

UNIVERSITY OF CALGARY

Worldwide Prevalence and Incidence of Non-Alcoholic Fatty Liver Disease (NAFLD) in the 21<sup>st</sup>  
Century: A Systematic Review and Meta-Analysis

by

Kiarash Riazi

A THESIS

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## **Abstract:**

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of lipid deposition in more than 5% of hepatocytes, also known as fatty liver, in the absence of any history of excessive alcohol use or secondary causes of fatty liver. NAFLD is the most common liver disorder worldwide and the leading cause of liver-related morbidity and mortality, mainly because of cirrhosis, end-stage liver disease, liver transplant, and an increased incidence of hepatocellular carcinoma.

The prevalence of NAFLD is constantly rising. Due to a silent presentation, high prevalence, and the detrimental outcomes associated with it, NAFLD is a major global health problem. Since there are currently no approved treatments for NAFLD, disease prevention is the only available option for reducing the disease burden. Since primary epidemiological data are the crucial cornerstones for every public health and preventive medicine attempt, updated and accurate epidemiological data are of utmost importance. There are currently a few existing research publications on the prevalence and incidence of NAFLD worldwide, which are either outdated, methodologically imperfect, or geographically restricted. Therefore, there is a need to update current data and accurately estimate NAFLD's worldwide prevalence and incidence.

We performed a systematic review to identify all the studies reporting on the prevalence or incidence of NAFLD among the general adult population of different world regions over the past two decades. We used a random-effects meta-analysis method to calculate overall or sex-specific pooled prevalence or incidence rates. We also created static and interactive choropleth maps of pooled data to highlight the geographic differences in NAFLD prevalence/incidence.

We estimated that the overall prevalence of NAFLD is appreciably higher than what had previously been assessed and is growing at an alarming rate. Furthermore, our findings show that the epidemiological reports regarding NAFLD from many parts of the world are missing. A dramatic rise in prevalence should drive enhanced awareness of NAFLD among primary care physicians, public health specialists, and health policy makers to encourage the development of more effective preventive policies.

## **Preface:**

This thesis is written in a manuscript-based format. Chapter 1 is an introductory chapter on Non-alcoholic fatty liver disease (NAFLD). The 2<sup>nd</sup> and 3<sup>rd</sup> chapters include the main research project and its knowledge translation implications, the study of NAFLD's worldwide prevalence and incidence.

**Chapter 1:** "Introduction to NAFLD" uses with permission, materials from a recently published co-authored narrative review article entitled: **Kiarash Riazi**, Maitreyi Raman, Lorian Taylor, Mark G. Swain, Abdel Aziz Shaheen. Dietary patterns and components in nonalcoholic fatty liver disease (NAFLD): What key messages can health care providers offer? *Nutrients*. 2019; 11: 2878.

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**Dedication:**

*To my Leyla, for her endless love, support, and encouragement.*

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## List of Abbreviations:

AASLD	American Association for the Study of Liver Diseases
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CAP	Controlled attenuation parameter
CKD	Chronic kidney disease
CVD	Cardiovascular diseases
DASH	Dietary Approaches to Stop Hypertension
EASD	European Association for the Study of Diabetes
EASL	European Association for the Study of the Liver
EASO	European Association for the Study of Obesity
FA	Fatty acid
FFA	Free fatty acid
FLI	Fatty liver index
GGT	Gamma-glutamyl-transferase
HCC	Hepatocellular carcinoma
HDL	High-density lipoprotein
HSI	Hepatic steatosis index
IHTGs	Intrahepatic triglycerides
IR	Insulin resistance
LC	Low carbohydrates
LF	Low fat

MAFLD	Metabolic dysfunction-associated fatty liver disease
MD	Mediterranean
MetS	Metabolic syndrome
MRS	Magnetic resonance spectroscopy
MUFA	Monounsaturated fatty acids
NAFL	Non-alcoholic fatty liver
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NEFAs	Non-esterified fatty acids
NHANES	National Health and Nutrition Examination Survey
PNPLA3	Patatin-like phospholipase domain-containing 3
PPAR $\gamma$	Peroxisome proliferator-activated receptor gamma
PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
T2DM	Type 2 diabetes mellitus
TG	Triglyceride
VLDL	Very low-density lipoprotein

## **Chapter 1: Introduction to Non-alcoholic fatty liver disease (NAFLD)**

### **1.1 What is NAFLD?**

Deposition of excessive fat inside the liver, also known as fatty liver, can occur as a consequence of many factors, some of which include excessive alcohol consumption, viral infections, medications, and may be observed with other medical diagnoses such as inflammatory bowel disease (1, 2). However, when the fatty liver is a consequence of over-nutrition and insulin resistance, the condition is referred to as NAFLD (3). NAFLD comprises a spectrum of liver histopathology ranging from the presence of a benign fatty liver, referred to as non-alcoholic fatty liver (NAFL), to fatty liver superimposed by inflammation, hepatocyte death, and fibrosis, also known as non-alcoholic steatohepatitis (NASH) (1). NAFLD is the most common liver disorder worldwide (4). Importantly, as recently reported, NASH is the second most common indication for being listed for liver transplants in the United States (5). The diagnosis of NAFLD requires evidence of hepatic steatosis, either by imaging or histology, and the absence of other causes of hepatic steatosis, like significant alcohol consumption, viral hepatitis, use of steatogenic medications, etc. (1). Most patients with NAFLD are either asymptomatic or experience non-specific symptoms, including fatigue and right upper quadrant abdominal discomfort. The diagnosis of NAFLD is frequently suspected incidentally following lab work identifying abnormal liver biochemistry, in the absence of any alternative explanation, or occasionally following hepatomegaly identification on physical examination, or sonographic features of steatosis (6). Therefore, the subtle presentation, high prevalence, and the hepatic and extra-hepatic detrimental outcomes associated with NAFLD make it a serious global health problem. While obesity and overweight are established risk factors for NAFLD and weight loss is recognized as effective therapy, long-term sustainability of weight loss is achievable only in a limited proportion of

patients (1). The objectives of this manuscript are to present an overview of the prevalence, risk factors, and pathophysiology of NAFLD, and focus on a detailed review of dietary patterns as a NAFLD treatment independent of weight loss and provide key dietary messages for health care providers to implement into clinical practice.

## 1.2 Natural History of NAFLD

Under normal circumstances, only a small amount of fat deposits are stored in hepatocytes (7). NAFL is diagnosed when there is evidence, either by imaging or histology, that over 5% of hepatocytes are filled with fat, in the absence of any history of excessive alcohol use or any secondary causes of fatty liver (1). The amount of alcohol is considered excessive when the patient reports drinking three units or more of alcohol per day for men and two units or more of alcohol per day for women (2). Each unit or standard drink is equivalent to 10 g of alcohol and is equal to 250 mL of beer, 200 mL of wine, or 30 mL of whiskey (2). Interestingly, the definition of a standard unit varies in different countries (8). The American Association for Study of Liver Diseases has defined significant alcohol consumption as >21 standard drinks per week for men and >14 standard drinks per week for women (1). In contrast, NASH is a pathological diagnosis, defined as the presence of NAFL complicated by inflammation and hepatocyte injury (e.g., ballooned hepatocytes or Mallory hyaline), with or without fibrosis (1). NASH may be a progressive diagnosis, resulting in advanced stages of fibrosis or cirrhosis (1). Aside from cirrhosis and the detrimental complications associated with it, NAFLD and NASH are concerning diagnoses due to the increased prevalence of hepatocellular carcinoma (HCC), especially with NASH (9, 10). The annual incidence of HCC in NAFLD patients is estimated to be 0.44 per 1000 person-years, whereas in patients with NASH, this incidence rate increases to 5.29 per 1000 person-years (4).

A recent meta-analysis of 86 studies from 22 countries with a total sample size of 8.5 million estimated the worldwide prevalence of NAFLD as 25.2% (95%CI: 22.1%–28.7%) (4). When stratified by geographical regions, the Middle East had the highest prevalence with 31.8%, followed closely by South America with 30.5%, Asia with 27.4%, Europe with 23.7%, and North America with 21.1%. The lowest prevalence was reported in Africa, with 13.5% (4). The gold standard to diagnose NASH is a liver biopsy, an invasive procedure with possible major complications, limiting a population estimate. The meta-analysis identified the prevalence of NASH to be 59.1% (47.6–69.7) among biopsied NAFLD patients and 6.7% (2.2%, 18.7%) among patients biopsied for reasons other than NAFLD (4). However, the global prevalence of NASH is estimated to be between 3% and 6% (11), while the prevalence of NASH cirrhosis is estimated to be 0.18% (12). The overall annual fibrosis progression rate from a baseline stage of no fibrosis (i.e., F0) in patients with NASH is estimated to be 0.09 stage (95% CI, 0.06–0.12), with 40.8% of patients showing fibrosis progression (4).

NAFLD is considered a component or hepatic manifestation of metabolic syndrome (MetS) (3). MetS is a cluster of metabolic abnormalities that result from insulin resistance and are often identified in obese and sedentary patients (13, 14). MetS is diagnosed when central obesity in addition to any two of the following components are present in an individual: High blood triglyceride (TG) levels, low high-density lipoprotein (HDL) cholesterol levels, hypertension, increased fasting blood glucose, or established type 2 diabetes mellitus (T2DM) (15). In Western countries, the prevalence of MetS is estimated to affect 20% of the adult population (16), and an increased prevalence of NAFLD has been reported in patients with MetS. For example, a meta-analysis of 80 studies from 20 countries in individuals with T2DM ( $n = 49,419$ ) demonstrated a prevalence of NAFLD of 55.5% (47.3%–63.7%) (17). Similarly, MetS or T2DM is present in as

high as 85% of NAFLD patients (18). Hypertension is also more common among NAFLD patients than in the control non-NAFLD group (OR = 1.24, 95% CI: 1.14–1.36) (19). Since the components of MetS are all risk factors for cardiovascular morbidity and mortality, cardiac-related comorbidity and death are increased in patients with NAFLD. Of major concern, cardiovascular disease is the main cause of mortality in patients with NAFLD (1), significantly surpassing liver-related mortality among NAFLD patients (48% vs. 7%, respectively) (20). Notably, only 0.1% of all deaths in the general population are liver-related (20). Although obesity and MetS are the major risk factors for NAFLD, up to 30% of NAFLD patients are neither obese nor have any of the MetS components (21). Lean NAFLD cases highlight a role for genetic susceptibility in the pathogenesis of NAFLD (21).

### 1.3 Pathogenesis of NAFLD and NASH

The pathophysiological hallmark of NAFLD is insulin resistance (IR) (22). IR leads to elevated portal and circulating free fatty acids, which subsequently result in increased hepatic uptake. When increased hepatic fatty acid input (uptake and biosynthesis) overcomes hepatic clearance by beta-oxidation or by secretion as very-low-density lipoprotein (VLDL), fatty acids accumulate within hepatocytes as triglycerides (TGs) (23-25). These TGs are secreted into the bloodstream in the form of VLDL to be transferred to adipose tissues. Under conditions where hepatocytes cannot fully secrete all TGs as VLDL, the TGs are stored as cytosolic lipid droplets within hepatocytes as lipid vesicles isolated from the cytoplasm by a phospholipid monolayer (26).

The source of fatty acid (FA) input to the liver is through either dietary fat intake, in the form of chylomicrons, or plasma-free FA pool (also known as circulating non-esterified fatty acids

(NEFAs), which are released from adipose tissue) (23). Additionally, the hepatic uptake of excess dietary carbohydrates can also be used as a substrate for FA biosynthesis and is another important source for FA input to the liver (24). The relative contribution of each source of FA is different between fed and fasting states. Donnelly *et al.* (2005) showed that in the fasting state, the contributions of peripheral NEFAs, FA biosynthesis, and dietary fats to hepatic fat content were 59% (45.1%–74.3%), 26.1% (12.7%–37%), and 14.9% (4.3%–28%), respectively (24). Therefore, NEFA originating from visceral adipose tissue may be the primary lipid source for increased hepatic fat content in NAFLD, followed by de novo biosynthesis and diet. Free fatty acids (FFAs) may be oxidized for routine use; however, in NAFLD, increased FFA oxidation causes oxidative stress that uncouples mitochondrial oxidation and phosphorylation and generates reactive oxygen species (ROS) (25). Mitochondria have several defense mechanisms against toxic by-products, such as ROS (e.g., anti-oxidants, glutathione, catalase, etc.); if these defense mechanisms are overcome, organelle damage may occur (27). Mitochondrial damage might result in decreased lipid oxidation or even cell death, which in turn can lead to inflammation, production of cytokines, recruitment of inflammatory cells, generation of fibrosis, and progression to NASH (25).

Although the pathophysiological outcomes of NASH progression are not entirely understood, it has been proposed that in NAFLD, the liver is vulnerable to parallel hits stemming from localized oxidative stress and pro-inflammatory mediators generated in peripheral tissues (28). The literature related to the gut–liver axis and its relationship to NAFLD is growing. Multiple preclinical and clinical studies have identified an increased prevalence of small intestinal bacterial overgrowth and changes in gut microbial composition and their influence on intestinal permeability in NAFLD/NASH (29, 30). Advanced microbiome assessment methods, such as shotgun metagenomic sequencing, have identified an association between gut microbiome

signatures and the presence of advanced NAFLD fibrosis (31). Furthermore, gut microbiome dysbiosis may contribute toward intestinal barrier damage, leading to hepatic inflammation in the setting of enriched dietary fat composition (32). High-fat diets and diets depleted in fiber lead to the reduced thickness of the intestinal mucous layer, redistribution of tight junction proteins, and low-grade inflammation. Moreover, bacterial components and metabolites derived from actions of the gut microbiome on dietary, environmental, and bile acid exposures can reach the portal vein and promote inflammation, further supporting targeting dietary composition as a therapeutic opportunity in NAFLD management (33).

#### 1.4 Risk Factors for NAFLD

The non-modifiable risk factors for NAFLD include age, sex, genetic background, ethnicity, and family history of fatty liver, T2DM, or premature cardiovascular disease (34). In general, the prevalence of NAFLD- and NAFLD-related fibrosis increases with age (35). For adults under 50, NAFLD is more prevalent among men (36). However, the prevalence of NAFLD in postmenopausal females is similar to males after 50 (36). Several gene variants predispose to NAFLD by enhancing fat accumulation in the liver, the most important of which is human patatin-like phospholipase domain-containing 3 (PNPLA3). PNPLA3 is a crucial regulator of lipid droplet turnover in hepatocytes and hepatic stellate cells (37). PNPLA3 rs738409 is a single nucleotide substitution of cytosine to guanine, which encodes for a nonsynonymous change, resulting in the substitution of isoleucine to methionine at position 148 (I148M) of the protein (38). This sequence variation is believed to be the most vital genetic determinant of the full spectrum of non-alcoholic fatty liver disease, from fatty liver formation to NASH and HCC (37-40).

Obesity, over-nutrition, dietary composition, and inactivity are important modifiable risk factors for NAFLD. Obesity, especially central obesity, is possibly the most important modifiable risk factor for NAFLD and results from over-nutrition and inactivity (34). Most patients with NAFLD are either obese or overweight. The overall prevalence of obesity among NAFLD patients worldwide is estimated at 51.3%, and 81.8% of NASH patients are obese (4). Results from 12,454 adult participants in the Third National Health and Nutrition Examination Survey (NHANES) describe the prevalence of NAFLD in various body mass index (BMI) categories: 7.5% in normal-weight men (BMI of 18.5–24.9), 38.6% in obesity class 1 (BMI of 30.0–34.9), and 56.6% in obesity class 2 (BMI 35 or above) (41). Similar trends have also been reported for women with NAFLD. Prevalence of NAFLD was 6.7% in women with a normal BMI compared to 24.7% in women with a BMI of 30.0 to 34.9, and 44.3% in those with a BMI of 35 and above (41). Central obesity, measured through waist circumference ( $\geq 102$  cm for men and  $\geq 88$  cm for women), is an independent risk factor for both NAFLD and MetS (42). It is a widely accepted hypothesis that visceral adipocytes are detrimental to the liver as they release more fatty acids, pro-inflammatory cytokines, and adipokines than peripheral adipose tissue pools (3).

Modern societies spend increasingly more time being sedentary and less time engaged in physical activity due to environments that minimize activity and require prolonged sitting times at work, in the home, and for transportation (43). Sedentary behavior is defined as low-energy expenditure in a sitting or reclining position during waking hours. It has been linked to chronic low-grade inflammation and contributes to obesity (44). High sitting times (e.g.,  $>8$  h/day) compared to low sitting times (e.g.,  $< 4$  h/d) almost double the risk of developing T2DM and increase the incidence and mortality risk associated with cancer and CVD by 10% to 20% (45). The association between sedentary lifestyle and obesity, MetS, and T2DM has been supported by

epidemiological research (44, 46, 47). Low levels of moderate-intensity physical activity and high amounts of sedentary time are associated with insulin resistance, T2DM (48), and NAFLD (49, 50).

The relationship between diet and the development of NAFLD is complex and extends beyond total energy intake. Certainly, over-nutrition results in obesity by altering the energy balance. However, dietary patterns may have an independent effect on NAFLD apart from energy density. A “Western” style diet is a well-recognized diet pattern associated with MetS and NAFLD (51-53). This pattern is generally hypercaloric; it consists of high intakes of animal products high in saturated fat, refined sugars, and grains; sugar-containing soft drinks; and high intakes of processed food high in trans-fats and low in fiber and phytochemicals (54). The high glycemic index associated with the Western dietary pattern results in a rapid increase in insulin and postprandial serum glucose levels and contributes to the induction of liver lipogenesis and VLDL secretion, resulting in obesity (54, 55). Besides the Western dietary pattern, the consumption of restaurant-based fast-foods is another example of an energy-dense, low-nutrient-rich diet (56). In a clinical trial in Sweden, 18 healthy volunteers were given at least two fast-food-based meals a day to double the regular caloric intake, in combination with adopting a sedentary lifestyle for four weeks, and were compared to a control group (55). The results showed an average 6.4-kg weight gain, a significant rise in serum alanine aminotransferase (ALT) levels, and an increase in average intrahepatic triglyceride levels from 1.1% to 2.8% compared to controls (55). Of note, eating patterns have also been associated with NAFLD. A prospective study of 2254 NAFLD-free subjects over three years found that individuals who habitually eat before bedtime had double the risk of developing NAFLD (57). Hyper-caloric snacking between meals results in increased liver fat; however, consuming these same snacks with meals did not cause liver fat accumulation (58).

## 1.5 NAFLD Management Overview

NAFLD management has focused on reducing insulin resistance, limiting oxidative stress, and modifying underlying risk factors (59, 60). Lifestyle modification remains the most effective treatment for NAFLD and NASH and is achieved through dietary changes and physical activity. Nevertheless, there are several challenges to achieving these outcomes (61-64). Aggressive weight loss can aggravate liver inflammation in subjects with NAFLD if it reaches a rate greater than 1.6 kg per week (65). Furthermore, weight loss maintenance is challenging, limiting long-term results. This weight gain is attributed to the challenges of maintaining a hypocaloric diet in an environment of overabundance and easy access to high-energy, palatable food options.

Consequently, it is essential to define which dietary components are most likely to induce NAFLD, allowing for the targeted increase in the availability of resources and programs to assist patients with long-term weight loss maintenance and achieving success. In NAFLD patients with more advanced liver disease stages and those with high genetic risk or diabetes, pharmacological treatment might be necessary to intensify lifestyle interventions. However, for the time being, there is no approved drug for the treatment of NAFLD.

### *1.5.1 Lifestyle Modification in NAFLD*

Weight loss in NAFLD is effective in improving liver disease severity. A recent meta-analysis evaluated the effect of weight loss on NAFLD-related biomarkers from 22 clinical trials (2588 combined subjects) using various interventions (66). These interventions included behavioral weight loss programs (15 studies), pharmacological (6 studies), and surgical procedures

(1 research). Results were compared to a lower-intensity weight loss intervention. In patients with NAFLD, weight loss (median -3.61 kg) achieved within the 6-month program was associated with significant improvements in ALT levels, liver steatosis, histologic NAFLD activity score, liver stiffness, and the disappearance of NASH (66). However, there was no significant effect on liver fibrosis score (66).

Most studies relating to the association of weight loss with NAFLD have focused exclusively on NASH, possibly due to the importance of NASH in developing adverse clinical outcomes, such as cirrhosis. For example, a randomized controlled trial by Wong *et al.* (2013) in a group of 154 NAFLD patients compared the effect of a dietitian-led lifestyle modification program for 12 months linked to a standard care protocol that included physician follow-up on remission of NAFLD. Remission was defined as a reduction in intrahepatic TG content to less than 5%. Findings indicated that a 10% weight reduction leads to remission of NAFLD in 97% of the patients, compared to only 13% in patients who lost less than 3% body weight (67). Another study examining the effect of the drug orlistat in patients with NASH found that at least a 5% weight reduction was required to improve the NASH activity score (NAS), liver fat content, and insulin sensitivity compared to those who did not lose weight (68). Vilar-Gomez and colleagues studied the effect of a 52-week weight loss program in 293 patients on histological features of NASH (69). They compared pre- and post-liver biopsy results and demonstrated that the degree of weight loss was independently associated with improvements in all NASH-related histological parameters. In their study, 90% of patients who lost  $\geq 10\%$  of body weight had a resolution of NASH, and 45% had liver fibrosis regression; however, only 10% of study participants achieved a 10% weight loss (69). The beneficial effects of weight loss on NASH-related liver parameters were still significant

even with a 5% to 10% weight loss, and 30% of participants were able to achieve this degree of weight loss (69).

### *1.5.2 The Effect of Exercise on NAFLD*

Physical activity has numerous beneficial effects on MetS. Increasing physical activity reduces intrahepatic triglyceride content and markers of hepatocellular injury in patients with NAFLD, independent of weight loss (70). A systematic review and meta-analysis from 17 studies on the impact of structured exercise training, and associated weight loss, on intrahepatic triglycerides (IHTGs) in individuals with NAFLD showed exercise reduced IHTG levels independent of significant weight change. However, the benefits achieved were substantially more significant when weight loss occurred (71). The guidelines from the European Associations for the Study of the Liver (EASL), Diabetes (EASD), and Obesity (EASO) recommend 150 to 200 min/week of moderate-intensity aerobic physical activity for NAFLD patients, in three to five sessions (72).

Since most clinical trials in NAFLD have focused on the effects of a combined physical activity and therapeutic diet approach in NAFLD, studies focusing on physical activity alone are less common. In a physical activity-only systematic review of 28 randomized clinical trials in a combined total of 1644 participants, increased physical activity was associated with a significant reduction in liver fat content and serum aminotransferase levels, a significant decrease in BMI, and improved peripheral insulin sensitivity (73). The effect of physical activity on hepatic liver fat content was more prominent in young patients and patients with a higher baseline body mass index (73). In line with these findings, a clinical trial by van der Heijden (74) also showed that a 12-week controlled moderate-intensity physical activity program without weight loss resulted in a

significant decrease in hepatic fat content and visceral fat content. However, insulin resistance decreased only in obese adolescents and not in lean adolescents. Considering the difficulty obese patients have in losing weight on a hypocaloric diet and the independent benefits of physical activity, setting realistic goals for increasing moderate-intensity activity provides another option for the patient with NAFLD.

Regarding the optimal type of physical activity training (aerobic vs. resistance), a systematic review and meta-analysis of 12 randomized clinical trials (13 aerobic and 4 resistance exercise protocols) showed that both aerobic and resistance exercise improve hepatic steatosis (75) and also significantly improve NAFLD.(70)

### *1.5.3 The Effect of Diet on NAFLD*

Long-term adoption of a low-calorie diet is associated with decreased liver fat and improved cardiovascular risk (76). Reducing caloric intake by at least 30% (or by approximately 750 to 1000 kcal/day) resulted in an improvement in IR and fatty liver. However, as discussed previously, the sustainability of such hypocaloric diets is low. The multifactorial origin of NAFLD and NASH has led to management strategies that incorporate a combination of dietary interventions aimed at tackling the multiple hits occurring during the onset and evolution of this disease. For example, dietary patterns that reduce the intake of high glycemic index foods, increase dietary fiber and resistant starch, reduce saturated fat, and promote mono-unsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) intake may have a positive effect on gut microbiota composition and function, intestinal barrier function, and IHTG accumulation.

Dietary patterns that leverage the above-described principles may reduce NASH development, possibly even in the absence of weight loss. Here, we discuss dietary regimens recommended for the management of NAFLD patients. The four most common dietary patterns include the low-carbohydrate diet, the low-fat diet, the Dietary Approaches to Stop Hypertension (DASH) diet, and the Mediterranean diet.

High-carbohydrate diets (50% to 65% of calories from carbohydrates) have been associated with increased insulin resistance and obesity and shifting benign fatty liver toward NASH (77). Low-carbohydrate diets are diets in which less than 40% of calories are from carbohydrates (78). NAFLD patients receiving low-carbohydrate diets had a significant weight loss only in the short term (6 months) than those consuming a conventional high-carbohydrate/low-fat diet. Still, at one year, no difference in weight loss was observed (78). Subjects on low-carbohydrate diets show improvements in biochemical parameters of MetS in comparison with those on high-carbohydrate diets. The ketogenic diet (8% carbohydrate, 30% protein, and 61% fat) is severely carbohydrate-restricted and induces weight loss, decreases serum TG levels, and increases HDL levels compared to a high-carbohydrate diet (in the short term), especially in men (79, 80). However, adherence rates to low-carbohydrate diets in the long term are poor, and the potential effects of ketogenic diets on micronutrient deficiencies and the gut microbiome are unknown (81). Adverse metabolic effects, such as thirst, fatigue, and even tachycardia, have been reported with the ketogenic diet (82).

The low-fat diet limits fat intake to 30%, with 50% of calories coming from carbohydrates and 20% from protein, and includes a restricted intake of trans-fat, saturated fat, and cholesterol (no more than 300 mg/day) (83, 84). Low-fat diets are intended to reduce conditions such as heart disease and obesity (85). In NAFLD, the comparison of low-fat and low-carbohydrate diets has

demonstrated inconclusive results. For example, when prescribed to overweight people, both diets are equally effective for weight loss (86). In contrast, one study in patients with NAFLD in Korea showed greater efficacy for a low-carbohydrate diet in reducing total energy intake and hepatic fat content (87). The authors explained their findings based on low-fat energy consumption as part of the habitual diet, with less than 20% of calories from fat (87). It is important to note that low-fat diets generally do not distinguish between the type of dietary fat consumed. Whether saturated, monounsaturated, or polyunsaturated, all fats are treated similarly, although the biological effects likely differ.

The DASH diet was first introduced to manage hypertension and focused on lowering energy-dense food items and decreasing total fat, saturated fat, and cholesterol. This dietary pattern is low in added sugars, sugar-sweetened beverages, and red and processed meats and is enriched in vegetables, fruits, low-fat dairy products, whole grains, poultry, fish, and nuts (83). It is rich in potassium, magnesium, calcium, and protein, and fibre (83). The DASH dietary pattern is associated with a reduced risk of cardiovascular disease (88). In a randomized controlled clinical trial including 60 overweight or obese adults with NAFLD diagnosed by ultrasonography, adherence to the DASH diet for 8 weeks, compared to a contemporary control diet, was significantly more effective in reducing weight and improving liver enzymes, markers of insulin resistance, serum triglycerides, and VLDL levels (89). A cross-sectional study in 3051 subjects between the ages of 40 and 75 showed that adherence to the DASH diet was independently associated with a significantly lower prevalence of NAFLD (90). The DASH diet has been demonstrated to improve blood pressure and hyperlipidemia, translating to protective effects in NAFLD (91). Nevertheless, despite the benefits of the DASH diet for T2DM and cardiovascular disease (88), the evidence for its efficacy in NAFLD is limited.

The Mediterranean (MD) diet has been recommended for NAFLD treatment by various clinical practice guidelines (92). It has the greatest evidence to prevent and manage MetS and its components, particularly T2DM and CVD (93). In the MD diet, high consumption of extra-virgin olive oil, vegetables (especially root and green varieties), fruits (particularly fresh), whole grains (cereals, bread, rice, or pasta), legumes, nuts (walnuts, hazelnuts, or almonds), and moderate consumption of fish (especially fatty fish rich in omega-3 fatty acids), low-fat dairy products, and red wine is recommended over the consumption of red and processed meat, processed and high-sugar food, refined carbohydrates and high-fat milk products (83). The MD diet has a higher fat content (about 40% of total calories), is low in saturated fat (less than 10% of total calories), high in monounsaturated fatty acids (MUFA) and omega-3 polyunsaturated fatty acids (PUFA), with a decreased omega-6 PUFA content, and high in fibre (25 and 35 g/day for women and men, respectively) (83). Substantial evidence supports the benefits of the MD diet in controlling body weight, fasting plasma glucose, serum TG, and blood pressure (88). Recently, Kaliora *et al.* studied the effect of the MD diet on clinical, biochemical, and inflammatory profiles in patients with simple NAFLD. Untreated NAFLD patients with no significant fibrosis ( $n = 44$ ) underwent a 24-week diet intervention after nutritional counselling to increase adherence to the MD diet. Commitment to the diet was associated with significant improvements in liver imaging, liver fibrosis score, blood pressure, fasting glucose, hemoglobin A1C, and several other biomarkers compared with pre-intervention values (94). In another study, 278 participants with abdominal obesity/dyslipidemia were randomly assigned to either a low fat (LF) or Mediterranean/low-carbohydrate (MD/LC + 28 g walnuts/day) diet for 18-months (95). The MD/LC diet induced a more significant reduction in hepatic fat content than the LF diet, which reached statistical significance after 18 months ( $-4.2\%$  vs.  $-3.8\%$ ,  $p = 0.04$ ). Interestingly, the advantageous effect

of the MD/LC diet in reducing hepatic fat content compared to that obtained with the LF diet was significant in both patients without NAFLD (hepatic fat content  $\leq 5\%$ ,  $p = 0.04$ ) and in NAFLD patients (hepatic fat content  $> 5\%$ ,  $p = 0.014$ ). Compared to the LF diet, the MD/LC diet induced a more significant increase in HDL and a greater decrease in blood pressure, triglycerides, and triglyceride/HDL ratio (95).

#### *1.5.4 Surgical and Pharmacological Treatment of NAFLD*

Bariatric surgery has been used to control obesity and, as a consequence, treat NAFLD. Significant improvement in the prevalence and severity of fatty liver occurs following bariatric surgery, although its benefits and safety in patients with NASH have yet to be established (96).

