WHAT YOU NEED TO KNOW:

BACKGROUND

The association of non-alcoholic fatty liver disease (NAFLD) with dietary factors is well established, but the role of specific nutritional components in the presence and severity of NAFLD has not been clarified.

FINDINGS

In our meta-analysis, we found that daily total caloric intake was significantly higher in NAFLD compared to healthy individuals.

There was no difference between NAFLD and healthy individuals in the daily intake of macronutrients (protein, fat and carbohydrate) as proportion of their total caloric intake.

There was no difference between NAFLD and healthy individuals in the daily intake of vitamins E, A and C.

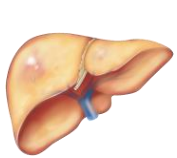
IMPLICATIONS FOR PATIENT CARE

NAFLD patients should pursue hypocaloric diets with no particular nutrient restrictions

Graphical abstract

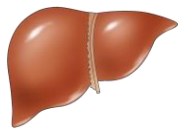
Systematic review and meta-analysis: The role of diet in NAFLD

Literature search 1985-2021: 19,891 studies → 60 studies included (100,621 patients)

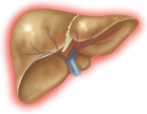


NAFLD patients

Higher daily total caloric intake (n=34 studies)

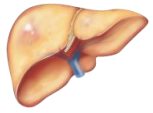


Healthy controls

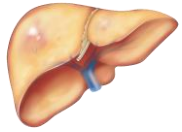


NASH patients

No significant difference in daily intake of total calories (n=5 studies) **or any macro-/micro-nutrient** (n=4-5 studies)



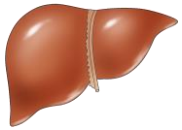
NAFLD patients



NAFLD patients

No significant difference in daily intake of

- Proteins (n=23 studies)
- Fat (n=12-28 studies) (total, saturated, MUFA, PUFA)
- Carbohydrates (total, fibre) (n=17-26 studies) & soft drinks (n=4)
- Vitamins E, A & C (n=7-9 studies)
- Caffeine (n=4 studies)



Healthy controls

Clinical Gastroenterology and Hepatology

Systematic review and meta-analysis: The role of diet in the development of non-alcoholic fatty liver disease

Short running title: **Association of diet with NAFLD**

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Specific author contributions: Elena Tsompanaki: systematic review of literature, data extraction and original manuscript drafting; Kessarin Thanapirom: systematic review of literature, data extraction, statistical analysis/metanalysis and original manuscript writing; Margarita Papatheodoridi: manuscript writing and revisions; Pathik Parikh: drafting the manuscript, revising for important intellectual concepts; Yasmin Chotai de Lima: drafting the manuscript, revising for important intellectual concepts; Emmanuel A. Tsochatzis: conception and design of the study, analysis and interpretation of the data, manuscript drafting and revisions for important intellectual content.

Statement for data availability: Data for this study will be available from the corresponding author after publication upon reasonable request.

ABSTRACT

Background: The association of non-alcoholic fatty liver disease (NAFLD) with dietary factors is well established but not thoroughly investigated. This systematic review and meta-analysis synthesizes available evidence regarding the effect of nutrition on the presence and severity of NAFLD.

Methods: A literature search was conducted identifying studies published between January 1985 and May 2021. We included studies with a dietary assessment and anthropometry based on validated tools, performed by a qualified dietitian or a trained health professional. We examined differences between NAFLD patients and healthy controls as well as patients with NAFLD and non-alcoholic steatohepatitis (NASH). Risk of bias was assessed with the Robin-I tool.

Results: There were 60 eligible studies with 100,621 patients. The risk of bias was moderate for the majority of studies (41/60, 68%). According to meta-analyses, total caloric intake was higher in NAFLD patients compared to controls (MD=78.08, 95%CI:41.03-115.13). Macronutrient (protein, fat and carbohydrate) consumption as proportion of total caloric intake and daily intake of fibre and vitamins E, A and C did not significantly differ between NAFLD and controls. Soft drink consumption had a trend towards association with the presence of NAFLD. However, the odds ratio was 4.4 and the confidence intervals very wide. Finally, there was no significant difference in any comparison between patients with NAFLD and NASH, however the number of patients was relatively small. All meta-analyses had significant heterogeneity.

Conclusions: Overall, despite high heterogeneity among studies, this meta-analysis demonstrated that higher caloric intake is positively associated with

NAFLD, while diet composition in macronutrients was not associated with the presence or severity of disease.

Keywords: Carbohydrates; protein; fatty acids; fructose; obesity; metabolic syndrome

Approximately 1 in 4 individuals worldwide has non-alcoholic fatty liver disease (NAFLD)[1], while non-alcoholic steatohepatitis (NASH), characterised by inflammation and ballooning, has a lower prevalence (5-10%) and may progress to fibrosis and cirrhosis[2]. Yet, no specific drug therapy for NAFLD is approved, despite numerous undergoing clinical trials; hence, lifestyle and dietary interventions are crucial in NAFLD management.

Hypocaloric diet with or without physical activity leading to weight loss improves hepatic steatosis, inflammation and fibrosis. Weight loss (5-7% of body weight) can improve necroinflammation[3], while weight loss >10% may lead to reversal of steatohepatitis and improvement of fibrosis[4]. However, the effect of certain diets or specific dietary patterns on NAFLD presence or severity is not clarified[5]. Current recommendations include non-specific hypocaloric diet modifications and physical activity. Given that weight loss and caloric deficit may be challenging not only to achieve but also to maintain[6], qualitative dietary aspects and specific nutritional components regarding NAFLD presence or progression should be considered in order to tailor dietary advice.

We therefore performed a systematic review to assess the role of nutrition in NAFLD development and severity and specifically the effect of macronutrients, micronutrients, food groups and dietary patterns.

METHODS

This systematic review was performed according to the PRISMA statement[7] (**Supplementary file "PRISMA Consort Checklist**").

Information sources

A comprehensive literature search was conducted, from January 1985 to May 2021 in EMBASE, Medline (Ovid version) and Web of Science databases (**Supplementary Appendix 1)**. The search was restricted to studies in humans and in English language. Reference lists of included studies were cross-searched for additional relevant studies.

Inclusion criteria

We included observational studies (prospective, cross-sectional, case-control studies and surveys), assessing the effect of nutrition on NAFLD in adults. We only included studies published as full text articles, with patients diagnosed with NAFLD either by imaging or histology and alcohol consumption <21 or <14 units/week for men or women respectively, for at least 2 years prior to study inclusion. Also, we included studies with a dietary assessment and anthropometry based on validated, internationally accepted tools, performed by a qualified dietitian or a trained health professional. We excluded studies that included patients with steatogenic medication use, or history of liver disease from other causes, and where the diagnosis of NAFLD was based solely on aminotransferase abnormalities without available imaging or histology.

Study selection and data extraction

Studies were selected by two independent authors (ET and KT). Data were extracted by these authors using a pre-specified form including study design, inclusion and exclusion criteria, number of participants, ethnicity, age, gender, body mass index (BMI), method of nutritional assessment and NAFLD/NASH diagnosis, type of nutrients investigated, and study limitations as free text. Any differences were arbitrated and resolved by EAT.

Risk of bias assessment

Risk of bias for each study was evaluated using the ROBIN-I tool[8] by two independent authors (ET and KT). This tool includes 7 domains classifying studies as low, moderate, serious or critical risk of bias. According to the ROBINS-I guidance, if a study is ranked low in all domains, it is considered low risk of bias; if it is ranked moderate, serious or critical in at least one domain, it is considered moderate, serious or critical risk of bias, respectively. (**Suppl. Appendix 1)**.

Evidence synthesis

All data were combined in a systematic review and, where appropriate, in meta-analyses. Three was the minimum number of studies for quantitative synthesis of data in a meta-analysis. Analysis was performed with random effect models because extracted data was expected to be heterogeneous due to relatively small sample sizes and study design diversity. Heterogeneity was assessed quantitatively using the I^2 statistic, with I^2 values >50% considered as highly heterogenous.

