Plasma Adiponectin and Fatty Acids in MAFLD: Associations with Liver Measurements, Type 2 Diabetes, and Liver Fibrosis

Thesis

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Abstract

A paradigm shift in the understanding of non-alcoholic fatty liver disease (NAFLD) has led to the introduction of metabolic dysfunction-associated fatty liver disease (MAFLD), emphasizing the role of metabolic dysfunction. MAFLD redefines the diagnostic framework, enhancing our understanding of its pathophysiology and enabling more targeted treatments. It is estimated that 35%-40% of U.S. adults are affected by MAFLD, highlighting the need for improved prevention and management strategies.

Adiponectin, the most abundant adipokine, is known for its anti-inflammatory properties and ability to reduce oxidative stress. It also plays a key role in regulating glucose and lipid metabolism and chronic inflammation. Additionally, lipidomic analyses have identified certain fatty acid fractions in plasma as biomarkers of lipid metabolism, closely linked to liver triglyceride composition.

This thesis presents a cross-sectional secondary analysis based on data from a larger randomized, placebo-controlled clinical trial involving 74 participants aged 22 to 80 years with a body mass index (BMI) between 20 and 55 kg/m² and reported MAFLD. After adjusting for covariates, the analysis revealed that each 1 µg/mL increase in plasma high molecular weight (HMW) adiponectin was associated with a 2.4% decrease in liver fat content. Furthermore, higher total plasma adiponectin was negatively associated with the presence of type 2 diabetes mellitus (T2DM) and liver fibrosis in individuals with MAFLD. Specifically, for each 1 µg/mL increase in total plasma adiponectin, the odds of

having both T2DM and liver fibrosis were significantly reduced (OR: 0.185, 95% CI: 0.040-0.877, $p = 0.033$). Additionally, higher levels of HMW adiponectin were associated with reduced odds of having T2DM alone (OR: 0.276, 95% CI: 0.089–0.856, $p = 0.026$). This study also found that higher plasma levels of linoleic acid (LA) and α-linolenic acid (α-LA) were inversely associated with the presence of T2DM in patients with MAFLD. Specifically, higher LA levels were linked to lower odds of T2DM (OR: 0.795, 95% CI: 0.638-0.990, $p = 0.041$), and higher α-LA levels were similarly associated with reduced odds of T2DM (OR: 0.001, 95% CI: 0.001-0.590, $p = 0.034$).

Dedication

This thesis is dedicated to my parents for their endless support and encouragement.

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List of Abbreviations

ACC	Acetyl-CoA Carboxylase
AgRP	Agouti-Related Peptide
ALD	Alcoholic Liver Disease
AMPK	AMP-Activated Protein Kinase
ApoB100	Apolipoprotein B100
BMI	Body Mass Index
CLD	Chronic Liver Disease
CVD	Cardiovascular Disease
DEXA	Dual-Energy X-ray Absorptiometry
DNL	De Novo Lipogenesis
FFA	Free Fatty Acids
FGF21	Fibroblast Growth Factor 21
GLP-1RAs	Glucagon-Like Peptide-1 Receptor Agonists
HCC	Hepatocellular Carcinoma
HDL-C	High-Density Lipoprotein Cholesterol
ALD	Alcoholic Liver Disease
HDL-C	High-density Lipoprotein Cholesterol
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
HSC	Hepatic Stellate Cells
JAK2	Janus Kinase 2
LDL	Low-Density Lipoprotein
MAFLD	Metabolic Associated Fatty Liver Disease
MAPK	Mitogen-Activated Protein Kinase
MASLD	Metabolic Dysfunction-Associated Steatotic Liver Disease
MCRs	Melanocortin Receptors
MRI	Magnetic Resonance Imaging
MS	Metabolic Syndrome
MUFA	Monounsaturated Fatty Acids
NAFLD	Non-Alcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Non-Alcoholic Steatohepatitis
NLRP3	NOD-Like Receptor Family Pyrin Domain Containing 3
NPY	Neuropeptide Y
PBMC	Peripheral Blood Mononuclear Cells
POMC	Pro-opiomelanocortin
PPAR	Peroxisome Proliferator-Activated Receptor

ROS	Reactive Oxygen Species
SNP	Single Nucleotide Polymorphism
SOCS	Suppressor of Cytokine Signaling
STAT3	Signal Transducer and Activator of Transcription 3
TG	Triglyceride

Chapter 1. Introduction

A paradigm shift in the understanding of fatty liver disease has emerged with the introduction of the term metabolic dysfunction-associated fatty liver disease (MAFLD) by a panel of international experts (1). By focusing on the impact of metabolic dysfunction, MAFLD redefines the diagnostic landscape, fostering a better understanding of the pathophysiology and enabling more targeted and effective interventions (2). MAFLD significantly increases the risk of severe liver-related outcomes, including progression to fibrosis, cirrhosis, end-stage liver disease, and liver transplantation (3, 4). Beyond its hepatic impact, MAFLD is associated with numerous extrahepatic complications, such as cardiovascular disease (CVD), and cancers, such as hepatocellular carcinoma (HCC) (3, 4). MAFLD currently affects an estimated 35%-40% of adults in the U.S. population, underscoring the urgent need for improved strategies to prevent and manage this condition (5).

An effective therapeutic strategy for MAFLD should adopt a multifaceted approach, targeting key aspects of the disease, including mitigating liver damage, reducing hepatic steatosis, and alleviating the metabolic dysfunction driving disease progression (2). Adiponectin, the most abundant circulating adipokine, is known for its anti-inflammatory effects and its capacity to reduce reactive oxygen species (ROS) production, which is crucial in reducing oxidative stress (6, 7). Moreover, adiponectin contributes to the regulation of glucose and lipid metabolism (7, 8).

The two major receptors for adiponectin, AdipoR1, and AdipoR2, are expressed in the liver, where the signaling pathways activated upon binding to these receptors play a protective role against a range of liver diseases (9). In an animal study using a high-fat diet-induced liver injury model, which is commonly used to study fatty liver progression, the development of hepatic fibrosis was significantly more pronounced in adiponectin knock-out mice compared to wild-type mice (10). In contrast, adiponectin-overexpressing transgenic mice demonstrated resistance to fibrosis induced by exposure to thioacetamide (11). In a population-based cohort study of 2215 participants, adiponectin levels showed an inverse association with hepatic steatosis (12).

In addition to adiponectin, lipidomic analyses have revealed that fatty acid (FA) fractions in plasma serve as biomarkers of lipid metabolism and closely reflect the composition of liver triglycerides (TGs) (13). Plasma FA levels are influenced by a combination of factors, including dietary fatty acid intake and the dynamic interplay between de novo lipogenesis (DNL), the storage of TGs, and the breakdown of these TGs through lipolysis (14). In a cross-sectional study of plasma FA composition and non-alcoholic fatty liver disease (NAFLD) fibrosis showed that higher levels of oleic acid (18:1n-9) in both liver and plasma have been associated with a lower degree of fibrosis (15). Conversely, another study found that higher levels of monounsaturated fatty acids (MUFA), such as palmitoleic acid and oleic acid, were associated with higher NAFLD Activity Scores (NAS) (16).

While several studies have explored the relationship between adiponectin and plasma fatty acid levels (12, 17-19), there is a notable lack of research investigating the associations between total and high molecular weight (HMW) adiponectin levels and

plasma fatty acid composition with outcomes related to fatty liver, as well as the presence of type 2 diabetes mellites (T2DM) and fibrosis in individuals with MAFLD.

1.1 Hypothesis

My overall hypothesis proposes that plasma HMW and total adiponectin, along with plasma FA composition are linked to MAFLD. I developed models using total and HMW adiponectin as independent variables to examine their association with MAFLD. To accurately assess this association, developing models that account for potential covariates is essential for observational studies.

1.2 Specific Aims

1.2.1 To examine the association between plasma HMW and total adiponectin levels with hepatic fat and liver stiffness. This aim involved developing unadjusted and multivariate linear regression models to assess these relationships and determine the influence of covariates on the observed associations in the entire study population.

1.2.2 To examine the association between plasma HMW and total adiponectin levels with the presence of T2DM alone and the coexistence of T2DM and liver fibrosis in individuals with MAFLD. For this purpose, multinomial logistic regression models were utilized. Participants were categorized into three groups: the reference group, comprising individuals with hepatic steatosis (>5% hepatic fat accumulation, n = 37); the second group, including those with hepatic steatosis and T2DM (n = 14); and the third group, consisting of participants with hepatic steatosis, T2DM, and fibrosis (n = 7).

1.2.3 To investigate the association between plasma FA composition with presence of T2DM in patients with MAFLD. I aimed to investigate the association between plasma levels FAs and the presence of T2DM in patients with MAFLD using logistic regression models. Additionally, further logistic regression analyses were performed to explore the relationship across tertiles of plasma FAs composition.

1.2.4 To explore the relationship between HMW and total plasma adiponectin levels and plasma fatty FA composition in patients with MAFLD. I aimed to determine whether HMW and total adiponectin levels can predict any of the FAs that showed a significant association with the presence of T2DM. Linear regression models were utilized for this purpose.

Chapter 2. Review of Literature

2.1 Background

Ludwig and colleagues first introduced the term NAFLD in 1980 to describe fatty liver disease that develops without substantial alcohol intake (20). In general, NAFLD represents a range of liver disorders defined by the presence of hepatic steatosis, with no identifiable causes for the secondary fat accumulation. This condition ranges from the relatively benign NAFLD to the more severe NASH (21, 22). In the case of NAFLD, hepatic steatosis occurs without signs of inflammation, while in NASH, hepatic steatosis is accompanied by lobular inflammation and apoptosis, which can ultimately lead to fibrosis and cirrhosis (23, 24).

NAFLD can be categorized into two distinct types. The first type is closely associated with metabolic syndrome (MS), with current understanding indicating that insulin resistance is the key pathophysiological mechanism in NAFLD, as individuals with the condition often exhibit one or more features of MS, including systemic dyslipidemia, hypertension, insulin resistance, and type 2 diabetes mellitus (T2DM) (25).

The development of insulin resistance is a complex process. In the context of MS, particularly for those with NAFLD/NASH, the increase in fat mass and the differentiation of fat cells are key factors in driving insulin resistance (26). The second type is linked to infectious conditions that can lead to liver steatosis. In this context, infections such as hepatitis C may contribute to the development of the disease (27, 28).

The complexity and diversity of NAFLD reflect a spectrum that spans from simple fat accumulation to advanced cirrhosis, the terminal phase of liver damage. Various factors play a role in driving metabolic alterations within the liver. For instance, an excessive

intake of nutrients can disrupt the gut microbiome, resulting in dysbiosis. This imbalance may cause microbial-associated molecular patterns to translocate to the liver via the portal vein and enter systemic circulation, exacerbated by increased intestinal permeability. Such changes can trigger inflammatory responses in the liver, contributing to the progression of the disease (29, 30).

2.2 Prevalence

In the United States, the prevalence of NAFLD is estimated to be 27% (95% CI: 24-30%) according to the most recent meta-analysis published in 2024 (31). This prevalence is higher among men, at 37% (95% CI: 33-41%), compared to 28% (95% CI: 25-32%) among women. This study included a total of 479 studies involving 78,001,755 participants from 38 countries were included. The global prevalence of NAFLD was estimated at 30.2% (95% CI: 28.7-31.7%). Regionally, the prevalence varied as follows: Asia 30.9% (95% CI: 29.2-32.6%), Australia 16.1% (95% CI: 9.0-24.8%), Europe 30.2% (95% CI: 25.6-35.0%), North America 29% (95% CI: 25.8–32.3%), and South America 34% (95% CI: 16.9-53.5%) (31).

An older meta-analysis was published in 2022 and included 72 publications for the prevalence analysis, involving a sample population of 1,030,160 individuals from 17 countries and 16 publications for the incidence analysis, representing 381,765 individuals from five countries. The global prevalence of NAFLD was estimated at 32.4% (95% CI 29.9-34.9). This rate has shown a significant increase over time, rising from 25.5% (20.1-31.0) before 2005 to 37.8% (32.4-43.3) in 2016 and beyond (32). According to another extensive epidemiological study, the worldwide prevalence of NAFLD is estimated at 32.16% (95% CI 18.40-50.14%), corresponding to a total of approximately

1,659,117,735 cases (range: 949,165,794 to 2,586,363,388) (33). These studies consistently suggest a similar estimate for the prevalence of NAFLD.

2.3 Introducing MAFLD and MASLD

The historical perspective on NAFLD showcases a shifting comprehension of this condition. The term was first coined 37 years ago to categorize liver steatosis unrelated to excessive alcohol consumption, thereby identifying only a specific patient group at that time (34). As research and clinical observations have progressed, our understanding of NAFLD has deepened, revealing its complex pathogenesis, varied clinical presentation, and related complications (35). This evolution indicates the terminology and diagnostic criteria may not adequately capture the full extent of the disease as the prevalence of NAFLD has increased dramatically (36).

In 2020, the term MAFLD was introduced, reflecting a consensus among international experts to highlight the significant role of metabolic dysfunction in the disease. This new terminology aims to better capture the underlying mechanisms contributing to liver health issues (3, 37). Accurately identifying individuals at high metabolic risk is essential, given the strong link between NAFLD and other complex metabolic disorders (38). In this context, CVD has been recognized as the leading cause of death among patients with NAFLD (39).

The link between NAFLD and CVD can be explained by various factors, such as obesity, diabetes mellitus, and atherogenic dyslipidemia (40). Obesity is a significant risk factor for both NAFLD and CVD. The accumulation of excess fat impacts not only the liver but also leads to systemic inflammation and metabolic disturbances, which greatly increase the risk of cardiovascular complications (41). So far, the term MAFLD has shown to be

more effective than NAFLD in identifying individuals with multiple risk factors and a greater likelihood of developing liver fibrosis (42).

In 2023, the term metabolic dysfunction-associated steatotic liver disease (MASLD) was introduced, which not only highlights the metabolic aspects of fatty liver disease but also aims to address the stigma attached to the condition. Additionally, it seeks to acknowledge the overlap between MASLD and alcoholic liver disease (ALD) (43). The introduction of the terms MASLD and MAFLD marks a pivotal advancement in establishing a clearer and more inclusive framework for diagnosing, researching, and managing this increasingly common liver disorder. These terms mark an important advancement in the field of hepatology, laying the groundwork for future advances in both diagnosis and treatment approaches (44).

2.4 MAFLD vs. MASLD?

MAFLD/MASLD is currently the leading cause of chronic liver disease (CLD), and its prevalence has been rising significantly over the past few decades (36). This rising trend has resulted in a higher incidence of cirrhosis, HCC, hepatic decompensation, and liver-related mortality linked to MAFLD/MASLD (45). Both terms address the metabolic aspect of the disease by offering diagnostic criteria that depend on the existence of underlying metabolic risk factors (43).

Based on an epidemiological cross-sectional analysis, the definition of MASLD seems to include a greater number of individuals, resulting in a higher prevalence of the disease (46). The transition from NAFLD to MAFLD was implemented to more precisely reflect the disease's metabolic etiology and to improve risk stratification. This redefinition underscores a more comprehensive understanding of the condition and its clinical

implications (44). Therefore, the primary issue is not just the prevalence of the disease, but the accuracy and, more critically, the clinical significance of identifying individuals who are at increased risk for negative outcomes.

(47, 48). Ramírez-Mejía et al. noted that the distinction between MAFLD and MASLD primarily pertains to the identification of lean individuals, which is affected by the number of metabolic risk factors needed for diagnosis.

Under the MASLD criteria, lean individuals are required to have at least one metabolic risk abnormality, whereas the MAFLD criteria mandate the presence of two metabolic abnormalities. Several studies indicate that NAFLD and MASLD identify the same patient groups in approximately 98% of cases, suggesting that MASLD may not provide superior risk stratification compared to NAFLD (46).

In our study, MAFLD was found to be a more appropriate terminology, as its definition requires the presence of metabolic risk factors. The participants included in our research exhibited several of these risk factors, which are detailed in the results section, which aligns with the thought that MAFLD provides a more comprehensive understanding of the disease's metabolic underpinnings, enhancing its clinical relevance in identifying individuals at greater risk for adverse outcomes associated with FLD.

2.5 Understanding MAFLD: Definition and Criteria

MAFLD is diagnosed based on the identification of hepatic steatosis, validated through imaging or liver biopsy, along with at least one of the following conditions: T2DM, obesity, or metabolic dysregulation (49). According to Eslam M, et al., Metabolic dysregulation is defined by having at least two of the following metabolic risk factors (49):

- "Waist circumference ≥ 102 cm for men or ≥ 88 cm for women in Caucasians, or ≥ 90 cm for men and ≥ 80 cm for women in Asians
- Blood pressure ≥ 130/85 mmHg or use of antihypertensive medication
- Plasma triglycerides ≥ 150 mg/dl or use of triglyceride-lowering medication
- Plasma high-density lipoprotein cholesterol (HDL-C) < 40 mg/dl for men and < 50 mg/dl for women, or use of lipid-lowering medication
- Prediabetes, indicated by fasting plasma glucose levels between 100–125 mg/dl, 2-hour post-load glucose levels between 140–199 mg/dl, or glycosylated hemoglobin (HbA1c) between 5.7–6.4%
- Homeostasis model assessment insulin resistance score (HOMA-IR) ≥ 2.5
- High-sensitivity C-reactive protein (CRP) levels > 2 mg/L".

To better understand the origin of MAFLD, we can focus on the term 'metabolic dysfunction'. Grasping the various ways this dysfunction manifests across different organs is crucial for understanding how liver steatosis develops in individuals without other clear causes of liver disease, such as alcohol use, infections, or chronic illnesses. In fact, a lack of metabolic health exacerbates the progression of all liver diseases, even when metabolic issues might not be the initial trigger, as seen with MAFLD (4). Despite a lack of extensive epidemiological studies estimating MAFLD prevalence under its new terminology, a recent meta-analysis indicated that MAFLD affects half of the global population. The prevalence is estimated at 50.7% worldwide and 34% in the United States, particularly among overweight and obese individuals (50).

The foundational framework for understanding metabolic health in individuals was established by Karelis et al. in 2004 (51). Their study defined metabolic health based on a

lipid profile, which included measurements of total cholesterol, triglycerides, HDL, low-density lipoprotein (LDL), and insulin sensitivity assessed through HOMA-IR. Consequently, the presence of metabolic dysfunction is linked to mechanisms that disrupt lipid and glucose metabolism, thereby increasing cardiovascular risk (3).

In clinical practice, laboratory tests can reveal signs of metabolic dysfunction, such as insulin resistance, type 2 diabetes, and dyslipidemia. However, significant changes in total serum levels of specific markers typically occur later, as smaller physiological changes at the cellular level happen first. This makes it challenging to detect metabolic dysfunction in its early stages. Consequently, assessing risk factors for metabolic dysfunction is crucial for effective MAFLD screening in the general population (4).

Risk factors for metabolic dysfunction are multifaceted and necessitate tailored approaches considering population demographics and individual characteristics. These factors include overweight and obesity, specific patterns of body fat distribution, dietary habits, lifestyle choices, pre-existing health conditions, and genetic predispositions shaped by family history and ethnicity (3).

In addition, acknowledging age-related variations in the prevalence of liver fibrosis and chronic liver disease is crucial. For instance, MAFLD primarily affects middle-aged and older adults, with increased mortality observed in those over 60 years of age (4, 52). In contrast, ALD is more prevalent in younger to middle-aged individuals, particularly between the ages of 40 and 50, with cases emerging as early as 19 years old (53). Therefore, prioritizing older age groups for MAFLD screening is advisable (4).

Moreover, the timing of screening for metabolic fatty liver should align with routine physician check-ups. Non-specific blood tests and anthropometric measurements during

these visits can indicate the need for further evaluation of MAFLD (4). Key metabolic syndrome markers, such as lipid levels, fasting glucose, and HbA1c values, can signal metabolic dysfunction, including insulin resistance, T2DM, or dyslipidemia conditions closely associated with FLD (54). If any of these parameters are abnormal, along with atypical anthropometric measurements, it becomes imperative to screen for MAFLD (4).

Table 1 Summary of key biological and metabolic risk factors for MAFLD

Risk factors	
Higher waist circumference	Hypertension
Hypertriglyceridemia	Genetic predisposition
Low HDL-cholesterol	Excessive alcohol consumption
Higher BMI (overweight, obesity)	Insulin resistance
Higher age	Physical inactivity
Gut microbiome dysbiosis	Some chronic viral infections, such as hepatitis C
Gender (male)	Dietary patterns (rich in saturated fats and sugars)
Type 2 diabetes	Environmental toxins

2.6 Approaches for Diagnosis of MAFLD

To diagnose MAFLD, it is crucial to confirm the presence of hepatic steatosis in addition to meeting the criteria for metabolic dysfunction. Assessing hepatic steatosis can be accomplished using clinical algorithms, imaging methods such as ultrasound (US),

computed tomography (CT), and magnetic resonance imaging (MRI), or by examining liver histology (55).

The traditional gold standard for diagnosing liver disease is a pathological biopsy, which is invasive, costly, and associated with potential postoperative complications (56). The most employed technique for detecting fatty liver is conventional brightness mode (B-mode) ultrasound, demonstrating a high level of accuracy for identifying moderate to severe steatosis (>33%). However, its sensitivity diminishes when detecting mild steatosis, especially in obese patients (57). Emerging sonographic techniques that evaluate backscatter and attenuation coefficients show promise for quantitatively assessing liver fat, though they may be less specific when significant liver fibrosis is present (58). The controlled attenuation parameter (CAP), utilized with FibroScan®, offers an area under the curve (AUC) of 0.81 for diagnosing fatty liver but faces challenges in accuracy with obesity and in distinguishing between different grades of steatosis (59). In contrast, MRI techniques such as proton-density fat fraction (PDFF) and volumetric fat fraction (VLFF) provide quantitative assessments with superior accuracy, capable of detecting a 5% change in steatosis grade (60, 61).