Since insulin resistance has been implicated in the pathogenesis of NAFLD, insulin-sensitizing agents have been investigated, with most studies examining the use of metformin and pioglitazone. Metformin is a biguanide drug that works through different mechanisms to lower blood glucose levels, including reducing postprandial and fasting glucose levels and the inhibition of hepatic and renal gluconeogenesis (97). In contrast, pioglitazone is a thiazolidinedione that inhibits hepatic gluconeogenesis and enhances glucose uptake in muscles and adipose tissue by activating peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) (59). A systematic review and meta-analysis done on the efficacy of these two medications in NASH patients demonstrated that metformin ( $n = 81$  patients) did not significantly improve liver histology scores for steatosis, ballooning, or fibrosis. At the same time, it significantly worsened lobular inflammation (98). On the other hand, Pioglitazone improved steatosis and lobular inflammation but did not improve fibrosis (98). Considering the recognized role of inflammation and oxidative stress in the pathogenesis of NASH, vitamin E, an antioxidant, has been studied as a treatment option in NASH.

Although the results indicated that while a daily dose of 800 to 1000 IU does not improve fibrosis, it significantly improves histologic scores, lobular inflammation, and ballooning in non-diabetic adults with biopsy-proven NASH (98). However, additional data is required to show that vitamin E is beneficial in other groups of NAFLD patients (76). While many new drugs are being investigated in NASH clinical trials, these pharmacological treatments are currently limited to patients with biopsy-proven NASH in the context of clinical trials (1).

### *1.5.5 Conclusion*

NAFLD is considered the hepatic manifestation of metabolic syndrome and is a rising epidemic worldwide. It will be the leading cause of cirrhosis, hepatocellular carcinoma, and liver transplant within the next decade. Behaviors, such as a sedentary lifestyle and consuming a Western diet, have led to substantial challenges in managing NAFLD patients. With no available curative pharmaceutical therapies, lifestyle modifications, including dietary changes and exercise, ultimately lead to weight loss remain the only effective therapy for NAFLD. With the evaluation of multiple diets, including low-carbohydrate, low-fat, DASH, and Mediterranean diets, it seems that NAFLD patients have shown better outcomes with a modified diet, such as the MD diet, where patients are encouraged to increase the consumption of fruits and vegetables, whole grains, and olive oil.

## **Chapter 2: Worldwide prevalence and incidence of Non-alcoholic fatty liver disease (NAFLD) in the 21<sup>st</sup> century: A systematic review and meta-analysis**

### 2.1 Introduction

The prevalence of NAFLD is constantly rising. Due to a silent presentation, high prevalence, and the detrimental hepatic and extra-hepatic outcomes associated with it, NAFLD is a serious global health problem. Since there are currently no approved treatments for NAFLD, disease prevention is the only available option for reducing the disease burden. From the public health perspective, by providing accurate assessments of the disease burden, epidemiological data are vital to targeting and implementing evidence-based control measures, to research priority setting, prevention and treatment programming, and policymaking. Understanding the extent to which the NAFLD prevalence and incidence have increased over time can provide useful information for various stakeholders to better understand the disease, predict NAFLD growth trends, and develop strategies to raise awareness and interventions to decrease NAFLD burden.

The only existing research publication on the prevalence and incidence of NAFLD at a global level was published in 2016 and estimated the global prevalence of NAFLD diagnosed by imaging at 25.2% (95% CI, 22.1 to 28.7). That report has been shown to have limitations and flaws, mainly because of including low quality publications. Since defining the burden of the disease requires updated and accurate epidemiological data, we felt the need for a systematic review and meta-analysis to provide updated and precise estimates of the NAFLD prevalence and incidence in the general adult population throughout different regions of the world.

## 2.2 Front Matter

Kiarash Riazi <sup>1,2</sup>, Hassan Azhari <sup>1</sup>, Jacob H. Charette <sup>1</sup>, Fox E. Underwood <sup>1,2,3</sup>, James A. King <sup>1,2,3</sup>, Elnaz Ehteshami Afshar <sup>1,2</sup>, Mark G. Swain <sup>1</sup>, Stephen E. Congly <sup>1,3</sup>, Gilaad G. Kaplan <sup>1,2,3</sup>, Abdel-Aziz Shaheen <sup>1,2,3,\*</sup>

<sup>1</sup> Division of Gastroenterology and Hepatology, Department of Medicine, Cumming School of Medicine, University of Calgary, Canada.

<sup>2</sup> Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Canada.

<sup>3</sup> O'Brien Institute for Public Health, Cumming School of Medicine, University of Calgary, Canada.

**Specific author contributions:** KR, FEU, JAK, MGS, SEK, GKG, and AAS designed the study. KR, HA, JHC, EEA, FEU, and JAK collected and analyzed the data. KR and FEU drafted the manuscript. All Authors interpreted the data and provided critical revisions of the manuscript for important intellectual contents. All authors have approved the final draft of the manuscript.

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## 2.3 Manuscript Abstract:

**Background:** Non-alcoholic fatty liver disease (NAFLD) is a significant public health concern worldwide. Defining the burden of the disease requires updated and accurate epidemiological data. We conducted a systematic review and meta-analysis to estimate the worldwide prevalence and incidence of NAFLD.

**Methods:** A search of MEDLINE and EMBASE databases was performed to find studies of the prevalence and incidence of NAFLD up to December 04, 2019. We included observational cross-sectional or longitudinal studies performed on samples representative of the general adult population and in which NAFLD was diagnosed using an imaging method in the absence of excessive alcohol consumption and viral hepatitis. Estimates were provided using a random-effects meta-analysis method.

**Results:** We screened 9860 records and included 49 papers with a population of 604,721 for prevalence calculations and 12 papers with a population of 265055 for incidence calculations. The overall worldwide prevalence of NAFLD was 32.3% (95% CI: 29.5, 35.1). The prevalence increased significantly over time, from 26.6% (95% CI: 19.6, 33.7) for 2000-2005, to 30.3% (95% CI: 25.7, 34.9) for 2006-2010, to 33.0% (95% CI: 29.1, 36.9) for 2011-2015, and to 44.1% (95% CI: 37.8, 50.3) for 2016 or later. The overall NAFLD incidence rate was 49.8 (36.7, 62.8) per 1000 person-years. The incidence rate in men was 78.2 (95% CI: 50.5, 105.8) and in women was 31.4 (95% CI: 19.6, 43.1) per 1000 person-years.

**Conclusion:** The worldwide prevalence of NAFLD is significantly higher than what had previously been estimated and is on an alarmingly constant rise.

## 2.4 Background

Nonalcoholic fatty liver disease (NAFLD) is defined as the presence of lipid deposition in more than 5% of hepatocytes, also known as hepatic steatosis, in the absence of the other causes of hepatic steatosis (1, 99). NAFLD is the most common liver disorder worldwide and the leading cause of liver-related morbidity and mortality, mainly associated with cirrhosis, end-stage liver disease, liver transplant, and an increased incidence of hepatocellular carcinoma (1, 4, 100, 101).

NAFLD includes a spectrum of liver pathologies ranging from simple steatosis, inflammation in the form of nonalcoholic steatohepatitis (NASH), and ultimately to advanced liver fibrosis and cirrhosis (1). NAFLD is associated with metabolic dysfunction in the body and is considered as the hepatic presentation of metabolic syndrome, a cluster of central obesity, high triglyceride levels, reduced high-density lipoprotein (HDL) cholesterol, hypertension, and prediabetes/type 2 diabetes mellitus (T2DM) (6, 102). Thus, it is also associated with the downstream consequences of metabolic syndrome, including T2DM (17), cardiovascular diseases (CVD) (103), and chronic kidney disease (CKD) (104). In fact, 40-48% of deaths in patients with NAFLD are due to CVD, making CVD the most common cause of mortality in NAFLD (20, 105).

The prevalence of NAFLD has constantly been rising and varies worldwide, usually paralleling the prevalence of obesity and T2DM (4). However, NAFLD can also occur in lean individuals who potentially comprise up to 20% of the NAFLD population, which indicates a genetic susceptibility or multiple etiologies for NAFLD (21, 35, 106). Due to its silent presentation, high prevalence, and detrimental hepatic and extra-hepatic outcomes, NAFLD is a serious global health problem (101, 107-109). Moreover, the incidence of hepatocellular carcinoma due to NAFLD is also continuously rising, and NAFLD-associated cirrhosis is rapidly surpassing hepatitis C as the most common indication for liver transplantation (35, 110). Since

there are currently no commercially approved treatments for NAFLD, the only option for reducing the disease burden is prevention. From the public health perspective, accurate data on temporal trends and geographical differences of NAFLD prevalence serve as the basis for research priority setting, prevention and treatment programming, and policymaking.

There are few existing research publications on the prevalence and incidence of NAFLD at a global level. The only current systematic review on the worldwide prevalence and incidence of NAFLD was published by Younossi *et al.* in 2016. This publication has been an outstanding reference for basic epidemiological data on NAFLD, not only for prevalence and incidence but also for associated risk factors and co-morbidities (4). They estimated the overall global prevalence of NAFLD in 2016 at 25.2%, with a steady upward trend (4). This publication nonetheless has some limitations. The incidence estimation was limited to 5 publications available (4). Two more recent NAFLD meta-analyses have been published and included more publications describing NAFLD incidence; however, they are geographically limited to Asia (111) and China (112). More importantly, previous reports have included a considerable number of publications in which study populations were either not representative of the general population (e.g., military personnel, company workers, male/female only samples, particular ethnicities, etc.) or had exclusion/inclusion criteria that inadvertently would affect prevalence estimates through inclusion or omission of sub-populations in which the distribution of NAFLD are known to be different from the general population (e.g., people with any components of the metabolic syndrome). For example, the prevalence of NAFLD in individuals with T2DM is over 55% (17), and the prevalence of NAFLD in overweight and obese patients is nearly five times that in patients with normal weight (52.3% vs. 11.8%) (111).

Updated and accurate NAFLD prevalence and incidence estimates of the general population are vital, particularly considering the ever-growing obesity and T2DM epidemics (4, 108, 109). Such epidemiological data will help stakeholders better understand the disease, predict NAFLD growth trends, and help develop strategies to increase awareness and interventions to decrease the NAFLD burden. Therefore, we conducted a systematic review and meta-analysis to systematically evaluate and summarize current knowledge and update current data and accurately estimate NAFLD's overall worldwide prevalence and incidence over the past two decades. We narrowed our study to the 21<sup>st</sup> century due to limited knowledge of NAFLD before that, and universal case definition and diagnostic criteria were only introduced during the late 90s (113). We applied criteria to select research publications that provided the most accurate estimates for the general adult population of different world regions. Since imaging is the recommended and most accurate available modality for diagnosing hepatic steatosis in epidemiological studies, we only examined reports based on diagnosing NAFLD using an imaging method (1, 4). We also stratified and analyzed point prevalence and incidence estimates by study year, geographical locations, sex, and other variables related to study methods (sample source, study setting, study size, and imaging method).

## 2.5 Methods

### *2.5.1 Search Strategy*

A systematic review and meta-analysis study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (114). A literature search of two databases, MEDLINE and EMBASE, was conducted using Ovid search

platforming in December 2019. The search terms, as represented in [Appendix A](#), were selected to help identify the observational studies that reported on the prevalence or incidence of NAFLD. No language or publication date restrictions were applied. The identified studies were cataloged using Endnote X8 (Clarivate Analytics). After excluding duplicates, the citations were independently screened by two of the authors (K.R., H.A., J.H.C., and E.E.A.) to provide a list of potentially relevant studies. The full texts of these studies were then obtained and examined independently in duplicates (K.R., H.A., J.H.C.) to determine their eligibility for inclusion in the systematic review. Any disagreements were resolved via consensus discussions.

Full texts not in English were translated using Google Translate (Google, Mountain View, CA, USA) or with the help of a native speaker colleague. A reference list search was also done on the previous systematic reviews for prevalence (4, 111, 115) and incidence (4, 111, 112), with the application of the same inclusion/exclusion criteria used for our search to identify any publications that were not captured in our database search.

### *2.5.2 Study Selection Criteria*

According to the American Association for the Study of Liver Diseases (AASLD) practice guidance, the definition of NAFLD requires evidence of hepatic steatosis, either by imaging or histology, and the absence of other causes of hepatic steatosis, like significant alcohol consumption, viral hepatitis, use of steatogenic medications, etc. (1). We included original descriptive research publications, including cross-sectional studies, or studies that contained cross-sectional data (e.g., a baseline assessment for NAFLD in a longitudinal incidence study). These studies were required to have reported crude data needed for prevalence or incidence calculations, including study date, study sample size, and description, number of NAFLD cases, diagnostic

method, average follow-up duration, etc. The study samples needed to be a close representation of the general adult population of the region. Regarding NAFLD diagnosis in the studies, publications were included if they reported a clear definition and diagnostic criteria for NAFLD (fatty liver in the absence of other causes). Hence, to be included, a study was required to have excluded other causes of fatty liver including excessive alcohol consumption and viral hepatitis (B or C) and to have used an imaging method (ultrasound, CT (computerized tomography) Scan, magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), or controlled attenuation parameter (CAP) by FibroScan®) to confirm the presence of fatty liver (116). Significant alcohol consumption has been variably defined in different countries (8) (e.g., > 21 standard drinks/week for men, and > 14 standard drinks/week for women; American Association for the Study of Liver Diseases (AASLD)(1)). Therefore, we did not restrict the criteria by volume, as long as a publication stated a history of excessive alcohol consumption was an exclusion. However, we have included excessive alcohol consumption thresholds in the quality assessment tables. If there were multiple studies from the same cohort, we only included data from one representative publication, which provided a complete dataset. If there were marginal temporal overlaps between studies of an ongoing cohort, we included all of them. For incidence publications, we also required a longitudinal study which included healthy non-drinker and viral hepatitis-free adults, a confirmation of the exclusion of NAFLD cases at baseline, and the provision of average follow-up duration, and a report of incidence/1000 person-years or included enough information to enable its' calculation.

Therefore, the exclusion criteria were:

- 1) Articles that were clinical trials, editorials, conference proceedings, letters to editors, review articles, or case reports.

- 2) Study samples that did not represent the general adult population, for reasons including that the study was in a pediatric population (<18 years old), or in a mix of pediatric and adult populations with inadequately provided data to calculate adult estimates, the study was in a specific subgroup of the general population, including those involving males or females only, in a limited age range, in particular ethnicities, in people holding particular jobs, or in specific social groups or medical conditions. If the study was conducted in patients with conditions known to be associated with NAFLD (overweight/obesity, prediabetes/T2DM, hypertension, hyperlipidemia, metabolic syndrome, cardiovascular diseases), or if the study was conducted on a sample population after excluding these conditions, the study was not included. Regarding the age range, NAFLD is associated with older ages (4, 111, 117), so we did not include studies that had a study sample with a narrow age range. We set an arbitrary minimum age range of 30-65 years as acceptable.
- 3) The fatty liver diagnosis was based on biopsy, autopsy, or biomarkers including transaminase levels, fatty liver index (FLI), hepatic steatosis index (HSI), etc., and/or the studies did not exclude cases with excessive alcohol use or viral hepatitis, and those missing any critical information on the diagnostic process.
- 4) The study was carried out before 2000.

### *2.5.3 Data Extraction*

Data were extracted by one of the authors (K.R.) and independently verified by another author (H.A.), and the discrepancies were discussed. The extracted data included first author and publication year, study location and period, setting (urban, rural), sample source (population-based, health checkup visitor-based, etc.), the total sample size and the number of NAFLD cases,

and their characteristics (mean age, sex distribution), diagnostic information (imaging modality and case ascertainment used to define NAFLD), and the average follow-up years (for incidence). Corresponding authors were contacted by email for clarifications or missing data. On occasions that we did not receive any response, and the missing data were critical for the inclusion of the publications, they were excluded.

#### *2.5.4 Quality assessment & data analysis*

Quality assessment of the included studies was performed independently in duplicate, based on a modified version of the Joanna Briggs Institute Prevalence Critical Appraisal Tool (118) which has a high methodologic rigor in addressing the most important items related to the methodological quality of prevalence studies (119), and an adapted version of the Newcastle-Ottawa Assessment Scale using criteria relevant to studies of incidence (120).

The statistical analyses were done using STATA 16.1 (STATA Corporation, TX, USA). All prevalence and incidence rates, and their associated standard errors, were recalculated. To yield a proper estimate, individuals with excessive alcohol consumption or living with viral hepatitis needed to be excluded from the study population before prevalence/incidence calculation. If these were not already excluded, we recalculated the prevalence/incidence values as well as, if possible, the age and sex distribution information. If the study period contained multiple years, the last year of the study period was used to calculate point prevalence. Sex-specific prevalence rates were calculated for publications that provided relevant data. Frequency weights were used in the calculations related to age and sex. Incidence rate was calculated for 1000 person-years. Since a high level of between-study heterogeneity was expected, we used a random-effects meta-analysis method with the restricted maximum-likelihood model to calculate overall or sex-specific pooled

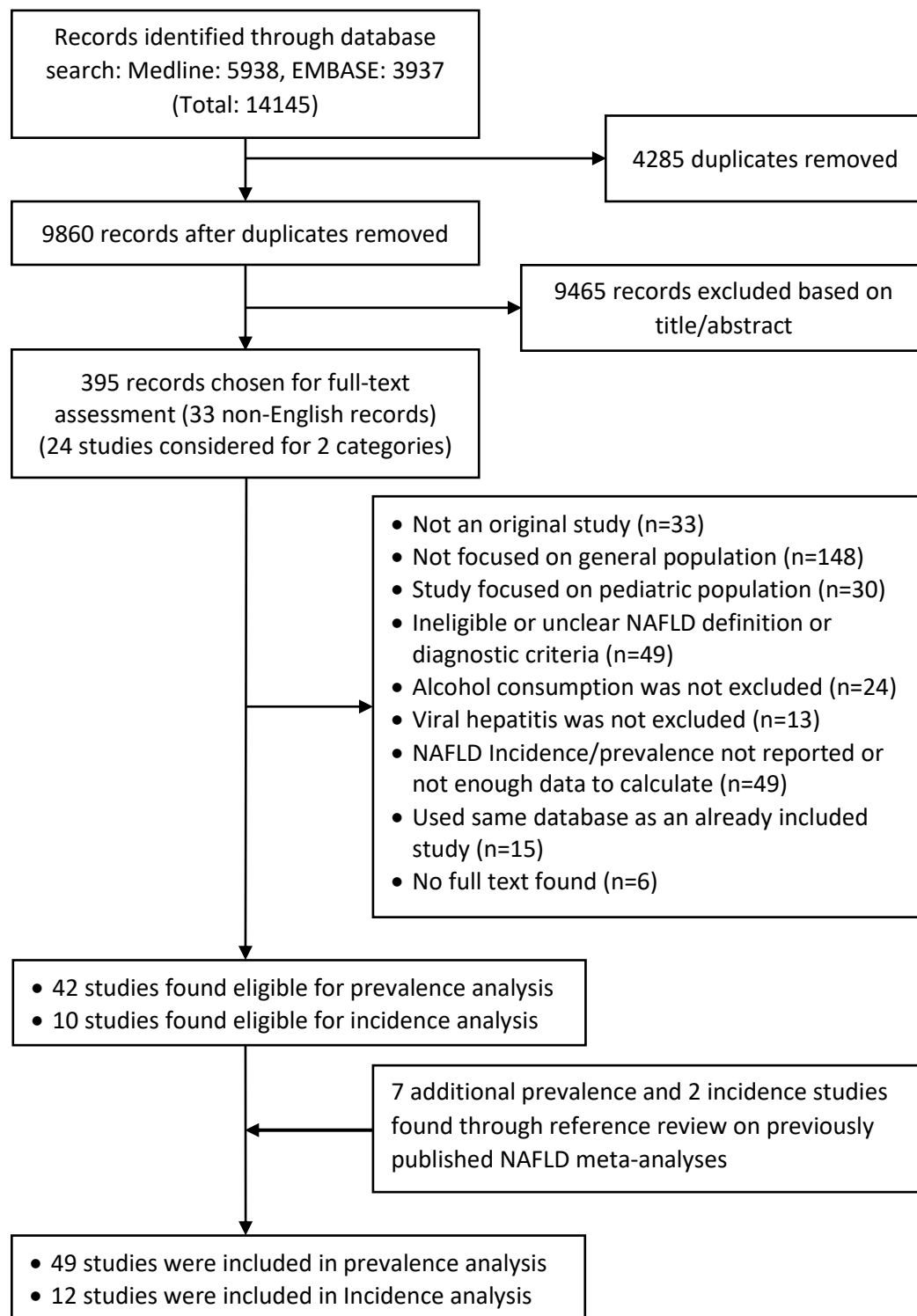
prevalence or incidence rates and their corresponding 95% confidence intervals. Heterogeneity between studies was assessed using  $I^2$  statistics, which reflects the percentage of total variation across studies due to heterogeneity rather than chance (121). To determine the contribution of moderator variables to the heterogeneity, we stratified the pooled outcome measures and ran univariate meta-regression analysis. Moderator variables included study year, geographical location (country, continent), sex, study setting (urban, rural), sample size, sample source (population-based, health check-up visitors, patients' companions), imaging modality, and specifically for incidence, follow up duration, and study design (retrospective, prospective). We generated funnel plots and ran Egger's test for publication bias assessment. Other statistical tests, if performed, were mentioned in the text. A p-value of less than 0.05 was considered statistically significant. To signify the geographic differences in prevalence, a static choropleth map of pooled prevalence was created using QGIS 3.44 (Open Source Geospatial Foundation, Chicago, Illinois, USA), classified as equal intervals. A web-based, interactive dashboard with maps and graphs was also created using ArcGIS Pro 2.4.1 (Environmental Systems Research Institute, Redlands, California, United States), similar to global reviews of other diseases (122-127). Shades of colours representing various levels of prevalence/incidence were determined through quartiles based on data from all periods.

## 2.6 Results:

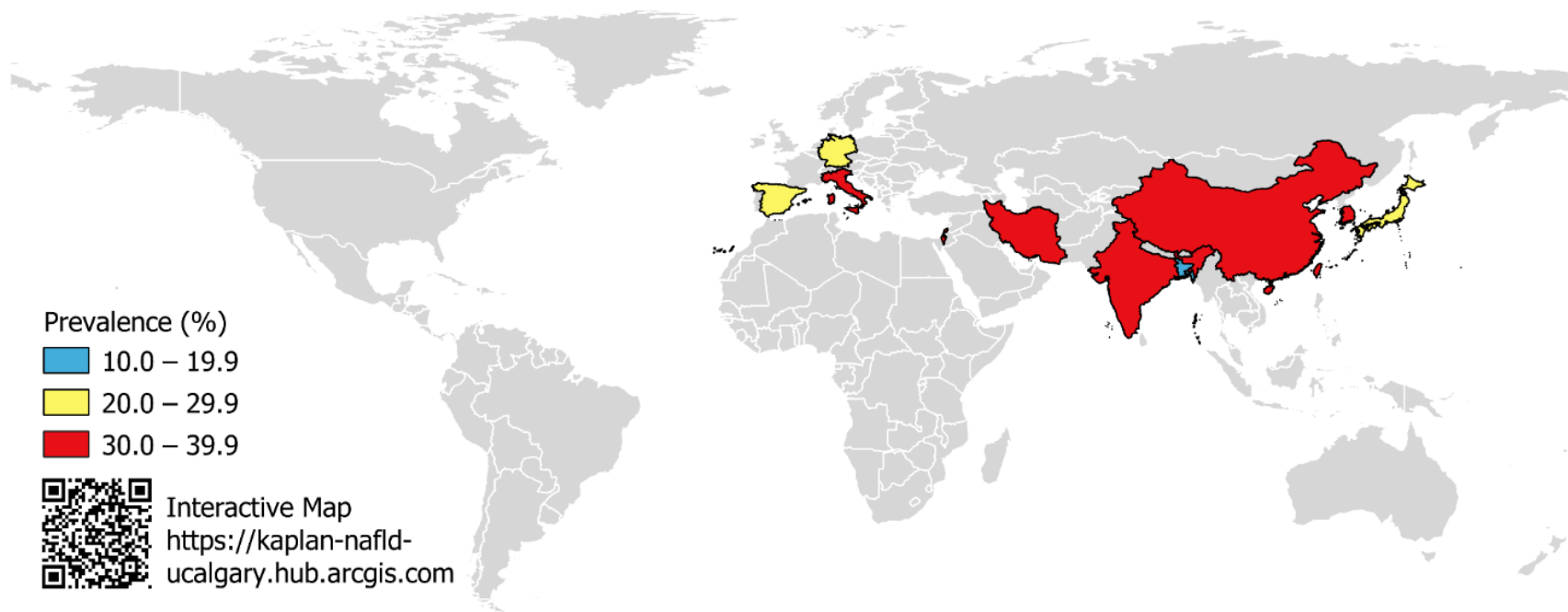
The initial database search resulted in 14145 records, out of which 4285 were duplicates, and 9465 were removed through title and abstract screening. The remaining 395 records (including 33 non-English records) were selected for full-text assessment. The process of study selection is presented in [Figure 1](#). We found 42 publications eligible for prevalence, and 10 for incidence

calculations. We also reviewed references from existing systematic reviews on NAFLD prevalence and incidence and retrieved 7 prevalence and 2 additional incidence references. Ultimately, we included 49 papers for NAFLD prevalence and 12 for incidence calculations. The characteristics of the included papers for prevalence are presented in [Appendix B](#) and for incidence in [Appendix C](#). Quality assessment for each study is also provided in Appendices [D1](#) and [D2](#) for prevalence studies and Appendices [E1](#) and [E2](#) for incidence studies. Generally, the risk of bias in included studies was low due to the application of strict inclusion/exclusion criteria with highly representative samples and adequate case definitions.

The Choropleth map presented as [Figure 2](#) illustrates the geographic differences in NAFLD prevalence in the 21<sup>st</sup> century. An interactive web-based map that illustrates NAFLD's prevalence and incidence worldwide is available online at the following link: <https://kaplan-nafld-ucalgary.hub.arcgis.com/>.



**Figure 1:** Flowchart outlining the study selection

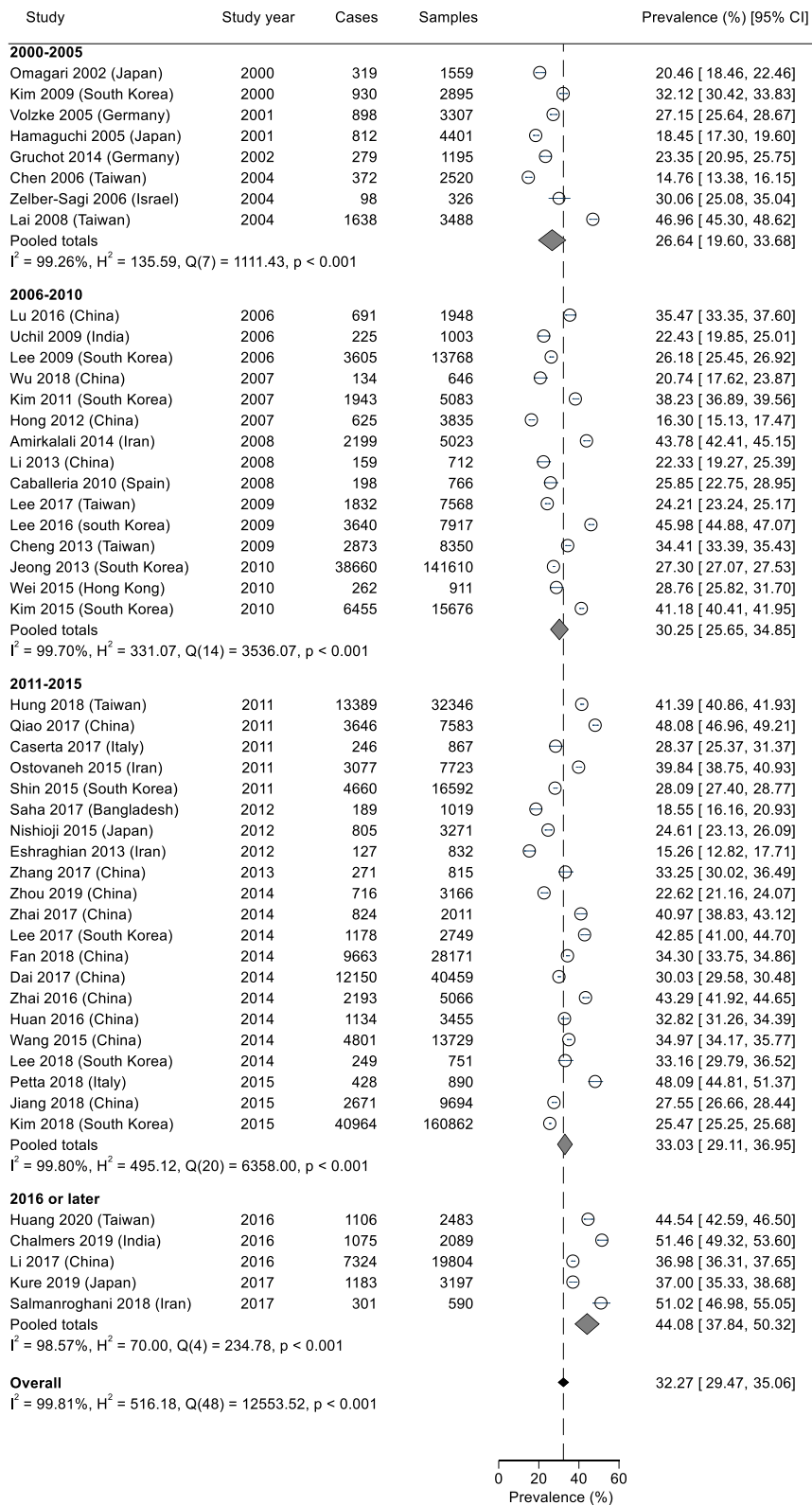


**Figure 2:** Geographic differences in the prevalence (%) of NAFLD in the 21<sup>st</sup> century. An interactive web-based map is available online: <https://kaplan-nafld-ucalgary.hub.arcgis.com/>.