Unadjusted data were entered in all meta-analyses. Comparisons of nutrient intake between NAFLD patients and healthy controls was made by the method of Hedges[9] and presented as mean differences (MD) with 95% Confidence Intervals (95%CI). Forest plots were created for each comparison. We further compared nutrient intake

between patients with biopsy-proven NAFLD and NASH. Data regarding soft drink consumption was scarce; hence, we decided to include all studies with available data and examine consumption as a qualitative variable. Pooled odds ratios (OR) with 95%CI of having any soft drink consumption vs. no consumption were presented in forest plots. Meta-regression analysis was performed to assess the relation between BMI and the presence of NAFLD. Finally, we performed sensitivity analyses for case-control studies and cross-sectional studies separately. All p-values were two-tailed with statistical significance set at 0.05. All analyses were performed with the use of Review Manager 5.3 (Copenhagen, Denmark).

RESULTS

In total, 19,891 studies were identified by the search strategy; 60 of them, including 100,621 patients, were eligible and included in the systematic review (**Suppl. Figure 1**). The main characteristics of all studies are summarized in **Suppl. Table 1**. Overall risk of bias was moderate for the majority of the studies (41/60, 68%), and serious for the rest (19/60, 32%) (**Suppl. Table 2)**.

1. Total Caloric Intake

Total caloric intake was evaluated in 34 studies[10-43] (18,463 NAFLD patients and 64,059 healthy controls) and was significantly higher in NAFLD patients compared to controls (MD=78.08, 95%CI: 41.03, 115.13, p<0.001) with significant heterogeneity (I^2=93%, P<0.001) (**Figure 1A)**. There was no significant difference in the caloric intake between patients with NASH and NAFLD (MD=-178.79, 95%CI: -366.44, 8.85, p=0.06; I^2=81%, p<0.001), although available data were limited in 4 studies[22,42,44-46] (622 NASH and 614 NAFLD patients) (**Figure 1B**).

2. Macronutrients

The mean difference in the daily intake of macronutrients, including proteins, fat and carbohydrates, as the percentage of daily caloric intake was assessed between NAFLD patients and healthy individuals and NASH vs. NAFLD. Data on the difference in the absolute intake of macronutrients are presented in **Suppl. Figure 2**.

2.1 Proteins

Protein intake has been investigated in 23 studies[8,12-14,16,17,23,24,26-28,32,35,36,39,42,47-53] (4,431 NAFLD patients vs. 7,145 controls) and the percentage of protein in total

caloric intake did not differ between NAFLD patients and controls (MD=0.09; 95%CI:-0.45, 0.64, p=0.73; I^2=96%, p<0.001 **Figure 2A)**[10-14,19-21,24-30,33-36,39,41,42,54]**.** Similarly, there was no difference in protein intake between patients with NAFLD and NASH (4 studies, 104 NASH vs. 105 patients with NAFLD, **Figure 2B**) (MD=-0.32; 95%CI: -0.97, 0.33, p=0.33; I^2=21%, p=0.28)[42,45,46,55].

2.2 Fat

Twenty-eight studies evaluated the effect of total fat and/or various types of fatty acids i.e. saturated, polyunsaturated [PUFA], monounsaturated [MUFA] and cholesterol on NAFLD patients.

There was no significant difference between NAFLD and controls in the consumption of total fat (MD=0.29, 95%CI: -0.26, 0.84, p=0.31; I^2=95%, p<0.001) (28 studies, 12,327 NAFLD patients vs. 33,377 controls)[10-14, 19-21, 24-30, 32-42, 54, 56 **(Figure 3A**), saturated fat (MD=0.03; 95%CI: -0.45, 0.52, p=0.89; I^2=92%, p<0.001) (14 studies, 5,381 NAFLD patients vs. 9,183 controls)[12,13,19-21,25,29,30,32,38,39,42,54,56 **Figure 3B**), PUFA and MUFA (for PUFA: MD=0.21; 95%CI: -0.17, 0.58, p=0.28; I^2=90%, p<0.0001, 12 studies, 3,654 NAFLD patients vs. 5,673 controls[12,13,17,19-21,25,29,30,39,42,56, **Figure 3C** and for MUFA: MD= 0.06; 95%CI: -0.47, 0.60, p=0.82; I^2=86%, p<0.001, 12 studies, 4,852 NAFLD patients vs. 4,465 controls[12,13, 17,19-21,25,29,30,39,42,56, **Figure 3D**) as percentage of total caloric intake. Total cholesterol intake did not differ between NAFLD and healthy controls, either (**Suppl. Figure 3**)

Total fat intake did not differ between patients with NASH and NAFLD (MD=0.57; 95%CI: -2.50, 3.64, p=0.72; I^2=75%, p<0.001) (5 studies, 148 NASH patients vs. 169 patients with NAFLD, **Figure 3E**)[42,44-46,55].

2.3. Carbohydrates

Twenty-six studies[10-14,19-21,24-30,32-36,39-42,54,56] investigated the association of carbohydrate intake and NAFLD. The percentage of caloric intake consumed as carbohydrates was not different in NAFLD patients compared to controls (MD=-0.09; 95%CI: -0.54, 0.37, p=0.71; I^2=75%, p<0.001) (9,298 NAFLD patients vs. 27,466 controls, **Figure 4A**)[10-14,19-21,24-30,32-36,39-42,54,56]. There was no difference in carbohydrate intake between patients with NAFLD and NASH (MD=0.83; 95%CI: -3.06, 7.72, p=0.68; I^2=83%, p<0.001) (5 studies, 148 NASH vs. 169 NAFLD patients, **Figure 4B**)[42,44-46,55]. Absolute fibre intake [11,13,19,25-27,31,32,43,45,52,55,57-61] had no effect in NAFLD presence (**Suppl. Figure 4).** Soft drink intake[11,14,52,62] had a trend towards association with NAFLD, however the OR was 4.4 and the confidence interval very wide (95% CI:0.8-24) (**Suppl. Figure 5**).

In individual studies, fructose intake was significantly higher in patients with NAFLD in one study[25], while other 2 large studies showed association of NAFLD with fructose-rich fruits (OR=1.45,p=0.005)[39] and fruit-juice intake (OR=1.07; 95%CI:1.01-1.13)[49] respectively. However, data was insufficient to perform a meta-analysis on fructose intake.

3. Micronutrients

Among micronutrients, meta-analytic comparisons were performed for vitamins E, A and C. There was no significant difference between NAFLD and controls in the daily intake of vitamin E (mg/day) (MD=-0.71, 95%CI: -2.00, 0.57, p=0.28; I^2=98%, p<0.001, 9 studies, 1,684 NAFLD patients vs. 3,280 controls[10,19,29,30,37,41,42,45,54], **Suppl. Figure 6A)**, vitamin A (µg/day) (MD=-102.22, 95%CI: -302.98, 98.54, p=0.32; I^2=100%, p<0.001, 7 studies, 680 NAFLD patients vs. 1,844 controls, **Suppl. Figure 6B**)[18,19,27,29,30,33,42] and C (mg/day) (MD=-5.53, 95%CI: -18.57, 7.51, p=0.41; I^2=93%, p<0.001, 8 studies, 1,686 NAFLD patients vs. 3,498 controls,

Suppl. Figure 6C)[10,19,27,30,33,37,42,54]. Out of 10 studies assessing vitamins intake, two studies excluded patients with vitamin supplements and one study included them, while in the remaining 7 studies this was not clarified.

