Nutrients. 2022 Apr; 14(7): 1335 (Quartile 1)

The present thesis is composed by four main chapters: 1. Introduction: theoretical background reviewing the most relevant evidence about the main topics of this work; 2. Aims: general and specific goals of this thesis; 3. Studies, the four studies cited above; 4. General discussion and conclusions, considering the main findings of each study and discussing the overall picture obtained here.

The field of NAFLD, and specially NAFLD coupled with HIV still has unanswered questions, particularly related to nutrition. I do hope I will be able to contribute with answers. In fact, I do believe that the work conducted in the last four years was of interest and raised awareness in the scientific community, allowing nutrition to be acknowledged in the management of these patients.

RESUMO

A presente tese caracteriza o estado clínico e nutricional dos doentes com doença hepática esteatósica, sob a definição clássica "Fígado Gordo não-alcoólico (FGNA)" e sob a recentemente proposta nova nomenclatura " Fígado Gordo associado a fatores metabólicos (MAFLD)", Adicionalmente, descreve os resultados e o impacto de uma intervenção nutricional sobre parâmetros antropométricos e clínicos em doentes com FGNA, com recurso a uma intervenção nutricional com métodos convencionais e com métodos remotos (telemedicina). Finalmente, é estudada a interação entre a infeção pelo vírus da imunodeficiência humana (VIH), o FGNA e o estado nutricional.

Estes estudos resultaram nas quatro publicações que incorporam esta tese.

O **estudo 1** reflete os resultados de uma intervenção nutricional em doentes com FGNA, gerida por uma equipa multidisciplinar numa intervenção direcionada para o estilo de vida durante 12 meses, onde demonstrámos que uma abordagem multidisciplinar baseada na modificação para um estilo de dieta mediterrânea, foi eficaz na perda de peso (PP), com 93,7% com PP aos 3 meses, 81,7% com PP aos 6 meses e 72,5% com PP aos 12 meses. Esta intervenção foi também eficaz na manutenção do peso perdido aos 12 meses, com 9,6% dos doentes a apresentarem uma PP superior a 10% aos12 meses.

O **estudo 2** descreve os fatores de risco, apresentação clínica, características e ingestão dietética de pacientes com infeção por VIH rastreados para FGNA em comparação com uma coorte de âmbito nacional. O grupo de doentes com VIH apresentou menos comorbilidades metabólicas como DMT2 e hipertensão arterial, mas maior prevalência de dislipidemia. Não obstante, a prevalência de FGNA foi superior no grupo VIH em comparação com a coorte nacional. Em conjunto com os fatores metabólicos, uma dieta de baixa qualidade nutricional, contribui para o desenvolvimento de FGNA em pacientes com VIH.

O **estudo 3** ilustra os resultados de um ensaio de intervenção nutricional realizado em doentes com VIH e FGNA usando a telemedicina, incluindo a descrição do impacto na perda de peso durante o encerramento nacional devido

à Covid-19. Observámos que o grupo de intervenção ganhou menos peso do que aquele que receberam apenas recomendações dietéticas gerais. Adicionalmente, este grupo, em oposição ao grupo de intervenção, também teve uma diminuição significativa na qualidade global do seu padrão alimentar, com aumento do apetite e da frequência de snacks. Este estudo demonstrou pela primeira vez, a eficácia de intervenções no estilo de vida com acompanhamento regular para atingir PP em doentes com VIH e FGNA. Além disso, devido às restrições do Covid-19, este também foi um estudo pioneiro no que diz respeito à aplicação da telemedicina a intervenções dietéticas e à caracterização de hábitos alimentares durante um bloqueio nacional.

O **estudo 4** caracteriza a ingestão alimentar e o teor inflamatório da dieta dos doentes com MAFLD explorando a apresentação clínica com base no consumo de álcool. Neste estudo observámos que o grupo com ingestão de álcool apresentou maior ingestão energética, de hidratos de carbono, carnes vermelhas, sobremesas doces, açúcar de adição e bebidas açucaradas. No grupo sem ingestão de álcool, uma ingestão alimentar mais pró-inflamatória foi associada a um aumento de 4 vezes na probabilidade de esteatose grave. No grupo com consumo excessivo de álcool, uma ingestão alimentar mais pró-inflamatória foi associada a um maior grau de fibrose, com todos os doentes com fibrose avançada a apresentarem um padrão de ingestão alimentar pró-inflamatória.

Em conclusão, consideramos que estes estudos no seu conjunto, contribuem para uma melhor caracterização dos doentes com doença hepática esteatósica, no que respeita ao estado nutricional, ingestão alimentar, bem como a relação entre estes dois fatores e apresentação da doença. Contribuem ainda para o conhecimento dos resultados de uma intervenção nutricional personalizada, quer na população em geral quer em populações específicas, tais como as pessoas que vivem com VIH.

Os nossos resultados podem traduzir-se numa melhor gestão de uma doença que é cada vez mais prevalente na prática clínica, em grande medida devido ao ambiente obesogénico.

PALAVRAS-CHAVE intervenção nutricional, ingestão alimentar, estado nutricional, FGNA, MAFLD

ABSTRACT

The present thesis characterizes the clinical and nutritional status of fatty liver disease patients, under the classic “Non-alcoholic fatty liver disease (NAFLD)” definition and the recently new proposed definition “Metabolic associated fatty liver disease (MAFLD)”. Furthermore, it describes the results and the impact of a dietary intervention on anthropometric and clinical parameters in NAFLD patients, through conventional and telemedicine methods. Finally, the interaction between Human Immunodeficiency virus (HIV) infection and NAFLD is studied.

These studies resulted in the four publications that incorporate this thesis.

Study 1 reflects the results of a dietary intervention in NAFLD patients from the general population managed on a combined 12-month lifestyle intervention by a multidisciplinary team, where we demonstrated that a multidisciplinary approach based on a modified Mediterranean diet, was effective in inducing weight loss (WL) with 93.7% of patients presenting WL at 3 months, 81.7% at 6 months and 72.5% at 12 months and maintaining that WL during the 12-month follow-up, with 9.6% of patients presenting WL higher than 10% at 12 months.

Study 2 describes the risk factors, clinical presentation, characteristics, and dietary intake of fatty liver disease patients screened within an HIV cohort in comparison with a nationwide cohort. The group of HIV patients presented less metabolic disturbances such as T2DM and HBP, but higher frequency of dyslipidaemia. Despite this, the prevalence of NAFLD was higher in the HIV-group compared to the national cohort. Coupled with metabolic factors, a low dietary quality contributes for NAFLD development in HIV patients, with these patients presenting overall lower median diet quality.

Study 3 illustrates the results of a dietary intervention trial conducted in HIV-NAFLD patients, including a description of the impact on weight loss during the national lockdown due to Covid-19, using telemedicine. We observed that patients on the intervention group gained a lower amount of weight than those that had only received generic dietary recommendations. Additionally, these group also had a significant decrease in the quality of their dietary pattern, with

increase in appetite and snack frequency during the day as opposed to the intervention group. This study demonstrated for the first time, to our knowledge, the efficacy of lifestyle interventions and regular follow-up to achieve WL in patients with HIV with NAFLD. Also, due to Covid-19 restrains, this was also a pioneer study regarding the application of telemedicine to dietary interventions and the characterization of dietary habits of patients with HIV during a national lockdown.

Study 4 characterizes the inflammatory content of the diet and clinical characteristics of MAFLD patients, taking in account the amount of alcohol intake. In this study we observed that the group of patients with alcohol intake presented higher intake of calories, carbohydrates, red meat, pastries, added sugar and sugar-sweetened beverages. In the group of patients without alcohol intake, a more pro-inflammatory dietary intake was associated with a 4-fold increase in the odds of severe steatosis. In the group of patients with harmful alcohol intake, a more pro-inflammatory dietary intake was associated with a higher degree of fibrosis, with all patients with advanced fibrosis presenting a pro-inflammatory dietary intake pattern.

In conclusion, we consider that these set of studies contribute to a better characterization of fatty liver patients, in terms of nutritional status, dietary intake and the relation between these two factors and disease presentation, and to a clarification of the results of a tailored dietary intervention either in the general population or in specific populations, such as PLWH. Our results may translate in a better disease management of a condition that is increasingly seen in clinical practise, in large measure due to the obesogenic environment.

KEYWORDS dietary intervention; dietary intake, nutritional status, fatty liver disease

LIST OF ABBREVIATIONS AND ACRONYMS

AIDS Acquired Immunodeficiency Disease Syndrome

BMI body mass index

CHO carbohydrates

CVD cardiovascular disease

cART combined antiretroviral therapy

CAP controlled attenuation-parameter

CI confidence interval

DII® Dietary Inflammatory Index

EASD European Association for the Study of Diabetes

EASO European Association for the Study of Obesity

ESPEN European Society for Clinical Nutrition and Metabolism

EASL European Society for the Study of the Liver

FLI Fatty Liver Index

FIB-4 Fibrosis-4

HCC Hepatocellular carcinoma

HDL High-density lipoprotein

HEI® Healthy Eating Index

HIV Human Immunodeficiency Virus infection

HOMA Homeostasis Model Assessment

*MedDie*t Mediterranean diet

MAFLD Metabolic dysfunction-associated fatty liver disease

MetS Metabolic syndrome

MUFA Monounsaturated fatty acids

NFS NAFLD fibrosis score

NAFLD Non-alcoholic fatty liver disease

NHANES National Health and Nutrition Examination Survey

NASH Non-alcoholic steatohepatitis

NCDs Non-communicable diseases

NITs Non-invasive tests

PNPLA 3 Patatin-like phospholipase domain-containing 3

PLWH People living with HIV

PY Person-years

PUFAs Polyunsaturated fatty acids

SFA Saturated fatty acids

SNPs Single-nucleotide polymorphism

TE Transient elastography

TM6SF2 Transmembrane 6 superfamily member 2

T2DM Type 2 diabetes mellitus

VLDL Very low-density lipoproteins

WL Weight loss

WHO World Health Organization

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1.

GENERAL INTRODUCTION

1.1. Obesity: a path to metabolic dysfunction

Overweight and obesity are defined by the World Health Organization (WHO) "as abnormal or excessive fat accumulation that presents a risk to health". According to WHO, individuals with a body mass index (BMI) over 25kg/m^2 are considered overweight, and over 30kg/m^2 are considered obese [1].

Obesity results from complex interactions between the genetic predisposition to weight gain and the environment [2–5]. Obesogenic environment, one of the key factors contributing to the development of obesity, is defined as the influence that some factors, such as the surroundings, the conditions of life and possibilities of healthy lifestyle choices have in the promoting obesity at the social and individual level, meaning that this concept encompasses all the environmental factors that influence energy intake and expenditure in individuals [1,5,6].

Such environmental factors that directly affect nutritional status are the exceeding availability and aggressive marketing of high energy dense foods (i.e., fast food restaurants and supermarkets) and adoption of unhealthy dietary patterns. This is coupled with an increase in the technology use and reduced number of facilities for physical activity, as well as a decrease in the regular daily physical activity thus reducing energy expenditure. Furthermore, the extent of influence of each factor is related to social economic status, sex and age [1,4,5,7]

The prevalence of obesity is increasing worldwide, with reports documenting that the prevalence rates of overweight and obesity have doubled since 1980 [1,3], with higher rates among women and in individuals with lower social economic status [1,8]. The increase in obesity prevalence is associated with higher all-cause mortality and substantial economic burden [1,9,10].

Recent WHO Reginal Obesity Report in Europe, demonstrates that almost a quarter (23%) of adults in the European region are obese, with the highest levels of overweight and obesity rates being found amongst Mediterranean and Eastern European countries [1].

On the national setting, Portugal is in line with this trend. Data from the last National Health Inquiry, from 2019, found that 53.6% of the population aged 18 or over, had a BMI≥25.kg/m^{2,} a value higher than in 2014 (Figure 1). Obesity was present in 16.9% of the population, affecting more women than men (17.4% e 16.4%, respectively) [11]. For 2025, the obesity prevalence is predicted to grow, with projections of prevalence for women of 26.3% (17.4-36.2%) and 28.1% (18.1-39.1%) for men [12].

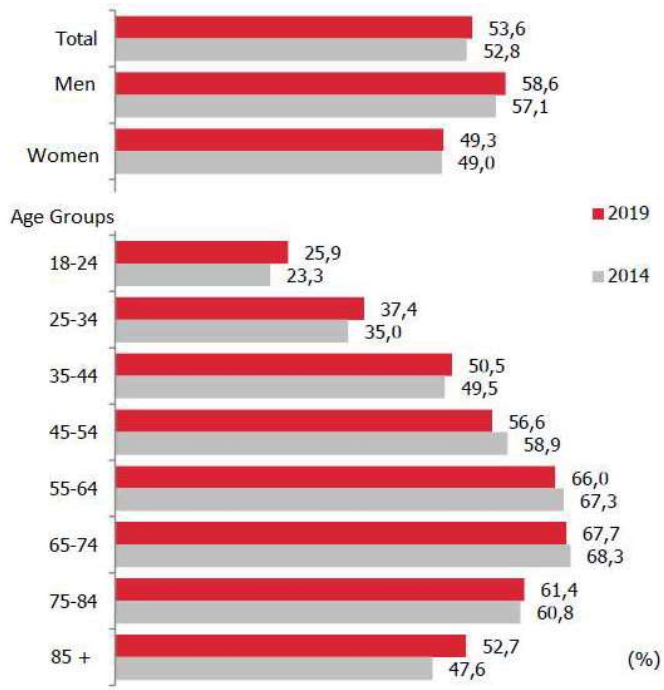

Figure 1: Overweight prevalence by sex and age group: comparison between national data of 2019 and 2014

Adapted from Ref.[11] with permission

Although the most used tool for diagnosing obesity is still the BMI, the last years focused on the issue of metabolic dysfunction in the presence of normal BMI. In fact, it has been demonstrated that even normal weight individuals when presenting some metabolic abnormalities have an increased risk for cardiometabolic disease [2,3].

This discussion is relevant, since the group of non-communicable diseases (NCDs) are currently the leading cause of disability and death worldwide and had proven to reduce life expectancy [3,13]. In the group of NCDs typically associated with obesity are included metabolic comorbidities, such as cardiovascular disease (CVD), type 2 diabetes mellitus (T2DM), hypertension, and fatty liver disease [1–5,14].

1.2 Fatty liver disease

The association between obesity and fatty liver disease is complex, with obese individuals presenting more elevated liver enzymes, a higher degree of hepatic steatosis and increased risk of liver fibrosis and metabolic comorbidities, meaning that obesity is a factor for the development of fatty liver disease and also an indicator of poor prognosis [15–18]. In a longitudinal study of a cohort of 77,425 metabolically healthy individuals without baseline fatty liver disease, overweight, was associated with a 2.2-fold increase in the risk of developing fatty liver disease, and obesity was associated with a 3.6-fold increase in the risk of developing fatty liver disease after a follow-up of 4.5 years [19], meaning that obesity is a key contributor to the development of fatty liver disease, independently of the presence of metabolic complications [16,19].

The mechanisms by which obesity promotes steatosis relate with the presence of adipocyte dysfunction and an increase in insulin resistance and lipolysis, thus leading to an increase in free fatty acids coupled with an increase in leptin, resulting in intrahepatic fat accumulation. When there is no regression in this condition, immune cells progressively infiltrate the liver resulting in a low-grade, but chronic inflammation that may lead to fibrosis of hepatic cells [16].

Non-alcoholic fatty liver disease (NAFLD) has become, in the last years, the most frequent cause of chronic liver disease, with an estimated global prevalence of 25%, increasing with age [20,21]. National data estimated an adjusted prevalence of NAFLD of around 17% [22]. Due to its rising prevalence, coupled with the increase in obesity, NAFLD is becoming one of the leading causes of end-stage-liver disease and liver transplantation, with increased disease and economic burden [20,21,23].

NAFLD is defined by the presence of steatosis in more than 5% of hepatocytes and in the absence of excessive alcohol consumption (≥30 g per day for men and ≥20 g per day for women or 21 standard drinks per week on average for men and 14 standard drinks on average for women) and after exclusion of other causes of liver disease [20,24,25].

The term NAFLD encompasses a broad range of clinicopathological findings (Figure 2), that include steatosis, with and without inflammation and a necro inflammatory subtype – non-alcoholic steatohepatitis (NASH) – when there is presence of steatosis, lobular inflammation, and hepatocyte ballooning with or without perisinusoidal fibrosis [20,23,25,26].

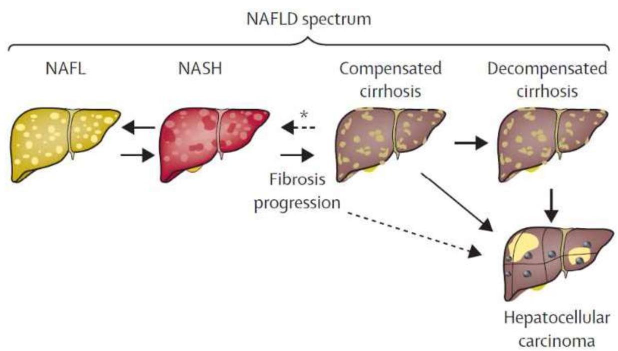

Figure 2: Spectrum of NAFLD

Adapted from Ref [20] with permission

NAFLD is a silent disease, and frequently patients can go undiagnosed for years. A key prognostic marker for liver-related outcomes and mortality is the presence of fibrosis, specially advanced fibrosis (stages 3 and 4, on a histological scale ranging from 0 to 4) [20,25,26]. Fibrosis progression is experienced in around 41% of NAFLD patients, with progression at a rate of approximately one stage per decade [21,25]. Progression from compensated to decompensated cirrhosis occurs at an estimated rate of 3-4% a year, cirrhosis being the strongest risk factor for the development of hepatocellular carcinoma (HCC) [20]. HCC in NAFDL patients has an estimated incidence of 0.44 per 1000 person-years (PY) and in NASH patients an estimated incidence of 5.29 per 1000 PY [21]. Liver specific and overall mortality incidence in NAFLD patients was estimated at 0.77 per 1000 PY and 15.4 per 1000 PY, respectively, and in NASH patients at 11.8 per 1000 PY and 25.6 1000 PY, respectively [21].

Despite this, the major cause of death among NAFLD patients is CVD, which includes fatal/non-fatal CVD events and other cardiac and arrhythmic complications, followed by extrahepatic malignancy [20,23,26–28]. This higher mortality from cardiovascular disease could be directly associated with the association with metabolic abnormalities, and pro-inflammatory mediators as well as higher visceral fat content [20,27–30].

1.2.1. Risk factors and disease progression

NAFLD is considered as the hepatic manifestation of the metabolic syndrome (MetS) due to its close association with metabolic abnormalities, which include obesity, dyslipidaemia, insulin resistance and T2DM [23,26,28,29]. NAFDL is an independent risk factor for incident metabolic comorbidities but is also a consequence of these metabolic comorbidities [28,29,31,32]. Pathways for development and progression of NAFLD are influenced by a wide range of factors, an hypothesis known as “multiple parallel hits” with recent evidence demonstrating that the underlying mechanisms can differ substantially by risk factor [33,34].

The overconsumption of calories, high intake of fat, sugar and fructose are directly associated with obesity, metabolic abnormalities and NAFLD [24,32,33,35–37].

Excess weight, particularly an increased visceral fat content is considered one of the main risk factors for NAFLD development, strongly associated with impaired insulin resistance and lipid profile alterations [23,24,32,36,38,39]. Also, this increase in the accumulation of ectopic fat, causes a proinflammatory state, due to the infiltration of macrophages in visceral adipose tissue [20,32,34,40]. Prevalence of obesity in NAFLD and NASH is estimated at 51.3% and 81.8%, respectively [21]. A high waist circumference and a high waist-to-hip ratio are considered good indicators of visceral adiposity and closely related to the development of NAFLD [23,32,36,38–40], independently of baseline BMI [25,40]. An android fat deposition pattern was also recently associated with higher fibrosis prevalence in women [40]. Independently of BMI, in NAFLD patients, increased waist circumference is associated with higher all-cause mortality [30].

Regarding metabolic factors, T2DM appears to be a key factor. The presence of insulin resistance, impairs adequate lipolysis and leads to the formation of lipotoxic lipids that contribute to cellular stress, and to an increase in inflammation, impairment of tissue regeneration and promotion of fibrinogenesis [20,32,41]. Prevalence of T2DM in NAFLD and NASH was estimated at 22.5% and 43.6%, respectively [21]. However, from a different perspective, in patients with T2DM, NAFLD prevalence was 55.5% and NASH prevalence was 37.3%, with estimates of advanced fibrosis, detected by liver biopsy, of 17% [41]. The triumvirate of NAFLD, obesity and insulin resistance was associated with a 14-fold increase in the risk of developing T2DM and the incidence rate of T2DM increases directly and progressively according to the severity of NAFLD, even after adjustment for multiple confounders [28]. Its presence is associated with more than a 2-fold increase in the risk of advanced fibrosis, cirrhosis-related complications, and liver disease mortality [20,23,41]. Also, T2DM in NAFLD patients contributes synergically to increased cardiovascular risk [41,42].

Other metabolic risk factors for the development and progression of liver disease are the presence of lipid abnormalities and hypertension [20,34].

Prevalence of dyslipidaemia in NAFLD and NASH was estimated at 69.2% and 72.1%, respectively [21], with lipid disturbances usually appearing early on [43]. Insulin resistance is closely related to the presence of lipoprotein abnormalities, leading to an increase in very low-density lipoproteins (VLDL), chylomicrons and hypertriglyceridemia and simultaneously to a decrease in high-density lipoprotein (HDL) cholesterol [43]. These variations are also closely linked to the higher CVD risk among NAFLD patients [43].

Prevalence of hypertension in NAFLD and NASH was estimated at 39.3% and 67.9%, respectively [21]. The systemic inflammation induced by NAFLD coupled with a malfunction in the renin-angiotensin system, independently of the presence of obesity or insulin resistance, is associated with the development of hypertension [44].

Given these metabolic abnormalities, MetS, a constellation of at least three metabolic abnormalities, is a common feature in NAFLD patients and a significant contributor to the increase CVD risk amongst these patients [28,44]. The relationship between MetS and NAFLD is bidirectional [26,44]. Prevalence of MetS in NAFLD and NASH was estimated at 42.5% and 70.6%, respectively [21].

Metabolic dysfunction worsens with increasing severity of NAFLD in patients with MetS [29,44] and patients with MetS had more than a 2-fold higher risk of developing cirrhosis or HCC [41].

Genetic factors associated with disease risk are the presence of some single-nucleotide polymorphism (SNPs) [20,25,33]. The most studied is patatin-like phospholipase domain-containing 3 (PNPLA 3), which appears to contribute to an increased susceptibility to NAFLD, followed by transmembrane 6 superfamily member 2 (TM6SF2) [20]. Recently it has been demonstrated that the presence of these SNP's impairs hepatic mitochondrial function, compromising the metabolization of substrates, such as sugar and fat, by the liver [33]. Beside susceptibility to NAFLD, genetic variants can also influence other metabolic domains, such as increase susceptibility to obesity or even a modification in CVD risk profile [20]. The variant of the PNPLA3 gene is associated with NAFLD histological severity and development of HCC, and consequently, liver-related and all-cause mortality [20].

NAFLD patients experience gut-microbiome alterations, such as a decrease in *Lactobacillus*, and increased abundance of *Roseburia* and *E. Coli* [34,42] This dysbiosis is possibly associated with impairment of the regulation of some intra hepatic and metabolic pathways, which can contribute to NAFLD development and progression [28,32]. Microbiome-related factors are still an ongoing debate but appears to associate with alterations in faecal-microbiome signature that associate with advanced fibrosis. [20,32,34,42]. Also, the disruption in microbiome composition could lead to a modification in factors produced by bacteria, such as an increase in circulating lipopolysaccharides (LPS) and other endotoxins, that contributes directly to systemic inflammation, and consequently to liver inflammation and disease progression [20,28,32,34,42].

Adding to these factors, the presence of low grade systemic inflammation, a possible consequence of the disruption of innate and adaptative immunity pathways has also been pointed out as a main driver in the development and progression of NAFDL [25,32,34]. This disruption is possibly linked to the interplay between the immune system and the metabolic, genetic and microbiome related factors mentioned previously [25,32,34].

Even though the major risk factors for hepatic fat and fibrosis development are currently understood, such as age above 50 years, obesity, insulin resistance and T2DM and the presence of PNPLA3 I148M polymorphism, the individual and synergic contribution of each of these factors to the progression is less understood [28,32].

1.2.2. Diagnosis and staging

The majority of NAFLD patients are asymptomatic until later stages of the disease, and monitoring liver enzymes is a common clinical practice used in some primary care settings to detect liver-related problems. However, in more than half of NAFLD patients their values are normal [20].

NAFLD is often diagnosed by imaging, with abdominal ultrasonography being the most used technique. However, it has some limitations, such as a low sensitivity when total fat content is low and, in the presence of advanced fibrosis, some typical imaging characteristics are lost [20,45,46].

Liver biopsy is the gold standard for the diagnosis of NAFLD, since it is the only technique that allows the characterization and quantification of histological features of steatosis, inflammation, hepatocyte ballooning, and fibrosis. Nonetheless, it is an invasive procedure with associated risks, not recommended for widespread use and also subjected to sampling bias [20,26,45–47].

Regarding prognosis, it has been well demonstrated that the degree of fibrosis is the major predictor of disease progression. Consequently, it became very important to develop and promote non-invasive tests (NITs), to improve screening of advanced stages of fibrosis and minimise the need for liver biopsy [20,25,26,46–48].

Non-patented tests or algorithms, such as serum fibrosis biomarkers like Fibrosis-4 (FIB-4) index, NAFLD fibrosis score (NFS) and aspartate aminotransferase-to-platelet ratio are amongst the most widely used, since they are very cheap, including demographic (i.e., age), clinical (i.e., BMI, presence of T2DM) and laboratory (i.e., alanine aminotransferase concentration, platelet count, albumin concentration) parameters that are commonly used in clinical practice. Although with moderate accuracy, influenced by factors such as age and comorbid conditions, and a proportion of around 30% of patients with indeterminate results globally, these scores have a high negative predictive values to exclude advanced liver fibrosis [20,45–48]. Patented tests, such as enhanced liver fibrosis (ELFTM) test, that among others Hyaluronic acid, FibroTest®, that includes haptoglobin, a2-macroglobulin, apolipoprotein-A, bilirubin, and GG, Fibrometer®, incorporating glucose, ferritin and body weight and Hepascore® that combines variables such as age and sex with parameters that include bilirubin, gamma-glutamyl transferase, hyaluronic acid, and a2-macroglobulin have also proved to accurately determine the presence of advanced fibrosis. However, due to their cost, are not widely used [47,48].

