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Running Head: IMPACT OF RENAMING NAFLD TO MAFLD

Impact of renaming NAFLD to MAFLD in an Australian regional cohort: Results from a prospective population-based study

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Abbreviations

NAFLD	Non-alcoholic fatty liver disease
MAFLD	Metabolic associated fatty liver disease
FLI	Fatty liver index
BMI	Body Mass Index
ALT	Alanine Transferase
NASH	non-alcoholic steatohepatitis
MetSyn	metabolic syndrome
AST	aspartate aminotransferase
GGT	gamma-glutamyltransferase
ULN	Upper limit of normal
VCTE	vibration controlled transient elastography
LSM	liver stiffness measurement
CAP	controlled attenuated parameter
HOMA-IR	Homeostasis model assessment of insulin resistance
ATP-III	Adult Treatment Panel III

Conflict of interest

The authors declare they have no conflict of interest, perceived or otherwise to declare in the conduct of this research or in the preparation of this manuscript

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ABSTRACT

Background and aims: Clinical and public health implications of the recent re-defining of non-alcoholic fatty liver (NAFLD) to metabolic associated fatty liver (MAFLD) remain unclear. We sought to determine the prevalence and compare MAFLD to NAFLD in a well-defined cohort.

Methods: A cross-sectional study was conducted in regional Victoria with participants from randomly selected households. Demographic and health-related clinical and laboratory data were obtained. Fatty liver was defined as a Fatty Liver Index (FLI)≥ 60 with MAFLD defined according to recent international expert consensus.

Results: 722 participants were included. Mean age was 59.3±16 years and 55.3% were women with a median BMI 27.8 kg/m^2. Most (75.2%) participants were overweight or obese. MAFLD was present in 341 participants giving an unadjusted prevalence of 47.2% compared to a NAFLD prevalence of 38.7%. 59 (17.5%) participants met the criteria of MAFLD but not NAFLD. The increased prevalence of MAFLD in this cohort was primarily driven by dual aetiology of fatty liver. All participants classified as NAFLD met the new definition of MAFLD. Compared to NAFLD subjects, participants with MAFLD had higher ALT (26.0 [14.0] U/L vs 30.0 [23] U/L, p = 0.024) but there were no differences in non-invasive markers for steatosis or fibrosis.

Conclusion: MAFLD is a highly prevalent condition within this large community cohort. Application of the MAFLD definition increased prevalence of fatty liver disease by including people with dual aetiologies of liver disease.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is an umbrella term encompassing a spectrum of disease from simple hepatic steatosis to a potentially progressive liver disease, non-alcoholic steatohepatitis (NASH). Histopathological features of this condition have been recognised since the mid ninetieth century however it was Ludwig in 1980 who introduced the term NASH(1). The prevalence of NAFLD parallels the obesity epidemic with current estimates putting the global prevalence at 24% although there remains significant geographic and ethnic difference in the burden of disease(2). Over time, concepts of NAFLD have evolved and it is now recognised as a complex condition with variable phenotypic expression resulting from the interplay of gender, ethnicity, genetics, environmental and metabolic risk factors(2, 3) and therefore may co-exist with other liver conditions.

Recent international expert consensus has endorsed a nomenclature change from NAFLD to metabolic associated fatty liver disease (MAFLD)(4). This reflects the central role metabolic dysfunction plays in the pathogenesis of this condition as well as recognising that fatty liver and its sequalae can coexist with other liver diseases. Such a change has support of representative patient groups(5) but has not been universally embraced(6-8). Despite this it is appreciated that there is significant overlap between the NAFLD and MAFLD populations(9), however the epidemiological and clinical implications of this important nomenclature change remain to be defined. Recent epidemiological studies on MAFLD indicate a global prevalence of over 50% in overweight or obese adults(10). Those subjects captured by the MAFLD definition but not the NAFLD definition have high rates of metabolic complications thereby underpinning the clinical utility of the MAFLD terminology(11, 12). Despite this, there remain concerns that a proportion of people with significant hepatic steatosis but without metabolic

dysfunction or excessive alcohol consumption are not captured by the MAFLD label and therefore potentially remain unrecognised(7).

There is limited robust data on the prevalence of NAFLD in the Australian community with current estimates extrapolated from international studies(13). Overall about 30% of the Australian population (about 7 million people) live in rural and remote areas and our group has recently reported the prevalence of NAFLD in rural communities in Victoria NAFLD of 38.9%(14) with high prevalence of NAFLD of 50%-80% observed in those with obesity, diabetes, dyslipidaemia and metabolic syndrome (MetSyn). The degree of overlap between NAFLD and MAFLD populations in Australia is unknown and furthermore the epidemiological and clinical relevance of the application of the newly accepted MAFLD definition to the Australian population is yet to be defined.

Therefore, the aim of our study was to use a large prospective, cross-sectional cohort to describe the prevalence of MAFLD in rural and regional Australia and to examine the concordance in patient characteristics between the NAFLD and MAFLD definitions.