From the remaining studies examining micronutrients, iron intake has been investigated in 6 studies[30,45,10,41,54], but did not differ between NAFLD or NASH patients and controls in all but one study[30]. On the other hand, zinc intake, assessed in 3 studies[45,41,54], was lower in 2 studies (one with 159 NAFLD patients vs. 158 controls[41] and another with 28 NASH and 18 NAFLD patients[45]), but no difference was reported in the last study[54]. Regarding differences in folate intake, results from two studies were contradictory[18,41], while 2 studies assessing copper intake among others did not show any differences between patients and controls[10,45]. Finally, several studies have included other micronutrients, such as vitamin K[21], carotenoids[46], manganese, selenium or vitamin B complex[41], but these were only single studies and therefore conclusions regarding their effect on NAFLD cannot be securely drawn.

4. **Coffee and tea consumption**

Four case-control studies[57,58,63,64] including 565 NAFLD patients and 679 controls were included in the meta-analysis regarding coffee consumption. Accordingly, mean daily caffeine intake in cups was not different between NAFLD patients and healthy controls (**Suppl. Figure 7)** (MD =-0.18; 95%CI: -0.67,0.32, p=0.49; I^2=83%, P<0.001). Notably, 3 out of 4 studies reported mean coffee intake <3 cups/day, whereas only one study reported high coffee intake (>3cups/day) in NAFLD or healthy individuals of the cohort[64.]

A further 4 studies were identified through the systematic review, but were not included in the coffee meta-analysis due to insufficient quantitative data. These studies showed a protective effect of coffee. In one of them that included 100 patients with NAFLD and 55 controls, coffee consumption was significantly lower in NAFLD[65]. In another study of 492 patients with NAFLD, coffee intake was also protective (OR= 0.74,p=0.001)[58]. Regarding NASH, in a case-control cohort (180 NAFLD patients, 126 controls), higher coffee consumption was correlated with lower NASH histological stage (r=-0.25, p=0.015) and with lower risk of fibrosis (p=0.044)[63]. Finally, in a study of 195 NAFLD patients, coffee consumption was associated with lower prevalence of significant fibrosis (OR= 0.75, p=0.035)[47].

Regarding tea consumption, green tea was not associated with NAFLD in a study performed in middle-aged men (492 NAFLD patients)[58], while the amount of tea drinking was not different between NAFLD patients and controls (134 NAFLD patients vs. 217 controls)[23]. However, when the role of tea was examined in a case-control Indian study (328 NAFLD patients vs. 100 controls)[52], tea consumption was significantly higher in the NAFLD group compared to healthy controls. There was no information on the sugar added to the tea by the participants.

5. **Sensitivity analyses**

In a meta-analysis of the case-control studies only, there was significantly higher total caloric intake in NAFLD patients compared to healthy controls (n=11 studies**,** 1,307 NAFLD patients vs. 11,612 controls), whereas the difference was not significant in cross-sectional studies only (n=14 studies, 5,049 NAFLD patients vs. 5283 controls) (data not shown).

We also performed a sensitivity analysis excluding studies with serious overall risk of bias (19/60 studies), but we observed no difference in our results, compared to including all 60 studies (data not shown).

Furthermore, we performed a meta-regression analysis to assess the role of BMI on the daily consumption of total calories and macronutrients, but no significant association was found between the mean difference in BMI and consumption of total calories or any of the macronutrients between NAFLD patients and controls (**Suppl. Table 3**).

Finally, the Egger's plot did not reveal the presence of significant publication bias (**Suppl. Table 4**). Funnel plots are also shown in **Suppl. Figure 8**.

6. Food groups, diets and other nutritional habits

Food groups

Overall, 5 studies were identified reporting comparisons of food group consumption between NAFLD patients and healthy controls. In particular, consumption of coarse cereals, potatoes, milk and derived products, vegetables and fruit was lower in 200 NAFLD patients compared to 200 controls[43]. Similarly, higher consumption of refined grains and lower consumption of whole grains and vegetables was found in 100 NAFLD patients compared to 100 controls in another cohort[24]. Another small study showed that poultry consumption was higher in NAFLD patients compared to controls, whereas butter, dairy products and saturated fat consumption was lower in NAFLD patients than controls[25]. Interestingly, 328 NAFLD participants reported significantly higher weekly consumption of fried, spicy and fast food than 100 healthy controls[52].

Prevention of NAFLD was significantly associated with seeds and nuts intake in male subjects (OR=3.66) and vegetables and vegetable products intake in female subjects (OR=4.11)[21] in a Korean study from Korea (348 patients). The protective role of nuts intake was also confirmed in a second study (OR=0.95) (134 patients and 217 controls). Finally, a large cross-sectional study from Taiwan (1,911 patients vs. 1,489 controls) showed a significant inverse correlation between NAFLD and vegetarian diets (OR=0.79) or whole grains intake (OR=0.96) assessed by semi-quantitative FFQ[49]. Significantly increased NAFLD risk was associated with moderate to high fat consumption and/or increased grilled food and white meat consumption in a case-control study (82 NAFLD patients, 198 controls)[50] and with consumption of refined starchy foods (OR=1.16), full fat cheese (OR=1.19), fast food (OR=1.35), sweet spreads and sugar (OR=1.19), sauces (OR=1.20) and fried food (OR=1.12) in another large case-control study (134 patients and 217 controls)[23]. Similar findings from a case-control study with 100 NAFLD patients and 55 controls suggested a significant aggravating role of sweets consumption in NAFLD (OR= 2.13)[65]. Finally, negative results regarding association of food items or groups with NAFLD were reported in 4 studies[62,50,66-53].

Diets

The role of diet in NAFLD was investigated in 4 relatively small studies. In a cross-sectional study including 358 NAFLD patients, a high fat/caloric diet was independently associated with NAFLD[67]. The effect of the Mediterranean diet was investigated in a Greek study of 73 patients and 58 controls, which showed that the dietary pattern was associated with the severity of steatosis but not the presence of NAFLD[68]. In a cross-sectional study of 82 NAFLD patients, higher adherence to Mediterranean diet based on the 14-item Mediterranean Diet Assessment Tool was associated with both lower risk of steatosis and steatohepatitis[69]. Finally, a case-

control study of 328 NAFLD patients and 100 controls revealed increased frequency of non-vegetarian meals in patients compared to controls (66% vs 50%, p=0.004)[52].

Other nutritional habits

The effect of particular nutritional habits in NAFLD has been assessed in 4 studies. First, in a study of 280 Caucasians (82 NAFLD patients and 198 controls) assessing other dietary habits using a questionnaire, the habit of dining out but that of not eating between meals or having a midnight snack was associated with increased NAFLD risk[50]. Alongside, data from a large Japanese cohort with 171 NASH, 29 NAFLD patients and 49 controls showed that short duration of dinner time (<30 min) was significantly more prevalent in NASH male patients compared to male controls, while increased weekly frequency of dining out was significantly more prevalent in NASH female patients compared to female controls[66]. Finally, shorter duration of meals was also associated with higher NAFLD risk in a Chinese cohort (200 NAFLD patients, 200 controls), but no correlation was found between skipping breakfast and NAFLD presence[43].

DISCUSSION

This systematic review included 60 studies evaluating the association of nutritional aspects with the presence and severity of NAFLD. We showed that total caloric intake was significantly higher in patients with NAFLD compared to controls. However, the contribution of individual macronutrients in the total caloric intake did not differ between patients with NAFLD and healthy controls or between those with NAFLD and NASH. Among micronutrients, vitamin A, C or E daily intake was not different between NAFLD and healthy individuals. Notably, heterogeneity was high in all meta-analyses, preventing the drawing of robust conclusions on the association of food composition and the risk of NAFLD.