Another method to estimate liver fibrosis in patients with NAFLD is to measure liver stiffness by ultrasound-based or magnetic-resonance based elastography techniques. From those, transient elastography (TE) has been extensively used, and due to its wide availability can be used as a point-of-care test, with established liver stiffness cut-offs for excluding advanced fibrosis and cirrhosis [20,45,47]. Adding to TE, it is also possible to determine the controlled attenuation-parameter (CAP), which allows quantification of hepatic steatosis during the measurement of liver stiffness [20,45,47]. Of note, TE has limitations for use in the presence of elevated BMI's, with reports documenting unreliable results. In such cases, the XL probe is recommended [26].

1.2.3. Lean NASH

Although overweight is implicated in the majority of NAFLD cases, in recent years, a growing prevalence of NAFLD in lean individuals is being observed [49,50]. This group is currently known as "lean NAFLD", with the definition of lean being based on normal BMI by ethnicity (BMI <25kg/m^2 in Caucasians and a BMI <23kg/m^2 in Asians) and can also present some of the histological features associated with increased severity of the disease, such as lobular inflammation, hepatocyte ballooning, and/or fibrosis, although to a lesser extent than their obese counterparts [17,18,49,50].

Prevalence of lean NAFLD vary substantially, depending on study design and diagnostic tools, but specially due to different cut-offs to define obesity between Asian and western population [49,50]. A recent meta-analysis estimated a prevalence in the general population of lean NAFLD of 12.1% (95% CI 9.3-15.6) and 5.1% of lean NASH (95% CI 3.7-7.0). The incidence of NAFLD in lean participants was 24.6 per 1000 PY [51]. The majority of studies uses ultrasound as a diagnostic tool, a technic with limitations in low steatosis grades, and consequently, the prevalence can be underestimated [50].

Some risk factors are similar to those present in overweight NAFLD patients. Lean NAFLD patients experience a higher rate of metabolic abnormalities, namely insulin resistance, in comparison with lean healthy individuals, which can contribute to a higher intrahepatic triglyceride content [49,50,52]. In addition, these patients, despite normal BMI, appear to have more visceral adipose tissue, which may contribute to the development of NAFLD. [49,50,52] Lifestyle factors, with high intake of energy and refined sugars, coupled with sedentary behaviour are also key contributors to lean NAFLD [49,52]. Finally, genetic predisposition, is also a risk factor, with SNPs on PNPLA3 and TM6SF2 being factors associated with higher risk of developing NAFLD [49].

In lean patients, the presence of NAFLD contributes to a high metabolic burden, with this group of patients being associated with a 5.4-fold greater risk of MetS and a 4.8-fold greater risk of T2DM in comparison with lean healthy individuals, which can contribute to an increased CVD risk [49,50,53]. CVD and all-cause mortality have been associated to NAFLD in lean patients, although lean NAFLD patients present globally a better prognosis than their obese counterparts [17,49,50,53]. In this modulation of CVD and all-cause mortality, the increased visceral fat content, measured by waist circumference, appears to be of relevance. In fact, it was demonstrated that the risk of CVD mortality in NAFLD lean patients but with greater waist circumference was higher than among NAFDL obese patients but with normal waist circumference [30] (Figure 3).

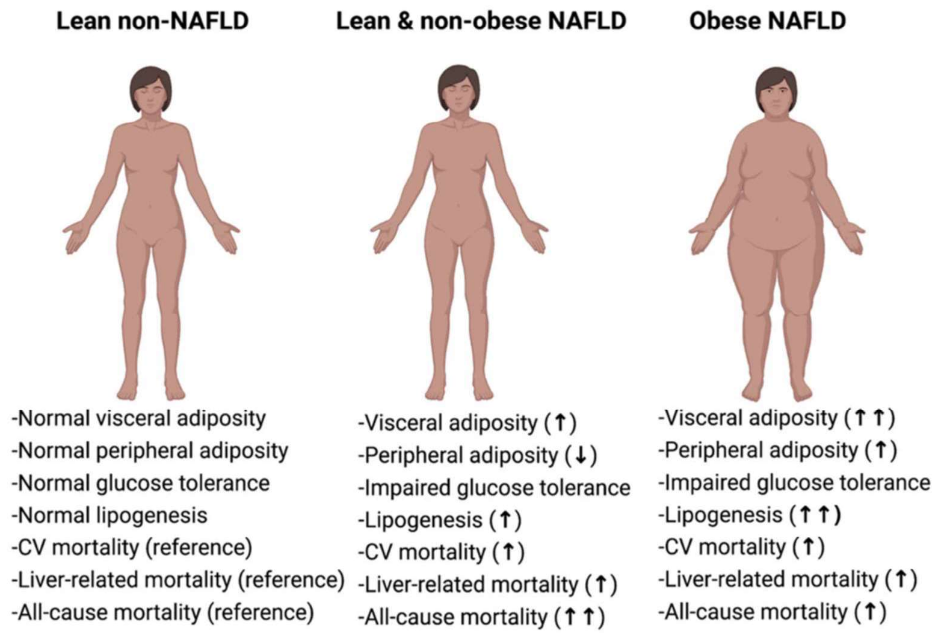

Figure 3: Lean and obese NAFLD phenotype

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Based on the prevalence and metabolic associated risks, NAFLD screening should include also lean individuals, with anthropometric measurements being an additional aid in risk stratifying [26,30,50,51].

1.2.4. Treatment

Given that NAFLD is a multisystemic disease, recommendations for its management should include a holistic approach, with a multidisciplinary team [37,54–57].

The composition of the multidisciplinary team is conditioned to health care setting and available resources. Ideally, besides hepatologists that are fundamental in adequate clinical diagnosis, staging and management of NAFLD, other healthcare professionals, such as dietitians, endocrinologists, and cardiologists to optimize metabolic comorbidities control, especially T2DM and, to ensure adequate management of cardiovascular risk might be involved [37,54–56]. In fact, the inclusion of a dietitian plays a fundamental role in implementing dietary recommendations and optimizing adherence to lifestyle changes [55,56,58] (Figure 4).

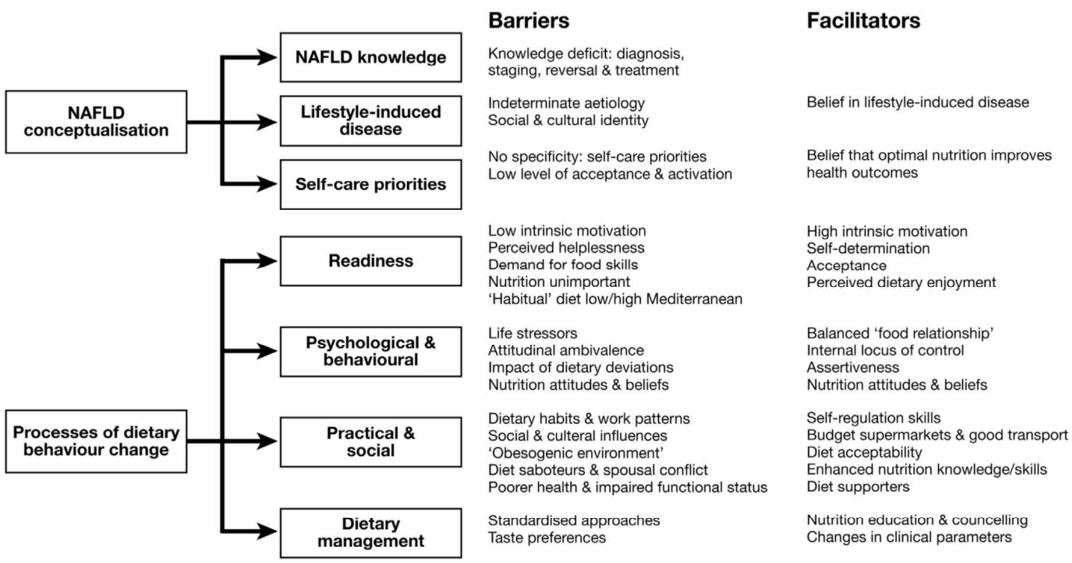

Figure 4: Management of NAFLD

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Studies conducted so far, document that this multidisciplinary team, besides providing nutritional information, can also address some of the barriers to diet maintenance reported by patients, such as poor understanding of causes of NAFLD, managing life stressors and understanding the influence of the obesogenic environment [55,58,59] This multidisciplinary approach has demonstrated a significant improvement in liver-related outcomes and cardiometabolic related health parameters [37,55,58,60].

However, it has been documented that even in high-resource settings, there is a great disparity in practices regarding lifestyle changes, with physicians rarely implementing or promoting them amongst these population [61]. The inclusion in a multidisciplinary team, could improve knowledge sharing and address clinicians barriers to adequate nutritional management [56,61].

Despite this recommended multidisciplinary approach, settings implementing this comprehensive model of care are few [56] and, data on long-term follow-up scarce. In fact, in Portugal, the are no defined pathways of care for these patients and no current national guidelines [56].

Currently, there is still no pharmacological treatment available for NAFLD and the solely available and effective treatment are lifestyle modifications, encompassing two major components: diet and physical activity [20,26,29,35,37,62].

The interventions aiming at weight loss (WL) coupled with a component of moderate-intense physical activity have demonstrated improvements in liver steatosis and fibrosis (Figure 5) , with a reduction in BMI of 5% being associated with a 25% reduction in liver steatosis, and 7-10% WL being associated with improvement in clinical features of NASH [20,24,35,37].

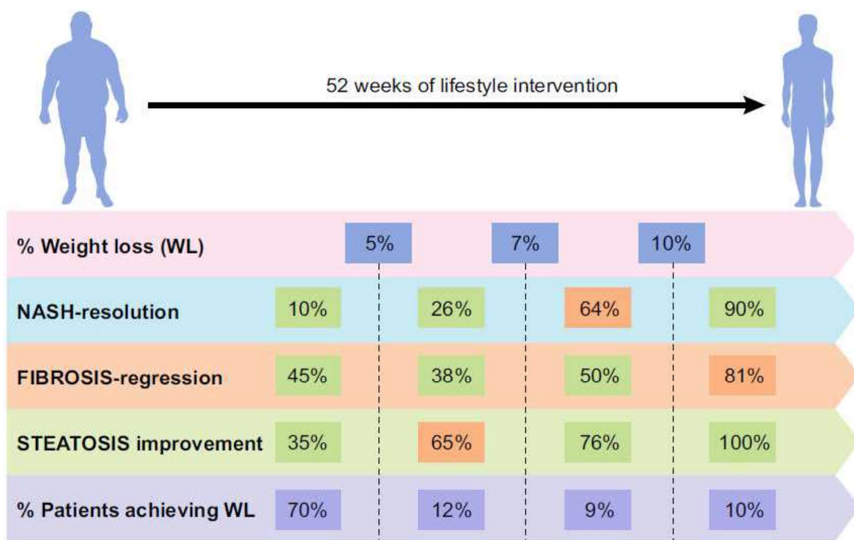

Figure 5: Improvement in liver related measures in patients with NASH according to % WL. Data from [60]

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Accordingly, it is recommended in overweight/obese NAFLD patients, to have a target WL of 7–10% for most lifestyle interventions [26]. For lean NASH the target WL should be from 3-5% [62].

The intervention, should last around 6 months, and at least a one-year follow-up is recommended, given the difficulty in compliance on the long term that has been described [20,24,37].

As for physical activity, it has been demonstrated that sedentary behaviour, such as prolonged sitting time, is an independent predictor for NAFLD [24,37,44,63] and that the increase in physical activity, potentiates the dietary intervention benefits, through further improvement of hepatic steatosis [24,44,64]. Also, physical activity *per se* improves insulin resistance and allows a significant reduction in visceral adipose tissue [37,44]. Although aerobic exercise has demonstrated better outcomes compared with resistance exercise in NAFLD, strategies documented to increase physical activity levels can range from an increase in daily activities to structured exercise programs, the choice pending to the modality that provides better patient engagement [24,44,62].

1.3. 2020: a change in the concept of fatty liver disease

Recently, an international panel of experts proposed a change in the nomenclature to partially replace the term NAFLD for Metabolic dysfunction-associated fatty liver disease (MAFLD) [65]. This definition has the advantage of being a positive definition and avoid the word “alcoholic” in the name.

In the context of increasing obesity prevalence, coupled with metabolic disturbances, such as diabetes, dyslipidaemia and high blood pressure, the authors proposed this new nomenclature, believing that it is more suitable to label the group of liver diseases associated with metabolic dysfunction. The diagnosis is based on the presence of hepatic steatosis plus the presence of another factor such as overweight, T2DM, or normal weight plus the presence of at least two metabolic abnormalities (Figure 6).

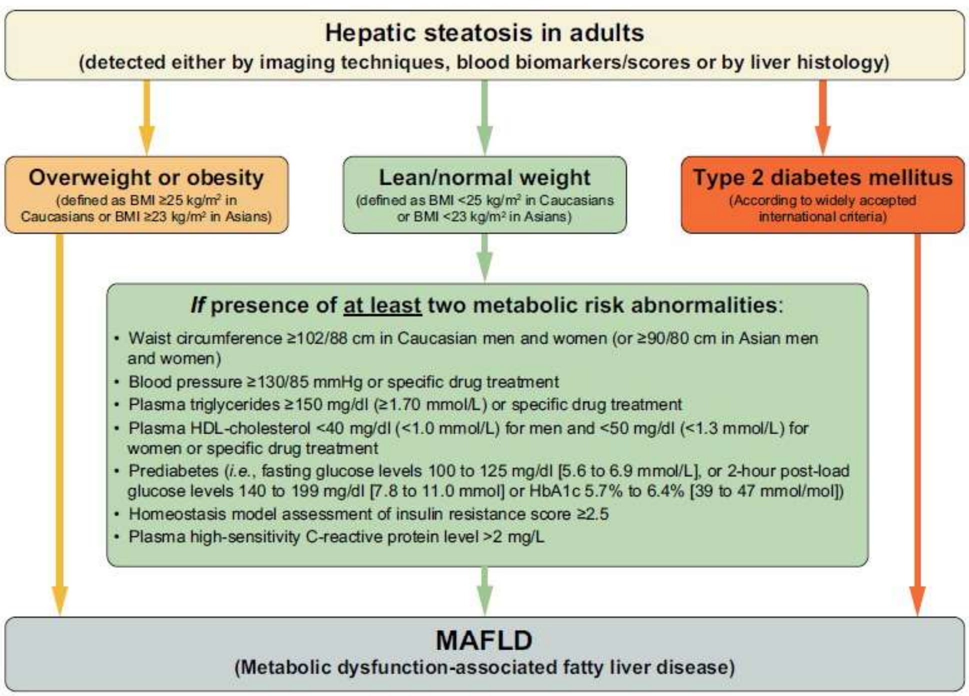

Figure 6: Flowchart for the proposed “positive” diagnostic criteria for MAFLD.

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This new proposed definition, according to the experts, can be applied in any clinical setting, and accounts for the heterogeneity in the underlying causes, taking in consideration different aetiologies. Furthermore it could increase the capacity to evaluate the contribution of frequent co-factors such as alcohol consumption [65].

Metabolic abnormalities are important factors in NAFLD progression, as well as in MAFLD patients, with insulin resistance being demonstrated to be a key factor in the progression of the disease [66]. This new definition allows the inclusion of patients with documented insulin resistance even in the presence of normal BMI [65], not limiting the diagnosis of liver disease frequently to those who are overweight.

MAFLD definition, considering a high waist circumference as an indicator of metabolic abnormalities is a good indicator of visceral fat mass content [16] and the inclusion of BMI values within the normal range for the diagnosis of MAFLD allows a broader inclusion of these group of patients, and a more premature diagnosis of a previously unknown metabolic dysfunction [13]. The group of patients with this phenotype has been also associated with lean NASH and associated with increased all-cause mortality [30]. MAFLD prevalence, in a recent meta-analysis, was estimated to be 39.22% [67].

Since the publication of this new concept, the debate is ongoing [68–70].

The first debate regarded the impact of the new definition on clinical outcomes. A first longitudinal study [71], aimed at comparing the fatal and non-fatal cardiovascular events between NAFLD and MAFLD. With 2985 participants and a follow-up of 7 years, the authors classified participants as having MAFLD or NAFLD according to definition, or as controls, if participants did not present fatty liver disease. The anthropometric characteristics and metabolic profiles of NAFLD and MAFDL patients were alike at baseline and both groups presented a higher incidence of cardiovascular events compared to controls, but between groups of fatty liver disease, the incidence of cardiovascular events was similar. Limitations associated with participant characteristics due to the study being conducted in participants from Sri-Lanka prevented a broader generalization of the results.

After this, in a large cohort [72], the investigators aimed to demonstrate if the terminology MAFLD was superior to NAFLD regarding long-term mortality risk and cause specific mortality risk. Using the Third National Health and Nutrition Examination Survey - NHANES III profiles, 12,480 eligible participants were included and followed up for an average of 22.8 years. The authors separated the cohort into four mutually exclusive groups based on definitions of MAFLD and NAFLD: Group M+N: participants who meet the diagnostic criteria for both MAFLD and NAFLD; Group N: participants solely with NAFLD; Group M: participants solely with MAFLD; participants excluded by both definitions were viewed as the control group. (Figure 7).

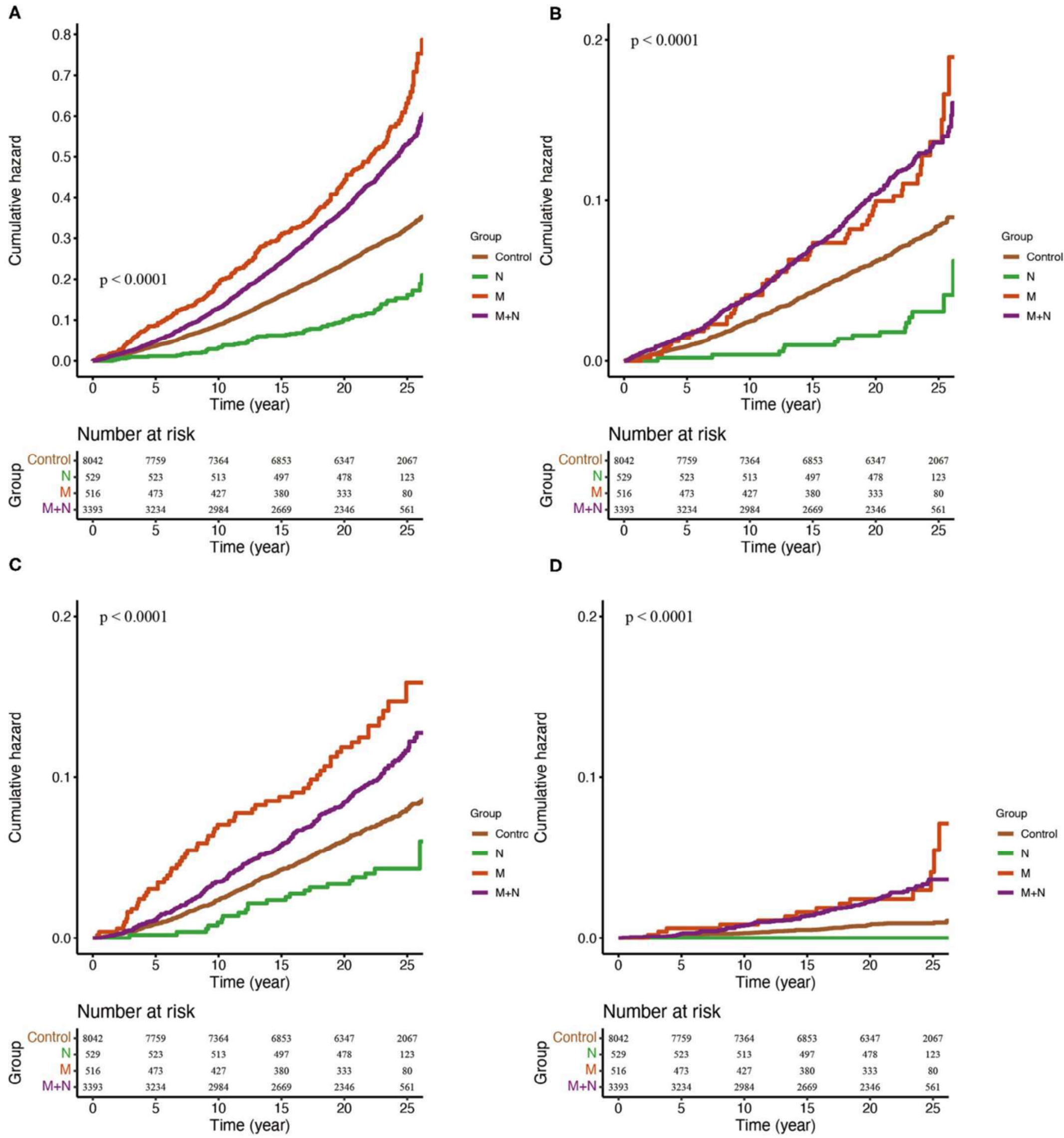

Figure 7: Kaplan-Meier estimates of overall (A), cardiovascular (B), neoplasm (C) and diabetes-related (D) mortality.

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In univariable models, MAFLD increased the risk for all-cause mortality by one-fold compared with non-MAFLD participants. Compared with patients with NAFLD, patients with MAFLD had an increased risk for all-cause mortality in a greater magnitude despite similar cardiovascular, neoplasm and diabetes-related mortality risks. The conclusions were afterwards corroborated by other others [73].

Although it has been reported that the new definition encompasses 80% of NAFLD patients [67] Huang and colleagues [72] suggested that the new terminology led to a higher inclusion of patients with higher mortality risk. Although some patients without metabolic abnormalities tended not to be diagnosed with fatty liver disease, those patients were the ones with a reduced mortality risk.

Also, it has been demonstrated that the new terminology, included patients with the worst metabolic profile, higher hepatic inflammation and liver fibrosis and with alcohol abuse [71,72,74]. The discussion regarding the inclusion of patients with different amounts of alcohol use is a topic of controversy in the MAFLD definition, since it does not exclude patients based on the amount of alcohol intake, but instead proposes to classify these patients as having dual aetiology [65]. Very frequently, hepatic steatosis and harmful alcohol consumption coexist [75], resulting in the fact that NAFLD definition is rather artificial, since it does not allow the inclusion of these group of patients, although they frequently present the metabolic abnormalities that can lead to a worse clinical course and prognosis [68,74,75]. Additionally, there is no consensual safe limit for alcohol intake in the presence of obesity and metabolic abnormalities since even in the presence of small amounts, alcohol intake can contribute to fibrosis progression and increased severity of liver disease [75,76].

This new definition is now being evaluated by an international group of experts, and it is expected that a consensus about the name is reached until the end of 2023.

1.4. Nutrition: a cornerstone in fatty liver disease

Nutrition plays a two-way role, both as one of the causes and as a treatment of this condition.

1.3.1 Nutrition as a cause

Besides weight gain, high energy intake is a leading risk factor for fatty liver disease [37,77,78]. The way that this high energy intake is consumed throughout the day is also of significance, with data showing that the intake of foods with high content of sugar and fat as a snack, as opposed to after main meals, leads to an increase in hepatic steatosis [37].

Although it appears that the proportion of macronutrients to the total energy intake may not pose a significant contribution to fatty liver disease development, the type and quality of these nutrients, especially fat and carbohydrates (CHO) appears to be of relevance [77,78] (Figure 8).

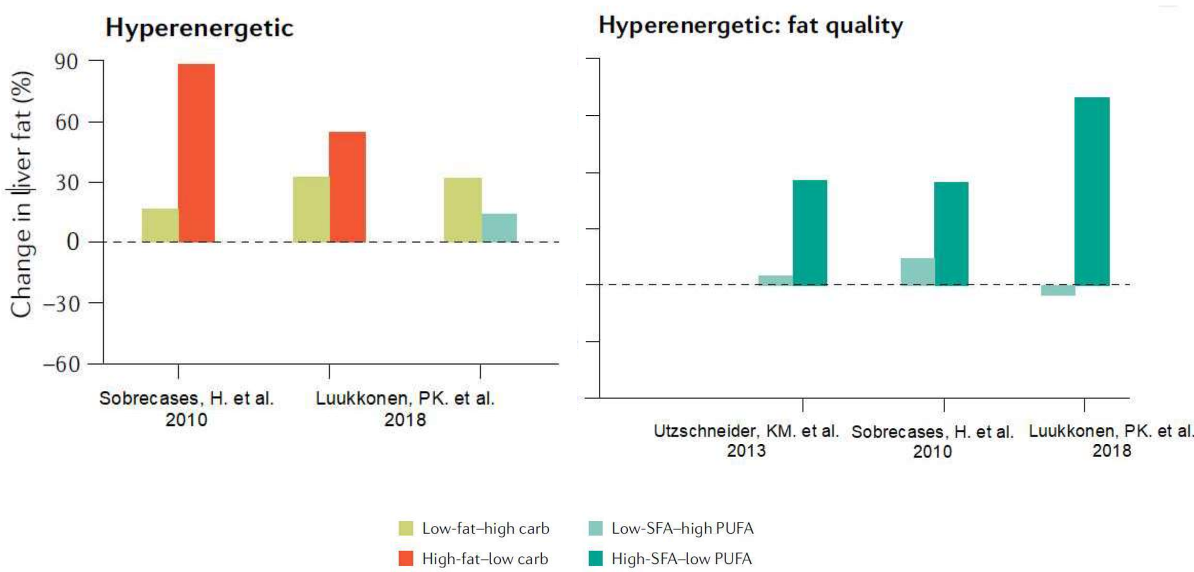

Figure 8: Effect of high/low fat, carbohydrates, SFA and PUFA diet content on liver fat

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High intake of saturated fatty acids (SFA) and cholesterol and poor intake of polyunsaturated fatty acids (PUFAs) is associated with increased risk for fatty liver disease; overconsumption of SFAs leads to an increase in liver fat content, with a 2-fold increase in visceral fat content when compared to PUFAs [24,37,80]. Regarding monounsaturated fatty acids (MUFA) and omega 3 PUFA although a protective effect on NAFDL development and progression have been identified, methodological considerations warrants care in using these nutrients as a strategy to prevent it [80].

As for CHO, added sugar, that includes refined sugars (i.e. sucrose, fructose, and high fructose corn syrup) and the one incorporated into food, fruit drinks, and beverages, has been linked to the development of NAFLD, particularly a high intake of sugar-sweetened beverages and fructose [24,36,37,79–81]. High intake of fructose is associated with an increase in hepatic synthesis of triglycerides and increased gut permeability with subsequent inflammation, leading to hepatic steatosis and fibrosis [37,79,82] although, it appears to have a different behaviour depending on the source, with mechanisms of NAFLD development more associated with soft drinks, high fructose corn syrup and sugar-sweetened beverages than with fruits or vegetables, possibly due to the balance of glucose/fructose [79–82]. Also, fruits and vegetables, have a high fiber content, associated with better metabolic control. In NAFDL patients, a negative association was identified between dietary intake of total, cereal, fruit, and vegetable fiber with NAFLD risk, even in stratified analysis by sex and age group [83].