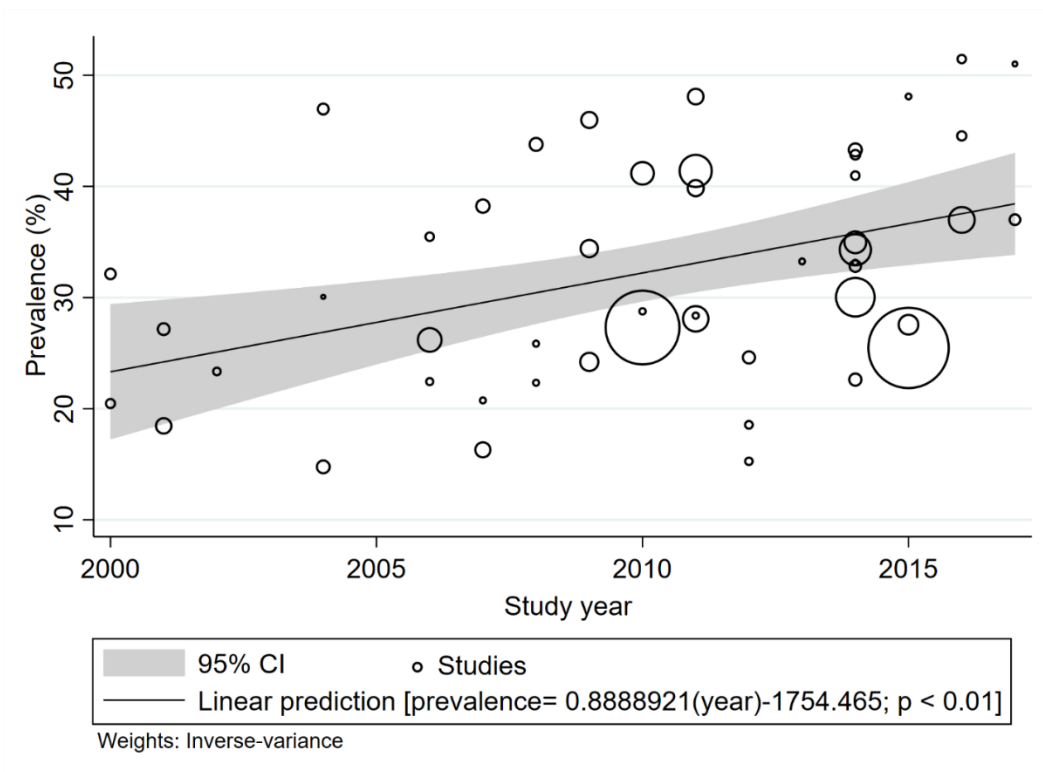
### 2.6.1 NAFLD prevalence

The 49 papers studied for NAFLD prevalence included 604,721 individuals (range 326-160862). Study years ranged from 2000 to 2017 and the publication years ranged from 2002 to 2020. On average, the manuscripts were published 4.2 (95% CI: 3.5, 4.9) years after the study year (Paired-sample t-test=11.86, df=48,  $p<0.001$ ). Most papers were from mainland China (n=15), followed by South Korea (n=10), Taiwan (n=6), Japan (n=4), and Iran (n=4). The mean age of the participants was 42.9 years (weighted average from 41 studies that reported the study sample mean age). Male individuals accounted for 52.8% of the participants (weighted average from 43 studies that reported the male percentage of the total sample). For the NAFLD cases, the mean age was 50.3 years (weighted average from 30 studies that reported the mean age in the NAFLD cases), and 69.4% were male (weighted average from 38 studies that reported the male percentage in the NAFLD cases).

The overall prevalence of NAFLD was estimated at 32.3% (95% CI: 29.5, 35.1). When stratified by study year groups, the pooled prevalence increased over time, from 26.6% (95% CI: 19.6, 33.7) for studies done in 2000-2005, to 30.3% (95% CI: 25.7, 34.9) for 2006-2010, to 33.0% (95% CI: 29.1, 36.9) for 2011-2015, and to 44.1% (95% CI: 37.8, 50.3) for 2016 or later ([Figure 3](#)). As shown in the bubble plot in [Figure 4](#), there has been a significant trend toward higher estimates of NAFLD prevalence since 2000 ( $p<0.01$ ).



**Figure 3:** Forrest plot using random-effects models for all included studies for NAFLD prevalence estimation stratified by study year.



**Figure 4:** Bubble plot of the association between study year and the prevalence of NAFLD. Circles represent estimates from each study, sized according to the estimate's precision (inverse of the prevalence variance).

36 studies reported sex-specific data allowing calculation and pooling of the sex-specific NAFLD prevalence. The overall prevalence of NAFLD in men was significantly higher than in women [39.1% (95% CI: 35.4, 42.8) vs. 25.5% (95% CI: 21.7, 29.2); difference: -13.6 (95% CI: -18.9, -8.4),  $p < 0.001$ ]. The NAFLD prevalence for both men and women showed a significantly increasing trend over time ([Table 1](#)). The NAFLD prevalence in males shows a steady time-dependant increase from 33.1% for 2000-2005 to 48.3% for 2016 or later, a 15.3% increase in less than two decades. The prevalence in females has been lower than males within every 5-year bracket but has reached 39.1% in reports of 2016 or later, a significant 21.6% increase in reports for 2000-2005.

**Table 1: Stratification and meta-regression analysis of the sex-specific estimates of NAFLD prevalence by study period.**

Period	Number of studies	Sample size	NAFLD prevalence (%) (95%CI)	Difference (95%CI)
<b>Male</b>				
2000-2005	4	6049	33.1 (17.6, 48.6)	Reference
2006-2010	14	116846	36.1 (29.8, 42.3)	3.0 (-9.0, 14.9)
2011-2015	13	66787	40.7 (36.2, 45.2)	7.6 (-4.5, 19.6)
2016 or later	5	18678	48.3 (41.1, 55.6)	15.3 (1.2, 29.4)*
Overall	36	208360	39.1 (35.4, 42.8)	
<b>Female</b>				
2000-2005	4	5555	18.4 (5.2, 31.6)	Reference
2006-2010	14	92887	22.4 (17.5, 27.4)	4.1 (-7.2, 15.4)
2011-2015	13	75237	25.4 (19.6, 31.2)	7.1 (-4.3, 18.4)
2016 or later	5	9485	39.8 (32.9, 46.7)	21.6 (8.2, 35.0)*
Overall	36	183164	25.5 (21.7, 29.2)	
<b>Total †</b>				
2000-2005	4	11604	25.7 (14.8, 36.6)	Reference
2006-2010	14	209733	29.2 (24.5, 33.9)	3.5 (-6.2, 13.2)
2011-2015	13	142024	33.0 (28.3, 37.7)	7.3 (-2.5, 17.1)
2016 or later	5	28163	44.1 (38.6, 49.6)	18.5 (6.9, 30.0)*
<b>Overall</b>	<b>36</b>	<b>391524</b>	<b>32.3 (29.2, 35.3)</b>	

\*Difference from the reference group is statistically significant (CI does not include zero).

† Totals here are from the same papers that provided sex-specific numbers (n=36).

Considerable heterogeneity was observed between NAFLD prevalence studies ( $I^2=99.80$  for overall population). We investigated the source of the homogeneity by stratifying the pooled data according to the moderator variables and performing meta-regression analysis ([Table 2](#)).

The NAFLD prevalence for Asia was 32.5% (95% CI: 29.5, 35.4; n=44) and for Europe was 30.5% (95% CI: 21.8, 39.2; n=5). We did not separate the Middle East from the rest of Asia; however, an estimate for the region was calculated from the 4 papers from Iran and 1 from Israel at 36.0% (23.6%, 48.1%) [ $I^2$  (%)=99.46, Egger's test p-value=0.93](data not shown). In Asia, NAFLD was most prevalent in Iran with 37.4%(95% CI: 22.3, 52.6), and in Europe, Italy had the highest prevalence with 38.2% (95% CI: 18.9, 57.5) (See Table 2 for the full list of country-specific NAFLD prevalence estimations). Meta-regression analysis did not show a significant difference between the pooled prevalence estimates from the populations-based studies and studies based on the health check up visitors [29.2 (95% CI: 24.5, 34.0) vs. 34.7 (95% CI: 31.4, 38.0)], however since a strong trend was indicated [difference= 5.5 (95% CI: -0.1, 11.0); p=0.06], we further explored the possible explanations for this observed difference ([Appendix F](#)).

The NAFLD prevalence reported from urban settings (n=29), rural settings (n=3), or mixed urban/rural settings (n=10) were not different. Similarly, no significant differences were observed in NAFLD prevalence by the sample size of the studies. Ultrasound was the most common imaging modality used to identify hepatic steatosis (n=44/49). The other imaging modalities (CAP, CT scan, or MRS) were used in 4 studies, and one study used both ultrasound and CT scan. No significant differences were observed in NAFLD prevalence based on the imaging modality used.

The R-squared statistics from the univariate meta-regression indicated that the study year and sex significantly explained the heterogeneity in the overall NAFLD prevalence estimate. If assessed categorically as periods, the study year would account for 16.2% (p= 0.007) and, if treated as a numerical variable, explained 16.0% of the heterogeneity (p= 0.002). Calculation of the effect of sex on the heterogeneity for overall NAFLD prevalence was not possible as not all the papers reported sex-specific data; however, for those that reported sex-specific data (n=36), sex

differences accounted for 26.4% of the heterogeneity. The rest of the moderator variables did not explain the heterogeneity. The funnel plot did not show any publication bias, but it showed a widened horizontal distribution of studies due to heterogeneity ([Appendix G](#)). However, Egger's test was not significant ( $p=0.977$ ).

**Table 2:** Stratification and meta-regression analysis of NAFLD prevalence estimates by moderator variables.

Moderator variable	Number of studies	Sample size	NAFLD prevalence (%) (95%CI)	I <sup>2</sup> (%)	Difference (95%CI)
<b>Overall</b>	49	604721	32.3 (29.5, 35.1)	99.81	-
<b>Period</b>					
2000-2005	8	19691	26.6 (19.6, 33.7)	99.26	Reference
2006-2010	15	214816	30.3 (25.7, 34.9)	99.70	3.6 (-4.2, 11.5)
2011-2015	21	342051	33.0 (29.1, 36.9)	99.80	6.4 (-1.1, 13.8)
2016 or later	5	28163	44.1 (37.8, 50.3)	99.57	17.5 (7.3, 27.7)*
<b>Continent</b>					
Asia	44	597696	32.5 (29.5, 35.4)	99.83	Reference
Europe	5	7025	30.5 (21.8, 39.2)	98.35	-1.9 (-11.3, 7.4)
<b>Country/Territory</b>					
China	15	141094	32.0 (27.5, 36.5)	99.67	Reference
South Korea	10	367903	34.0 (29.4, 38.7)	99.87	2.0 (-6.2, 10.3)
Taiwan	6	56755	34.4 (24.3, 44.5)	99.82	2.4 (-7.4, 12.1)
Japan	4	12428	25.1 (17.0, 33.3)	99.12	-6.8 (-18.2, 4.5)
Iran	4	14168	37.4 (22.3, 52.6)	99.66	5.4 (-6.0, 16.8)
India	2	3092	37.0 (8.5, 65.4)	99.65	5.0 (-10.3, 20.2)
Bangladesh	1	1019	18.5 (16.2, 20.9)	-	-13.5 (-34.4, 7.5)
Hong Kong	1	911	28.8 (25.8, 31.7)	-	-3.2 (-24.3, 17.8)
Israel	1	326	30.1 (25.1, 35.0)	-	-1.9 (-23.3, 19.5)
Italy	2	1757	38.2 (18.9, 57.5)	98.66	6.2 (-9.1, 21.5)
Germany	2	4502	25.4 (21.6, 29.1)	85.54	-6.7 (-22.0, 8.5)
Spain	1	766	25.8 (22.7, 28.9)	-	-6.2 (-27.2, 14.9)

<b>Sex</b>					
Male	36	208360	39.1 (35.4, 42.8)	99.61	Reference
Female	36	183164	25.0 (21.7, 29.2)	99.73	-13.6 (-18.9, -8.4)*
<b>Sample source</b>					
Population-based	19	54144	29.2 (24.5, 34.0)	99.33	Reference
Health check-up visitors	29	549558	34.7 (31.4, 38.0)	99.84	5.5 (-0.1, 11.0)
Patients' companions	1	1019	18.5 (16.2, 20.9)	-	-10.7 (-30.1, 8.7)
<b>Study setting</b>					
Urban	29	391980	32.6 (28.9, 36.3)	99.82	Reference
Rural	3	7986	29.5 (14.3, 44.7)	99.60	-3.1 (-15.3, 9.1)
Mix	10	177436	32.5 (25.1, 39.8)	99.80	-0.2 (-7.5, 7.2)
Unclear	7	27319	31.9 (28.1, 35.6)	97.13	-0.9 (-9.4, 7.6)
<b>Sample size</b>					
<1000	11	8106	30.6 (24.2, 37.0)	97.71	
1000-10000	28	113598	32.8 (28.7, 36.9)	99.57	2.2 (-4.9, 9.3)
>10000	10	483017	32.6 (28.8, 36.3)	99.86	2.0 (-6.7, 10.7)
<b>Imaging modality</b>					
Ultrasound	44	593170	32.3 (29.5, 35.2)	99.81	Reference
CAP	2	3639	45.3 (40.2, 50.4)	86.53	13.1 (-0.6, 26.8)
MRS	1	911	28.8 (25.8, 31.7)	-	-9.7 (-28.7, 9.3)
CT	1	3166	22.6 (21.2, 24.1)	-	-3.6 (-22.8, 15.6)
Ultrasound and CT	1	3835	16.3 (15.1, 17.5)	-	-16.0 (-35.0, 3.0)

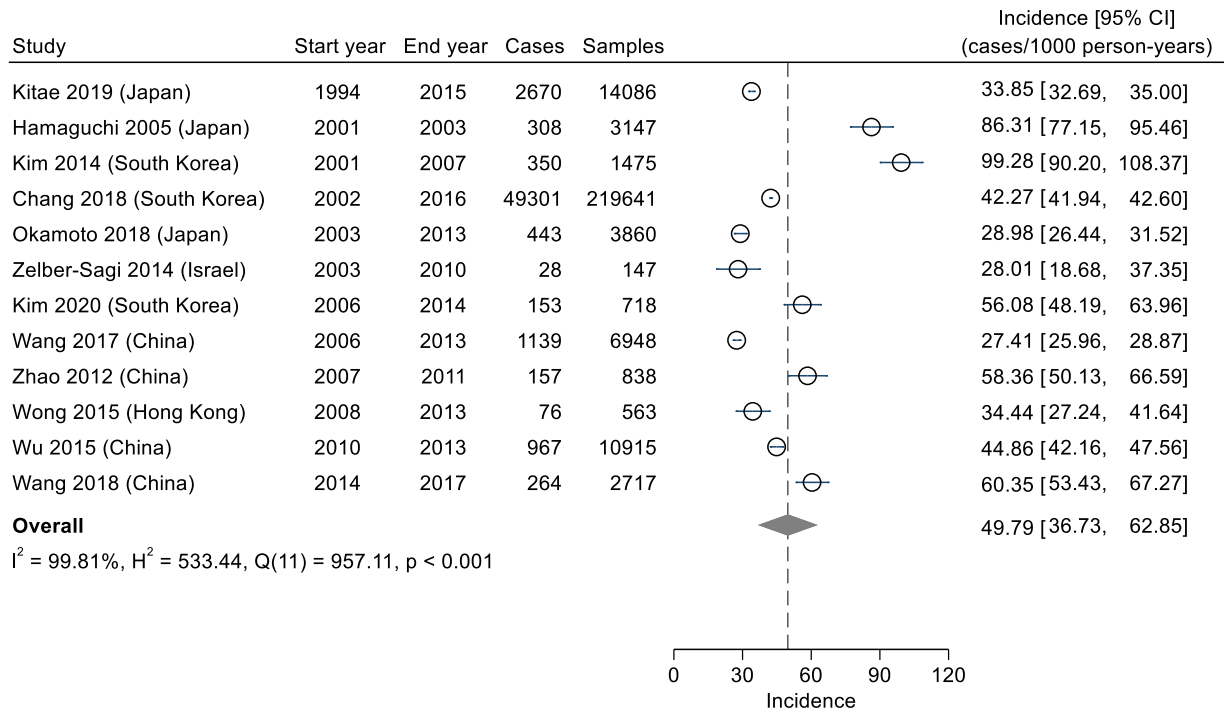
\* Statistically significant.

### 2.6.2 NAFLD incidence

The 12 studies included for incidence analysis comprised a sample size of 265055 (range 147- 219641, median 2932). The mean age of the participants was  $42.2 \pm 2.9$  years (weighted average from 11 studies that reported the study sample mean age at the beginning of the follow-up). Men accounted for 44.1% of the participants. All the included publications for incidence estimation were from Asia, with 4 from mainland China, 3 from South Korea, 3 from Japan, and 1 from Hong Kong and Israel each. Ultrasound was the imaging method for 11 publications, and MRS was used in 1.

The overall pooled NAFLD incidence rate was estimated at 49.8 (36.7, 62.8) per 1000 person-years ([Figure 5](#)). 8 studies reported the sex-specific data allowing the calculation and pooling of the sex-specific NAFLD incidence. We calculated the overall incidence rate of NAFLD for males at 78.2 (95% CI: 50.5, 105.8) per 1000 person-years and females at 31.4 (95% CI: 19.6, 43.1) per 1000 person-years. This difference was statistically significant [difference= -46.1 (95%CI: -75.9, -16.4); p= 0.002].

Considerable heterogeneity was observed between NAFLD incidence studies ( $I^2=99.81$ ) which we investigated through stratification and univariate meta-regression analysis ([Table 3](#)) and R-squared calculation. None of the geographical location, sample size, sample source, and imaging modality explain the heterogeneity, however, mean follow-up duration significantly explained 50.6% of the heterogeneity (p<0.001). For the 8 papers which reported sex-specific data, sex differences accounted for 36.5% of the heterogeneity (p=0.002). The funnel plot for incidence data is presented in [Appendix H](#).



**Figure 5:** Forrest plot using random-effects models for all included studies for NAFLD incidence estimation.

**Table 3: Stratification and meta-regression analysis of pooled NAFLD incidence by moderator variables.**

<b>Moderator variable</b>	<b>Studies</b>	<b>Sample size</b>	<b>NAFLD incidence (cases/1000 person-years)(95%CI)</b>	<b>I<sup>2</sup> (%)</b>	<b>Difference (95%CI)</b>
<b>Overall</b>	12	265055	49.8 (36.7, 62.8)	99.81	-
<b>Countries/Territories</b>					
China	4	21418	47.4 (32.4, 62.4)	98.74	Reference
South Korea	3	221834	65.7 (32.1, 99.3)	99.95	18.0 (-19.7, 55.7)
Japan	3	21093	49.5 (13.7, 85.2)	99.81	1.7 (-35.9, 39.3)
Hong Kong	1	563	34.4 (27.2, 41.6)	-	-13.2 (-68.4, 42.1)
Israel	1	147	28.0 (18.7, 37.3)	-	-19.6 (-75.2, 36.0)
<b>Sex</b>					
Male	8	116919	78.2 (50.5, 105.8)	99.88	Reference
Female	8	148136	31.4 (19.6, 43.1)	99.68	-46.1 (-75.9, -16.4)*
<b>Sample size</b>					
<1000	4	2266	44.3 (29.4, 59.2)	92.58	Reference
1000-10000	5	18147	60.2 (31.6, 88.8)	99.68	15.7 (-15.4, 46.8)
>10000	3	244642	40.3 (33.8, 46.8)	99.03	-3.9 (-39.1, 31.3)
<b>Sample source</b>					
Population-based	2	710	31.9 (25.8, 38.1)	12.36	Reference
Health check-up visitors	10	264345	53.5 (38.8, 68.1)	99.85	20.4 (-11.0, 51.8)
<b>Imaging modality</b>					
Ultrasound	11	264492	51.2 (37.2, 65.2)	99.84	Reference
MRS	1	563	34.4 (27.2, 41.6)	-	-39.1 (-124.4, 46.2)

\* Statistically significant.

## 2.7 Discussion

### 2.7.1 Increasing NAFLD prevalence trends

NAFLD prevalence is rising in parallel with the global increase of obesity and T2DM. In this systematic review and meta-analysis, we studied 49 papers encompassing a total sample size of 604,721. We estimate that the overall prevalence of NAFLD is 32.3%. The studies used in calculating this number were performed during 18 years from 2000 to 2017. An increase in NAFLD prevalence was observed over time, with the prevalence steadily increasing from 26.6% for 2000-2005 to 30.3% for 2006-2010, to 33.0% for 2011-2015, and an alarming 44.1% for 2016 or later. Hence, a 17.5% increase in the NAFLD prevalence between those reported in 2000-2005 and those in 2016 or later.

The increasing trend observed in our study is in line with previous reports. The report by Younossi *et al.* showed a 6.5% increase, from 20.1% to 26.8%, between the years 2000 to 2015 (4), and the report by Li *et al.*, studying only Asia, showed a jump from 25.3% to 33.9% within the same period (111). In this study, like the study by Li *et al.*, we used the study period instead of the publication year as used by Younossi *et al.*, which according to our data, has an average lag of 4.2 years following the study date (4, 111).

### 2.7.2 Sex and gender considerations in NAFLD prevalence

Our study shows that men have a significantly higher prevalence of NAFLD than women (39.1% vs. 25.5%). Such difference in prevalence is also reflected in the incidence rate difference between men and women (78.2 vs. 31.4 per 1000 person-years). Our study's analysis of temporal trends indicates that the NAFLD prevalence in both male- and female-specific are significantly

rising, getting as high as 48.3% for men and 39.8% for women after 2016. Out of 49 publications selected for prevalence analysis, 36 publications provided separate data for males and females, while 8 out of 12 incidence papers contained sex-specific data. We did not include single-sex studies in calculating sex-specific estimates and only analyzed publications that included both males and females. We believe this provides a more accurate comparison between the sex-specific data. Studying men and women in parallel could help control the research-associated factors, like geographical location, ethnicity, age range, healthcare access, etc.

NAFLD prevalence is commonly reported higher in men by the existing systematic reviews (111, 112, 128). Most of the individual prevalence studies have reported a nearly similar NAFLD prevalence difference between men and women (34). However, there have been some studies that reported a higher NAFLD prevalence in women. Out of the 36 publications we analyzed for sex-specific prevalence estimates, 4 reported higher prevalence in females (data not shown)(129-132). Such unexpected reports might signify the importance of gender-related factors in NAFLD, despite the considerable literature indicating a protective role for female sex against NAFLD.

The difference between the NAFLD prevalence among men and women can be examined based on either sex- or gender-related differences in the determinants of disease. Sex and gender differences produce fundamental biological variations in the disease and its progression (133). Sex differences refer to biological and physiological characteristics of males or females, including anatomy, physiology, genes, and hormones. Gender, on the other hand, is a multidimensional social construct and refers to the socially constructed characteristics, including norms, behaviours and roles associated with being a man or woman (133, 134). The experience of gender is always linked to the social and political context. As such, gender is also intimately connected to social and economic status in systems (135). Differences in socio-cultural characteristics, such as dietary

patterns, exercise, and quality of life, are equally crucial in NAFLD as sex differences (136, 137). However, gender and sex, while separate concepts, are intimately linked and reciprocally influence each other. While research on the differences between the sexes is essential, it is critical to explore further how sex and gender operate collectively to influence health outcomes and behaviours (135).

#### *2.7.2.1 Sex-differences in NAFLD prevalence*

Numerous studies have sex differences in NAFLD's risk, onset, progression, and treatment response (138, 139). Despite considerable advances, the molecular mechanisms by which sex modulates NAFLD's pathogenesis and clinical outcome are not entirely understood, and most of the available information comes from animal studies (138). Profound differences have been indicated in lipid metabolism, glucose homeostasis, oxidative stress, inflammation, and fibrosis (137, 138, 140). The liver shows sexual dimorphisms in a complex set of genes related to lipid metabolism, drug metabolism, and glucose homeostasis (138). Moreover, Sex is a major determinant of body composition, energy storage and partitioning (140). For example, compared to men and postmenopausal women, women of fertile age are typically spared from storing fat in the visceral and ectopic compartments, are more insulin-sensitive, and display a higher capacity to secrete insulin and incretin, being relatively protected against the development of T2D and NAFLD (140). Central obesity or visceral fat, excess fat in the abdomen, is often associated with the development and progression of NAFLD or more advanced forms of liver disease (141). A recent study shows that a lower threshold of total body fat (26.73% vs. 32.23%) or abdominal fat (13.76% vs. 21.42%) is needed to increase the risk of developing NAFLD in men compared to women, respectively (142). Furthermore, other major risk factors for NAFLD, including MetS, gut

microbiome, and T2DM, also display profound sex differences (138). Therefore, a better understanding of the molecular mechanisms and the role of sexual dimorphism is of paramount importance to improve both prevention and therapeutic strategies of NAFLD. The comprehensive discussion of sex-differences in NAFLD pathogenesis is beyond the scope of this paper, however we have briefly reviewed the role of sex hormones as a major factor to the development and progression of NAFLD.

#### *2.7.2.2 Role of sex hormones in NAFLD*

The prevalence of NAFLD is higher in men than women, but it is also higher among postmenopausal women compared to women of reproductive age (138). Hormone replacement therapy in postmenopausal women lowers the prevalence of NAFLD (143). Moreover, oral contraceptive pills are associated with a reduction in insulin sensitivity (144). All such evidence suggests that estrogens may protect females against insulin resistance. The role of estrogen signalling in the metabolism of lipids and glucose has been shown in several publications (reviewed in (145)). In vitro and animal studies strongly suggest that estradiol has a protective effect in females by enhancing fatty acid oxidation and reducing hepatic lipogenesis (146). Estrogens are believed to regulate systemic insulin sensitivity and glucose tolerance through modulation of the metabolic function of adipose tissue, glucose homeostasis in skeletal muscle and liver, central regulation of energy balance, and protective actions on beta-cell preservation and maintenance of insulin secretion (146). Such helpful regulatory effects of estrogens are mediated mainly through ER-alpha receptors (146). In postmenopausal women, estrogen deficiency accelerates visceral obesity, IR and T2DM, while estradiol therapy improves insulin sensitivity and glucose homeostasis (146, 147).

Similar to estrogens effects in women, androgens in men exert positive metabolic effects by improving insulin sensitivity and lowering body fat (148). Although primarily a male hormone, testosterone is also produced in women's ovaries, adrenal glands, and peripheral tissues in far less quantities than men (149). In men, a low level of testosterone is associated with the features of MetS, and its supplementation causes favourable effects (150). Conversely, in women, insulin resistance and obesity are associated with increased testosterone levels (151). A systematic review of the association of testosterone and NAFLD has shown that NAFLD is associated with lower serum testosterone levels in men and higher serum testosterone levels in women (152). Similar associations have been reported between testosterone levels and T2DM (153). Low testosterone levels also correlate with the development of NASH and increasing fibrosis stage (154).

### *2.7.2.3 Gender influence on NAFLD prevalence*

Gender includes socially constructed roles, values, relationships, behaviours, attitudes, relative power, and influence that society assigns to the two sexes on a differential basis (133, 134). Gender roles, the behavioural norms applied to men and women in society, do not exist in isolation and are dependent on time and location (133, 134).

Gender-focused epidemiologic studies regarding NAFLD are currently limited. This warrants the need for research to generate a systematic body of knowledge on the influence of gender on NAFLD since gender differences are as important as sex differences in human health and influence communities, clinicians, and patients (133). The evidence on gender differences in the determinants of chronic non-communicable diseases has been reviewed by Vlassoff (2007)(133). In particular, the section on the gender differences in social determinants of nutrition reasonably applies to NAFLD since diet plays a crucial role in the development of NAFLD (155).

A healthy diet, sufficient physical activity, and weight reduction, which are currently the foundations of NAFLD prevention and treatment, are all influenced by gender. Gender differences in eating behaviours have historically been reported, with women experience more pressure to be thin and attempt more dieting behaviour even from early adolescence when compared to men (156, 157). Unhappiness with body weight and shape is more common among women than men (156). Compared to men, women are more likely to have stronger beliefs in healthy eating, avoid high-fat foods, and eat fruit and fibre (157).

Gender influences the social determinants of nutrition patterns and hence on health outcomes (133, 158). As an example from the developing countries, in women, social factors such as the degree of education, income, or participation in public life, affect their knowledge about health problems and how to prevent and treat them, which positively affects the nutritional status of themselves and their children, as well as preventing gender bias in children food allocation (159). Gender bias in children's food allocation refers to the preferential food allocation to males over females in families in developing countries, mainly for their future gender role as families' primary source of income (160). Another example of a family's lack of access to nutritional food due to gender roles is that while women are selected for nutritional education because they are responsible for the preparation of meals, they often lack access to nutritional food because men, as breadwinners, generally make decisions about buying and making food without receiving any education about nutrition (161).

Gender can influence the amount of physical activity as well. For instance, in many societies, the physical activity of women remains low due to barriers resulting from culturally imposed gender norms and roles (162, 163). This way, a gender norm can influence NAFLD

prevalence since physical activity is directly associated with both NAFLD development and treatment.

These differences, besides many more, indicate that gender-related differences in lifestyle (157, 164, 165) are crucial in explaining NAFLD prevalence differences between males and females. Gender differences, however, are not limited to nutrition. Gender constructs affect many other social determinants of diseases, including access to health care as well as the behaviour of the community, clinicians, and patients in a healthcare system (133).

### *2.7.3 Diagnostic modalities for fatty liver in NAFLD prevalence/incidence studies*

According to the AASLD practice guidance, the definition of NAFLD requires evidence of hepatic steatosis, either by imaging or histology, and the absence of other causes of hepatic steatosis, like significant alcohol consumption, viral hepatitis, use of steatogenic medications, etc. (1). Therefore, a crucial part of NAFLD diagnosis is the confirmation of hepatic steatosis, hence detecting and quantifying the amount of fat inside the liver (76).

In our study, ultrasound was the most common imaging modality used for the diagnosis of fatty liver. Among imaging modalities used for diagnosing NAFLD, ultrasound is the most common as it is safe, relatively cheap, and widely available. It is considered an accurate method in the diagnosis of fatty liver with an AUROC of 0.93 and high sensitivity (85%) and specificity (94%), especially with steatosis grades over 20% (166, 167). However, the sensitivity drops considerably when steatosis is <20%, and it also tends to be inaccurate in the presence of fibrosis, iron overload, morbid obesity, and renal disease (166, 168, 169). Nonetheless, it is recommended as the first-line diagnostic procedure for imaging of NAFLD by European Associations for the

Study of Liver (EASL), Diabetes (EASD), and Obesity (EASO), considering its overall pros and cons (170).

The gold standard for diagnosis of hepatic steatosis is a liver biopsy. Yet, it is not feasible for prevalence studies of the general population nor for initial screening for NAFLD, as it is invasive, expensive, and with complications (171, 172), ranging from pain (20%) to less common but potentially severe complications including hemorrhage, bile leak and visceral perforation that happen in around 1% of the procedures with 0.1% mortality rate (172-174). Biopsies are performed on a limited subset of the population. The indications for liver biopsy include a histological demonstration of the disease (as in auto-immune hepatitis), a part of diagnostic workup (in cholestatic liver diseases or storage diseases), and an assessment of inflammation and fibrosis (e.g., for NASH, or viral hepatitis) (174). Thus, retrospective pathology-based studies cannot be used as prevalence estimation reference as their study sample would never represent the general population (e.g., Mohanty *et al.* (175) in (4)). For this reason, the prevalence studies generally use non-invasive methods to ascertain fatty liver, which includes imaging modalities or panels of serum biomarkers.