Regarding macronutrients, total protein consumption in NAFLD patients was not significantly different compared to healthy controls. However, data suggest that meat protein intake may be higher, whereas fish protein intake could be lower in patients with NAFLD compared to controls[12,61,70] and that animal protein intake might be associated with increased NAFLD risk[33]. Consequently, the role of protein source in these patients remains unclear. Moreover, we found that fat consumption, either as total fat or different types of fat, was not different in patients with NAFLD compared to controls, potentially implying that low fat diets in NAFLD would not be more beneficial that any other hypo-caloric diet. Carbohydrates consumption as a percentage of caloric intake was also not higher in NAFLD patients compared to controls. Studies have shown that there is a U-shaped association of carbohydrate consumption with mortality, with minimal risk observed at a 50-55% intake[51]. Although soft drink consumption had a trend towards association with the presence of NAFLD, the odds ratio was 4.4 and the confidence intervals were very wide, implying the possibility of a type II error. This could also be due to the fact that we

were only able to compare no intake versus any intake, potentially diluting the effects of higher consumption. Finally, although there is convincing evidence on the effect of fructose on NAFLD, there were not enough data to perform a meta-analysis. Importantly, the absence of differences in daily consumption of macronutrients between NAFLD and NASH patients was most likely due to limited number of studies and small sample size, suggesting the need for larger studies in this setting.

Regarding coffee consumption, this did not significantly differ between patients with NAFLD and controls. Previous meta-analyses exploring the association of coffee consumption and NAFLD prevalence have also been inconclusive. Three metanalyses so far have examined the same question[53,71,72], but only the most recent one from Hayat et al.[72] including 7 studies, (two of which were common to the ones included in our meta-analysis), showed significant association between NAFLD and coffee consumption. Moreover, Shen[59] et al. have demonstrated that regular coffee consumption was associated with NAFLD risk in a metanalysis of 2 studies only, while Chen et al.[73] found a dose-dependent association between coffee and NAFLD. The different inclusion criteria are a possible reason for this discrepancy. Moreover, our outcome was the presence of NAFLD rather than the severity of fibrosis as in other studies[74]. Although there is a well-documented non-linear association of coffee with most health outcomes[75], this could not be explored for NAFLD with the available data.

Finally, we reviewed the potential impact of food groups, diets and habits on NAFLD, but data was not sufficient for meta-analysis or conclusive results. The findings of a recent meta-analysis by He et al.[62] suggest that consumption of red meat and soft drinks is associated with increased NAFLD risk, while nut consumption may have a

protective role[70]. Among dietary patterns, Mediterranean diet has been extensively studied and found to be protective against the metabolic syndrome and insulin resistance[76-78] and therefore would be expected to have similar effect in NAFLD. However, no conclusive results could be drawn from the 2 studies identified in our systematic review[79,80]. Earlier this year, two meta-analyses have addressed this issue and were inconclusive on the effect of Mediterranean diet on NAFLD[81,82]. In another meta-analysis, NAFLD risk was significantly lower in individuals on Mediterranean than western-type diet[59]. Finally, a sub-analysis of participants of the Framingham Heart study has shown that higher diet quality as determined by the Mediterranean-style diet score (MDS) and Alternative Healthy Eating Index (AHEI), is associated with lower liver fat accumulation, especially in individuals with genetic predisposition for NAFLD[83]. Similarly, in a study including elderly individuals, a plant-based high-fibre, low-fat diet was associated with regression of NAFLD[84].

Our study has some limitations. Those include heterogeneity in the study design, and in the nutritional intake assessment tools, including 24-hour recall, semi-quantitative FFQs or 3-days food records, which represent methods inevitably limited by recall bias. Another limitation is the lack of uniformity in control groups across the studies included. Moreover, this meta-analysis was not performed on a patient level but collected aggregate data from studies of different cohorts, while there was complete paucity of randomized controlled trials in the setting. Finally, the number of patients included in the comparisons between NAFLD and NASH was relatively small, therefore a type II error cannot be excluded.

In conclusion, this systematic review and meta-analysis assessed several nutritional aspects in NAFLD, despite high heterogeneity and significant risk of bias among studies. We showed that individuals with NAFLD consume more calories than those

without, probably leading to weight gain and increasing steatosis. The percentage of energy intake from any major nutrient was not significantly different between patients with NAFLD and healthy controls or NASH. Because of significant heterogeneity in all comparisons, these results should be approached with caution. The dietary advice in NAFLD should also take into account that the main risk in the majority of these patients is cardiovascular and therefore should be tailored accordingly until more liver-specific data become available[85,86]. Finally, we should underline that studies on the consumption of specific food groups and food items were limited; hence, their effect in NAFLD merits further in-depth research, ideally with randomized controlled trials[80].

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Figure Legends

Figure 1. Forest plot showing total calorie intake in (A) non-alcoholic fatty liver disease (NAFLD) patients versus healthy controls and (B) non-alcoholic steatohepatitis (NASH) vs NAFLD patients.

Figure 2. Forest plots showing (A) total protein intake as proportion of total calorie intake (%) in non-alcoholic fatty liver disease (NAFLD) patients versus healthy controls and (B) non-alcoholic steatohepatitis (NASH) vs NAFLD patients.

Figure 3: Forest plots showing (A) total, (B) saturated, (C) polyunsaturated and (D) monounsaturated fat intake as proportion of total calorie intake (%) in non-alcoholic fatty liver disease (NAFLD) patients versus healthy controls and (E) total fat in non-alcoholic steatohepatitis (NASH) vs NAFLD patients.

Figure 4. Forest plots showing (A) total carbohydrate intake as proportion of total calorie intake (%) in non-alcoholic fatty liver disease (NAFLD) patients versus controls and (B) in non-alcoholic steatohepatitis (NASH) vs NAFLD patients.

Fig 1A

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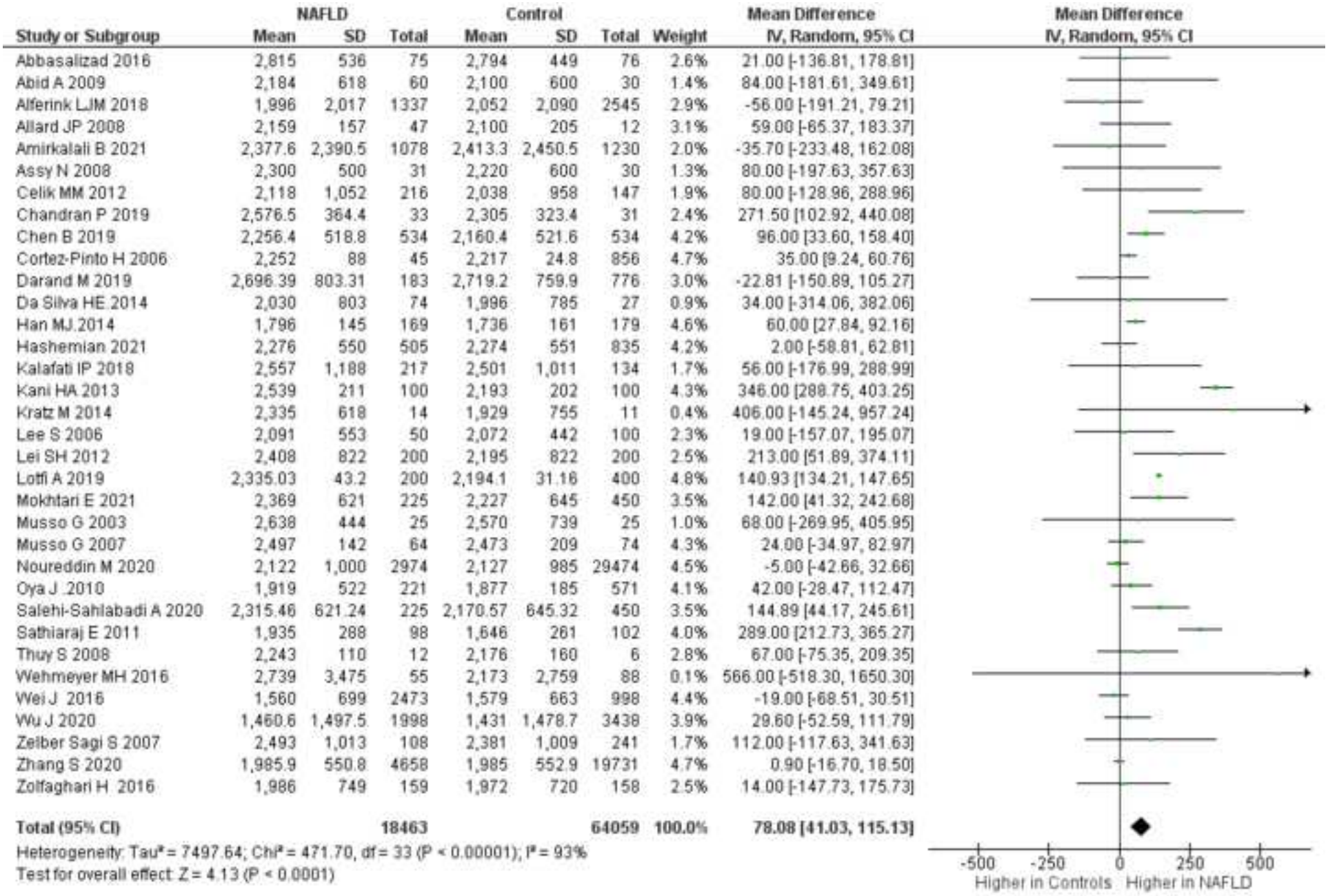