After diagnosing MAFLD, clinicians must assess the severity of the condition and evaluate the risk of liver-related complications. The primary focus is often on liver fibrosis, as it has a well-established correlation with adverse liver outcomes (62). Most guidelines recommend the Fibrosis-4 index (FIB-4) for initial assessment. FIB-4 is a straightforward calculation based on age, platelet count, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), and it has shown higher accuracy than several simple fibrosis scores in identifying advanced fibrosis (63, 64). The positive

predictive value (PPV) of FIB-4 is relatively limited, highlighting the importance of performing a follow-up test to verify the presence of advanced fibrosis (65). Ultrasound elastography, such as VCTE, is commonly used as the second test (66, 67). In addition to VCTE, magnetic resonance elastography (MRE) offers the advantage of assessing the entire liver within a brief acquisition period, a notable improvement over ultrasound elastography (68). Additionally, MRE exhibits reduced dependence on operator skill, ensuring greater consistency and repeatability in both diagnosing and quantitatively staging liver fibrosis (68). Research has demonstrated that MRE provides the highest accuracy in detecting stage 4 fibrosis, along with superior intra- and inter-observer reproducibility (69).

2.7 Pathophysiology

Some authors have developed a pathophysiological model that is based on the "two-hit hypothesis". According to this hypothesis, the “first hit” involves the buildup of TGs in hepatic cells and the development of hepatic insulin resistance, which makes the liver more susceptible to additive lipid accumulation and subsequent liver damage. The “second hit” arises from the secondary damage resulting from the first hit, manifested as altered adipokine production, heightened inflammation, oxidative stress, apoptosis, and liver fibrosis (70).

However, the original two-hit hypothesis is now considered insufficient to account for all the molecular and metabolic changes. The mechanism is now understood to be more complex, with growing support for the "multiple hit" hypothesis. MAFLD develops when TG synthesis in the liver exceeds the catabolism of non-esterified fatty acids, relying on

mitochondrial oxidation and the export of these TGs to very low-density lipoproteins (VLDL) (71-73).

As a result, it has been updated to the "multiple hit" hypothesis, which offers a more comprehensive explanation of the disease. This revised model identifies insulin resistance as a key factor driving increased DNL and lipolysis in adipose tissue, leading to an influx of free fatty acids (FFAs) into the liver via the portal vein. This hypothesis suggests that TG accumulation in the liver does not inherently induce hepatotoxicity (74); rather, it may serve as a protective mechanism to mitigate the excess of FFAs, as evidenced by findings in murine models (75, 76). Yamaguchi et al. demonstrated that inhibiting Diacylglycerol O-acyltransferase 2 (DGAT2), a critical enzyme in triglyceride synthesis, leads to decreased intrahepatic TG levels (77). This reduction is accompanied by increased FFA oxidation and exacerbation of steatohepatitis in murine models; therefore, elevated TG concentrations should be viewed as an epiphenomenon that coincides with the generation of toxic metabolites, lipo-toxicity, and liver damage as a contributor to MAFLD (78).

2.8 Glucose and Lipid Metabolism and the Role of Insulin Resistance in MAFLD

Chronic hyperglycemia, often seen in individuals with T2DM, contributes to a range of pathological mechanisms, such as persistent low-grade inflammation, steatosis, and apoptosis (2). The pathological reaction to an excess of carbon flux from energy-rich nutrients like carbohydrates and lipids underscores the vulnerability of metabolic pathways in handling surplus intake (79, 80). This vulnerability primarily stems from systemic insulin resistance, a key early predictor of dysregulated lipid and glucose

metabolism. In patients with MAFLD and NASH, insulin resistance is commonly observed alongside visceral adiposity, elevated TG levels, and reduced HDL-C (81-83). Insulin resistance disrupts both anabolic processes (such as de novo synthesis and lipid accumulation) and catabolic processes (including oxidation and secretion) in lipid and glucose metabolism (84).

A primary immediate effect of insulin resistance is the diminished capacity of peripheral skeletal muscle to effectively dispose of glucose by converting it into glycogen (85, 86). Under physiological conditions, insulin binding and the subsequent phosphorylation of insulin receptor substrates (IRS) initiate a downstream cascade of reactions that results in the translocation of the glucose transporter type 4 (GLUT 4) to the plasma membrane, enhancing glucose uptake by skeletal muscle (87, 88). However, in an insulin-resistant state, GLUT 4 fails to translocate to the membrane in skeletal muscle, impeding the uptake of plasma glucose (89). This leads to the depletion of glycogen stores in the muscle, which is one of the earliest signs of insulin resistance (90). The resulting energy depletion reserves in skeletal muscle contribute to muscle wasting and sarcopenia in patients with T2DM as well as in those with NAFLD (91, 92).

Insulin resistance results in enhanced lipolysis of TGs from adipose tissue and increased gluconeogenesis. This heightened lipolysis elevates plasma concentrations of non-esterified fatty acids (NEFA) and glycerol (93-95). When NEFA enter hepatocytes, they are esterified into TGs through the action of glycerol-3-phosphate acyltransferase (GPAT) and DGAT. Glycerol, another product of adipose tissue lipolysis, is converted into glycerol-3-phosphate (G3P) in the liver by glycerol-3-kinase (G3K). The increased

flow of glycerol to the liver enhances gluconeogenic flux, contributing to hyperglycemia (93, 96, 97).

In hepatic steatosis, G3P is funneled into the glycolytic and tricarboxylic acid (TCA) cycles, where it undergoes oxidation to produce oxaloacetate (OAA) (98). Next, OAA is subsequently converted to phosphoenolpyruvate (PEP) by mitochondrial phosphoenolpyruvate carboxykinase (PEPCK), which acts as the rate-limiting enzyme in gluconeogenesis, thereby enhancing hepatic glucose production (84). Additionally, the reductive equivalents generated during the TCA cycle and their subsequent oxidation contribute to endoplasmic reticulum (ER) stress and the formation of ROS, which play a role in the development of steatohepatitis (99-101). G3P not only provides carbon for gluconeogenesis but also serves as a carbon backbone for the esterification of acyl chains through the actions of enzymes such as GPAT and DGAT; therefore, promoting lipid synthesis (98, 102).

Prolonged hyperglycemia leads to increased insulin secretion from the beta cells of the pancreas, which is a hallmark of disrupted glucose metabolism (81, 86, 103, 104). This condition is commonly associated with NAFLD (84). Sustained hyperinsulinemia can desensitize insulin signaling pathways in skeletal muscle, hepatocytes, and adipocytes, exacerbating systemic insulin resistance (85, 86).

Alongside the pathways mentioned earlier, increased lipid uptake contributes significantly to the accumulation of lipids in MAFLD (2). The hepatic lipid uptake is regulated by fatty acid transport proteins (FATPs) along with cluster of differentiation 36 (CD36). Notably, isoforms 2 and 5 of FATP are the predominant types present in hepatic tissue (105). Elevated levels of FATP5 expression in humans have been associated with

greater hepatic steatosis in male patients with MAFLD (106). Moreover, Fatty acid binding protein 1 (FABP1) is primarily expressed in the liver, where it plays a key role in transporting fatty acids between organelles (105). Evidence suggests that mice deficient in the FABP1 gene exhibited a reduced response in hepatic triglyceride uptake and oxidation during fasting, resulting in lower hepatic lipid uptake (107).

Some studies have highlighted the role of transcription factors in the development of hepatic steatosis (108, 109). For instance, Sterol regulatory element-binding protein 1c (SREBP1c) and carbohydrate response element-binding protein (ChREBP) are responsible for regulating DNL through their influence on fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 (SCD1) (110). Overexpression of SREBP-1c has been associated with the enhanced expression of critical enzymes involved in DNL, leading to increased hepatic lipid accumulation (110). In contrast, ChREBP plays a vital role in orchestrating the normal lipogenic response triggered by carbohydrate intake (111). Targeted inhibition of ChREBP in the liver has been shown to alleviate hepatic steatosis and enhance insulin sensitivity in ob/ob mice (112). Increased DNL is likely a key factor driving lipid accumulation in MAFLD. This process not only leads to the storage of excess fat in the liver but also promotes the accumulation of toxic lipid species, such as ceramides. These ceramides can disrupt cellular function and contribute to inflammation, facilitating the transition to NASH (2).

Other critical aspects to consider in the context of hepatic steatosis are the oxidation of fatty acids and the export of hepatic lipids (2). When the liver experiences lipid overload due to a high-fat diet, it faces challenges in managing excessive lipid levels. In this scenario, ω-oxidation by cytochrome P450 enzymes becomes a critical pathway for fatty

acid metabolism. However, this process also generates considerable amounts of ROS, which can induce oxidative stress and trigger inflammatory responses (113). Peroxisome proliferator-activated receptor-α (PPARα) is a well-known regulator of fatty acid oxidation across cytochrome, mitochondria, and peroxisome systems (114, 115). Research involving mice with liver-specific knockout of PPARα revealed significant steatosis and inflammation when these mice were subjected to a high-fat diet, in contrast to their wild-type counterparts (65). This observation indicates that the lack of PPARα disrupts normal lipid metabolic pathways, resulting in heightened activation of the ω-oxidation pathway (2).

In the ER, the formation of VLDL particles occurs, facilitated by the action of microsomal triglyceride transfer protein (MTTP), which catalyzes the lipidation of apolipoprotein B100 (ApoB100) (114). VLDL particles are subsequently secreted into the bloodstream through ApoB100-mediated mechanisms. However, during episodes of increased fat intake, increased FA levels can induce ER stress, which interferes with the secretion of ApoB100 (116). This impairment in ApoB100 export disrupts normal lipid metabolism, contributing to the accumulation of lipids within the liver and promoting the development of steatosis (2). Patients with NASH exhibit reduced synthesis rates of apoB100, which contributes to the progression of advanced steatosis (117). Research has shown that hepatic steatosis can develop within a few days following the consumption of a high-fat diet, observed in both rodent studies and human cases (118, 119). Also, Skogsberg et al. mice that lack apoB100 demonstrate increased oxygen consumption and enhanced lipid oxidation. This suggests that without apoB100, the metabolic pathways

responsible for breaking down fats become more active, potentially as a compensatory response to the impaired lipid transport (120).

2.9 Genetics Contribution to MAFLD

Genetic variations in specific genes may contribute to the development of MAFLD. Genome-wide and exome-wide association studies have identified single nucleotide polymorphisms (SNPs) associated with the disease (121). Carrying the I148M variant (rs738409) in the PNPLA3 gene enhances genetic predisposition to developing hepatic steatosis (122). The I148M variant in PNPLA3 alters FA metabolism in hepatocytes, resulting in the buildup of PNPLA3 on lipid droplets (123). This accumulation occurs because the mutated protein is less efficiently degraded via the ubiquitin-proteasome pathway, compared to the wild-type form (123, 124). In an experimental mouse model of steatosis, reducing the levels of this protein effectively resolves the condition, suggesting that targeting the knockout or inhibition of the enzyme could offer a potential therapeutic strategy for hepatic steatosis (125).

The gene MBOAT7, which encodes a protein in the endoplasmic reticulum membrane, has been linked to an increased risk of MAFLD through the rs651738 C > T variant (121). This variant contributes to the development of steatosis and fibrosis, as demonstrated in a study showing that individuals carrying the variant had higher levels of liver fat, greater liver damage, and an elevated risk of fibrosis compared to those without the variant (121).

The FNDC5 gene encodes a protein that is cleaved in muscle cells to produce irisin, which is released into the bloodstream and has been linked to lower liver triglyceride levels in steatosis (121). In a recent study of 987 Caucasian patients with MAFLD,

the FNDC5 rs3480 variant was associated with more severe steatosis but had no significant impact on inflammation or fibrosis (126). Furthermore, serum irisin levels were found to be inversely correlated with the degree of hepatic steatosis (126). These results highlight the potential of FNDC5 as a therapeutic target for treating MAFLD (2). Fibroblast growth factor 21 (FGF21) is a hepatokine that plays a role in regulating metabolic balance. However, its effects on metabolism remain a topic of debate, with conflicting evidence on whether FGF21 has a truly beneficial impact on metabolic processes (127). A recent study identified that the FGF21 rs838133 variant is associated with higher serum FGF21 levels and increased hepatic inflammation in individuals with MAFLD (128). The underlying mechanism suggests that the minor allele of the rs838133 variant enhances FGF21 translation and protein stability, resulting in elevated serum FGF21 levels and promoting subsequent liver inflammation (128).

2.10 Adipokines and MAFLD

Adipose tissue is increasingly understood not just as the primary reservoir for surplus energy from food consumption, but also as an active endocrine organ that plays a key role in metabolic regulation (129). This highly adaptable organ works alongside the liver, widely regarded as the body's primary metabolic regulator to preserve homeostasis (130). Adipose tissue achieves this through the secretion of adipokines, most of which could potentially induce chronic low-grade inflammation, pleiotropic effects, and altering metabolism (131).

The discovery of leptin in 1994 was a novel advancement in obesity research, providing insights into the molecular mechanisms that regulate body weight control (130, 132). Leptin exerts a wide range of pleiotropic effects, influencing not only the regulation of

neuroendocrine function and energy balance but also contributing to processes like cognitive function, angiogenesis, and immune response (133). In whole body leptin gene knock-out (ob/ob) mice, the absence of leptin was linked to the development of severe, early-onset obesity (130). Administration of leptin normalized body weight and reversed several associated dysfunctions, including excessive food intake (hyperphagia), insulin resistance, immune deficiencies, lowered metabolic rate, and reduced body temperature (134-136).

Leptin acts as a signal of adiposity and helps to regulate adipose tissue mass to ensure survival during periods of negative energy balance that protects individuals from the risks associated with both excessive and insufficient fat accumulation (137). Studies have shown that leptin modulates appetite by inhibiting neural pathways activated by orexigenic signals, thereby reducing energy intake. Leptin also activates anorexigenic pathways, which suppress appetite and contribute to the regulation of energy intake (138, 139). More specifically, leptin alters the expression of pro-opiomelanocortin (POMC) leading to the release of α-MSH (alpha-melanocyte-stimulating hormone) (140). This neuropeptide binds to melanocortin receptors (MCRs) in neurons particularly MC4R, initiating a signaling cascade that results in the inhibition of appetite (141, 142). Additionally, leptin suppresses the synthesis of neuropeptide Y (NPY) and agouti-related peptide (AgRP) in neurons, which diminishes the antagonistic effect of AgRP on MCRs, further promoting appetite suppression (140, 141).

In hepatocytes, leptin primarily exerts its effects through the long isoform of the leptin receptor (LepRb), triggering activation of the Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3) pathway; therefore, leading to

activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and STAT5 pathways (143). At the same time, STAT3 activation induces the transcription and expression of suppressor of cytokine signaling 3 (SOCS-3), which serves as a feedback regulator, dampening LepRb signaling (143).

Leptin may work in conjunction with insulin to lower the synthesis of VLDL and may also improve insulin resistance while reducing liver fat accumulation, as observed in studies with lipodystrophic mice (144-146). Further supporting its therapeutic potential, Hackl et al. showed that leptin signaling in the brain helps prevent ectopic fat accumulation, suggesting it could be an effective strategy for addressing obesity-related liver steatosis independently of caloric intake (147).

In contrast to the protective role of leptin in reducing the risk of NAFLD, a significant percentage of NAFLD patients are obese, and evidence suggests that hyperleptinemia is closely linked to obesity, leading to the suggestion that elevated leptin levels play a role in the pathogenesis of hepatic steatosis and NAFLD (148-150). The severity of hepatic steatosis is closely linked to serum leptin levels, especially in patients with a high BMI (151). Leptin levels are higher in patients with more severe liver fibrosis, especially those with NAFLD. In contrast, lean individuals usually have lower leptin levels and less liver fibrosis and inflammation (151). The inability of elevated leptin levels to resolve hepatic steatosis is due to the development of leptin resistance (152). This resistance is driven by several mechanisms, such as the phosphorylation of Tyrosine 985 (Tyr985) in obesity receptor b (Ob-Rb) and increased expression of SOCS-3, both of which dampen leptin signaling after leptin binds to Ob-Rb in hepatic cells (153). These changes promote

cellular leptin resistance, particularly in the arcuate nucleus, and are common in obesity (153).

Adiponectin (also known as Acrp30, GBP-28, apM1, and AdipoQ) is the most abundant adipokine in plasma with physiological concentrations ranging from 5 to 30 μg/mL, primarily secreted by white adipose tissue (WAT) (154, 155). The human adiponectin protein is encoded by the *AdipoQ* gene, located on chromosome 3q27 and spanning approximately 15.8 kb (155). The full-length adiponectin protein comprises a sequence of 244 amino acids and is organized into four main regions: an N-terminal signal sequence of 18 amino acids, a variable region with 24 amino acids, a collagenous domain containing 65 amino acids, and a C-terminal globular domain of 137 amino acids (156, 157).

Following synthesis and post-translational modification, adiponectin forms three main oligomeric isoforms: a low-molecular-weight (LMW) trimer, a medium-molecular-weight (MMW) hexamer, and a high-molecular-weight (HMW) multimer (158). The LMW isoform (60 KDa) is structured as a trimer composed of three adiponectin monomers, linked through the C-terminal globular domain and collagen-like domain (159). These trimers can further assemble into MMW hexamers (150 kDa) and HMW multimers (420 kDa), which contain 12–32 monomers (159). MMW and HMW forms are the main isoforms of adiponectin, whereas LMW monomers are scarcely present and detectable only at very low concentrations in human plasma (159).

Adiponectin receptors, AdipoR1, and AdipoR2 have an intracellular NH2-terminal domain and an extracellular COOH-terminal domain, with seven transmembrane regions that set them apart from conventional G-protein-coupled receptors. Although AdipoR1

and AdipoR2 are highly homologous, sharing 67% amino acid identity, they differ in their binding affinities for full-length and globular adiponectin forms (160). In addition to AdipoR1 and AdipoR2, T-cadherin is another receptor that is abundantly expressed in the cardiovascular system. The interaction between adiponectin and T-cadherin has been shown to contribute to the reduction of atherosclerosis and provide protection to the cardiovascular system (161, 162). Research using mammalian cell models has suggested that T-cadherin acts as the main binding partner for native adiponectin in the bloodstream (162).

Adiponectin regulates glucose and lipid metabolism in hepatocytes by reducing gluconeogenesis, while simultaneously enhancing glycolysis and promoting fatty acid oxidation. In hepatocytes, the breakdown of FA is driven by both the adiponectin-AMPK axis and the PPARα signaling pathway, which works in synergy with AMPK to boost fatty acid oxidation (163). In MAFLD and NASH progression, adiponectin exerts anti-inflammatory actions by stimulating interleukin 10 (IL-10) release and downregulating NF-κβ and tumor necrosis factor-alpha (TNF-α) in the liver (164). In addition, adiponectin controls hepatic stellate cells (HSC) in migration, proliferation, and apoptosis (165). Ding et al. showed that adiponectin can inhibit liver fibrosis by reducing HSC activation and inhibiting the expression of genes that lead to fibrogenesis, such as transforming growth factor-beta 1 (TGF-β1), alpha-smooth muscle actin (α-SMA), and collagen I (165).

Despite the protective roles of adiponectin in MAFLD and hepatic steatosis, two key challenges persist that impede its development as a viable therapeutic option (166). The insolubility of the C-terminal domain and larger peptide fragments presents a significant

obstacle to the development of adiponectin-based therapies (167-169). Additionally, the varying structural isoforms of adiponectin complicate the consistency of results in both in vitro and in vivo models (160, 170, 171). Nevertheless, alternative candidates have been developed to address these previously mentioned challenges (166). AdipoRon, a non-peptide oral agonist for adiponectin receptors, has attracted interest as a potential therapeutic agent, including applications in cancer treatment (160, 172). AdipoRon features a structural arrangement with three key functional groups: a 1-benzyl, 4-substituted six-membered cyclic amine moiety, a central carbonyl group, and a terminal aromatic ring (172).

A key feature of AdipoRon is its ability to bind to both AdipoR1 and AdipoR2, potentially modulating pathways linked to each receptor (172) in several tissues. Okada-Iwabu et al. demonstrated that AdipoRon activates the AMPK and PPAR pathways to improve insulin resistance and glucose intolerance in mice fed a high-fat diet. These effects were partially preserved in AdipoR1$^{-/-}$ or AdipoR2$^{-/-}$ single knockout mice, while no effects were observed in the AdipoR1$^{-/-}$ and AdipoR2$^{-/-}$ double-knockout mice. This finding suggests that AdipoRon could be a promising therapeutic approach for obesity-related diseases, including T2D (173). Zhao et al. showed that AdipoRon treatment could be a potential therapeutic agent for supporting hepatic lipid homeostasis and mitochondrial function during the transition period and has a beneficial impact on lipid metabolism and mitochondrial dysfunction in response to the NEFA challenge (174). In a study involving three groups of mice monitored for up to 62 weeks, one group was given a normal diet (ND), another received a high-fat diet (HFD), and a third group received HFD along with AdipoRon orally for nearly a year. Results showed that AdipoRon

reduced liver steatosis and cellular ballooning, reflected in a 30% reduction in the NAS. Furthermore, AdipoRon substantially reversed intramyocellular lipid (IMCL) buildup (175). In addition, administration of AdipoRon significantly alleviated body weight loss and muscle wasting while also restoring muscle strength in both C26 tumor-bearing and ApcMin/+ mice (176). These findings suggest that AdipoRon not only improves metabolic parameters but also holds potential as a therapeutic agent for conditions associated with chronic inflammation, such as cachexia and muscle wasting (176). Another synthetic adiponectin-mimetic short peptide, ADP355, is unique because it includes non-natural amino acids. Although initially developed for cancer therapy, ADP355's ability to activate the adiponectin receptor-mediated AMPK pathway suggests its potential as an adiponectin receptor agonist (168). ADP355 significantly alleviated necroinflammation and liver fibrosis caused by thioacetamide (TAA) exposure. Treatment with ADP355 increased liver glycogen levels, reduced alkaline phosphatase activity, and serum alanine transaminase level, and promoted body weight gain by enhancing cell proliferation and reducing apoptosis (177). Additionally, ADP355 administration inhibited the activation of hepatic stellate cells and macrophages in acute liver injury and chronic fibrosis induced by TAA in a mouse model suggesting it could alleviate necroinflammation and liver fibrosis (177).