Alcohol, in the context of NAFLD, is still an ongoing debate. Although some data report benefits in NAFLD and NASH prevalence associated with alcohol intake of <20g/day for women and <30g/day for men, the studies do not have adequate management of confounders [84]. In recent years, the deleterious effects of alcohol, even in moderation, have been highlighted and guidelines currently recommend complete abstinence in patients with fatty liver disease [26,75,84].

In a recent umbrella review, from 11 dietary factors studied (coffee, sugar-sweetened beverages, red meat, soft drinks, nuts, refined grains, fish, fruits, vegetables, dairy, and legumes) only three demonstrated to be associated with increased risk for NAFLD: sugar-sweetened beverages (random effect ES [95% CI], 1.48 [1.29–1.69]), soft drinks (random effect ES [95% CI], 1.32 [1.17–1.48]), and red meat (random effect ES [95% CI], 1.22 [1.06–1.41]), while intake of nuts (random effect ES [95% CI], 0.87 [0.78–0.98]) was determined as a protective factor [36].

The role of dietary composition on the progression of NAFLD has scarce data. A high intake of fish, vegetables and fiber coupled with a low intake of sugar is associated with lower odds of liver cancer [37,80]. Coffee intake, with its hepatoprotective effects linked to caffeine and polyphenolic fractions, has also been linked to a reduced risk of liver fibrosis and HCC, mostly in cross-sectional studies [37,81,84,85]. However, the optimum quantity of intake has not yet been determined [37,84,85]. Regarding micronutrients, choline, an essential component of cell membranes, when in deficit has also been associated with fibrosis [37,78]. Vitamin D deficiency is common in NAFLD patients, but the association with NAFLD outcomes and disease severity is still controversial, with results demonstrating either no association, when adjusting for adiposity levels, or a direct relation with increased lobular inflammation, ballooning and fibrosis [37,78].

Given that nutrients or even foods are not consumed alone, in recent years, the focus has changed to explore the association of NAFLD with different dietary patterns, allowing a more comprehensive approach and reflection of the possible synergistic effects of food and nutrients [80,86,87].

The “western dietary pattern” characterized by a high intake of soft drinks, refined grains, salty and sweet snacks, processed meat, and fast food has been associated with an increased risk for NAFLD development [79,80,86,87], whereas a more “prudent dietary pattern” characterized by high intake of whole grains, vegetables, fruit, legumes, fish and low-fat dairy being associated with lower risk [80,86,87].

Regarding MAFLD, only two groups of authors have studied the dietary characteristic of MAFLD patients.

Taheri and colleagues [88] in a cross-sectional study with 1932 Iranian participants aimed at exploring the association between a more pro-inflammatory lifestyle and dietary pattern and MAFLD. The lifestyle inflammatory score included data on three components: smoking status, physical activity and BMI. The authors demonstrated that those with a more pro-inflammatory dietary pattern had an odd of NAFLD 84% significantly higher and those with a more pro-inflammatory lifestyle score had an odd of NASH 96% significantly higher. However, the participants included in this study had no alcohol intake, which constitutes a major limitation in the results, considering the diagnostic criteria for MAFLD.

Guveli and colleagues [89], in a retrospective analysis of 106 MAFLD patients, of whom 25.5% presented with advanced fibrosis, documented that the dietary intake of cholesterol and saturated fatty acid was independently associated with the presence of advanced fibrosis. However, there is no data regarding the intake of alcohol, and this represents a major bias in the results.

1.3.2. Nutrition as treatment

The first longitudinal study with a higher length of follow-up (12 months) was conducted in 2015 by Vilar-Gomez and colleagues to assess the impact on histological features of NASH in a large cohort of patients in a program of lifestyle change using a structured dietary intervention aiming at WL [60], as discussed above. The results, report a directly dose-response relationship between WL percentage and overall histological changes [60]. Weight reductions of 10% are required for NAS, but modest WL (7–10%) reduces steatosis, lobular inflammation and ballooning [60].

Joint Clinical Practice Guidelines for the management of NAFLD from the European Society for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO) recommend a WL of 7-10% in overweight NAFLD patients as a target of most lifestyle interventions, with improvement in liver enzymes and histology (Grade B1) [26].

To achieve this degree of WL, a hypocaloric diet, with a caloric deficit of 500-1000kcal/day is recommended [26,84].

Currently, the Mediterranean diet (MedDiet) is the dietary pattern recommended by EASL-EASD-EASO Clinical Practice Guidelines (Grade: B1), [26] and is also the dietary pattern endorsed by the European Society for Clinical Nutrition and Metabolism (ESPEN) for the management of chronic liver disease [90].

Although subject to variations due to cultural differences, MedDiet is a dietary pattern characterized by a high intake of fruits, legumes, vegetables, fish, nuts and olive oil [37,57,91]. Even though the majority of calories in MedDiet are derived from fat, comprising 35-45% of the total daily energy intake, they derived mostly from MUFA and omega-3 PUFA, with dietary regimens rich in omega 3 PUFA demonstrating a reduction in insulin resistance and intrahepatic triglyceride content [37,57,91]. Also, MedDiet has higher fiber content, with benefits in gut microbiota modulation, reduction in insulin resistance and improvement in dyslipidaemia [78,91]. MedDiet has a low content of red and processed meat, which translated into a low intake of saturated fat and cholesterol and has also a low intake of high-sugar content foods. Wine intake is allowed in moderation (1-2 glasses/day) [37,57,78,91].

Although MedDiet-based dietary interventions in NAFDL patients present some degree of heterogeneity, namely, in caloric intake, number of fruit portions allowed, that influence the fructose content of the diet, and amount of alcohol intake permitted, this dietary pattern has been consistently associated, by numerous systematic reviews and meta-analysis, to the improvement of cardiovascular risk factors, decrease in weight and BMI, reduction in liver enzymes and reduction in hepatic steatosis [57,78,84,91–95].

Besides a reduction in cardiovascular mortality, MedDiet may also reduce the risk of HCC and liver-related death, although high-quality trials are necessary to clarify the magnitude of this reduction [57,84,91].

Other dietary approaches have been applied to NAFLD, such as manipulation of macronutrient composition with a “low carb” or “low fat” diet (Figure 9).

A “low carb” diet is defined as a content of less than 26% of total energy intake from CHO, being fat the nutrient that contributes the most to total energy intake.

When this CHO content is lower than 10% the term “ketogenic diet” or very-low-carbohydrate diet” are used [78,96]. In this “low carb” approach, the type of sugar restricted, seems to be of importance, with results demonstrating a higher degree of reduction in hepatic steatosis with a reduction in isolated fructose in comparison with other forms of digestible CHO with lower glycaemic index [78]. The “ketogenic diet”, although with short-term interventions, has demonstrated an improvement in insulin resistance, with subsequent reduction in lipogeneses and liver fat content, although the possible metabolic effects may be associated with WL [78,91,96]. In the “low fat” diet, fat content is less than 30% of total energy intake [96,97].

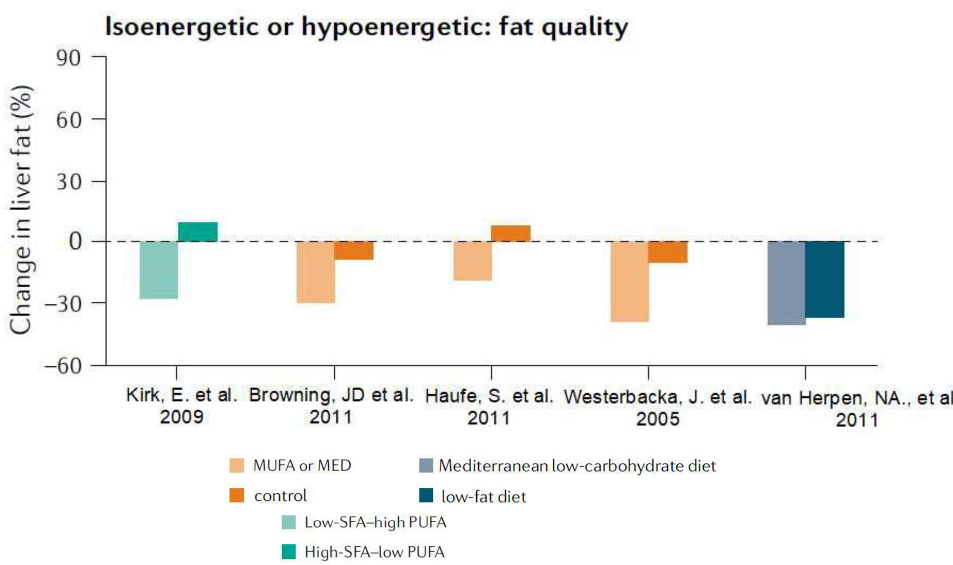

Figure 9: Effect of different types of iso or hypocaloric diet in liver fat

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Recent systematic reviews with meta-analyses demonstrate that, no significant differences were found between “low carb” and “low fat” diet, with benefits possibly mainly associated with the global caloric restriction of the diet, although both resulted in a reduction of hepatic steatosis, liver enzymes and improvement of anthropometric parameters, [96,97]. Besides high heterogeneity of trials, for clinical practice it is also necessary to explore the long-term feasibility and safety associated with these dietary regimens [91].

A recent meta-analysis, analysing the effect of isolated dietary interventions without coupled physical activity, demonstrated that hypocaloric dietary interventions (either low fat or low carb) favour the normalization of liver enzymes and weight loss, similar to MedDiet approach [98]. However, it is still inferior to the MedDiet in the reduction of hepatic steatosis, with the impossibility of determining the impact of dietary intervention on fibrotic NASH due to the reduced number of studies included [98].

In recent years, different patterns of fasting have become a dietary restriction modality associated with better metabolic control, reduction in body weight and improvement in liver function tests in healthy individuals [78,91].

Intermittent fasting refers to a period during a day with low or null caloric intake, followed by a normal feeding period [78,99]. Alternate day fasting refers to a modality where during a defined period of the week the daily intake is less than 25% of requirements followed by normal intake on the remaining days [78,100]. Periodic fasting, is a different modality, with an intake of less than 250 kcal/day for a period from 2 to 21 days [78,101]. These modalities in NAFLD patients, have demonstrated a decrease in liver enzymes, hepatic steatosis, and body weight, with higher adherence from participants [99–101]. Reported adverse effects might include light headache and constipation, with mixed results on food craving [99,101]. A major limitation associated with trials using these fasting regimens is the reduced intervention period and, in most studies, the absence of an adequate control group [78,91], which makes this strategy, to this day, just a possible complement or short time alternative to more common dietary restriction modalities, even to MedDiet, the most widely accepted dietary pattern for the management of NAFLD.

Nonetheless, this method of dietary intervention is, to date, the one that has been studied for MAFLD. Rozanski et colleagues [102], conducted a systematic review regarding the effect of intermittent fasting on biochemical and anthropometric parameters among patients with MAFLD. Eight studies, comprising 498 participants were included. The authors were not able to determine the effects of different types of intermittent fasting on anthropometric and biochemical parameters but suggest that this dietary intervention can have some therapeutic effect. Of note, however, six of the included studies in the systematic review excluded patients with alcohol intake above the recommended threshold for NAFLD, which again constitutes a major limitation in the application of the results to the MAFLD population.

However, since the new proposed definition, dietary studies are scarce and to date, no study has addressed dietary intake in relation to the severity of steatosis and fibrosis in MAFLD patients, also considering the amount of alcohol intake.

1.5. Fatty liver disease in HIV patients: a growing concern but overlooked condition?

Human Immunodeficiency Virus infection (HIV) continues to be a public health issue. The Joint United Nations Programme on HIV/AIDS estimates that globally 37.7 million [30.2 million–45.1 million] people are currently living with HIV (PLWH) [103]. National data estimates around 55.000 PLWH, with an annual incidence of 8.7 cases per 10^5 inhabitants, with PLWH being mostly heterosexual males under 40 years of age [104].

The management of HIV infection witnessed a turning point with the introduction of combined antiretroviral therapy (cART) treatment, which allowed almost a complete restauration of the immune system and prevented the progression to Acquired Immunodeficiency Disease Syndrome (AIDS) [105].

Currently, 90% of those on cART are virally suppressed and with sustained adherence to this drug regimen, PLWH have an increased life expectancy, made possible by the reduced morbidity and mortality from opportunistic infections and malignancies [103,105–108]

This increased life expectancy, raisedhigher the incidence and prevalence of non-infectious comorbidities in PLWH, with higher prevalence than the general population [105,107,109,110]. These include cardiovascular disease, hypertension, diabetes mellitus, and liver disease [105,107,109,110]

Liver disease remains one of the highest causes of mortality in PLWH, with increasing rates in recent years, surpassing cardiovascular disease [108,111–114].

With the associated chronicity of HIV infection and the rising prevalence of obesity and MetS in PLWH, the first scarce references before 2010 documented an increase in hepatic steatosis in this population, associated mostly with lipodystrophy syndrome, metabolic abnormalities or with the co-existence of viral hepatitis [115–119], but with limited information regarding its management.

Presently, high rates of coinfection with viral hepatitis and excessive alcohol consumption, remain as leading causes of liver disease in PLWH. However, with the increasing availability of effective antiviral therapy for viral hepatitis, and also with the recognition of the burden of liver disease in HIV mono-infected, a major interest in NAFLD as a manageable cause of liver disease in this population has emerged [108,111–114,120,121].

Some metabolic comorbidities are more frequent in PLWH than in the general population [113,121]. Traditional risk factors, such as high BMI, high waist circumference, T2DM and hypertension are highly associated with NAFLD in PLWH [108,113,114,122–125] (Figure 10).

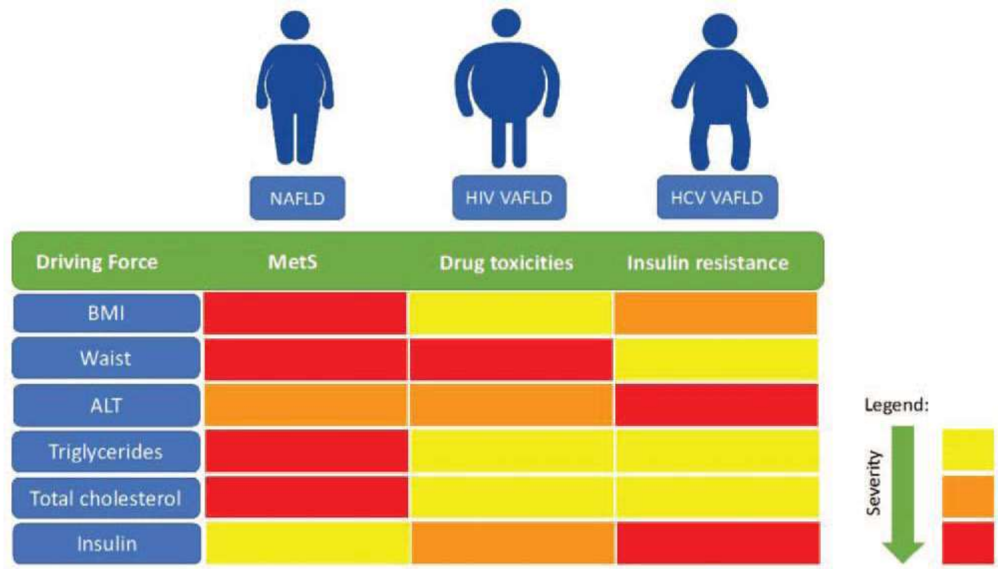

Figure 10: Predictors of fatty liver: NAFLD versus NAFLD-HIV and HCV-NAFLD

Adapted from Ref [121] with permission

Higher BMI and presence of insulin resistance have also been associated with liver fibrosis development [122,126–128]. However, mechanisms of interaction may be different from the general population, since generally NAFLD occurs in PLWH in lower BMI values and a recent study in 1511 PLWH, from whom 57.4% were lean, estimated a NAFLD prevalence of 25% in the latter [111,114,129]. Also, contributions of specific demographic characteristics such as sex and ethnicity could contribute to an increased risk of developing NAFLD, explained by the higher risk of weight gain, poorly managed comorbidities and higher exposure to food insecurity [130–132].

Also, one must consider HIV-associated factors. Length of exposure to HIV, immune activation, the presence of low-grade inflammation, and increased production of oxygen reactive species are substantial contributors to insulin resistance, that diminish with successful viral suppression, but are still present in chronic HIV infection, and can contribute to NAFLD development and progression [111,113,114,121,123,125] (Figure 11).

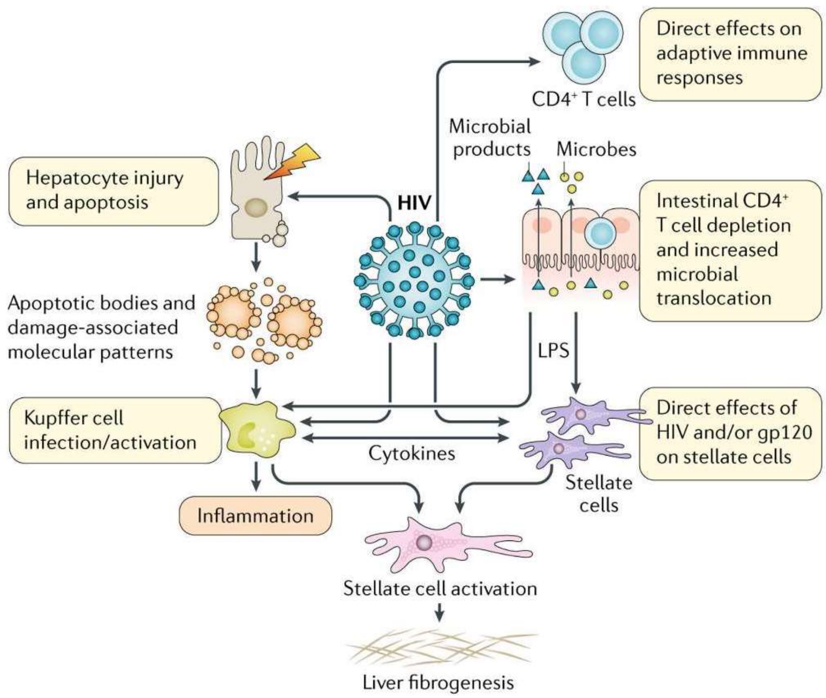

Figure 11: Promotion of hepatic inflammation and fibrosis by HIV

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Also, cART can contribute to insulin resistance, lipid profile alterations, and mitochondrial dysfunction, factors that can lead to hepatic steatosis development and fibrosis progression, although conflicting data still exists because of different types of drug profiles within cART classes and hepatic different possible profiles of hepatic toxicity [111,113,114,127,134–138]. A direct relation between time of exposure to cART and hepatic steatosis has not been demonstrated, despite some of the more cART toxic agents being older, but higher TCD4+ count emerged as a factor associated with NAFLD in PLWH, despite lower TCD4+ count before cART being associated with higher odd of significant weight gain

[114,122]. Associated with cART, is lipodystrophy, a syndrome characterized by body composition alterations, with an increase in visceral adipose tissue and the presence of metabolic abnormalities, such as insulin resistance and dyslipidaemia, and also a specific factor associated with increased odd of NAFLD [108,113,114,136].

Finally, and similar to what has been described in the general population, the genetic background may also be of importance in PLWH, with SNPs such as PNPLA3 gene (rs738409; I148M) non-CC genotype to be associated with a higher prevalence of fatty liver disease in PLWH [113,127].

To date, there are still conflicting results on the classical and HIV associated factors that are key to the development and progression of NAFLD (Figure 12).

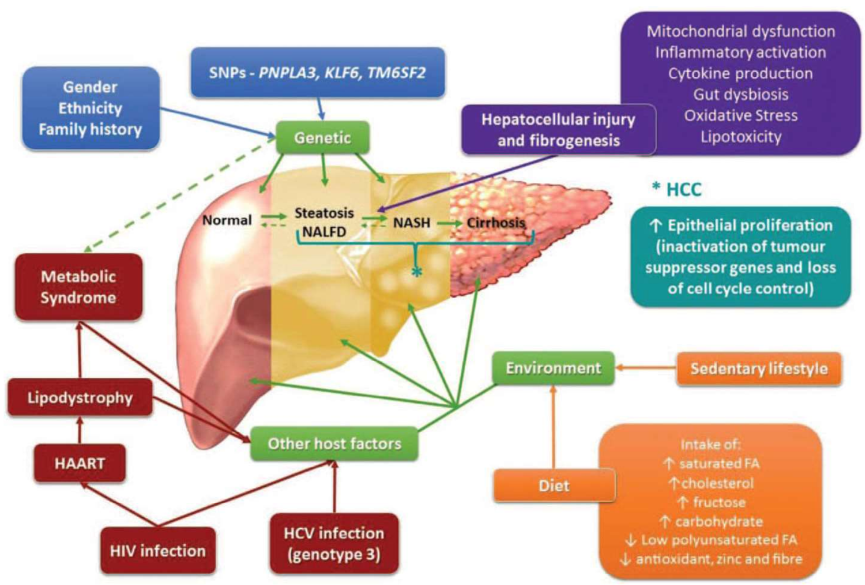

Figure 12: Risk factors associated with NAFLD and liver disease progression in PLWH

Adapted from Ref [121] with permission

The gold standard for diagnosis NASH is liver biopsy, however, due to its associated risk and practical constraints, NITs have been proposed to PLWH. Serological biomarkers such as FIB-4, NAFLD fibrosis score, and Fatty Liver Index (FLI) have been applied to PLWH and shown good accuracy, high specificity and negative predictive value [108,114,139–141]. TE with determination of CAP has also been demonstrated to be a useful diagnostic tool in PLWH [113,140–143].

The estimated prevalence for NAFLD in PLWH is between 28-45%, with estimates varying depending on the study population and diagnostic tools [108,111,113,123,140,144] with a recent meta-analysis with imaging-based diagnosis and excluding hepatitis C co-infection and high alcohol intake patients, estimating the prevalence in 35% [122], a higher value than the one documented for the general population. The prevalence of NASH presents also conflicting data in HIV-mono infected patients, with estimates ranging from around 10% to more than 50% in patients with metabolic abnormalities and persistent elevation of liver enzymes, depending on diagnostic tools [113,122].

This natural history of NAFLD in PLWH is also not completely established, with evidence indicating that PLWH present more features of hepatocyte injury, higher rates of NASH, and higher risk of fibrosis progression in comparison with the general population with NAFLD, and even compared to HIV/HCV co-infected patients [108,112,113,123]. Also, there are no specific features that can aid in the distinction between primary NAFLD and HIV-NAFLD [114]. In longitudinal studies, the incidence of NAFLD in PLWH is estimated at 6.9 per 100 persons-years (PY), with the presence of metabolic abnormalities, specially higher BMI and the presence of T2DM, being associated with a higher risk of progression to cirrhosis [113,128,142,145–148], with a longitudinal study determining a progression to liver fibrosis or liver cirrhosis at a rate of 12.7 per 100 PY, [123,147] higher than the general population.

Although data in HIV-mono infected patients is lower than for viral hepatitis, NAFLD and NASH have been associated with increased incidence of metabolic comorbidities, such as T2DM and dyslipidaemia, and increased cardiovascular risk, even in the presence of normal BMI and at a younger age [113,121,148–152], which can lead to higher mortality from cardiovascular disease in PLWH [108,113,121,148,151,152]. Frailty, a clinical condition that increases the risk of multimorbidity and disability, has also been associated with NAFLD and fibrosis in PLWH [121,153]. Liver related events (decompensation, hepatocellular carcinoma and death) are estimated in a rate of 8.6 per 1000 PY in PLWH [113].

In recent years, an effort has been made to develop algorithms for optimizing NAFLD screening in PLWH, to reduce the disease burden associated with late diagnosis, especially mortality [113,114,121,154,155]. Estimates point that one in five HIV-mono infected would benefit from a detailed assessment for NAFLD and disease severity, with an implementation of a two-tier pathway, with NITs and TE, to optimize resources and reduce costs [114,155,156].

Despite this effort in improving screening, and considering that NAFLD management in the general population resides in lifestyle changes, data on therapeutic strategies and lifestyle changes implementation in PLWH is still scarce, with recommendations deriving mostly from general guidelines on NAFLD management [24,37,90,114,120,157,158]. In the management of chronic HIV infection, UNAIDS established a 90-90-90 target to countries: 90% of PLWH are knowledgeable about their diagnosis, 90% of those diagnosed are on cART and 90% of patients on cART have sustained suppressed viral load. The additional "fourth 90", establishes that at least 90% of PLWH under cART and virologically supressed have a good quality of life [120]. The presence of NAFLD and other metabolic comorbidities has been demonstrated to impair health related quality of life in PLWH, especially in the dimensions of mobility and pain/discomfort [159].

To reach the "fourth 90" goal, and improve quality of life it is essential an adequate assessment of risk factors, and nutritional intervention plays a key role in the management of chronic HIV infection, NAFLD and related metabolic complications [120,159].

Lifestyle intervention trials in PLWH, have focused on the management or prevention of cardiovascular disease or metabolic comorbidities. Five trials with a period of intervention ranging from 8 weeks to 12 months, with dietary modifications, (reduced caloric content, reduction in saturated fat and refined sugar, increase in polyunsaturated fat and fiber) coupled with smoking cessation and increased in physical activity, demonstrated a decrease in total weight and body fat, improvement in blood lipid profile (decrease in total cholesterol and triglycerides) and improvement in markers of insulin resistance (decrease in glucose and insulin) [160]. Only two trials have assessed solely the impact of dietary modifications. Reeds and colleagues demonstrated that weekly sessions of nutritional counselling in HIV obese women allowed 68% of them to achieve between 6-8% of weight loss and Stradling and colleagues, in 60 participants with LDL cholesterol ≥116mg/dl and triweekly sessions of nutritional counselling, demonstrated a decrease in LDL levels and an improvement in adherence to Mediterranean diet recommendations [160–162]. Only recently, the focus on NAFLD has emerged. In a cross-sectional study with 451 non-obese PLWH, mostly under cART, using two non-consecutive 24h dietary recall for dietary intake assessment and TE for liver disease assessment, it was demonstrated that a higher intake of total fat was associated with higher odd for NAFLD [aOR=1.91 (95% CI 1.06–3.44)] and higher intake of n-6 polyunsaturated fatty acids was associated with higher odd of fibrosis [aOR = 2.45 (95% CI 1.12–5.32)]. On the opposite, higher fiber intake was associated with lower odd of NAFLD [aOR = 0.88 (95% CI 0.48–1.60)] and fibrosis [aOR = 0.95 (95% CI 0.47–1.95)] [163].

Despite this recent interest, the impact of dietary modification strategies on liver related outcomes in PLWH is largely unknown.

2.