Fig 1B

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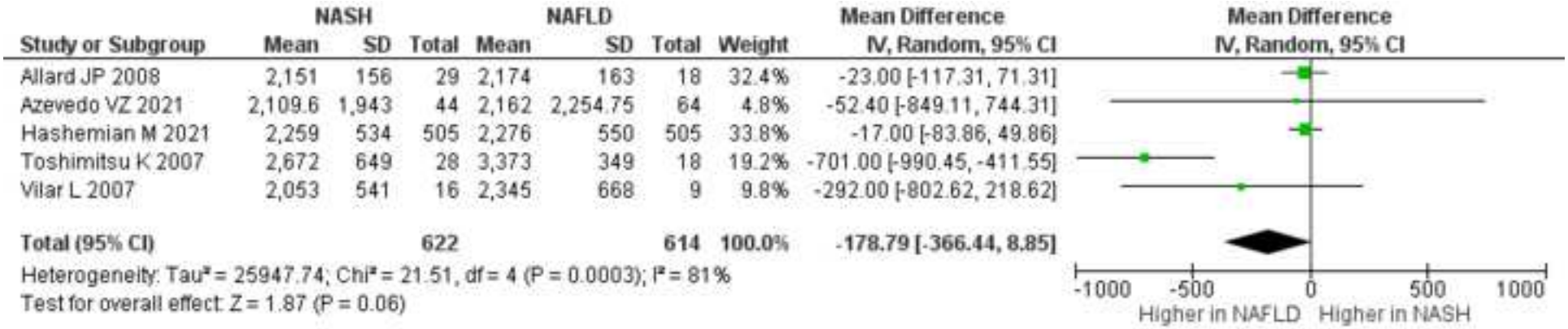

Fig 2A

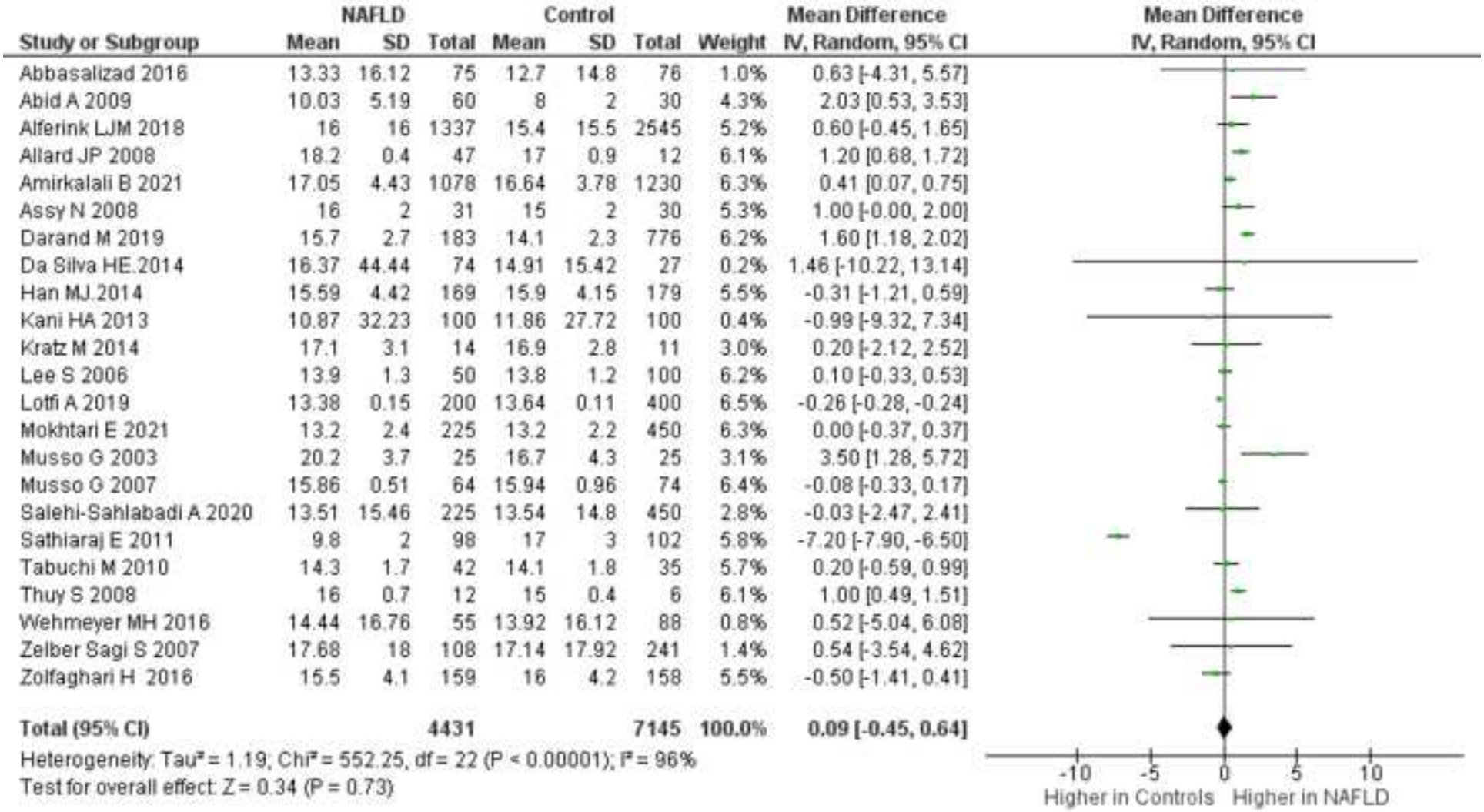

Fig 2B

Study or Subgroup	NASH Mean	NASH SD	NASH Total	Steatosis Mean	Steatosis SD	Steatosis Total	Weight	Mean Difference IV, Random, 95% CI
Allard JP 2008	18.2	0.1	29	18.2	0.7	18	70.0%	0.00 [-0.33, 0.33]
Kang H 2006	15	3	31	16	3	60	19.2%	-1.00 [-2.30, 0.30]
Toshimitsu K 2007	12.18	2.22	28	13	4.52	18	7.6%	-0.82 [-3.06, 1.42]
Vilar L 2007	18.3	3.9	16	20.4	4.6	9	3.2%	-2.10 [-5.66, 1.46]
Total (95% CI)			**104**			**105**	**100.0%**	**-0.32 [-0.97, 0.33]**

Heterogeneity: Tau² = 0.13; Chi² = 3.80, df = 3 (P = 0.28); I² = 21%

Test for overall effect: Z = 0.97 (P = 0.33)