Methods

Study design

For this study we used data obtained from the CrossRoads-II dataset which is a large cross sectional epidemiological study across four towns in the Goulburn Valley, a rural region of Victoria, Australia 100-300 km north of metropolitan Melbourne. The CrossRoads-II methods have been published in detail elsewhere(14, 15)(Figure 1.). Briefly, in CrossRoads-II a face-to-face survey was conducted of 1,895/3,122 (60.9%) randomly selected households from residential address lists from local government organisations across four regional towns of population sizes 6,300-49,800 in the Northern

Victoria. Self-reported health, health behaviour and health service information verified and supplemented in a nested sub-study of 1233 randomly selected adult participants in testing clinics conducted across four towns between October 2016 and December 2018. One non-pregnant adult participant (≥18 years) was selected by a computer-generated random number protocol from each participating household to attend the clinic that involved additional health questionnaires related to diet, exercise, smoking and alcohol history, as well biophysical measurements. The latter included anthropometric measurements, blood pressure, oral glucose tolerance testing, and blood tests including liver biochemistry (ie. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT)), serum lipids (ie. total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), trigycerides (TG)), and viral hepatitis B and C serology. The presence or absence of other causes for liver disease were noted from the history. For ALT the upper limit of normal (ULN) was defined 20 U/L for females and 30 U/L for males. In addition, vibration controlled transient elastography (VCTE) with Fibroscan® (Echosens, Paris) was performed when available to assess for asymptomatic liver disease. Both the CrossRoads II study and this sub-study were approved by the Goulburn Valley Health Human Research Ethics Committee

Determination of Hepatic Steatosis

The presence of hepatic steatosis was determined by a fatty liver index (FLI) score ≥60. The FLI score is based on waist circumference, body mass index (BMI), and serum triglyceride and GGT levels (16). This scoring system has been previously validated and used in other epidemiological and population studies on NAFLD (16-20) including publications in relation to the CrossRoads-II study(14) and is endorsed as an appropriate biomarker for detection of steatosis in large epidemiological studies by the international expert consensus statement on MAFLD(21).

Liver stiffness

VCTE (Fibroscan®; models 402, or 530 compact, M- or XL-probes) was performed to determine liver stiffness measurement (LSM) (kPa) and controlled attenuated parameter (CAP) (dB/m) by one of three hepatologists with experience of over 1000 procedures. The liver was localised with a KX5100 portable ultrasound (Kaixin®, China) and used to determine the skin to capsule distance. The XL-probe was used when skin to capsule distance exceeded 20-25mm. CAP was measured with the 530 compact machine when available. Reliable readings of median LSM (kPa) required a minimum of 10 valid readings with ≥ 60% success and a median to interquartile range ratio of <30%. A LSM <7.0 kPa was considered normal while a cut-off <8 kPa was used to exclude advanced fibrosis(22).

Definition of NAFLD

NAFLD was primarily defined in this study as a FLI score ≥60 in the absence of excess alcohol intake (ie. females ≥20 g/d, males ≥30 g/d) and viral hepatitis or other cause of liver disease. Later in the study when the Fibroscan® 530 compact model became available to measure CAP, NAFLD was defined as CAP >302 dB/m (23) in the absence of excess alcohol, viral hepatitis or other cause of liver disease.

Definition of MAFLD

MAFLD was defined in accordance with the international consensus statement(21) as hepatic steatosis in combination with one or more of: (1) overweight/obesity (defined as BMI ≥25 kg/m^2 in Caucasians or BMI ≥23 kg/m^2 in Asian participants; (2) type 2 diabetes mellitus; or (3) two or more markers of metabolic dysregulation including: a)waist circumference ≥102 in Caucasian men and ≥ 88 cm in Caucasian women (or ≥90 in Asian men or ≥80 cm in Asian women); b) Blood pressure

≥130/85 mmHg or on anti-hypertensive treatment; c) Plasma triglycerides ≥1.70 mmol/L or on lipid lowering treatment; d) Plasma HDL-cholesterol <1.0 mmol/L for men and <1.3 mmol/L for women on lipid lowering treatment; e) Prediabetes (i.e. fasting glucose levels 5.6 to 6.9 mmol/L, or 2-hour post-load glucose levels 7.8 to 11.0 mmol or HbA1c 5.7% to 6.4% (39-46 mmol/mol); f) Homeostasis model assessment of insulin resistance (HOMA-IR) score ≥2.5; or g) Plasma high-sensitivity C-reactive protein level >2 mg/L.

Definition of metabolic syndrome

Metabolic syndrome (MetSyn) was defined according to the National Cholesterol Education Program Adult Treatment Panel III (ATP III) criteria (24). Dyslipidaemia was defined according to standard criteria (25). Obesity was defined as a BMI ≥30 kg/m^2. Hypertension was defined as a blood pressure >130 mmHg systolic, and/or 85 mmHg diastolic or requiring anti-hypertensive treatment.

Statistical analysis

Descriptive statistics of the cohort were performed with continuous variables assessed for normality and expressed as mean ± standard deviation (SD) for parametric data, and median and interquartile range [IQR] for nonparametric data. Categorical variables were summarised using frequencies or proportions. Comparison of those with and without MAFLD and NAFLD was made with t-test for normally distributed continuous variables or Wilcoxon rank-sum (Mann-Whitney) test for non-parametric data. Chi-squared test or Fishers exact test used for categorical variables as appropriate. All reported P values are two-tailed and P <0.05 indicated statistical significance. Logistic regression analysis was undertaken to determine the independent variables associated with a diagnosis of steatosis defined by a FLI score ≥60 excluding the four components of the FLI score. Data were entered

into RedCap and analyses were performed using Stata software v14.1 (StataCorp, College Station, TX, USA).