AIMS

- Assess the magnitude of weight loss with a dietary intervention program in either NAFLD patients from the general population or NAFLD-HIV patients.
- Compare the amount of weight loss between patients managed by a multidisciplinary team and patients managed solely by the hepatologist.
- Determine the prevalence of NAFLD in a cohort of HIV patients.
- Compare the presence of risk factors for NAFLD between HIV patients and a national cohort.
- Explore the possible contribution of HIV-related factors for the presence of NAFLD.
- Assess the dietary intake of NAFLD-HIV patients in comparison with a national cohort.
- Characterize the dietary pattern alterations during a 3-month Covid-19 national lockdown.
- Assess the impact of dietary intervention on weigh evolution during a 3-month Covid-19 national lockdown, and the use of telemedicine.
- Explore the dietary intake of fatty liver disease patients based on the new proposed definition of MAFLD.
- Explore the relation between the inflammatory content of the diet and MAFLD.

3.

STUDIES

3.1.

STUDY 1

NAFLD Nutritional Management: Results from a Multidisciplinary Approach

Policarpo S, Machado MV, Barreira D, Cortez-Pinto H. NAFLD Nutritional Management: Results from a Multidisciplinary Approach. GE Port J Gastroenterol doi: 10.1159/000519932 Epub 2019 Oct 22 *Reproduced with permission*.

NAFLD NUTRITIONAL MANAGEMENT: RESULTS FROM A MULTIDISCIPLINARY APPROACH

ABSTRACT

Introduction: Lifestyle changes are the mainstay treatment of Non-Alcoholic Fatty Liver Disease (NAFLD). We aimed to assess the magnitude of weight loss in a group of NAFLD patients followed on a combined lifestyle intervention by a multidisciplinary team.

Methods: Patients were assessed before and after a 12-month dietary intervention (Mediterranean diet aiming weight loss). Patients who received a structured dietary plan along with general lifestyle recommendations were designated Multidisciplinary treatment (MdT) group. Patients who declined follow-up still received general lifestyle recommendations, and were designated as conventional treatment group, being used as a control group.

Results: From the 77 patients with documented NAFLD, 31.2% of patients were overweight and 55.8% obese; 66 patients constituted the MdT group and 11 the conventional treatment group. After 3 months, 89% of patients lost weight; at 6 months, 75.4% maintained weight lost. At 12 months, 65% of patients still decreased their weight, with 92.2% of patients in the MdT group still maintaining a lower weight than baseline vs. just 50% on conventional group; p=0.008. Only patients on MdT group presented a weight loss higher than 10% (9.6%; n=6). At 12 months patients in MdT group presented an average reduction of 4.2kg vs. reduction of just 0.6kg on the conventional treatment group (p=0.016). MdT group, but not the conventional group, presented significant differences in liver enzymes at 12 months compared to baseline.

Conclusion: Adherence to a multidisciplinary approach, compared to management solely by an Hepatologist, in NAFLD patients, is effective with greater weight loss after a 12-month follow-up and a lower rate of weight gain recurrence.

Keywords: dietary intervention, Mediterranean diet, NAFLD, multidisciplinary team, weight loss

RESUMO

Introdução: Mudanças no estilo de vida são a base do tratamento do Fígado Gordo não-alcoólico (FGNA). O nosso objetivo foi avaliar a magnitude da perda de peso num grupo de doentes com FGNA sujeitos a intervenção nutricional e acompanhados por uma equipa multidisciplinar.

Métodos: Os doentes foram avaliados antes e após uma intervenção nutricional de 12 meses (dieta mediterrânica com objetivo de perda ponderal). Os doentes que recusaram follow-up multidisciplinar, receberam recomendações gerais de estilo de vida e foram designados como grupo de tratamento convencional e usados como comparação com o grupo de tratamento multidisciplinar (MD), que em adição às recomendações gerais, recebeu plano nutricional personalizado

Resultados: Dos 77 pacientes com FGNA documentado, 31,2% dos pacientes estavam em pré-obesidade e 55,8% eram obesos; 66 pacientes constituíram o grupo MD e 11 o grupo de tratamento convencional. Após 3 meses, 89% dos pacientes perderam peso, aos 6 meses, 75,4% mantiveram a perda de peso. Aos 12 meses, 65% dos pacientes ainda diminuíram seu peso, com 92,2% dos pacientes no grupo MD ainda mantendo um peso inferior ao baseline vs. 50% no grupo convencional; p=0,008. Apenas os pacientes do grupo MD, conseguiram uma perda de peso superior a 10% (9,6%; n=). Aos 12 meses, os pacientes do grupo MD apresentaram redução média de 4,2kg vs. redução de 0,6kg no grupo de tratamento convencional (p = 0,016). O grupo MD apresentou diferenças significativas nas enzimas hepáticas aos 12 meses em comparação com baseline.

Conclusão: A abordagem multidisciplinar em pacientes com FGNA é efetiva, com maior perda de peso durante o acompanhamento de 12 meses e maior taxa de perda de peso mantida, em comparação com acompanhamento exclusivamente médico.

Palavras-chave: intervenção nutricional, dieta mediterrânica, FGNA, equipa multidisciplinar, perda ponderal

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent cause of chronic liver disease in the Western world, coupled with the worldwide increase in the prevalence of obesity and diabetes mellitus (1,2) Obesity not only associates with an increased risk for developing NAFLD (3.4) it also associates with a more severe presentation, a higher risk for advanced liver disease, and liver cancer (3,5)

It is estimated that the global prevalence of NAFLD has been increasing in the past decade and is currently around 24% of the general population worldwide (1)

One of the most relevant factors associated with the development of NAFLD is imbalanced dietary habits (1,4,6,7) In fact, NAFLD seems to associate with a high intake of calories, sodium, sugar, and fat, and a lower amount of nutritional dense foods present in their diet (6,8,9) as well as with a sedentary behavior (1,8).

NAFLD guidelines recommend that the assessment of dietary and physical activity habits should be a part of the initial evaluation of NAFLD patients (10)

The Mediterranean diet is a dietary pattern strongly recommended for the management of NAFLD patients (4,7,10). This dietary pattern is rich in fiber and polyunsaturated fatty acids. It is characterized by a high intake of fruit and vegetables, a moderate intake of low-fat dairy products and fish, and a low intake of red and processed meat (11). This dietary pattern has demonstrated effectiveness in lowering body weight, improving clinical features of the metabolic syndrome and reducing the severity of liver disease (6,12)

For the treatment of NAFLD a tailored approach combining diet and physical activity should be implemented (4,7,8,10) However, low adherence to lifestyle changes, specifically to dietary changes, is commonly reported amongst these patients (8). Even though physician advice has demonstrated positive effects on weight lost, ideally the treatment of NAFLD patients should be managed by a multidisciplinary team, which provides strategies to prevent relapses and weight gain (8,13)

We aimed to assess the magnitude of weight loss in a group of NAFLD patients managed on a combined lifestyle intervention by a multidisciplinary team. We hypothesized that patients managed by the multidisciplinary team would achieve a higher weight loss compared to those managed solely by the Hepatologist.

MATERIALS/SUBJECTS AND METHODS

Subjects

During six-months, patients referred from primary care to the outpatient Hepatology Ambulatory Clinic of a tertiary university hospital, with the diagnosis of NALFD, were enrolled. The diagnosis of NAFLD was made on the presence of liver steatosis on ultrasound, after exclusion of significant alcohol intake, (more than 20 grams per day in women and 30 grams per day in men) or of other causes of liver disease: chronic viral hepatitis B or C, primary biliary cholangitis, autoimmune hepatitis, primary sclerosing cholangitis, Wilson's disease, hemochromatosis or a1-antitripsin deficiency. Patients were excluded if there was history of: treatment with potentially steatogenic drugs (such as steroids, high dose estrogen, tamoxifen, methotrexate or amiodarone) within six months of enrolment: gastrointestinal bypass surgery or segmental small bowel resection. The severity of liver disease was evaluated by liver enzyme tests and transient hepatic elastography (10,14) Clinical data including liver biochemistry was collected.

NAFLD Fibrosis Score (NFS) and Fibrosis 4 score (FIB4) were calculated to evaluate fibrosis severity, and advanced fibrosis was considered wen FIB4>2.67 and NFS>0.675 (15,16). Advanced fibrosis was considered if liver stiffness measurement (LSM), measured by transient elastography (FibroScan®), was ≥11.1kPa (17)

Lifestyle counselling and nutritional assessment

All NAFLD patients were invited to be managed in a multidisciplinary group (hepatologist, dietitian and psychologist – MdT group). Clinical data was collected by two experienced hepatologists, who used a standardized protocol to minimize bias on data collection. At baseline, a dietician performed a complete nutritional assessment. General lifestyle recommendations were given and included written resources containing healthy eating guidelines and tips, basic information on portion size, exchange tips and general dietary recommendations. Other specifications of dietary advice included alcohol abstinence.

Baseline physical activity was assessed using the International Physical Activity Questionnaire (18) Patients were also encouraged to increase their physical activity, with an incremental approach of exercise in everyday life, aiming at 150m/week (10).

Patients who agreed to enrol on the multidisciplinary approach (MdT) also received a structured personalized dietary plan, with 5 to 7 meals a day, aiming at caloric restriction (-500kcal) to promote weight loss. This structured nutritional plan was based on the Mediterranean diet and was individualized according to nutritional needs, the presence of other dietary restrictions alongside NAFLD, and the personal preferences and food habits of the patients. Patients who declined long-term management by the dietitian still received general lifestyle recommendations and were designated as conventional treatment group, maintaining management solely by the hepatologist. This group was used as a comparison with MdT group.

Regular appointments at 1, 3, 6 and 12months, were provided by a dietitian. During appointments, adherence to recommendations was monitored (19) Psychological support was offered during follow-up to increase the motivation to adopt lifestyle changes and to help copping with difficulties.

Anthropometric data was collected with participants wearing light clothes and bare foot; current weight was measured using a calibrated scale, and height was assessed using a stadiometer. History of weight loss in the previous six months was recorded. Waist circumference (WC) was measured at halfway between the inferior rib and the iliac crest and abdominal circumference was measured at the umbilicus level (20) . Body mass index (BMI) was defined as an individual's weight in kilograms divided by the square of height in meters (kg/m2) and classified according to World Health Organization. Body fat mass (BFM, %) was assessed using a single frequency bioimpedance analyser (OMNRON BF350).

During follow-up, patients that presented a clinical condition that implied a modification of dietary pattern and/or had a clinical condition that impacted directly on nutritional (i.e., pregnancy, newly diagnosed cancer or gastrointestinal disease, hospital stay, bariatric surgery) status were excluded. Patients who did not comply with scheduled appointments (MdT or conventional treatment group) were also excluded from the analysis.

Statistical Analysis

To determine a significant difference in both groups for α=0.05 and a power of 0.8 we estimated a minimum sample of 21 participants in each group. Normal distribution was assessed using histograms, boxplots and Shapiro-Wilk test. Continuous variables were summarized as mean and standard deviation (mean±SD) or median and Interquartile range (IQR) when the distribution was not normal. Categorical variables were summarized using frequency and percentage. For normal distributed variables, Chi-square or Fisher-exact Test for 2x2 tables were used for independence of categorical variables and Student T-Test or ANOVA, with multiple comparisons adjusted with Bonferroni correction, was used for continuous variables. For each analysis, unadjusted and adjusted odds ratio (OR) and the corresponding 95% confidence interval (CI) and p-value were reported. The significance level was set at 5%. All data analysis was performed with IBM® SPSS® software, version 26.0.

RESULTS

Population characterization

From the 139 patients assessed, 50 were excluded for having other identifiable causes of liver disease. During follow-up, 8 patients were excluded due to the occurrence of pregnancy, scheduled bariatric surgery and diagnosis of cancer.

77 patients with NAFLD were enrolled, of whom 51 were males (66.3%). At baseline, BMI was 31.5±5.8 kg/m2, with 31.2% of patients being overweight and 55.8% obese. Advanced fibrosis was present in 15.7% of patients when assessed by elastography, and in 11.4% and 18,6% of patients, according to FIB4 and NFS respectively (Table 1).

Table 1: Baseline patient characteristics

	Baseline (n=77)
Sex (%)	
Male	66.2
Female	33.8
Age (years)	54.4±12.7
Type 2 diabetes mellitus (%)	45.5
High blood pressure (%)	55.8
Dyslipidaemia (%)	58.4
Weight (kg)	88.7±16.5
Body fat mass (%)	30.8±9.1
Body fat mass (kg)	27.4±10.7
BMI (kg/m^2)	31.5±5.8
BMI category (%)	
Normal (18.5-24.9kg/m^2)	13.0
Overweight (25-29.9kg/m^2)	31.2
Obesity (30-39.9kg/m^2)	42.8
Obesity (≥40kgm^2)	13.0
Waist circumference (cm)	103.9±11.9
Abdominal circumference (cm)	108.5±12.9
AST (IU)	44.6±28.0
ALT (IU)	68.9±49.5
GGT (IU)	113.3±108.0
Weight status prior to enrolment (%)	
Increase	37.7
Decrease	26.2
Stable	36.1
Elastography (%)	
F0 (≤5.0kPa)	27.1
F1-F2 (5.1-11kPa)	57.2
F3-F4 (≥11.1kPa)	15.7
FIB-4 >2.67 (%)	11.4
NFS >0.675 (%)	18.6

Values presented as % for categorical values or mean ± standard deviation for continuous variables; BMI: Body Mass Index; AST: Aspartate transaminase, ALT: Alanine aminotransferase, GGT: gamma-glutamyl transferase.

From the 77 patients, 14.3% (n=11) declined to be managed by the MdT (conventional treatment group). There were no significant differences in baseline characteristics, namely age, sex, comorbidities, baseline BMI and severity of liver disease, between groups.

At baseline, none of the patients included in both groups presented moderate or vigorous physical activity levels.

Anthropometric evolution

After 3 months, 89% of patients lost weight, with a mean weight loss of 3.1kg. At 6 months, 75.4% maintained weight lost, with a mean additional weight loss of 0.7kg. At 12 months, 65% of patients decreased their weight, but only with a mean additional reduction of 0.3kg. When comparing groups, we witness a higher degree of weight loss in the MdT group. Indeed, in the MdT group, at 3 months, 93.7% of patients lost weight at 3 months (vs. 60% on conventional group; p = 0.001); 81.7% of patients continued to lose weight at 6 months (vs. 33.3% on conventional group; p=0.005) and 72.5% at 12 months (vs. 22% on conventional group; p=0.006). Also, in the MdT group, comparing with baseline, 95.2% of patients had lower weight at 6 months (vs. 70% on conventional group; p=0.003), and 92.2% at 12 months (vs. 50% on conventional group; p=0.008).

At 12 months, 32.7% of patients in the MdT group had a weight reduction between 5-10%, as compared to only 12.5% of patients in the conventional treatment group (p=0.007). None of the patients in the conventional treatment group presented a weight loss higher than 10%, compared with 9.6% of patients in the MdT group at 12 months (Table 2).

Table 2: Anthropometric evolution during follow-up

	MdT-Group (n=66)			Conventional Group (n=11)		
	3-Months	**6-Months**	**12-Months**	**3-Months**	**6-Months**	**12-Months**
Weight (kg)	85.2±14.2	83.5±13.4	83.4±13.6	89.7±24.0	92.6±22.3	94.7±24.7
Reduction in weight from baseline (kg)	-3.2±2.8**	-4.2±4.0**	-4.2±3.6**	-1.7±3.9	-1.2±4.1	-0.6±4.0
Reduction in weight from previous assessment (kg)		-1.0±1.9	-0.4±1.7		-1.1±3.1	0.3±1.1
Decrease from baseline (% of patients)	93.7*	95.2*	92.2**	60	70.0	50.0
Decrease from previous assessment (%)		81.7**	72.5**		33.3	22
Weight loss (% of patients)						
<5%	60.9	47.6	50	50	60	37.5
5-10%	28.1	41.3	32.7	10	10	12.5
>10%	1.6	4.8	9.6			
BMI (kg/m^2)	30.3±5.2	29.9±5.1	29.6±5.3	31.9±7.3	32.1±7.6	34.0±7.5
Mean decrease in BMI from baseline (kg/m^2)	-1.2±0.9*	-1.5±1.4*	-1.5±1.3*	-0.6±1.3	-0.3±1.4	-0.1±1.5
BMI category (% of patients)						
Normal (18.5-24.9kg/m^2)	14.1	15.9	21.2	20.0	20.0	12.5
Overweight (25-29.9kg/m^2)	42.2	42.9	40.4	30.0	30.0	25.0
Obesity (30-39.9kg/m^2)	35.9	34.9	32.6	30.0	30.0	37.5
Obesity (≥40kgm^2)	7.8	6.3	5.8	20.0	20.0	25.0
Reduction in BMI category (% of patients)	33.9	34.9	42.9	22.2	30	38.5

Values presented as % for categorical values or mean ± SD for continuous variables (SW test); * p <0.05 when compared to the conventional group; ** p<0.01 when compared to the conventional group (Fisher-exact test for categorical values and Student T-Test for continuous variables); MdT Group: Multidisciplinary treatment group; BMI: Body Mass Index

The presence of comorbidities (diabetes, high blood pressure or dyslipidaemia) was not associated with weight variation or percentage of weight loss. Also, age, sex, presence of comorbidities and severity of liver disease were not associated with the magnitude of weight loss at 12 months. Furthermore, there was no association between previous reported weight loss or baseline BMI, with the magnitude of weight loss achieved during the intervention period, although there was a tendency for patients with a higher baseline BMI to achieve higher weight loss (Figure 1).

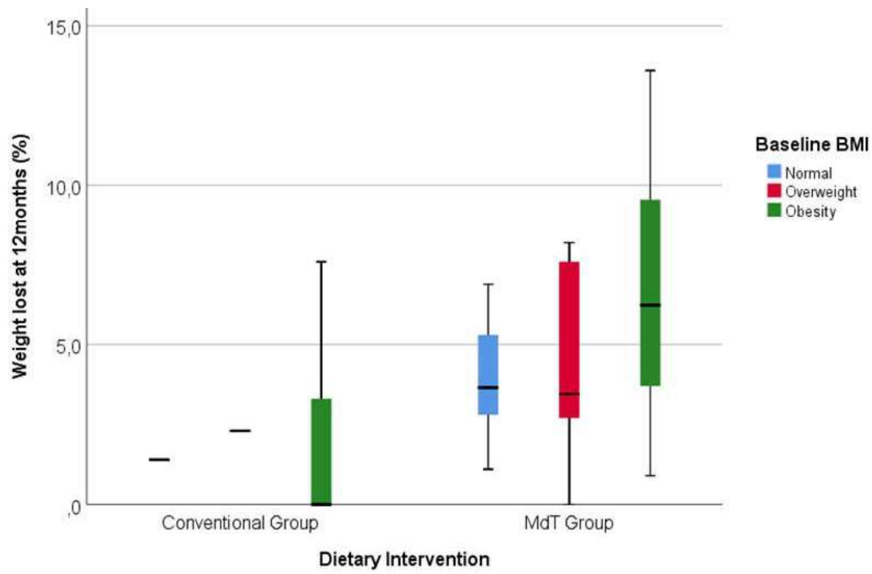

Fig 1: Weigh loss at 12 months by baseline BMI

Evolution of makers of liver disease activity, and evidence of fibrosis severity by elastography

Liver enzymes decreased in most patients, being the effect attenuated over time: decrease in AST in 81.3%, 79.2% and 81% of patients, ALT in 77.1%, 79.2% and 50%, and GGT in 75%, 68.8% and 42.9%, at 3, 6 and 12 months, respectively.

MdT and conventional treatment group did not show any significant difference in baseline liver enzymes. However, although both groups experienced reduction in liver enzymes, the mean values at 3 months, 6 months and 12 months improved only in the MdT group, with significant reductions. The group of patients on the MdT group that presented weight loss >10% at 12 months, presented a lower median ALT (20 IU) when compared with patients with lower weight loss (29 IU 5-10%; 43 IU <5%, 41 IU in patients with no weight loss; p=0.042). Globally, 52.2% of patients showed an improvement in liver fibrosis measured by FIB4 and 49.2% by NFS, without significant differences between MdT or conventional treatment group (p>0.05) (Table 3).

Table 3: Liver enzymes evolution during follow-up

	MdT-Group (n=66)			Conventional Group (n=11)		
	3-Months	6-Months	12-Months	3-Months	6-Months	12-Months
AST (IU)	35.0±16.0*	31.5±25.0*	30.2±14.2	25.5±4.6*	35.2±13.3	26.1±10.4
Reduction from baseline (IU)	-17.6±30.2	-14.2±34.1	-21.1±35.5	-5.8±6.7	-1.5±6.3	-4.1±11.3
Decrease from baseline (% of patients)	81.0	81.0	79.4	83.3	66.7	87.5
ALT (IU)	53.0±31.6*	37.5±16.8*	39.9±23.3*	33.3±14.6*	61.8±37.5	42.8±26.5
Reduction from baseline (IU)	-29.7±56.2	-30.6±47.8	-11.3±38.6	-9.8±10.3	-0.7±17.5	12.5±26.2
Decrease from baseline (% of patients)	76.2	78.6	52.9	83.3	83.3	37.5
GGT (IU)	85.2±76.8*	90.0±76.0*	72.3±60.0*	43.5±23.6	80.7±70.6	63.0±45.4
Reduction from baseline (IU)	-38.2±67.9	-36.4±88.8**	-21.1±70.4	-23.3±71.4	9.5±16.8	32.8±52.1
Decrease from baseline (% of patients)	78.6	73.8	41.2	50.0	33.3	50
FIB-4 >2.67 (% of patients)	17.1	12.5	11.8	-	-	-
NFS >0.675 (% of patients)	29.3*	22.5	17.6	33.3	-	25.0

Values presented as % for categorical values or mean ± SD for continuous variables; * p <0.05 when compared to baseline; **p<0.05 when compared with conventional group (Fisher exact test for categorical values and Student T-Test for continuous variables); MdT Group: Multidisciplinary treatment group; AST: Aspartate transaminase, ALT: Alanine aminotransferase, GGT: gamma-glutamyl transferase.

In a group of 24 patients that performed more than one transient elastography during follow-up, an improvement was verified in 70.8% of patients, with significant reduction of mean values between baseline and 6 months (10.1±6.5 vs. 8.5±6.7kPa; p=0.048) in the MdT group. In the conventional treatment group, all patients remained an F2-F3 classification and in the MdT group F0 patients increased from 23.8% at baseline to 38.1% with a reduction in the F1-F2 from 52.4% to 42.9% and F3-F4 from 23.8% to 19% of patients after 6 months (p>0.05).

DISCUSSION

Our results suggest that a multidisciplinary approach is effective in inducing weight loss and in maintaining the weight loss during the 12-month follow-up.

In this study we evaluated the efficacy of a multidisciplinary team on the implementation of lifestyle changes, regarding the adoption of a more balanced dietary pattern and an increase in physical activity. The results of the present study suggest that a multidisciplinary approach is effective. However, regular follow-up by the multidisciplinary team is needed since patients who had only one nutritional appointment and maintained management solely by the hepatologist presented lower weight loss and regained almost all the lost weight.

Regarding an effect on liver fibrosis, in the subset of 24 patients that performed more than one transient elastography, we witnessed a trend to a decrease in mean kPa. We could not demonstrate an improvement of liver fibrosis with the non-invasive scores FIB-4 and NFS. However, the time interval of 12 months is probably not enough to accomplish a significant reduction in fibrosis degree.

Our results are consistent with previous studies (21,22) that have demonstrated that tailored nutritional intervention integrated in a multidisciplinary approach is effective in reducing body weight and improving liver enzyme tests and steatosis in NAFLD patients.

It has been demonstrated that fat or carbohydrates content of the dietary intervention has different influences on liver disease severity scores and assessment of steatosis (23) However, instead of an intervention of specific nutrient, dietary patterns have emerged as a better option to study the impact of dietary intervention on the development or progression of liver disease (24).

Although some dietary strategies have demonstrated benefits in weight loss, such as the classic hypocaloric low-fat/low-carb diet (25,26), and recently ketogenic diet (27), and intermittent fasting (28) the Mediterranean dietary pattern is the one recommended by the European Association for the Study of the Liver in NAFLD guidelines. Indeed, several epidemiological and interventional studies assessing the Mediterranean diet showed a beneficial effect on BMI reduction and long-term maintenance of body weight, as well as on triglycerides, cholesterol and glycaemia, and liver enzymes (7,29-32) However, directives on how to implement it and the magnitude of caloric restriction required remains unclear. Also, additional changes to the traditional pattern, such as recommendations on alcohol intake, remain unclear (33).

In this study, we were able to demonstrate that with the modification of the traditional Mediterranean dietary pattern, the group of patients intervened demonstrated a higher weight loss, a higher reduction in liver enzymes and a higher and more sustained weight loss in comparison with patients with general nutritional counselling.

One of the strengths of this study is the demonstration that a multidisciplinary approach is more effective than standard counselling alone. However, and although it has been demonstrated that educational interventions improve readiness to change dietary habits, (34) we cannot exclude the degree of bias resulting from the fact that those on the conventional treatment group could be the less motivated for a change in their lifestyle. Additionally, our study has a relatively small sample size. Another limitation of this study is the inability to quantify the motivation for change in both groups, even though a psychological intervention was carried out.

Previous studies with liver biopsy were able to demonstrate that weight loss was very effective in improving all histological parameters of NAFLD, much more significantly than any pharmacological intervention (35). In the present study, because performing a liver biopsy for the sole purpose of this research was considered unethical, histology was not available, what precludes us from evaluating in more detail the effect of the MdT.

This study also contributes to explain the reason why the placebo arms of the NAFLD/NASH treatments frequently have a significant improvement in their liver disease. In fact, patients enrolled in critical trials, often start to receive more attentive lifestyle counselling, and gain motivation to change their lifestyle with consequent weight loss (36). Education interventions improve anthropometric parameters, metabolic syndrome and cardiovascular disease, liver disease, and also health-related quality of life (13,34).

In conclusion, this study demonstrates that a multidisciplinary approach is effective in promoting a Mediterranean dietary pattern, reducing body weight, and maintaining that weight loss.

Even though differences in baseline motivation cannot be excluded, patients on the conventional treatment group with one session of nutritional counselling were able to achieve weight loss. This study shows that one session might not be enough to guarantee the amount of weight loss necessary to improve liver steatosis/ fibrosis. This reinforces the need to maintain/create adequate multidisciplinary groups to guarantee an adequate care and management of these patients, since often clinicians do not have neither time nor resources to support behavior change (37)

Further studies incorporating motivational, and quality of life questionnaires coupled with dietary intervention are needed to better characterize NAFLD patients and to delineate more tailored lifestyle interventions.

Author Contributions: All authors contributed to the design of the work. SP and MVM were responsible for analysis and interpretation of data. All authors contributed to the draft and revision of the paper.

Conflict of Interest The authors declare no conflict of interest.

Statement of Ethics: The study complies with the guidelines for human studies and was conducted in accordance with the World Medical Association Declaration of Helsinki. Written informed consent was obtained from all participants and the study protocol was approved by the local Hospital Human Ethics Committee.

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3.2.