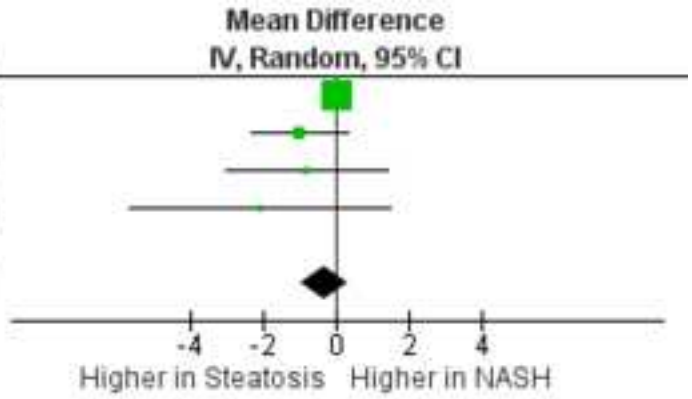

Fig 3A

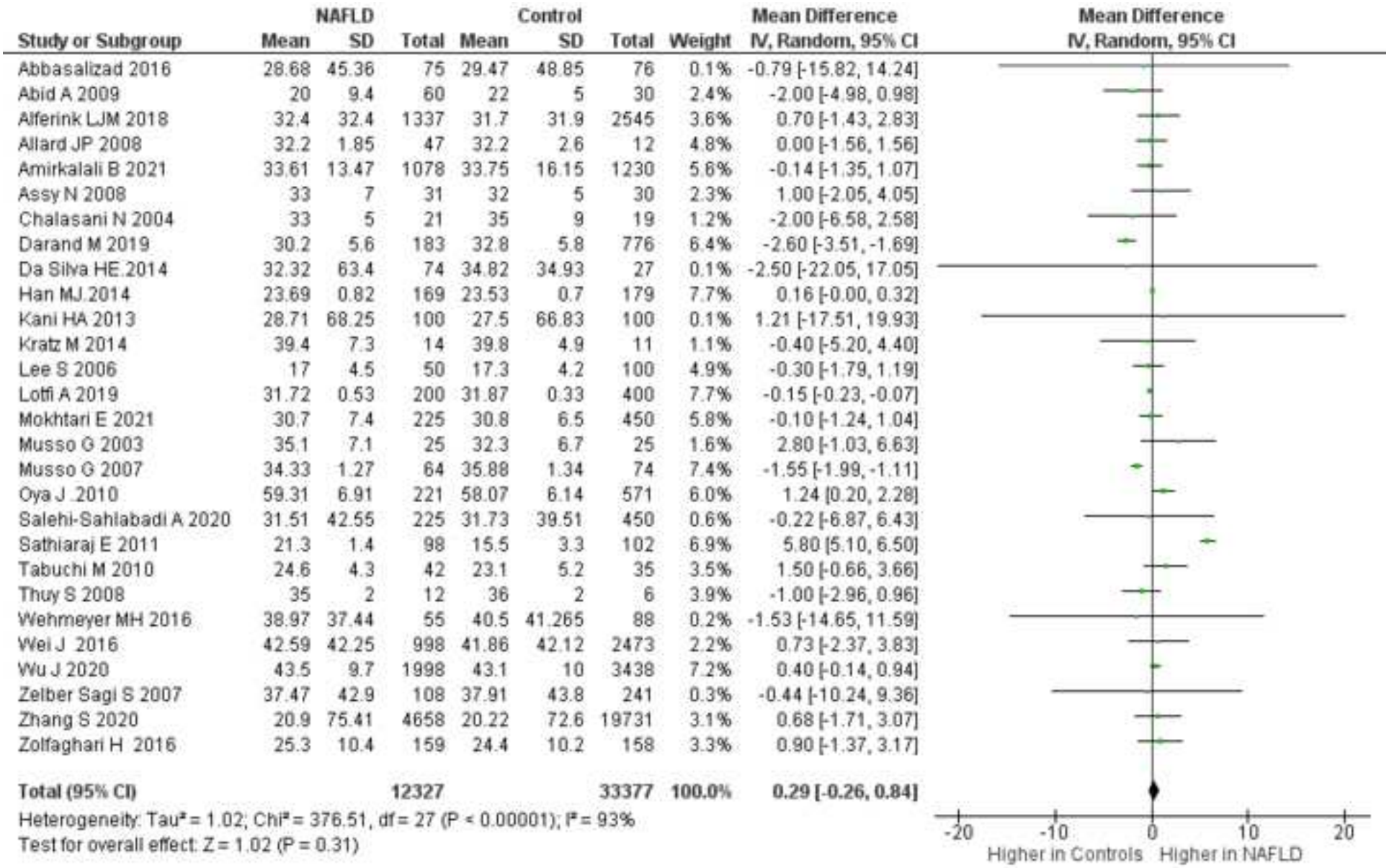

Fig 3B

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Study or Subgroup	NAFLD Mean	NAFLD SD	NAFLD Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Alferink LJM 2018	11.6	11.7	1337	11.4	11.5	2545	9.1%	0.20 [-0.57, 0.97]
Allard JP 2008	10.38	0.8	47	9.9	1.3	12	9.1%	0.48 [-0.29, 1.25]
Amirkalali B 2021	12.28	3.79	1078	12.03	3.32	1230	11.3%	0.25 [-0.04, 0.54]
Chalasani N 2004	12	2.4	21	13	3.4	19	4.4%	-1.00 [-2.84, 0.84]
Darand M 2019	10.5	4.2	183	12.96	6.2	776	9.2%	-2.46 [-3.21, -1.71]
Da Silva HE.2014	10.01	10.22	74	11.45	10.84	27	1.0%	-1.44 [-6.15, 3.27]
Han MJ.2014	9.33	1.21	169	8.19	1	179	11.5%	1.14 [0.91, 1.37]
Kratz M 2014	12.5	3.4	14	15.3	2.7	11	3.0%	-2.80 [-5.19, -0.41]
Musso G 2003	13.7	3.1	25	10	2.1	25	5.7%	3.70 [2.23, 5.17]
Musso G 2007	11.8	0.56	64	11.82	0.47	74	11.6%	-0.02 [-0.19, 0.15]
Oya J .2010	6.44	1.66	221	6.63	1.59	571	11.4%	-0.19 [-0.44, 0.06]
Tabuchi M 2010	13.2	3.8	42	12.2	37	35	0.2%	1.00 [-11.31, 13.31]
Wu J 2020	6.1	6.2	1998	6.1	6.1	3438	11.2%	0.00 [-0.34, 0.34]
Zelber Sagi S 2007	12.27	16.61	108	12.55	16.95	241	1.4%	-0.28 [-4.07, 3.51]
Total (95% CI)			**5381**			**9183**	**100.0%**	**0.03 [-0.45, 0.52]**

Heterogeneity: $Tau^2 = 0.52$; $Chi^2 = 157.62$, df = 13 ($P < 0.00001$); $I^2 = 92\%$

Test for overall effect: $Z = 0.14$ ($P = 0.89$)