2.11 The Role of Diet and FA Levels in MAFLD

The development of MAFLD and insulin resistance is intricately linked to various types of fats, such as dietary fatty acids, TG, and cholesterol (178). Also, visceral adipose tissue (VAT) may have a role through the hepatic portal vein in fat accumulation and metabolic processes that contribute to FLD (179). Dietary fatty acids contribute

approximately 15% to TG synthesis, linking them to hepatic lipogenesis (180). Diets high in saturated fatty acids are particularly associated with increased liver fat, primarily due to enhanced lipolysis driven by inflammation within adipose tissue (181).

Yet, their precise influence on the progression or resolution/prevention of hepatic fat accumulation remains inconclusive (182). Therefore, understanding the specific types of dietary fatty acids that contribute to hepatic fat accumulation is crucial for addressing hepatic steatosis progression (183).

With the exception of dietary weight loss approaches, current research does not definitively endorse a particular dietary approach for MAFLD. It is notable that many patients with MAFLD tend to consume high-calorie diets with increased intake of trans fat, saturated fats, and cholesterol that lack essential micronutrients like fiber, green vegetables, fresh fruits, and omega-3 polyunsaturated fatty acids (n-3 PUFAs) (2, 181, 183, 184). As a result, the Mediterranean diet is often recommended for patients with hepatic steatosis which emphasizes higher consumption of vegetables, fruits, whole grains, and olive oil, along with reduced carbohydrate intake and increased levels of monounsaturated and omega-3 fatty acids (185).

Lipidomic analyses have shown a gradual reduction in hepatic PUFAs corresponding to the increasing severity of hepatic steatosis where both n-3 and n-6 PUFAs were reduced in hepatic biopsies from patients with NAFLD (186, 187). Evidence indicates that intake of unsaturated fatty acids may reduce lipolysis and limit hepatic fat accumulation (188). Essential PUFAs cannot be synthesized in the body and must be obtained through dietary sources (189). In North America, the Adequate Intake (AI) levels for linoleic acid (LA, C18:2n6), have been established based on typical daily consumption, as there was

insufficient evidence to determine an estimated average requirement (EAR) or recommended dietary allowance (RDA) (190). At present, AIs for LA are 17 and 12 grams per day for men and women respectively, accounting for roughly 6% of the total daily caloric intake (191, 192).

Research has shown dietary LA is the predominant source of the body's n-6 PUFA reservoir, with arachidonic acid (ARA, C20:4n-6) being the second major contributor (189). While circulating LA in compartments such as plasma phospholipids and erythrocytes is primarily influenced by dietary intake, the composition of other fatty acids in these compartments is shaped by a combination of diet, de novo lipogenesis (DNL), and factors such as sex and age (193, 194).

Dietary LA has a negative association with visceral fat, liver fat, total body fat, insulin resistance, and inflammatory markers (195, 196). Supplementation with n-3 PUFAs particularly (α-LA, C18:3n-3), along with a higher ratio of monounsaturated fat relative to total dietary fat, has been shown to effectively improve hepatic enzyme levels, reduce steatosis scores, and decrease hepatic fat accumulation in numerous meta-analyses and controlled trials (188, 197-202), suggesting that PUFAs intake may alleviate hepatic steatosis (198, 199). In addition, n-3 PUFAs serve as precursors to several lipid mediators with anti-inflammatory properties, including resolvins, protectins, and eicosanoids (203). In a longitudinal study participants with elevated serum levels of LA had significantly reduced Fatty Liver Index (FLI) and decreased likelihood of hepatic steatosis. Also, in cross sectional analyses n-3 PUFAs showed an inverse association with hepatic steatosis (204).

PUFAs and their derived eicosanoids can bind to transcription factors, triggering shifts in gene expression (205). Studies shown that PUFAs can suppress SREBP1c and increase the activity of PPAR*α,* thereby reducing lipogenesis while increasing fatty acid oxidation (206, 207). A recent study on alcohol-induced steatohepatitis found that n-3 PUFAs helped protect against hepatic steatosis by interacting with GRP120 (FFA4), identified as a receptor for n-3 PUFAs (206).Specifically, the anti-inflammatory effects of n-3 PUFAs were linked to the FFA4/α-arrestin pathway, which inhibited the activation of TGF-α activated kinase 1 in macrophages (208, 209).

2.12 Management of MAFLD

Although several pharmaceutical compounds are currently under investigation for the effective treatment of MAFLD, none have been specifically approved for this purpose. Therefore, identifying new management strategies for MAFLD remains essential to prevent disease progression to more advanced stages and to ultimately reverse the severity of MAFLD (2, 210). An effective approach to managing MAFLD should encompass various elements, such as lifestyle modifications, weight loss, and increased physical activity, all of which could contribute to reducing hepatic steatosis, minimizing liver damage, alleviating metabolic complications linked to the disease, and addressing associated cardiovascular risks (210).

Lifestyle intervention programs and weight reduction can lead to decreases in liver fat, resolution of steatohepatitis and fibrosis, and improvements in quality of life, with outcomes improving proportionally to the extent of weight loss (211). In studies, a weight loss of 7% has been associated with significant histological improvements in NAFLD /NASH, while a 10% decrease in body weight results in a 45% reduction in liver fat

content (212-214). A meta-analysis showed that a 7-10% reduction in weight through caloric restriction can lead to significant improvements in liver steatosis. This is evidenced by reductions in liver enzyme levels and improvements in histological markers of steatosis and inflammation, although its effect on fibrosis remains less conclusive (2, 215). According to the Asian Pacific Association for the Study of the Liver (APASL) guideline recommendations, weight loss can be advantageous for both overweight/obese and nonobese individuals with MAFLD. For overweight or obese individuals, targeting a weight reduction of 7%–10% through lifestyle changes is often recommended. Integrating diet and exercise strategies proves to be more effective in normalizing liver enzyme levels, reducing hepatic steatosis, and improving histology compared to either approach alone (211).

Unfortunately, sustaining long-term behavioral changes to maintain weight loss demands continuous effort. Even the most effective short-term interventions are unlikely to produce lasting positive results without ongoing support to sustain reduced body weight (216). Maintaining weight loss over the long term is much more difficult, with weight regain being common (217, 218). A meta-analysis of 29 long-term weight loss US studies found that over half of the lost weight was typically regained within two years, and by the five-year mark, more than 80% of the lost weight was usually recovered (219).

As previously mentioned, insulin resistance is implicated in the development of MAFLD, prompting the use of insulin-sensitizing agents as a potential therapeutic approach for individuals with MAFLD (2, 220). Pioglitazone is recommended for patients with confirmed metabolic-associated steatohepatitis, whereas a recent meta-analysis indicated that metformin can significantly improve body composition and liver function even in

non-diabetic MAFLD patients (220). Bugianesi et al. found that metformin had a more pronounced effect on improving AST levels in patients with NAFLD compared to a treatment group receiving vitamin E (221). Metformin could exert its effect in the liver primarily by activating AMPK, which inhibits ACC. This leads to a reduction in malonyl-CoA levels and consequently enhances fatty acid oxidation in the mitochondria (222-224).

Moreover, emerging anti-diabetic therapies, including sodium-glucose cotransporter 2 (SGLT2) inhibitors and glucagon-like peptide-1 receptor agonists (GLP-1RAs), are being explored for their ability to reduce liver fat accumulation and prevent the advancement of severe fibrosis (220, 225). SGLT2 inhibitors reduce the renal reabsorption of glucose, thereby improving glycemic control. Research has shown that these inhibitors positively affect MAFLD by improving liver enzyme levels, such as AST and ALT, and reducing hepatic fat accumulation and bodyweight (214, 226, 227). Empagliflozin, a SGLT2 inhibitor, has demonstrated effectiveness in reducing hepatic fat accumulation, improving liver steatosis and fibrosis, and lowering liver enzymes like AST and ALT in MAFLD patients (228-230). In a NASH mouse model, it was observed to reduce the expression of key inflammatory markers such as TNF-α, IL-6, and monocyte chemoattractant protein-1 (MCP-1) (231).

GLP-1RAs are typically prescribed for diabetes management, act by mimicking the incretin hormone GLP-1, which is naturally released from the L cells of the small intestine post-meal (227). GLP-1 stimulates insulin secretion, inhibits glucagon production, and slows gastric emptying, helping to regulate blood glucose levels (232). It has been suggested that GLP-1RAs offer benefits beyond weight loss and blood sugar

control (233). Preclinical studies have shown that GLP-1 analogs can mitigate NAFLD by suppressing the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome through the enhancement of autophagy and mitophagy pathways, reducing macrophage recruitment and activation, and boosting antioxidant defenses in the liver (234).

Additionally, GLP-1RAs improve insulin sensitivity by decreasing c-Jun N-terminal kinase (JNK) phosphorylation and enhancing the expression and activity of PPARγ (235). Budd et al. demonstrated that GLP-1RAs hold promise in reducing major cardiovascular risks and improving liver histology in patients with NAFLD (236). In animal studies, treatment with GLP-1RAs has been found to reduce liver inflammation and could potentially hinder hepatic steatosis progression (237, 238). Also, in patients with diabetes with increased liver enzymes and hepatic steatosis, GLP-1RAs enhanced liver and lipid metabolism (239). In conclusion, while SGLT2, GLP-1RAs, and metformin show potential to manage hepatic steatosis, additional research is needed to establish their long-term efficacy in managing MAFLD in both diabetic and non-diabetic individuals (240, 241).

Chapter 3. Study Design and Methodology

This study is a secondary analysis of a large randomized, placebo-controlled clinical trial aimed at evaluating changes in hepatic lipid accumulation, visceral adipose tissue, postprandial lipid profiles, inflammatory markers, and energy metabolism. This cross-sectional study was conducted in accordance with protocols approved by The Ohio State University Institutional Review Board. Data were collected from participants during their first clinical visit, following consent, and before any interventions took place.

3.1 Inclusion and Exclusion Criteria

The inclusion criteria for participants were as follows:

BMI Range: Participants had a BMI between 20 and 55 kg/m². The rationale behind setting the BMI range is to include individuals with varying degrees of adiposity, as a higher BMI increases the risk of cardiometabolic diseases and NAFLD. Additionally, the upper BMI limit was established to ensure that the anthropometric measurements could be performed accurately, as individuals with a BMI above 55 kg/m² may pose challenges in measurement precision. Other criteria include: potential hepatic steatosis, assessed through a review of medical records and/or self-reported data from the screening questionnaire, and patients aged 22-80 years.

Participants were excluded if they met any of the following criteria: unstable management of heart failure or recent cardiovascular events (e.g., stroke or heart attack) within the past 3 months, planned heart surgeries or procedures, use of pacemakers or defibrillators, severe kidney failure (GFR <55), liver cirrhosis, autoimmune hepatitis, chronic hepatitis, alpha-1 antitrypsin deficiency, cystic fibrosis, tuberculosis, pneumonia, emphysema, severe or uncontrolled pulmonary diseases (e.g., COPD, chronic bronchitis,

asthma), severe or uncontrolled circulatory diseases (excluding hypo- or hypertension), autoimmune diseases (excluding rheumatoid arthritis, psoriasis, and lupus), current or recent cancer treatment (excluding non-melanoma skin cancer), type 1 diabetes, recent use of Vitamin E supplements, Actos, or Glucagon-like Peptide-1 medications within 1 month prior to enrollment, gastrointestinal disorders, hyperthyroidism, dietary restrictions incompatible with study foods, current use of weight loss supplements or medications, recent use of high linoleic acid supplements, pregnancy or lactation, alcohol or drug abuse, and presence of metal implants or metallic foreign objects in the body. After the screening process, 74 patients aged 22 to 80 years with a BMI ranging from 20 to 55 kg/m^2 were recruited from the Columbus, OH region, with the recruitment process designed to ensure representation of the region's diversity. One participant identified as non-binary, and their data were included in the analysis based on their sex at birth.

3.2 Body Composition and Measurement of MAFLD

Lean mass, appendicular lean mass, total adipose mass, and trunk adipose mass were evaluated using dual-energy X-ray absorptiometry (DEXA) (242) with the Lunar iDEXA system, which includes the CoreScan program for assessing visceral adiposity (Lunar Corp, Madison, WI). Previous research has reported coefficients of variation for lean mass and trunk adipose mass in obese adults as 0.37% and 1.8% CV, respectively, using this DEXA instrument (243, 244).

3.3 Magnetic Resonance Imaging (MRI) with Proton Density Fat Fraction (PDFF)

To quantify liver fat and visceral adipose tissue, PDFF MRI was employed. This technique used a multi-echo VIBE Dixon sequence with a 3-Tesla MRI scanner (Prisma, Siemens, Erlangen, Germany) (245, 246) The following parameters were used: 6 echo

times (TEs) of 1.05, 2.46, 3.69, 4.92, 6.15, and 7.38 ms; a repetition time (TR) of 9.15 ms; slice thickness of 3.5 mm; a flip angle of 5° to minimize the T1 effect; a matrix size of 160×95; and a field-of-view (FOV) of 420×315 mm. The bandwidth was set at 1,040 Hz/Px, and a parallel acceleration technique with an acceleration factor of 2 was applied. A Levenberg-Marquardt nonlinear fitting was applied to fit the magnitude of the complex signal from the multi-echo data. In-line reconstruction was carried out by correcting for confounding factors such as field inhomogeneity, eddy currents, T1 bias, T2* decay, and spectral complexity, enabling the generation of a fat fraction map through automatic pixel-by-pixel fitting. Multiple regions of interest (ROIs) were drawn in the liver parenchyma to calculate the mean fat fraction, and visceral adipose tissue was quantified. For visceral adipose tissue, axial slices from T12 to L4 were scanned using the same multi-echo VIBE Dixon method (247).

Reconstruction included corrections for field inhomogeneity, eddy currents, T1 bias, T2 decay, and spectral complexity, and mean fat fractions were calculated from multiple ROIs in the liver and visceral adipose tissue (248, 249). Liver fibrosis was measured using MR elastography (MRE), a noninvasive technique that quantifies liver stiffness through shear wave propagation. The vibration produced by an MRE driver on any tissue in the human body is below the EU guidelines that a human body can experience. The stiffness values were expressed in kilopascals (kPa), and recommended thresholds were used for staging liver fibrosis. Specifically, 3.0 kPa as the cutoff for detecting F1 fibrosis or higher, 3.5 kPa for F2 or higher, 4.0 kPa for F3 or higher, and 5.0 kPa for F4, indicating the presence of cirrhosis (250). All scans were evaluated under blinded conditions by the same technician.

3.4 Blood Collection and Processing

Fasting whole blood was collected in Cell Preparation Tubes with Sodium Citrate (BD Vacutainer® CPT™, Franklin Lakes, NJ) for peripheral blood mononuclear cells (PBMC) at baseline. According to the manufacturer's instructions, a blank tube was first used to fill the butterfly line with blood, ensuring the collection of 8ml of whole blood (with no air) and a proper blood-to-media ratio for PBMC sample preparation. The blank tube was discarded. Fasting whole blood was then collected from one lithium heparin tube, one 10ml and one 6ml serum separation tube, and one 10ml and one 3ml EDTA tube (BD Vacutainer® EDTA™, Franklin Lakes, NJ). The total amount of fasting blood collected at baseline was approximately 66ml (about 4 tablespoons), while the total amount of postprandial blood collected was 54ml (about 3.5 tablespoons), for a total of about 126ml. For all remaining study visits, approximately 25ml (about 1.6 tablespoons) was collected. Immediately after collection, the CPT tubes were inverted 8-10 times and stored upright at room temperature until centrifugation at 2700 rpm for 30 minutes at room temperature.

The mononuclear layer was removed and washed with 1x PBS CMF, followed by centrifugation at 1700 rpm to remove contaminants. The washing steps were repeated, and the final cell pellet was either stored at -80°C for later analysis. The serum separator tubes were inverted 8-10 times and incubated for 30-60 minutes at room temperature to allow clotting, while the 10ml EDTA tube was inverted 8-10 times and stored upright on ice. Both the serum separator and EDTA tubes were centrifuged at 1932g for 10 minutes at 4°C. For the EDTA tubes, after the plasma layer was removed, the buffy coat layer was discarded. The plasma aliquots were stored at -80°C until analysis. All blood samples

were processed within one to two hours of collection. The 3ml EDTA and all lithium heparin tubes were transported to the OSUWMC lab for analysis. If participants consented to the data and specimen repository study (2015H0294), any unused samples and all collected data were stored for the repository study. Both plasma and RBC fatty acid compositions have been linked to dietary fat intake (251).

One aliquot of plasma, PBMC, and RBC samples was thawed on ice. Total lipids were extracted from the plasma using a 2:1 (v/v) chloroform: methanol solution, followed by washing with 0.88% KCl (252). Fatty acid methyl esters (FAMEs) were prepared by adding 5% hydrochloric acid in methanol (253) and heating at 76°C. For RBCs, fatty acids were extracted and methylated using boron trifluoride, with samples heated at 100°C (254, 255). Fatty acid methyl esters from all samples were analyzed via gas chromatography on a 30-meter Omegawax™ 320 fused silica capillary column (Supelco, Bellefonte, PA). The oven temperature began at 175°C and was increased by 3°C per minute until reaching 220°C. Retention times of the samples were compared to standards for fatty acid methyl esters (Matreya, LLC, Pleasant Gap, PA, and Nu-Check Prep Inc., Elysian, MN) (256).

3.5 Data Analysis

Statistical analyses were conducted using Stata version 17 (Stata Press, College Station, TX), GraphPad Prism 9, and Python. Python, along with libraries such as pandas, numpy, seaborn, and matplotlib, was utilized to create heatmaps for visualizing correlations between variables. Descriptive statistics for baseline characteristics were summarized as mean (standard deviation) or median (interquartile range) for continuous variables and as frequency (percentage) for categorical variables.

Differences between groups were evaluated using chi-square tests for categorical data, and either t-tests or Mann–Whitney U tests for continuous data, depending on data distribution.Univariate and multivariate linear regression analyses were performed to examine associations between liver fat fraction, liver stiffness, and plasma fatty acids with adiponectin levels, presenting β regression coefficients and 95% confidence intervals. Multinomial logistic regression models were used to investigate the relationship between adiponectin levels with the presence of diabetes and significant liver fibrosis. These models provided adjusted odds ratios and corresponding confidence intervals to account for potential confounding variables. The predictive performance of two models created in Stata was assessed using the area under the curve (AUC) of their receiver operating characteristic (ROC) curves. The AUC values provided a measure of the model's ability to distinguish between outcomes, with higher AUC values indicating better discriminative performance. A p-value of less than 0.05 was considered statistically significant throughout the analysis. To identify relevant covariates, we first assessed the impact of each covariate on the regression model by observing the change in the regression coefficients. Covariates that caused a change of more than 10% in the coefficient were considered for inclusion in the final models. Among these, only the covariates that produced the most significant changes to the model's coefficients were retained in the final models. Participants with missing data for any of the variables included in the analysis were excluded from the statistical models. Specifically, cases with missing values for the primary outcomes or covariates were not considered in the analysis. This approach was chosen to ensure the validity of the results and avoid biases that could arise from imputing missing data.

Chapter 4. Results

4.1 Baseline Characteristics of the Participants

Data from 74 participants aged 22 to 80 years with a BMI between 20-55 kg/m² were included in the analysis. Among the participants, 43 (58%) were female, and 31 (42%) were male (Table 2). Of these, 14 females (32.5%) and 13 males (42%) had T2DM (Table 2). The median BMI for both groups was categorized within the obesity category, with a median (quartile interval) of 35.59 (31.76–39.20) kg/m². Males had higher total tissue (kg) (p = 0.020), total lean (kg) (p = <0.001), appendicular lean mass (kg) (p = <0.001), visceral fat (kg) (p = 0.008), appendicular lean mass/BMI (p = <0.001), and appendicular lean mass/ height (kg/m2) (p = <0.001) compared to the female participants. No significant differences were observed between male and female participants in terms of WC (cm) (p = 0.056), BMI (kg/m2) (p = 0.453), total adipose tissue (kg) (p = 0.111) and trunk fat (kg) (p = 0.823).

The median (quartile interval) fasting glucose and insulin levels were 99.66 (86.62-112.92) mg/dL and 18.46 (12.82-23.77) uIU/mL, respectively. HOMA-IR was slightly higher in males, with a median (quartile interval) of 4.66 (3.34-6.54) compared to 4.06 (2.9-5.97) in females, though the difference was not statistically significant (p = 0.296).

Table 2. Anthropometric characteristics of the participants stratified by sex

Variable	Male	Female	Total	*P*-value
Diabetes (n, %)[1]	13/31 (42)	14/43 (32.5)	27/74 (36.5)	0.408
Age (years)	50 (36-56)	54 (49-63)	53 (43-62)	**0.047**
Weight (kg)	103.9 (91.75-119.55)	95.15 (84.15 -101.75)	97.2 (85.75-112.7)	**0.009**
BMI (kg/m^2)	35.31 (29.78-39.04)	36.21 (33.39-39.27)	35.59 (31.76-39.20)	0.453
WC (cm)	116.1 (104.7- 131.57)	111.09 (11.87)	114.19 (15.90)	0.056
Total tissue (kg)	99.50 (88.17- 115.72)	92.62 (82.13-98.13)	93.42 (83.92-107.94)	**0.020**
Total lean (kg)	61.12 (54.12-69.67)	43.40 (40.48-49.80)	50.87 (42.81-60.80)	**<0.001**
ALM (kg)	29.37 (26.09-35.02)	19.53 (17.92-22.78)	23.69 (18.96-28.59)	**<0.001**
Total adipose (kg)	40.62 (29.52- 50.98)	46.28 (39.3-52.78)	43.64 (35.89-50.98)	0.111
Visceral fat (kg)	2.62 (1.68-3.54)	1.90 (1.40 – 2.30)	2.14 (1.47-2.79)	**0.008**
Trunk fat (kg)	24.82 (17.61-33.71)	25.66 (20.58 – 30.18)	25.51 (20.48-31.23)	0.823
ALM/BMI	0.88 (0.79-0.92)	0.56 (0.5-0.62)	0.66 (0.55-0.87)	**<0.001**
ALM/ height (kg/m^2)	9.73 (8.66-11.02)	7.81 (6.93-8.38)	8.44 (7.46-9.73)	**<0.001**

[1] number and percentage of individuals with diabetes in each group
Values expressed as number (%), mean (SD), or median (quartile interval).
Statistical significance is defined as a *p*-value < 0.0
BMI, body mass index; ALM, appendicular lean mass

Visceral fat and hepatic characteristics, as measured by MRI-PDFF, are presented in Table 3. Fatty liver is defined as a liver fat fraction of 5% or greater (257). Out of 74 participants, 61 (82%) had a liver fat fraction greater than 5%. The average liver fat fraction for both groups fell within the category of hepatic steatosis, with a value of 16.29 (7.80) %. The median (quartile interval) values for liver stiffness measurement (LSM), iron content, and visceral adipose volumes were 2.25 (2.01-2.69) KPa, 18.19 (16.08-20.92) μmol Fe/g, and 428,154 (317,021-571,932) mm^3, respectively. There were no significant differences in MRI PDFF measurements between males and females.