STUDY 2

Dietary intake as a risk factor for NAFLD in HIV patients: a comparison with a nationwide cohort

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DIETARY INTAKE AS A RISK FACTOR FOR NAFLD IN HIV PATIENTS: A COMPARISON WITH A NATIONWIDE COHORT

ABSTRACT

Objective: Factors associated with NAFLD in HIV patients still present conflicting results. Our study aimed to investigate the relationship between metabolic abnormalities, HIV-related factors, dietary intake, and NAFLD in a cohort of HIV patients and a national cohort, with a particular focus on dietary factors.

Methods: NAFLD diagnosis was based on the presence of liver steatosis on ultrasound, after exclusion of other causes of liver disease; the severity of liver disease was evaluated by transient hepatic elastography (Fibroscan®). A national representative cohort collected from a cross-sectional, multicentre study (e_COR) was used as comparison. Clinical, anthropometric (weight and waist circumference) and dietary data (food frequency questionnaire, Healthy Eating Index (HEI®) score and Mediterranean diet adherence score) were collected.

Results: In the general population, 660 participants and in the HIV group, 97 patients were included. The prevalence of NAFLD was higher in the HIV-group (54.8% vs. 33.3%, $p<0.001$). Participants with NAFLD in the e_COR group were more frequently overweight [OR: 1.258 (95%CI: 1.038-1.526); $p=0.002$], diabetic [OR: 3.091 (95%CI: 1.688-5.660); $p<0.001$] and with history of HBP [OR: 1.446 (95%CI: 1.024-2.043); $p=0.0017$] compared with patients with NAFLD in the HIV group. BMI≥30kg/m2 (aOR: 10.370 [95%CI 1.983-54.225]; $p=0.006$) and the presence of dyslipidaemia (aOR: 5.329 [95%CI 1.599-17.765]; $p=0.006$) were the factors that were associated with higher odd of NAFLD in HIV patients.

Conclusions: Low dietary quality, appears to be associated with NAFLD, but only in the HIV group. Also, in the HIV group dyslipidaemia, elevated BMI and the presence of insulin resistance demonstrated an association with NAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), a condition defined by the presence of steatosis in more than 5% of hepatocytes in the absence of excessive alcohol consumption or other liver diseases, has been increasingly recognized in the past decades as the primary cause of chronic liver disease, with an estimated prevalence in the adult population of around 25% [1]. A close relation with metabolic abnormalities, such as overweight, increased waist circumference, diabetes mellitus, dyslipidaemia and hypertension are well established in the development of this condition. Additionally, these metabolic disorders can promote the progression of NAFLD. [1,2]

The burden of liver disease amongst people living with Human Immunodeficiency Virus Infection (PLWH) has been growing considerably in the past few years. [3,4] Although viral hepatitis and excessive alcohol consumption are still important causes of liver disease, the prevalence of NAFLD is increasing among this population, associated with the rising prevalence of obesity and metabolic syndrome. [3,4]

In PLWH, the prevalence of NAFLD is at least 35%, with significant discrepancies between published studies [4,5]. Non-alcoholic steatohepatitis (NASH), the fibroinflammatory subtype, is estimated to be around 25% [6], increasing to 55% when considering patients with persistent liver enzymes elevation [7].

Factors that have been associated with NAFLD in PLWH are the classic metabolic factors, such as the presence of obesity, type 2 diabetes and hypertension [4,8–10]. However, HIV associated factors may also play a role, such as immunologic status, some antiretroviral drugs (cART) and persistent systemic inflammation [3,4,6,10–13]. Noteworthy, controversies still exist on the contribution of each factor to the development and progression of this disease in PLWH. Additionally, some aspects of dietary intake are also linked to the development of NAFLD, such as high sugar and fat content of the diet, with a less healthy dietary pattern [14–16], when studying risk factors associated with NAFLD, there is no data regarding dietary intake in PLWH.

Given that known risk factors for fatty liver disease in PLWH have differed and dietary data, to the best of our knowledge, is absent, our aim was to compare the presence of risk factors and dietary intake between HIV patients and a nationwide cohort assessed for metabolic risk factors and presence of fatty liver disease randomly selected from the non-infected population.

METHODS

This observation study was conducted comparing two different groups.

Study population

Patients with HIV diagnosis, under ART, with undetectable viral load and no switch in ART in the previous 6 months, without known liver disease, and without conditions that affected dietary intake (such as recent hospitalization, active opportunistic disease) were recruited from an outpatient Infectious Diseases Clinic to be screened for NAFLD with the aim of being enrolled in a lifestyle program aiming at weight loss [17]. The diagnosis of NAFLD was based on the presence of liver steatosis on ultrasound, after exclusion of significant alcohol intake and of other liver diseases, including viral hepatitis. (Figure 1) The severity of liver disease was evaluated by transient hepatic elastography (Fibroscan©), with determination of Controlled Attenuation Parameter (CAP) and Liver Stiffness Measurement (LSM) [18,19]. CAP classification was based on a recent threshold proposed for HIV patients [20].

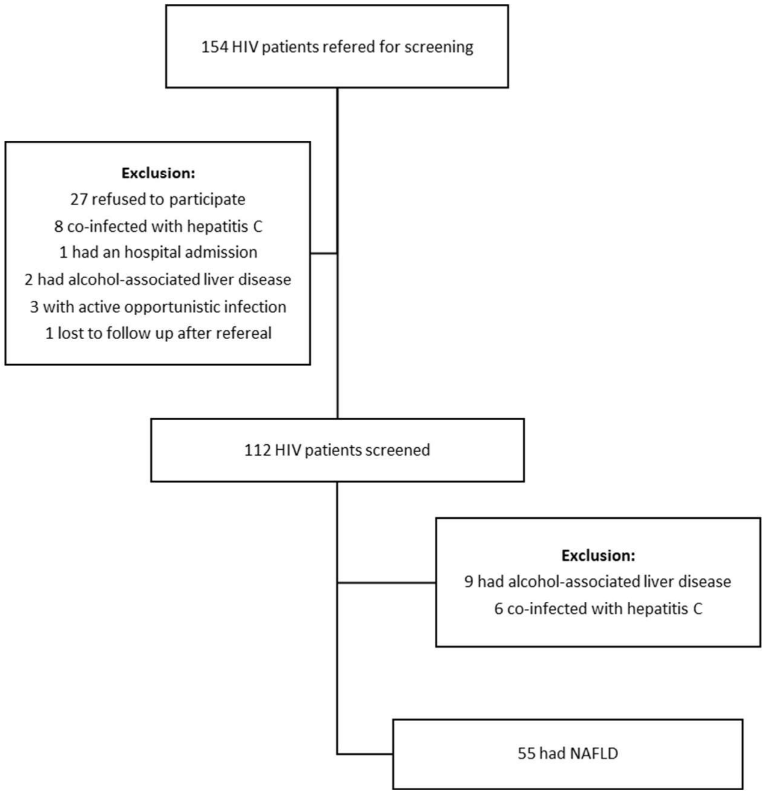

Figure 1 – Flowchart of inclusion of patients in the HIV group

A national representative cohort from a study for the determination of cardiovascular risk factors that also underwent evaluation of fatty liver disease (e_COR study), was used as comparison. Briefly, e_COR was a cross-sectional, population-based multicentre study that included 1688 randomly selected participants to assess the prevalence of cardiovascular risk factors. From those, 834 participants with complete data (18-79 years) were included in a sub analysis to evaluate the presence of fatty liver using ultrasound. Clinical (alcohol consumption, and associated diseases and past clinical history), anthropometric (weight and waist circumference) and dietary data (food frequency questionnaire) were collected. NAFLD was diagnosed after confirmation of the presence of steatosis on ultrasound, without alcohol intake and after exclusion of viral infections [21] (Figure 2).

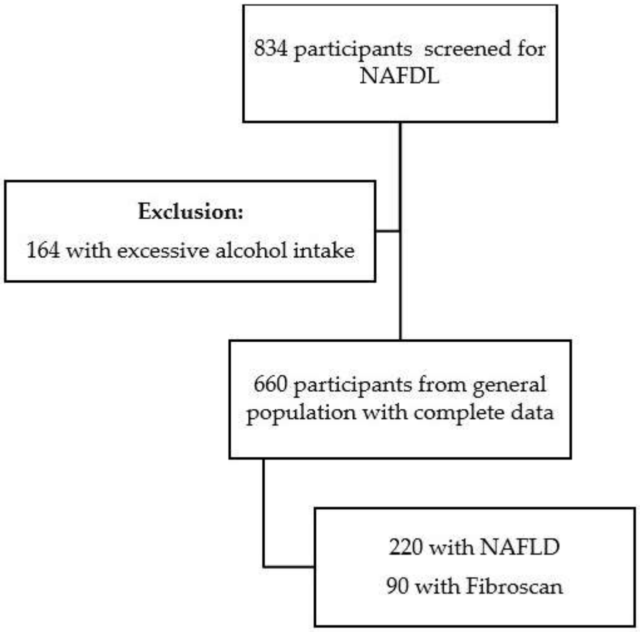

Figure 2 – Flowchart of inclusion of patients in the e_COR group

This group of participants, designated from now on as e_COR group was compared with the HIV group for the presence of metabolic risk factors (presence of diabetes, dyslipidaemia, high blood pressure – HBP - and overweight - defined as body mass index – BMI > 25kg/m2) and dietary intake characterization. A subset of 90 participants in this nationwide study, besides ultrasound, also conducted transient elastography (Fibroscan®) and were used as a comparison with the HIV patients in terms of severity of liver fibrosis [22].

Study procedures

Anthropometric data were collected with participants wearing light clothes and bare foot. Weight was measured using a calibrated scale and height was assessed using a stadiometer. Waist circumference (WC) was measured at halfway between the inferior rib and the iliac crest. BMI was defined as an individual's weight in kilograms divided by the square of height in meters (kg/m2).

A validated food frequency questionnaire developed for the national population (FFQ) was applied by trained investigators. The FFQ is a semi-quantitative questionnaire comprising 89 food items, distributed by 8 categories (dairy; eggs, meat and fish; fat; cereals; sweat and pastries; vegetables and legumes; fruits and drinks), ranging from daily to monthly frequency and allows the quantification of energy and 44 nutrients [23]. Dietary data was then coded to apply the Healthy Eating Index (HEI®), an index that allows the classification of the overall dietary quality, ranging from 0 to 100, based on the intake of fruit, vegetables, whole and refined grains, seafood, dairy, fatty acids, sodium, added sugars and saturated fat [24]. Higher scores represent higher adherence to the principles of healthy eating, namely, an adequate amount of fruits, whole grains, greens and beans, dairy, seafood and fatty acids, and a limited amount of saturated fats, added sugars and sodium and have been associated with positive health outcomes [24,25]. The FFQ used was the same in both groups.

In the HIV group only, the Mediterranean diet adherence score – PREDIMED – was applied [26].

Fibrosis was classified based on the proposed cut-off for NAFLD screening in primary care, as absent or mild (<8kPa) or significant fibrosis (≥8kPa) [27].

On the fasting state (12h fasting), blood samples were collected in an accredited laboratory using standard methodology for the determination of glucose, insulin, HbA1c, total cholesterol, triglycerides (TG), HDL-cholesterol (HDL-c) and liver enzymes (AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGT - gamma glutamyl transpeptidase). LDL-cholesterol (LDL-c) was calculated using the Friedewald formula in mg/dL [c-LDL= CT – (c-HDL + TG/5)]. The presence of dyslipidaemia was defined as total cholesterol ≥190mg/dl or LDL-c≥120mg/d or TG≥150mg/dl or by presence of specific drug therapy. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated based on glucose and insulin levels.

All participants' participation was voluntary, and both studies were approved by the local Human Ethics Committee - Ethical Committee of Faculty of Medicine, University of Coimbra (CE-15/2012) and Ethical Committee of Medical Academic

Center, University of Lisbon (CE 136/18). Written informed consent was obtained from all participants. This study was conducted according to the guidelines of the Declaration of Helsinki.

Statistical analysis

Data are expressed as mean±standard deviation (SD) or as median [interquartile range] in continuous variables and percentage in categorical data. Un-paired t-tests (Student's t test or one-way ANOVA) were used to compare distribution across continuous variables between study groups. When variables did not follow a normal distribution, assessed by Shapiro-Wilk test and inspection of distribution graphs, the non-parametric test alternative was used (Mann Whitey or Kruskal–Wallis). Chi-Squared test was used for categorical variables to test differences between different groups. OR and 95% CI summarising the association between metabolic, anthropometric and dietary characteristics and severe steatosis and advanced liver fibrosis were calculated.

The following variables were adjusted in the logistic regression model: age, sex and BMI and all those that presented a significant contribution in univariate analysis.

A sub analysis only with HIV patients was conducted to explore the factors associated with NAFLD. In this subgroup, fibrosis degree was further classified as absent (F0), mild (F1≥5.5kPa), moderate (F2-F3≥7kPa) and advanced (F4 ≥10.5 kPa) based on LSM [28]. Exposure to antiretroviral therapy (ART) was defined as positive for a drug when patients have recorded a treatment with a length of at least 3 months. Length of treatment was categorized into quartiles. In this second regression model the following variables were adjusted: age, sex, BMI, duration of HIV infection, duration of ART use, and all those that presented a significant contribution in univariate analysis.

A P-value < 0.05 was used as the cut-off for statistical significance. All statistical analysis was conducted using SPSS® (IBM SPSS Statistics 26).

RESULTS

From the nationwide cohort, 660 participants were considered eligible and in the HIV group, 97 were considered eligible for this study.

The baseline characteristics of study participants and comparison between eCOR and HIV are shown in Table 1. Globally, compared to the e_COR group, HIV patients were more frequently male, older, with lower BMI and less metabolic disturbances such as T2DM and HBP, but higher frequency of dyslipidaemia. Despite this, the prevalence of NAFLD was higher in the HIV-group compared to the e_COR subgroup (54.8% vs. 33.3%, p<0.001). (Table 2)

Table 1: Baseline patient characteristics

	e_COR group (660)	HIV patients (n=97)
Sex (%, n)		
Male	45.5 (300)	74.2 (72)
Female	54.5 (360)	25.8 (25)
Age (years)	48.3±17.1	54.6±12.1
Weight (kg)	72.8±14.6	75.4±11.6
BMI (kg/m2)	26.9±4.7	86.5±4.3
BMI category (%, n)		
Normal (18.5-24.9kg/m^2)	34.8 (230)	37.1 (36)
Pre-obese (25-29.9kg/m^2)	42.6 (281)	45.4 (44)
Obesity (≥30kg/$m^{2)}$	22.6 (149)	17.5 (17)
Waist circumference (cm)	92.4±13.5	95.4±10.6
AST above threshold (%, n)	2.9 (19)	12.9 (12)
ALT above threshold (%, n)	8.8 (58)	22.6 (21)
Type 2 diabetes mellitus (%, n)	31.8 (210)	17.5 (17)
High HOMA score (%, n)	34.6 (222)	42.2 (41)
High blood pressure (%, n)	40 (264)	42.3 (41)
Dyslipidaemia (%, n)	35.5 (234)	45.4 (44)

Data presented as % (n) or mean±SD; BMI – body mass index; AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGT - gamma glutamyl transferase

Table 2: Characteristics of e_COR and HIV groups based on NAFLD

	Non-NAFLD e_COR group (n=440)	NAFLD e_COR group (n=220)	Non-NAFLD HIV patients (n=42)	NAFLD HIV-Patients (n=51)	p-value*
Sex (%, n)					
Male	40.0 (176)	56.4 (124)	76.2 (32)	76.5 (39)	**0.008**
Female	60.0 (264)	43.6 (96)	23.8 (10)	23.5 (12)	
Age (years)	46.3±17.3	52.2±15.9	55.1±13.7	54.2±10.9	0.274
Weight (kg)	68.7±11.8	81.2±15.6	71.5±12.0	78.3±10.4	0.230
BMI (kg/m2)	25.6±4.0	29.6±4.9	24.7±3.4	27.9±4.4	**0.011**
BMI category (%, n)					
Normal (18.5-24.9kg/m^2)	45.5 (200)	13.6 (30)	47.6 (20)	31.4 (16)	**0.004**
Pre-obese (25-29.9kg/m^2)	42.3 (186)	43.2 (95)	47.6 (20)	43.1 (22)	
Obesity (≥30kg/$m^{2)}$	12.3 (54)	43.2 (95)	4.8 (2)	25.5 (13)	
Waist circumference (cm)	88.6±11.6	99.9±13.9	91.3±9.9	98.3±10.2	0.307
AST above threshold (%, n)	1.8 (8)	5 (11)	16.7 (7)	10.9 (6)	0.190
ALT above threshold (%, n)	5.2 (23)	15.9 (35)	16.7 (7)	27.3 (15)	**0.054**
Type 2 diabetes mellitus (%, n)	20.4 (90)	54.5 (120)	19.0 (8)	15.4 (9)	**<0.001**
High HOMA score (%, n)	23.1 (99)	57.5 (123)	36.8 (14)	61.4 (27)	0.6034
High blood pressure (%, n)	30.2 (133)	59.5 (131)	40.5 (17)	43.6 (24)	**0.017**
Dyslipidaemia (%, n)	31.1 (137)	44.1 (97)	35.7 (15)	52.7 (29)	0.253

Data presented as % (n) or mean±SD; * p p-value for comparison between NAFLD patients in the e_COR group and NAFLD-HIV patients; BMI – body mass index; AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGT - gamma glutamyl transferase

When participants with NAFLD from the e_COR group were compared with patients with NAFLD from the HIV group, they were more frequently female [OR: 1.855(95%CI: 1.106-3.110); p=0.008], overweight [OR: 1.258 (95%CI: 1.038-1.526); p=0.002], diabetic [OR: 3.091 (95%CI: 1.688-5.660); p<0.001] and with history of HBP [OR: 1.446 (95%CI: 1.024-2.043); p=0.0017].

The only significant difference between non-NAFLD HIV patients and NAFLD HIV patients was mean BMI, with non-NAFLD HIV patients presenting a lower mean value (24.7±3.4 vs. 27.9±4.4. p=0.023)

Indeed, when we evaluated separately the effects of metabolic factors on both cohorts, in multivariate analysis, in the e_COR group in multivariate analysis, having diabetes [adjusted OR - aOR: 2.683 (95%CI: 1.808-3.970); p<0.001], HBP [aOR: 1.992 (95%CI: 1.331-2.981); p<0.001], BMI≥30kg/m2 [aOR: 3.549 (95%CI: 2.284-5.513); p<0.001) and being male [aOR: 1.972 (95%CI: 1.354-2.871); p<0.001] were factors associated with higher odds of NAFLD. In the HIV population, with the same model of adjustment, having dyslipidaemia [aOR: 4.118 (95%CI: 1.207-14.051); p=0.024], an increased HOMA score [aOR: 3.368 (95%CI: 1.176-9.642); p=0.024]and BMI≥30kg/m2 [aOR: 6.509 (95%CI: 1.186-36.833); p=0.031] were associated with the presence of NAFLD.

Dietary Intake

Dietary intake was significantly different between groups (Table 3). NAFLD participants in the e_COR group presented higher caloric, protein, carbohydrate (CHO) and sugar intake and lower dietary fiber intake compared with both groups of HIV patients. However, regarding fat intake, only the non-NAFLD HIV group differed from the e_COR group, presenting lower intakes of total fat (p=0.009) and saturated fat (p=0.011).

Table 3: Nutritional intake in the e_COR and HIV groups

	No-NAFLD e_COR group (n=440)	NAFLD e_COR group (n=220)	Non NAFLD HIV patients (n=42)	NAFLD HIV- Patients (n=51)	**p-value***
Total energy intake (g/day)	2482±802	2585±953	1987±526	2098±504	<0.001
Total fat intake (g/day)	97.5±38.7	101.3±44.7	84.4±31.1	92.9±28.9	0.034
Saturated fat intake (g/day)	27.6±11.2	28.2±13.4	22.3±8.0	25.7±8.3	0.011
MUFA intake (g/day)	46.6±21.3	48.5±23.5	41.6±18.4	45.4±15.3	0.143
PUFA intake (g/day)	15.4±6.7	16.4±8.3	13.8±4.8	14.7±5.1	0.070
Omega 3 (g/day)	1.7±0.6	1.7±0.7	1.4±0.4	1.4±0.3	<0.001
Omega 6 (g/day)	11.9±5.8	12.7±7.1	10.9±4.2	11.5±4.1	0.153
Total protein intake (g/day)	109.8±36.7	114.4±42.8	90.2±24.1	89.9±25.4	<0.001
Total CHO intake (g/day)	294.8±105.9	304.5±125.2	219.5±60.8	224.6±64.5	<0.001
Sugar (g/day)	135.3±60.9	134.5±65.3	96.1±29.7	103.9±41.1	<0.001
Dietary fiber intake (g/day)	28.7±12.1	30.6±13.5	21.4±8.1	19.4±7.9	<0.001

Data presented as mean±SD; * p p-value for comparison between NAFLD patients in the e_COR group, non-NAFLD HIV patients and NAFLD-HIV patients; MUFA – Monounsaturated fatty acids; PUFA - Polyunsaturated fatty acids; CHO – Carbohydrates

Comparing HEI between groups, HIV-NAFLD patients presented the lower median diet quality (46[13] points), compared with e_COR NAFLD (48[15.75] points; p=0.006), e_COR non-NALFD (49[15] points; p=0.012) and HIV non-NAFLD (51[13.5] points; p= 0.011). In the e_COR NAFLD group, there was a trend for participants with advanced fibrosis to present lower median HEI score (46 points; p=0.068), compared with mild (48[14] points) and moderate fibrosis (59[15.25] points). HIV NAFLD patients had higher intakes of snacks, such as chips (p=0.028), cookies (p<0.001) and salty pastry (p=0.007), and higher intake of sugar-sweetened beverages (p=0.027), in comparison with e_COR NAFLD patients.

In the HIV group, a HEI below 50 points was associated with a higher odd of having NAFLD (OR: 1.647 [95%CI: 1.088-2.494]; p=0.012). In a multivariate analysis with metabolic comorbidities (DM2, insulin resistance, dyslipidaemia, HEI score and obesity), a HEI below 50 points, although modestly, maintains its association with NAFLD (OR: 0.285 [95%CI: 0.101-0.810]; p=0.018) in the HIV group. This association is not present in the general population (data not shown).

NAFDL HIV patients presented non-significant trend to have lower adherence to the Mediterranean diet when compared with the remaining HIV patients (6.7±2.6 vs. 7.7±2.2 points; p =0.086).

Liver Disease

From the 220 participants from the e_COR study with NAFLD, 90 had data on transient elastography. Participants presented a mean CAP of 276.3±65.0dB/m and a mean LSM of 5.7±2.5kPA, with no differences between e-COR and HIV groups.

In the e_COR group 8.9% (n=8) participants presented significant fibrosis and in the HIV group 13.7% (n=7) presented significant fibrosis. However, these differences in the prevalence of significant fibrosis did not have statistical significance.

The risks factors associated for significant fibrosis differed in both groups: in the e_COR group, only BMI ≥30kg/m2 (OR: 1.195 [95%CI: 1.027 - 1.391]; p=0.008) was associated with the presence of significant fibrosis with participants with significant fibrosis presenting higher mean BMI (35.8±9.3 vs. 29.2±4.1kg/m2; p<0.001) and in the HIV group only presence of HBP (OR: 1.353 [95%CI: 1.024-1.788]; p=0.015) was associated with significant fibrosis.

When all NAFLD individuals were analysed together, in the logistic regression analysis, only BMI was associated with significant fibrosis (OR: 1.123 [95%CI: 1.011-1.247]; p=0.030).

Sub analysis: HIV Group

Comparing both groups of HIV patients, no significant differences were found between groups on HIV infection characteristics (Table 4).

However, patients with NAFLD were associated with TCD4+ count ≥ 500 cel/mm3 (OR: 3.265 [95%CI 1.033-10.321]; p=0.037). As for metabolic comorbidities and cART, there was an association with T2DM in individuals exposed to zidovudine (OR: 3.729 [95%CI 0.986-14.100]; p=0.042), indinavir (OR: 4.200 [95%CI 1.253-14.081]; p=0.014) and stavudine (OR: 4.130 [95%CI 1.345-12.684; p=0.009]; an association with HBP in individuals exposed to lamivudine (OR: 3.239 [95%CI 1.001-10.827]; p=0.042), abacavir (OR: 4.045 [95%CI 1.707-9.581]; p=0.001); and an association with dyslipidaemia in individuals exposed to zidovudine (OR: 2.396 [95%CI 1.030-5.577]; p=0.041); lamivudine (OR: 3.849 [95%CI 1.171-12.651]; p=0.020), stavudine (OR: 3.261 [95%CI 1.355-7.849; p=0.007] and abacavir (OR: 2.524 [95%CI 1.105-5.764]; p=0.027). However, no specific ART class, either currently or previously exposure, was associated with the presence of NAFLD.

Table 4: HIV group sub analysis

	Non NAFDL HIV (n=42)	NAFLD HIV (n=51)	**p-value**
Timing of diagnosis (years)	22.5 (16)	17.5 (10.7)	0.829
Duration of ART (months)	236.5 (190)	170 (142)	0.980
Known detectable viral load (months)	36 (65)	24 (59)	0.620
TCD4/TCD8 Ratio >0.75 (%, n)	61.9 (26)	74.5 (38)	0.192
TCD4+ cell ≥500cel/mm3 (%, n)	73.8 (31)	90.2 (46)	0.037
Current ART (%, n)			
NRTI	83.5 (35)	90.0 (45)	0.370
NNRTI	52.4 (22)	50.0 (25)	0.837
PI	31.0 (13)	36.0 (18)	0.610
ISTI	35.7 (15)	24.0 (12)	0.255
Nadir TCD4+ (cell/mm3)	160 (238)	231 (264)	0.572
Past opportunistic infections (%, n)	11.9 (5)	16.0 (8)	0.574
Past ART (%, n)			
NRTI	97.6 (41)	100 (50)	0.273
NNRTI	83.3 (35)	80 (40)	0.790
PI	66.7 (28)	60.0 (30)	0.509
ISTI	42.9 (18)	28 (14)	0.136

Data presented as median (interquartile range) * p-value for comparison between groups; NRTI - Nucleoside Analogue Reverse Transcriptase Inhibitor; NNRTI – Non-Nucleoside Analogue Reverse Transcriptase Inhibitor; PI – protease inhibitor; INSTI - integrase strand transfer inhibitors

Combining HIV factors and the metabolic factors previously described, in the multivariate analysis, BMI≥30kg/m2 (aOR: 10.370 [95%CI 1.983-54.225]; p=0.006) and the presence of dyslipidaemia (aOR: 5.329 [95%CI 1.599-17.765]; p=0.006) were the factors that maintained association with higher odds of NAFLD.

From the group of patients that presented NAFLD and fibrosis, those with dyslipidaemia presented higher LSM (10.7±4.2 vs. 6.2±0.6kPa; $p<0.001$). There was also a difference in length of ART, with patients with mild fibrosis presenting lower length of treatment (109.9±76.3months) compared with those with moderate (161.5±68.9months) and advanced fibrosis (225.0±67.1months; $p=0.041$). There was no significant association between metabolic factors and degree of fibrosis in this group.