CT scan has shown better accuracy than ultrasound for the diagnosis of hepatic steatosis; however, its disadvantages include radiation exposure and low sensitivity for mild grades of steatosis (166, 176). MRS is shown to have a high correlation with biopsy and has a sensitivity and specificity of 80% for steatosis at grades as low as 5% (177); however, it is expensive, scarce, and complicated (166, 171). CAP is a novel physical parameter that measures hepatic steatosis with ultrasound attenuation using FibroScan® (178). While it is a rapid, non-invasive, and relatively low-cost procedure, it has limited efficacy due to the lack of optimal threshold for detecting hepatic steatosis, high failure rates in obese patients, and mediocre sensitivity (166, 178).

Panels of surrogate biomarkers of liver steatosis are acceptable alternatives for the diagnosis of steatosis. They are non-invasive and are based on aggregate scores from individual anthropometric and biochemical components that can be routinely measured in clinical practice and, therefore, easy to employ (171, 179). Biomarker-based panels are recommended when the imaging tools are not available or logistically possible (e.g., large epidemiological studies) (170). The two most used panels in prevalence studies are the Fatty Liver Index (FLI) and Hepatic Steatosis Index (HSI). FLI is calculated out of body mass index (BMI), waist circumference, and serum triglyceride and gamma-glutamyl-transferase (GGT) levels. In comparison, HSI is calculated using serum transaminases ratio (ALT/AST), BMI, and the existence of T2DM (180, 181). Although these methods have been recommended for epidemiological studies on NAFLD, and they have acceptable diagnostic accuracy for detection of steatosis, the existing evidence points to their limitations in quantifying steatosis as defined by MRI or liver biopsy; besides; their accuracy can be affected by the presence of steatohepatitis and fibrosis (171, 182). Previous systematic reviews have shown that the biomarker-based prevalence reports underestimated the NAFLD prevalence compared to imaging, roughly by half. For instance, Younossi *et al.* reported that the pooled global prevalence by FLI was 9-13% for different continents, compared to overall 25.2% by imaging (4). Similarly, Li *et al.* reported a prevalence of 15.8% among patients diagnosed with FLI or HSI versus 30.6% by ultrasound and 25% with CT scan or MRI in Asia (111). Therefore, since imaging has been a more reliable method than the biomarker-based panels, we only included the imaging-based studies in our analysis.

Using disease-identifying codes like ICD codes is another method that is sometimes used to estimate NAFLD prevalence. However, these parameters tend to underestimate the prevalence rates mainly because NAFLD is a profoundly under-diagnosed condition and is under-registered

in health record databases. It is estimated that over 75% of patients with NAFLD remain undiagnosed (183, 184). A study of the health records from four major European countries, the UK, Italy, Spain, and the Netherlands, showed a pooled NAFLD prevalence of 1.9% in 2014 (185), which is hugely lower than the 30.5% prevalence that we estimated for Europe. Another example is the ICD-based 2.92% NAFLD prevalence extracted from the paper by Sanyal *et al.* (186) in Supplementary Fig 1D in Younossi *et al.* (4) compared to the estimated 28.3% prevalence estimated for the same period in the USA (187).

#### *2.7.4 NAFLD prevalence in different regions of the world*

Most of the reports in our study were from Asia (44 out of 49 papers), with an overall Asian NAFLD prevalence of 32.5%. Among the Asian countries with multiple reports, the highest NAFLD prevalence was from Iran with 37.4%, followed by Taiwan (34.4%), South Korea (34%), and China (32%), while Japan had a lower prevalence at 25.1%. Our results are comparable to the recent report by Li *et al.* (111), who estimated a NAFLD prevalence at 38.1%, 33.3%, 32.9%, 29.8%, and 22.3% for Iran, Taiwan, South Korea, China, and Japan, respectively, with an overall ultrasound-based prevalence of 30.6% for the whole continent (111). The study by Younossi *et al.* also reported an overall NAFLD prevalence of 27.4% (4), indicating a steady rise in the prevalence of NAFLD in this region. The highest prevalence for NAFLD, as reported by Younossi *et al.*, was from the Middle East with 32% (compared with a 25.2% global rate) (4). That estimation was based on three publications, 2 being population-based studies (188, 189) and another from Turkey, which studied a sample of non-obese gastroenterology and internal medicine outpatients, which neither represented the general population nor were appropriate for NAFLD prevalence study for their selected weight range (190). Excluding the Turkish publication, the estimated NAFLD

prevalence would have been ~20%. Nevertheless, our estimated prevalence of 36% from the papers from the region is in line with their claim, although we refrain from generalizing our finding to the whole region of the Middle East since we only have data from two countries, against some 18 countries associated with the region. Apart from Iran, for which several recent reports have been published on NAFLD prevalence (111, 191), there has been no new data from the region. A relatively recent study estimated the all-age prevalence of NAFLD at 24.8% for Saudi Arabia and 23.7% for the United Arab Emirates for 2015, using modeling based on changes in prevalence rates of adult obesity and T2DM (192). Similarly, we estimated the overall NAFLD prevalence for Europe at 30.5%, which is higher than the 23.7% reported by Younossi *et al.* (4), suggesting an increasing NAFLD prevalence trend in Europe.

We were not successful in including any papers from North America. While conventionally estimated NAFLD prevalence data from Canada are not available, a recently published modeling study estimated NAFLD prevalence to be over 20%, based on BMI trends (193). For the USA, however, there are a substantial number of published prevalence studies. However, many of the NAFLD research publications in the USA have focused on the National Health and Nutrition Examination Survey (NHANES) cohort, a population-based program of studies designed to assess the health and nutritional status of adults and children in the United States. The NHANES study is based on a complex multistage, stratified, clustered probability-sample design that yields a representative sample of the US population (41). NHANES III was a cross-sectional study from 1988-1994 containing ultrasound imaging information that allowed imaging-based NAFLD assessment (41). Of the 13 publications analyzed by Younossi *et al.*, eight were from that database (4). All these publications showed a NAFLD prevalence of about 34% for mild to severe grades of steatosis (determined by ultrasound) and a prevalence in the low 20s for moderate to severe

liver steatosis (4, 194). The other four publications evaluated subpopulations and hence did not represent the general population (4). Since NHANES III was outside the temporal scope of our study, we did not include any of those publications. The ongoing phase of NHANES started in 1999. Although it does not include imaging data anymore, NAFLD prevalence is calculated using biomarker-based panels, especially the US-FLI, a version of FLI that includes ethnicity and age coefficients to improve accuracy for the American population (195). Based on US-FLI, the prevalence of NAFLD is estimated at 28.3% for 1999-2004, 33.2% for 2009-2012, and 31.9% for 2013-2019 (187). Such high estimates are not unexpected considering the 68% estimated prevalence of overweight and obese individuals reported for the USA in 2016 (196).

South America was reported by Younossi *et al.* as having the second-highest prevalence of NAFLD in the world at 30%, slightly behind the Middle East (4). We could not verify that claim, as neither of the two South American publications met the criteria for inclusion in our analysis. The Columbian report by Perez *et al.* only studied males aged 29–54 years enlisted in the Colombian Air Force (197), and the Brazilian report by Karnikowski *et al.* only recruited subjects aged 55 years or older (198), which may explain why the estimated NAFLD prevalence was so high in that study. Therefore, the prevalence of NAFLD remains unknown in South America because of insufficient epidemiological data. However, considering the high prevalence of overweight and obesity in most countries in South America (e.g., Brazil (56.6%), Argentina(62.7%), Colombia (59.0%), Chile (63%), etc.) (196), and a growing rate of T2DM in that region (199), the NAFLD prevalence is likely high.

Previous estimates had suggested Africa had the lowest NAFLD prevalence worldwide at 13.5%, roughly half the global average (4). However, we were unable to confirm this finding since neither of the two papers used for that estimation met our inclusion criteria, and there were no

more recent publications available for analysis. The publication by Onyekwere *et al.* from Nigeria was a case-control study, and the population used to calculate NAFLD prevalence were non-diabetic hospital workers and students who were age and sex-matched to the case group (200). That population did not represent the general population. The publication by Almobarak *et al.* from Sudan did not exclude viral hepatitis cases (201), even though hepatitis B is highly endemic in Africa (202). Hence, the low reported prevalence of NAFLD in Africa is probably a result of limited available information (203). The obesity data provided by WHO estimates the prevalence of overweight and obesity at 31.1% and rising for Africa, which is lower than the global estimate of 39% (196). Similarly, the prevalence of T2DM in Africa has risen steadily at the same rate as the global average (204), and a few limited available studies indicate that the prevalence of NAFLD among patients with T2DM in Africa is similar to those reported globally (203). Therefore, the prevalence of NAFLD in Africa is likely similar to, or maybe slightly less than, the global average. However, accurate estimates of NAFLD prevalence in Africa await future reliable studies.

#### *2.7.5 Definition limitations in NAFLD prevalence estimation: NAFLD vs. MAFLD*

Currently, a NAFLD diagnosis requires the exclusion of other causes of hepatic steatosis, including excessive alcohol use, viral and autoimmune hepatitis, etc. (1). This has resulted in an inherent limitation both in NAFLD diagnosis and in proper NAFLD prevalence estimation. The current method for prevalence estimation requires that subjects with excessive alcohol use and viral hepatitis be excluded from the study sample size before NAFLD prevalence ratios can be calculated, as there is no way to determine the exact cause of fatty liver (4). This exclusion is often done assuming that the distribution of NAFLD in these populations is similar to the general

population, and their exclusion would not affect the estimation, an assumption with little literature support. It is worth noting that hepatic steatosis due to multiple etiologies is possible and even frequent (205, 206). For example, individuals with NAFLD might have excessive alcohol intake and may contract hepatitis, and vice versa. For that reason, exclusion of cases with alternative causes of hepatic steatosis can negatively affect the accuracy of NAFLD prevalence estimates, even more so in places where such alternative causes are prevalent. For example, in the studies from Japan by Omagari *et al.* (207), Nishioji *et al.* (208), and Hamaguchi *et al.* (209), 55%, 31%, and 25% of the original study samples respectively were excluded due to daily alcohol consumption. Moreover, individual patient alcohol consumption reporting is usually subjective and prone to underestimation (210). In countries where alcohol consumption is outlawed, there is always a possibility of under-reporting due to concerns for retribution or stigma (211, 212). Like excessive alcohol intake, the exclusion of subjects with hepatitis B in Hong Kong would result in the exclusion of nearly 8% of the general population from the NAFLD prevalence studies (213). To address such diagnostic limitations, a recent attempt was made to define specific positive diagnostic criteria for NAFLD and introduce the term metabolic dysfunction-associated fatty liver disease (MAFLD) to replace NAFLD (214, 215). The diagnostic criteria for MAFLD are based on evidence of fatty liver, in addition to one of the following three criteria, overweight/obesity, presence of T2DM, or evidence of at least two metabolic abnormalities if the person is lean (214). MAFLD is not a diagnosis of exclusion but is diagnosed based on the presence of metabolic dysfunction (214). It supports the existence of multiple etiologies for fatty liver and is not based on the exclusion of individuals based on subjective parameters like alcohol consumption, although it is not yet widely accepted by the hepatology community.

### 2.7.6 NAFLD incidence rate

Our study highlights the high NAFLD incidence rate of 49.8/1000 person-years. The incidence rate is higher in males than females, at 78.2 and 31.4/1000 person-years, respectively. Unfortunately, although NAFLD publications were sought worldwide, the publications found to be suitable for analysis were all from Asia. Therefore, it is not surprising that our NAFLD estimates were close to Zhou *et al.* (112), at 50.0/1000 person-years based on studies from China and Li *et al.* at 50.9/1000 person-years for the whole of Asia (111). Published studies examining the worldwide incidence of NAFLD in the general adult population are few, and to the best of our knowledge, only three meta-analysis papers exist in the literature, all of which have similarly only included papers from Asia (4, 111, 112). Lack of published robust data from outside Asia is an important issue that undermines the generalizability of estimated NAFLD incidence rates to anywhere outside of Asia. To the best of our knowledge, our study is the first systematic review to examine sex differences in NAFLD incidence.

To accurately report the NAFLD incidence rate, the study cohort needs to include healthy adults free of fatty liver and negative for alternative causes of hepatic steatosis at the beginning of the follow-up period. Since there is no exact definition of a healthy adult, this is interpreted differently in publications and ranges from the exclusion of cases with other medical conditions, including a history of any malignancy, cardiovascular diseases, and stroke, thyroid disease, or chronic renal disease (216) to studies only focusing on liver-related health with exclusion limited to autoimmune liver disease, drug-induced hepatitis, liver malignancies, infections and biliary tract infections (217). This variability could lead to considerable heterogeneity among the studies.

### *2.7.7 Strengths and limitations of this study*

Our study has several strengths. The epidemiological data provided through our meta-analysis and systematic review are the most up-to-date on NAFLD prevalence and incidence globally. Due to the application of strict inclusion/exclusion criteria, the reports that we have included from the existing literature provide the most accurate available estimates that best represent the general adult population for specific regions. We selected publications that included a wide age range to improve the generalizability of our estimates to the overall adult population. Moreover, sex-specific trends in NAFLD prevalence are reported for the first time.

There are several limitations to this study. Our data was limited to 12 countries from two continents, Asia and Europe. This stemmed from a lack of available quality data from many parts of the world and the high reporting standards used by us to include reports. We chose quality over quantity and think this limitation is a trade-off for more accurate estimates of the global NAFLD situation. In addition, our analysis includes many publications with sub-national level geographical coverage, e.g., from small towns or districts, which may not be generalizable to the whole country. We hope our report highlights the need for more accurate data to be generated from everywhere in the world to improve geographical coverage. Moreover, our meta-analysis demonstrated a significant level of heterogeneity. We have investigated variables that might explain the heterogeneity, including the geographical location, study time, sex ratio, sample source, size of the study population, and diagnostic modality; however, we could not fully explain the heterogeneity.

### *2.7.8 Conclusion and implications*

In conclusion, NAFLD is a major public health concern worldwide. Our findings highlight that the worldwide prevalence of NAFLD is appreciably higher than what had previously been

estimated and is growing at an alarming rate. Furthermore, epidemiological reports regarding NAFLD from many parts of the world, including Africa, South America, or Oceania, are scarce or non-existent. Bearing in mind the hepatic and extrahepatic health burden associate with NAFLD, together with the lack of any available effective treatment, a dramatic rise in prevalence should drive enhanced awareness of NAFLD at the level of primary care physicians, public health specialists, and health policymakers, to encourage the development of more effective preventive policies. Since basic epidemiological data are the crucial cornerstones for every public health and preventive medicine attempt, continuous updates of the trend will be of the utmost importance.

## 2.8 Appendices:

### 2.8.1 Appendix A: MEDLINE and EMBASE search terms for citation selection.

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**Search terms**

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1. incidence/ or incidence\*.mp. or prevalence/ or prevalence\*.mp.
  2. nafld.mp. or non-alcoholic fatty liver disease.mp.
  3. fatty liver/ or fatty liver.mp. or hepatic steatosis.mp. or nonalcoholic fatty liver/
  4. Non-alcoholic steatohepatitis.mp. or NASH.mp.
  5. 2 or 3 or 4
  6. 1 and 5
  7. limit 6 to animals
  8. limit 7 to humans
  9. 6 not 7
  10. 8 or 9
  11. limit 10 to conference abstracts
  12. 10 not 11
- 

Lines 7-10 were used to exclude animal studies.

Lines 11-12 were only used in EMBASE to exclude conference abstracts.

## 2.8.2 Appendix B: Included studies for NAFLD prevalence analysis

Study	Continent	Country	Study years	Sample size	Mean age $\pm$ SD (y)	%male	Sample source	Imaging modality	NAFLD prevalence (%)
Amirkalali 2014 (131)	Asia	Iran	2008	5023	45.35 $\pm$ 15.9	56.70	population-based	Ultrasound	43.78
Caballeria 2010 (218)	Europe	Spain	2007-2008	766	53 $\pm$ 14.3	42.17	population-based	Ultrasound	25.8
Caserta 2017 (219)	Asia	Italy	2009-2011	867	-	-	population-based	Ultrasound	28.37
Chalmers 2019 (220)	Asia	India	2013-2016	2089	47.2 $\pm$ 11.6 <sup>†</sup>	38.87	population-based	Ultrasound	51.46
Chen 2006 (221)	Asia	Taiwan	2003-2004	2520	-	45.32	population-based	Ultrasound	14.76
Cheng 2013 (222)	Asia	Taiwan	2004-2009	8350	45.4 $\pm$ 11.7 <sup>†</sup>	54.18	health checkup visitors	Ultrasound	34.41
Dai 2017 (223)	Asia	China	2014	40459	43.7 $\pm$ 14.1	45.32	health checkup visitors	Ultrasound	30.03
Eshraghian 2013 (188)	Asia	Iran	2011-2012	832	38.7 $\pm$ 18.4 <sup>†</sup>	38.70	population-based	Ultrasound	15.26
Fan 2018 (224)	Asia	China	2013-2014	28171	48.3 $\pm$ 15	51.08	health checkup visitors	Ultrasound	34.30
Gruchot 2014 (225)	Europe	Germany	2002	1195	40.8 $\pm$ 12.3	47.62	population-based	Ultrasound	23.3
Hamaguchi 2005 (209)	Asia	Japan	2001	4401	47.6 $\pm$ 8.8	58.44	health checkup visitors	Ultrasound	18.45
Hong 2012 (226)	Asia	China	2007	3835	46	45.92	population-based	Ultrasound and CT	16.30
Huan 2016 (227)	Asia	China	2013-2014	3455	-	-	population-based	Ultrasound	32.82
Huang 2020 (228)	Asia	Taiwan	2005-2016	2483	46.9 $\pm$ 13.4	30.37	health checkup visitors	Ultrasound	44.54
Hung 2018 (229)	Asia	Taiwan	2005-2011	32346	50.4 $\pm$ 12.3	52.11	health checkup visitors	Ultrasound	41.39
Jeong 2013(230)	Asia	South Korea	2009-2010	141610	41.6 $\pm$ 8.6	57.16	health checkup visitors	Ultrasound	27.30
Jiang 2018 (231)	Asia	China	2014-2015	9694	53.7 $\pm$ 14.1 <sup>†</sup>	41.18	population-based	Ultrasound	27.55
Kim 2009 (232)	Asia	South Korea	2000	2895	47 $\pm$ 8.7	51.88	health checkup visitors	Ultrasound	32.12
Kim 2011 (233)	Asia	South Korea	2006-2007	5083	47.1 $\pm$ 11.8 <sup>†</sup>	50.21	health checkup visitors	Ultrasound	38.23
Kim 2015 (234)	Asia	South Korea	2008-2010	15676	47.9 $\pm$ 11.5 <sup>†</sup>	53.03	health checkup visitors	Ultrasound	41.18
Kim 2018 (235)	Asia	South Korea	2011-2015	160862	36.1 $\pm$ 7.2	51.74	health checkup visitors	Ultrasound	25.47
Kure 2019 (236)	Asia	Japan	2007-2017	3197	56.8 $\pm$ 10.5 <sup>†</sup>	53.89	health checkup visitors	Ultrasound	37.00
Lai 2008 (237)	Asia	Taiwan	2001-2004	3488	49.4 $\pm$ 12.4	50.63	health checkup visitors	Ultrasound	46.96
Lee 2009 (238)	Asia	South Korea	2005-2006	13768	-	53.12	health checkup visitors	Ultrasound	26.18
Lee 2016 (239)	Asia	South Korea	2007-2009	7917	49 $\pm$ 12.8 <sup>†</sup>	44.84	health checkup visitors	Ultrasound	45.98
Lee 2017 (240)	Asia	South Korea	2013-2014	2749	54	58.24	health checkup visitors	CAP	42.85
Lee 2017 (241)	Asia	Taiwan	2006-2009	7568	52.6 $\pm$ 12.1 <sup>†</sup>	59.36	health checkup visitors	Ultrasound	24.21
Lee 2018 (242)	Asia	South Korea	2012-2014	751	49.5 $\pm$ 11.3	39.95	health checkup visitors	Ultrasound	33.16
Li 2013 (243)	Asia	China	2008	712	46.5 $\pm$ 12.7 <sup>†</sup>	39.04	population-based	Ultrasound	22.33
Li 2017 (244)	Asia	China	2016	19804	46.76 $\pm$ 14.1 <sup>†</sup>	76.08	health checkup visitors	Ultrasound	36.98
Lu 2016 (245)	Asia	China	2006	1948	41.3 $\pm$ 11.4 <sup>†</sup>	65.86	health checkup visitors	Ultrasound	35.47

<b>Nishioji 2015 (208)</b>	Asia	Japan	2011-2012	3271	56.8 <sup>†</sup>	44.24	health checkup visitors	Ultrasound	24.61
<b>Omagari 2002 (207)</b>	Asia	Japan	2000	1559	-	-	health checkup visitors	Ultrasound	20.46
<b>Ostovaneh 2015 (246)</b>	Asia	Iran	2008-2011	7723	39.8 ± 19.9 <sup>†</sup>	54.75	population-based	Ultrasound	39.84
<b>Petta 2018 (129)</b>	Europe	Italy	2015	890	53 ± 14	48.09	health checkup visitors	CAP	48.09
<b>Qiao 2017 (247)</b>	Asia	China	2009-2011	7583	45.1 ± 9.1 <sup>†</sup>	65.79	health checkup visitors	Ultrasound	48.08
<b>Saha 2017 (248)</b>	Asia	Bangladesh	2012	1019	38.2 ± 13.3 <sup>†</sup>	31.01	patients' companions	Ultrasound	18.55
<b>Salmanroghani 2019 (249)</b>	Asia	Iran	2017	590	44.8 ± 12.3	54.58	health checkup visitors	Ultrasound	51.02
<b>Shin 2015 (250)</b>	Asia	South Korea	2009-2011	16592	48.3 ± 10.6 <sup>†</sup>	40.87	health checkup visitors	Ultrasound	28.1
<b>Uchil 2009 (251)</b>	Asia	India	2006	1003	-	56.33	health checkup visitors	Ultrasound	22.43
<b>Volzke 2005 (252)</b>	Europe	Germany	1997-2001	3307	-	-	population-based	Ultrasound	27.15
<b>Wang 2015 (253)</b>	Asia	China	2014	13729	-	-	health checkup visitors	Ultrasound	34.97
<b>Wei 2015 (254)</b>	Asia	Hong Kong	2008-2010	911	48 ± 11	41.93	population-based	MRS	28.76
<b>Wu 2018 (255)</b>	Asia	China	2007	646	46 ± 11.9 <sup>†</sup>	41.95	population-based	Ultrasound	20.74
<b>Zelber-Sagi 2006 (256)</b>	Europe	Israel	2003-2004	326	50.5 ± 10.3	-	population-based	Ultrasound	30.06
<b>Zhai 2016 (257)</b>	Asia	China	2014	5066	52.2 ± 14	31.64	population-based	Ultrasound	43.29
<b>Zhai 2017 (130)</b>	Asia	China	2014	2011	54 ± 13	26.11	population-based	Ultrasound	40.97
<b>Zhang 2017 (258)</b>	Asia	China	2013	815	46.31 ± 10.3 <sup>†</sup>	70.80	health checkup visitors	Ultrasound	33.25
<b>Zhou 2019 (259)</b>	Asia	China	2012-2014	3166	49.6 ± 11.9 <sup>†</sup>	39.10	population-based	CT	22.62

† Mean age was calculated by us from the provided data.

### 2.8.3 Appendix C: Included studies for NAFLD incidence analysis.

Study	Country/ Territory	Study period	Sample size	Mean age $\pm$ SD (y)	%Male	Sample source	Imaging	follow up (y)	NAFLD Incidence (/1000 person- years)
<b>Chang 2018 (260)</b>	South Korea	2002- 2016	219641	-	43.19	health checkup visitors	US	5.31	42.27
<b>Hamaguchi 2005 (209)</b>	Japan	2001- 2003	3147	47.2 $\pm$ 8.8 <sup>†</sup>	53.83	health checkup visitors	US	1.134	86.31
<b>Kim 2014 (261)</b>	South Korea	2001- 2007	1475	51.1 $\pm$ 11.1 <sup>†</sup>	35.59	health checkup visitors	US	2.39	99.28
<b>Kim 2020 (216)</b>	South Korea	2006- 2014	718	42 $\pm$ 8.8 <sup>†</sup>	48.05	health checkup visitors	US	3.8	56.08
<b>Kitae 2019 (262)</b>	Japan	1994- 2015	14086	40 $\pm$ 9.6 <sup>†</sup>	48.44	health checkup visitors	US	5.6	33.85
<b>Okamoto 2018 (263)</b>	Japan	2003- 2013	3860	41.1 $\pm$ 10.1 <sup>†</sup>	39.17	health checkup visitors	US	3.96	28.98
<b>Wang 2017 (264)</b>	China	2006- 2013	6948	44.5 $\pm$ 12.8 <sup>†</sup>	64.02	health checkup visitors	US	5.98	27.41
<b>Wang 2018 (265)</b>	China	2014- 2017	2717	40.6 $\pm$ 12	51.09	health checkup visitors	US	1.61	60.35
<b>Wong 2015 (266)</b>	Hong Kong	2008- 2013	563	48 $\pm$ 10	37.12	Population-based	MRS	3.92	34.44
<b>Wu 2015 (267)</b>	China	2010- 2013	10915	40.9 $\pm$ 12.4 <sup>†</sup>	42.38	health checkup visitors	US	1.975	70.16
<b>Zelber-Sagi 2014 (189)</b>	Israel	2003- 2010	147	51.0 $\pm$ 9.8	47.62	Population-based	US	6.8	28.01
<b>Zhao 2012 (217)</b>	China	2007- 2011	838	46.5 $\pm$ 14.2	49.40	health checkup visitors	US	3.21	58.36

† Mean age was calculated by us from the provided data.

## 2.8.4 Appendix D1: Quality assessment of analyzed studies for prevalence estimation

<b>Publication</b>	<b>Was the study's target population a truly or somewhat representation of the general population</b>	<b>Was the sample size adequate?</b>	<b>Were valid methods used for the identification of the condition?</b>
Amirkalali 2014 (131)	Yes	Yes	Yes
Caballeria 2010 (218)	Yes	Yes	Yes
Caserta 2017 (219)	Yes	Yes	Yes
Chalmers 2019 (220)	Yes	Yes	Yes
Chen 2006 (221)	Yes	Yes	Yes
Cheng 2013 (222)	Yes	Yes	Yes
Dai 2017 (223)	Yes	Yes	Yes
Eshraghian 2013 (188)	Yes	Yes	Yes
Fan 2018 (224)	Yes	Yes	Yes
Gruchot 2014 (225)	Yes	Yes	Yes
Hamaguchi 2005 (209)	Yes	Yes	Yes
Hong 2012 (226)	Yes	Yes	Yes
Huan 2016 (227)	Yes	Yes	Yes
Huang 2020 (228)	Yes	Yes	Yes
Hung 2018 (229)	Yes	Yes	Yes
Jeong 2013(230)	Yes	Yes	Yes
Jiang 2018 (231)	Yes	Yes	Yes
Kim 2009 (232)	Yes	Yes	Yes
Kim 2011 (233)	Yes	Yes	Yes
Kim 2015 (234)	Yes	Yes	Yes
Kim 2018 (235)	Yes	Yes	Yes
Kure 2019 (236)	Yes	Yes	Yes
Lai 2008 (237)	Yes	Yes	Yes
Lee 2009 (238)	Yes	Yes	Yes
Lee 2016 (239)	Yes	Yes	Yes
Lee 2017 (240)	Yes	Yes	Yes
Lee 2017 (241)	Yes	Yes	Yes
Lee 2018 (242)	Yes	Yes	Yes
Li 2013 (243)	Yes	Yes	Yes
Li 2017 (244)	Yes	Yes	Yes
Lu 2016 (245)	Yes	Yes	Yes
Nishioji 2015 (208)	Yes	Yes	Yes
Omagari 2002 (207)	Yes	Yes	Yes
Ostovaneh 2015 (246)	Yes	Yes	Yes
Petta 2018 (129)	Yes	Yes	Yes
Qiao 2017 (247)	Yes	Yes	Yes

Saha 2017 (248)	Yes	Yes	Yes
Salmanroghani 2019 (249)	Yes	Yes	Yes
Shin 2015 (250)	Yes	Yes	Yes
Uchil 2009 (251)	Yes	Yes	Yes
Volzke 2005 (252)	Yes	Yes	Yes
Wang 2015 (253)	Yes	Yes	Yes
Wei 2015 (254)	Yes	Yes	Yes
Wu 2018 (255)	Yes	Yes	Yes
Zelber-Sagi 2006 (256)	Yes	Yes	Yes
Zhai 2016 (257)	Yes	Yes	Yes
Zhai 2017 (130)	Yes	Yes	Yes
Zhang 2017 (258)	Yes	Yes	Yes
Zhou 2019 (259)	Yes	Yes	Yes

### 2.8.5 Appendix D2: Quality assessment of analyzed studies for prevalence estimation (continued)

Publication	Was the condition measured in a standard, reliable way for all participants?	How much alcohol was excessive?	Hep B Ag positive excluded	Hep C Ab positive excluded	Info Source for viral hep	Age range of the sample:	Other exclusion criteria
Amirkalali 2014 (131)	Yes	> 30 g/day in men and > 20 g/day in women	Yes	Yes	lab test	18-90	patients with chronic liver diseases, autoimmune hepatitis or Wilson disease, patients with cognitive diseases, and individuals who were incapable of communicating.
Caballeria 2010 (218)	Yes	> 30 g/d in men and > 20 g/d in women	Yes	Yes	lab test	17-83	known chronic liver disease, individuals with incapacitating diseases or cognitive deterioration, institutionalized patients, or those with no fixed address in any of the centers participating in the study.