The clinical and biochemical characteristics of participants are presented in Table 3. Regarding liver enzymes, ALT was significantly higher in males, with a median (quartile interval) of 49 (27-71) IU/L compared to 28 (19-43) IU/L in females ($p = 0.013$). Among adipokines, leptin levels were higher in females, with a median (quartile interval) of 0.039 (0.029-0.039) compared to 0.017 (0.01-0.03) in males ($p < 0.001$). Similarly, both HMW and total adiponectin were higher in females, with a median (quartile interval) of 1.76 (1.19-2.49) μg/mL compared to 0.90 (0.64-1.14) μg/mL and 4.16 (3.15-5.74) μg/mL compared to 2.45 (1.91-3.45) μg/mL, respectively ($p < 0.001$).

TG levels were higher in males, with a median (quartile interval) of 194 (156-237) mg/dL compared to 140 (86-193) mg/dL in females ($p = 0.021$). However, HDL-C was higher in females ($p < 0.001$). No significant differences were observed in the following variables: HSI ($p = 0.520$), AST (IU/L) ($p = 0.107$), GGT (IU/L) ($p = 0.558$), sICAM-1 (ng/mL) ($p = 0.402$), total cholesterol (mg/dL) ($p = 0.078$), LDL-C (mg/dL) ($p = 0.074$), sCD-14 (μg/mL) ($p = 0.215$), OxLDL (U/L) ($p = 0.160$), TNFR2 (pg/mL) ($p = 0.269$), saliva

cortisol (µg/mL) (p = 0.560), IL-6 (pg/mL) (p = 0.370), TNF-α (pg/mL) (p = 0.260), and CRP (mg/dL) (p = 0.097).

The differences in plasma fatty acid composition are presented in Table 4. Males had a higher plasma composition of oleic acid (C18:1, n-9), with a mean (SD) of 22.79 (2.25) %, compared to 20.25 (2.56) % in females ($p < 0.001$). Similarly, α-linolenic acid (α-LA, C18:3, n-3) was significantly higher in males, with a median (quartile interval) of 0.76% (0.66-0.88), compared to 0.56% (0.48-0.71) in females ($p < 0.001$). In contrast, females had higher plasma percentages of dihomo-γ-linolenic acid (DHLA, C20:3, n-6) at 1.66% (1.35-1.86) compared to 1.31% (1.15-1.66) in males (p = 0.012), arachidonic acid (AA, C20:4, n-6) at 7.46% (6.21-9.51) compared to 6.53% (5.39-7.90) in males (p = 0.009), and docosapentaenoic acid (DPA, C22:5, n-6) at 0.15% (0.12-0.21) compared to 0.12% (0.09-0.17) in males (p = 0.026).

Table 3 Clinical, biochemical characteristics of participants

Variable	Male	Female	Total	P-value
Fasting glucose (mg/dL)	101.12 (86.62- 126.26)	98.23 (85.65- 109.63)	99.66 (86.62-112.92)	0.297
Insulin (uIU/mL)	19.17 (14.03-25.65)	16.77 (11.62- 23.02)	18.46 (12.82- 23.77)	0.359
HOMA-IR	4.66 (3.34-6.54)	4.06 (2.9- 5.97)	4.37 (3.22-6.42)	0.296
Liver fat fraction (%)	17.58 (1.28)	15.53 (8.53)	16.29 (7.80)	0.308
Liver stiffness (KPa)	2.46 (2.11-3.076)	2.13 (2-2.5)	2.25 (2.01-2.69)	0.071
Liver iron content (μmol/g)	19.65 (16.66- 21.32)	17.28 (15.53-20.45)	18.19 (16.08-20.92)	0.063
Visceral adipose (mm3)	487116 (365765-680923)	401094 (304322-513530)	428154 (317021-571932)	0.165
HSI	49.27 (9.34)	48.6 (6.14)	48.6 (7.69)	0.520
ALT (IU/L)	49 (27-71)	28 (19-43)	31.5 (21-56)	**0.013**
AST (IU/L)	27 (21- 42)	23 (19-31)	23 (20-38)	0.107
GGT (IU/L)	29 (25-50)	30 (22-52)	29.5 (22-50)	0.558
Leptin (μg/mL)	0.017 (0.01 - 0.03)	0.039 (0.029- 0.039)	0.03 (0.02-0.05)	**<0.001**
sICAM-1 (ng/mL)	225.37 (199.14-272.59)	236.5 (188.38- 301.37)	231.56 (189.10-281.41)	0.402
Cholesterol (mg/dL)	149 (113-187)	170 (140 - 195)	166 (131-192)	0.078
TG (mg/dL)	194 (156-237)	140 (86 -193)	173 (113-223)	**0.021**
LDL-C (mg/dL)	77.87 (36.43)	93.28 (34.37)	86.46 (35.87)	0.074
HDL-C (mg/dL)	36 (30- 43)	47 (41-59)	42.5 (35-49)	**<0.001**
sCD-14 (μg/mL)	1.4 (1.22- 1.53)	1.47 (1.26- 1.62)	1.46 (1.24-1.54)	0.215
OxLDL (U/L)	68.19 (22.74)	77.09 (27.66)	73.35 (25.90)	0.160
Total adiponectin (μg/mL)	2.45 (1.91-3.45)	4.16 (3.15 – 5.74)	3.47 (2.38- 4.74)	**<0.001**
HMW adiponectin (μg/mL)	0.90 (0.64-1.14)	1.76 (1.19-2.49)	1.26 (0.77- 2.01)	**<0.001**
TNFR2 (pg/mL)	10673 (8448- 15139)	12052 (9685-14545)	11546 (9232-15140)	0.269
Saliva cortisol (μg/mL)	0.17 (0.11-0.24)	0.15 (0.01-0.24)	0.16 (0.103-0.24)	0.560
IL-6 (pg/mL)	2.20 (1.5 - 3.28)	2.56 (1.89- 2.81)	2.26 (1.72-3.048)	0.370
TNF-α (pg/mL)	0.85 (0.79-1.02)	0.92 (0.79-1.15)	0.88 (0.79-1.08)	0.260
CRP (mg/dL)	0.35 (0.09 - 0.62)	0.47 (0.29-0.89)	0.44 (0.15-0.8)	0.097

Values expressed as mean (SD), or median (quartile interval).
Statistical significance is defined as a *p*-value < 0.05.
HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; HSI, Hepatic Steatosis Index; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; GGT, Gamma-Glutamyl Transferase; sICAM-1, Soluble Intercellular Adhesion Molecule 1; TG, Triglycerides; LDL-C, Low-Density Lipoprotein Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; OxLDL, Oxidized Low-Density Lipoprotein; HMW adiponectin, High Molecular Weight Adiponectin

Table 4 Plasma fatty acid composition

Variable	Male	Female	Total	*P*-value
Palmitic acid (C16:0)	22.71 (21.58- 23.82)	22.23 (20.65- 22.91)	22.38 (21.24- 23.30)	0.079
Oleic acid (C18:1, n-9)	22.79 (2.25)	20.25 (2.56)	21.36 (2.72)	**<0.001**
LA (C18:2, n-6)	28.05 (3.63)	28.26 (4.37)	28.17 (4.04)	0.830
α-LA (C18:3, n-3)	0.76 (0.66-0.88)	0.56 (0.48-0.71)	0.65 (0.51-0.81)	**<0.001**
GLA (C18:3, n-6)	0.44 (0.3-0.49)	0.49 (0.38- 0.58)	0.47 (0.35-0.55)	0.057
DHLA (C20:3, n-6)	1.31 (1.15-1.66)	1.66 (1.35-1.86)	1.52 (1.23-1.82)	**0.012**
AA (C20:4, n-6)	6.53 (5.39 -7.90)	7.46 (6.21-9.51)	6.94 (5.93-8.34)	**0.009**
DTA (C22:4, n-6)	0.18 (0.01)	0.19 (0.05)	0.18 (0.05)	0.462
EPA (C20:5, n-3)	0.39 (0.27- 0.5)	0.42 (0.36-0.61)	0.42 (0.32-0.58)	0.179
DPA (C22:5, n-6)	0.12 (0.94- 0.17)	0.15 (0.12-0.21)	0.14 (0.11-0.18)	**0.026**
DHA (C22:6n-3)	1.25 (1.09- 1.44)	1.19 (0.95-1.48)	1.23 (1.05-1.46)	0.467
EPA-DPA-DHA	2.19 (1.77- 2.5)	2.04 (1.84-2.42)	2.09 (1.81-2.45)	0.785

Expressed as the mean (SD) or median (interquartile range), the values represent the percentage of total identified fatty acids, calculated based on the area of the peaks. Statistical significance is defined as a p-value < 0.05.
LA, Linoleic acid; α-LA, α-linoleic acid; GLA, γ-linolenic Acid; DHLA, Dihomo-γ-linolenic acid; AA, Arachidonic acid; DTA, Docosatetraenoic acid; EPA, Eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, docosahexaenoic acid

4.2 Relationship between HMW and Total Adiponectin with Liver Fat Fraction

The first objective of this study was to examine the associations between HMW and total adiponectin with hepatic fat fraction and liver stiffness using multivariate linear regression in the entire study population. Plasma HMW adiponectin was a significant inverse predictor of liver fat fraction in the crude model (Table 5; $p = 0.022$). However, this association became non-significant after adjusting for age and sex (Table 4; $p = 0.139$). In the fully adjusted model (Model 2), which accounted for potential confounding variables, every 1 µg/mL increase in plasma HMW adiponectin was associated with a 2.83% decrease in hepatic fat fraction (Table 5; $p = 0.020$). In contrast, plasma total adiponectin showed no significant association with hepatic fat fraction in the crude model or in adjusted models 1 and 2 (Table 5; $p = 0.358$, $p = 0.903$, and $p = 0.612$, respectively).

Table 5 Relationship between plasma adiponectin levels and liver fat

Predictor	Coeff	Std Err	95% CI	*P*-value
Unadjusted Model				
Total Adiponectin (µg/mL)	-0.449	0.485	(-1.421, 0.522)	0.358
Adjusted Model 1				
Total Adiponectin (µg/mL)	-0.065	0 .536	(-1.009, 1.141)	0.903
Adjusted Model 2				
Total Adiponectin (µg/mL)	-0.308	0.603	(-1.521, 0.904)	0.612
Unadjusted Model				
HMW adiponectin (µg/mL)	-2.398	1.023	(-4.446, -0.351)	**0.022**

Continued

Adjusted Model 1				
HMW adiponectin (µg/mL)	-1.709	1.139	(-3.991, 0.573)	0.139
Adjusted Model 2				
HMW adiponectin (µg/mL)	-2.830	1.173	(-5.188, -0.472)	**0.020**

Model 1 is adjusted for age and sex.
Model 2 is adjusted for age, sex, Homa-IR, TG, AST, ALT, GGT, Appendicular lean Mass/BMI, and Race.
Statistical significance is defined as a *p*-value < 0.05.

4.3 Relationship between HMW and Total Adiponectin with Liver Stiffness

Concerning liver stiffness, plasma total adiponectin exhibited a negative relationship only in the crude model, where each 1 µg/mL increase was associated with a 0.086 kPa reduction in liver stiffness (Table 6; $p = 0.023$). However, no significant relationship was observed between plasma HMW adiponectin and liver stiffness in the models.

Table 6 Relationship between plasma adiponectin levels and liver stiffness

Predictor	Coeff	Std Err	95% CI	*P*-value
Unadjusted Model				
Total Adiponectin (µg/mL)	-0.086	0.037	(-0.161, -0.012)	**0.023**
Adjusted Model 1				
Total Adiponectin (µg/mL)	-0.084	0.042	(-0.169, -0.001)	0.050
Adjusted Model 2				
Total Adiponectin (µg/mL)	-0.060	0.045	(-0.153, 0.031)	0.190
Unadjusted Model				
HMW adiponectin (µg/mL)	-0.151	1.023	(-0.317, 0.014)	0.072
Adjusted Model 1				
HMW adiponectin (µg/mL)	-1.709	1.139	(-3.991, 0.573)	0.139
Adjusted Model 2				
HMW adiponectin (µg/mL)	-0.001	0.095	(-0.193, 0.191)	0.990

Model 1 is adjusted for age and sex.
Model 2 is adjusted for age, sex, Homa-IR, TG, AST, ALT, GGT, Appendicular lean Mass/BMI, and Race.
Statistical significance is defined as a *p*-value < 0.05.

4.4 Relationship between Adiponectin with the Presence of T2DM and Liver Fibrosis

The second objective of this study was to examine the relationship between HMW and total adiponectin levels and the presence of T2DM and liver fibrosis in patients with MAFLD. To achieve this, I performed multinomial logistic regression, using patients with MAFLD without T2DM (diagnosed with hepatic steatosis and metabolic risk factors according to MAFLD criteria) (49) and without liver fibrosis (determined by LSM measured via MRE) as the reference group for the analysis (n = 37). In the unadjusted regression model, plasma total adiponectin in patients with MAFLD was not significantly associated with the presence of T2DM alone (p = 0.238). However, 1 µg/mL increase in plasma total adiponectin was significantly associated with a 77.7% reduction in the odds of having both T2DM and fibrosis concurrently in patients with MAFLD (Table 7; OR: 0.223, 95% CI: 0.065-0.762, p = 0.017). Similarly, after adjusting for age and sex, total adiponectin did not show a significant association with the presence of T2DM alone. However, 1 µg/mL increase in plasma total adiponectin was significantly associated with 81.5% reduction in the odds of both T2DM and fibrosis concurrently in patients with MAFLD (Table 7; OR: 0.185, 95% CI: 0.040-0.877, p = 0.033).

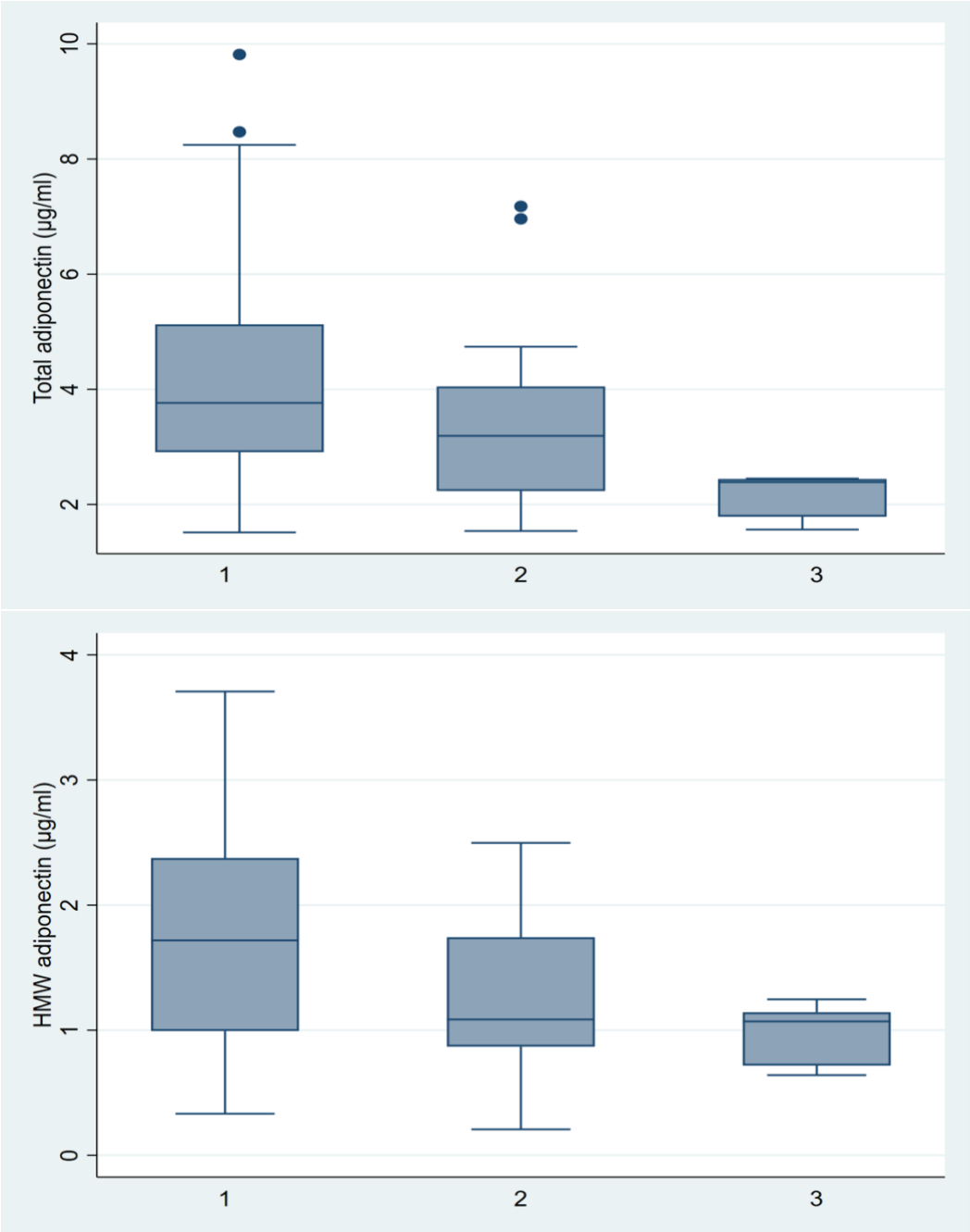
In addition, in the unadjusted model, plasma HMW adiponectin was not significantly associated with either the presence of T2DM alone or the coexistence of T2DM and fibrosis (Table 7; OR: 0.490, p = 0.116 for T2DM; OR: 0.254, p = 0.054). After adjusting for sex and age, higher plasma HMW adiponectin levels were associated with a 72.4% reduction in the odds of T2DM alone (Table 7; OR: 0.276, 95% CI: 0.089-0.856, p = 0.026). However, no significant relationship was observed between HMW adiponectin

levels and the coexistence of T2DM and fibrosis (Table 7; OR: 0.328, 95% CI: 0.054-1.968, $p = 0.222$). Figure 1 shows the total and HMW adiponectin levels by groups used in multinomial logistic regression.

Table 7 Relationship between adiponectin and the presence of T2DM or its coexistence with liver fibrosis

	Reference [1]	Patients with hepatic steatosis and T2DM			Patients with hepatic steatosis, T2DM and fibrosis		
		OR	(95% CI)	*P*-value	OR	(95% CI)	*P*-value
Unadjusted model							
Total Adiponectin (μg/mL)	*Ref*	0.791	(0.535, 1.168)	0.238	0.223	(0.065, 0.762)	**0.017**
Adjusted model 1							
Total Adiponectin (μg/mL)	*Ref*	0.622	(0.384, 1.010)	0.055	0.185	(0.040, 0.877)	**0.033**
sex	*Ref*	3.254	(-.458, 2.819)	0.158	0.273	(0.023, 3.228)	0.303
Age (years)	*Ref*	1.039	(0.632, 16.743)	0.171	1.053	(0.978, 1.135)	0.164
Unadjusted model							
HMW adiponectin (μg/mL)	*Ref*	0.490	(0.201, 1.193)	0.116	0.254	(0.063, 1.026)	0.054
Adjusted model 1							
HMW adiponectin (μg/mL)	*Ref*	0.276	(0.089, 0.856)	**0.026**	0.328	(0.054, 1.968)	0.222
Sex	*Ref*	3.737	(0.688, 20.192)	0.127	0.170	(0.014, 2.038)	0.162
Age (years)	*Ref*	1.044	(0.985, 1.106)	0.143	1.046	(0.976, 1.123)	0.206

[1]Patients with MAFLD without T2DM and liver fibrosis
Statistical significance is defined as a *p*-value <0.05.



1. Patients with hepatic steatosis (Reference)
2. Patients with hepatic steatosis and T2DM
3. Patients with hepatic steatosis, T2DM, and fibrosis

Figure 1 Total and HMW adiponectin levels by groups

4.5 Association between Plasma Fatty Acids and T2DM

The third objective of this study was to examine the relationship between plasma fatty acid composition and T2DM in patients with MAFLD. In the multivariate logistic regression analysis, plasma fatty acids showed varying associations with the presence of T2DM. LA (C18:2, n-6) was significantly associated with reduced odds of T2DM, both in the adjusted model 1 (Table 8; OR: 0.818; 95% CI: 0.707–0.945; $p = 0.006$) and after adjustment for more covariates (Table 8; OR: 0.795; 95% CI: 0.638–0.990; $p = 0.041$). Similarly, α-LA (C18:3, n-3) exhibited an inverse association in the adjusted model 2 (Table 7; OR: 0.001; 95% CI: 0.001–0.590; $p = 0.034$). Other fatty acids, including C16:0 (Table 7; OR = 1.079; $p = 0.809$), C18:1 (n-9) (Table 8; OR = 1.140; $p = 0.400$), C18:3 (n-6) (Table 7; OR = 1.876; $p = 0.600$), C20:3 (n-6) (Table 8; OR = 1.353; $p = 0.715$), C20:4 (n-6) (Table 7; OR = 1.373; $p = 0.117$), C20:5 (n-3) (Table 8; OR = 3.893; $p= 0.255$), and C22:4 (n-6) (Table 8; OR = 0.533; $p = 0.586$), did not demonstrate statistically significant associations with T2DM in either adjusted models. The predictive performance of the models in identifying the presence of T2DM was assessed using the AUC of their ROC curves. As shown in Figure 2, the α-LA model had an AUC of 0.875, while the LA model had an AUC of 0.882.