DISCUSSION

This study aimed to compare NAFLD prevalence and associated metabolic and dietary factors, between a nationwide representative cohort and a group of HIV patients.

Recent meta-analysis, estimated prevalence of NAFLD in the HIV population, using non-invasive imaging methods of around 35% [6], which is not in agreement with our cohort in which over half of the HIV patients, presented NAFLD (prevalence of 54.8%). One explanation for this difference may be the fact that patients with current or past chronic viral hepatitis were excluded, which could have led to an overestimation of the prevalence of NAFLD.

Other striking unexpected finding was the weaker association in the HIV group between classical metabolic disturbances known to associate with NAFLD in the general population.

Dyslipidaemia, as a component of the metabolic syndrome, has been associated with the presence of NAFLD in other studies, and BMI has been demonstrated to be a key factor in the development of NAFLD in the HIV population [9]. However, the strongest documented association is of insulin resistance/diabetes with NAFLD [6,9,29]. Our data suggest an association between dyslipidaemia, obesity and insulin resistance with NAFLD, but when these metabolic factors are combined with HIV related factors, only dyslipidaemia and obesity remain associated with the presence of NAFLD, what may suggest different or least added pathways to the development of NAFLD in HIV patients as compared to the general population. Dyslipidaemia is closely associated with cardiovascular risk, but in the HIV population with NAFLD may be a result of disruption of hepatic mechanisms promoted by HIV per se such as hepatocyte injury and increased inflammation [30].

Regarding dietary factors, a recent study, from Brazil, in the HIV population, suggested that a higher intake of total fat was associated with higher odds of NAFLD [31].

Our study did not corroborate those results. Indeed, when we compared the HIV group with the e_COR group, HIV patients globally presented lower caloric, protein, and CHO intake. Furthermore, in the HIV group, no association was found between specific macronutrient intake and higher odds of NAFLD. Interestingly, despite the HIV group lower intake of macronutrients, those patients presented lower dietary quality scores as compared to the e_COR group, and to previous published quality scores in HIV patients [32]. The association with low dietary quality was previously described by other groups for the general population [25,33], but this is the first study reporting an association in the HIV population. Low dietary quality maintained its association with NAFLD even when adjusted for the presence of metabolic comorbidities.

Similarly, there was also a non-significant trend for patients with HIV that developed NAFLD to present lower adherence to the Mediterranean diet. The aggregate data suggests that in this group of patients, more than the number of calories consumed, the dietary pattern can possibly be a major contributor to NAFLD.

Furthermore, in the PLWH a low overall quality of the diet has been associated with food insecurity [34]. Food insecurity could lead to an increased intake of more affordable foods, traditionally high energy dense foods but with low nutritional value [32,34]. Recently, in a study conducted in a low-income setting, among middle aged adults, including HIV infected participants, food insecurity was associated with higher prevalence of NAFLD [35]. The data regarding dietary factors and NAFLD in HIV patients is still scarce and further extensive characterization is needed.

Finally, regarding specific HIV factors, we did not find any significant association between duration of HIV infection, HIV viral load and nadir TCD4+ cell count. This association could have been impaired by the reduced sample size, but is in line with some published studies [6]. We did find a significant association between length of ART treatment and fibrosis severity ; however, the group with fibrosis had a reduced number of patients which may limit the results. A better immunological status, measured by a TCD4+ count higher than 500 cel/mm3, in adjusted analysis was associated with higher odds of NAFLD, but lacked statistical significance in the multivariate analysis. In this study, no association was found between ART class and NAFLD. The association of ART treatment with development and/or progression of NAFLD is an ongoing debate [12].

We recognize that the reduced number of patients in the HIV group, with and without NAFLD, is a limitation in this study, with small groups of patients within each of the studied factors, limiting the power of statistical significance and not allowing a robust extrapolation of the results. However, the comparison between the subgroup of the national cohort and HIV patients has a statistical power of 78%, for a 95%CI which translates into a relatively robust extrapolation of the findings of our study, allowing us to corroborate some of the metabolic findings previously described in the HIV population. Adding to this, the exploration of dietary factors, unveiled a relationship between the overall diet quality and the presence of NAFLD, not yet described for the HIV population and a risk factor that can be modified with a timely dietary intervention.

In conclusion, we did find that the most important factor associated with NAFLD in HIV patients was dyslipidaemia, which should be aggressively addressed. Furthermore, patients with HIV develop NAFLD at lower BMI as compared to the general population, and more frequently in the absence of type 2 diabetes mellitus; as such, screening programs as well as clinical trials should take those differences into account. Furthermore, patients with HIV that develop NAFLD eat less calories/macronutrients as compared to the general population but present less healthy dietary patterns that might be easier to address in clinical practice.

Since no specific HIV-NAFLD management recommendations exist, besides scarce ones associated with screening, our data, reinforces the need for a multidisciplinary management, such as the one recommended for the overall NAFLD population [36]. The findings of this study contribute to the knowledge of the metabolic, HIV related, and dietary factors associated with NAFLD, and provide information regarding dietary intake, essential for an adequate nutritional management, to this date, still the solely available treatment for NAFLD patients.

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3.3.

STUDY 3

Telemedicine as a tool for dietary intervention in NAFLD-HIV patients during the COVID-19 lockdown: A randomized controlled trial

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TELEMEDICINE AS A TOOL FOR DIETARY INTERVENTION IN NAFLD-HIV PATIENTS DURING THE COVID-19 LOCKDOWN: A RANDOMIZED CONTROLLED TRIAL

ABSTRACT

Background & Aims: Given reports of changes in dietary habits during covid-19 lockdown, our aim was to assess weight changes, over a 3-month Covid-19 national lockdown in a cohort of NAFLD-HIV patients on a dietary intervention trial.

Methods: After NAFLD screening in an outpatient Infectious Diseases Clinic, NAFLD patients were randomly allocated to general dietary recommendations (SC group) or to a structured dietary intervention based on the Mediterranean diet (intervention group). During lockdown, follow-up consultations in the intervention group were done by video and/or phone. After 3 months of lockdown, all patients (intervention and SC group) consented to a telephone interview which aimed to characterize eating habits and lifestyle changes and evaluate stress and depression. Biochemical data when available, was compared between the peri-period of confinement.

Results: One hundred and twelve patients were screened. From the 55 NAFDL identified, 27 were allocated to dietary intervention and 28 to SC and were followed before lockdown for a mean period of 5.0±1.5months in which SC group gained a median of 0.65kg vs. a median loss of 1.5kg in the intervention group ($p<0.001$). During lockdown, 93.3% of patients in the SC group referred that "diet got worse" *vs.* 6.7% in the intervention group $p<0.01$), and 35.3% *vs.* 15.7% ($p=0.014$) reported increase in appetite, respectively. Both groups gained weight, SC group vs. 0.7±1.7kg in the intervention group, $p<0.001$). Higher weight gain was associated with changes in the dietary pattern (3.8 ±2.1kg vs. 2.0±1.3kg in "no change in dietary pattern"; $p=0.002$). Glucose blood levels increased after lockdown in the SC group, with a mean increase of 15mg/dl ($p=0.023$). The remaining metabolic parameters remained unchanged.

Conclusion: The maintenance of dietary intervention, using telemedicine, can mitigate the adverse change in dietary habits and physical activity pattern, preventing a substantial increase in body weight.

Keywords: dietary intervention, Covid-19 lockdown, NAFLD, nutrition

INTRODUCTION

The pandemic caused by SARS-Cov2 has originated an unprecedent global lockdown, with every nation struggling with their own challenges and repercussions in social well-being (1). Currently there are still some countries in this situation. From previous experiences, mandatory quarantines can result in psychological distress, generating depressive symptoms, irritability, anger, and insomnia (2,3).

Stressful events are recognized as triggers for alterations in eating patterns (2)An increase in staying at home time could lead to a higher intake of alcohol and frequent snacking of comfort food and processed foods (1,2,4-6) An increase in a sedentary behaviour motivated by social isolation and decreased outdoor time, further contribute to variations in body weight (1,2,7) Recent studies have demonstrated that during lockdown, there was a significant reduction in physical activity and a change in dietary habits (2,6,8) In particular, lockdown associates with an increase in dietary intake (6)mostly based on ultra-processed foods, (5) comfort food (4,6) and drinking, which has been described as a way of dealing with anxiety and distress (3,4). Additionally, changes in dietary habits may be a consequence of a lower access to grocery stores/supermarkets (9). This change in dietary habits seems to promote weight gain and the development of obesity and eating disorders (1,2,4,7). In fact, previous studies showed that patients already obese tend to gain a significant amount of weigh during lockdown (7,10). Also, the disruption of circadian rhythms can lead to metabolic abnormalities, such as insulin resistance (2)

Non-alcoholic fatty liver disease (NAFLD) is a multisystemic disease that occurs in association with metabolic syndrome and in the absence of significant alcohol intake (11-13). Currently there is no pharmacological treatment available, and lifestyle changes are the cornerstone of treatment of NAFLD (13,14). In the last decade, there is a growing interest in NAFLD in patients living with human immunodeficiency virus (PLHIV). However, data on metabolic characteristics of this population and the efficacy of dietary intervention in PLHIV is scarce (15-18).

Given that an increased compliance to dietary guidelines can possible mitigate metabolic disturbances, (1) our aim was to assess the impact of a dietary intervention, on weight variation during a 3-month Covid-19 national lockdown in a cohort of NAFLD-HIV patients undergoing a dietary intervention trial, as well as the impact on metabolic data.

MATERIAL & METHODS

Patients

Patients recruited from an outpatient Infectious Diseases Clinic were screened for NAFLD. The diagnosis was based on the presence of liver steatosis on ultrasound, after exclusion of significant alcohol intake (defined as more than 20 grams per day in women and 30 grams per day in men), and of other liver diseases, namely chronic hepatitis B or C, primary biliary cholangitis, autoimmune hepatitis, primary sclerosing cholangitis, Wilson's disease, hemochromatosis or α1-antitripsin deficiency. Patients were also excluded if there were under medication with potentially steatogenic drugs such as steroids, high dose estrogen, tamoxifen, methotrexate, or amiodarone within six months of enrolment, and if there were an history of gastrointestinal bypass surgery or segmental small bowel resection. The severity of liver disease was evaluated by non-invasive scores of fibrosis, FIB-4, as well as transient hepatic elastography (Fibroscan©) (19,20)

The study was approved by the local Human Ethics Committee and written informed consent was obtained from all participants.

Lifestyle counselling

NAFLD patients were consecutively and randomly allocated 1:1 to general dietary recommendations or to a structured dietary intervention that included general dietary recommendations and a nutritional plan. General recommendations, based on Mediterranean diet, consisted in the delivery of written resources containing healthy eating guidelines and tips. The structured dietary intervention included these and a detailed nutritional plan aiming at caloric restriction and weight loss, based on the Mediterranean diet and was individualized according to nutritional needs, with a caloric deficit of 500kcal, and taking in to account the presence of other dietary restrictions alongside NAFLD, and the personal preferences and habits of the patient. With the nutritional plan, information on portion size, culinary specifications and exchange tips were provided. Other specifications of dietary advice included alcohol abstinence, and a limitation in total fruit content of the diet when the intake was above 5 units/day. Regarding physical activity, the aim was to stimulate an increase in baseline physical activity, with an incremental approach of exercise in everyday life. The intervention was conducted at baseline, 1 month, 3 months and 6 months by a single dietitian, all of them before lockdown. During appointments, adherence to recommendations was evaluated using PREDIMED, with maximum score of 14 (21,22). All patients (SC and intervention group) had their weight monitored at 1, 3 and 6 months after baseline.

Clinical assessment and laboratorial tests

Anthropometric data were collected with participants wearing light clothes and bare foot. Weight was measured using a calibrated scale and height was assessed using a stadiometer. Waist circumference (WC) was measured at halfway between the inferior rib and the iliac crest. Body mass index (BMI) was defined as an individual's weight in kilograms divided by the square of height in meters (kg/m^2). Body fat mass (BFM, %) was assessed using a single frequency analyser (OMNRON BF350).

Clinical data including liver biochemistry, abdominal ultrasound and transient hepatic elastography, were collected. FIB-4 score was calculated.

Lockdown assessment

Given the restrictions to clinical visits in the hospital, during the 3 months confinement, follow-up consultations in the intervention group were done by video and/or phone and included a review of dietary advice. Weight was measured using the patient's household equipment, with a measurement of error, using a standardized measure, conducted on the first video contact. Body composition during the confinement was unavailable in the participants' households.

After 3 months of lockdown, all patients (intervention and SC group) consented to a telephone interview which aimed to characterize eating habits and lifestyle changes during COVID-19 lockdown, using methodology previously published (23). Also, four questions, previously validated for the Portuguese population, were included to characterize stress ("I found myself getting agitated"; "I found it difficult to relax", "I had a hard time calming down") and depression ("I felt discouraged and melancholic") and asked the participants to define during this period, if this sentence applied or not to them. Questions were scored from zero to three (0-did not apply to me; 3-applied most of the times) (24).

Clinical data when available, was compared between the peri-period of confinement.

Statistical Analysis

To determine a significant difference in both groups for $\alpha=0.05$ and a power of 0.8 we estimated a minimum sample of 21 participants in each group. Continuous variables were summarized as mean and standard deviation (mean±SD) and categorical variables were summarized using frequency and percentage. Parametric and non-parametric tests were applied based on the different variable distribution. Chi-square was used for independence of categorical variables and Student T-Test or ANOVA, were used for continuous variables. Multiple comparisons adjusted with the Bonferroni correction. For each analysis, unadjusted and adjusted odds ratio (OR) and the corresponding 95% confidence interval (CI) and p-value were reported. For the analysis of peri-period of lockdown pairwise comparisons were used. The significance level was set at 5%. All statistical analyses were performed with IBM® SPSS® software, version 24.0.

RESULTS

From the 148 HIV-positive patients referred for screening from the Infectious Disease Outpatient Clinic, 27 refused to participate in this study; 8 were excluded prior to screening due to the presence of co-infection with hepatitis C, 1 patient had an hospital admission, 2 patients had alcohol-associated liver disease, 3 patients had an opportunistic infection after referral, and 1 patient was lost to follow up. After screening, 15 additional patients were excluded due to the presence of previously undiagnosed alcohol-associated liver disease (in 9) and co-infection with viral hepatitis (in 6).

Ninety-seven patients were enrolled, mostly males (n=72, 74.2%), Caucasian (n=81, 83.5%) with a mean age of 54.6±12.6 years and with a mean time since HIV diagnosis of 17.9±8.3 years; 83.5% (n=82) had T-CD4+≥ 500 cel/mm^3; all presented undetectable viral load. As for metabolic risk factors: 17.5% (n=17) had type 2 diabetes mellitus, 42.3% (n=42) had hypertension, 45.4% (n=44) had dyslipidaemia and 82.5% (n=61) were overweight (BMI≥25kg/m^2). (Table 1) Regarding alcohol intake, 57.7% (n=52) did not consume any alcoholic beverages.

At baseline screening, 56.7% (n=55) had hepatic steatosis on abdominal ultrasound, 12.4% (n=13) and 22.7% (n=22) had increased serum levels of AST and ALT, respectively; 78.4% (n=76) presented non-invasive score FIB4 with low probability of liver fibrosis.

NAFLD was associated with higher BMI, with BMI>30kg/m^2 in 88.2% of patients in the NAFLD group, vs. 11.8% in non-NAFLD patients; p=0.005. However, NAFLD did not associate with metabolic syndrome comorbidities (Table 1).

FIB-4 presented low probability for liver fibrosis in 87.3% (n=48). Regarding Fibroscan©: 63.6% (n=35) had no evidence of fibrosis, 27.3% (n=15) had a moderate degree of fibrosis (F2-F3) and 9.1% (n=5) had severe fibrosis (F4).

A subset of patients with NAFLD were randomly assigned to dietary intervention (n=27) or standard of care – SC (n=28). This subset of patients did not differ in baseline characteristics to the global cohort (Table 1).

Table 1: Baseline patient characteristics

		Non NAFLD Patients (n=42)	NAFLD Patients (n=55)	p-value	NAFLD Standard of Care Group (n=28)	NAFLD Intervention Group (n=27)	p-value
Sex (%)	Male Female	76.2 (32) 23.8 (10)	72.7(40) 27.3 (15)	>0,05	71.4 (20) 28.6 (8)	74.1 (20) 25.9 (7)	>0,05
Age (years)		55.1±13.7	54.2±10.9	>0,05	55.9±11.7	52.6±9.9	>0,05
Weight (kg)		71.5±12.0	78.3±10.4	0.04	77.8±9.9	78.9±11.1	>0,05
BMI (kg/m2)		24.7±3.4	27.9±4.4	0.001	28.1±4.0	27.7±4.9	>0,05
BMI category (%) Normal (18.5-24.9kg/m^2) Overweight (25-29.9kg/m^2) Obesity (≥30kg/m^2)		 47.6 (20) 47.6 (20) 4.8 (2)	 29.1 (16) 43.6 (24) 27.3 (15)	0.01	 25.0 (7) 46.4 (13) 28.6 (8)	 33.3 (9) 40.7 (11) 25.9 (7)	>0,05
Waist circumference (cm)		91.3±9.9	98.3±10.2	0.005	98.1±10.6	98.5±9.9	>0,05
AST % above threshold		23.3±7.1 16.7 (7)	24.1±9.1 10.9 (6)	>0,05 >0,05	23.5±7.7 7.1 (2)	24.7±10.5 14.8 (4)	 >0,05
ALT % above threshold		25.4±9.8 16.7 (7)	30.9±10.1 27.3 (15)	>0,05 >0,05	28.8±16.1 28.6 (8)	33.0±19.8 25.9 (7)	 >0,05
HIV diagnosis time (years)		17.9±9.2	18.0±7.6	>0,05	17.2±7.1	18.4±8.1	>0,05
T-CD4+cell ≥500cel/mm3		73.8 (31)	90.9 (50)	0.025	82.1 (23)	100 (27)	0.021
Type 2 diabetes mellitus (%)		19.0 (8)	15.4 (9)	>0,05	17.9 (5)	14.8 (4)	>0,05
High blood pressure (%)		40.5 (17)	43.6 (24)	>0.05	53.6 (15)	33.3 (9)	>0,05
Dyslipidaemia (%)		35.7 (15)	52.7 (29)	0.095	50.0 (14)	55.6 (15)	>0,05

Values presented as % (n) or mean±SD; BMI: Body Massa Index; AST: Aspartate transaminase, ALT: Alanine aminotransferase; NAFDL: non-alcoholic fatty liver disease;HIV: human immunodeficiency virus

Anthropometric Evolution before the Lockdown period

From the 55 NAFLD patients, 2 did not comply with regular appointments during face-to-face follow up, 1 was diagnosed with anal cancer and 1 with an opportunistic disease (Kaposi´s Sarcoma) and were excluded from the analysis.

Patients were followed for a mean period of 5.0±1.5months before lockdown. Globally, 51% of patients lost weight, with a mean weight loss of 1.7±1.4kg. We witness that the standard of care (SC) group gained a median of 0.65kg vs. a median loss of 1.5kg in the intervention group ($p<0.001$).

Lockdown period

At the beginning of the lockdown period, mean BMI was not significantly different between SC group and intervention group (28.3±0.85kg/m^2 vs. 26.8±0.88kg/m^2; $p=0.139$).

After the 3 month-lockdown, on the telephone interview, 41.2% (n=21) of patients reported that there was no change in their professional situation, but 35.5% (n=18) changed to telework and 23.5% (n=12) became unemployed or experienced a significant reduction in household budget due to lay-off. This demographic characteristic did not differ between SC and intervention group ($p=0.835$)

As for psychological impact, stress was reported in a significant number of patients, with 90.2% (n=46) referring agitation, 88.2% (n=45) difficulty in relaxing and 92.2% (n=47) difficulty in calming down. Feelings associated with depression were reported by 62.6% (n=28) patients.

There were no differences between groups. A positive score in the depression question was associated with higher weight gain during lockdown (2.6±1.8kg vs. 0.7±1.9kg, $p=0.003$).

Even though dietary and lifestyle behaviours changed in both groups, with a decrease in physical activity in 62.7% (n=32) and changes in dietary pattern in 52.9% (n=27), they were more dramatic in the SC group as compared to the intervention group.

As such, 93.3% of patients in the SC group referred that "diet got worse" *vs.* 6.7% in the intervention group p<0.01), and 35.3% *vs.* 15.7% (p=0.014) reported increase in appetite, respectively. The SC group also presented a more prevalent increase in snack frequency during the day (41.2 *vs.* 19.6% in the intervention group, p=0.02).

Mean Mediterranean diet score during lockdown was 7.4±2.8 points, with patients on the intervention group presenting higher scores (9.2±1.9 *vs.* 5.6±2.4 points in the STD group, p<0.01). Higher adherence to Mediterranean diet was associated with lower weight gain (r=-0,397, p=0.004). Changes in dietary pattern during lockdown reported by patients are represent in figure 1.

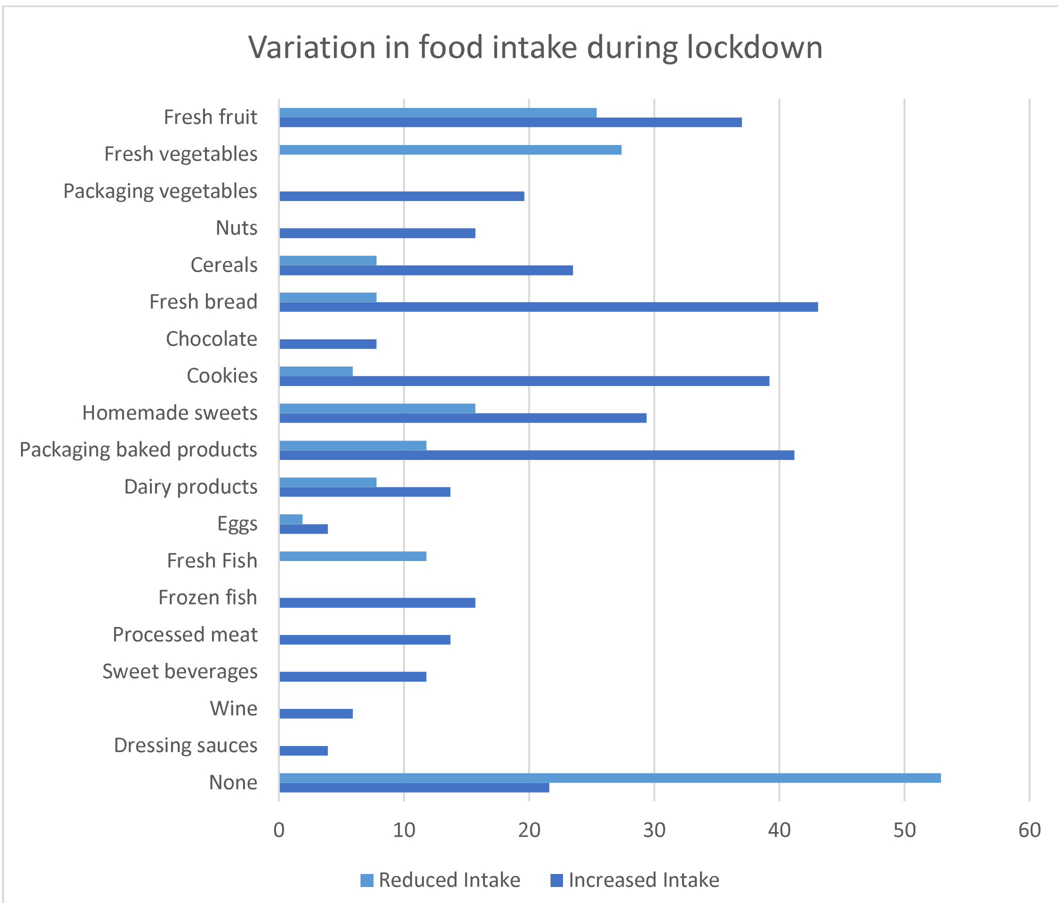

Fig. 1 Variation in food intake during lockdown in overall patients

As for physical activity, more patients in SC group, reported a decrease in activity compared with the intervention group (73.1%, n=19, vs 52%, n=13 respectively; p=0.009).

Changes in dietary pattern did not associate with the presence of T2DM, dyslipidaemia, degree of fibrosis, liver enzymes, previous weight loss before lockdown or changes in professional situation. Of note, BMI and hypertension at baseline were predictors of changes in the dietary pattern. Patients with hypertension had a higher likelihood of changing their dietary pattern (OR=1.995 CI95% [1.005-3.961]; p=0.031) as well as patients with BMI above 25kg/m^2 (OR=2.188 CI95% [1.276-3.750]; p=0.007). However, in subgroup analysis, BMI above 25kg/m^2 remained a predictor of dietary change only in SC group (OR=2.714 CI95% [1.119-6.586]; p=0.036).

During lockdown, both groups gained weight, which was significantly higher in the SC group (3.1±1.6kg vs. 0.7±1.7kg in the intervention group, p<0.001) (Fig. 2).

Fig. 2 Weight variation during lockdown between groups (SC vs. nutritional intervention)

Weight gain was higher in patients that reported “more appetite (2.4±2.1kg vs. “no change” 0.7±1.3kg; p=0.003). Comparing BMI category at the end of lockdown with BMI category of the last face-to-face evaluation, we witnessed worsening in the BMI category in 15.7% (n=8) patients, with no differences between groups. However, mean BMI at the end of lockdown was significantly higher in the SC group compared with the intervention group (29.5kg/m^2 vs. 27.0kg/m^2, p=0.035).

Weight gain during the intervention period was significantly correlated with weight gain during lockdown (r=0.4999, p <0.001). A decrease in physical activity during lockdown was also associated with weight gain in both groups, more pronounced in the SC group (3.6±1.5kg vs. “no change” 1.6±0.9kg; p=0.015) compared with the intervention group (1.6±1.5kg vs. “no change” 0.8±1.5kg; p=0.008). Variation of weight during lockdown was also associated with changes in the dietary pattern, but only in SC group, with patients presenting higher weigh gain (3.8 ±2.1kg) vs. “no change in dietary pattern” (2.0±1.3kg; p=0.002).

Neither metabolic comorbidities, degree of fibrosis or liver enzyme alterations, or previous weight loss before lockdown were associated with weight variation during lockdown.

Immediately after the end of national lockdown, 12 patients in the STD group and 13 patients in the intervention group had laboratory data available. Blood glucose levels increased after lockdown in the SC group, with a mean increase of 15mg/dl (95±15.4 vs. 110±31.0mg/dL, p=0.023). The remaining metabolic parameters remained unchanged (Table 2).

In the SC group, triglyceride levels after lockdown were directly correlated with a positive variation in weight during lockdown (r=0.675 p=0.016).