Results

For the nested sub-study, 1233 participants were randomly selected; of these, 741 (60%) attended for clinic review, and 722 (97%) completed all the required questionnaires, clinical, and laboratory evaluations. These patient characteristics are shown in Table 1. Most participants were Caucasian (92.9%), Australian born (85.0%), and overweight or obese (75.2%). There was a slight preponderance of females (55.3%), and the mean age of participants was 59.3±16.0 years. A substantial number of patients had features of metabolic dysregulation, including dyslipidaemia (44.3%), elevated waist circumference (58.5%), diabetes mellitus (9.7%), hypertension (54.9%), and/or the metabolic syndrome (29.9%) (Table 1). Moreover, 101 (14.1%) of the participants reported they consumed alcohol in excess (defined here as ≥20g/day for females, ≥30g/day for males).

Characteristics and comparison of the MAFLD and non-MAFLD cohort

Overall, 341 participants fulfilled the definition of MAFLD giving a prevalence of 47.2%. The characteristics of the MAFLD patients are presented in Table 1; in brief, when compared to non-MAFLD subjects, MAFLD patients were more likely to be male, older, obese, and have clinical and biochemical features of metabolic dysregulation or the metabolic syndrome. Additionally, 57 (16.9%) of the MAFLD cohort reported excess alcohol consumption, compared with 38 (10.3%) of the non-MAFLD cohort ($p = 0.042$). Across all age groups, the prevalence of MAFLD was higher in males than in females (Figure 2). Subgroup analysis by demographic features showed particularly high prevalence of MAFLD in men and in those aged ≥ 60 years, with 59.1% of men aged over 60 years having MAFLD.

Similarly, prevalence rates of over 50% were seen in patients with major metabolic risk factor(s) such as obesity, dyslipidaemia, a history of diabetes, and/or the metabolic syndrome (Table 2).

Compared to the non-MAFLD cohort, biochemical evidence of liver dysfunction was more prevalent in the MAFLD cohort, with a significant difference in both ALT levels (27 [15] U/L vs 20 [11] U/L, $p < 0.001$) as well as the number of patients with an ALT more than 1.5 x ULN (19.7% vs 7.6%, $p < 0.001$). Non-invasive assessment of fibrosis in the 385 patients who underwent VCTE assessment was also consistent with this – the MAFLD subgroup had a significantly higher median LSM (6.4 ± 5.1 kPa vs 5.1 ± 1.8 kPa, $p < 0.001$), and significantly more of the MAFLD group were unable to have advanced liver fibrosis excluded non-invasively as they had LSM measurements ≥ 8.0 kPa (28 (15.5%) vs 13 (6.5%), $p = 0.007$). Of the 72 subjects (18.7%) who had CAP assessed, subjects with MAFLD had significantly higher CAP scores than subjects in the non-MAFLD group (303.8 ± 64.2 dB/m vs 250.5 ± 49.8 dB/m, $p < 0.001$). Furthermore, all subjects with MAFLD who had evidence of hepatic steatosis based on a CAP cut-off of ≥302 dB/m were in the overweight/obese category. Interestingly, the FIB-4 scores between groups were not different ($p = 0.874$), suggesting a lack of utility for FIB-4 calculations when evaluating for fibrosis in a MAFLD patient group.

Comparison between MAFLD and NAFLD

Of the 341 patients meeting MAFLD criteria, 4 did not complete the alcohol questionnaire data and were excluded from the analysis comparing MAFLD and NAFLD. Of the remaining cohort 278 (82.5%) met the criteria for both MAFLD as well as NAFLD. However, a further 59 (17.5%) subjects met the criteria for MAFLD but not NAFLD. There were no patients who met criteria for NAFLD but did not fulfill the MAFLD case definition. Alcohol excess was almost universal (96.6%) in the MAFLD/not-NALFD group but absent in the NAFLD cohort (by definition). In addition, the MAFLD patient group

also had higher HDL-C, higher GGT (47 [62] U/L vs 29 [26] U/L, p < 0.001), and higher ALT (30 [23] U/L vs 26 [14] U/L, p = 0.024) (Table 3). Despite these factors there was no difference in age, gender, BMI or markers of metabolic dysfunction between NAFLD and MAFLD cohorts. Independent variables associated with the presence of steatosis (FLI score ≥60) were determined by logistic regression analysis. In addition to alcohol excess [OR 2.04, 95%CI 1.23–3.38; p=0·005], both fasting blood glucose level ≥5.6mmol/L [OR 1.42, 95%CI 1.12–1.80; p=0·003] and the presence of Metabolic syndrome [OR 4.40, 95%CI 2.76-7.01; p<0·001] were independently associated with the presence of hepatic steatosis whereas HDL was protective [OR 0.22, 95%CI 0.13–0.38; p=0·005]. Of note, there was no differences in the non-invasive markers of hepatic fibrosis (FIB-4 or NAFLD Fibrosis score (NFS)) or steatosis between MAFLD and NAFLD.

Discussion

Metabolic-associated fatty liver disease (MAFLD) is the single largest cause of liver disease world-wide, fuelled by the growing epidemics of both obesity and impaired metabolic function. MAFLD is defined as the presence of hepatic steatosis in the setting of type 2 diabetes mellitus, being overweight/obese, or the presence of at least two markers of metabolic dysfunction(21). Previously, non-alcoholic fatty liver disease (NAFLD) was the preferred nomenclature for an analogous entity – however, recent acknowledgement that hepatic manifestations of lifestyle and/or dysregulated metabolic processes may occur in tandem with alcohol excess or co-exist with other forms of liver disease has led to the reclassification of NAFLD to MAFLD(4). This important change in terminology serves to provide a more holistic approach to patient management as well as directing public health policy while avoiding potentially pejorative terminology such as obesity or even “non-alcoholic” related fatty liver(26).