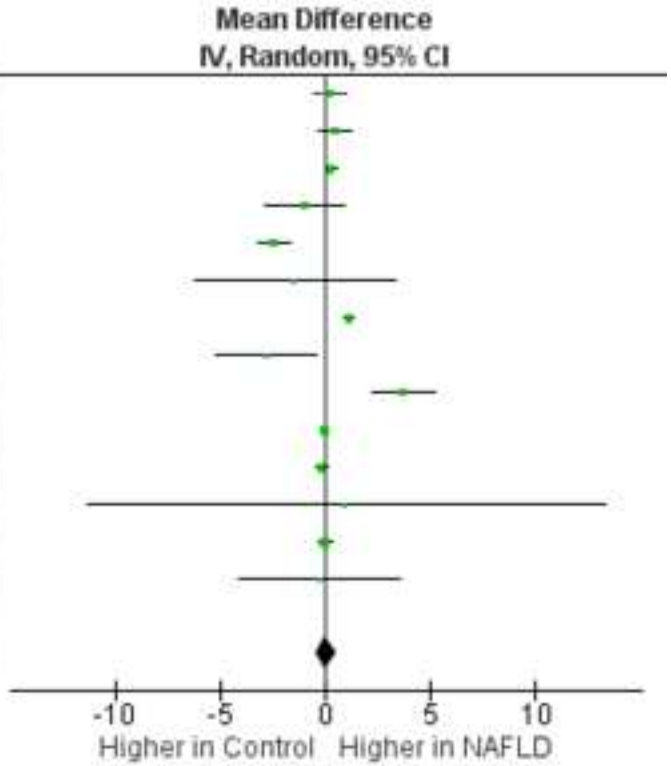

Fig 3C

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Study or Subgroup	NAFLD Mean	NAFLD SD	NAFLD Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Alferink LJM 2018	6.4	6.5	1337	6.4	6.5	2545	12.6%	0.00 [-0.43, 0.43]
Allard JP 2008	5.2	0.5	47	6.1	0.7	12	12.7%	-0.90 [-1.32, -0.48]
Amirkalali B 2021	6.32	2.17	1078	6.26	1.98	1230	14.7%	0.06 [-0.11, 0.23]
Chalasani N 2004	6.3	2	21	6.3	2	19	5.7%	0.00 [-1.24, 1.24]
Chen B 2019	10.25	45.1	534	9.58	40.2	534	0.5%	0.67 [-4.45, 5.79]
Darand M 2019	15.79	4.47	183	12.14	5.83	776	9.2%	3.65 [2.88, 4.42]
Da Silva HE.2014	5.4	5.44	74	5.07	5.38	27	2.1%	0.33 [-2.05, 2.71]
Han MJ.2014	8.13	0.53	169	8.11	0.98	179	14.7%	0.02 [-0.14, 0.18]
Kratz M 2014	8.7	3.2	14	6.6	2.5	11	2.4%	2.10 [-0.13, 4.33]
Musso G 2003	3.5	1.3	25	4.7	2	25	7.8%	-1.20 [-2.14, -0.26]
Musso G 2007	4.44	0.39	64	4.4	0.26	74	14.9%	0.04 [-0.07, 0.15]
Zelber Sagi S 2007	7.73	8.79	108	7.82	9.81	241	2.7%	-0.09 [-2.16, 1.98]
Total (95% CI)			**3654**			**5673**	**100.0%**	**0.21 [-0.17, 0.58]**

Heterogeneity: Tau² = 0.24; Chi² = 114.62, df = 11 (P < 0.00001); I² = 90%

Test for overall effect: Z = 1.08 (P = 0.28)

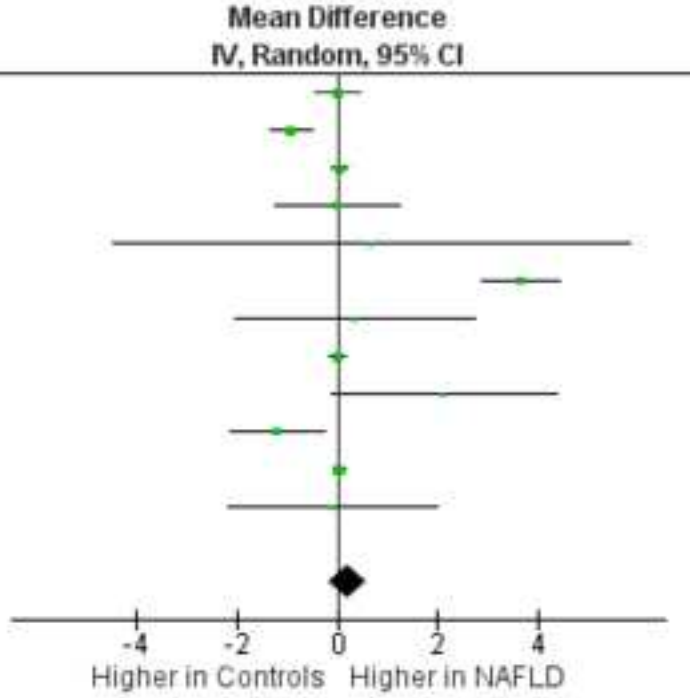

Fig 3D

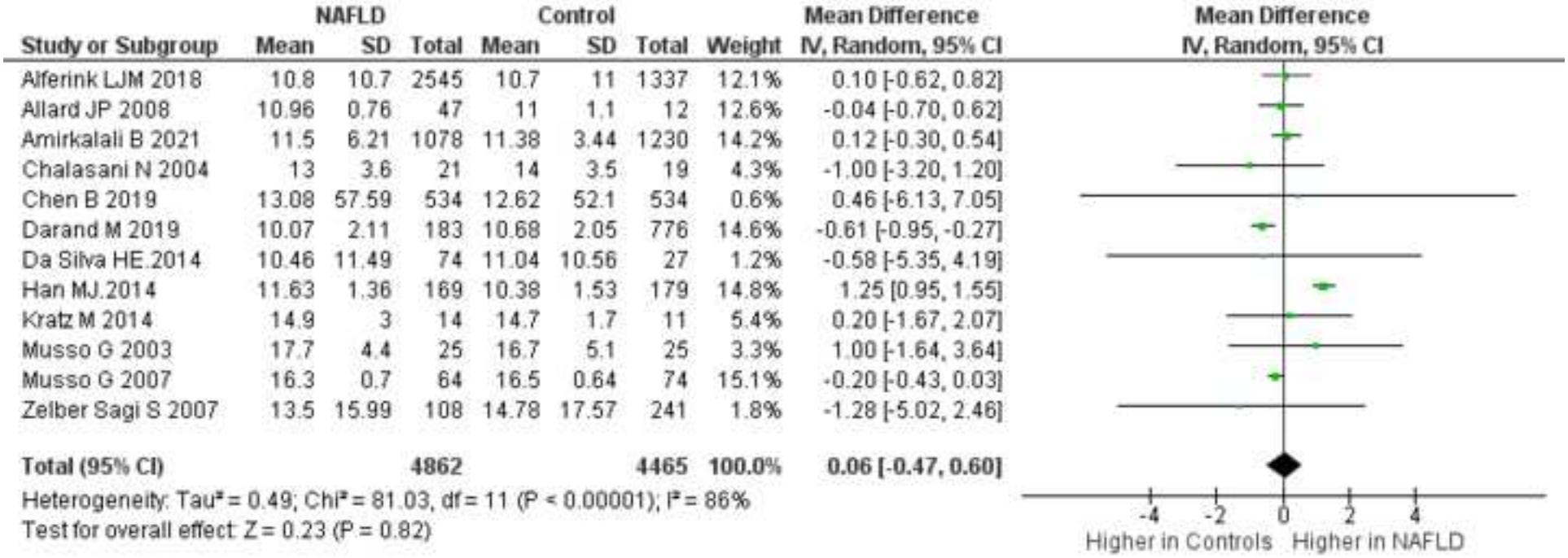

Fig 3E

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Study or Subgroup	NASH Mean	NASH SD	NASH Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Allard JP 2008	33.2	1.1	29	30.6	1.7	18	30.2%	2.60 [1.72, 3.48]
Azevedo V 2021	35.4	8.3	44	37.1	7.4	64	23.7%	-1.70 [-4.75, 1.35]
Kang H 2006	40	10	31	39	9	60	19.6%	1.00 [-3.19, 5.19]
Toshimitsu K 2007	27.29	8.49	28	33.56	11.93	18	13.3%	-6.27 [-12.62, 0.08]
Vilar L 2007	37.5	8	16	31.2	7.8	9	13.1%	6.30 [-0.13, 12.73]
Total (95% CI)			**148**			**169**	**100.0%**	**0.57 [-2.50, 3.64]**

Heterogeneity: Tau² = 7.95; Chi² = 15.78, df = 4 (P = 0.003); I² = 75%

Test for overall effect: Z = 0.36 (P = 0.72)