Caserta 2017 (219)	Yes	>140 g/week for women and > 210 g/week for men over a 2-year period before the study	Yes	Yes	lab test	18-75	Other causes of chronic liver disease and not assuming potentially steatogenic drugs
Chalmers 2019 (220)	Hepatitis excluded, but how they diagnosed hepatitis not mentioned	>21 units per week	Yes	Yes	did not say	≥25	alternate causes of an echo-bright liver such as a history of viral hepatitis or other liver diseases)
Chen 2006 (221)	yes	women: >20 g/week, men: >30 g/week	Yes	Yes	lab test	≥18	known etiologies of liver disease. Subjects were excluded from NAFLD diagnosis when they ingested drugs known to produce fatty liver diseases, such as steroids, estrogens, amiodarone, tamoxifen, or other chemotherapeutic agents within the previous 6 months.
Cheng 2013 (222)	yes	>20 g/d	Yes	Yes	lab test	≥20	clinical, biochemical, and ultrasound findings consistent with liver cirrhosis and other chronic liver diseases, taking amiodarone or other drugs known to promote fatty liver disease.
Dai 2017 (223)	yes	≥140 g for men and ≥70 g for women	Yes	Yes	history	18-94	schistosomiasis liver disease or other chronic liver diseases, a history of taking steatogenic medications, pregnant, a history of taking lipid-lowering medications.
Eshraghian 2013 (188)	yes	>20g/day	Yes	Yes	lab test	>18	history of liver cirrhosis, underlying liver diseases such as

							autoimmune or viral-induced hepatitis, hepatobiliary cancers, medications known to produce fatty liver disease during the last six months before the study, and individuals receiving antithyroid medications.
Fan 2018 (224)	yes	>140 g/week for men and 70 g/week for women	Yes	Yes	history	adults	auto-immune hepatitis or other forms of chronic liver disease, a history of respiratory, heart failure, or renal diseases
Gruchot 2014 (225)	yes	> 20 g/d in women and > 40 g/d in men	Yes	Yes	lab test	18-65	positive history of liver diseases, chronic renal disorders; questionable hepatitis, hemochromatosis, and hypothyroidism (basal TSH > 4 mIU/liter); pharmacological treatment with beta-blockers or diuretics
Hamaguchi 2005 (209)	yes	>20 g/d	Yes	Yes	lab test	21-80	known liver diseases, including viral, genetic, autoimmune, and drug-induced. Current use of medication.
Hong 2012 (226)	yes	>40 g per week	Yes	Yes	lab test	≥18	other liver diseases. People who had abnormal autoantibodies, ceruloplasmin, and iron tests.
Huan 2016 (227)	Hepatitis excluded but based on self-report	> 5 years, ≥40 g/d in males and ≥20 g/d in females	Yes	Yes	questionnaire	20-82	-
Huang 2020 (228)	Yes	>140 g/week	Yes	Yes	medical records	20-70	-
Hung 2018 (229)	Yes	>20 g/d in men and >10 g/d in women	Yes	Yes	history or lab test	≥20	a history of liver or pancreas surgery

Jeong 2013(230)	Yes	>210 g/ week in men and >140 g/ week in women	Yes	Yes	lab test	18-80	liver cirrhosis, liver transplantation, malignant tumor, or other hepatobiliary diseases including cholecystitis, Clonorchis Sinensis, or chronic pancreatitis
Jiang 2018 (231)	Yes	>40 g/day for men or >20 g/day for women	Yes	Yes	lab test	≥18	history of cirrhosis or hepatic carcinoma, long-term medications related to NAFLD (corticosteroids, estrogen, amiodarone, and methotrexate)
Kim 2009 (232)	Yes	> 3 times a week	Yes	Yes	lab test	20 - 79	Lack of on drinking habits, hepatic enzyme concentrations greater than 3 times the normal upper limit, those with overt hypo- or hyperthyroidism, and those with any cancer or sonographic evidence of other chronic liver diseases at baseline.
Kim 2011 (233)	Yes	≥30 g/d for men ≥20 g/d for women	Yes	Yes	lab test	≥20	-
Kim 2015 (234)	Yes	>140g/week for males and >70g/week for females	Yes	Yes	history	>20	human immunodeficiency virus, presence of thyroid disease, including hyperthyroidism, hypothyroidism, or thyroid hormone replacement therapy, abnormal ultrasonographic liver findings (i.e., suspected hepatocellular carcinoma, hepatic mass, or signs of Clonorchis Sinensis)
Kim 2018 (235)	Yes	>140 g/week for men and >70	Yes	Yes	lab test	≥18	a history of HCC

		g/week for women					
Kure 2019 (236)	Yes	>20g per day	Yes	Yes	medical records	22-74	-
Lai 2008 (237)	Yes	did not say	Yes	Yes	lab test	20-87	-
Lee 2009 (238)	Yes	≥20 g/day	Yes	Yes	lab test	adults	biliary disease, liver cirrhosis, or malignant disease
Lee 2016 (239)	Yes	> 20g/d	Yes	Yes	lab test	>20	liver cirrhosis, or hepatocellular carcinoma, elevated liver enzyme [aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≥200 IU/l], and elevated gamma-glucuronyl transpeptidase (GGT) (≥1,000 IU/l), participants who had been taking medicine due to liver disease.
Lee 2017 (240)	Yes	>30 g/day for men and >20 g/day for women	Yes	Yes	lab test	22-91	autoimmune hepatitis or other cause of chronic or acute liver disease, right-sided heart failure
Lee 2017 (241)	Yes	>40 g/day for males or >20 g/day for females	Yes	Yes	medical records	adults	-
Lee 2018 (242)	Yes	>140 g/week for men and >70 g/week for women	Yes	Yes	lab test	adults	more than 3 times the normal serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), or γ-glutamyltransferase (GGT), history of chronic liver diseases, such as cirrhosis and malignancy
Li 2013 (243)	Alcohol use was assessed but	habitual drinking	Yes	Yes	lab test	20-79	specific diseases that could result in fatty liver

	based on self-report						
Li 2017 (244)	Yes	20 g/day for males and 10 g/day for females	Yes	Yes	history	adults	autoimmune hepatitis, drug-induced liver disease, or any other chronic liver disease
Lu 2016 (245)	Yes	men >140 g/week, women >70 g/week	Yes	Yes	lab test	>20	a history of autoimmune hepatitis or other chronic liver diseases with clear causes; and absence of uncontrolled biliary diseases (e.g., bile ductal stone, stenosis, biliary dilatation).
Nishioji 2015 (208)	Yes	≥20 g/d	Yes	Yes	lab test	18-92	autoimmune liver diseases (e.g., autoimmune hepatitis, primary biliary cirrhosis); uncontrolled biliary diseases (e.g., bile ductal stone, stenosis, biliary dilatation).
Omagari 2002 (207)	Yes	>23 g/day, 5–7 times per week	Yes	Yes	lab test	24-68	-
Ostovaneh 2015 (246)	Yes	> 20 g/d	Yes	Yes	lab test	≥18	decompensated liver disease; active malignancy; and history of hemochromatosis, celiac disease, Wilson's disease, or autoimmune hepatitis.
Petta 2018 (129)	Yes	in the last year of >20 g/d in females and >30 g/d in males	Yes	Yes	lab test	18-90	Cases with no data about alcohol drinking
Qiao 2017 (247)	Yes	>20 g/d	Yes	Yes	lab test	Adults	absence of a history of liver or gallbladder surgery; autoimmune hepatitis, gallbladder polyps, or inflammatory bowel disease; and complete abdominal ultrasound (US) results, physical

							examination data, and clinical indicators.
Saha 2017 (248)	Yes	>30g/d for men >20 g/d for women	Yes	Yes	Interview and medical records	18-80	Pregnant women, malignancies, severe illnesses, known hypothyroidism, fatty liver-related medications.
Salmanroghani 2019 (249)	Yes	>20 g/day for men and 10 g/day for women	Yes	Yes	history	≥18	known cases of Wilson disease, hemochromatosis, and Cushing syndrome; with a recent effort at losing weight (during the last 6 months); with prolonged use of estrogen or regular consumption of drug associated with fatty liver diseases, such as corticosteroid, methotrexate, tamoxifen, and amiodarone; and with thyroid disease, such as goiter or uncontrolled hypothyroidism or hyperthyroidism that may interfere with NC measurement.
Shin 2015 (250)	Yes	> 20 g/d	Yes	Yes	lab test	≥30	a history of chronic liver disease, previous coronary artery disease, impaired renal function (estimated glomerular filtration rate [GFR] < 60 mL/min/1.73 m <sup>2</sup> ), and out of normal range of albumin corrected calcium levels (Cac)
Uchil 2009 (251)	Yes	>20 grams/day	Yes	Yes	lab test	18-80	drugs, toxins, infectious diseases, or any other identifiable exogenous causes.
Volzke 2005 (252)	Yes	moderate (20-30 g/d in women, 20-60 g/d in men) or heavy	Yes	Yes	lab test	20-79	liver cirrhosis

		(>30 g/d in women, >60 g/d in men)					
Wang 2015 (253)	Yes	>140 g/week for men and 70 g/week for women	Yes	Yes	history	adults	autoimmune hepatitis, or other forms of chronic liver disease
Wei 2015 (254)	Yes	≥140 g/week men and ≥70 g/week in women	Yes	Yes	lab test	≥18	active malignancy, metallic implants, or other contraindications to MRI, secondary causes of fatty liver (e.g., consumption of amiodarone and tamoxifen), and liver decompensation
Wu 2018 (255)	Yes	≥140 g/week in men and ≥70 g/week in women during the past month	Yes	Yes	lab test	20-75	those with other liver diseases or on medications for liver disease
Zelber-Sagi 2006 (256)	Yes	>30 g/day in men or 20 g/day in women	Yes	Yes	lab test	24-70	Hepatotoxic-drugs, IBD, relevant surgery
Zhai 2016 (257)	Hepatitis excluded but based on self-report, same for alcohol consumption	history of excessive alcohol consumption	Yes	Yes	Self-reported or from history	≥18	patients taking vitamin supplements and without vitamin D results were excluded
Zhai 2017 (130)	Hepatitis excluded but based on self-report	male > 20 g/d, female >10 g/d	Yes	Yes	self-reported	≥18	-
Zhang 2017 (258)	Hepatitis excluded, but how they diagnosed	male >140 g/week, female >70 g/week	Yes	Yes	did not say	21-90	cirrhosis, polycystic liver disease, primary liver cancer, drug-induced liver disease, total parenteral nutrition, liver peas?

	hepatitis not mentioned						nucleus degeneration, autoimmune liver disease, etc., can lead to specific fatty liver Disease.
Zhou 2019 (259)	Yes	men >210 g/week, women >140 g/week	Yes	Yes	lab test	26-76	Subjects with missing CT scan data, including women pregnant or tested positive for pregnancy.

### 2.8.6 Appendix E1: Quality assessment of analyzed studies for incidence estimation

Publication	Representativeness: Was the study's target population a true or close representation of the general population	Age range of the sample:	Demonstration that outcome of interest was not present at the start of study	Was follow-up long enough for outcomes to occur
Chang 2018 (260)	Yes	Adults	Yes	Yes
Hamaguchi 2005 (209)	Yes	21-80	Yes	Yes
Kim 2014 (261)	Yes	>18	Yes	Yes
Kim 2020 (216)	Yes	>18	Yes	Yes
Kitae 2019 (262)	Yes	Adults	Yes	Yes
Okamoto 2018 (263)	Yes	18-80	Yes	Yes
Wang 2017 (264)	Yes	Adults	Yes	Yes
Wang 2018 (265)	Yes	Adults	Yes	Yes
Wong 2015 (266)	Yes	≥18	Yes	Yes
Wu 2015 (267)	Yes	18-65	Yes	Yes
Zelber-Sagi 2014 (189)	Yes	24–70	Yes	Yes
Zhao 2012 (217)	Yes	19-85	Yes	Yes

### 2.8.7 Appendix E2: Quality assessment of analyzed studies for incidence estimation (continued)

Publication	Age range of the sample:	How much alcohol was excessive?	Hep B Ag and Hep C Ab positive excluded	Info Source for viral hep	Other exclusion criteria	Exclusion criteria throughout the study
Chang 2018 (260)	Adults	≥30 g/day for men or ≥20 g/day for women	Yes	lab test	history of malignancy, known liver disease, cirrhosis; or use of medications associated with NAFLD within the past year	-
Hamaguchi 2005 (209)	21-80	> 20 g/d	Yes	lab test	reported a history of known liver disease (290), including viral, genetic, autoimmune, and drug-induced liver disease	New start of medications
Kim 2014 (261)	>18	> 20 g/d	Yes	lab test	known liver disease because of another etiology and subjects taking medications for diabetes, hypertension, and hyperlipidemia.	-

Kim 2020 (216)	>18	>30 g for men and >20 g for women	Yes	lab test	history of any malignancy, severe cardiovascular diseases (unstable angina, myocardial infarction, and stroke), thyroid disease, and hormone therapy, subjects with established liver disease such as chronic hepatitis or cirrhosis, AST or ALT serum levels three times higher than normal upper limits and estimated glomerular filtration rate <60 mL/min/1.73 m <sup>2</sup> .	New cirrhosis.
Kitae 2019 (262)	Adults	>30 g/day in men and >20 g/day in women	Yes	lab test	current use of medication, autoimmune, genetic, viral, and drug-induced liver disease	-
Okamoto 2018 (263)	18-80	>210 g/week for male or >140 g/week for female	Yes	lab test	currently being treated with antidiabetic, antihypertensive, or lipid-lowering agents	any change in alcohol consumption or smoking status
Wang 2017 (264)	Adults	> 140 g/week for men and >70 g/week for women	Yes	history	autoimmune hepatitis, or other forms of chronic liver disease, those with body mass index (BMI) less than 18.5 kg/m <sup>2</sup>	
Wang 2018 (265)	Adults	≥ 30 g/day for men and ≥20 g/day for women	Yes	lab test	a serum aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio > 2, an ALT level more than twice the upper limit of normal, liver cirrhosis, or malignancy at baseline; patients who were treated with lipid-lowering agents or who had previous clinical cardiovascular diseases or diabetes.	-
Wong 2015 (266)	≥18	> 20 g/d (140 g/week) for men and > 10 g/d (70 g/week) for women	Yes	lab test	active malignancy, metallic implants or other contraindications to magnetic resonance imaging, secondary causes of fatty liver (e.g., amiodarone and tamoxifen), and decompensated liver disease	New-onset heavy drinking

Wu 2015 (267)	18-65	>140 g/week for men & >70 g/week for women	Yes	Not menti oned	systolic/diastolic blood pressure <90/60mmHg or >130/85mmHg; those taking antihypertensive, lipid-lowering agents, or with a history of CVD (including a self- reported or diagnosed history of myocardial infarction, stroke, arterial revascularization, heart failure, arrhythmia); any other known potential causes of chronic liver disease, such as viral or autoimmune hepatitis or those using hepatotoxic medications	-
Zelber-Sagi 2014 (189)	24-70	≥ 30 g/d in men or ≥ 20 g/d in women	Yes	lab test	fatty liver suspected to be secondary to hepatotoxic drugs, inflammatory bowel disease	
Zhao 2012 (217)	19-85	male> 40 g/week, female> 20 g/week	Yes	lab test	autoimmune liver disease, drug- induced hepatitis, liver malignancies, infections, and biliary tract infections	

### **2.8.8 Appendix F:** Comparison of the NAFLD prevalence estimates of population-based and health checkup visitor-based studies

As reported earlier in the results, the NAFLD prevalence from population-based studies was 29.2 (95% CI: 24.5, 34.0), and from health-checkup visitor-based studies were 34.7 (95% CI: 31.4, 38.0). Although the difference was not quite statistically significant, a strong trend was indicated ( $p=0.06$ ). We further explored the possible explanations for this observed difference.

Since NAFLD is less prevalent in younger people and females, we hypothesized that the health-checkup visitor study subjects were older and/or included a higher number of males than population-based samples. The average age of the participants was not statistically different between population-based studies and the health checkup visitor-based studies [47.4 years (95% CI: 44.7, 50.1) (15 studies) vs. 47.8 years (95% CI: 45.9, 49.8) (25 studies), respectively; independent t-test,  $t=-2.6$ ,  $p>0.05$ ], but the proportion of male participants were indeed significantly lower in population-based studies [42.0% (95% CI: 37.8%, 46.3%) (15 studies) vs 53.3% (95% CI: 49.5%, 57.1%)(27 studies), respectively; independent t-test,  $t=-3.88$ ,  $p>0.001$ ].

We then questioned if estimated sex-specific prevalence was different based on the sample source. We stratified the pooled NAFLD prevalence estimates of population-based and health checkup visitor-based studies by sex ([Table 4](#): Comparison of NAFLD prevalence estimates of population-based and health checkup visitor-based studies stratified by moderator variables.). While the reported prevalence among females were no different [25.1 (95% CI: 18.4, 31.8) vs. 26.2 (95% CI: 21.4, 30.9), respectively], the reported prevalence among males was significantly lower in population-based studies [32.3 (95% CI: 25.5, 39.1) vs. 43.2 (95% CI: 39.6, 46.8)].

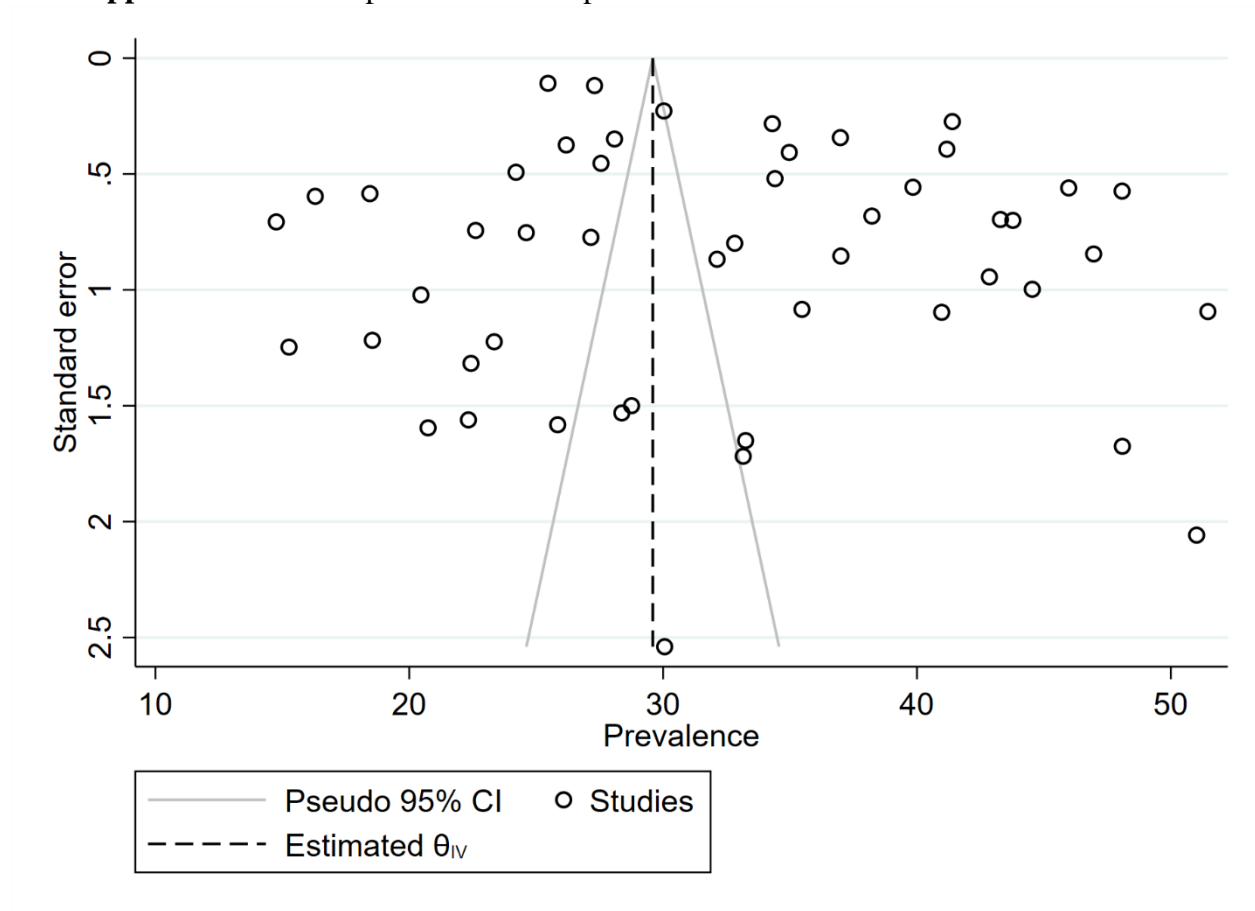
To summarize, population-based studies recruit fewer male participants than health checkup visitor-based studies. Although studies of both sample sources similarly estimate the female-specific NAFLD prevalence, the male-specific NAFLD prevalence is estimated significantly higher in health checkup visitor-based studies. The combination of both above findings can explain an overall higher estimate of NAFLD by health checkup visitor-based studies.

**Table 4:** Comparison of NAFLD prevalence estimates of population-based and health checkup visitor-based studies stratified by moderator variables.

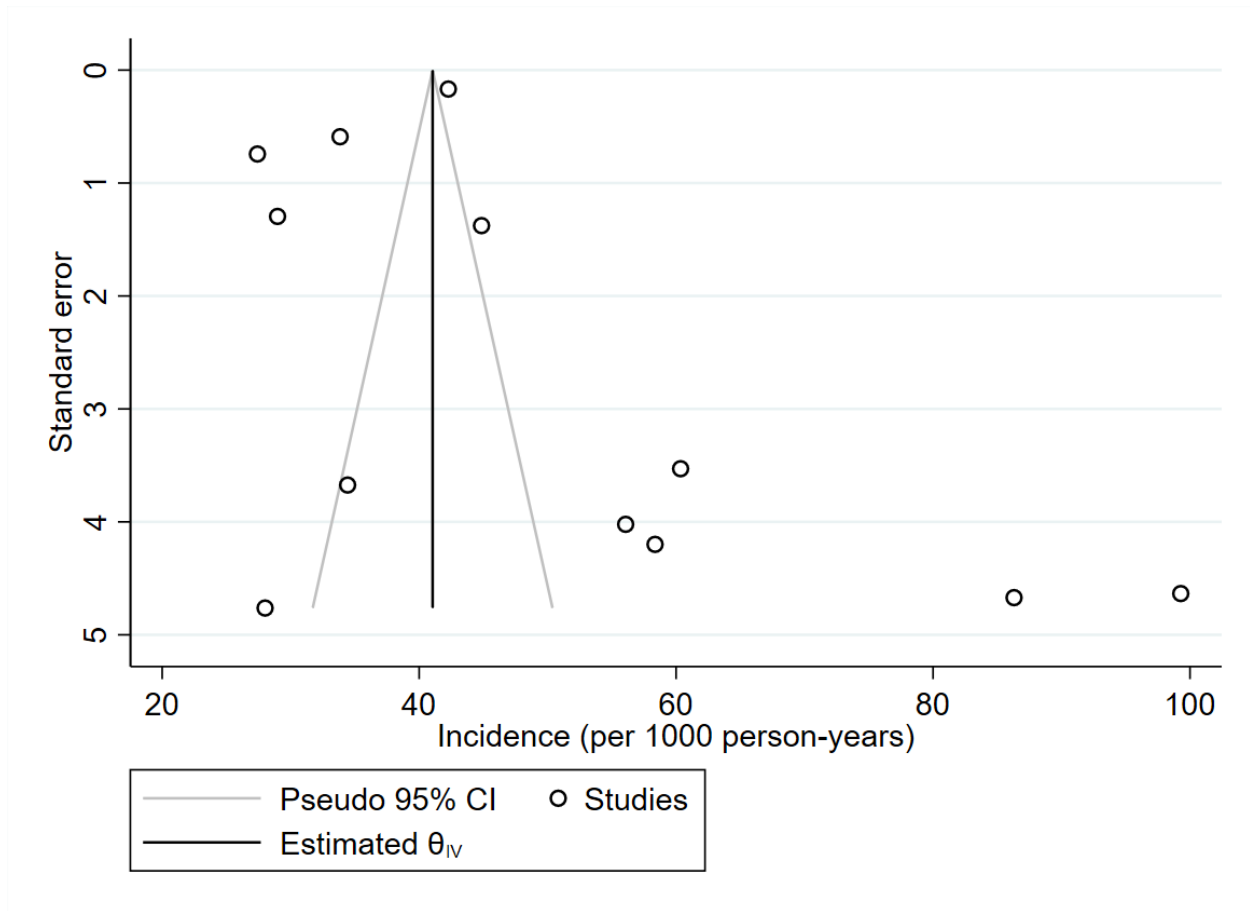
Moderator variables	NAFLD Incidence (cases/1000 person-years)(95%CI)(n)				Difference (95%CI)
	Population-based	I <sup>2</sup> (%)	Health check-up visitor-based	I <sup>2</sup> (%)	
<b>Overall</b>	29.2 (24.5, 34.0)(19)	99.33	34.7 (31.4, 38.0)(29)	99.84	5.5 (-0.1, 11.0)
<b>Period</b>					
≤2005	23.6 (17.1, 30.2)(4)	97.58	29.5 (16.6, 42.3)(4)	99.62	5.7 (-8.7, 20.2)
2006-2010	26.3 (18.6, 34.1)(6)	98.82	32.8 (27.5, 38.2)(9)	99.75	6.5 (-2.6, 15.6)
2011-2015	31.4 (24.6, 38.1)(8)	99.41	35.3 (30.7, 39.9)(12)	99.83	4.0 (-3.9, 11.8)
2016-2020	51.5 (49.3, 53.6)(1)	-	42.2 (35.7, 48.7)(4)	98.39	-
<b>Sex</b>					
Male	32.3 (25.5, 39.1)(12)	98.75	43.2 (39.6, 46.8)(23)	99.55	10.9 (3.9, 17.9)*
Female	25.1 (18.4, 31.8)(12)	99.14	26.2 (21.4, 30.9)(23)	99.81	1.1 (-7.1, 9.3)

\* Statistically significant.

**2.8.9 Appendix G:** Funnel plot for NAFLD prevalence studies.



**2.8.10 Appendix H:** Funnel plot for NAFLD incidence studies.



Egger's test p-value= 0.021

## **Chapter 3: Knowledge translation implications of the systematic review of the worldwide prevalence of Non-alcoholic fatty liver disease (NAFLD)**

Our systematic review of the worldwide prevalence of NAFLD specified three important implications, an overall high and growing prevalence of NAFLD in the past two decades, a considerable heterogeneity among included studies that could not be fully explained, and a relative scarcity of high-quality reports on the prevalence of disease from around the world. Updated prevalence data on NAFLD is essential to enhance awareness of the disease burden in all stakeholders, including patients, primary care physicians, public health specialists, and health policymakers.

Addressing heterogeneity is important because it might limit the generalizability of research findings to other settings and populations (268). In this chapter, we discuss the role of diversity of study participants as a likely source of heterogeneity. Further, we provide directives aimed toward curbing the prevalence of NAFLD.

### **3.1 Introduction:**

Non-alcoholic fatty liver disease (NAFLD) is a significant public health concern in many countries, especially in the western world (101, 269). Although associated with metabolic syndrome, NAFLD is not widely recognized as a public health issue as much as the more common metabolic syndromes such as diabetes, obesity, and cardiovascular diseases (270). NAFLD is one of the leading causes of chronic liver disease, and an increased prevalence of NAFLD and consequently of NASH could result in a rise in the cases of cirrhosis and HCC, which add to the burden of disease and the overall cost of health care (101, 269, 271).

The most effective treatment strategies against NAFLD remain preventive measures through regular physical activity, low caloric intake, and weight loss (101). A key public health concern with NAFLD is that the disease remains undiagnosed in the majority of the patients (272). When the disease advances to NASH, for which there is no proven medical therapy, the prognosis worsens with an increased risk of developing end-stage liver disease and HCC (271, 273). Furthermore, the co-existence of metabolic comorbidities like T2DM, increases the risk of NAFLD progression to NASH (274). Therefore, early screening and diagnosis of NAFLD, besides modifying its metabolic risk factors, are essential to reversing the progression of the disease (275).

Our systematic review of the currently available data on the worldwide NAFLD prevalence and incidence shows that the overall prevalence in the past two decades has been 32.3%, with a constantly rising trend. NAFLD prevalence was significantly higher in men than in women (39.1% vs. 25.5%). The overall NAFLD incidence rate was 49.8 per 1000 person-years. Similar to prevalence trends, men showed a higher incidence rate than women (78.2 vs. 31.4 per 1000 person-years).

The epidemiology and demographic characteristics of NAFLD vary from place to place. Furthermore, a lack of epidemiological studies from many countries has resulted in an unknown prevalence status there. The worldwide pooled prevalence of our research came from 49 publications from only 12 countries, which might limit the generalizability of our estimates to elsewhere. While epidemiological reports on NAFLD prevalence from many parts of the world, including Africa, North and South America, or Oceania, are sparse, given the trends in the prevalence of diabetes and obesity, the prevalence of NAFLD and its consequences are expected to show an increasing trend worldwide (276). However, such an assumption requires targeted research to verify.

Another observation from our study was that the meta-analysis of the data demonstrated a considerable heterogeneity indicating variation in the study outcomes between the included studies in both prevalence and incidence studies. Exploring the heterogeneity is vital because it might limit the generalizability of research findings (268, 277-279). In this chapter, we discuss the possible sources of heterogeneity with a particular focus on the role of diversity of study participants.

### 3.2 Generalizability limitations of the worldwide prevalence/incidence estimates

Epidemiologic studies provide a foundation for controlling the direction of the actions to monitor and prevent diseases. As a fundamental component of public health, epidemiologic research generates information that can be used in policy development to promote, prevent, and control health problems (280). Knowledge translation, as defined by the Canadian Institutes of Health Research as "a dynamic and iterative process that includes the synthesis, dissemination, exchange, and ethically sound application of knowledge to improve health, provide more effective health services and products, and strengthen the health care system" (281), relies on the information provided by both descriptive and analytical epidemiological studies.

Systematic reviews provide the best evidence for healthcare management and policy-making (282, 283). In systematic reviews, meta-analysis techniques are often used to combine data and calculate pooled point estimates, accompanied by a measure of precision (95% confidence interval). Such estimates are often incorporated by clinical practice guidelines (282). However, a limitation of many systematic reviews is that such estimates are not necessarily always valuable for decision-makers due mainly to concerns for generalizability (284, 285). Also referred to as applicability or external validity, generalizability describes whether and to what extent the results from a study can be generalized to different patient populations, outcomes, settings, as well as to

individual patients with varying risks and prognostic factors (286). Identifying variables that affect the estimated prevalence or incidence of a disease (i.e., effect-measure modifiers) is important to all stakeholders, including patients, health care providers, and policymakers, because it helps them recognize who is possibly more or less at risk of the disease, which in turn will define the focus of the preventive measures and help appropriately tailor individualized treatments (277). Examples of such effect-measure modifiers might include demographic variables like age, sex, income, comorbidities, or other disease risk factors. The risk of NAFLD might not be equal among every individual from the general population. For example, the young, the old, the females, or the people with diabetes have specific susceptibility to the disease (277). The precision and generalizability of the estimates from systematic reviews depend on heterogeneity, defined as the variation in the study outcomes between the included studies (287). There are several possible sources of heterogeneity among included studies in the meta-analyses. Clinical heterogeneity is the variability in the participants, the types or timing of outcome measurements, and intervention characteristics; Methodological heterogeneity is the variability in study design and how the studies were conducted, or the study's risk of bias; and Statistical heterogeneity refers to the variability in summary treatment effects between trials which can be tested and measured using statistical tests (287). Methodological and clinical sources of heterogeneity contribute to the magnitude and presence of statistical heterogeneity (288, 289).