Table 8 Plasma fatty acid composition and the presence of T2DM

	OR	(95% CI)	*P*-value	OR	(95% CI)	*P*-value
		Model 1 [1]			Model 2 [2]	
Palmitic acid (C16:0)	1.324	(0.974-1.801)	0.073	1.079	(0.579-2.010)	0.809
Oleic acid (C18:1, n-9)	1.171	(0.938-1.462)	0.163	1.140	(0.839-1.551)	0.400
LA (C18:2, n-6)	0.818	(0.707-0.945)	**0.006**	0.795	(0.638-0.990)	**0.041**
α-LA (C18:3, n-3)	0.052	(0.002-1.29)	0.071	0.001	(0.001-0.590)	**0.034**
GLA (C18:3, n-6) [3]	1.619	(0.346-7.563)	0.540	1.876	(0.178-19.762)	0.600
DHLA (C20:3, n-6)	1.014	(0.295-3.484)	0.982	1.353	(0.266-6.869)	0.715
AA (C20:4, n-6)	1.182	(0.918-1.523)	0.194	1.373	(0.923-2.043)	0.117
EPA (C20:5, n-3) [3]	3.282	(0.722-14.905)	0.124	3.893	(0.373-40.548)	0.255
DTA (C22:4, n-6) [3]	1.041	(0.223-4.856)	0.959	0.533	(0.055-5.117)	0.586

[1] Model 1 is adjusted for age and sex.
[2] Model 2 is adjusted for age, sex, Homa-IR, total lean, trunk fat, AST, ALT, GGT, BMI, and WC.
[3] Data were log transformed for maintaining normality.
LA, Linoleic acid; α-LA, α-linoleic acid; GLA, γ-linolenic Acid; DHLA, Dihomo-γ-linolenic acid; AA, Arachidonic acid; DTA, Docosatetraenoic acid; EPA, Eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, docosahexaenoic acid
Statistical significance is defined as a p-value < 0.05.

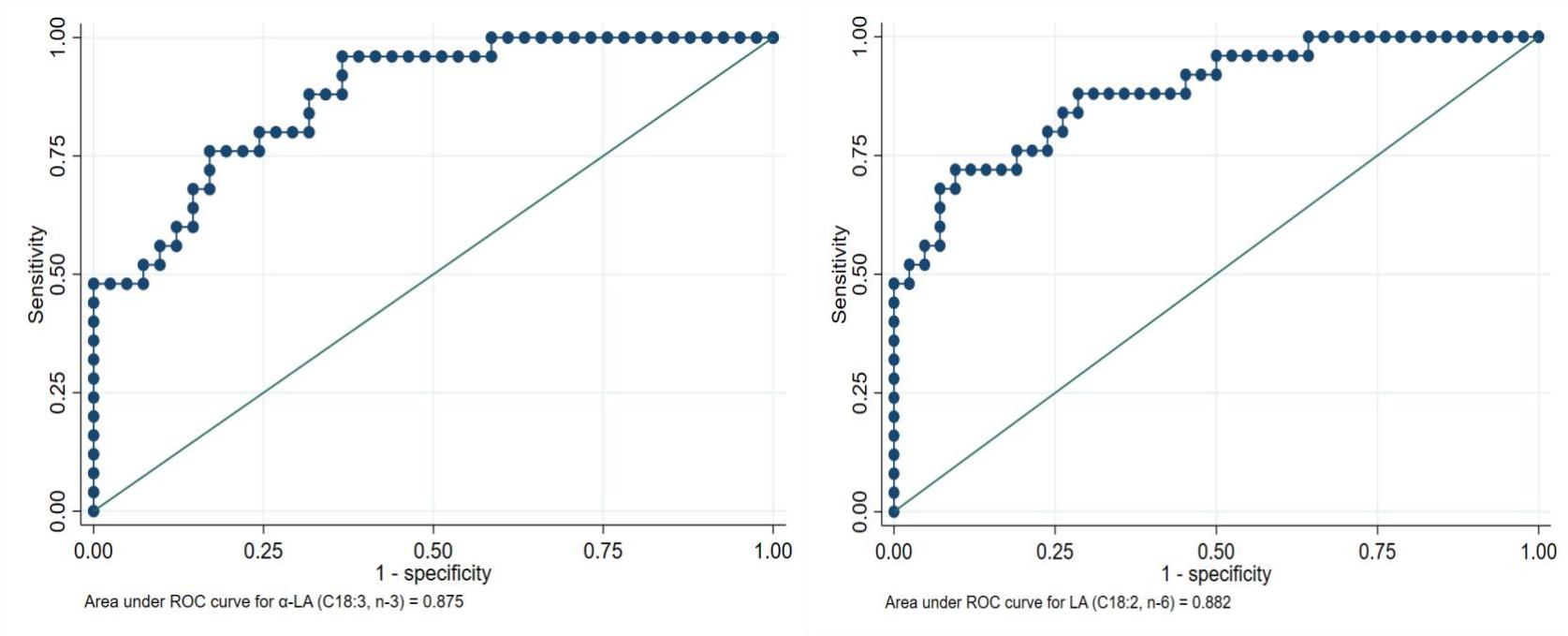


Figure 2 ROC curve for T2DM models

After observing a significant inverse association between plasma α-LA and LA levels with the presence of T2DM in the study population, a further logistic regression analysis was conducted to explore the relationships in more detail. Tertiles of fatty acids were used to assess their association with T2DM, allowing for the examination of potential nonlinear effects across different levels of plasma fatty acids. In the analysis, plasma LA showed a significant negative association with the presence of T2DM in both models compared to the reference. In adjusted model 1, the second tertile (T2: 26.00-30.56%), the OR was 0.227 (Table 9; 95% CI: 0.063-0.818, $p = 0.023$), and in the third tertile (T3: ≥ 30.57%), the OR was 0.120 (Table 9; 95% CI: 0.029-0.486, $p = 0.003$). Specifically, for each 1 % increase in plasma LA, there is a 77.3% reduction in the likelihood of having T2DM in T2 and an 88% reduction in T3 compared to the reference group (T1: ≤ 25.99%). In adjusted model 2, in the second tertile (T2: 26.00-30.56%), OR was 0.089 (Table 9; 95% CI: 0.012-0.647, $p = 0.017$), and in the third tertile (T3: ≥ 30.57%), the OR was 0.071 (Table 9; 95% CI: 0.007-0.657, $p = 0.020$). Specifically, participants in T2 had 91.1% lower likelihood of having T2DM, and those in T3 had a 92.9% lower likelihood of the presence of T2DM compared to the reference group (T1: ≤ 25.99%). Similarly, plasma α-LA exhibited a significant negative association with the odds of T2DM compared to the reference. These findings suggest that higher levels of α-LA are associated with reduced odds of T2DM, with 79% and 96.2 % reduction in the odds of T2DM in T3 compared to the reference group (T1: ≤ 0.55 %) in models 1 and 2, respectively. The cross-tabulation of diabetes status across tertiles of plasma fatty acid levels is presented in Table 10.

Table 9 Tertiles of plasma fatty acid and the presence of T2DM

	OR	(95% CI)	P-value	OR	(95% CI)	P-value
		Model 1 [1]			Model 2 [2]	
Palmitic acid (C16:0)						
T1 (≤ 21.58)	1.00	1.00		1.00	1.00	
T2 (21.59-22.90)	1.487	(0.418-5.285)	0.539	0.692	(0.131-3.649)	0.664
T3 (≥22.91)	2.926	(0.770-11.122)	0.115	0.802	(0.100- 6.433)	0.836
Oleic acid (C18:1, n-9)						
T1 (≤20.02)	1.00	1.00		1.00	1.00	
T2 (20.03-22.67)	1.749	(0.480-6.375)	0.396	3.686	(0.548-24.758)	0.179
T3 (≥22.68)	2.380	(0.603-9.386)	0.215	2.683	(0.329-21.879)	0.356
LA (C18:2, n-6)						
T1 (≤25.99)	1.00	1.00		1.00	1.00	
T2 (26.00-30.56)	0.227	(0.063-0.818)	**0.023**	0.089	(0.012- 0.647)	**0.017**
T3 (≥30.57)	0.120	(0.029-0.486)	**0.003**	0.071	(0.007-0.657)	**0.020**
α-LA (C18:3, n-3)						
T1 (≤0.55)	1.00	1.00		1.00	1.00	
T2 (0.56-0.73)	0.635	(0.168-2.392)	0.502	0.44	(0.060-3.306)	0.432
T3 (≥0.74)	0.210	(0.045-0.975)	**0.046**	0.038	(0.002-0.614)	**0.021**
GLA (C18:3, n-6)						
T1 (≤0.38)	1.00	1.00		1.00	1.00	
T2 (0.39-0.50)	1.844	(0.235- 3.029)	0.796	1.557	(0.242-10.001)	0.640
T3 (≥0.51)	1.238	(0.349- 4.382)	0.741	2.07	(0.321- 13.347)	0.444
DHLA (C20:3, n-6)						
T1 (≤1.29)	1.00	1.00		1.00	1.00	
T2 (1.3-1.72)	0.856	(0 .248- 2.948)	0.806	0.557	(0.083- 3.718)	0.546
T3 (≥1.73)	0.926	(0.255- 3.355)	0.907	1.113	(0.176-7.036)	0.909
AA (C20:4, n-6)						
T1 (≤6.19)	1.00	1.00		1.00	1.00	
T2 (6.2-7.94)	0.985	(0.278-3.492)	0.982	0.486	(0.077- 3.033)	0.440
T3 (≥7.95)	1.866	(0.527-6.601)	0.333	1.510	(0.218-10.425)	0.676
EPA (C20:5, n-3)						
T1 (≤0.36)	1.00	1.00		1.00	1.00	
T2 (0.37-0.48)	2.586	(0.691-9.671)	0.158	1.975	(0.562-8.564)	0.356
T3 (≥0.49)	2.472	(0.641- 9.523)	0.188	1.899	(0.611- 7.658)	0.658
DTA (C22:4, n-6)						
T1 (≤0.15)	1.00	1.00		1.00	1.00	
T2 (0.15-0.20)	0.634	(0.184-2.178)	0.470	0.255	(0.029-2.176)	0.212
T3 (≥0.21)	1.102	(0.327-3.709)	0.876	0.317	(0.045-2.245)	0.251

[1] Model 1 is adjusted for age and sex. 2 Model 2 is adjusted for age, sex, Homa-IR, total lean, trunk fat, AST, ALT, GGT, BMI, and WC. Statistical significance is defined as a p-value < 0.05.

Table 10 Cross-tabulation of diabetes status across tertiles of plasma fatty acid levels

	T2DM (No)	T2DM (Yes)	Total
Palmitic acid (C16:0)			
T1 (≤ 21.58)	17 (71)	7 (29)	24 (100)
T2 (21.59-22.90)	15 (65)	8 (35)	23 (100)
T3 (≥22.91)	12 (52)	11 (48)	23 (100)
Oleic acid (C18:1, n-9)			
T1 (≤20.02)	18 (75)	6 (25)	24 (100)
T2 (20.03-22.67)	14 (61)	9 (39)	23 (100)
T3 (≥22.68)	12 (52)	11 (48)	23 (100)
LA (C18:2, n-6)			
T1 (≤25.99)	9 (37.5)	15 (62.5)	24 (100)
T2 (26.00-30.56)	16 (70)	7 (30)	23 (100)
T3 (≥30.57)	19 (83)	4 (17)	23 (100)
α-LA (C18:3, n-3)			
T1 (≤0.55)	13 (54)	11 (46)	24 (100)
T2 (0.56-0.73)	14 (61)	9 (39)	23 (100)
T3 (≥0.74)	17 (74)	6 (26)	23 (100)
GLA (C18:3, n-6)			
T1 (≤0.38)	14 (58)	10 (42)	24 (100)
T2 (0.39-0.50)	16 (70)	7 (30)	23 (100)
T3 (≥0.51)	14 (61)	9 (39)	23 (100)
DHLA (C20:3, n-6)			
T1 (≤1.29)	14 (58)	10 (42)	24 (100)
T2 (1.3-1.72)	15 (65)	8 (35)	23 (100)
T3 (≥1.73)	15 (65)	8 (35)	23 (100)
AA (C20:4, n-6)			
T1 (≤6.19)	16 (67)	8 (33)	24 (100)
T2 (6.2-7.94)	16 (70)	7 (30)	23 (100)
T3 (≥7.95)	12 (52)	11 (48)	23 (100)
EPA (C20:5, n-3)			
T1 (≤0.36)	18 (75)	6 (25)	24 (100)
T2 (0.37-0.48)	13 (57)	10 (43)	23 (100)
T3 (≥0.49)	13 (56)	10 (44)	23 (100)
DTA (C22:4, n-6)			
T1 (≤0.15)	14 (58)	10 (42)	24 (100)
T2 (0.15-0.20)	16 (70)	7 (30)	23 (100)
T3 (≥0.21)	14 (61)	9 (39)	23 (100)

values are presented as counts (percentages) within each tertile.

4.6 The Relationship between HMW and Total Adiponectin with LA

The final objective of this study was to investigate whether plasma levels of HMW and total adiponectin can predict LA and α-LA levels. In the unadjusted model, total adiponectin was not significantly associated with plasma LA levels (Table 11; coefficient = 0.444, 95% CI: -0.011 to 0.901, $p = 0.056$). After adjusting for sex and age in Model 1, total adiponectin showed a significant positive association with plasma LA levels (Table 11; coefficient = 0.603, 95% CI: 0.064 to 1.142, $p = 0.029$). However, in model 2, the association was no longer statistically significant (Table 11; coefficient = 0.389, 95% CI: -0.216 to 0.995, $p = 0.203$). For HMW adiponectin (µg/mL), no significant associations with plasma LA levels were observed in any of the models. The association remained non-significant in both Model 1 (Table 11; coefficient = 0.787, 95% CI: -0.420 to 1.995, $p = 0.197$) and Model 2 (Table 11; coefficient = 0.426, 95% CI: -0.883 to 1.736, $p = 0.517$).

Table 11 The association between HMW and total adiponectin and LA (C18:2, n-6)

Predictor	Coeff	Std Err	95% CI	*P*-value
Unadjusted Model				
Total Adiponectin (µg/mL)	0.444	0.228	(-.011, .901)	0.056
Adjusted Model 1				
Total Adiponectin (µg/mL)	0.603	0.270	(0.064, 1.142)	**0.029**
Adjusted Model 2				
Total Adiponectin (µg/mL)	0.389	0.303	(-0.216, 0.995)	0.203
Unadjusted Model				
HMW adiponectin (µg/mL)	0.593	0.513	(-0.429, 1.617)	0.251
Adjusted Model 1				
HMW adiponectin (µg/mL)	0.787	0.604	(-0.420, 1.995)	0.197
Adjusted Model 2				
HMW adiponectin (µg/mL)	0.426	0.653	(-0.883, 1.736)	0.517

Model 1 is adjusted for age and sex. Statistical significance is defined as a p-value < 0.05.
Model 2 is adjusted for age, sex, Homa-IR, TG (mg/dL), AST, ALT, GGT, Appendicular lean Mass/BMI, Race.

4.7 The Relationship between HMW and Total Adiponectin and α-LA

Neither total adiponectin nor HMW adiponectin showed significant associations with α-LA across all models. In the unadjusted model, total adiponectin had a coefficient of -0.046 (Table 12;95% CI: -0.093 to 0.000, $p = 0.052$), and this association was not significant after adjusting for potential confounders. Similarly, HMW adiponectin did not demonstrate any significant relationship with α-LA in any model, with coefficients of -0.084 ($p = 0.251$), -0.020 ($p = 0.737$), and -0.015 ($p = 0.808$) in the unadjusted and adjusted models, respectively (Table 12). The relationships between the variables were further examined using a Spearman correlation matrix, as shown in Figure 3.

Table 12 The association between HMW and total adiponectin and α-LA (C18:3, n-3)

Predictor	Coeff	Std Err	95% CI	*P*-value
Unadjusted Model				
Total Adiponectin (μg/mL)	-0.046	0.023	(-0.093, 0.000)	0.052
Adjusted Model 1				
Total Adiponectin (μg/mL)	-0.019	0.027	(-0.074, 0.034)	0.469
Adjusted Model 2				
Total Adiponectin (μg/mL)	-0.018	0.031	(-0.081, 0.043)	0.547
Unadjusted Model				
HMW adiponectin (μg/mL)	-0.084	0.052	(-0.188, 0.020)	0.251
Adjusted Model 1				
HMW adiponectin (μg/mL)	-0.020	0.060	(-0.014, 0.099)	0.737
Adjusted Model 2				
HMW adiponectin (μg/mL)	-0.015	0.064	(-0.145, 0.114)	0.808

Model 1 is adjusted for age and sex.
Model 2 is adjusted for age, sex, Homa-IR, TG (mg/dL), AST, ALT, GGT, Appendicular lean Mass/BMI, Race.
Statistical significance is defined as a p-value < 0.05.

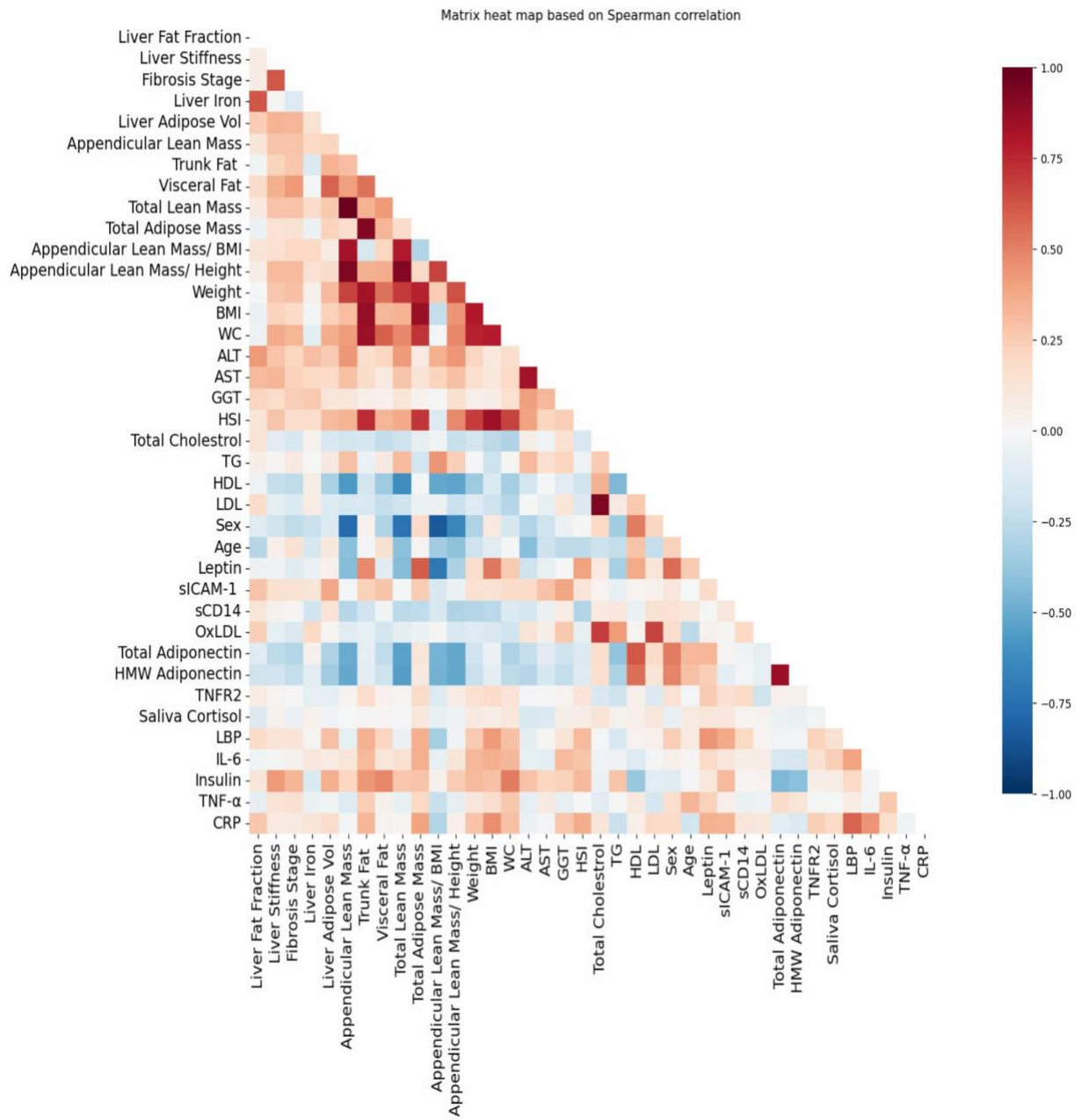


Figure 3 Matrix heatmap of variables

Chapter 5. Discussion

Several studies have previously reported an inverse relationship between serum adiponectin levels and conditions such as obesity, T2DM, and CVD (258-260). However, the relationship between liver fat, liver stiffness, and plasma total/HMW adiponectin in patients with MAFLD remains insufficiently understood. This cross-sectional study analyzed data from a randomized, placebo-controlled clinical trial involving 74 participants from the Columbus, Ohio area. After adjusting for covariates, the results showed that each 1 μg/mL increase in HMW adiponectin was associated with a 2.4% decrease in liver fat content ($p = 0.02$). In contrast, no significant relationship was observed between total plasma adiponectin and liver fat content ($p = 0.612$). Furthermore, after controlling for confounding variables, no significant associations were observed between plasma HMW/total adiponectin and liver stiffness ($p = 0.99$ and 0.190), respectively.