Table 2: Patient characteristics before and after lockdown

	NAFLD SC Group (n=28)	p-value**	NAFLD Intervention Group (n=27)	p-value**
Weight (kg)				
Before lockdown	79.1±10.7	<0.001	77.0±11.7	0.099
After lockdown	82.2±11.4		77.6±12.3	
BMI (kg/m2)				
Before lockdown	28.3±4.4	<0.001	26.8±4.4	0.094
After lockdown	29.5±4.7		27.0±4.6	
BMI Obesity category (%)				
Before lockdown	30.8	>0.05	24	>0.05
After lockdown	34.6		28	
Glycemia*				
Before lockdown	95.7±15.4	0.023	94.6±13.7	>0.05
After lockdown	110.2±31.0		97.6±10.4	
Triglycerides*				
Before lockdown	174.8±56.5	>0.05	148.9±58.5	>0.05
After lockdown	201.0±83.2		126.2±44.4	
Total Cholesterol*				
Before lockdown	220.6±49.8	>0.05	216.6±41.3	>0.05
After lockdown	214.8±63.6		214.6±45.7	
HDL cholesterol*				
Before lockdown	52.9±16.3	>0.05	52.2±11.8	>0.05
After lockdown	50.3±16.9		53.4±9.4	
FIB-4 low probability of fibrosis* (%)				
Before lockdown	80.8	>0.05	100	0.038
After lockdown	91.7		69.2	

Values presented as % of patients or mean±SD; BMI: Body Massa Index; AST: Aspartate transaminase, ALT: Alanine aminotransferase; NAFDL: non-alcoholic fatty liver disease; HIV: human immunodeficiency virus. * available from 12 patients in the STD group and 13 patients in the intervention group; ** reported comparison between before and after lockdown.

DISCUSSION

During Covid-19 lockdown each individual had a sole responsibility to ensure an adequate diet to maintain a healthy weight and preserve overall health. This study demonstrates that a population of NAFDL patients with HIV, underwent an increase in their body weight during a period of forced confinement. However, to the best of our knowledge this is the first published study that demonstrates that this weight gain under these circumstances can be mitigated by telemedicine, namely dietary intervention.

This is an important achievement, since for NAFLD patients, an increase in body weight may lead to progression of the disease (14) and in HIV patients, weight gain has been associated with the onset of cardiovascular disease (25).

Recently published studies documented an increase in body weight during lockdown, around 3kg. In our study SC group presented a mean weigh gain similar to other observational studies (8,26,27) (around 3kg), and higher than the weight gain on the intervention group (less than 1kg). Also, given the recent reports describing an increase in alcohol consumption during lockdown (28) in patients with hepatic disease, dietary intervention can improve adherence to alcohol abstinence. Experiencing mood disorders, with reports of increased anxiety and/or stress seemed to have an impact on weight gain, consistent with observational cross-sectional studies (6,29).

Also, a change in dietary habits during lockdown was demonstrated in our study, with patients referring an increase in snack and processed food intake, as documented in other observational cohort studies (3,6,9,29,30). However, this change in dietary pattern was less pronounced in the intervention group, who maintained higher adherence to the Mediterranean pattern, and who had less than 10% of patients reporting a worse dietary pattern.

Physical activity decreased in this group of patients, and it has been reported that physical active people experienced a reduction in their physical activity time during lockdown (8,29). However, more patients in the intervention group maintained their physical activity pattern.

The aggregate data showed that patients on the intervention group presented less weight gain and lower alterations in dietary habits and were able to maintain their physical activity pattern during lockdown. Our study suggests a beneficial effect of regular dietary follow up by telemedicine in promoting healthy lifestyle and weight control during lockdown.

This study has some limitation. Given that this was an ongoing dietary trial, the number of patients allocated to both groups was small and the Fibroscan® that was planned for 6 months after the beginning of the intervention was not performed, due to national and local constraints. The maintenance of these constraints also prevented a new Fibroscan and biochemical data collection in some patients.

In conclusion, this study shows the impact on body weight, dietary habits, physical activity, and mood of a national lockdown in a group of NAFLD-HIV patients and demonstrates that the maintenance of dietary intervention, using telemedicine, can mitigate the change in dietary habits and physical activity pattern, preventing a substantial increase in body weight. Given the recent data on the pandemic evolution this data reinforces the need to maintain health services, namely nutritional appointments, in future lockdowns, to guarantee adherence to dietary recommendations.

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3.4.

STUDY 4

Do MAFLD Patients with Harmful Alcohol Consumption Have a Different Dietary Intake?

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DO MAFLD PATIENTS WITH HARMFUL ALCOHOL CONSUMPTION HAVE A DIFFERENT DIETARY INTAKE?

Abstract: The term metabolic-associated fatty liver disease (MAFLD) has been proposed to define positively fatty liver disease in the form associated with metabolic risk factors. The aim of this study was to assess the dietary intake of MAFLD and explore a possible relationship between its inflammatory characteristics (assessed by Dietary Inflammatory Index—DII®), the degree of liver fibrosis (assessed by transient elastography), and the amount of alcohol intake. MAFLD patients were included (n = 161) and were classified, according to the amount of alcoholic intake, as MAFLD without alcohol intake (n = 77) and MAFLD with alcohol intake (n = 84), with 19 presenting harmful alcoholic consumption. Dietary intake was 1868±415 kcal/day and did not present differences in energy or nutrient intake based on the presence of metabolic comorbidities. Patients with MAFLD and alcohol intake consumed significantly more energy and presented a tendency for higher intake of carbohydrates and sugar. Patients with harmful alcohol intake presented a higher intake of total fat and cholesterol compared with moderate alcohol intake. There were no differences in DII® based on fibrosis severity or the amount of alcohol consumption. This work contributes to the characterization of baseline dietary intake in MAFLD patients, paving the way to design more suited dietary interventional trials.

Keywords: dietary inflammatory index; sugar; fat; steatosis; advanced fibrosis

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a multisystemic disease that occurs in association with the metabolic syndrome and in the absence of harmful alcohol intake. The cornerstone of NAFLD treatment is lifestyle changes, since there is no pharmacological treatment available (1,3)

Recently, an international panel of experts proposed a change in nomenclature to metabolic dysfunction-associated fatty liver disease (MAFLD) (4). This new designation emphasizes the heterogeneity in the underlying causes and manifestations, clinical course and outcome, also avoiding a definition based on a negative. According to the authors, it is more suitable to label the group of liver diseases associated with metabolic dysfunction (4,5) . In addition, regarding nutritional status, the positive criteria for MAFLD includes lean patients, who often have body composition alterations that can affect the progression to a more severe form of the disease [6,7]. Still, the proposed definition remains controversial [8].

Diet influences liver fat content [9], and although numerous nutritional approaches have proven effective in promoting weight loss in NAFLD, the Mediterranean diet has been more robustly associated with better metabolic outcomes, improvement in insulin resistance, and better lipid profile, independently of weight loss [3,10–12]—possibly because of the diet's anti-inflammatory properties, which can contribute to mitigate hepatic stress [12–15].

Since the human diet consists of foods and nutrients that can be predominantly pro-inflammatory or anti-inflammatory, resulting in different levels of inflammatory biomarkers [16], a dietary pattern approach acknowledges the complex interactions between dietary components, and it has advantages over individual foods or nutrients when studying associations with disease [17].

The Dietary Inflammatory Index (DII®) is a literature-derived, population-based index that was developed to predict the inflammatory potential of diet [18]. The DII® has been validated with various inflammatory markers. A more pro-inflammatory dietary pattern has been associated with higher mortality risk [19–21].

A less pro-inflammatory diet has been demonstrated to be associated with less liver fat [22–24]. However, studies were conducted mostly with non-invasive scores of liver fat, and there are no data, to our knowledge, regarding DII® and stages of liver fibrosis, the latter strongly implicated in the outcome of fatty liver diseases. In addition, different degrees of alcohol intake can, possibly, have different inflammatory properties.

Since MAFLD has only recently been proposed, with scarce information regarding the impact of dietary pattern in the development and progression of fatty liver disease [25], the aim of the present study was to characterize the dietary intake in MAFLD patients, focusing on the possible relationship between dietary pattern—assessed by DII®, and degree of liver fibrosis, as well as on the effect of harmful alcohol consumption in this equation.

2. MATERIALS AND METHODS

This is a cross-sectional study, conducted at the outpatient Hepatology Clinic of a tertiary university hospital, where patients are referred due to suspected fatty liver disease by general practitioners or by other medical specialists within the hospital.

2.1. Participants

During a six-month period, patients with a first appointment for suspected fatty liver disease were included in the analysis. Nutritional assessment, conducted by a single dietitian, included anthropometric and dietary data. Clinical data were recorded by a group of trained hepatologists, who used a standardized protocol to minimize bias on data collection. All data were collected in the first appointment. Patients who were unable to complete full nutritional assessment (detailed in following sections) or who did not present liver steatosis (detailed in Section 2.4) were excluded.

On the fasting state (12 h fasting), blood samples were collected in an accredited laboratory using standard methodology for the determination of fasting glucose (Hexokinase UV test, Cobas 8000 c702 (Roche Diagnostics, Mannheim, Germany)), insulin (ECLIA sandwich assay, Cobas 8000 e801 (Roche Diagnostics, Mannheim, Germany)), HbA1c (Boronate affinity HPLC, Premier Hb9210 (A. Menarini Diagnostics, Florence, Italy)), total cholesterol (Enzymatic colorimetric method, Cobas 8000 c702 (Roche Diagnostics, Mannheim, Germany)), triglycerides (TG) (Enzymatic colorimetric method, Cobas 8000 c702 (Roche Diagnostics, Mannheim, Germany)), HDL-cholesterol (HDL-c) (Homogeneous enzymatic colorimetric assay, Cobas 8000 c702 (Roche Diagnostics, Mannheim, Germany)), and liver enzymes (AST—aspartate aminotransferase and ALT—alanine aminotransferase; Enzymatic IFCC modified—without pyridoxal phosphate activation, Cobas 8000 c702 (Roche Diagnostics, Mannheim, Germany) and GGT—gamma glutamyl transferase; Enzymatic-Szasz, Cobas 8000 c702 (Roche Diagnostics, Mannheim, Germany)). LDL-cholesterol (LDL-c) was calculated using the Friedewald formula in mg/dL (c-LDL = CT (c-HDL +TG/5)).

2.2. Anthropometric Parameters

Anthropometric data were collected with participants wearing light clothes and bare feet. Weight was measured using a calibrated scale and height was assessed using a stadiometer. Waist circumference (WC) was measured halfway between the inferior rib and the iliac crest. Body mass index (BMI) was defined as an individual's weight in kilograms divided by the square of height in meters (kg/m2). Body fat mass was determined using bioelectrical impedance analysis (BIA—Biodynamics BIA 450 Bioimpedance Analyzer).

2.3. Dietary Intake and DII®

A validated food frequency questionnaire developed for the national population (FFQ) was applied by a trained investigator on the first appointment. The FFQ is a semiquantitative questionnaire comprising 89 food items, distributed by 8 categories (dairy; eggs, meat and fish; fat; cereals; sweat and pastries; vegetables and legumes; fruits and drinks), ranging from daily to monthly frequency and allows the quantification of energy and 44 nutrients, reflecting the dietary intake of the previous year [26].

Shivappa et al. [16] documented that a total of 45 specific foods and nutrients were associated with one or more inflammatory markers: IL-1b, IL-6, TNF-_, or CRP. They scored the inflammatory potential for each food parameter according to its relation to inflammatory markers: increased, decreased or no effect. Then, based on 11 data sets from 11 countries worldwide, they calculated the mean and standard deviation for each of the 45 food parameters. Subsequent studies acknowledged that it is possible to assess DII® with less food items [18].

Dietary data derived from the FFQ were used to calculate DII® scores for all participants. As documented previously [18], we calculated DII® score based on 28 food parameters from the FFQ: energy, protein, carbohydrates, fiber, total fat, saturated fat, mono-unsaturated fat, polyunsaturated fat, omega-3, omega-6, trans fat, thiamine, folic acid, niacin, riboflavin, vitamin B12, vitamin B6, vitamin C, vitamin A, vitamin D, vitamin E, iron, selenium, magnesium, zinc, alcohol, and caffeine. To calculate the DII® score for each participant, a z score for a given food parameter was calculated by subtracting the "standard global mean" from the amount consumed by each participant and dividing this value by the "global standard deviation". Then, this value was converted to a centered percentile score. For each participant, this score was then multiplied by the respective food parameter effect score [25]. The overall DII® score was calculated for each participant by summing up all DII® scores from each calculated FFQ item. A higher and positive DII® score indicates a more inflammatory diet, and a lower and negative DII® score indicates a less inflammatory diet. The DII® score was categorized into quartiles and coded as more anti-inflammatory or pro-inflammatory based on the patients' distribution.

2.4. Assessment of MAFLD

On the initial evaluation of patients with suspected MAFLD, the presence of liver steatosis on ultrasound, which uses sound waves to evaluate the size and shape of the liver, as well as blood flow through the liver, was assessed, and a transient hepatic elastography (Fibroscan®) evaluation, which measures, in the middle of the liver, the movement of the liver caused by ultrasound wave and allows the determination of its stiffness (or elasticity), was conducted. Fibroscan® allows the determination of Controlled Attenuation Parameter (CAP) as an indicator of the degree of steatosis, and it measures liver stiffness measurement (LSM) as an indicator of liver fibrosis [27–29]. The values chosen to indicate steatosis as absent (S0), mild (S1), moderate (S2), and severe (S3) were: S0 < 236 dB/m, S1

≥236 dB/m, S2 ≥270 dB/m, and S3 ≥302 dB/m [30]. Fibrosis was defined as absent (F0), mild (F1 ≥5.5 kPa), moderate (F2–F3 ≥7 kPa), and advanced (F4 ≥10.5 kPa) [29].

The patient was considered as having MAFLD if liver steatosis was present on ultrasound, plus the presence of one of three factors: overweight (BMI ≥25 kg/m2) or type 2 diabetes (T2D if fasting glucose levels, HbA1c ≥6.5% or) or lean BMI (BMI < 25 kg/m2) plus the presence of two metabolic risk abnormalities (waist circumference ≥102/88 cm in Caucasian men and women, blood pressure ≥130/85 mmHg or specific drug treatment, plasma triglycerides ≥150 mg/dL (≥1.70 mmol/L) or specific drug treatment, plasma HDL-cholesterol < 40 mg/dL (<1.0 mmol/L) for men and <50 mg/dL (<1.3 mmol/L) for women or specific drug treatment, prediabetes (i.e., fasting glucose levels 100 to 125 mg/dL (5.6 to 6.9 mmol/L), or 2 h post-load glucose levels 140 to 199 mg/dL (7.8 to 11.0 mmol), or HbA1c 5.7% to 6.4% (39 to 47 mmol/mol)), homeostasis model assessment of insulin resistance score ≥2.5 or plasma high-sensitivity C-reactive protein level > 2 mg/L) [4]. MAFLD patients were further classified as MAFLD with alcohol intake and MAFLD without alcohol intake, according to alcoholic beverage consumption, as previously described [5].

Metabolic syndrome was considered when 3 of 5 criteria were present [31]: elevated waist circumference ≥94/88 cm in Caucasian men and women, elevated blood pressure 130/85 mmHg or specific drug treatment, elevated plasma triglycerides ≥150 mg/dL or specific drug treatment, reduced plasma HDL-cholesterol < 40 mg/dL for men and <50 mg/dL for women or specific drug treatment, fasting glucose ≥100 mg/dL or specific drug treatment.

Patients who presented positivity to hepatitis B virus surface antigen; positivity to anti-hepatitis C virus; other type of liver diseases (i.e., primary biliary cholangitis, autoimmune hepatitis, primary sclerosing cholangitis, Wilson's disease, hemochromatosis, or a1-antitripsin deficiency); and treatment with potentially steatogenic drugs such as steroids, high-dose estrogen, tamoxifen, methotrexate, or amiodarone were classified as having dual etiology [4].

Patients were further classified, according to the amount of alcoholic consumption, as MAFLD without alcohol intake and MAFLD with alcohol intake. Those who presented alcohol intake higher than 20 g/day for women and 30 g/day for men were considered further as MAFLD with harmful alcohol intake and compared with those with moderate intake (alcohol intake below 20 g/day for women and 30 g/day).

2.5. Statistical Analysis

Continuous variables were summarized using mean and standard deviation (SD). Categorical data are represented using frequency tables. Unpaired t-tests (Student's t test or one-way ANOVA) were used to compare distribution across continuous variables. When variables did not follow a normal distribution, as assessed by Shapiro–Wilk test and inspection of distribution graphs, the non-parametric test alternative was used (Mann–Whitney or Kruskal–Wallis) Chi-Squared test was used for categorical variables to test differences between different groups. To verify the association between continuous variables, Pearson's correlation coefficient was used. The odds ratio (OR) and 95% CI summarizing the association between DII® and severe steatosis and advanced liver fibrosis were calculated. The following variables were adjusted in the logistic regression model: age, BMI, metabolic syndrome, dyslipidemia, HBP, diabetes, and sex. A p-value < 0.05 was used as the cut-off for statistical significance. All statistical analysis was conducted using SPSS® (IBM SPSS Statistics 26).

3. RESULTS

From the 187 patients screened, 161 had complete nutritional and clinical assessment, and MAFLD diagnosis was established. None of the patients presented dual etiology.

From the 161 patients, 47.8% (n = 77) presented MAFLD without alcohol intake and 52.2% (n = 84) presented MAFLD with alcohol intake; from these, 22.6% (n = 19) presented harmful alcoholic consumption (Figure 1).

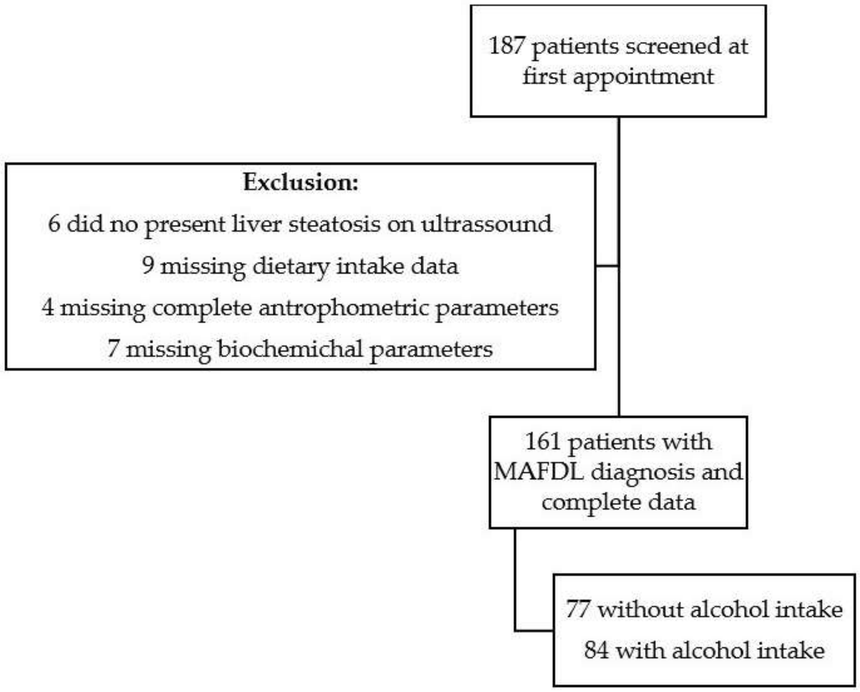


Fig. 1: Flowchart of patient inclusion

Patients were mostly males (59.6%; n=96); 91,9% (n=148) presented overweight (BMI≥ 25kg/m^2) When comparing MAFLD patients with or without alcohol intake, there was a trend for patients without alcohol intake to present a higher prevalence of high blood pressure and metabolic syndrome (Table 1).

Table 1. Baseline patient characteristics

		MAFLD patients (n=161)	MAFLD without alcohol (n=77)	MAFLD with alcohol (n=84)	p-value*
Sex (%)	Male	59.6 (96)	42.9 (33)	75.0 (63)	**<0.001**
	Female	40.4 (65)	57.1 (44)	25.0 (21)	
Age (years)		55.9±12.7	55.2±13.3	56.5±12.2	0.534
Weight (kg)		86.3±16.5	83.5±16.9	89.0±15.7	**0.035**
BMI (kg/m2)		30.9±5.3	30.9±5.3	30.8±5.3	0.909
BMI	Normal (<25kg/m^2)	8.1 (13)	6.5 (5)	9.5 (8)	0.732
	Pre-obese (≥25-29.9kg/m^2)	42.2 (68)	41.6 (32)	42.9 (36)	
	Obese (≥30kg/m^2)	49.7 (80)	51.9 (40)	47.6 (40)	
Waist circumference (cm)		102.9±10.8	100.9±9.8	104.6±11.4	0.109
Body fat mass (kg)		28.1±11.4	29.4±12.4	26.9±10.5	0.228
AST above threshold		40.6 (65)	39.0 (30)	42.2 (35)	0.680
ALT above threshold		59.4 (95)	57.1 (44)	61.4 (51)	0.580
GGT above threshold		56.9 (87)	58.9 (43)	55.0 (44)	0.626
Type 2 diabetes mellitus (%)		41.0 (66)	45.5 (35)	36.9 (31)	0.271
High blood pressure (%)		58.4 (94)	66.2 (51)	51.2 (43)	**0.053**
Dyslipidemia (%)		59.6 (96)	57.1 (44)	61.9 (52)	0.538
Metabolic Syndrome (%)		50.3 (81)	57.1 (44)	44 (37)	**0.097**

Data presented as % (n) or mean±SD; * p-value for comparison between MAFLD without alcohol and MAFLD with alcohol (independent samples T-test for continuous variables; Chi-Squared test for categorical variables); BMI – body mass index; AST - aspartate aminotransferase; ALT - alanine aminotransferase; GGT - gamma glutamyl transferase

Male patients were significantly associated with the presence of MAFLD with alcohol intake (OR: 2.286 (IC95%: 1.505–3.471); $p < 0.001$). No association between BMI category (BMI < 25 kg/m2 vs. BMI ≥25 kg/m2) and alcohol intake was found.

MAFLD patients with metabolic syndrome were older (61.0±9.7 vs. 50.7±13.4years; p<0.001) and presented higher mean levels of BMI (32.5±5.4 vs. 29.3±4.7kg/m2; p<0.001) and weight circumference (110±12.1 vs. 103.9±9.8cm; p=0.011). Patients with moderate alcohol intake did not present significant differences in mean age, BMI, or metabolic comorbidities prevalence, when compared with patients with harmful alcohol intake.

3.1. Dietary Intake and DII®

Patients reported a mean dietary caloric intake of 1868±415kcal/day. Patients with any alcohol intake, presented higher intake of calories, higher median global daily intake of red meat (35.7 vs. 46g/day; p=0.024), pastries (13 vs. 15g/day; p=0.038), added sugar (5.8 vs. 7.6g/day; p=0.019) and sugar sweetened beverages (26.5 vs. 48.2ml/day; p=0.045). This group with any alcohol intake also presented a tendency for higher intake of carbohydrates and sugar (Table 2).

Table 2. Dietary intake in MAFLD patients

	MAFLD patients (n=161)	**MAFLD without alcohol (n=77)**	**MAFLD with alcohol (n=84)**	**p-value***
Total energy intake (g/day)	1868±415	1777±381	1945±429	**0.013**
Total fat intake (g/day)	74.2±19.0	74.6±18.4	73.9±19.6	0.861
Saturated fat intake (g/day)	21.4±6.3	21.3±6.7	21.5±6.1	0.687
MUFA intake (g/day)	34.3±9.5	34.7±9.3	33.9±9.7	0.828
PUFA intake (g/day)	12.6±3.9	12.5±13.9	12.6±3.9	0.575
Total protein intake (g/day)	83.2±18.6	83.6±18.3	83.1±18.9	0.719
Total CHO intake (g/day)	209.8±56.8	201.6±56.0	216.7±56.8	**0.096**
Sugar (g/day)	89.9±37.5	86.2±39.7	92.9±35.5	**0.094**
Fiber intake (g/day)	19.2±6.4	18.9±6.5	19.5±6.3	0.425

Data presented as mean±SD; * p-value for comparison between MAFLD without alcohol and MAFLD with alcohol (independent samples T-test) MUFA – Monounsaturated fatty acids; PUFA - Polyunsaturated fatty acids; CHO – Carbohydrates

Comparing further only the patients with alcohol intake, those with moderated alcohol intake when compared with harmful alcohol intake presented higher median global daily intake of total fat (76.8 vs. 63.1 g/day; p = 0.008), saturated fat (22.2 vs. 18.7 g/day; p = 0.032), MUFA (35.3 vs. 28.7 g/day; p = 0.09), PUFA (13.1 vs. 10.4 g/day; p = 0.007), and cholesterol (295.7 vs. 247.7 mg/day; p = 0.027), even though there was no significant difference in mean caloric, protein, or carbohydrates intake between groups. In addition, there was a tendency for patients with moderated alcohol intake to present higher median intakes of iron (14.5 vs. 12.2 mg/day; p = 0.068), magnesium (337 vs. 289 mg/day; p = 0.077), and omega-6 fatty acids (9.4 vs. 8.0 g/day; p = 0.074) compared with those with harmful alcohol intake. Patients with harmful alcohol intake presented lower median daily intake of dairy (62.0 vs. 131.3 mL/day; p = 0025) and chicken (27.4 vs. 40.4 g/day; p = 0.030) when compared with the group with moderated alcohol intake.

Patients with harmful alcohol intake presented a higher mean energy intake compared with non-drinkers (2256 _ 929 kcal; p = 0.004) with no other nutritional difference.

Based on BMI, there were no differences in dietary intake in the group of patients with no alcohol intake and moderated alcohol intake. In patients with harmful alcohol intake, there was a significant difference in sugar intake from pre-obese patients and obese patients (122.8 g/day vs. 70.7 g/day; p = 0.028) but not between lean patients and the remaining.

MAFLD patients with metabolic syndrome did not present any significant differences in dietary intake compared with the remaining group.

Mean DII® was 0.0042 (□3.92; 4.58); 50.3% (n = 81) presented a predominantly anti-inflammatory DII® and 49.7% (n = 80) presented a more pro-inflammatory DII®. There were no differences in BMI category, or the presence of metabolic syndrome based on DII® quartiles.

3.2. Liver Disease

Patients presented a mean CAP of 298.9±62.9 dB/m, with 56.1% (n = 88) presenting severe steatosis, 48.7% (n = 78) presenting moderate fibrosis, and 21.3% (n = 34) presenting advanced fibrosis.

Based on BMI category, there were no significant differences in mean CAP, but mean LSM differed significantly between BMI categories, with obese patients presenting higher mean LSM (10.0 ±6.8 kPa) compared with lean patients (5.9±1.9 kPa; p = 0.038) and pre-obese patients (7.0±4.1 kPa; p = 0.004).