Our systematic review of the worldwide prevalence of NAFLD aimed mainly at the available reports on the prevalence and incidence of NAFLD that were the overall representatives of the disease status in the general adult population from different countries at different timepoints. We applied carefully designed inclusion/exclusion criteria and selected a group of papers with similar research protocols to minimize heterogeneity. The included studies' methodological quality

was determined using robust appraisal tools, the Joanna Briggs Institute Prevalence Critical Appraisal Tool (118) for prevalence studies and an adapted version of the Newcastle-Ottawa Assessment Scale using criteria relevant to studies of incidence (120). Yet, we observed a significant amount of heterogeneity, evident by detecting a significant statistical heterogeneity using  $I^2$  statistics calculations. We investigated the heterogeneity using subgroups analysis followed by meta-regression to examine the influence of the possible effect modifiers and explained some of the heterogeneity associated with sex (26%) and study year (16%). However, a considerable portion of heterogeneity remained unexplained. We think that for some of the explanatory variables, including study setting (rural vs urban) and diagnostic imaging modality, our analysis might have lacked enough statistical power due to some groups' low number of studies. Nevertheless, despite clearly defined eligibility criteria, included studies often differ for population characteristics, settings, methodological rigour, outcome measurement, or specific elements in the study design (277).

### 3.3 Clinical heterogeneity (diversity of study participants) in NAFLD prevalence/incidence studies

Clinical heterogeneity arises from differences in participant characteristics (e.g., sex, age, baseline disease severity, ethnicity, comorbidities), types or timing of outcome measurements, and intervention characteristics (e.g., dose and frequency of dose) (288). This heterogeneity can cause significant statistical heterogeneity, biased point estimates and associated conclusions, and consequently mislead the stakeholders (288). Diversity in participant characteristics can imply diversity in disease susceptibility, NAFLD risk profiles, and possibly treatment responsiveness. Ignoring such diversity can challenge the proper identification of high-risk individuals and

personalized preventive/therapeutic strategies, thus hindering attempts to inhibit this ever-increasing health hazard (277, 288). In this section, we discuss the heterogeneity associated with the diversity of the study participants.

The general population in any specific geographical location is a heterogeneous group of people. It thus is expected to show heterogeneity in terms of NAFLD risk profiles when studied for NAFLD prevalence. Risk factors associated with NAFLD disease include but are not limited to age, sex, comorbidities including metabolic syndrome (or any one of its components, including obesity/overweight, T2DM, hypertension, and dyslipidemias), genetic factors, ethnicity, and environmental factors affecting dietary habits and physical activity (290).

### *3.3.1 Sex and age*

The overall prevalence of NAFLD in adult men is significantly higher than in women (39.1% vs. 25.5% from our study). The incidence rate of NAFLD among men is almost double that in women (78.2 vs. 31.4 per 1000 person-years). In our study, a meta-regression analysis followed by calculation of  $R^2$  statistics showed that sex explained a considerable 26% of the heterogeneity observed with meta-analysis. The effect of sex on NAFLD prevalence, however, is age-dependent (271). When considering the age, the higher prevalence in men is only seen when the age is less than 50. However, the prevalence is like males in postmenopausal women (291). This observation has been attributed to the protective effects of sex hormones in premenopausal females (138). The sex differences in the prevalence of NAFLD have been discussed in detail in chapter 2. A subgroup analysis following stratification based on age group as performed by Younossi et al. (2016) and Zhou et al. (2019) will help explore the effect of age (4, 112). However, not all included publications provide enough information to enable such analysis.

### 3.3.2 *Metabolic comorbidities*

The most well-known comorbidities associated with NAFLD are metabolic syndrome and its components (292, 293). Like NAFLD, the prevalence of T2DM is increasing globally and correlates with the increased prevalence of NAFLD (294). It is reported that the prevalence of NAFLD in patients with T2DM ranged between 50 to 75% and is also dependent on the patients' ethnicities (295). Lee (2010) showed that MetS at baseline increased the risk of developing NAFLD by almost six times (296), and even one or two components of MetS at baseline made the risk 2.4-fold (296). T2DM is a significant risk factor for the development of NAFLD; it accelerates the progression of NAFLD to advanced liver disease, and increases mortality risk (274). The prevalence of NAFLD/ NASH among T2DM is over 60% (297). Hypertension is another risk factor for NAFLD and has a bidirectional association with NAFLD (298). The severity and progression of NAFLD have been associated with hypertension, but also NAFLD is a possible risk factor for the development of hypertension (298-300). Similar associations exist between dyslipidemias, including hypercholesterolemia and hypertriglyceridemia with NAFLD (221, 299, 300).

The strong association of NAFLD with obesity is well known. Obesity is believed to be the most significant modifiable risk factor for NAFLD (101). While NAFLD prevalence is around 30% of the general population, it climbs to 50-90% in obese subjects (301). Both obesity and NAFLD are associated with over-nutrition, a Western-type diet and a sedentary lifestyle, and NAFLD prevalence correlates with the obesity prevalence to a large degree (101, 302). Besides, the prevalence of NAFLD also correlates with the degree of obesity as well, NAFLD has a

prevalence of 65% and 85% in patients with grade I-II obesity (BMI = 30-39.9 kg/m<sup>2</sup>), and grade III obesity (BMI = 40-59 kg/m<sup>2</sup>), respectively (302).

Since the comorbidity profile is not a component of NAFLD definition and based on the high prevalence of metabolic syndrome and its components, it can result in considerable diversity in susceptibility to NAFLD. Planning and considering such a source of diversity will help collect pertinent diagnostic data from study subjects and provide the opportunity to explore the effect measure modification capabilities of each comorbidity properly. For example, a recent comparison of NAFLD prevalence among overweight and obese patients with nonobese subjects in Asia shows that the prevalence is significantly higher among overweight/obese patients (26.3% vs 11.8%)(111). Some authors limit the study subjects to individuals with one or more comorbidities (for example (303)), which provides valuable information related to that subgroup and might help explain some of the heterogeneity. Still, such restriction could result in limited generalizability of the research results (287).

### *3.3.3 Diet and physical activity*

The association of diet and physical activity with NAFLD has already been exclusively discussed in chapter 1 (also (101)). Several dietary elements and dietary habits have been associated with NAFLD. A western-type diet containing high amounts of saturated fats, fructose, cholesterol-rich sources, and sugar-packed soft drinks predisposes individuals to NAFLD directly or through contribution to weight gain (101). Conversely, modified diets like a Mediterranean diet rich in fruits, vegetables, whole grains, nuts, legumes, and fish but low in red and processed meat, high sugar foods, and refined carbohydrates improves NAFLD (101). Similarly, sedentary behaviour has been increasingly accepted as an independent risk factor for NAFLD (304), and

physical activity like aerobic exercise can protect against NAFLD by decreasing body fat (305). The systematic reviews of the association of diet and exercise with NAFLD prevalence are limited. Zhou et al. (2019) showed that in China, a lifestyle that included exercise was associated with a significant decrease in the odd of NAFLD (OR=0.78 (95%CI: 0.68-0.89)) based on the results of 10 publications (112).

#### *3.3.4 Genetic susceptibility*

The etiology of NAFLD is complex, with multiple factors contributing to NAFLD development and its progression (306). Besides metabolic and environmental factors, many genetic changes have been identified by the extensive research of the last decade that may be associated with the development of NAFLD and NASH (306, 307). There is ample epidemiological evidence on the role of genetic susceptibility in NAFLD, mainly coming from studies that show clustering of cases of NAFLD among families and the observation of differences in the prevalence and severity of NAFLD among ethnicities (308). Variations in genetic susceptibility may explain a significant portion of heterogeneity observed in the NAFLD prevalence/incidence estimates from different geographical regions. They can also explain variability in disease severity among individuals. The four genes most reported to be associated with NAFLD include PNPLA3, TM6SF2, MBOAT7, and GCKR (309).

Patatin-like phospholipase domain-containing protein 3 (PNPLA3) is a lipid droplet-associated protein that hydrolyses triglycerides and retinyl esters (310) in adipocytes and hepatocytes, where it helps regulate both lipogenesis and lipolysis (7, 311). The expression of the PNPLA3 gene decreases during fasting and increases after eating, suggesting a role for PNPLA3 protein in processing and storing fats in the diet (312). A variant of the PNPLA3 gene is rs738409

which is a single nucleotide substitution of cytosine (C allele) to guanine (G allele), resulting in the replacement of isoleucine with methionine at position 148 (I148M) of the protein (313). This nonsynonymous change in encoding results in a loss of function in the PNPLA3 protein, leading to increased accumulation of triglycerides in lipid droplets within hepatocytes (314). It has been shown that the G allele (either heterozygous or homozygous) is not only associated with NAFLD development but also with a more advanced liver fibrosis and NASH (311, 315). This gene variant is believed to be the most important genetic determinant of the full spectrum of NAFLD, from development to progression to NASH and HCC (40).

Transmembrane 6 superfamily 2 (TM6SF2) is a gene that codes a lipid transporter with transmembrane domains in the endoplasmic reticulum and ER-Golgi intermediate compartment in the hepatocytes (308). TM6SF2 activity is required for normal VLDL secretion, and its impaired function contributes to NAFLD (308). The rs58542926 variant is a Glu167Lys missense mutation that alters serum lipid profiles in humans. As TM6SF2 controls hepatic lipid efflux, decreased effect by deletions or mutations diminishes lipoprotein secretion, causing triglyceride accumulation within the hepatocyte (316). After the PNPLA3 I148M variant, the TM6SF2 E167K (rs58542926) genetic variant has the most significant effect on NAFLD susceptibility (317). Patients with the TM6SF2 E167K variant have severe fatty liver disease, a decreased apolipoprotein B100 levels, and lower circulating lipids (308).

Membrane-Bound O-acyltransferase Domain Containing 7 (MBOAT7) gene encodes an enzyme involved in hepatic phospholipid remodelling by transferring polyunsaturated fatty acids to lysophospholipids (318). Genetic studies indicate that low levels of MBOAT7 in a human's liver cells increase the severity of NAFLD and an inactivating mutation increases the risk of fat accumulating in the liver (318). A polymorphism (rs641738) in the locus carrying the membrane-

bound MBOAT7 gene has been associated with the risk and severity of NAFLD through suppression of MBOAT7 at the messenger RNA and protein levels, and altered phosphatidylinositol profiles (319). It is both associated with NAFLD development and with progress and severity of fibrosis and HCC in patients without cirrhosis (320).

The glucokinase regulator (GCKR) gene codes a regulatory protein in the hepatocytes, which negatively regulates glucokinase in response to fructose-1-phosphate, modulating glucose uptake in the liver (320). The GCKR rs1260326 variant is a loss-of-function mutation that increases hepatic glucose uptake and malonyl-CoA concentration, providing more substrates for de novo lipogenesis. The variant has been linked to NAFLD development and fatty liver in obese youths (320).

Besides what was explained above, more genetic variants have now been recognized to affect the susceptibility to NAFLD. Such differences in the genetic components not only can explain heterogeneity associated with the diversity of susceptibility to NAFLD, but they can act as reliable susceptibility markers to help identify at-risk individuals at earlier ages for better management of the disease by timely patient education early interventions, and lifestyle changes.

### *3.3.5 Race/ethnicity*

NAFLD has a complex etiology and is caused by a combination of environmental and genetic factors. Research has shown differences in susceptibility to NAFLD among races and ethnicities. Many have attributed the ethnic differences to genetic factors or other variables like lifestyle, dietary habits, comorbidity profiles, access to health care, socioeconomic status, or a combination of multiple factors (321, 322).

The comparative epidemiologic research on NAFLD prevalence among different ethnicities is relatively scarce and limited to only a couple of countries, especially the USA. In the USA, a systematic review has shown that in the population-based cohorts, 23% of Hispanics have NAFLD, compared to 14% of Caucasians and 13% of African Americans (323). Available data suggest that NAFLD may be less prevalent in African Americans than White and Latino populations, despite a similar prevalence of metabolic syndrome (324). In China, a recent systematic review has shown that NAFLD is significantly more prevalent among Uygur ethnics compared to Han people (46.6% vs 29.3%) (112).

Genetic factors have been suggested as one of the underlying causes for ethnic differences in susceptibility to NAFLD. Differences have been recognized among different ethnicities in the expression of NAFLD-associated genes. PNPLA3 rs738409 variant has been implicated in various ethnicities, including Hispanics, African Americans, East Asians, and South Asians (305). This variant has primarily been associated with a lean NAFLD phenotype from Asia (305). In those with GG alleles, compared to those with wild-type genotype, the risk of having NAFLD was similar in Asians and Caucasians (3-fold) and Hispanics (4-fold) but was much higher in Black patients (9-fold) (325). A considerable bulk of literature supports genetic, metabolic, and dietary differences among African, Hispanic, and Asian ethnicities (reviewed in (326)) though most studies were done in the USA. In Hispanics with American ancestry (Mexican-, Central-, and South American), the PNPLA3 rs738409 G allele frequency is higher than those from European or Afro-Caribbean backgrounds (327). The rs738409 variant was found to be more common in Hispanics (49%) compared to African Americans (17%) and Europeans (23%), corresponding to a high prevalence of NAFLD in Hispanics and low prevalence in African Americans (310). On the other hand, the TM6SF2-T allele mutation E167K had similar low frequencies between Hispanics

(327) and those from European ancestry, and was strongly associated with ALT levels (328). In individuals of European descent, a T mutation in the MBOAT7 gene (rs641738) has been associated with severity of NAFLD in those with TT homozygosity (329).

Research done outside the USA shows that polymorphisms in PNPLA3 are frequent in Asian Indians with NAFLD, likely contributing to their increased prevalence of NAFLD (330). A study by Bale et al. (2017) (331) from India, comparing two distinct Indian ethnicities, showed that in South Indian ethnics, higher susceptibility to NAFLD was associated with TM6SF2 rs58542926 variant (OR=2.7), while for North-East Indian ethnicities, an rs2281135 variant of PNPLA3 gene was associated with higher susceptibility to NAFLD (OR=2).

Dietary differences are another factor that has been linked to ethnic disparities in association with NAFLD (305, 332). Differences in exercise and dietary behaviours exist in various ethnic groups (305, 333). However, the association of NAFLD risk in ethnicity with their dietary habits is not yet well supported. It is known that some aspects of diet and weight control would predispose specific populations to NAFLD (101). For example, the Mediterranean diet inspired by the eating habits of the people in the Mediterranean region has been associated with a lower prevalence of NAFLD or even have been recommended as a preventive solution for NAFLD (101); however, several Mediterranean countries have some of the highest NAFLD prevalence estimates in the world, including Turkey with estimates between 48.3-60.1% (334), Greece with 41% (335), and Italy with 38.2% (our systematic review). Similarly, the traditional Chinese diet, while being high in carbohydrates, is vegetable-rich and believed to be low-risk for NAFLD (336), yet in China, NAFLD is a seriously surging health burden (112). Some authors associate such discrepancies with increased popularity and the dissemination of Western-type dietary behaviours worldwide (305). Assuming rural communities being less affected by the western type dietary

changes than urban communities, one could hypothesize that the prevalence of NAFLD is lower in rural than urban areas. Neither our study nor the study by Li et al. (2019) (111) showed a significant difference; however, the number of rural prevalence reports was disproportionately low in both studies (n=3 in either study).

Interesting recent research by Nouredin et al. (2020) on a diverse ethnic population in the USA showed that diets high in consumption of cholesterol-rich foods like red and processed meats and low in fibre were associated with NAFLD independent of race and ethnic groups (337). This might indicate that the more substantial effect of lifestyle changes and environmental factors resulting in unhealthy dietary habits and lack of physical activity might conceal the more subtle racial/ethnic differences in NAFLD susceptibility. As already implicated in the obesity epidemic, several environmental factors promote the rise of metabolic diseases like obesity and NAFLD through encouraging energy consumption and discouraging energy expenditure (338, 339). The prevalence of western type diets that are mainly composed of large portions of readily available, good-tasting, inexpensive, energy-dense foods, besides reduction in energy expenditure in the form of jobs with decreased physical labour, easy commute, decreased physical activity at school and in daily living, and an increase in time spent on sedentary activities such as watching television, and web surfing (338, 339). This necessitates research on lifestyle modifications and the environmental policies for their effects on the health status of specific racial, ethnic and socioeconomic groups toward NAFLD treatment.

A common challenge with medical literature on race and ethnicity is that individuals' race, ethnicity, and geographical origin have often been used synonymously to differentiate between populations. While studies tend to group participants as Latino, Black, Asian, White, or Indigenous, it is essential to acknowledge that these racial classifications are subjective or not

clearly defined (340). As noted by Bonacini et al. (2021), "ethnicity and race are fluid constructs that have no clear-cut definition" (325). While race might imply biological differences among groups, ethnicity distinguishes people more culturally and socially (325, 340); however, the historical biological-based racial groupings are now considered obsolete (341). Nevertheless, racial diversity among ethnicities, or ethnicities living in diverse geographical areas, will result in uncertainties of applicability of race- or ethnicity-focused research results (341, 342). Environmental and genetic factors differ considerably within these pooled ethnic groups, impacting study results and limiting inferences drawn based on ethnicity or race. The generalizability of described ethnic differences in disease prevalence, progression of the disease, and response to treatment is therefore limited by the many intrinsic geographic and socioeconomic disparities within ethnic groups. A study by Fleischman et al. (2014) showed that in the USA, the prevalence of NAFLD in Hispanics of Dominican origin (16%), and Hispanics of Puerto Rican origin (18%), was significantly lower than the Hispanics of Mexican origin (33%) (342). This observation raises the question whether the inferences regarding the high prevalence of NAFLD among Hispanics in the USA have been made with considerations of diversity within the pooled ethnicity (342).

### 3.4 Addressing heterogeneity

The meta-analysis in our systematic review showed significant statistical heterogeneity among studies that could not be fully explained by sub-group or meta-regression analyses. Recognizing the source of heterogeneity is crucial because it influences generalization (284, 285). However, our finding is not unique since similar systematic reviews on NAFLD prevalence reported considerable statistical heterogeneity (111, 112, 128). In general, heterogeneity is

commonly encountered by systematic reviews of the incidence or prevalence of any disease in diverse environments since they collect and analyze highly heterogeneous studies (279). Attempts to explore the statistical heterogeneity include applying pre-planned subgroup analysis, post hoc sensitivity analysis, and meta-regression assessment of the impact of study-level covariates (277). Several researchers have tried "restriction" of the study subjects to narrower subgroups, study settings, or a specific outcome measurement method, which would help address clinical heterogeneity; however, it also limits the generalizability of the study findings only to populations with similar conditions to the studied subgroup (287). For example, Liu et al. (2021) have limited their analysis of worldwide NAFLD prevalence to overweight and obese adults (343), while Lu et al. (2020) limited their systematic review to lean subjects (344). Nonetheless, even in these studies or after extensive exploration of the heterogeneity, the significant heterogeneity remains (111, 112, 128, 343, 344), possibly due to the numerous known or unknown effect measure modifiers working simultaneously. The two common recommendations in the literature in regard to addressing clinical heterogeneity in systematic reviews, include identifying factors that may cause clinical or methodological heterogeneity during protocol development stage, and performing subgroup analyses (287). To be able to draw causal inferences, subgroup analyses have to be defined deductively at the protocol writing stage (287).

A limitation of the available data to address heterogeneity and generalizability of NAFLD prevalence estimates is that the available data on the prevalence of NAFLD is still considerably scarce, and the reports of prevalence are from only very few countries. This could restrict the generalizability of the findings in two ways. First, available data from some countries are not generalizable to other countries due to correctly presumed environmental, ethnic, and risk profile differences between different individuals, and second, lack of enough high-quality research could

limit the power of biostatistical methods to recognize the effect-measure modifiers that might explain some of the heterogeneity in the pooled data. The sparsity of quality reports on NAFLD could result in the desperate inclusion of lower quality papers to systematic reviews resulting in biased inferences.

### 3.5 Conclusion

NAFLD is the most prevalent cause of liver disease worldwide, currently affecting 1 in 3 people. Its high prevalence should boost NAFLD awareness in all stakeholders, from patients to primary care physicians, specialists, and policymakers. Lifestyle change, promoted by population-level measures, is successful in preventing obesity and achieving weight loss, which has proven efficacy in treating NAFLD. Obesity prevention strategies require the engagement of all stakeholders, from patients to health care providers and health policymakers. Though, there is a constant need to develop more effective preventive measures to treat NAFLD and decrease the burden of the disease. Since the susceptibility to the development of NAFLD and response to treatment might not be equal among every individual, such measures require updated epidemiological data that could be generalized to the target population.

The overall worldwide estimates of NAFLD prevalence/incidence offer a general appraisal of the current burden of the disease. Yet, such statistics cannot equally apply to every individual from the general population (284, 285). Although weight loss through appropriate dietary measures and increased physical activity remains the only effective preventive measure for NAFLD for everybody, recognizing diversity in NAFLD risk profiles, treatment responsiveness, and their associated factors might help tailor individual treatment protocols with higher success rates or more realistic expectations. While several of the risk factors associated with NAFLD like

age, sex, obesity, lifestyle, and metabolic comorbidities have been recognized, many still await well-constructed future research to be discovered.

Currently, there is a stern need for epidemiologic publications on basic NAFLD prevalence/incidence data worldwide, with considerations for heterogeneity in mind. It is recommended that researchers from all over the world initiate high-quality research to produce reliable reports on the prevalence/incidence of NAFLD and associated risk factors, especially from locations where the data is scant, including Africa, South America, or Oceania. Uniform research protocols regarding disease definition, inclusion/exclusion criteria, and diagnostic modality will decrease the heterogeneity, at least partially. Comprehensive data collection will help include more studies into systematic reviews and therefore contribute to the production of reliable data and the assessment of possible risk factors for the disease.

## References:

1. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2018;67(1):328-57.
2. Abd El-Kader SM, El-Den Ashmawy EM. Non-alcoholic fatty liver disease: The diagnosis and management. *World J Hepatol*. 2015;7(6):846-58.
3. Yki-Jarvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol*. 2014;2(11):901-10.
4. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016;64(1):73-84.
5. Samji NS, Verma R, Satapathy SK. Magnitude of Nonalcoholic Fatty Liver Disease: Western Perspective. *J*. 2019;9(4):497-505.
6. Tsochatzis EA, Newsome PN. Non-alcoholic fatty liver disease and the interface between primary and secondary care. *Lancet Gastroenterol Hepatol*. 2018;3(7):509-17.
7. Alves-Bezerra M, Cohen DE. Triglyceride Metabolism in the Liver. *Comprehensive Physiology*. 2017;8(1):1-8.
8. Kalinowski A, Humphreys K. Governmental standard drink definitions and low-risk alcohol consumption guidelines in 37 countries. *Addiction (Abingdon, England)*. 2016;111(7):1293-8.
9. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, et al. Risk of Hepatocellular Cancer in Patients With Non-Alcoholic Fatty Liver Disease. *Gastroenterology*. 2018;155(6):1828-37.e2.
10. Younossi Z, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A, et al. Nonalcoholic Steatohepatitis Is the Fastest Growing Cause of Hepatocellular Carcinoma in Liver Transplant Candidates. *Clin Gastroenterol Hepatol*. 2019;17(4):748-55.e3.
11. 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.
12. Kabbany MN, Conjeevaram Selvakumar PK, Watt K, Lopez R, Akras Z, Zein N, et al. Prevalence of Nonalcoholic Steatohepatitis-Associated Cirrhosis in the United States: An Analysis of National Health and Nutrition Examination Survey Data. *Am J Gastroenterol*. 2017;112(4):581-7.
13. Clifton P. Metabolic Syndrome-Role of Dietary Fat Type and Quantity. *Nutrients*. 2019;11(7).
14. Kassi E, Pervanidou P, Kaltsas G, Chrousos G. Metabolic syndrome: definitions and controversies. *BMC Med*. 2011;9:48.
15. Alberti KG, Zimmet P, Shaw J, Group IDFETFC. The metabolic syndrome--a new worldwide definition. *Lancet*. 2005;366(9491):1059-62.
16. Beltran-Sanchez H, Harhay MO, Harhay MM, McElligott S. Prevalence and trends of metabolic syndrome in the adult U.S. population, 1999-2010. *J Am Coll Cardiol*. 2013;62(8):697-703.
17. 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.
18. Ortiz-Lopez C, Lomonaco R, Orsak B, Finch J, Chang Z, Kochunov VG, et al. Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). *Diabetes Care*. 2012;35(4):873-8.
19. Wu S, Wu F, Ding Y, Hou J, Bi J, Zhang Z. Association of non-alcoholic fatty liver disease with major adverse cardiovascular events: A systematic review and meta-analysis. *Sci*. 2016;6:33386.
20. Younossi Z, Henry L. Contribution of Alcoholic and Nonalcoholic Fatty Liver Disease to the Burden of Liver-Related Morbidity and Mortality. *Gastroenterology*. 2016;150(8):1778-85.
21. Kim D, Kim WR. Nonobese Fatty Liver Disease. *Clin Gastroenterol Hepatol*. 2017;15(4):474-85.

22. Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World J Hepatol.* 2017;9(16):715-32.
23. Caldwell SH, Chang CY, Nakamoto RK, Krugner-Higby L. Mitochondria in nonalcoholic fatty liver disease. *Clin Liver Dis.* 2004;8(3):595-617, x.
24. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest.* 2005;115(5):1343-51.
25. Machado MV, Cortez-Pinto H. Non-alcoholic fatty liver disease: what the clinician needs to know. *World J Gastroenterol.* 2014;20(36):12956-80.
26. Wang DQ, Portincasa P, Neuschwander-Tetri BA. Steatosis in the liver. *Compr Physiol.* 2013;3(4):1493-532.
27. Noureddin M, Sanyal AJ. Pathogenesis of NASH: The Impact of Multiple Pathways. *Curr Hepatol Rep.* 2018;17(4):350-60.
28. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology.* 2010;52(5):1836-46.
29. Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology.* 2009;49(6):1877-87.
30. Boursier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology.* 2016;63(3):764-75.
31. Looma R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, et al. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab.* 2017;25(5):1054-62 e5.
32. Luck H, Tsai S, Chung J, Clemente-Casares X, Ghazarian M, Revelo XS, et al. Regulation of obesity-related insulin resistance with gut anti-inflammatory agents. *Cell Metab.* 2015;21(4):527-42.
33. Albillos A, Gottardi A, Rescigno M. The gut-liver axis in liver disease: pathophysiological basis for therapy. *J Hepatol.* 2019.
34. Iqbal U, Perumpail BJ, Akhtar D, Kim D, Ahmed A. The Epidemiology, Risk Profiling and Diagnostic Challenges of Nonalcoholic Fatty Liver Disease. *Medicines (Basel).* 2019;6(1).
35. 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.
36. Yang JD, Abdelmalek MF, Pang H, Guy CD, Smith AD, Diehl AM, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. *Hepatology.* 2014;59(4):1406-14.
37. Valenti LVC, Baselli GA. Genetics of Nonalcoholic Fatty Liver Disease: A 2018 Update. *Curr Pharm Des.* 2018;24(38):4566-73.
38. 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.
39. Burza MA, Pirazzi C, Maglio C, Sjöholm K, Mancina RM, Svensson PA, et al. PNPLA3 I148M (rs738409) genetic variant is associated with hepatocellular carcinoma in obese individuals. *Dig Liver Dis.* 2012;44(12):1037-41.
40. Pingitore P, Romeo S. The role of PNPLA3 in health and disease. *Biochimica et biophysica acta Molecular and cell biology of lipids.* 2019;1864(6):900-6.
41. Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol.* 2013;178(1):38-45.
42. Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the

- American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *Circulation*. 2014;129(25 Suppl 2):S102-38.
43. Wagner A, Dallongeville J, Haas B, Ruidavets JB, Amouyel P, Ferrieres J, et al. Sedentary behaviour, physical activity and dietary patterns are independently associated with the metabolic syndrome. *Diabetes Metab*. 2012;38(5):428-35.
  44. Hamilton MT, Hamilton DG, Zderic TW. Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. *Diabetes*. 2007;56(11):2655-67.
  45. Vallance JK, Gardiner PA, Lynch BM, D'Silva A, Boyle T, Taylor LM, et al. Evaluating the Evidence on Sitting, Smoking, and Health: Is Sitting Really the New Smoking? *Am J Public Health*. 2018;108(11):1478-82.
  46. Dunstan DW, Salmon J, Healy GN, Shaw JE, Jolley D, Zimmet PZ, et al. Association of television viewing with fasting and 2-h postchallenge plasma glucose levels in adults without diagnosed diabetes. *Diabetes care*. 2007;30(3):516-22.
  47. Healy GN, Dunstan DW, Salmon J, Shaw JE, Zimmet PZ, Owen N. Television time and continuous metabolic risk in physically active adults. *Med Sci Sports Exerc*. 2008;40(4):639-45.
  48. Snowling NJ, Hopkins WG. Effects of different modes of exercise training on glucose control and risk factors for complications in type 2 diabetic patients: a meta-analysis. *Diabetes care*. 2006;29(11):2518-27.
  49. Perseghin G, Lattuada G, De Cobelli F, Ragogna F, Ntali G, Esposito A, et al. Habitual physical activity is associated with intrahepatic fat content in humans. *Diabetes Care*. 2007;30(3):683-8.
  50. Ryu S, Chang Y, Jung HS, Yun KE, Kwon MJ, Choi Y, et al. Relationship of sitting time and physical activity with non-alcoholic fatty liver disease. *J Hepatol*. 2015;63(5):1229-37.
  51. Assy N, Nasser G, Kamayse I, Nseir W, Beniashvili Z, Djibre A, et al. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. *Can J Gastroenterol*. 2008;22(10):811-6.
  52. Hosseini Z, Whiting SJ, Vatanparast H. Current evidence on the association of the metabolic syndrome and dietary patterns in a global perspective. *Nutr Res Rev*. 2016;29(2):152-62.
  53. Stephenson K, Kennedy L, Hargrove L, Demieville J, Thomson J, Alpini G, et al. Updates on Dietary Models of Nonalcoholic Fatty Liver Disease: Current Studies and Insights. *Gene Expr*. 2018;18(1):5-17.
  54. Oddy WH, Herbison CE, Jacoby P, Ambrosini GL, O'Sullivan TA, Ayonrinde OT, et al. The Western dietary pattern is prospectively associated with nonalcoholic fatty liver disease in adolescence. *Am J Gastroenterol*. 2013;108(5):778-85.
  55. Kechagias S, Ernerson A, Dahlqvist O, Lundberg P, Lindstrom T, Nystrom FH, et al. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. *Gut*. 2008;57(5):649-54.
  56. Yasutake K, Kohjima M, Kotoh K, Nakashima M, Nakamuta M, Enjoji M. Dietary habits and behaviors associated with nonalcoholic fatty liver disease. *World J Gastroenterol*. 2014;20(7):1756-67.
  57. Nishi T, Babazono A, Maeda T, Imatoh T, Une H. Effects of Eating Fast and Eating Before Bedtime on the Development of Nonalcoholic Fatty Liver Disease. *Popul Health Manag*. 2016;19(4):279-83.
  58. Koopman KE, Caan MW, Nederveen AJ, Pels A, Ackermans MT, Fliers E, et al. Hypercaloric diets with increased meal frequency, but not meal size, increase intrahepatic triglycerides: a randomized controlled trial. *Hepatology*. 2014;60(2):545-53.
  59. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. *World J Gastroenterol*. 2018;24(30):3361-73.
  60. Pallayova M, Taheri S. Non-alcoholic fatty liver disease in obese adults: clinical aspects and current management strategies. *Clinical Obesity*. 2014;4(5):243-53.