Modelling suggests that a combination of the aging population and rising diabetes mellitus and obesity rates will result in significant increase in the prevalence of NAFLD/NASH and liver disease morbidity and mortality over the coming decade (27). These variables also underpin the new MAFLD definition and increases in MAFLD rates have already been demonstrated in the USA(28). To that end, an appreciation of current disease burden particularly in Western countries where obesity rates are high is imperative. There is a paucity of data regarding the true prevalence of fatty liver disease in Australia(13) and no data on the prevalence of MAFLD. Our group has previously demonstrated the high unadjusted and age- and sex-adjusted prevalence rates of NAFLD in regional Victoria of 39% and 35% respectively(14). Of significant public health concern, this current study found a high prevalence of overweight/obesity (75%) and of the metabolic syndrome (almost 30%). In parallel with these findings was the high prevalence of MAFLD of 47.2% which was significantly greater than the NAFLD prevalence of 38.7% within the same cohort. This is primarily related to reclassification of subjects with dual aetiology of alcohol and fatty liver. While some studies have found no significant difference in disease prevalence between NAFLD and MAFLD(11, 29) others have seen a significant increase in disease prevalence with application of the MAFLD definition(30). Consistent with our findings is a recent study on global prevalence of MAFLD amongst overweight or obese subjects where the prevalence of MAFLD was 50.7% based on a meta-analysis of 116 studies and over 2.6 million participants(10).

A concern raised by the transition of terminology from NAFLD to MAFLD is that a subgroup of patients with the potential for progressive liver disease will not be captured under the MAFLD definition. In particular, this subgroup includes lean NAFLD without diabetes mellitus or metabolic dysregulation. Of reassurance therefore is the finding of our study that in a cohort of 722 adult subjects all NAFLD subjects were also classified as MAFLD. Our study used the FLI score algorithm incorporating body

mass index (BMI), waist circumference, gamma-glutamyl transferase (GGT), and triglyceride (TG) levels to diagnosis hepatic steatosis. As the FLI score incorporates both waist circumference and BMI, it may result in bias against the detection of lean NAFLD. However, using the alternative definition of NAFLD with a CAP ≥302 dB/m to define hepatic steatosis there were no cases of lean or normal weight NAFLD or MAFLD. Furthermore, an additional 59 participants were also captured under the MAFLD definition thereby highlighting the more inclusive nature of the MAFLD definition. The dominant factor excluding these participants from meeting a definition of NAFLD was the presence of significant alcohol intake. Indeed, logistic regression analysis indicated that alcohol excess, in addition to the metabolic syndrome and raised FGTT were independent predictors of hepatic steatosis defined by the FLI score whereas HDL was protective against hepatic steatosis. Previous cohort studies including participants of various ethnicities have found a relatively modest 1-5% people with NAFLD do not fulfill the MAFLD definition (11, 29-32). It is possible that these non-MAFLD patients with fatty liver maybe too young to have developed a metabolic disorder and thus would not be diagnosed with MAFLD yet still be subject to the consequence of metabolic dysfunction(7). Aligned with this concept is the findings from a Hong Kong study(29) demonstrating MAFLD classified fewer young patients (especially men younger than 40 years) as MAFLD compared to NAFLD and of further reassurance, subjects who fulfilled the definition of NAFLD but not MAFLD, all had no or mild metabolic conditions. Furthermore, consistent with our study, Zheng and colleagues(31) also demonstrated that factors often associated with fibrosis progression including age, gender, comorbid diseases (i.e. diabetes mellitus and hypertension), variant distribution of the PNPLA3 gene, and metabolic profile were not significantly different between NAFLD and MAFLD individuals. While our study demonstrated high degrees of concordance in non-invasive markers of liver disease, Yamamura (33) and colleagues demonstrated that the MAFLD definition better identifies a group with fatty liver and significant fibrosis evaluated

by non-invasive markers amongst a Japanese cohort. A recent study by Younes et al(34) found lean patients with NAFLD were younger, with male preponderance and a lower prevalence of diabetes and similar distribution of PNLA3. Furthermore, their histological severity of steatosis, lobular inflammation, ballooning and fibrosis was lower. Despite this, the incidence of liver decompensation and HCC development as well as non-hepatic outcomes such as diabetes and cardiovascular events were all numerically lower (but not statistically significant) in lean patients than non-lean patients. This data does highlight that lean NAFLD is not a benign condition but underpins the importance of additional longitudinal data to determine if the small yet potentially significant cohort of subjects with non-MAFLD NAFLD are at increased risk of liver disease progression.

The impact of the high prevalence of MAFLD in rural communities on clinical endpoints remains to be determined. However previous data from Melbourne, Australia indicated the NAFLD was the dominant risk factor for HCC in 14% of cases(35). Furthermore, Australian Bureau of Statistics data indicates Australians living in regional communities are more likely to die from coronary artery disease (44%) or stroke (31%) than those living in major cities. A recent publication group comparing the prevalence of diabetes in rural communities from 2001–2003 (Crossroads) and 2016–2018 (Crossroads-II) indicated the age standardised prevalence of diagnosed diabetes increased from 5.0(4.4–5.7)% to 7.7(6.7–8.6)%(36). These data highlight the importance of NAFLD/MAFLD and its risk factors within rural communities and the wider Australian population.