In a study involving 65 patients with biopsy-confirmed advanced fibrosis (stages 3-4) and 54 patients with mild fibrosis (stages 0-1), no significant differences were found in liver fat content or adiponectin levels. However, in patients with advanced NASH, each 4 μg/L increase in adiponectin was associated with an OR of 2.0 (95% CI: 1.3-3.0, $p < 0.01$) for a 5% reduction in hepatic fat (261). Also, in a cross-sectional study involving 1,200 participants, where the severity of hepatic steatosis was assessed using ultrasound and adjusted for various risk factors, adiponectin emerged as the strongest independent predictor of liver fat with an OR of 0.963 (95% CI: 1.3–3.0, $p = 0.02$) (262). In a recent cohort study conducted in Beijing, China, with a 10-year follow-up, For every 1

μg/mL increase in ln-adiponectin, the odds of having NAFLD decreased by 47% (OR = 0.53; 95% CI = 0.33–0.85) (263). Moreover, in a cohort study, low adiponectin levels were found to be linked to an increased risk of developing NAFLD in middle-aged and older adults (264).

It has been proposed that the interplay between elevated leptin and resistin levels, along with reduced adiponectin, may contribute to the development and progression of NAFLD (265). The protective role of adiponectin in NAFLD is attributed to its ability to suppress DNL and gluconeogenesis and promote fatty acid oxidation, which in turn enhances insulin sensitivity and lowers the risk of CVD (266, 267). The two adiponectin receptors trigger distinct downstream signaling pathways: adipoR1 activates AMPK, while adipoR2 enhances the PPAR-α pathway (268). Gluconeogenesis is suppressed through the inhibition of key enzymes, such as glucose 6-phosphatase (G6Pase) PEPCK, by activated AMPK (269). AMPK activation also phosphorylates Ser 372 on SREBP-1c, leading to the inhibition of this transcription factor, which in turn reduces the expression of genes involved in DNL (270, 271). Additionally, AMPK inhibits ACC, decreasing the production of malonyl-CoA. Malonyl-CoA plays a critical role in regulating fatty acid entry into the mitochondria by inhibiting CPT-I (270, 271).

Next, in our study, the relationship between HMW and total adiponectin levels and the presence of T2DM and liver fibrosis was examined. The analysis revealed that higher total plasma adiponectin levels were negatively associated with the presence of T2DM and liver fibrosis in individuals with MAFLD. Specifically, for each 1 μg/mL increase in total plasma adiponectin, the odds for the coexistence of T2DM and liver fibrosis were significantly lower (OR: 0.185, 95% CI: 0.040–0.877, p = 0.033). In contrast, higher

levels of HMW adiponectin were more strongly associated with reduced odds of having T2DM alone (OR: 0.276, 95% CI: 0.089–0.856, p = 0.026). Due to the small sample size for this aim of the study, which consisted of 37 participants with MAFLD but without diabetes or fibrosis (reference group), 14 participants with both MAFLD and T2DM, and just 7 participants with MAFLD, T2DM, and fibrosis, the models were only adjusted for age and sex. Therefore, the results should be interpreted with caution, as the limited sample size may affect the reliability and generalizability of the findings.

In line with the findings of this study, in a cross-sectional study of 79 participants, individuals with hepatic steatosis alone or with significant fibrosis had notably lower levels of HMW adiponectin compared to those without NAFLD. Furthermore, lower plasma HMW adiponectin levels were strongly linked to approximately threefold and sixfold higher odds of having hepatic steatosis alone or NAFLD with significant fibrosis (272). Similarly, another cross-sectional study found that plasma adiponectin levels were significantly reduced in patients with biopsy-proven NASH compared to those without NASH (261). In addition, a meta-analysis of 27 studies found that patients with NASH had lower adiponectin levels compared to those with NAFLD (19 studies, random-effects WMD (95% CI) = 1.81 (1.09-2.53))(273). These findings suggest that reduced adiponectin levels may be associated with the severity or progression of NAFLD to NASH (272, 273).

In addition, our study showed that higher plasma levels of LA and α-LA were both inversely associated with the presence of T2DM in patients with MAFLD.

As previously mentioned, plasma FA levels are influenced by both dietary FA intake and the dynamic balance between de novo synthesis, storage TGs, and the breakdown of

these TGs through lipolysis (14). Elevated levels of LA in blood or tissue have been found to correlate positively with improved insulin sensitivity, thereby reducing the risk of developing insulin resistance and T2DM (195, 274-276). In a study that included 3,377 post-myocardial infarction (MI) patients, plasma LA was associated with a significantly lower risk of T2DM (HR: 0.45; 95% CI: 0.27 - 0.76; $P = 0.002$) compared to the reference group (277). In a comprehensive case-cohort study involving 12,132 individuals with T2DM and 15,919 participants from a sub-cohort, an inverse relationship between plasma phospholipid levels of α-LA and the risk of developing T2DM was found (278). In addition, among the n-6 PUFAs, LA (HR: 0.80; 95% CI 0.77-0.83) and EDA (HR: 0.89; 95% CI 0.85-0.94) plasma levels were found to have an inverse relationship with T2DM (271).

Research has demonstrated that supplementation with LA-rich oils can enhance insulin sensitivity, improve glycemic control, and reduce central obesity (195, 242, 279, 280). The exact mechanisms through which LA minimizes the risk of insulin resistance remain unclear (281). While it is likely that its effects on dyslipidemia play a role, this alone may not fully account for its benefits. One possible explanation is that LA, along with its oxylipin metabolites, may influence glucose and insulin metabolism by activating PPARs (282-284).

In addition to T2DM, it is suggested that n-3 PUFA supplementation could be particularly beneficial in the early stages of NAFLD (185). A recent systematic review found that n-3 PUFA supplementation when combined with a hypocaloric or heart-healthy diet low in SFA, improved liver enzyme levels and reduced steatosis scores (178). However, n-3 PUFA supplementation in systematic reviews and meta-analyses

showed no effect on lobular inflammation score, hepatocellular ballooning score, fibrosis score, or NAS (178, 198).

Epilogue

Limitations

This study has some limitations that are noteworthy of mention. Firstly, the study's cross-sectional design prevents the assessment of causal relationships between variables. Secondly, the sample size is insufficient to generalize the findings to the broader MAFLD population. Additionally, the participants may not fully represent the wider MAFLD population, as they were recruited from a specific region, potentially limiting the study's generalizability. Furthermore, in our study, the number of females (43) exceeded that of males (31), and females had higher adiponectin levels. Although the models were adjusted for potential confounding factors, this gender imbalance could have contributed to variations in the results. Moreover, since almost all participants were in the overweight or obesity category, the results may not apply to lean individuals with MAFLD. Finally, patients were allowed to take omega-3 supplementation, which could have influenced the fatty acid composition and may have impacted the results of analyses that included these variables.

Conclusions and Future Directions

In conclusion, this cross-sectional study revealed that, after adjusting for potential confounding factors, higher levels of HMW adiponectin were associated with lower liver fat content and a reduced presence of T2DM alone. Furthermore, higher total adiponectin levels were negatively associated with the coexistence of T2DM and liver fibrosis. Additionally, elevated plasma levels of LA and α-LA were inversely linked to the presence of T2DM in patients with MAFLD.

These findings emphasize the potential role of adiponectin isoforms/receptors in MAFLD, highlighting their significance as potential targets for halting disease progression or mitigating adverse outcomes. Future research should focus on validating these relationships in larger, more diverse cohorts while exploring the distinct roles of adiponectin isoforms in the pathophysiology of MAFLD. Furthermore, understanding the differential contributions of AdipoR1 and AdipoR2 signaling pathways and developing receptor agonists, such as AdipoRon, to activate these pathways may open new avenues for innovative strategies to attenuate MAFLD in metabolically vulnerable populations.

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Bibliography

1. Eslam M, Sanyal AJ, George J. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology. 2020;158(7):1999-2014.e1.
2. Habibullah M, Jemmieh K, Ouda A, Haider MZ, Malki MI, Elzouki AN. Metabolic-associated fatty liver disease: a selective review of pathogenesis, diagnostic approaches, and therapeutic strategies. Front Med (Lausanne). 2024;11:1291501.
3. Eslam M, El-Serag HB, Francque S, Sarin SK, Wei L, Bugianesi E, et al. Metabolic (dysfunction)-associated fatty liver disease in individuals of normal weight. Nat Rev Gastroenterol Hepatol. 2022;19(10):638-51.
4. Pal SC, Méndez-Sánchez N. Screening for MAFLD: who, when and how? Ther Adv Endocrinol Metab. 2023;14:20420188221145650.
5. Ciardullo S, Perseghin G. Prevalence of NAFLD, MAFLD and associated advanced fibrosis in the contemporary United States population. Liver Int. 2021;41(6):1290-3.
6. Giannessi D, Maltinti M, Del Ry S. Adiponectin circulating levels: a new emerging biomarker of cardiovascular risk. Pharmacol Res. 2007;56(6):459-67.
7. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548-56.
8. Yanai H, Yoshida H. Beneficial Effects of Adiponectin on Glucose and Lipid Metabolism and Atherosclerotic Progression: Mechanisms and Perspectives. Int J Mol Sci. 2019;20(5).
9. Park PH, Sanz-Garcia C, Nagy LE. Adiponectin as an anti-fibrotic and anti-inflammatory adipokine in the liver. Curr Pathobiol Rep. 2015;3(4):243-52.
10. Asano T, Watanabe K, Kubota N, Gunji T, Omata M, Kadowaki T, et al. Adiponectin knockout mice on high fat diet develop fibrosing steatohepatitis. J Gastroenterol Hepatol. 2009;24(10):1669-76.
11. Shafiei MS, Shetty S, Scherer PE, Rockey DC. Adiponectin regulation of stellate cell activation via PPARγ-dependent and -independent mechanisms. Am J Pathol. 2011;178(6):2690-9.
12. Turer AT, Browning JD, Ayers CR, Das SR, Khera A, Vega GL, et al. Adiponectin as an independent predictor of the presence and degree of hepatic steatosis in the Dallas Heart Study. J Clin Endocrinol Metab. 2012;97(6):E982-6.
13. Rosqvist F, Fridén M, Vessby J, Hockings P, Hulthe J, Gummesson A, et al. Fatty acids in multiple circulating lipid fractions reflects the composition of liver triglycerides in humans. Clin Nutr. 2022;41(4):805-9.
14. A ISS, C AB, A JS. Changes in Plasma Free Fatty Acids Associated with Type-2 Diabetes. Nutrients. 2019;11(9).
15. Fridén M, Rosqvist F, Ahlström H, Niessen HG, Schultheis C, Hockings P, et al. Hepatic Unsaturated Fatty Acids Are Linked to Lower Degree of Fibrosis in Non-alcoholic Fatty Liver Disease. Front Med (Lausanne). 2021;8:814951.

16. Miyake T, Furukawa S, Matsuura B, Yoshida O, Miyazaki M, Shiomi A, et al. Plasma Fatty Acid Composition Is Associated with Histological Findings of Nonalcoholic Steatohepatitis. Biomedicines. 2022;10(10).
17. Fernández-Real JM, Vendrell J, Ricart W. Circulating adiponectin and plasma fatty acid profile. Clin Chem. 2005;51(3):603-9.
18. Xia Z, Shi S, Ma X, Li F, Li X, Gaisano HY, et al. Mediating effect of adiponectin between free fatty acid and tumor necrosis factor-α in patients with diabetes. Nutr Diabetes. 2024;14(1):45.
19. Bersch-Ferreira Â C, Sampaio GR, Gehringer MO, Torres E, Ross-Fernandes MB, da Silva JT, et al. Association between plasma fatty acids and inflammatory markers in patients with and without insulin resistance and in secondary prevention of cardiovascular disease, a cross-sectional study. Nutr J. 2018;17(1):26.
20. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc. 1980;55(7):434-8.
21. Ahmed A, Wong RJ, Harrison SA. Nonalcoholic Fatty Liver Disease Review: Diagnosis, Treatment, and Outcomes. Clin Gastroenterol Hepatol. 2015;13(12):2062-70.
22. Machado MV, Diehl AM. Pathogenesis of Nonalcoholic Steatohepatitis. Gastroenterology. 2016;150(8):1769-77.
23. Nasr P, Ignatova S, Kechagias S, Ekstedt M. Natural history of nonalcoholic fatty liver disease: A prospective follow-up study with serial biopsies. Hepatol Commun. 2018;2(2):199-210.
24. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018;15(1):11-20.
25. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology. 2001;121(1):91-100.
26. Pouwels S, Sakran N, Graham Y, Leal A, Pintar T, Yang W, et al. Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC Endocr Disord. 2022;22(1):63.
27. Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. Semin Liver Dis. 2001;21(1):27-41.
28. Fromenty B, Pessayre D. Impaired mitochondrial function in microvesicular steatosis. Effects of drugs, ethanol, hormones and cytokines. J Hepatol. 1997;26 Suppl 2:43-53.
29. Méndez-Sánchez N, Bugianesi E, Gish RG, Lammert F, Tilg H, Nguyen MH, et al. Global multi-stakeholder endorsement of the MAFLD definition. Lancet Gastroenterol Hepatol. 2022;7(5):388-90.
30. Tilg H, Adolph TE, Dudek M, Knolle P. Non-alcoholic fatty liver disease: the interplay between metabolism, microbes and immunity. Nat Metab. 2021;3(12):1596-607.
31. Amini-Salehi E, Letafatkar N, Norouzi N, Joukar F, Habibi A, Javid M, et al. Global Prevalence of Nonalcoholic Fatty Liver Disease: An Updated Review Meta-Analysis comprising a Population of 78 million from 38 Countries. Arch Med Res. 2024;55(6):103043.
32. Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2022;7(9):851-61.

33. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. Hepatology. 2023;77(4):1335-47.
34. Schaffner F, Thaler H. Nonalcoholic fatty liver disease. Prog Liver Dis. 1986;8:283-98.
35. Chan KE, Koh TJL, Tang ASP, Quek J, Yong JN, Tay P, et al. Global Prevalence and Clinical Characteristics of Metabolic-associated Fatty Liver Disease: A Meta-Analysis and Systematic Review of 10 739 607 Individuals. J Clin Endocrinol Metab. 2022;107(9):2691-700.
36. Ramírez-Mejía MM, Méndez-Sánchez N. What Is in a Name: from NAFLD to MAFLD and MASLD—Unraveling the Complexities and Implications. Current Hepatology Reports. 2023;22(4):221-7.
37. Eslam M, Alkhouri N, Vajro P, Baumann U, Weiss R, Socha P, et al. Defining paediatric metabolic (dysfunction)-associated fatty liver disease: an international expert consensus statement. Lancet Gastroenterol Hepatol. 2021;6(10):864-73.
38. Pipitone RM, Ciccioli C, Infantino G, La Mantia C, Parisi S, Tulone A, et al. MAFLD: a multisystem disease. Ther Adv Endocrinol Metab. 2023;14:20420188221145549.
39. Wen W, Li H, Wang C, Chen C, Tang J, Zhou M, et al. Metabolic dysfunction-associated fatty liver disease and cardiovascular disease: A meta-analysis. Front Endocrinol (Lausanne). 2022;13:934225.
40. Targher G. Concordance between MAFLD and NAFLD diagnostic criteria in 'real-world' data. Liver Int. 2020;40(11):2879-80.
41. Gutiérrez-Cuevas J, Santos A, Armendariz-Borunda J. Pathophysiological Molecular Mechanisms of Obesity: A Link between MAFLD and NASH with Cardiovascular Diseases. Int J Mol Sci. 2021;22(21).
42. Lim GEH, Tang A, Ng CH, Chin YH, Lim WH, Tan DJH, et al. An Observational Data Meta-analysis on the Differences in Prevalence and Risk Factors Between MAFLD vs NAFLD. Clin Gastroenterol Hepatol. 2023;21(3):619-29.e7.
43. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Hepatology. 2023;78(6):1966-86.
44. Kawaguchi T, Tsutsumi T, Nakano D, Torimura T. MAFLD: Renovation of clinical practice and disease awareness of fatty liver. Hepatol Res. 2022;52(5):422-32.
45. Yip TC, Vilar-Gomez E, Petta S, Yilmaz Y, Wong GL, Adams LA, et al. Geographical similarity and differences in the burden and genetic predisposition of NAFLD. Hepatology. 2023;77(4):1404-27.
46. Ramírez-Mejía MM, Jiménez-Gutiérrez C, Eslam M, George J, Méndez-Sánchez N. Breaking new ground: MASLD vs. MAFLD-which holds the key for risk stratification? Hepatol Int. 2024;18(1):168-78.
47. Huang Q, Zou X, Wen X, Zhou X, Ji L. NAFLD or MAFLD: Which Has Closer Association With All-Cause and Cause-Specific Mortality?-Results From NHANES III. Front Med (Lausanne). 2021;8:693507.
48. Liang Y, Chen H, Liu Y, Hou X, Wei L, Bao Y, et al. Association of MAFLD With Diabetes, Chronic Kidney Disease, and Cardiovascular Disease: A 4.6-Year Cohort Study in China. J Clin Endocrinol Metab. 2022;107(1):88-97.
49. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. J Hepatol. 2020;73(1):202-9.

50. Liu J, Ayada I, Zhang X, Wang L, Li Y, Wen T, et al. Estimating Global Prevalence of Metabolic Dysfunction-Associated Fatty Liver Disease in Overweight or Obese Adults. Clin Gastroenterol Hepatol. 2022;20(3):e573-e82.
51. Karelis AD, Brochu M, Rabasa-Lhoret R. Can we identify metabolically healthy but obese individuals (MHO)? Diabetes Metab. 2004;30(6):569-72.
52. Frith J, Day CP, Henderson E, Burt AD, Newton JL. Non-alcoholic fatty liver disease in older people. Gerontology. 2009;55(6):607-13.
53. Mellinger JL. Epidemiology of Alcohol Use and Alcoholic Liver Disease. Clin Liver Dis (Hoboken). 2019;13(5):136-9.
54. Neeland IJ, Lim S, Tchernof A, Gastaldelli A, Rangaswami J, Ndumele CE, et al. Metabolic syndrome. Nat Rev Dis Primers. 2024;10(1):77.
55. Chan WK, Wong VW, Adams LA, Nguyen MH. MAFLD in adults: non-invasive tests for diagnosis and monitoring of MAFLD. Hepatol Int. 2024;18(Suppl 2):909-21.
56. Castera L, Friedrich-Rust M, Loomba R. Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2019;156(5):1264-81.e4.
57. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology. 2011;54(3):1082-90.
58. Jang JK, Choi SH, Lee JS, Kim SY, Lee SS, Kim KW. Accuracy of the ultrasound attenuation coefficient for the evaluation of hepatic steatosis: a systematic review and meta-analysis of prospective studies. Ultrasonography. 2022;41(1):83-92.
59. Petroff D, Blank V, Newsome PN, Shalimar, Voican CS, Thiele M, et al. Assessment of hepatic steatosis by controlled attenuation parameter using the M and XL probes: an individual patient data meta-analysis. Lancet Gastroenterol Hepatol. 2021;6(3):185-98.
60. Harry S, Lai LL, Nik Mustapha NR, Abdul Aziz YF, Vijayananthan A, Rahmat K, et al. Volumetric Liver Fat Fraction Determines Grade of Steatosis More Accurately Than Controlled Attenuation Parameter in Patients With Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol. 2020;18(4):945-53.e2.
61. Qadri S, Vartiainen E, Lahelma M, Porthan K, Tang A, Idilman IS, et al. Marked difference in liver fat measured by histology vs. magnetic resonance-proton density fat fraction: A meta-analysis. JHEP Rep. 2024;6(1):100928.
62. Taylor RS, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM, et al. Association Between Fibrosis Stage and Outcomes of Patients With Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. Gastroenterology. 2020;158(6):1611-25.e12.
63. Mózes FE, Lee JA, Selvaraj EA, Jayaswal ANA, Trauner M, Boursier J, et al. Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients with NAFLD: an individual patient data meta-analysis. Gut. 2022;71(5):1006-19.
64. Wong VW, Tak WY, Goh GBB, Cheng PN, Lawitz EJ, Younossi ZM, et al. Performance of Noninvasive Tests of Fibrosis Among Asians, Hispanic, and non-Hispanic Whites in the STELLAR Trials. Clin Gastroenterol Hepatol. 2023;21(1):90-102.e6.
65. Stec DE, Gordon DM, Hipp JA, Hong S, Mitchell ZL, Franco NR, et al. Loss of hepatic PPARα promotes inflammation and serum hyperlipidemia in diet-induced obesity. Am J Physiol Regul Integr Comp Physiol. 2019;317(5):R733-r45.
66. Chan WK, Treeprasertsuk S, Goh GB, Fan JG, Song MJ, Charatcharoenwitthaya P, et al. Optimizing Use of Nonalcoholic Fatty Liver Disease Fibrosis Score, Fibrosis-4 Score, and Liver

Stiffness Measurement to Identify Patients With Advanced Fibrosis. Clin Gastroenterol Hepatol. 2019;17(12):2570-80.e37.
67. Wong VW, Adams LA, de Lédinghen V, Wong GL, Sookoian S. Noninvasive biomarkers in NAFLD and NASH - current progress and future promise. Nat Rev Gastroenterol Hepatol. 2018;15(8):461-78.
68. Ozturk A, Olson MC, Samir AE, Venkatesh SK. Liver fibrosis assessment: MR and US elastography. Abdom Radiol (NY). 2022;47(9):3037-50.
69. Imajo K, Honda Y, Kobayashi T, Nagai K, Ozaki A, Iwaki M, et al. Direct Comparison of US and MR Elastography for Staging Liver Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol. 2022;20(4):908-17.e11.
70. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology. 1998;114(4):842-5.
71. Shabalala SC, Dludla PV, Mabasa L, Kappo AP, Basson AK, Pheiffer C, et al. The effect of adiponectin in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and the potential role of polyphenols in the modulation of adiponectin signaling. Biomed Pharmacother. 2020;131:110785.
72. Santos RD, Valenti L, Romeo S. Does nonalcoholic fatty liver disease cause cardiovascular disease? Current knowledge and gaps. Atherosclerosis. 2019;282:110-20.
73. Boccatonda A, Andreetto L, D'Ardes D, Cocco G, Rossi I, Vicari S, et al. From NAFLD to MAFLD: Definition, Pathophysiological Basis and Cardiovascular Implications. Biomedicines. 2023;11(3).
74. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65(8):1038-48.
75. Koliwad SK, Streeper RS, Monetti M, Cornelissen I, Chan L, Terayama K, et al. DGAT1-dependent triacylglycerol storage by macrophages protects mice from diet-induced insulin resistance and inflammation. J Clin Invest. 2010;120(3):756-67.
76. Musso G, Gambino R, Cassader M. Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. Prog Lipid Res. 2013;52(1):175-91.
77. Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology. 2007;45(6):1366-74.
78. Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. Hepatology. 2010;52(2):774-88.
79. Loomba R, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, et al. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology. 2012;56(3):943-51.
80. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol. 2019;71(4):793-801.
81. Bril F, Lomonaco R, Orsak B, Ortiz-Lopez C, Webb A, Tio F, et al. Relationship between disease severity, hyperinsulinemia, and impaired insulin clearance in patients with nonalcoholic steatohepatitis. Hepatology. 2014;59(6):2178-87.
82. Chalasani N, Deeg MA, Persohn S, Crabb DW. Metabolic and anthropometric evaluation of insulin resistance in nondiabetic patients with nonalcoholic steatohepatitis. Am J Gastroenterol. 2003;98(8):1849-55.