61. Palmer M, Schaffner F. Effect of weight reduction on hepatic abnormalities in overweight patients. *Gastroenterology*. 1990;99(5):1408-13.
62. Kugelmas M, Hill DB, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. *Hepatology*. 2003;38(2):413-9.
63. Hickman IJ, Jonsson JR, Prins JB, Ash S, Purdie DM, Clouston AD, et al. Modest weight loss and physical activity in overweight patients with chronic liver disease results in sustained improvements in alanine aminotransferase, fasting insulin, and quality of life. *Gut*. 2004;53(3):413-9.
64. Tamura Y, Tanaka Y, Sato F, Choi JB, Watada H, Niwa M, et al. Effects of diet and exercise on muscle and liver intracellular lipid contents and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab*. 2005;90(6):3191-6.
65. Andersen T, Gluud C, Franzmann MB, Christoffersen P. Hepatic effects of dietary weight loss in morbidly obese subjects. *J Hepatol*. 1991;12(2):224-9.
66. 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.
67. Wong VW, Chan RS, Wong GL, Cheung BH, Chu WC, Yeung DK, et al. Community-based lifestyle modification programme for non-alcoholic fatty liver disease: a randomized controlled trial. *J Hepatol*. 2013;59(3):536-42.
68. Harrison SA, Fecht W, Brunt EM, Neuschwander-Tetri BA. Orlistat for overweight subjects with nonalcoholic steatohepatitis: A randomized, prospective trial. *Hepatology*. 2009;49(1):80-6.
69. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, et al. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology*. 2015;149(2):367-78 e5; quiz e14-5.
70. Kwak MS, Kim D. Non-alcoholic fatty liver disease and lifestyle modifications, focusing on physical activity. *Korean J Intern Med*. 2018;33(1):64-74.
71. Sargeant JA, Gray LJ, Bodicoat DH, Willis SA, Stensel DJ, Nimmo MA, et al. The effect of exercise training on intrahepatic triglyceride and hepatic insulin sensitivity: a systematic review and meta-analysis. *Obes Rev*. 2018;19(10):1446-59.
72. Marchesini G, Day CP, Dufour JF, Canbay A, Nobili V, Ratziu V, et al. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol*. 2016;64(6):1388-402.
73. Orzi LA, Gariani K, Oldani G, Delaune V, Morel P, Toso C. Exercise-based Interventions for Nonalcoholic Fatty Liver Disease: A Meta-analysis and Meta-regression. *Clin Gastroenterol Hepatol*. 2016;14(10):1398-411.
74. van der Heijden GJ, Wang ZJ, Chu ZD, Sauer PJ, Haymond MW, Rodriguez LM, et al. A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. *Obesity (Silver Spring)*. 2010;18(2):384-90.
75. Hashida R, Kawaguchi T, Bekki M, Omoto M, Matsuse H, Nago T, et al. Aerobic vs. resistance exercise in non-alcoholic fatty liver disease: A systematic review. *J Hepatol*. 2017;66(1):142-52.
76. Younossi ZM, Loomba R, Anstee QM, Rinella ME, Bugianesi E, Marchesini G, et al. Diagnostic modalities for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. *Hepatology*. 2018;68(1):349-60.
77. Grundy SM, Abate N, Chandalia M. Diet composition and the metabolic syndrome: what is the optimal fat intake? *Am J Med*. 2002;113 Suppl 9B:25S-9S.
78. York LW, Puthalapattu S, Wu GY. Nonalcoholic fatty liver disease and low-carbohydrate diets. *Annu Rev Nutr*. 2009;29:365-79.
79. Sharman MJ, Kraemer WJ, Love DM, Avery NG, Gomez AL, Scheett TP, et al. A ketogenic diet favorably affects serum biomarkers for cardiovascular disease in normal-weight men. *J Nutr*. 2002;132(7):1879-85.

80. Volek J, Sharman M, Gomez A, Judelson D, Rubin M, Watson G, et al. Comparison of energy-restricted very low-carbohydrate and low-fat diets on weight loss and body composition in overweight men and women. *Nutr Metab (Lond)*. 2004;1(1):13.
81. Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, et al. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med*. 2003;348(21):2082-90.
82. Asrih M, Jornayvaz FR. Diets and nonalcoholic fatty liver disease: the good and the bad. *Clin Nutr*. 2014;33(2):186-90.
83. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Houston Miller N, Hubbard VS, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2014;129(25 Suppl 2):S76-99.
84. Properzi C, O'Sullivan TA, Sherriff JL, Ching HL, Jeffrey GP, Buckley RF, et al. Ad Libitum Mediterranean and Low-Fat Diets Both Significantly Reduce Hepatic Steatosis: A Randomized Controlled Trial. *Hepatology*. 2018;68(5):1741-54.
85. Schwartz MW, Seeley RJ, Zeltser LM, Drewnowski A, Ravussin E, Redman LM, et al. Obesity Pathogenesis: An Endocrine Society Scientific Statement. *Endocr Rev*. 2017;38(4):267-96.
86. Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med*. 2009;360(9):859-73.
87. Jang EC, Jun DW, Lee SM, Cho YK, Ahn SB. Comparison of efficacy of low-carbohydrate and low-fat diet education programs in non-alcoholic fatty liver disease: A randomized controlled study. *Hepatol Res*. 2018;48(3):E22-E9.
88. Chiavaroli L, Vigiulouk E, Nishi SK, Blanco Mejia S, Rahelic D, Kahleova H, et al. DASH Dietary Pattern and Cardiometabolic Outcomes: An Umbrella Review of Systematic Reviews and Meta-Analyses. *Nutrients*. 2019;11(2).
89. Razavi Zade M, Telkabadi MH, Bahmani F, Salehi B, Farshbaf S, Asemi Z. The effects of DASH diet on weight loss and metabolic status in adults with non-alcoholic fatty liver disease: a randomized clinical trial. *Liver international : official journal of the International Association for the Study of the Liver*. 2016;36(4):563-71.
90. Xiao ML, Lin JS, Li YH, Liu M, Deng YY, Wang CY, et al. Adherence to the Dietary Approaches to Stop Hypertension (DASH) diet is associated with lower presence of non-alcoholic fatty liver disease in middle-aged and elderly adults. *Public Health Nutr*. 2019:1-9.
91. Miller ER, 3rd, Erlinger TP, Appel LJ. The effects of macronutrients on blood pressure and lipids: an overview of the DASH and OmniHeart trials. *Curr Atheroscler Rep*. 2006;8(6):460-5.
92. Bugianesi E. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease: disease mongering or call to action? *Diabetologia*. 2016;59(6):1145-7.
93. de la Iglesia R, Loria-Kohen V, Zulet MA, Martinez JA, Reglero G, Ramirez de Molina A. Dietary Strategies Implicated in the Prevention and Treatment of Metabolic Syndrome. *Int J Mol Sci*. 2016;17(11).
94. Kaliora AC, Gioxari A, Kalafati IP, Diolintzi A, Kokkinos A, Dedoussis GV. The Effectiveness of Mediterranean Diet in Nonalcoholic Fatty Liver Disease Clinical Course: An Intervention Study. *J Med Food*. 2019;22(7):729-40.
95. Gepner Y, Shelef I, Komy O, Cohen N, Schwarzfuchs D, Bril N, et al. The beneficial effects of Mediterranean diet over low-fat diet may be mediated by decreasing hepatic fat content. *J Hepatol*. 2019;71(2):379-88.
96. Fakhry TK, Mhaskar R, Schwitalla T, Muradova E, Gonzalvo JP, Murr MM. Bariatric surgery improves nonalcoholic fatty liver disease: a contemporary systematic review and meta-analysis. *Surg Obes Relat Dis*. 2019;15(3):502-11.
97. Wang YW, He SJ, Feng X, Cheng J, Luo YT, Tian L, et al. Metformin: a review of its potential indications. *Drug Des Devel Ther*. 2017;11:2421-9.

98. Said A, Akhter A. Meta-Analysis of Randomized Controlled Trials of Pharmacologic Agents in Non-alcoholic Steatohepatitis. *Ann Hepatol.* 2017;16(4):538-47.
99. Wong VW, Chan WK, Chitturi S, Chawla Y, Dan YY, Duseja A, et al. Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017-Part 1: Definition, risk factors and assessment. *J Gastroenterol Hepatol.* 2018;33(1):70-85.
100. Mikolasevic I, Filipec-Kanizaj T, Mijic M, Jakopcic I, Milic S, Hrstic I, et al. Nonalcoholic fatty liver disease and liver transplantation - Where do we stand? *World J Gastroenterol.* 2018;24(14):1491-506.
101. 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).
102. Parikh RM, Mohan V. Changing definitions of metabolic syndrome. *Indian J Endocrinol Metab.* 2012;16(1):7-12.
103. Kasper P, Martin A, Lang S, Kütting F, Goeser T, Demir M, et al. NAFLD and cardiovascular diseases: a clinical review. *Clinical research in cardiology : official journal of the German Cardiac Society.* 2020.
104. Behairy MA, Sherief AF, Hussein HA. Prevalence of non-alcoholic fatty liver disease among patients with non-diabetic chronic kidney disease detected by transient elastography. *Int Urol Nephrol.* 2021.
105. Maurice J, Manousou P. Non-alcoholic fatty liver disease. *Clin Med.* 2018;18(3):245-50.
106. Younes R, Bugianesi E. NASH in Lean Individuals. *Semin Liver Dis.* 2019;39(1):86-95.
107. Boursier J, Tsochatzis EA. Case-finding strategies in non-alcoholic fatty liver disease. *JHEP reports : innovation in hepatology.* 2021;3(2):100219.
108. Perumpail BJ, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol.* 2017;23(47):8263-76.
109. Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. *J Hepatol.* 2019;70(3):531-44.
110. Thandra KC, Barsouk A, Saginala K, Aluru JS, Rawla P, Barsouk A. Epidemiology of non-alcoholic fatty liver disease and risk of hepatocellular carcinoma progression. *Clin Exp Hepatol.* 2020;6(4):289-94.
111. Li J, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999-2019: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* 2019;4(5):389-98.
112. Zhou F, Zhou J, Wang W, Zhang XJ, Ji YX, Zhang P, et al. Unexpected Rapid Increase in the Burden of NAFLD in China From 2008 to 2018: A Systematic Review and Meta-Analysis. *Hepatology.* 2019;70(4):1119-33.
113. Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: From steatosis to cirrhosis. *Hepatology.* 2006;43(2 SUPPL. 1):S99-S112.
114. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Bmj.* 2009;339:b2535.
115. Zhu JZ, Dai YN, Wang YM, Zhou QY, Yu CH, Li YM. Prevalence of Nonalcoholic Fatty Liver Disease and Economy. *Dig Dis Sci.* 2015;60(11):3194-202.
116. Pastori D, Sciacqua A, Marcucci R, Farcomeni A, Perticone F, Del Ben M, et al. Prevalence and Impact of Nonalcoholic Fatty Liver Disease in Atrial Fibrillation. *Mayo Clin Proc.* 2019;18:18.
117. Gan L, Chitturi S, Farrell GC. Mechanisms and Implications of Age-Related Changes in the Liver: Nonalcoholic Fatty Liver Disease in the Elderly. *Current Gerontology and Geriatrics Research.* 2011;2011:831536.
118. The Joanna Briggs Institute Critical Appraisal tools for use in JBI Systematic Reviews: Checklist for Prevalence Studies <http://joannabriggs.org/research/critical-appraisal-tools.html> (accessed March 08, 2021).

119. Migliavaca CB, Stein C, Colpani V, Munn Z, Falavigna M. Quality assessment of prevalence studies: a systematic review. *J Clin Epidemiol*. 2020;127:59-68.
120. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. Available at: [http://www.ohri.ca/programs/clinical\\_epidemiology/oxford.asp](http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) Accessed September 2020. 2014.
121. Melsen WG, Bootsma MC, Rovers MM, Bonten MJ. The effects of clinical and statistical heterogeneity on the predictive values of results from meta-analyses. *Clin Microbiol Infect*. 2014;20(2):123-9.
122. Coward S, Clement F, Benchimol EI, Bernstein CN, Avina-Zubieta JA, Bitton A, et al. Past and Future Burden of Inflammatory Bowel Diseases Based on Modeling of Population-Based Data. *Gastroenterology*. 2019;156(5):1345-53 e4.
123. King JA, Jeong J, Underwood FE, Quan J, Panaccione N, Windsor JW, et al. Incidence of Celiac Disease Is Increasing Over Time: A Systematic Review and Meta-analysis. *Am J Gastroenterol*. 2020;115(4):507-25.
124. Ferris M, Quan S, Kaplan BS, Molodecky N, Ball CG, Chernoff GW, et al. The Global Incidence of Appendicitis: A Systematic Review of Population-based Studies. *Ann Surg*. 2017;266(2):237-41.
125. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology*. 2017;153(2):420-9.
126. Kotze PG, Underwood FE, Damiao A, Ferraz JGP, Saad-Hossne R, Toro M, et al. Progression of Inflammatory Bowel Diseases Throughout Latin America and the Caribbean: A Systematic Review. *Clin Gastroenterol Hepatol*. 2020;18(2):304-12.
127. Ng SC, Shi HY, Hamidi N, Underwood FE, Tang W, Benchimol EI, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*. 2017;390(10114):2769-78.
128. Cholongitas E, Pavlopoulou I, Papatheodoridi M, Markakis GE, Bouras E, Haidich AB, et al. Epidemiology of nonalcoholic fatty liver disease in Europe: a systematic review and meta-analysis. *Ann Gastroenterol*. 2021;34(3):404-14.
129. Petta S, Di Marco V, Pipitone RM, Grimaudo S, Buscemi C, Craxi A, et al. Prevalence and severity of nonalcoholic fatty liver disease by transient elastography: Genetic and metabolic risk factors in a general population. *Liver Int*. 2018;38(11):2060-8.
130. Zhai H, Chen C, Wang N, Chen Y, Nie X, Han B, et al. Blood lead level is associated with non-alcoholic fatty liver disease in the Yangtze River Delta region of China in the context of rapid urbanization. *Environ Health*. 2017;16(1):93.
131. Amirkalali B, Poustchi H, Keyvani H, Khansari MR, Ajdarkosh H, Maadi M, et al. Prevalence of Non-Alcoholic Fatty Liver Disease and Its Predictors in North of Iran. *Iran J Public Health*. 2014;43(9):1275-83.
132. Hong Z, Yanfang J, Shumei H, Jie S, Qing G, Xiangwei F, et al. Relationship between serum aminotransferase levels and metabolic disorders in northern China. *Turk J Gastroenterol*. 2012;23(6):699-707.
133. Vlassoff C. Gender differences in determinants and consequences of health and illness. *J Health Popul Nutr*. 2007;25(1):47-61.
134. World Health Organization. Gender and Health. [https://www.who.int/health-topics/gender#tab=tab\\_1](https://www.who.int/health-topics/gender#tab=tab_1). Accessed Aug 19, 2021.
135. Johnson JL, Greaves L, Repta R. Better science with sex and gender: Facilitating the use of a sex and gender-based analysis in health research. *International journal for equity in health*. 2009;8:14.
136. Haghghian Roudsari A, Vedadhir A, Amiri P, Kalantari N, Omidvar N, Eini-Zinab H, et al. Psycho-Socio-Cultural Determinants of Food Choice: A Qualitative Study on Adults in Social and Cultural Context of Iran. *Iranian journal of psychiatry*. 2017;12(4):241-50.

137. Lonardo A, Nascimbeni F, Ballestri S, Fairweather D, Win S, Than TA, et al. Sex Differences in Nonalcoholic Fatty Liver Disease: State of the Art and Identification of Research Gaps. *Hepatology*. 2019;70(4):1457-69.
138. Ballestri S, Nascimbeni F, Baldelli E, Marrazzo A, Romagnoli D, Lonardo A. NAFLD as a Sexual Dimorphic Disease: Role of Gender and Reproductive Status in the Development and Progression of Nonalcoholic Fatty Liver Disease and Inherent Cardiovascular Risk. *Adv Ther*. 2017;34(6):1291-326.
139. Lorbek G, Urlep Ž, Rozman D. Pharmacogenomic and personalized approaches to tackle nonalcoholic fatty liver disease. *Pharmacogenomics*. 2016;17(11):1273-88.
140. Lonardo A, Suzuki A. Sexual Dimorphism of NAFLD in Adults. Focus on Clinical Aspects and Implications for Practice and Translational Research. *J Clin Med*. 2020;9(5).
141. Abenavoli L, Di Renzo L, De Lorenzo A. Body Composition and Non-alcoholic Fatty Liver Disease. *Journal of lifestyle medicine*. 2016;6(1):47-8.
142. Ariya M, Koochpayeh F, Ghaemi A, Osati S, Davoodi SH, Razzaz JM, et al. Assessment of the association between body composition and risk of non-alcoholic fatty liver. *PLoS ONE*. 2021;16(4):e0249223.
143. Florentino GS, Cotrim HP, Vilar CP, Florentino AV, Guimarães GM, Barreto VS. Nonalcoholic fatty liver disease in menopausal women. *Arq Gastroenterol*. 2013;50(3):180-5.
144. Cortés ME, Alfaro AA. The effects of hormonal contraceptives on glycemic regulation. *The Linacre quarterly*. 2014;81(3):209-18.
145. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev*. 2013;34(3):309-38.
146. Foryst-Ludwig A, Kintscher U. Metabolic impact of estrogen signalling through ERalpha and ERbeta. *The Journal of steroid biochemistry and molecular biology*. 2010;122(1-3):74-81.
147. Yan H, Yang W, Zhou F, Li X, Pan Q, Shen Z, et al. Estrogen Improves Insulin Sensitivity and Suppresses Gluconeogenesis via the Transcription Factor Foxo1. *Diabetes*. 2019;68(2):291-304.
148. Yu IC, Lin HY, Sparks JD, Yeh S, Chang C. Androgen receptor roles in insulin resistance and obesity in males: the linkage of androgen-deprivation therapy to metabolic syndrome. *Diabetes*. 2014;63(10):3180-8.
149. Tyagi V, Scordo M, Yoon RS, Liporace FA, Greene LW. Revisiting the role of testosterone: Are we missing something? *Reviews in urology*. 2017;19(1):16-24.
150. Yassin A, Doros G. Testosterone therapy in hypogonadal men results in sustained and clinically meaningful weight loss. *Clin Obes*. 2013;3(3-4):73-83.
151. Salvoza NC, Giraudi PJ, Tiribelli C, Rosso N. Sex differences in non-alcoholic fatty liver disease: hints for future management of the disease. *Explor Med*. 2020;1:51-74.
152. Jaruvongvanich V, Sanguankeo A, Riangwiwat T, Upala S. Testosterone, Sex Hormone-Binding Globulin and Nonalcoholic Fatty Liver Disease: a Systematic Review and Meta-Analysis. *Ann Hepatol*. 2017;16(3):382-94.
153. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *Jama*. 2006;295(11):1288-99.
154. Sarkar M, Yates K, Suzuki A, Lavine J, Gill R, Ziegler T, et al. Low Testosterone Is Associated With Nonalcoholic Steatohepatitis and Fibrosis Severity in Men. *Clin Gastroenterol Hepatol*. 2021;19(2):400-2.e2.
155. Mirmiran P, Amirhamidi Z, Ejtahed HS, Bahadoran Z, Azizi F. Relationship between Diet and Non-alcoholic Fatty Liver Disease: A Review Article. *Iran J Public Health*. 2017;46(8):1007-17.
156. Rolls BJ, Fedoroff IC, Guthrie JF. Gender differences in eating behavior and body weight regulation. *Health Psychol*. 1991;10(2):133-42.
157. Wardle J, Haase AM, Steptoe A, Nillapun M, Jonwutiwes K, Bellisle F. Gender differences in food choice: the contribution of health beliefs and dieting. *Ann Behav Med*. 2004;27(2):107-16.
158. Phillips SP. Defining and measuring gender: a social determinant of health whose time has come. *International journal for equity in health*. 2005;4:11.

159. Doan RM, Bisharat L. Female autonomy and child nutritional status: the extended-family residential unit in Amman, Jordan. *Social science & medicine* (1982). 1990;31(7):783-9.
160. Borooah VK. Gender bias among children in India in their diet and immunisation against disease. *Social science & medicine* (1982). 2004;58(9):1719-31.
161. Salehi M, Kimiagar SM, Shahbazi M, Mehrabi Y, Kolahi AA. Assessing the impact of nutrition education on growth indices of Iranian nomadic children: an application of a modified beliefs, attitudes, subjective-norms and enabling-factors model. *Br J Nutr*. 2004;91(5):779-87.
162. Segar M, Jayaratne T, Hanlon J, Richardson CR. Fitting fitness into women's lives: effects of a gender-tailored physical activity intervention. *Womens Health Issues*. 2002;12(6):338-47.
163. Backstrand JR, Allen LH, Peltó GH, Chavez A. Examining the gender gap in nutrition: an example from rural Mexico. *Social science & medicine* (1982). 1997;44(11):1751-9.
164. Vari R, Scazzocchio B, D'Amore A, Giovannini C, Gessani S, Masella R. Gender-related differences in lifestyle may affect health status. *Ann Ist Super Sanita*. 2016;52(2):158-66.
165. Craft BB, Carroll HA, Lustyk MK. Gender Differences in Exercise Habits and Quality of Life Reports: Assessing the Moderating Effects of Reasons for Exercise. *Int J Lib Arts Soc Sci*. 2014;2(5):65-76.
166. Zhou JH, Cai JJ, She ZG, Li HL. Noninvasive evaluation of nonalcoholic fatty liver disease: Current evidence and practice. *World J Gastroenterol*. 2019;25(11):1307-26.
167. Zelber-Sagi S, Webb M, Assy N, Blendis L, Yeshua H, Leshno M, et al. Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification. *World J Gastroenterol*. 2013;19(1):57-64.
168. Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J Hepatol*. 2009;51(6):1061-7.
169. 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.
170. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *Diabetologia*. 2016;59(6):1121-40.
171. Fedchuk L, Nascimbeni F, Pais R, Charlotte F, Housset C, Ratziu V, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. *Aliment Pharmacol Ther*. 2014;40(10):1209-22.
172. Starekova J, Reeder SB. Liver fat quantification: where do we stand? *Abdominal radiology (New York)*. 2020;45(11):3386-99.
173. Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol*. 2014;20(2):475-85.
174. Tannapfel A, Dienes HP, Lohse AW. The indications for liver biopsy. *Dtsch Arztebl Int*. 2012;109(27-28):477-83.
175. Mohanty SR, Troy TN, Huo D, O'Brien BL, Jensen DM, Hart J. Influence of ethnicity on histological differences in non-alcoholic fatty liver disease. *J Hepatol*. 2009;50(4):797-804.
176. Schwenzer NF, Springer F, Schraml C, Stefan N, Machann J, Schick F. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. *J Hepatol*. 2009;51(3):433-45.
177. Chan WK, Nik Mustapha NR, Wong GL, Wong VW, Mahadeva S. Controlled attenuation parameter using the FibroScan® XL probe for quantification of hepatic steatosis for non-alcoholic fatty liver disease in an Asian population. *United European Gastroenterol J*. 2017;5(1):76-85.
178. Caussy C, Alqiraish MH, Nguyen P, Hernandez C, Cepin S, Fortney LE, et al. Optimal threshold of controlled attenuation parameter with MRI-PDFF as the gold standard for the detection of hepatic steatosis. *Hepatology*. 2018;67(4):1348-59.
179. Huang X, Xu M, Chen Y, Peng K, Huang Y, Wang P, et al. Validation of the Fatty Liver Index for Nonalcoholic Fatty Liver Disease in Middle-Aged and Elderly Chinese. *Medicine (Baltimore)*. 2015;94(40):e1682.

180. Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Dig Liver Dis.* 2010;42(7):503-8.
181. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol.* 2006;6:33.
182. Lonardo A, Nascimbeni F, Maurantonio M, Marrazzo A, Rinaldi L, Adinolfi LE. Nonalcoholic fatty liver disease: Evolving paradigms. *World J Gastroenterol.* 2017;23(36):6571-92.
183. Dokmak A, Lizaola-Mayo B, Trivedi HD. The Impact of Nonalcoholic Fatty Liver Disease in Primary Care: A Population Health Perspective. *Am J Med.* 2021;134(1):23-9.
184. Blais P, Husain N, Kramer JR, Kowalkowski M, El-Serag H, Kanwal F. Nonalcoholic fatty liver disease is underrecognized in the primary care setting. *Am J Gastroenterol.* 2015;110(1):10-4.
185. Alexander M, Loomis AK, Fairburn-Beech J, van der Lei J, Duarte-Salles T, Prieto-Alhambra D, et al. Real-world data reveal a diagnostic gap in non-alcoholic fatty liver disease. *BMC Med.* 2018;16(1):130.
186. Sanyal A, Poklepovic A, Moyneur E, Barghout V. Population-based risk factors and resource utilization for HCC: US perspective. *Curr Med Res Opin.* 2010;26(9):2183-91.
187. Younossi ZM, Stepanova M, Younossi Y, Golabi P, Mishra A, Rafiq N, et al. Epidemiology of chronic liver diseases in the USA in the past three decades. *Gut.* 2019;31:31.
188. Eshraghian A, Dabbaghmanesh MH, Eshraghian H, Fattahi MR, Omrani GR. Nonalcoholic fatty liver disease in a cluster of Iranian population: thyroid status and metabolic risk factors. *Arch Iran Med.* 2013;16(10):584-9.
189. Zelber-Sagi S, Salomone F, Yeshua H, Lotan R, Webb M, Halpern Z, et al. Non-high-density lipoprotein cholesterol independently predicts new onset of non-alcoholic fatty liver disease. *Liver Int.* 2014;34(6):e128-35.
190. Kasapoglu B, Turkay C, Yalcin KS, Carlioglu A, Sozen M, Kaktener A. Low vitamin D levels are associated with increased risk for fatty liver disease among non-obese adults. *Clin Med.* 2013;13(6):576-9.
191. Moghaddasifar I, Lankarani KB, Moosazadeh M, Afshari M, Ghaemi A, Aliramezany M, et al. Prevalence of Non-alcoholic Fatty Liver Disease and Its Related Factors in Iran. *International Journal of Organ Transplantation Medicine.* 2016;7(3):149-60.
192. Alswat K, Aljumah AA, Sanai FM, Abaalkhail F, Alghamdi M, Al Hamoudi WK, et al. Nonalcoholic fatty liver disease burden - Saudi Arabia and United Arab Emirates, 2017-2030. *Saudi journal of gastroenterology : official journal of the Saudi Gastroenterology Association.* 2018;24(4):211-9.
193. Swain MG, Ramji A, Patel K, Sebastiani G, Shaheen AA, Tam E, et al. Burden of nonalcoholic fatty liver disease in Canada, 2019-2030: a modelling study. *CMAJ open.* 2020;8(2):E429-e36.
194. Kim D, Kim WR, Kim HJ, Therneau TM. Association between noninvasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology.* 2013;57(4):1357-65.
195. Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States National Health and Nutrition Examination Survey. *Aliment Pharmacol Ther.* 2015;41(1):65-76.
196. World Health Organization Global Health Observatory data repository. Prevalence of overweight among adults, BMI  $\geq$  25, age-standardized Estimates by WHO Region <https://apps.who.int/gho/data/view.main.GLOBAL2461A?lang=en>.
197. Perez M, Gonzales L, Olarte R, Rodriguez NI, Tabares M, Salazar JP, et al. Nonalcoholic fatty liver disease is associated with insulin resistance in a young Hispanic population. *Prev Med.* 2011;52(2):174-7.
198. Karnikowski M, Cordova C, Oliveira RJ, Karnikowski MG, Nobrega Ode T. Non-alcoholic fatty liver disease and metabolic syndrome in Brazilian middle-aged and older adults. *Sao Paulo Med J.* 2007;125(6):333-7.