It is important to note our study is not without limitations. Participants from this study were from four townships in regional and rural Victoria and of limited ethnic diversity with few Aboriginal and Torres Strait Islander people. This provides the opportunity to study the epidemiology of MAFLD in a predominantly Caucasian Australian population. However, whilst approximately 30% of Australian

residents live in regional/remote communities, these data cannot be extrapolated to other rural or metropolitan communities. Furthermore, the study sample size was powered for the main study and this subgroup analysis is potentially subject to a type II error. In addition, there were insufficient numbers of participants to compare subgroups of MAFLD subjects. Finally, as previous highlighted, the design of the CrossRoads-II study is subject to selection bias as subjects with concern about their health are more likely to accept invitations to participate in health check-ups, although our reasonable (>60%) participation rate mitigates this risk somewhat.

In conclusion, this cross-sectional epidemiological study of participants from regional Victoria, Australia demonstrates a high prevalence of MAFLD paralleling high rates of overweight/obesity and metabolic risk factors. Furthermore, the recently endorsed MAFLD definition captures all subjects previously diagnosed with NAFLD but also captures additional subject with dual liver disease aetiologies. Our findings support the adoption of MAFLD as the preferred terminology for fatty liver disease due to its recognition of the key pathogenesis of fatty liver and the inclusive nature of the definition due to its compatibility to co-exist with other liver disease.

Table 1. Participant characteristics of CrossRoads II study.

Characteristics	Overall (n = 722)	MAFLD (n = 341)	Not MAFLD (n = 381)	*P*[*]
Demographics				
Gender: male, n (%)	323 (44.7%)	177 (51.9%)	146 (38.3%)	**< 0.001**
Age, years	59.3 (± 16.0)	61.2 (± 15.0)	57.6 (± 16.8)	**0.003**
Australian born, n (%) [n = 721]	613 (85.0%)	288 (84.6%)	324 (85.3%)	0.834
Ethnicity, n (%) [n = 707]				0.336
Caucasian	665 (92.9%)	320 (93.8%)	345 (92.0%)	
Asian	30 (4.2%)	10 (2.9%)	20 (5.3%)	
Aboriginal or Torres Strait Islander	6 (0.9%)	2 (0.6%)	4 (1.1%)	
Other	15 (2.1%)	9 (2.6%)	6 (1.6%)	
Clinical features				
Weight, kg	80.5 [22.4]	94.3 (± 17.3)	71.0 (± 12.0)	**< 0.001**
BMI, kg/m^2	27.8 [7.3]	33.1 (± 5.8)	25.2 (± 3.3)	**< 0.001**
BMI category, n (%)				**< 0.001**
Underweight/normal (<25)	179 (24.8%)	9 (2.6%)	174 (45.7%)	
Overweight (25 - <30)	286 (39.6%)	101 (29.6%)	182 (47.8%)	
Obese (≥30)	257 (35.6%)	231 (67.7%)	25 (6.6%)	
Waist circumference, cm	98.5 [18.9]	108 [12.0]	90 [13.5]	**< 0.001**
High waist circumference[†], n (%)	422 (58.5%)	297 (87.1%)	125 (32.8%)	**< 0.001**
Hypertension, n (%) [n = 718]	394 (54.9%)	222 (65.5%)	172 (45.4%)	**< 0.001**
Dyslipidaemia, n (%)	320 (44.3%)	212 (62.4%)	108 (28.3%)	**< 0.001**
Diabetes Mellitus, n (%) [n = 699]	68 (9.7%)	52 (15.8%)	16 (4.3%)	**< 0.001**
Alcohol excess[‡], n (%) [n = 717]	101 (14.1%)	57 (16.9%)	44 (11.6%)	**0.040**
Metabolic Syndrome, n (%) [n = 705]	211 (29.9%)	173 (51.5%)	38 (10.3%)	**< 0.001**
Laboratory features				
ALT, U/L	22.5 [14]	27 [15]	20 [11]	**< 0.001**
ALT > 1.5 x ULN, U/L	96 (13.3%)	67 (19.7%)	29 (7.6%)	**< 0.001**
γ-Glutamyltransferase, mean U/L	22 [21]	31 [30]	17 [11]	**< 0.001**
Fasting glucose, mmol/L [n = 721]	5.1 [0.8]	5.3 [1.0]	4.9 [0.6]	**< 0.001**
Fasting glucose ≥ 5.6 mmol/L, n (%) [n = 721]	155 (21.5%)	115 (33.8%)	41 (10.5%)	**< 0.001**
HbA1c, %	5.3 [0.55]	5.5% [0.73]	5.2% [0.46]	**< 0.001**
Total cholesterol, mmol/L	4.9 [1.4]	4.8 [1.5]	5.0 [1.3]	0.180
LDL-Cholesterol, mmol/L [n = 713]	2.8 [1.3]	2.8 [1.3]	2.8 [1.3]	0.114
HDL-Cholesterol, mmol/L	1.4 [0.57]	1.2 [0.45]	1.5 [0.53]	**< 0.001**
Triglycerides, mmol/L	1.2 [0.8]	1.6 [1.0]	1.0 [0.5]	**< 0.001**
Low HDL-Cholesterol level[§], n (%)	187 (25.9%)	139 (40.8%)	48 (12.6%)	**< 0.001**
Triglycerides ≥ 1.7 mmol/L, n (%)	198 (27.4%)	151 (44.3%)	47 (12.3%)	**< 0.001**
Non-invasive testing				
FibroScan - Median LSM, kPA [n = 385]	5.7 (± 3.8)	6.4 (± 5.1)	5.1 (± 1.8)	**< 0.001**
FibroScan - LSM < 7 kPa, n (%) [n = 385]	318 (82.6%)	144 (77.4%)	174 (87.4%)	**0.010**
FibroScan - LSM ≥ 8 kPa, n (%) [n= 385]	41 (10.7%)	28 (15.5%)	13 (6.5%)	**0.007**
CAP value, dB/m [n = 72]	274.1 (± 62.2)	303.8 (± 64.2)	250.5 (± 49.8)	**< 0.001**