MAFLD patients with metabolic syndrome presented higher mean levels of CAP (316.3 vs. 282.3 dB/m; p = 0.001) and fibrosis (10.1 vs. 6.6 kPa; p < 0.001) compared with the remaining.

Some metabolic factors were associated with a higher risk of presenting advanced fibrosis: namely, the presence of diabetes (OR: 7.250 (IC: 3.014–17.441); p < 0.001), high blood pressure (OR: 2.369 (IC: 1.024–5.480); p = 0.040), metabolic syndrome (OR: 4.333 (IC: 1.820–10.317); p = 0.001), and BMI ≥30 kg/m2 (OR: 2.531 (IC: 1.109–5.574); p = 0.025).

Mean CAP and LSM did not present differences from MAFLD patients with alcohol intake, regardless of the amount consumed, compared with MAFLD patients without alcohol intake. The prevalence of severe steatosis or advanced fibrosis between groups also did not present significant differences. When adjusting for baseline BMI, in the group of patients with moderate alcohol intake, there was a significant difference in mean LSM, with obese patients presenting higher mean LSM (9.6±6.1 kPa) compared with lean patients (5.4±1.3 kPa; p = 0.032) and pre-obese patients (6.4±2.9 kPa; p = 0.007). There was no significant difference in mean CAP or LSM, based on BMI in the group of patients with no alcohol intake and with harmful alcohol intake. The prevalence of severe steatosis or advanced fibrosis between groups of alcohol intake based on BMI category also did not present significant differences.

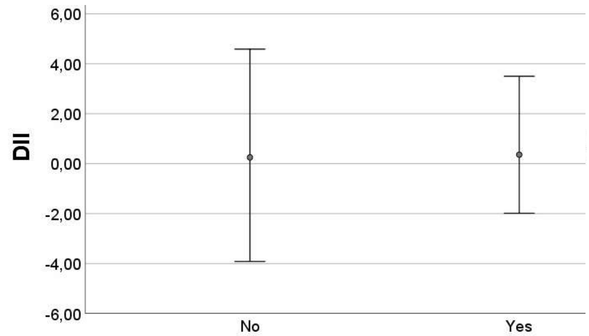
3.3. Dietary Intake, DII®, and Liver Disease

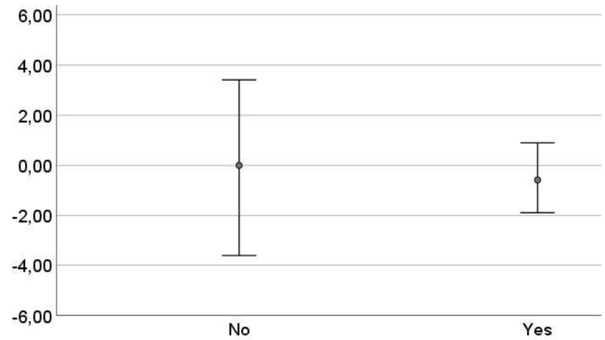
Patients did not present significant differences in caloric or nutrient intake (protein, CHO, total fat and fractions, fiber, sugar, and caffeine) based on the presence of metabolic comorbidities or metabolic syndrome.

Patients with severe steatosis presented higher median intakes of simple carbohydrates (109.8 vs. 85.0 g/day; p = 0.046) and a tendency to lesser folic acid intake (compared with the remaining). When compared with patients with mild steatosis, patients with severe steatosis presented higher median intake of added sugar (8.0 vs. 1.0 g/day; p = 0.001) and a tendency for a higher median intake of pastries (9.4 vs. 5.8 g/day; p = 0.083) and sugar sweetened beverages (20.9 vs. 10.0 mL/day; p = 0.081).

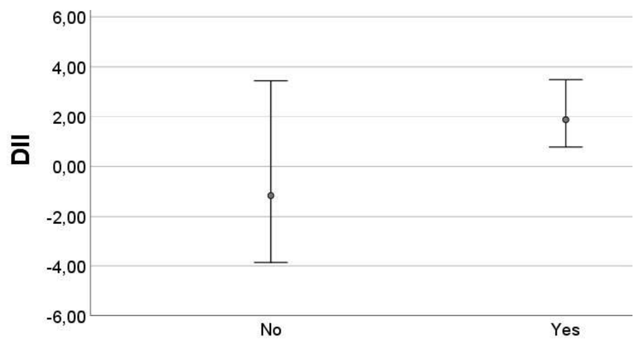
Patients with advanced fibrosis presented lower median intake of total fat (65.4 vs. 77.3 g/day; p = 0.024), saturated fat (18.2 vs. 22.5 g/day; p = 0.014), and MUFA (30.4 vs. 35.6 g/day; p = 0.025) and a tendency for lower caloric intake (1687 vs. 1927 kcal/day; p = 0.014) and PUFA intake (11.4 vs. 13.1 g/day; p = 0.014) compared with the remaining. In addition, they presented lower median intakes of riboflavin (1.4 vs. 1.7 mcg/day; p = 0.022), calcium (575 vs. 778 mg/day; p = 0.009), and zinc (8.9 vs. 10.6 mg/day; p = 0.017).

No correlation was found between raw and quartiles of DII® and liver enzymes, liver steatosis (measured by CAP), or LSM. However, in the group of patients with no alcohol intake, those who presented a more pro-inflammatory DII® more frequently had severe steatosis (87.5% vs. 12.5%), with a predominantly pro-inflammatory diet associated with a a four-fold increase in the odds of having severe steatosis (OR: 4.000 (95%CI 1.129–14.175); $p = 0.026$). In patients with harmful alcohol intake, a more pro-inflammatory DII® was associated with a higher degree of fibrosis, with all patients with advanced fibrosis presenting a pro-inflammatory DII® ($p = 0.027$) (Figure2).





(a) (b)



(c)

Figure 2. Mean DII based on the presence of advanced fibrosis in MAFLD patients (a) without alcohol intake; (b) with moderate alcohol intake; (c) with harmful alcohol intake

In a multinomial logistic regression model, in patients that did not present alcohol intake, after adjustment, a more pro-inflammatory diet maintained its association with a higher probability of presenting severe steatosis (OR: 4.789 [95%CI 1.102-20.807]; p=0.037).

4. DISCUSSION

The aim of this study was to examine the dietary intake of MAFLD patients and explore possible links with DII® and the degree of liver fibrosis. To the best of our knowledge this is the first study conducted assessing DII® in MAFLD using transient hepatic elastography.

Although in prospective studies of NAFLD patients, a higher DII® has been associated with higher probability of steatosis, based on non-invasive scores, such as the fatty liver index [22,24], as well as with higher risk of metabolic syndrome [33], in this cross-sectional study, with MAFLD patients, we did not find such correlation, nor a significant correlation with BMI. A recent study, comparing 968 MAFLD patients with 964 controls, found that a more pro-inflammatory dietary score was associated with higher risk of MAFLD [34], although no investigation was conducted on the severity of the disease. In our study, we observed that MAFLD patients with no alcohol intake, had a more pro-inflammatory diet, and this was associated with a higher likelihood of having severe steatosis. This association prevailed after adjustment for the major metabolic comorbidities. Also, all patients with harmful alcohol intake and advanced fibrosis presented a pro-inflammatory DII®.

In this study, the inflammatory component of the diet appears to be not so relevant in patients with alcohol intake, independently of the amount consumed. In patients with NAFLD, a moderate alcohol consumption is allowed, and with MAFLD there is currently no defined threshold for alcohol intake [4]. Although the impact of chronic alcohol consumption on the liver is very well demonstrated [35], the health benefits of moderate alcohol consumption, compared with abstainers and heavy drinkers, is an ongoing debate, with findings being inconsistent and varying depending on the studied outcome [33]. In this study, the inflammatory degree of the diet was of most relevance in non-drinkers. It is possible that in the absence of alcohol consumption, diet becomes more relevant to the risk of developing fatty liver disease. However, all patients with harmful alcohol intake and advanced fibrosis presented a more pro-inflammatory DII®, suggesting that in this circumstance alcohol and a pro-inflammatory diet may have a synergistic effect in the progression of liver disease.

We also found that, globally, patients with MAFLD present an unbalanced dietary intake, with higher intakes of added sugar, sugar sweetened beverages and pastries. In our study, similar to the results of a recent meta-analysis [37], that included 60 eligible studies with 100,621 patients, we did not find any correlation between nutrient intake and the degree of steatosis and fibrosis.

This study has some limitations. The reduced sample size of patients with harmful alcohol intake does not allow us to extrapolate the data and can possibly limit the amount of significance achieved. Also, the use of dietary scores, like the DII®, although useful to compare different populations, are based on a selection of specific aspects of the diet and cannot describe with complete accuracy overall dietary pattern [38]. Since MAFLD is a new concept, harmful alcohol intake could also be incorporated in dual aetiology [8]. However, in the present study the contribution of this aspect was within the aim of the study and since the dietary intake of these patients can have some differences from the NAFLD patients, it may be easier to evaluate what may represent the additive effect of harmful alcohol intake.

In the patient-centred, multidisciplinary management, recommended for these patients [39], an accurate knowledge of the dietary intake and is essential to tailor a successful lifestyle intervention. The findings of this study contribute to the characterization of baseline dietary intake and inflammatory degree of the diet in MAFLD patients, paving the way to design more suited dietary interventional trials.

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4.

GENERAL DISCUSSION & CONCLUSIONS

Obesity is globally rising, with implications in metabolic health. Fatty liver disease accompanies this increase, as shown in a recent meta-analysis estimating prevalence of NAFLD in the overweight population of 69.9% [164].

Nutrition plays a key role in fatty liver disease. An unbalanced diet, particularly with high sugar and fat content, is one of the major factors for its development. As such, nutritional intervention is a crucial component of fatty liver disease management, and although many drugs are being tested in clinical trials, dietary intervention is the sole treatment approved so far for this condition. This treatment aims at WL, with the improvement of outcomes directly related with its magnitude.

Unmet needs in this area, motivated the development of the present thesis, and the studies presented here. The aim is to contribute to the existing knowledge, either by consolidating therapeutic approaches or by exploring nutritional status characteristics associated with fatty liver disease.

A detailed discussion of each study included in this thesis is provided within the individual manuscripts, and so the main purpose of this chapter is to overview and general discussion of the main results, identify limitations and strengths of the present work and, additionally, implications for future research and for clinical practice.

4.1. Summary of results and general discussion

Given that dietary intervention is cornerstone in NAFLD treatment, our first study (study 1) aimed to test the hypothesis that this intervention conducted within a multidisciplinary team (hepatologist, dietitian and psychologist) with personalized dietary advice, would have better results in terms of WL compared with an approach conducted solely by an hepatologist.

Although we witness WL in both groups, it was only in the multidisciplinary group that a WL higher than 10% was observed, which represents the lower limit recommend by EASL to improve liver fibrosis.

Ideally, NAFLD clinical management should cover a wide range of aspects, i.e., NAFLD knowledge, priorities of care, lifestyle induced factors for disease and dietary intervention. Given that consultations usually have a short length, physicians tend to not have the time needed to conduct this holistic approach. Consequently, the inclusion of a nutritionist/dietitian plays a fundamental role. Nutritionists/dietitians are specialized in nutritional care, and in outpatients' population, the focus is on increasing heath related nutrition literacy and implementing dietary intervention to assure adequate clinical outcome. As such, it has been proven that the inclusion of a dietitian is fundamental to optimize adherence to lifestyle changes. Accordingly, we were able to demonstrate that a dietary intervention alongside a multidisciplinary approach is effective in significantly reducing body weight and improving liver enzymes in NAFLD patients.

To date, and despite EASL advocate for a multidisciplinary management of these patients, only two studies have been published documenting the effects of this multidisciplinary approach in anthropometric and liver related markers, being those studies conducted in single centre locations in Cuba [60] and in the United Kingdom [55]. The first study, by Vilar-Gomez and colleagues, although paved the way to lifestyle intervention in NAFLD, had a standardized dietary intervention limited to a low-fat hypocaloric diet (-750kcal). The second study, has limited information regarding the characteristics of lifestyle changes applied, particularly concerning dietary intervention.

Currently, EASL does not recommend specifically limiting fat intake, but advocates for an adoption of the Mediterranean dietary pattern [26] characterized by a fat content of around 35-45%, substantially higher than the one applied by Vilar-Gomez and colleagues. Furthermore, EASL recommends a dietary restriction of 500-1000kcal, but the guidelines to implement dietary intervention are scarce in further details, namely the proportion of macronutrients.

In our dietary intervention we used a modified version of the Mediterranean diet, with a caloric deficit of 500kcal, tailored to patient's nutritional needs and preferences. By doing so, we were able to reduce aminotransferases in more than 50% of patients, and moreover improving liver fibrosis (assessed by NITs), even in the lower limit of caloric restriction.

Although with multiple identified benefits [165], long-term dietary restriction has low adherence rate, possibly because of the obesogenic environment that characterizes nowadays society. With a lower extent of caloric restriction, it is possible to achieve the needed results to improve clinical outcomes and maintain patient's adherent and engaged on their clinical management, minimizing the risks of long-term nutritional deficits due to an imbalanced dietary intake.

Given the increase frequency of fatty liver disease in the HIV population and the associated high disease burden, we aimed to assess the prevalence of this condition and the related factors.

As such, we developed a screening referral algorithm and implemented it in an outpatient HIV clinic of a tertiary university hospital. Due to major discrepancies in NAFLD prevalence in HIV patients, we assumed a prevalence of 25% of NAFLD, described in the international literature, [108] and our aim was to screen 252 patients to obtain a representative sample. Due to Covid-19 restrains on data collection, only 140 HIV-positive patients were referred for screening, and from those only 118 were screened (46% of planned). We then compared the HIV patients clinical and demographic characteristics with a subgroup of a nationwide representative cohort (e_COR) [22].

We observed an estimated NAFLD prevalence of 54.8% in HIV patients. This prevalence was significantly higher than the one observed in the subgroup of the national cohort (33.3%). Only two studies have assessed NAFLD prevalence based on screening; Guaraldi and colleagues [117], in a cohort of 225 Italian HIV patients, documented a NAFLD prevalence of 36.9% and a recent study of Michel and colleagues [166], in a cohort of 282 German HIV patients documented a NAFLD prevalence of 27%, both substantial lower than we observed. The difference in prevalence may be attributable to a higher prevalence of overweight in our national cohort, with the Italian cohort presenting a mean BMI of 23.75±3.59kg/$m^{2,}$ and the German cohort a median BMI of 25kg/m^2, both significantly lower than our cohort (26.9±4.7kg/m^2).

As for metabolic risk factors, although rather prevalent, surprisingly, the only ones associated with the presence of NAFLD in the HIV population were obesity, dyslipidaemia, and an increased Homeostasis Model Assessment (HOMA) score. In the three cohorts (study 2, [117,166]) T2DM prevalence was within a range of 15.9-18.7%, lower than the recent 22.5% estimated prevalence in NAFLD patients [21] which may have limited the ability to establish this expected association.

Additionally, no anthropometric parameters, such as greater waist circumference demonstrated association with NAFLD in HV patients.

Finally, since, an unbalanced dietary intake is also a risk factor for NAFLD and dietary data in the NAFLD-HIV population is non-existent/ limited, we additionally aimed to compare dietary intake between HIV patients and this national cohort. Besides evaluating dietary intake, we analysed dietary data quality, using HEI® an index that ranges from zero to 100, with higher scores being associated with higher dietary quality [167]. We observed that, in PLWH, a HEI below 50 points was associated with a higher odd of having NAFLD and these group of patients presented the lower diet quality, a higher intake of CHO, fat and sugar, and lower intake of dietary fiber, all of which are known dietary risk factors for NAFLD development (revised in chapter 1). The association of a low quality/ high pro-inflammatory diet content and NAFLD had already been demonstrated in some groups of the general population [168–170] but this was the first study assessing diet quality in HIV NAFLD patients.

Our data corroborated previous findings regarding the association between obesity and NAFLD in HIV patients, and we found a high proportion of NAFLD patients with increasing comorbidities. Although advocating that, ideally, all patients with HIV should have access to a registered dietitian, recommendations for nutritional care of PLWH, pose obesity as a moderate risk factor for nutritional compromise and focus still on the wasting syndrome and associated factors [171] as priorities of management. Nonetheless, obesity prevalence has been steadily growing amongst PLWH, with known health related implications. Also, in our work, we demonstrated that despite the presence of HIV related factors modulating the inflammatory processes, dietary intake characteristics are of importance. Taken together, we believe that these aspects corroborate that nutritional education and intervention should not be a secondary component in the prevention and management of fatty liver disease in this group of patients.

Taking these findings (study 2), we furthermore aimed to determine if the strategy and subsequent findings observed in the general population (study 1) applied to PLWH, namely if the dietary intervention aiming at WL that was designed would achieve the same magnitude of WL and improvement in liver enzymes as documented in the general population.

To do that, we develop an intervention study, aiming to include a minimum of 44 patients and randomly allocated 1:1 to, either general dietary recommendations (referred as standard of care) or to a structured dietary intervention. We randomly assigned 27 patients to dietary intervention and 28 to standard of care. The intervention was planned to have periodic nutritional assessment during 12 months but due to the national lockdown, patients were followed prior to lockdown for a mean period of 5.0±1.5months, with 51% of patients losing weight in this period.

We then, maintained follow-up remotely and additionally aimed to assess the impact of this dietary intervention, on weight variation of these group of NAFLD HIV patients during lockdown, since pioneer data emerged regarding the modification of dietary habits with repercussions on weight gain and mental health during lockdown periods [172–174]. After 3 months of lockdown, a significantly higher proportion of patients in the standard of care group reported a decrease in their dietary pattern quality with a parallel increase in appetite. Although both groups gained weight, this increase was significantly higher in the standard of care group. Due to restrains in clinical practice we were unable to verify if this WL translated into an improvement of liver related parameters.

However, even with this constrains, we were able to demonstrate that HIV NAFLD patients benefit from a tailored dietary intervention, like the one applied to the general population. As such, clinical management of NAFLD-HIV patients, should be conducted with the inclusion of a nutritionist/dietitian. Additionally, in a pioneer way, we also demonstrated that this dietary intervention is also effective when performed remotely and provided a characterization of dietary habits in the presence of a unprecedent national and worldwide lockdown.

One additional interesting finding in this work, is that patients, either in the general population or PLWH that were in the general recommendations arm of the dietary intervention, also achieved some degree of WL. This is, possibly, due to an increased awareness regarding their health status and an increase motivation to change their lifestyle, since it has been demonstrated that educating NAFLD patients regarding their condition leads to a better disease management with a parallel improvement in clinical outcomes [175,176].This may be an explanation to the significant improvement in liver disease outcomes observed on the placebo arm of clinical trials.

Last, and due to a release of a new proposed definition for fatty liver disease during the course of this work, we decided to incorporate it and explore, in a pioneer way, the nutritional status characteristics associated with MAFLD.

Given that there is a higher degree of heterogeneity of MAFLD aetiology, as compared to NAFLD, and that the impact of alcohol intake on nutritional status is known to be deleterious, increasing with the number of alcoholic beverages consumed [177], we aimed to assess the impact of alcohol intake on the clinical presentation and nutritional status characteristics of MAFLD patients. As such, patients fulfilling MAFLD criteria, were classified according to the amount of alcoholic consumption. Moreover, considering that MAFLD definition emphasises the metabolic alterations with underlining inflammatory processes, we evaluated the degree of inflammation of the diet, using the Dietary Inflammatory Index-DII® [178] a score that classifies the diet as more pro or anti-inflammatory.

We observed that MAFLD patients with no alcohol intake had a significantly more pro-inflammatory diet, and this was associated with a higher likelihood of having severe steatosis, even after adjustment for metabolic comorbidities.

Recently, Tian and colleagues assessed in the 2017-2018 National Health and Nutrition Examination Survey (NHANES) cohort the inflammatory characteristics of the diet and observed that the presence of MAFLD was associated with a higher pro-inflammatory score [179]. In our study, all patients with harmful alcohol intake and advanced fibrosis presented a pro-inflammatory dietary intake. We cannot compare our data with Tian and colleagues since patients were not divided based on alcohol intake but based on liver fibrosis and also because heavy drinkers were excluded from the analysis of disease severity [179]. However, our data suggests that the inflammatory component of the diet appears to be not so relevant in patients with alcohol intake, independently of the amount consumed, which could imply that alcohol per se has a deleterious effect on the liver that may possibly overcome the inflammatory characteristics of the diet.

Given the association between fatty liver disease and inflammation, and the finding of this work, showing that inflammatory content of the diet is associated with the odd of steatosis, the assessment of dietary habits and its inflammatory potential are a key factor for adequate dietary intervention.

4.2. Limitations and Strengths

Specific limitations have been addressed in the discussion section of each article. Globally, there are three major limitations of this work.

First one is the representativeness of the samples in the studies. This was a single centre study which can impact the characteristics of included patients, namely social and demographic, and limit the generalization of the results. Additionally, the low screening rate of HIV patients for NAFLD may also contribute to this limitation. Sample size was inferior to the initially predicted upon the development of the research project, but data recently published documented a prevalence of NAFLD in the Portuguese population of 17% [22] inferior to the one reported in the international literature (25%), which translates into a reduction of 22% of the estimated number of patients that needed to be screened. Taken together, this information reveals that, our study had a population screening that was in fact 55% of the required one.

The second aspect is the inexistence of liver biopsy to document the histological improvement due to WL in the intervention studies (study 1 and study 3).

However, as referred in the discussion section of each article, it was considered unethical to perform liver biopsy solely for research purposes. Additionally, due to Covid-19 restrains, an assessment, such as TE, was not possible to obtain in all patients.

Finally, the cross-sectional design of the studies does not allow to draw conclusions regarding the nature of the studied associations and prevented to investigate the temporal relation between outcomes and risk factors.

Nevertheless, this work has also several strengths. First, it was pioneer, not only in characterizing nutritional status in Portuguese NAFLD patients (study 1), but also documenting their response to a nutritional intervention. Additionally, it is one of the few studies published in NAFLD patients, investigating the results of a multidisciplinary team approach. This is especially true for HIV-NAFLD patients, given that the present work (study 3) was the first one published worldwide regarding the results of a nutritional intervention in this specific population, and one of the few reporting clinical outcomes after dietary intervention in PLWH.

Second, part of this work was developed under a global pandemic. This led to an adaptation of the clinical practice to Covid-19 restrains, which driven the follow-up dietary intervention (study 3) to be conducted remotely. Apart from the characterization of eating habits during a national lockdown, this strategy allowed us to demonstrate that nutritional follow-up is also a key factor that maintain global health when an extreme condition such as global lockdown was implemented. Additionally, we demonstrated that telemedicine can be effective in managing dietary intervention and this was also the first study published using this remote intervention in fatty liver disease.

Third, and since a PhD is a work in progress, the incorporation of a study characterizing dietary intake based on the recent new proposed definition of MAFLD, and considering that is still an ongoing debate, study 4 contributes to a better characterization of nutritional status based on liver disease severity in MAFLD patients and provides some insight on the impact of alcohol on nutritional intake and disease severity.

4.3. Implications and Future studies

This work contributes to the current guidance in the field regarding the impact of lifestyle changes and rate of adherence to them amongst fatty liver disease patients and contributes to an increased awareness regarding the clinical characteristics of fatty liver disease patients and the impact of dietary interventions on their clinical outcome. Given that hepatic cirrhosis is the 10^{th} national mortality cause, this is of special importance, since, to date, no pharmacological treatment is available for fatty liver disease, and lifestyle changes are a relative affordable strategy to implement and help to delay the natural progression of the disease.

Additionally, this work sheds light regarding disease burden in the national HIV population, scarcely characterized despite the incidence and prevalence of HIV infection in Portugal, and, to the best of our knowledge, provides the first data on the impact of a dietary intervention on liver related outcomes in this group. Also, due to the current knowledge of obesity and fatty liver disease related prevalence and morbidity in PLWH, this work supports the need of a possible revision in the recommendations categories of nutritional risk, with implications in nutritionist/dietitian timing of nutritional intervention.

As for clinical practice, a recent meta-analysis, that aimed to describe the effects of dietary interventions based on Mediterranean diet and caloric restriction on NAFLD surrogate markers, concluded that clarification on the specifications of dietary intervention is needed, since the authors verified a high degree of heterogeneity in the studies reviewed [180]. This work provides new tools regarding dietary intervention specifications which can be applied easily in the clinical management of these patients by dietitians/ nutritionists and other members of multidisciplinary teams.

Deriving from this premise, since it is one of the few studies documenting the impact of a multidisciplinary approach, the present work could change the perception of clinical physicians regarding the management of these patients. Recently, a global survey in 40 countries, assessed physician knowledge about NAFLD [181]. This survey, that included hepatologists, gastroenterologists, endocrinologists and primary care physicians, demonstrated that, accurate knowledge concerning NAFLD management was lacking, and physicians reported that the major barrier identified was adherence to lifestyle recommendations [181]. Multidisciplinary teams, as demonstrated in our study, achieve higher gains in terms of health-related outcomes, so, additionally, this work could also contribute to the development of other dedicated multidisciplinary teams within the national health system.

As for future studies, and given the high disease burden associated with fatty liver disease, one additional interesting study would be to incorporate the modifications witnessed in liver related markers or liver disease stage in patients submitted to the dietary intervention in the new recent mathematical model developed to assess the natural history of NAFLD [182]. The NAFLD simulator is an analytical model based on 1 000 000 simulated patients that allows to estimate risk of 10-year mortality, decompensation of cirrhosis and development of HCC. Although the model has still some limitations, since it does not take into account the presence of other comorbidities, such as T2DM and obesity, it would be of interest to assess the additional impact of lifestyle changes in the natural history of the disease of that particular patient.

Last, the work regarding the characterization of dietary patterns of MAFLD reinforces the need of an accurate quantification of alcohol intake in these patients. Since, alcohol report differs depending on the method of quantification used (ex. self-report vs. structured questionnaire) and food frequency questionnaires are time consuming, the application or further development of a tool specifically designed for fatty liver disease patients is needed to minimize this bias. As a baseline characterization of dietary habits of MAFLD patients, this work also paves the way for more suited clinical dietary interventions, that will also clarify the impact of alcohol intake on WL.

Some questions remain that still need to be addressed and represent further research opportunities in this field, i.e. how is the long-term WL maintained? How is disease progression affected in the long term by cyclic WL? Do HIV patients benefit in the long-term from WL and how is the interaction between nutrition, cART and WL? Considering the new proposed definition, do MAFLD patients need the same amount of WL to achieve similar clinical outcome?

From the findings of the present work, a long-term prospective study could clarify some of these aspects. Also, incorporating motivational and quality of life scales, microbiota characterization and genetic background of patients, could refine some of the individual impact in this WL and contribute to an improvement in care of patients with fatty liver disease.

So, in summary, the findings from the present work contribute to better characterization of fatty liver patients and to a clarification of the results of dietary intervention either in the general population or in specific populations, such as PLWH which may translate into a better disease management with relevant health gains.

5.

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