199. Gallardo-Rincón H, Cantoral A, Arrieta A, Espinal C, Magnus MH, Palacios C, et al. Review: Type 2 diabetes in Latin America and the Caribbean: Regional and country comparison on prevalence, trends, costs and expanded prevention. *Prim Care Diabetes*. 2021;15(2):352-9.
200. Onyekwere CA, Ogbera AO, Balogun BO. Non-alcoholic fatty liver disease and the metabolic syndrome in an urban hospital serving an African community. *Ann Hepatol*. 2011;10(2):119-24.
201. Almobarak AO, Barakat S, Khalifa MH, Elhoweris MH, Elhassan TM, Ahmed MH. Non alcoholic fatty liver disease (NAFLD) in a Sudanese population: What is the prevalence and risk factors? *Arab J Gastroenterol*. 2014;15(1):12-5.
202. Kramvis A, Kew MC. Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. *Hepatology research : the official journal of the Japan Society of Hepatology*. 2007;37(s1):S9-s19.
203. Paruk IM, Pirie FJ, Motala AA. Non-alcoholic fatty liver disease in Africa: a hidden danger. *Glob Health Epidemiol Genom*. 2019;4:e3.
204. Group NCDRFC-AW. Trends in obesity and diabetes across Africa from 1980 to 2014: an analysis of pooled population-based studies. *Int J Epidemiol*. 2017;46(5):1421-32.
205. Boyle M, Masson S, Anstee QM. The bidirectional impacts of alcohol consumption and the metabolic syndrome: Cofactors for progressive fatty liver disease. *J Hepatol*. 2018;68(2):251-67.
206. Brunt EM, Ramrakhiani S, Cordes BG, Neuschwander-Tetri BA, Janney CG, Bacon BR, et al. Concurrence of histologic features of steatohepatitis with other forms of chronic liver disease. *Mod Pathol*. 2003;16(1):49-56.
207. Omagari K, Kadokawa Y, Masuda J, Egawa I, Sawa T, Hazama H, et al. Fatty liver in non-alcoholic non-overweight Japanese adults: incidence and clinical characteristics. *J Gastroenterol Hepatol*. 2002;17(10):1098-105.
208. Nishioji K, Sumida Y, Kamaguchi M, Mochizuki N, Kobayashi M, Nishimura T, et al. Prevalence of and risk factors for non-alcoholic fatty liver disease in a non-obese Japanese population, 2011-2012. *J Gastroenterol*. 2015;50(1):95-108.
209. Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann Intern Med*. 2005;143(10):722-8.
210. Livingston M, Callinan S. Underreporting in alcohol surveys: whose drinking is underestimated? *Journal of studies on alcohol and drugs*. 2015;76(1):158-64.
211. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol*. 2020;5(3):245-66.
212. Gual A, Ángel Arbesú J, Zarco J, Balcells-Oliveró MLM, López-Pelayo H, Miquel L, et al. Risky Drinkers Underestimate their Own Alcohol Consumption. *Alcohol Alcohol*. 2017;52(4):516-7.
213. Mak LY, Yuen MF, Seto WK. Letter regarding "A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement". *J Hepatol*. 2020;73(6):1573-4.
214. 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.
215. 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.
216. 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.
217. Zhao H, Hu B, Yu SJ, Liu Y, Leng S, Sun GH. Relationship between components of metabolic syndrome and incidence of non - Alcoholic fatty liver disease. [Chinese]. *Journal of Dalian Medical University*. 2012;34(6):586-9.

218. Caballeria L, Pera G, Auladell MA, Toran P, Munoz L, Miranda D, et al. Prevalence and factors associated with the presence of nonalcoholic fatty liver disease in an adult population in Spain. *Eur J Gastroenterol Hepatol.* 2010;22(1):24-32.
219. Caserta CA, Mele A, Surace P, Ferrigno L, Amante A, Messineo A, et al. Association of non-alcoholic fatty liver disease and cardiometabolic risk factors with early atherosclerosis in an adult population in Southern Italy. *Ann Ist Super Sanita.* 2017;53(1):77-81.
220. Chalmers J, Ban L, Leena KB, Edwards KL, Grove JL, Aithal GP, et al. Cohort profile: the Trivandrum non-alcoholic fatty liver disease (NAFLD) cohort. *BMJ Open.* 2019;9(5):e027244.
221. Chen CH, Huang MH, Yang JC, Nien CK, Yang CC, Yeh YH, et al. Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of taiwan: metabolic significance of nonalcoholic fatty liver disease in nonobese adults. *J Clin Gastroenterol.* 2006;40(8):745-52.
222. Cheng H-Y, Wang H-Y, Chang W-H, Lin S-C, Chu C-H, Wang T-E, et al. Nonalcoholic Fatty Liver Disease: Prevalence, Influence on Age and Sex, and Relationship with Metabolic Syndrome and Insulin Resistance. *International Journal of Gerontology.* 2013;7(4):194-8.
223. Dai H, Wang W, Chen R, Chen Z, Lu Y, Yuan H. Lipid accumulation product is a powerful tool to predict non-alcoholic fatty liver disease in Chinese adults. *Nutr Metab (Lond).* 2017;14:49.
224. Fan N, Peng L, Xia Z, Zhang L, Wang Y, Peng Y. Helicobacter pylori Infection Is Not Associated with Non-alcoholic Fatty Liver Disease: A Cross-Sectional Study in China. *Front Microbiol.* 2018;9:73.
225. Gruchot M, Graeter T, Oeztuerk S, Haenle MM, Koenig W, Imhof A, et al. Fasting time and lipid parameters: association with hepatic steatosis--data from a random population sample. *Lipids health dis.* 2014;13:18.
226. Hong Z, Yanfang J, Shumei H, Jie S, Qing G, Xiangwei F, et al. Relationship between serum aminotransferase levels and metabolic disorders in northern China. *Turk J Gastroenterol.* 2012;23(6):699-707.
227. Huan S, Hao X, Sun X, Ren Q, Gu Y, Zhu W. Prevalence and risk factors of fatty liver disease in urban adult residents of qingdao: An epidemiological survey. *Int J Clin Exp Med.* 2016;9(8):16476-83.
228. Huang JF, Tsai PC, Yeh ML, Huang CF, Huang CI, Hsieh MH, et al. Risk stratification of non-alcoholic fatty liver disease across body mass index in a community basis. *J Formos Med Assoc.* 2020;119(1 Pt 1):89-96.
229. Hung CS, Tseng PH, Tu CH, Chen CC, Liao WC, Lee YC, et al. Increased Pancreatic Echogenicity with US: Relationship to Glycemic Progression and Incident Diabetes. *Radiology.* 2018;287(3):853-63.
230. Jeong EH, Jun DW, Cho YK, Choe YG, Ryu S, Lee SM, et al. Regional prevalence of non-alcoholic fatty liver disease in Seoul and Gyeonggi-do, Korea. *Clin Mol Hepatol.* 2013;19(3):266-72.
231. Jiang B, Gu T, Zhou K, Zheng Y, Guo Y, Lu Y. Fatty Liver as a Potential Surrogate for Waist Circumference in the Diagnosis of Metabolic Syndrome: A Population-Based Study among Chinese Adults. *Int.* 2018;2018:7903982.
232. Kim HK, Park JY, Lee KU, Lee GE, Jeon SH, Kim JH, et al. Effect of body weight and lifestyle changes on long-term course of nonalcoholic fatty liver disease in Koreans. *Am J Med Sci.* 2009;337(2):98-102.
233. Kim SH, Lee JW, Hwang HJ. Associations between combinations of body mass index plus non-alcoholic fatty liver disease and diabetes mellitus among Korean adults. *Asia Pac J Clin Nutr.* 2011;20(1):14-20.
234. Kim G, Lee YH, Park YM, Kim J, Kim H, Lee BW, et al. Use of a Diabetes Self-Assessment Score to Predict Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. *Medicine (Baltimore).* 2015;94(27):e1103.
235. Kim NH, Jung YS, Hong HP, Park JH, Kim HJ, Park DI, et al. Association between cotinine-verified smoking status and risk of nonalcoholic fatty liver disease. *Liver Int.* 2018;38(8):1487-94.

236. Kure T, Mawatari S, Imamura Y, Oda K, Kumagai K, Hiramane Y, et al. Nonalcoholic fatty liver disease is associated with both subcutaneous and visceral adiposity: A cross-sectional study. *Medicine (Baltimore)*. 2019;98(46):e17879.
237. Lai HC, Lin T, Lai SW, Liao KF. Association between nonalcoholic fatty liver disease and cardiovascular risk factors in a hospital-based study. *Tzu Chi Medical Journal*. 2008;20(3):213-7.
238. Lee K, Sung JA, Kim JS, Park TJ. The roles of obesity and gender on the relationship between metabolic risk factors and non-alcoholic fatty liver disease in Koreans. *Diabetes/Metabolism Research and Reviews*. 2009;25(2):150-5.
239. Lee S, Ko BJ, Gong Y, Han K, Lee A, Han BD, et al. Self-reported eating speed in relation to non-alcoholic fatty liver disease in adults. *Eur J Nutr*. 2016;55(1):327-33.
240. Lee HW, Kim BK, Kim SU, Park JY, Kim DY, Ahn SH, et al. Prevalence and Predictors of Significant Fibrosis Among Subjects with Transient Elastography-Defined Nonalcoholic Fatty Liver Disease. *Dig Dis Sci*. 2017;62(8):2150-8.
241. Lee SW, Lee TY, Yang SS, Peng YC, Yeh HZ, Chang CS. The association of non-alcoholic fatty liver disease and metabolic syndrome in a Chinese population. *Hepatobiliary Pancreat Dis Int*. 2017;16(2):176-80.
242. Lee SH, Kim MJ, Kim YS, Chun H, Won BY, Lee JH, et al. Low hair copper concentration is related to a high risk of nonalcoholic fatty liver disease in adults. *J Trace Elem Med Biol*. 2018;50:28-33.
243. Li H, Dong K, Fang Q, Hou X, Zhou M, Bao Y, et al. High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China. *J Hepatol*. 2013;58(3):557-63.
244. Li L, You W, Ren W. The ZJU index is a powerful index for identifying NAFLD in the general Chinese population. *Acta Diabetol*. 2017;54(10):905-11.
245. Lu ZY, Shao Z, Li YL, Wulasihan M, Chen XH. Prevalence of and risk factors for non-alcoholic fatty liver disease in a Chinese population: An 8-year follow-up study. *World J Gastroenterol*. 2016;22(13):3663-9.
246. Ostovaneh MR, Zamani F, Ansari-Moghaddam A, Sharafkhan M, Saeedian FS, Rohani Z, et al. Nonalcoholic Fatty Liver: The Association with Metabolic Abnormalities, Body Mass Index and Central Obesity--A Population-Based Study. *Metab*. 2015;13(7):304-11.
247. Qiao QH, Zhu WH, Yu YX, Huang FF, Chen LY. Nonalcoholic fatty liver was associated with asymptomatic gallstones in a Chinese population. *Medicine (Baltimore)*. 2017;96(38):e7853.
248. Saha M, Nahar K, Parveen I, Mahmuduzzaman M, Hosen MA, Mutsuddy P, et al. Sonographically Detected Non Alcoholic Fatty Liver Disease in Apparently Healthy Adults and Associated Factors. *Mymensingh Med J*. 2017;26(1):109-16.
249. Salmanroghani H, Salmanroghani R, Nourian M, Khayarn K, Lahmi F, Iravani S. Evaluation of neck circumference as an easy and reliable predictor for non-alcoholic fatty liver disease. *Turk J Gastroenterol*. 2019;30(2):163-70.
250. Shin JY, Kim MJ, Kim ES, Mo EY, Moon SD, Han JH, et al. Association between serum calcium and phosphorus concentrations with non-alcoholic fatty liver disease in Korean population. *Journal of Gastroenterology and Hepatology (Australia)*. 2015;30(4):733-41.
251. Uchil D, Pipalia D, Chawla M, Patel R, Maniar S, Narayani, et al. Non-alcoholic fatty liver disease (NAFLD)--the hepatic component of metabolic syndrome. *J Assoc Physicians India*. 2009;57:201-4.
252. Volzke H, Robinson DM, Kleine V, Deutscher R, Hoffmann W, Ludemann J, et al. Hepatic steatosis is associated with an increased risk of carotid atherosclerosis. *World J Gastroenterol*. 2005;11(12):1848-53.
253. Wang J, Xu C, Xun Y, Lu Z, Shi J, Yu C, et al. ZJU index: a novel model for predicting nonalcoholic fatty liver disease in a Chinese population. *Sci Rep*. 2015;5:16494.

254. Wei JL, Leung JCF, Loong TCW, Wong GLH, Yeung DKW, Chan RSM, et al. Prevalence and Severity of Nonalcoholic Fatty Liver Disease in Non-Obese Patients: A Population Study Using Proton-Magnetic Resonance Spectroscopy. *Am J Gastroenterol*. 2015;110(9):1306-14.
255. Wu J, He S, Xu H, Chi X, Sun J, Wang X, et al. Non-alcoholic fatty liver disease incidence, remission and risk factors among a general Chinese population with a 6-year follow-up. *Sci*. 2018;8(1):7557.
256. Zelber-Sagi S, Nitzan-Kaluski D, Halpern Z, Oren R. Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. *Liver Int*. 2006;26(7):856-63.
257. Zhai HL, Wang NJ, Han B, Li Q, Chen Y, Zhu CF, et al. Low vitamin D levels and non-alcoholic fatty liver disease, evidence for their independent association in men in East China: a cross-sectional study (Survey on Prevalence in East China for Metabolic Diseases and Risk Factors (SPECT-China)). *Br J Nutr*. 2016;115(8):1352-9.
258. Zhang Q, Yu Y, Yang M, Lin X, Liu Y, Xu X. Association between glycosylated hemoglobin and non-alcoholic fatty liver disease in health examination population. [Chinese]. *Zhong nan da xue xue bao*. 2017;Yi xue ban = Journal of Central South University. Medical sciences. 42(5):529-35.
259. Zhou X, Li Y, Zhang X, Guan YY, Puentes Y, Zhang F, et al. Independent markers of nonalcoholic fatty liver disease in a gentrifying population-based Chinese cohort. *Diabetes Metab Res Rev*. 2019;35(5):e3156.
260. Chang Y, Noh YH, Suh BS, Kim Y, Sung E, Jung HS, et al. Bidirectional Association between Nonalcoholic Fatty Liver Disease and Gallstone Disease: A Cohort Study. *J Clin Med*. 2018;7(11):21.
261. Kim NH, Kim JH, Kim YJ, Yoo HJ, Kim HY, Seo JA, et al. Clinical and metabolic factors associated with development and regression of nonalcoholic fatty liver disease in nonobese subjects. *Liver Int*. 2014;34(4):604-11.
262. Kitae A, Hashimoto Y, Hamaguchi M, Obora A, Kojima T, Fukui M. The Triglyceride and Glucose Index Is a Predictor of Incident Nonalcoholic Fatty Liver Disease: A Population-Based Cohort Study. *Can J Gastroenterol Hepatol*. 2019;2019:5121574.
263. Okamoto M, Miyake T, Kitai K, Furukawa S, Yamamoto S, Senba H, et al. Cigarette smoking is a risk factor for the onset of fatty liver disease in nondrinkers: A longitudinal cohort study. *PLoS ONE*. 2018;13(4):e0195147.
264. Wang J, Zhu W, Huang S, Xu L, Miao M, Wu C, et al. Serum apoB levels independently predict the development of non-alcoholic fatty liver disease: A 7-year prospective study. *Liver Int*. 2017;37(8):1202-8.
265. Wang K, Shan S, Zheng H, Zhao X, Chen C, Liu C. Non-HDL-cholesterol to HDL-cholesterol ratio is a better predictor of new-onset non-alcoholic fatty liver disease than non-HDL-cholesterol: a cohort study. *Lipids health dis*. 2018;17(1):196.
266. Wong VW, Wong GL, Yeung DK, Lau TK, Chan CK, Chim AM, et al. Incidence of non-alcoholic fatty liver disease in Hong Kong: a population study with paired proton-magnetic resonance spectroscopy. *J Hepatol*. 2015;62(1):182-9.
267. Wu SJ, Zou H, Zhu GQ, Wang LR, Zhang Q, Shi KQ, et al. Increased levels of systolic blood pressure within the normal range are associated with significantly elevated risks of nonalcoholic fatty liver disease. *Medicine (Baltimore)*. 2015;94(19):e842.
268. West SL, Gartlehner G, Mansfield AJ, Poole C, Tant E, Lenfestey N, et al. AHRQ Methods for Effective Health Care. Comparative Effectiveness Review Methods: Clinical Heterogeneity. AHRQ Methods for Effective Health Care. Rockville (MD): Agency for Healthcare Research and Quality (US); 2010.
269. Fotbolcu H, Zorlu E. Nonalcoholic fatty liver disease as a multi-systemic disease. *World J Gastroenterol*. 2016;22(16):4079-90.
270. Godoy-Matos AF, Silva Júnior WS, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. *Diabetol Metab Syndr*. 2020;12:60.

271. Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology*. 2018;67(1):123-33.
272. Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. *Frontline gastroenterol*. 2014;5(3):211-8.
273. Mitra S, De A, Chowdhury A. Epidemiology of non-alcoholic and alcoholic fatty liver diseases. *Transl Gastroenterol Hepatol*. 2020;5:16.
274. Tomah S, Alkhoury N, Hamdy O. Nonalcoholic fatty liver disease and type 2 diabetes: where do Diabetologists stand? *Clin Diabetes Endocrinol*. 2020;6:9.
275. Zhang JZ, Cai JJ, Yu Y, She ZG, Li H. Nonalcoholic Fatty Liver Disease: An Update on the Diagnosis. *Gene Expr*. 2019;19(3):187-98.
276. Kumar R, Priyadarshi RN, Anand U. Non-alcoholic Fatty Liver Disease: Growing Burden, Adverse Outcomes and Associations. *J Clin Transl Hepatol*. 2020;8(1):76-86.
277. Gartlehner G, West SL, Mansfield AJ, Poole C, Tant E, Lux LJ, et al. Clinical heterogeneity in systematic reviews and health technology assessments: synthesis of guidance documents and the literature. *Int J Technol Assess Health Care*. 2012;28(1):36-43.
278. West SL, Gartlehner G, Mansfield AJ, Poole C, Tant E, Lenfestey N, et al. Comparative Effectiveness Review Methods: Clinical Heterogeneity. *AHRQ Methods for Effective Health Care*. Rockville (MD)2010.
279. Imrey PB. Limitations of Meta-analyses of Studies With High Heterogeneity. *JAMA Netw Open*. 2020;3(1):e1919325.
280. Aldrich MC, Hidalgo B, Widome R, Briss P, Brownson RC, Teutsch SM. The role of epidemiology in evidence-based policy making: a case study of tobacco use in youth. *Ann Epidemiol*. 2015;25(5):360-5.
281. Straus SE, Tetroe J, Graham I. Defining knowledge translation. *Cmaj*. 2009;181(3-4):165-8.
282. Gopalakrishnan S, Ganeshkumar P. Systematic Reviews and Meta-analysis: Understanding the Best Evidence in Primary Healthcare. *J Family Med Prim Care*. 2013;2(1):9-14.
283. Perrier L, Mrklas K, Lavis JN, Straus SE. Interventions encouraging the use of systematic reviews by health policymakers and managers: a systematic review. *Implement Sci*. 2011;6:43.
284. Glasgow RE, Green LW, Klesges LM, Abrams DB, Fisher EB, Goldstein MG, et al. External validity: we need to do more. *Ann Behav Med*. 2006;31(2):105-8.
285. Green LW. Making research relevant: if it is an evidence-based practice, where's the practice-based evidence? *Fam Pract*. 2008;25 Suppl 1:i20-4.
286. Kukull WA, Ganguli M. Generalizability: the trees, the forest, and the low-hanging fruit. *Neurology*. 2012;78(23):1886-91.
287. Higgins JPT TJ, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). *Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (updated February 2021)*.
288. Chess LE, Gagnier JJ. Applicable or non-applicable: investigations of clinical heterogeneity in systematic reviews. *BMC medical research methodology*. 2016;16:19.
289. West SL GG, Mansfield AJ, et al. . Comparative Effectiveness Review Methods: Clinical Heterogeneity [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2010 Sep. 3, Results. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK53317/>.
290. Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. *Frontline gastroenterol*. 2014;5(3):211-8.
291. DiStefano JK. NAFLD and NASH in Postmenopausal Women: Implications for Diagnosis and Treatment. *Endocrinology*. 2020;161(10).
292. Bang KB, Cho YK. Comorbidities and Metabolic Derangement of NAFLD. *Journal of lifestyle medicine*. 2015;5(1):7-13.
293. Glass LM, Hunt CM, Fuchs M, Su GL. Comorbidities and Nonalcoholic Fatty Liver Disease: The Chicken, the Egg, or Both? *Federal practitioner : for the health care professionals of the VA, DoD, and PHS*. 2019;36(2):64-71.

294. Rhee EJ. Nonalcoholic Fatty Liver Disease and Diabetes: An Epidemiological Perspective. *Endocrinol Metab (Seoul)*. 2019;34(3):226-33.
295. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. *Dig Liver Dis*. 2015;47(3):181-90.
296. Lee K. Metabolic syndrome predicts the incidence of hepatic steatosis in Koreans. *Obes Res Clin Pract*. 2010;4(3):e163-246.
297. Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. *J Hepatol*. 2019;70(3):531-44.
298. Zhao YC, Zhao GJ, Chen Z, She ZG, Cai J, Li H. Nonalcoholic Fatty Liver Disease: An Emerging Driver of Hypertension. *Hypertension (Dallas, Tex : 1979)*. 2020;75(2):275-84.
299. Ma J, Hwang SJ, Pedley A, Massaro JM, Hoffmann U, Chung RT, et al. Bi-directional analysis between fatty liver and cardiovascular disease risk factors. *J Hepatol*. 2017;66(2):390-7.
300. Wainwright P, Byrne CD. Bidirectional Relationships and Disconnects between NAFLD and Features of the Metabolic Syndrome. *Int J Mol Sci*. 2016;17(3):367.
301. Divella R, Mazzocca A, Daniele A, Sabbà C, Paradiso A. Obesity, Nonalcoholic Fatty Liver Disease and Adipocytokines Network in Promotion of Cancer. *Int J Biol Sci*. 2019;15(3):610-6.
302. Divella R, Mazzocca A, Daniele A, Sabba C, Paradiso A. Obesity, Nonalcoholic Fatty Liver Disease and Adipocytokines Network in Promotion of Cancer. *Int J Biol Sci*. 2019;15(3):610-6.
303. 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.
304. Hallsworth K, Thoma C, Moore S, Ploetz T, Anstee QM, Taylor R, et al. Non-alcoholic fatty liver disease is associated with higher levels of objectively measured sedentary behaviour and lower levels of physical activity than matched healthy controls. *Frontline gastroenterol*. 2015;6(1):44-51.
305. Han MAT, Yu Q, Tafesh Z, Pysopoulos N. Diversity in NAFLD: A Review of Manifestations of Nonalcoholic Fatty Liver Disease in Different Ethnicities Globally. *J Clin Transl Hepatol*. 2021;9(1):71-80.
306. Robinson KE, Shah VH. Pathogenesis and pathways: nonalcoholic fatty liver disease & alcoholic liver disease. *Transl Gastroenterol Hepatol*. 2020;5:49.
307. Sookoian S, Pirola CJ. Genetic predisposition in nonalcoholic fatty liver disease. *Clin Mol Hepatol*. 2017;23(1):1-12.
308. Choudhary NS, Duseja A. Genetic and epigenetic disease modifiers: non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD). *Transl Gastroenterol Hepatol*. 2021;6:2.
309. Danford CJ, Yao ZM, Jiang ZG. Non-alcoholic fatty liver disease: a narrative review of genetics. *J Biomed Res*. 2018;32(5):389-400.
310. Dong XC. PNPLA3-A Potential Therapeutic Target for Personalized Treatment of Chronic Liver Disease. *Front Med (Lausanne)*. 2019;6:304.
311. Salari N, Darvishi N, Mansouri K, Ghasemi H, Hosseinian-Far M, Darvishi F, et al. Association between PNPLA3 rs738409 polymorphism and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *BMC Endocr Disord*. 2021;21(1):125.
312. Hoekstra M, Li Z, Kruijt JK, Van Eck M, Van Berkel TJ, Kuiper J. The expression level of non-alcoholic fatty liver disease-related gene PNPLA3 in hepatocytes is highly influenced by hepatic lipid status. *J Hepatol*. 2010;52(2):244-51.
313. 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.
314. Pingitore P, Pirazzi C, Mancina RM, Motta BM, Indiveri C, Pujia A, et al. Recombinant PNPLA3 protein shows triglyceride hydrolase activity and its I148M mutation results in loss of function. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*. 2014;1841(4):574-80.

315. Grimaudo S, Pipitone RM, Pennisi G, Celsa C, Cammà C, Di Marco V, et al. Association Between PNPLA3 rs738409 C>G Variant and Liver-Related Outcomes in Patients With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol*. 2020;18(4):935-44.e3.
316. Holmen OL, Zhang H, Fan Y, Hovelson DH, Schmidt EM, Zhou W, et al. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet*. 2014;46(4):345-51.
317. Prill S, Caddeo A, Baselli G, Jamialahmadi O, Dongiovanni P, Rametta R, et al. The TM6SF2 E167K genetic variant induces lipid biosynthesis and reduces apolipoprotein B secretion in human hepatic 3D spheroids. *Sci Rep*. 2019;9(1):11585.
318. Helsley RN, Varadharajan V, Brown AL, Gromovsky AD, Schugar RC, Ramachandiran I, et al. Obesity-linked suppression of membrane-bound O-acyltransferase 7 (MBOAT7) drives non-alcoholic fatty liver disease. *elife*. 2019;8.
319. Mancina RM, Dongiovanni P, Petta S, Pingitore P, Meroni M, Rametta R, et al. The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent. *Gastroenterology*. 2016;150(5):1219-30.e6.
320. Fougerat A, Montagner A, Loiseau N, Guillou H, Wahli W. Peroxisome Proliferator-Activated Receptors and Their Novel Ligands as Candidates for the Treatment of Non-Alcoholic Fatty Liver Disease. *Cells*. 2020;9(7).
321. Krishnarao A, Punzalan CS. National and Global Ethnicity Differences in Non-alcoholic Fatty Liver Disease. *Curr*. 2020;19(4):429-36.
322. Sherif ZA, Saeed A, Ghavimi S, Nouria SM, Laiyemo AO, Brim H, et al. Global Epidemiology of Nonalcoholic Fatty Liver Disease and Perspectives on US Minority Populations. *Dig Dis Sci*. 2016;61(5):1214-25.
323. Rich NE, Oji S, Mufti AR, Browning JD, Parikh ND, Odewole M, et al. Racial and Ethnic Disparities in Nonalcoholic Fatty Liver Disease Prevalence, Severity, and Outcomes in the United States: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol*. 2018;16(2):198-210.e2.
324. 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.
325. Bonacini M, Kassamali F, Kari S, Lopez Barrera N, Kohla M. Racial differences in prevalence and severity of non-alcoholic fatty liver disease. *World J Hepatol*. 2021;13(7):763-73.
326. Szanto KB, Li J, Cordero P, Oben JA. Ethnic differences and heterogeneity in genetic and metabolic makeup contributing to nonalcoholic fatty liver disease. *Diabetes Metab Syndr Obes*. 2019;12:357-67.
327. Kallwitz ER, Tayo BO, Kuniholm MH, Cai J, Daviglius M, Cooper RS, et al. American Ancestry Is a Risk Factor for Suspected Nonalcoholic Fatty Liver Disease in Hispanic/Latino Adults. *Clin Gastroenterol Hepatol*. 2019;17(11):2301-9.
328. Moore JX, Chaudhary N, Akinyemiju T. Metabolic Syndrome Prevalence by Race/Ethnicity and Sex in the United States, National Health and Nutrition Examination Survey, 1988-2012. *Prev Chronic Dis*. 2017;14:E24.
329. Mancina RM, Dongiovanni P, Petta S, Pingitore P, Meroni M, Rametta R, et al. The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent. *Gastroenterology*. 2016;150(5):1219-30 e6.
330. Bhatt SP, Nigam P, Misra A, Guleria R, Pandey RM, Pasha MA. Genetic variation in the patatin-like phospholipase domain-containing protein-3 (PNPLA-3) gene in Asian Indians with nonalcoholic fatty liver disease. *Metab Syndr Relat Disord*. 2013;11(5):329-35.
331. Bale G, Steffie AU, Ravi Kanth VV, Rao PN, Sharma M, Sasikala M, et al. Regional differences in genetic susceptibility to non-alcoholic liver disease in two distinct Indian ethnicities. *World J Hepatol*. 2017;9(26):1101-7.

332. Pan JJ, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. *World J Hepatol.* 2014;6(5):274-83.
333. August KJ, Sorkin DH. Racial/ethnic disparities in exercise and dietary behaviors of middle-aged and older adults. *J Gen Intern Med.* 2011;26(3):245-50.
334. Kaya E, Yilmaz Y. Non-alcoholic fatty liver disease: A growing public health problem in Turkey. *Turk J Gastroenterol.* 2019;30(10):865-71.
335. Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, et al. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. *Hepatology.* 2019;69(6):2672-82.
336. Liu X, Peng Y, Chen S, Sun Q. An observational study on the association between major dietary patterns and non-alcoholic fatty liver disease in Chinese adolescents. *Medicine (Baltimore).* 2018;97(17):e0576.
337. Noureddin M, Zelber-Sagi S, Wilkens LR, Porcel J, Boushey CJ, Le Marchand L, et al. Diet Associations With Nonalcoholic Fatty Liver Disease in an Ethnically Diverse Population: The Multiethnic Cohort. *Hepatology.* 2020;71(6):1940-52.
338. Walker B, Mays VM, Warren R. The changing landscape for the elimination of racial/ethnic health status disparities. *J Health Care Poor Underserved.* 2004;15(4):506-21.
339. Hill JO, Wyatt HR, Reed GW, Peters JC. Obesity and the environment: where do we go from here? *Science.* 2003;299(5608):853-5.
340. Lear SA, Gasevic D. Ethnicity and Metabolic Syndrome: Implications for Assessment, Management and Prevention. *Nutrients.* 2019;12(1).
341. Templeton AR. Biological races in humans. *Stud Hist Philos Biol Biomed Sci.* 2013;44(3):262-71.
342. Fleischman MW, Budoff M, Zeb I, Li D, Foster T. NAFLD prevalence differs among hispanic subgroups: the Multi-Ethnic Study of Atherosclerosis. *World J Gastroenterol.* 2014;20(17):4987-93.
343. 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.* 2021.
344. Lu FB, Zheng KI, Rios RS, Targher G, Byrne CD, Zheng MH. Global epidemiology of lean non-alcoholic fatty liver disease: A systematic review and meta-analysis. *J Gastroenterol Hepatol.* 2020;35(12):2041-50.

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The following co-authors gave permission for Kiarash Riazi to use the manuscripts in his MSc thesis at the University of Calgary in the Department of Community Health Sciences.

- For manuscript entitled: Dietary patterns and components in nonalcoholic fatty liver disease (NAFLD): What key messages can health care providers offer?
  - Dr. Maitreyi Raman
  - Dr. Lorian Taylor
  - Dr. Mark Swain
  - Dr. Abdel Aziz Shaheen
  
- For manuscript entitled: Worldwide prevalence and incidence of non-alcoholic fatty liver disease (NAFLD) in the 21st century: A systematic review and meta-analysis.
  - Dr. Hassan Azhari
  - Dr. Jacob Charette
  - Fox Underwood
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