CAP value > 302 dB/m, n (%) [n = 72]	25 (34.7%)	20 (62.5%)	5 (12.5%)	**< 0.001**
Fatty Liver Index (FLI), units	53.6 (± 30.9)	81.1 (± 12.7)	28.2 (± 17.3)	**< 0.001**
FIB-4, units [total = 659]	1.3 (± 0.7)	1.3 (± 0.7)	1.3 (± 0.7)	0.874

Continuous data expressed as mean (±SD) or median [IQR] unless otherwise stated.* Comparison MAFLD vs not MAFLD; MAFLD= Metabolic associated fatty liver disease; BMI= Body mass index; ALT = Alanine Transferase; LDL = low density lipoprotein; HDL = high density lipoprotein; FLI= Fatty liver index; SD = standard deviation. †Women, > 88 cm; men, > 102 cm. ‡ Women, ≥ 20 g/day; men, ≥ 30 g/day. § Women, < 1.3 mmol/L; men, < 1.04 mmol/L.

Table 2: Prevalence of MAFLD according to risk factors stratified by gender and age group.

Risk Factor	Male		Female		Overall					
	< 60 years (n = 120)	**≥ 60 years** (n = 203)	**< 60 years** (n = 189)	**≥ 60 years** (n = 210)	**< 60 years** (n = 309)	**95% CI** (n)	**≥ 60 years** (n = 413)	**95% CI** (n)	*P*	*P**
BMI, kg/m², n (%)									< 0.001	< 0.001
< 25	0/29 (0.0%)	5/37 (13.5%)	1/59 (1.7%)	3/58 (5.2%)	1/88 (1.1%)	0.03 - 5.42	8/95 (8.4%)	3.52 - 15.12		
25-29	24/57 (42.1%)	53/103 (51.5%)	7/54 (13.0%)	17/69 (24.6%)	31/111 (27.9%)	22.01 - 41.34	70/172 (40.7%)	57.25 - 83.3		
≥ 30	33/34 (97.1%)	62/63 (98.4%)	65/76 (85.5%)	71/83 (85.5%)	98/110 (89.1%)	89.89 - 103.66	133/146 (91.1%)	124.47 - 138.95		
Diabetes, n (%)									< 0.001	0.005
Yes	12/13 (92.3%)	26/36 (72.2%)	7/11 (63.6%)	20/33 (60.6%)	19/24 (79.1%)	13.88 - 22.29	46/69 (66.7%)	37.46 - 53.52		
No	45/107 (42.1%)	94/167 (56.3%)	66/178 (37.1%)	71/177 (40.1%)	111/285 (39.0%)	94.77 - 127.89	165/344 (48.0%)	146.47 - 183.65		
Hypertension, n (%)									< 0.001	0.02
Yes	39/65 (60.0%)	91/140 (65.0%)	37/66 (56.1%)	55/123 (44.7%)	76/131 (58.0%)	64.30 - 87.22	146/263 (55.5%)	129.95 - 161.59		
No	18/55 (32.7%)	29/62 (46.8%)	34/120 (28.3%)	36/87 (41.4%)	52/175 (29.7%)	40.35 - 64.88	65/149 (43.6%)	52.93 - 77.45		
Dyslipidaemia, n (%)									< 0.001	< 0.001
Yes	42/56 (75.0%)	64/91 (70.3%)	47/83 (56.6%)	59/90 (65.6%)	89/139 (64.0%)	77.09 - 100.06	123/181 (68.0%)	109.74 - 135.18		
No	15/64 (23.4%)	56/112 (50.0%)	26/106 (24.5%)	32/120 (26.7%)	41/170 (24.1%)	30.43 - 53.15	88/232 (37.9%)	73.46 - 103.27		
MetSyn, n (%)									< 0.001	< 0.001
Yes	29/30 (96.7%)	60/72 (83.3%)	30/40 (75.0%)	54/69 (78.3%)	59/70 (84.3%)	51.53 - 64.32	114/140 (80.9%)	103.58 - 122.49		
No	27/87 (31.0%)	57/123 (46.3%)	42/145 (29.0%)	37/139 (26.6%)	69/232 (29.7%)	55.53 - 83.69	94/262 (35.9%)	78.78 - 110.07		

†Metabolic syndrome (MetSyn) was defined according to the National Cholesterol Education Program Adult Treatment Panel III (ATP III) criteria(24); ‡Dyslipidaemia was defined according to standard criteria(25); §Hypertension was defined as a blood pressure >130 mmHg systolic, and/or 85 mmHg diastolic or requiring anti-hypertensive treatment. BMI = body mass index; numbers in brackets represent percentages. 95% CI = 95% confidence intervals for estimation of prevalence. *P* = comparison of MAFLD prevalence among those < 60 years in overall cohort (Chi-square). *P** = comparison of MAFLD prevalence among those ≥ 60 years in overall cohort (Chi-square)