83. Sakurai Y, Kubota N, Yamauchi T, Kadowaki T. Role of Insulin Resistance in MAFLD. Int J Mol Sci. 2021;22(8).
84. Bhat N, Mani A. Dysregulation of Lipid and Glucose Metabolism in Nonalcoholic Fatty Liver Disease. Nutrients. 2023;15(10).
85. DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, et al. Type 2 diabetes mellitus. Nat Rev Dis Primers. 2015;1:15019.
86. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. N Engl J Med. 2014;371(23):2237-8.
87. Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harb Perspect Biol. 2014;6(1).
88. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care. 2009;32 Suppl 2(Suppl 2):S157-63.
89. Kashyap SR, Belfort R, Berria R, Suraamornkul S, Pratipranawatr T, Finlayson J, et al. Discordant effects of a chronic physiological increase in plasma FFA on insulin signaling in healthy subjects with or without a family history of type 2 diabetes. Am J Physiol Endocrinol Metab. 2004;287(3):E537-46.
90. Miyake K, Ogawa W, Matsumoto M, Nakamura T, Sakaue H, Kasuga M. Hyperinsulinemia, glucose intolerance, and dyslipidemia induced by acute inhibition of phosphoinositide 3-kinase signaling in the liver. J Clin Invest. 2002;110(10):1483-91.
91. Mesinovic J, Zengin A, De Courten B, Ebeling PR, Scott D. Sarcopenia and type 2 diabetes mellitus: a bidirectional relationship. Diabetes Metab Syndr Obes. 2019;12:1057-72.
92. Emhmed Ali S, Nguyen MH. Sarcopenic Obesity in Non-Alcoholic Fatty Liver Disease-The Union of Two Culprits. Life (Basel). 2021;11(2).
93. Perry RJ, Camporez JG, Kursawe R, Titchenell PM, Zhang D, Perry CJ, et al. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. Cell. 2015;160(4):745-58.
94. Vatner DF, Majumdar SK, Kumashiro N, Petersen MC, Rahimi Y, Gattu AK, et al. Insulin-independent regulation of hepatic triglyceride synthesis by fatty acids. Proc Natl Acad Sci U S A. 2015;112(4):1143-8.
95. Petersen MC, Madiraju AK, Gassaway BM, Marcel M, Nasiri AR, Butrico G, et al. Insulin receptor Thr1160 phosphorylation mediates lipid-induced hepatic insulin resistance. J Clin Invest. 2016;126(11):4361-71.
96. Kalemba KM, Wang Y, Xu H, Chiles E, McMillin SM, Kwon H, et al. Glycerol induces G6pc in primary mouse hepatocytes and is the preferred substrate for gluconeogenesis both in vitro and in vivo. J Biol Chem. 2019;294(48):18017-28.
97. Nurjhan N, Consoli A, Gerich J. Increased lipolysis and its consequences on gluconeogenesis in non-insulin-dependent diabetes mellitus. J Clin Invest. 1992;89(1):169-75.
98. Jin ES, Browning JD, Murphy RE, Malloy CR. Fatty liver disrupts glycerol metabolism in gluconeogenic and lipogenic pathways in humans. J Lipid Res. 2018;59(9):1685-94.
99. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology. 2001;120(5):1183-92.
100. Miele L, Grieco A, Armuzzi A, Candelli M, Forgione A, Gasbarrini A, et al. Hepatic mitochondrial beta-oxidation in patients with nonalcoholic steatohepatitis assessed by 13C-octanoate breath test. Am J Gastroenterol. 2003;98(10):2335-6.

101. Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell Metab. 2015;21(5):739-46.
102. Yu J, Loh K, Song ZY, Yang HQ, Zhang Y, Lin S. Update on glycerol-3-phosphate acyltransferases: the roles in the development of insulin resistance. Nutr Diabetes. 2018;8(1):34.
103. Uehara K, Sostre-Colón J, Gavin M, Santoleri D, Leonard KA, Jacobs RL, et al. Activation of Liver mTORC1 Protects Against NASH via Dual Regulation of VLDL-TAG Secretion and De Novo Lipogenesis. Cell Mol Gastroenterol Hepatol. 2022;13(6):1625-47.
104. Okada T, Liew CW, Hu J, Hinault C, Michael MD, Krtzfeldt J, et al. Insulin receptors in beta-cells are critical for islet compensatory growth response to insulin resistance. Proc Natl Acad Sci U S A. 2007;104(21):8977-82.
105. Ipsen DH, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. Cell Mol Life Sci. 2018;75(18):3313-27.
106. Auinger A, Valenti L, Pfeuffer M, Helwig U, Herrmann J, Fracanzani AL, et al. A promoter polymorphism in the liver-specific fatty acid transport protein 5 is associated with features of the metabolic syndrome and steatosis. Horm Metab Res. 2010;42(12):854-9.
107. Newberry EP, Xie Y, Kennedy S, Han X, Buhman KK, Luo J, et al. Decreased hepatic triglyceride accumulation and altered fatty acid uptake in mice with deletion of the liver fatty acid-binding protein gene. J Biol Chem. 2003;278(51):51664-72.
108. Ansari RA, Husain K, Rizvi SA. Role of Transcription Factors in Steatohepatitis and Hypertension after Ethanol: The Epicenter of Metabolism. Biomolecules. 2016;6(3).
109. Kim JY, Park KJ, Hwang JY, Kim GH, Lee D, Lee YJ, et al. Activating transcription factor 3 is a target molecule linking hepatic steatosis to impaired glucose homeostasis. J Hepatol. 2017;67(2):349-59.
110. Knebel B, Haas J, Hartwig S, Jacob S, Köllmer C, Nitzgen U, et al. Liver-specific expression of transcriptionally active SREBP-1c is associated with fatty liver and increased visceral fat mass. PLoS One. 2012;7(2):e31812.
111. Iizuka K, Takao K, Yabe D. ChREBP-Mediated Regulation of Lipid Metabolism: Involvement of the Gut Microbiota, Liver, and Adipose Tissue. Front Endocrinol (Lausanne). 2020;11:587189.
112. Dentin R, Benhamed F, Hainault I, Fauveau V, Foufelle F, Dyck JR, et al. Liver-specific inhibition of ChREBP improves hepatic steatosis and insulin resistance in ob/ob mice. Diabetes. 2006;55(8):2159-70.
113. Moreno-Fernandez ME, Giles DA, Stankiewicz TE, Sheridan R, Karns R, Cappelletti M, et al. Peroxisomal β-oxidation regulates whole body metabolism, inflammatory vigor, and pathogenesis of nonalcoholic fatty liver disease. JCI Insight. 2018;3(6).
114. Hinds TD, Jr., Hosick PA, Chen S, Tukey RH, Hankins MW, Nestor-Kalinoski A, et al. Mice with hyperbilirubinemia due to Gilbert's syndrome polymorphism are resistant to hepatic steatosis by decreased serine 73 phosphorylation of PPARα. Am J Physiol Endocrinol Metab. 2017;312(4):E244-e52.
115. Francque S, Verrijken A, Caron S, Prawitt J, Paumelle R, Derudas B, et al. PPARα gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. J Hepatol. 2015;63(1):164-73.
116. Ota T, Gayet C, Ginsberg HN. Inhibition of apolipoprotein B100 secretion by lipid-induced hepatic endoplasmic reticulum stress in rodents. J Clin Invest. 2008;118(1):316-32.

117. Charlton M, Sreekumar R, Rasmussen D, Lindor K, Nair KS. Apolipoprotein synthesis in nonalcoholic steatohepatitis. Hepatology. 2002;35(4):898-904.
118. Lindeboom L, Nabuurs CI, Hesselink MK, Wildberger JE, Schrauwen P, Schrauwen-Hinderling VB. Proton magnetic resonance spectroscopy reveals increased hepatic lipid content after a single high-fat meal with no additional modulation by added protein. Am J Clin Nutr. 2015;101(1):65-71.
119. Turner N, Kowalski GM, Leslie SJ, Risis S, Yang C, Lee-Young RS, et al. Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. Diabetologia. 2013;56(7):1638-48.
120. Skogsberg J, Dicker A, Rydén M, Aström G, Nilsson R, Bhuiyan H, et al. ApoB100-LDL acts as a metabolic signal from liver to peripheral fat causing inhibition of lipolysis in adipocytes. PLoS One. 2008;3(11):e3771.
121. Zhu X, Xia M, Gao X. Update on genetics and epigenetics in metabolic associated fatty liver disease. Ther Adv Endocrinol Metab. 2022;13:20420188221132138.
122. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008;40(12):1461-5.
123. BasuRay S, Smagris E, Cohen JC, Hobbs HH. The PNPLA3 variant associated with fatty liver disease (I148M) accumulates on lipid droplets by evading ubiquitylation. Hepatology. 2017;66(4):1111-24.
124. Ruhanen H, Perttilä J, Hölttä-Vuori M, Zhou Y, Yki-Järvinen H, Ikonen E, et al. PNPLA3 mediates hepatocyte triacylglycerol remodeling. J Lipid Res. 2014;55(4):739-46.
125. BasuRay S, Wang Y, Smagris E, Cohen JC, Hobbs HH. Accumulation of PNPLA3 on lipid droplets is the basis of associated hepatic steatosis. Proc Natl Acad Sci U S A. 2019;116(19):9521-6.
126. Metwally M, Bayoumi A, Romero-Gomez M, Thabet K, John M, Adams LA, et al. A polymorphism in the Irisin-encoding gene (FNDC5) associates with hepatic steatosis by differential miRNA binding to the 3'UTR. J Hepatol. 2019;70(3):494-500.
127. Eslam M, George J. Genetic contributions to NAFLD: leveraging shared genetics to uncover systems biology. Nat Rev Gastroenterol Hepatol. 2020;17(1):40-52.
128. Bayoumi A, Elsayed A, Han S, Petta S, Adams LA, Aller R, et al. Mistranslation Drives Alterations in Protein Levels and the Effects of a Synonymous Variant at the Fibroblast Growth Factor 21 Locus. Adv Sci (Weinh). 2021;8(11):2004168.
129. Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. Int J Mol Sci. 2014;15(4):6184-223.
130. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994;372(6505):425-32.
131. Chang ML, Yang Z, Yang SS. Roles of Adipokines in Digestive Diseases: Markers of Inflammation, Metabolic Alteration and Disease Progression. Int J Mol Sci. 2020;21(21).
132. Palou A, Serra F, Bonet ML, Picó C. Obesity: molecular bases of a multifactorial problem. Eur J Nutr. 2000;39(4):127-44.
133. Dalamaga M, Chou SH, Shields K, Papageorgiou P, Polyzos SA, Mantzoros CS. Leptin at the intersection of neuroendocrinology and metabolism: current evidence and therapeutic perspectives. Cell Metab. 2013;18(1):29-42.

134. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science. 1995;269(5223):546-9.
135. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 1995;269(5223):543-6.
136. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 1995;269(5223):540-3.
137. Friedman JM. Leptin and the endocrine control of energy balance. Nat Metab. 2019;1(8):754-64.
138. Fruhwürth S, Vogel H, Schürmann A, Williams KJ. Novel Insights into How Overnutrition Disrupts the Hypothalamic Actions of Leptin. Front Endocrinol (Lausanne). 2018;9:89.
139. Ahima RS. Revisiting leptin's role in obesity and weight loss. J Clin Invest. 2008;118(7):2380-3.
140. Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, et al. Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. Neuron. 1999;23(4):775-86.
141. Cowley MA, Smart JL, Rubinstein M, Cerdán MG, Diano S, Horvath TL, et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature. 2001;411(6836):480-4.
142. Timper K, Brüning JC. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. Dis Model Mech. 2017;10(6):679-89.
143. Robertson SA, Leinninger GM, Myers MG, Jr. Molecular and neural mediators of leptin action. Physiol Behav. 2008;94(5):637-42.
144. Huang W, Metlakunta A, Dedousis N, Ortmeyer HK, Stefanovic-Racic M, O'Doherty RM. Leptin augments the acute suppressive effects of insulin on hepatic very low-density lipoprotein production in rats. Endocrinology. 2009;150(5):2169-74.
145. Wang MY, Chen L, Clark GO, Lee Y, Stevens RD, Ilkayeva OR, et al. Leptin therapy in insulin-deficient type I diabetes. Proc Natl Acad Sci U S A. 2010;107(11):4813-9.
146. Cortés VA, Cautivo KM, Rong S, Garg A, Horton JD, Agarwal AK. Leptin ameliorates insulin resistance and hepatic steatosis in Agpat2-/- lipodystrophic mice independent of hepatocyte leptin receptors. J Lipid Res. 2014;55(2):276-88.
147. Hackl MT, Fürnsinn C, Schuh CM, Krssak M, Carli F, Guerra S, et al. Brain leptin reduces liver lipids by increasing hepatic triglyceride secretion and lowering lipogenesis. Nat Commun. 2019;10(1):2717.
148. Adolph TE, Grander C, Grabherr F, Tilg H. Adipokines and Non-Alcoholic Fatty Liver Disease: Multiple Interactions. Int J Mol Sci. 2017;18(8).
149. Polyzos SA, Kountouras J, Zavos C, Deretzi G. The potential adverse role of leptin resistance in nonalcoholic fatty liver disease: a hypothesis based on critical review of the literature. J Clin Gastroenterol. 2011;45(1):50-4.
150. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34(3):274-85.

151. Rotundo L, Persaud A, Feurdean M, Ahlawat S, Kim HS. The Association of leptin with severity of non-alcoholic fatty liver disease: A population-based study. Clin Mol Hepatol. 2018;24(4):392-401.
152. Jiménez-Cortegana C, García-Galey A, Tami M, Del Pino P, Carmona I, López S, et al. Role of Leptin in Non-Alcoholic Fatty Liver Disease. Biomedicines. 2021;9(7).
153. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell. 2012;148(5):852-71.
154. Obata Y, Yamada Y, Takahi Y, Baden MY, Saisho K, Tamba S, et al. Relationship between serum adiponectin levels and age in healthy subjects and patients with type 2 diabetes. Clin Endocrinol (Oxf). 2013;79(2):204-10.
155. Guerre-Millo M. Adipose tissue hormones. J Endocrinol Invest. 2002;25(10):855-61.
156. Fang H, Judd RL. Adiponectin Regulation and Function. Compr Physiol. 2018;8(3):1031-63.
157. Liu M, Liu F. Regulation of adiponectin multimerization, signaling and function. Best Pract Res Clin Endocrinol Metab. 2014;28(1):25-31.
158. Magkos F, Sidossis LS. Recent advances in the measurement of adiponectin isoform distribution. Curr Opin Clin Nutr Metab Care. 2007;10(5):571-5.
159. Parker-Duffen JL, Nakamura K, Silver M, Kikuchi R, Tigges U, Yoshida S, et al. T-cadherin is essential for adiponectin-mediated revascularization. J Biol Chem. 2013;288(34):24886-97.
160. Wang ZV, Scherer PE. Adiponectin, the past two decades. J Mol Cell Biol. 2016;8(2):93-100.
161. Maeda N, Funahashi T, Matsuzawa Y, Shimomura I. Adiponectin, a unique adipocyte-derived factor beyond hormones. Atherosclerosis. 2020;292:1-9.
162. Kita S, Fukuda S, Maeda N, Shimomura I. Native adiponectin in serum binds to mammalian cells expressing T-cadherin, but not AdipoRs or calreticulin. Elife. 2019;8.
163. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest. 2006;116(7):1784-92.
164. Santos J, Maio MC, Lemes MA, Laurindo LF, Haber J, Bechara MD, et al. Non-Alcoholic Steatohepatitis (NASH) and Organokines: What Is Now and What Will Be in the Future. Int J Mol Sci. 2022;23(1).
165. Ding X, Saxena NK, Lin S, Xu A, Srinivasan S, Anania FA. The roles of leptin and adiponectin: a novel paradigm in adipocytokine regulation of liver fibrosis and stellate cell biology. Am J Pathol. 2005;166(6):1655-69.
166. Barbalho SM, Méndez-Sánchez N, Fornari Laurindo L. AdipoRon and ADP355, adiponectin receptor agonists, in Metabolic-associated Fatty Liver Disease (MAFLD) and Nonalcoholic Steatohepatitis (NASH): A systematic review. Biochem Pharmacol. 2023;218:115871.
167. Heiker JT, Klöting N, Blüher M, Beck-Sickinger AG. Access to gram scale amounts of functional globular adiponectin from E. coli inclusion bodies by alkaline-shock solubilization. Biochem Biophys Res Commun. 2010;398(1):32-7.
168. Kim S, Lee Y, Kim JW, Son YJ, Ma MJ, Um JH, et al. Discovery of a novel potent peptide agonist to adiponectin receptor 1. PLoS One. 2018;13(6):e0199256.

169. Otvos L, Jr., Haspinger E, La Russa F, Maspero F, Graziano P, Kovalszky I, et al. Design and development of a peptide-based adiponectin receptor agonist for cancer treatment. BMC Biotechnol. 2011;11:90.
170. Jasinski-Bergner S, Büttner M, Quandt D, Seliger B, Kielstein H. Adiponectin and Its Receptors Are Differentially Expressed in Human Tissues and Cell Lines of Distinct Origin. Obes Facts. 2017;10(6):569-83.
171. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem. 2003;278(41):40352-63.
172. Nigro E, Daniele A, Salzillo A, Ragone A, Naviglio S, Sapio L. AdipoRon and Other Adiponectin Receptor Agonists as Potential Candidates in Cancer Treatments. Int J Mol Sci. 2021;22(11).
173. Okada-Iwabu M, Yamauchi T, Iwabu M, Honma T, Hamagami K, Matsuda K, et al. A small-molecule AdipoR agonist for type 2 diabetes and short life in obesity. Nature. 2013;503(7477):493-9.
174. Zhao C, Wu B, Li J, Jiang Q, Loor JJ, Liu M, et al. AdipoRon alleviates fatty acid-induced lipid accumulation and mitochondrial dysfunction in bovine hepatocytes by promoting autophagy. J Dairy Sci. 2023;106(8):5763-74.
175. Selvais CM, Davis-López de Carrizosa MA, Nachit M, Versele R, Dubuisson N, Noel L, et al. AdipoRon enhances healthspan in middle-aged obese mice: striking alleviation of myosteatosis and muscle degenerative markers. J Cachexia Sarcopenia Muscle. 2023;14(1):464-78.
176. Massart IS, Kouakou AN, Pelet N, Lause P, Schakman O, Loumaye A, et al. Administration of adiponectin receptor agonist AdipoRon relieves cancer cachexia by mitigating inflammation in tumour-bearing mice. J Cachexia Sarcopenia Muscle. 2024;15(3):919-33.
177. Wang H, Zhang H, Zhang Z, Huang B, Cheng X, Wang D, et al. Adiponectin-derived active peptide ADP355 exerts anti-inflammatory and anti-fibrotic activities in thioacetamide-induced liver injury. Sci Rep. 2016;6:19445.
178. Das A, Tang YLM, Althumiri NA, Garcia-Larsen V, Schattenberg JM, Alqahtani SA. Fatty acid composition but not quantity is an important indicator of non-alcoholic fatty liver disease: a systematic review. Eur J Clin Nutr. 2023;77(12):1113-29.
179. Mattace Raso G, Simeoli R, Russo R, Iacono A, Santoro A, Paciello O, et al. Effects of sodium butyrate and its synthetic amide derivative on liver inflammation and glucose tolerance in an animal model of steatosis induced by high fat diet. PLoS One. 2013;8(7):e68626.
180. Ferramosca A, Zara V. Modulation of hepatic steatosis by dietary fatty acids. World J Gastroenterol. 2014;20(7):1746-55.
181. Zelber-Sagi S, Ivancovsky-Wajcman D, Fliss Isakov N, Webb M, Orenstein D, Shibolet O, et al. High red and processed meat consumption is associated with non-alcoholic fatty liver disease and insulin resistance. J Hepatol. 2018;68(6):1239-46.
182. Kuchay MS, Choudhary NS, Mishra SK. Pathophysiological mechanisms underlying MAFLD. Diabetes Metab Syndr. 2020;14(6):1875-87.
183. Luukkonen PK, Sädevirta S, Zhou Y, Kayser B, Ali A, Ahonen L, et al. Saturated Fat Is More Metabolically Harmful for the Human Liver Than Unsaturated Fat or Simple Sugars. Diabetes Care. 2018;41(8):1732-9.
184. Mouzaki M, Allard JP. The role of nutrients in the development, progression, and treatment of nonalcoholic fatty liver disease. J Clin Gastroenterol. 2012;46(6):457-67.