Table 3. Comparison on MAFLD & NAFLD versus MAFLD not NAFLD cohorts

Characteristics	Fatty Liver Disease (n = 337)	MAFLD & NAFLD (n = 278)	MAFLD not NAFLD (n = 59)	*P*[*]
Demographics				
Gender: male, n (%)	174 (51.6%)	138 (49.6%)	36 (61.0%)	0.112
Age, years	61.2 (± 15.1)	61.4 (± 15.4)	60.1 (± 13.6)	0.546
Australian born, n (%)	286 (84.9%)	236 (84.9%)	50 (84.8%)	0.977
Ethnicity, n (%) [n = 334]				0.769
Caucasian	314 (94.0%)	258 (93.8%)	56 (94.9%)	
Asian	10 (3.0%)	9 (3.3%)	1 (1.7%)	
Aboriginal or Torres Strait Islander	2 (0.6%)	2 (0.7%)	0 (0.0%)	
Other	8 (2.4%)	6 (2.2%)	2 (3.4%)	
Clinical features				
Weight, kg	91.6 [18.0]	91.2 [17.9]	92.6 [18.2]	0.858
BMI, kg/m^2	32.4 [6.6]	32.6 [7.0]	31.1 [5.3]	0.101
BMI category, n (%)				0.307
Underweight/normal (<25)	8 (2.3%)	5 (1.8%)	3 (5.1%)	
Overweight (25 - <30)	100 (29.7%)	82 (29.5%)	18 (30.5%)	
Obese (≥ 30)	229 (68.0%)	191 (68.7%)	38 (64.4%)	
Waist circumference, cm	108 [12.0]	108 [12.0]	109 [12.0]	0.989
High waist circumference[†], n (%)	295 (87.5%)	244 (87.8%)	51 (86.4%)	0.779
Hypertension, n (%) [n = 335]	219 (65.3%)	182 (65.7%)	37 (63.8%)	0.781
Dyslipidaemia, n (%)	210 (62.3%)	175 (63.0%)	35 (59.3%)	0.602
Diabetes, n (%)	51 (15.1%)	42 (15.1%)	9 (15.3%)	0.977
Alcohol excess[‡], n (%) [n = 336]	58 (17.3%)	0 (0.0%)	56 (96.6%)	**< 0.001**
Metabolic Syndrome, n (%) [n = 332]	171 (51.5%)	145 (52.9%)	26 (44.8%)	0.263
Laboratory features				
ALT, U/L	27 [15]	26 [14]	30 [23]	**0.024**
ALT > 1.5 x ULN, U/L	65 (19.3%)	49 (17.6%)	16 (27.1%)	0.093
γ-Glutamyltransferase, mean U/L	31 [30]	29 [26]	47 [62]	**< 0.001**
Fasting glucose, mmolL [n = 336]	5.3 [1.0]	5.3 [1.0]	5.4 [1.0]	0.237
Fasting glucose ≥ 5.6 mmol/L, n (%) [n = 336]	113 (33.6%)	92 (33.2%)	21 (35.6%)	0.725
HbA1c, mean %	5.5 [0.7]	5.5 [0.7]	5.4 [0.6]	0.375
Total cholesterol, mmol/L	4.8 [1.5]	4.8 [1.5]	4.9 [1.7]	0.362
LDL-Cholesterol, mmol/L [n = 328]	2.8 [1.3]	2.7 [1.3]	2.8 [1.5]	0.833
HDL-Cholesterol, mmol/L	1.2 [0.5]	1.2 [0.4]	1.3 [0.6]	**0.013**
Triglycerides, mmol/L	1.6 [0.9]	1.6 [1.0]	1.5 [0.9]	0.602
Low HDL-Cholesterol level[§], n (%)	138 (41.0%)	123 (44.2%)	15 (25.4%)	**0.008**
Triglycerides ≥ 1.7 mmol/L, n (%)	149 (44.2%)	123 (44.2%)	26 (44.1%)	0.980
Non-invasive testing				
FibroScan LSM, kPa [n= 185]	6.4 (± 5.1)	6.5 (± 5.5)	6.1 (± 2.9)	0.687
LSM < 7 kPa, n (%) [n= 185]	144 (77.8%)	116 (79.5%)	28 (71.8%)	0.306
LSM ≥ 8 kPa, n (%) [n= 185]	27 (14.6%)	18 (12.3%)	9 (23.1%)	0.091

CAP value, dB/m [n = 32]	303.8 (± 64.2)	300.0 (± 66.8)	330.0 (± 36.8)	0.871
CAP value > 302 dB/m, n (%) [n = 32]	20 (62.5%)	17 (60.7%)	3 (75%)	0.581
Fatty Liver Index (FLI), units	82.2 (± 12.0)	81.7 (± 12.3)	84.6 (± 10.4)	0.088
FIB4, units [total = 300]	1.3 (± 0.7)	1.3 (± 0.6)	1.4 (± 0.8)	0.497

Continuous data expressed as mean (±SD) or median [IQR] unless otherwise stated.* Comparison MAFLD & NAFLD vs MAFLD not NALFD; MAFLD= Metabolic associated fatty liver disease; BMI= Body mass index; ALT = Alanine Transferase; LDL = low density lipoprotein; HDL = high density lipoprotein; FLI= Fatty liver index; SD = standard deviation. †Women, > 88 cm; men, > 102 cm. ‡ Women, ≥ 20 g/day; men, ≥ 30 g/day. § Women, < 1.3 mmol/L; men, < 1.04 mmol/L.

Figure 1: Study participant selection process

Figure 2. Proportion (%) of study participants with MAFLD stratified by age group and gender

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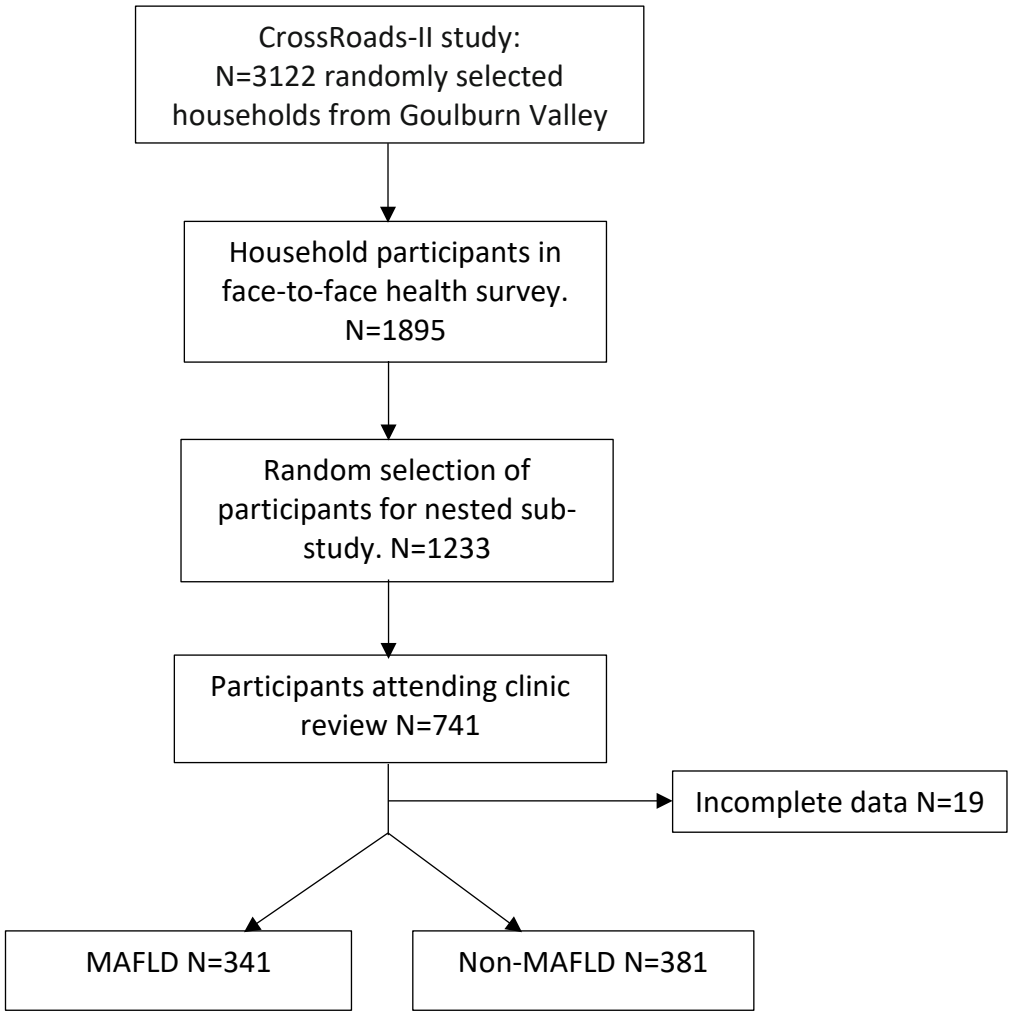
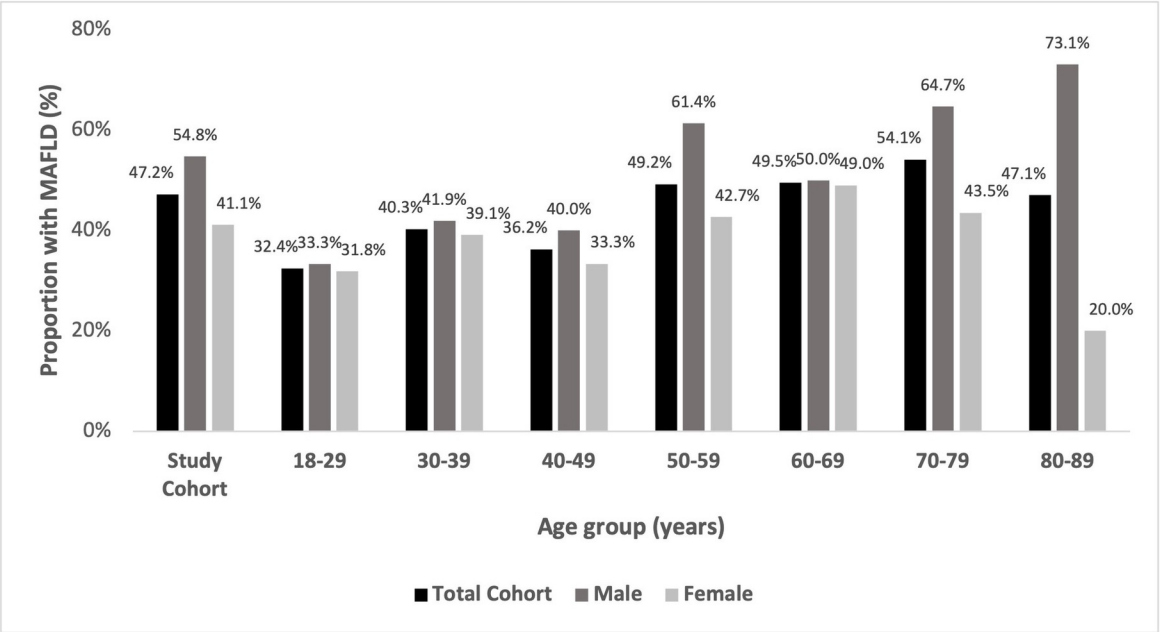
Figure 1: Study participant selection process





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