185. Riazi K, Raman M, Taylor L, Swain MG, Shaheen AA. Dietary Patterns and Components in Nonalcoholic Fatty Liver Disease (NAFLD): What Key Messages Can Health Care Providers Offer? Nutrients. 2019;11(12).
186. Puri P, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, et al. A lipidomic analysis of nonalcoholic fatty liver disease. Hepatology. 2007;46(4):1081-90.
187. Chiappini F, Coilly A, Kadar H, Gual P, Tran A, Desterke C, et al. Metabolism dysregulation induces a specific lipid signature of nonalcoholic steatohepatitis in patients. Sci Rep. 2017;7:46658.
188. Guo XF, Yang B, Tang J, Li D. Fatty acid and non-alcoholic fatty liver disease: Meta-analyses of case-control and randomized controlled trials. Clin Nutr. 2018;37(1):113-22.
189. Kawashima H. Intake of arachidonic acid-containing lipids in adult humans: dietary surveys and clinical trials. Lipids Health Dis. 2019;18(1):101.
190. National Research Council Subcommittee on the Tenth Edition of the Recommended Dietary A. The National Academies Collection: Reports funded by National Institutes of Health. Recommended Dietary Allowances: 10th Edition. Washington (DC): National Academies Press (US)
Copyright © 1989 by the National Academy of Sciences.; 1989.
191. Heymsfield SB, Shapses SA. Guidance on Energy and Macronutrients across the Life Span. N Engl J Med. 2024;390(14):1299-310.
192. Raatz SK, Conrad Z, Jahns L. Trends in linoleic acid intake in the United States adult population: NHANES 1999-2014. Prostaglandins Leukot Essent Fatty Acids. 2018;133:23-8.
193. Pertiwi K, Kok DE, Wanders AJ, de Goede J, Zock PL, Geleijnse JM. Circulating n-3 fatty acids and linoleic acid as indicators of dietary fatty acid intake in post-myocardial infarction patients. Nutr Metab Cardiovasc Dis. 2019;29(4):343-50.
194. Yamada K, Mizukoshi E, Sunagozaka H, Arai K, Yamashita T, Takeshita Y, et al. Characteristics of hepatic fatty acid compositions in patients with nonalcoholic steatohepatitis. Liver Int. 2015;35(2):582-90.
195. Rosqvist F, Bjermo H, Kullberg J, Johansson L, Michaëlsson K, Ahlström H, et al. Fatty acid composition in serum cholesterol esters and phospholipids is linked to visceral and subcutaneous adipose tissue content in elderly individuals: a cross-sectional study. Lipids Health Dis. 2017;16(1):68.
196. Belury MA, Cole RM, Bailey BE, Ke JY, Andridge RR, Kiecolt-Glaser JK. Erythrocyte linoleic acid, but not oleic acid, is associated with improvements in body composition in men and women. Mol Nutr Food Res. 2016;60(5):1206-12.
197. Yan JH, Guan BJ, Gao HY, Peng XE. Omega-3 polyunsaturated fatty acid supplementation and non-alcoholic fatty liver disease: A meta-analysis of randomized controlled trials. Medicine (Baltimore). 2018;97(37):e12271.
198. Musa-Veloso K, Venditti C, Lee HY, Darch M, Floyd S, West S, et al. Systematic review and meta-analysis of controlled intervention studies on the effectiveness of long-chain omega-3 fatty acids in patients with nonalcoholic fatty liver disease. Nutr Rev. 2018;76(8):581-602.
199. Lu W, Li S, Li J, Wang J, Zhang R, Zhou Y, et al. Effects of Omega-3 Fatty Acid in Nonalcoholic Fatty Liver Disease: A Meta-Analysis. Gastroenterol Res Pract. 2016;2016:1459790.
200. Garcêz LS, Avelar CR, Fonseca NSS, Costa PRF, Lyra AC, Cunha CM, et al. Effect of dietary carbohydrate and lipid modification on clinical and anthropometric parameters in

nonalcoholic fatty liver disease: a systematic review and meta-analysis. Nutr Rev. 2021;79(12):1321-37.
201. Capanni M, Calella F, Biagini MR, Genise S, Raimondi L, Bedogni G, et al. Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study. Aliment Pharmacol Ther. 2006;23(8):1143-51.
202. Parker HM, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Hepatol. 2012;56(4):944-51.
203. Czumaj A, Śledziński T. Biological Role of Unsaturated Fatty Acid Desaturases in Health and Disease. Nutrients. 2020;12(2).
204. Mäkelä TNK, Tuomainen TP, Hantunen S, Virtanen JK. Associations of serum n-3 and n-6 polyunsaturated fatty acids with prevalence and incidence of nonalcoholic fatty liver disease. Am J Clin Nutr. 2022;116(3):759-70.
205. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated Fatty acids. J Nutr Metab. 2012;2012:539426.
206. Kang S, Koh JM, Im DS. N-3 Polyunsaturated Fatty Acids Protect against Alcoholic Liver Steatosis by Activating FFA4 in Kupffer Cells. Int J Mol Sci. 2024;25(10).
207. Echeverría F, Ortiz M, Valenzuela R, Videla LA. Long-chain polyunsaturated fatty acids regulation of PPARs, signaling: Relationship to tissue development and aging. Prostaglandins Leukot Essent Fatty Acids. 2016;114:28-34.
208. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, et al. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med. 2005;11(1):90-4.
209. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell. 2010;142(5):687-98.
210. Qu W, Ma T, Cai J, Zhang X, Zhang P, She Z, et al. Liver Fibrosis and MAFLD: From Molecular Aspects to Novel Pharmacological Strategies. Front Med (Lausanne). 2021;8:761538.
211. Eslam M, Sarin SK, Wong VW, Fan JG, Kawaguchi T, Ahn SH, et al. The Asian Pacific Association for the Study of the Liver clinical practice guidelines for the diagnosis and management of metabolic associated fatty liver disease. Hepatol Int. 2020;14(6):889-919.
212. Raza S, Rajak S, Upadhyay A, Tewari A, Anthony Sinha R. Current treatment paradigms and emerging therapies for NAFLD/NASH. Front Biosci (Landmark Ed). 2021;26(2):206-37.
213. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64(6):1388-402.
214. Mundi MS, Velapati S, Patel J, Kellogg TA, Abu Dayyeh BK, Hurt RT. Evolution of NAFLD and Its Management. Nutr Clin Pract. 2020;35(1):72-84.
215. Koutoukidis DA, Astbury NM, Tudor KE, Morris E, Henry JA, Noreik M, et al. Association of Weight Loss Interventions With Changes in Biomarkers of Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis. JAMA Intern Med. 2019;179(9):1262-71.
216. Hall KD, Kahan S. Maintenance of Lost Weight and Long-Term Management of Obesity. Med Clin North Am. 2018;102(1):183-97.
217. Loveman E, Frampton GK, Shepherd J, Picot J, Cooper K, Bryant J, et al. The clinical effectiveness and cost-effectiveness of long-term weight management schemes for adults: a systematic review. Health Technol Assess. 2011;15(2):1-182.

218. Wing RR, Phelan S. Long-term weight loss maintenance. Am J Clin Nutr. 2005;82(1 Suppl):222s-5s.
219. Anderson JW, Konz EC, Frederich RC, Wood CL. Long-term weight-loss maintenance: a meta-analysis of US studies. Am J Clin Nutr. 2001;74(5):579-84.
220. Abou Assi R, Abdulbaqi IM, Siok Yee C. The Evaluation of Drug Delivery Nanocarrier Development and Pharmacological Briefing for Metabolic-Associated Fatty Liver Disease (MAFLD): An Update. Pharmaceuticals (Basel). 2021;14(3).
221. Bugianesi E, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, et al. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. Am J Gastroenterol. 2005;100(5):1082-90.
222. Zang M, Zuccollo A, Hou X, Nagata D, Walsh K, Herscovitz H, et al. AMP-activated protein kinase is required for the lipid-lowering effect of metformin in insulin-resistant human HepG2 cells. J Biol Chem. 2004;279(46):47898-905.
223. Hinds TD, Jr., Burns KA, Hosick PA, McBeth L, Nestor-Kalinoski A, Drummond HA, et al. Biliverdin Reductase A Attenuates Hepatic Steatosis by Inhibition of Glycogen Synthase Kinase (GSK) 3β Phosphorylation of Serine 73 of Peroxisome Proliferator-activated Receptor (PPAR) α. J Biol Chem. 2016;291(48):25179-91.
224. Steinberg GR, Macaulay SL, Febbraio MA, Kemp BE. AMP-activated protein kinase--the fat controller of the energy railroad. Can J Physiol Pharmacol. 2006;84(7):655-65.
225. Xian YX, Weng JP, Xu F. MAFLD vs. NAFLD: shared features and potential changes in epidemiology, pathophysiology, diagnosis, and pharmacotherapy. Chin Med J (Engl). 2020;134(1):8-19.
226. Pereira MJ, Eriksson JW. Emerging Role of SGLT-2 Inhibitors for the Treatment of Obesity. Drugs. 2019;79(3):219-30.
227. Badmus OO, Hillhouse SA, Anderson CD, Hinds TD, Stec DE. Molecular mechanisms of metabolic associated fatty liver disease (MAFLD): functional analysis of lipid metabolism pathways. Clin Sci (Lond). 2022;136(18):1347-66.
228. Taheri H, Malek M, Ismail-Beigi F, Zamani F, Sohrabi M, Reza Babaei M, et al. Effect of Empagliflozin on Liver Steatosis and Fibrosis in Patients With Non-Alcoholic Fatty Liver Disease Without Diabetes: A Randomized, Double-Blind, Placebo-Controlled Trial. Adv Ther. 2020;37(11):4697-708.
229. Lai LL, Vethakkan SR, Nik Mustapha NR, Mahadeva S, Chan WK. Empagliflozin for the Treatment of Nonalcoholic Steatohepatitis in Patients with Type 2 Diabetes Mellitus. Dig Dis Sci. 2020;65(2):623-31.
230. Sattar N, Fitchett D, Hantel S, George JT, Zinman B. Empagliflozin is associated with improvements in liver enzymes potentially consistent with reductions in liver fat: results from randomised trials including the EMPA-REG OUTCOME® trial. Diabetologia. 2018;61(10):2155-63.
231. Jojima T, Tomotsune T, Iijima T, Akimoto K, Suzuki K, Aso Y. Empagliflozin (an SGLT2 inhibitor), alone or in combination with linagliptin (a DPP-4 inhibitor), prevents steatohepatitis in a novel mouse model of non-alcoholic steatohepatitis and diabetes. Diabetol Metab Syndr. 2016;8:45.
232. Kenny PR, Brady DE, Torres DM, Ragozzino L, Chalasani N, Harrison SA. Exenatide in the treatment of diabetic patients with non-alcoholic steatohepatitis: a case series. Am J Gastroenterol. 2010;105(12):2707-9.

233. Clark L. GLP-1 receptor agonists: A review of glycemic benefits and beyond. Jaapa. 2024;37(4):1-4.
234. Shao N, Yu XY, Ma XF, Lin WJ, Hao M, Kuang HY. Exenatide Delays the Progression of Nonalcoholic Fatty Liver Disease in C57BL/6 Mice, Which May Involve Inhibition of the NLRP3 Inflammasome through the Mitophagy Pathway. Gastroenterol Res Pract. 2018;2018:1864307.
235. Svegliati-Baroni G, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, et al. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. Liver Int. 2011;31(9):1285-97.
236. Budd J, Cusi K. Role of Agents for the Treatment of Diabetes in the Management of Nonalcoholic Fatty Liver Disease. Curr Diab Rep. 2020;20(11):59.
237. Daniels SJ, Leeming DJ, Detlefsen S, Bruun MF, Hjuler ST, Henriksen K, et al. Biochemical and histological characterisation of an experimental rodent model of non-alcoholic steatohepatitis - Effects of a peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist and a glucagon-like peptide-1 analogue. Biomed Pharmacother. 2019;111:926-33.
238. Trevaskis JL, Griffin PS, Wittmer C, Neuschwander-Tetri BA, Brunt EM, Dolman CS, et al. Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. Am J Physiol Gastrointest Liver Physiol. 2012;302(8):G762-72.
239. Fan H, Pan Q, Xu Y, Yang X. Exenatide improves type 2 diabetes concomitant with non-alcoholic fatty liver disease. Arq Bras Endocrinol Metabol. 2013;57(9):702-8.
240. Bril F, Kalavalapalli S, Clark VC, Lomonaco R, Soldevila-Pico C, Liu IC, et al. Response to Pioglitazone in Patients With Nonalcoholic Steatohepatitis With vs Without Type 2 Diabetes. Clin Gastroenterol Hepatol. 2018;16(4):558-66.e2.
241. Loomba R, Abdelmalek MF, Armstrong MJ, Jara M, Kjær MS, Krarup N, et al. Semaglutide 2·4 mg once weekly in patients with non-alcoholic steatohepatitis-related cirrhosis: a randomised, placebo-controlled phase 2 trial. Lancet Gastroenterol Hepatol. 2023;8(6):511-22.
242. Norris LE, Collene AL, Asp ML, Hsu JC, Liu LF, Richardson JR, et al. Comparison of dietary conjugated linoleic acid with safflower oil on body composition in obese postmenopausal women with type 2 diabetes mellitus. Am J Clin Nutr. 2009;90(3):468-76.
243. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Glaser R. Omega-3 supplementation lowers inflammation and anxiety in medical students: a randomized controlled trial. Brain Behav Immun. 2011;25(8):1725-34.
244. Kiecolt-Glaser JK, Belury MA, Andridge R, Malarkey WB, Hwang BS, Glaser R. Omega-3 supplementation lowers inflammation in healthy middle-aged and older adults: a randomized controlled trial. Brain Behav Immun. 2012;26(6):988-95.
245. Yu H, McKenzie CA, Shimakawa A, Vu AT, Brau AC, Beatty PJ, et al. Multiecho reconstruction for simultaneous water-fat decomposition and T2* estimation. J Magn Reson Imaging. 2007;26(4):1153-61.
246. Yoo YH, Kim HS, Lee YH, Yoon CS, Paek MY, Yoo H, et al. Comparison of Multi-Echo Dixon Methods with Volume Interpolated Breath-Hold Gradient Echo Magnetic Resonance Imaging in Fat-Signal Fraction Quantification of Paravertebral Muscle. Korean J Radiol. 2015;16(5):1086-95.

247. Wald D, Teucher B, Dinkel J, Kaaks R, Delorme S, Boeing H, et al. Automatic quantification of subcutaneous and visceral adipose tissue from whole-body magnetic resonance images suitable for large cohort studies. J Magn Reson Imaging. 2012;36(6):1421-34.
248. Yokoo T, Serai SD, Pirasteh A, Bashir MR, Hamilton G, Hernando D, et al. Linearity, Bias, and Precision of Hepatic Proton Density Fat Fraction Measurements by Using MR Imaging: A Meta-Analysis. Radiology. 2018;286(2):486-98.
249. Middleton MS, Heba ER, Hooker CA, Bashir MR, Fowler KJ, Sandrasegaran K, et al. Agreement Between Magnetic Resonance Imaging Proton Density Fat Fraction Measurements and Pathologist-Assigned Steatosis Grades of Liver Biopsies From Adults With Nonalcoholic Steatohepatitis. Gastroenterology. 2017;153(3):753-61.
250. Moura Cunha G, Fan B, Navin PJ, Olivié D, Venkatesh SK, Ehman RL, et al. Interpretation, Reporting, and Clinical Applications of Liver MR Elastography. Radiology. 2024;310(3):e231220.
251. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. Am J Clin Nutr. 2007;86(1):74-81.
252. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957;226(1):497-509.
253. Stoffel W, Chu, F, Ahrens, EH. Analysis of long-chain fatty acids by gas-liquid chromatography. Anal Chem. 1959;31: p. 307-8.
254. Whelan J. The health implications of changing linoleic acid intakes. Prostaglandins Leukot Essent Fatty Acids. 2008;79(3-5):165-7.
255. Morrison WR, Smith LM. PREPARATION OF FATTY ACID METHYL ESTERS AND DIMETHYLACETALS FROM LIPIDS WITH BORON FLUORIDE--METHANOL. J Lipid Res. 1964;5:600-8.
256. Belury MA, Kempa-Steczko A. Conjugated linoleic acid modulates hepatic lipid composition in mice. Lipids. 1997;32(2):199-204.
257. Lin SC, Heba E, Wolfson T, Ang B, Gamst A, Han A, et al. Noninvasive Diagnosis of Nonalcoholic Fatty Liver Disease and Quantification of Liver Fat Using a New Quantitative Ultrasound Technique. Clin Gastroenterol Hepatol. 2015;13(7):1337-45.e6.
258. Ebrahimi-Mamaeghani M, Mohammadi S, Arefhosseini SR, Fallah P, Bazi Z. Adiponectin as a potential biomarker of vascular disease. Vasc Health Risk Manag. 2015;11:55-70.
259. Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol. 2003;23(1):85-9.
260. Hashimoto N, Kanda J, Nakamura T, Horie A, Kurosawa H, Hashimoto T, et al. Association of hypoadiponectinemia in men with early onset of coronary heart disease and multiple coronary artery stenoses. Metabolism. 2006;55(12):1653-7.
261. van der Poorten D, Samer CF, Ramezani-Moghadam M, Coulter S, Kacevska M, Schrijnders D, et al. Hepatic fat loss in advanced nonalcoholic steatohepatitis: are alterations in serum adiponectin the cause? Hepatology. 2013;57(6):2180-8.
262. Pisto P, Ukkola O, Santaniemi M, Kesäniemi YA. Plasma adiponectin--an independent indicator of liver fat accumulation. Metabolism. 2011;60(11):1515-20.

263. Yi X, Han L, Li L, Zhu H, Li M, Gao S. Adipokine/hepatokines profiling of fatty liver in adolescents and young adults: cross-sectional and prospective analyses of the BCAMS study. Hepatol Int. 2024.
264. Kim YS, Lee SH, Park SG, Won BY, Chun H, Cho DY, et al. Low levels of total and high-molecular-weight adiponectin may predict non-alcoholic fatty liver in Korean adults. Metabolism. 2020;103:154026.
265. Polyzos SA, Kountouras J, Mantzoros CS. Adipokines in nonalcoholic fatty liver disease. Metabolism. 2016;65(8):1062-79.
266. Khoramipour K, Chamari K, Hekmatikar AA, Ziyaiyan A, Taherkhani S, Elguindy NM, et al. Adiponectin: Structure, Physiological Functions, Role in Diseases, and Effects of Nutrition. Nutrients. 2021;13(4).
267. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med. 2002;8(11):1288-95.
268. Mandal P, Pritchard MT, Nagy LE. Anti-inflammatory pathways and alcoholic liver disease: role of an adiponectin/interleukin-10/heme oxygenase-1 pathway. World J Gastroenterol. 2010;16(11):1330-6.
269. Zhou L, Deepa SS, Etzler JC, Ryu J, Mao X, Fang Q, et al. Adiponectin activates AMP-activated protein kinase in muscle cells via APPL1/LKB1-dependent and phospholipase C/Ca2+/Ca2+/calmodulin-dependent protein kinase kinase-dependent pathways. J Biol Chem. 2009;284(33):22426-35.
270. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology. 2010;52(5):1836-46.
271. Combs TP, Marliss EB. Adiponectin signaling in the liver. Rev Endocr Metab Disord. 2014;15(2):137-47.
272. Mantovani A, Zusi C, Csermely A, Salvagno GL, Colecchia A, Lippi G, et al. Association between lower plasma adiponectin levels and higher liver stiffness in type 2 diabetic individuals with nonalcoholic fatty liver disease: an observational cross-sectional study. Hormones (Athens). 2022;21(3):477-86.
273. Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Metabolism - Clinical and Experimental. 2011;60(3):313-26.
274. Warensjö E, Sundström J, Lind L, Vessby B. Factor analysis of fatty acids in serum lipids as a measure of dietary fat quality in relation to the metabolic syndrome in men. Am J Clin Nutr. 2006;84(2):442-8.
275. Chiva-Blanch G, Giró O, Cofán M, Calle-Pascual AL, Delgado E, Gomis R, et al. Low Percentage of Vegetable Fat in Red Blood Cells Is Associated with Worse Glucose Metabolism and Incidence of Type 2 Diabetes. Nutrients. 2022;14(7).
276. Alsharari ZD, Risérus U, Leander K, Sjögren P, Carlsson AC, Vikström M, et al. Serum Fatty Acids, Desaturase Activities and Abdominal Obesity - A Population-Based Study of 60-Year Old Men and Women. PLoS One. 2017;12(1):e0170684.
277. Harbers MC, Pertiwi K, Soedamah-Muthu SS, de Goede J, Mölenberg FJ, Wanders AJ, et al. Abstract P215: Plasma and Dietary Linoleic Acid and Diabetes Incidence After Myocardial Infarction. Circulation. 2018;137(suppl_1):AP215-AP.

278. Forouhi NG, Imamura F, Sharp SJ, Koulman A, Schulze MB, Zheng J, et al. Association of Plasma Phospholipid n-3 and n-6 Polyunsaturated Fatty Acids with Type 2 Diabetes: The EPIC-InterAct Case-Cohort Study. PLoS Med. 2016;13(7):e1002094.
279. Asp ML, Collene AL, Norris LE, Cole RM, Stout MB, Tang SY, et al. Time-dependent effects of safflower oil to improve glycemia, inflammation and blood lipids in obese, post-menopausal women with type 2 diabetes: a randomized, double-masked, crossover study. Clin Nutr. 2011;30(4):443-9.
280. Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML, et al. Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. Diabetologia. 2002;45(3):369-77.
281. Jackson KH, Harris WS, Belury MA, Kris-Etherton PM, Calder PC. Beneficial effects of linoleic acid on cardiometabolic health: an update. Lipids Health Dis. 2024;23(1):296.
282. Shearer GC, Walker RE. An overview of the biologic effects of omega-6 oxylipins in humans. Prostaglandins Leukot Essent Fatty Acids. 2018;137:26-38.
283. Belury MA. Linoleic acid, an omega-6 fatty acid that reduces risk for cardiometabolic diseases: premise, promise and practical implications. Curr Opin Clin Nutr Metab Care. 2023;26(3):288-92.
284. Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. Cell. 1998;93(2):229-40.