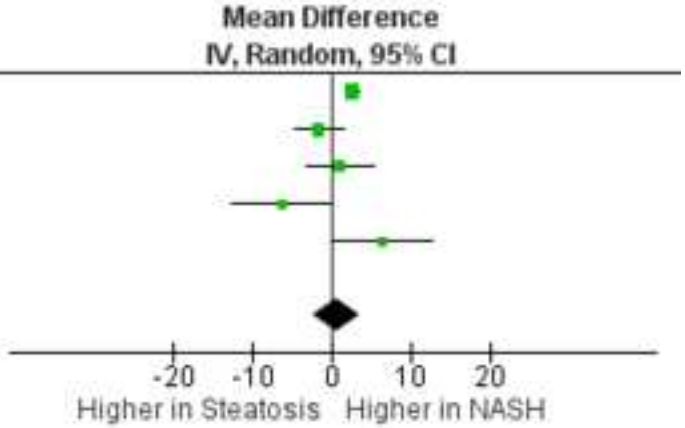

Fig 4A

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Study or Subgroup	NAFLD Mean	NAFLD SD	NAFLD Total	Control Mean	Control SD	Control Total	Weight	Mean Difference IV, Random, 95% CI
Abbasalizad 2016	62.14	66.9	75	62.76	70.95	76	0.0%	-0.62 [-22.61, 21.37]
Abid A 2009	69.9	12.9	60	70	6	30	1.2%	-0.10 [-4.01, 3.81]
Alferink LJM 2018	45.5	45.9	1337	46.6	46.6	2545	1.9%	-1.10 [-4.15, 1.95]
Allard JP 2008	50.67	2.2	47	51.7	2.8	12	4.7%	-1.03 [-2.73, 0.67]
Amirkalali B 2021	11.2	11.2	1078	11.3	11.2	1230	9.0%	-0.10 [-1.02, 0.82]
Assy N 2008	49	7	31	47.8	6	30	1.7%	1.20 [-2.07, 4.47]
Chalasani N 2004	52	6	21	49	11	19	0.6%	3.00 [-2.57, 8.57]
Darand M 2019	57.99	6.4	183	57.76	8.19	776	7.8%	0.23 [-0.86, 1.32]
Da Silva HE 2014	47.7	12.29	41	50.3	12.02	27	0.6%	-2.60 [-8.49, 3.29]
Han MJ 2014	60.17	1.01	169	60.86	1	179	13.8%	-0.69 [-0.90, -0.48]
Kani HA 2013	62.23	72.04	100	59.64	69.31	100	0.1%	2.59 [-17.00, 22.18]
Kratz M 2014	42.9	6.1	14	42.3	6.6	11	0.8%	0.60 [-4.44, 5.64]
Lee S 2006	69.1	5.5	50	68.8	5.2	100	4.3%	0.30 [-1.53, 2.13]
Lotfi A 2019	57.08	0.53	200	56.98	0.34	400	14.2%	0.10 [0.02, 0.18]
Mokhtari E 2021	55.9	7.4	225	55.9	6.5	450	7.5%	0.00 [-1.14, 1.14]
Musso G 2003	44.7	8.7	25	48.6	9.1	25	0.8%	-3.90 [-8.84, 1.04]
Musso G 2007	42.09	7.89	64	44.18	6.58	74	2.8%	-2.09 [-4.54, 0.36]
Oya J 2010	25.19	5.24	221	26.29	4.78	571	9.9%	-1.10 [-1.89, -0.31]
Salehi-Sahlabadi A 2020	57.3	64.05	225	57.36	61.37	450	0.2%	-0.06 [-10.17, 10.05]
Sathiaraj E 2011	69.9	2.3	98	67.4	3.5	102	9.7%	2.50 [1.68, 3.32]
Tabuchi M 2010	61.9	5.9	42	62.8	6.2	35	2.3%	-0.90 [-3.62, 1.82]
Thuy S 2008	49	1	12	49	3	6	2.7%	0.00 [-2.47, 2.47]
Wehrmeyer MH 2016	41.64	43.96	55	41.08	39.58	88	0.1%	0.56 [-13.70, 14.82]
Zelber Sagi S 2007	47.41	54.94	108	47.39	49.83	241	0.1%	0.02 [-12.10, 12.14]
Zhang S 2020	71.74	258.53	4658	71.96	258.35	19731	0.3%	-0.22 [-8.47, 8.03]
Zolfaghari H 2016	59.1	10.8	159	59.5	10.6	158	2.9%	-0.40 [-2.76, 1.96]
Total (95% CI)			**9298**			**27466**	**100.0%**	**-0.09 [-0.54, 0.37]**

Heterogeneity: Tau² = 0.38; Chi² = 100.58, df = 25 (P < 0.00001); I² = 75%

Test for overall effect: Z = 0.38 (P = 0.71)

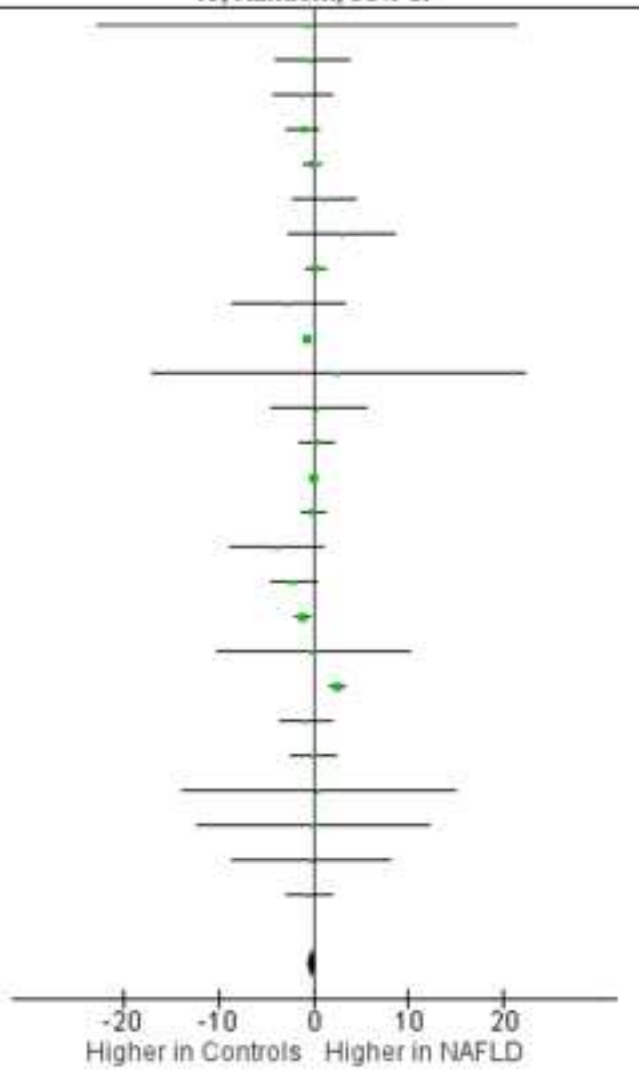
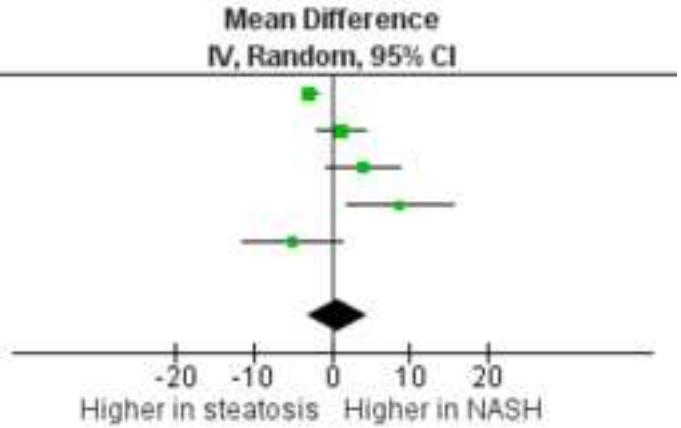


Fig 4B

Study or Subgroup	NASH Mean	NASH SD	NASH Total	Steatosis Mean	Steatosis SD	Steatosis Total	Weight	Mean Difference IV, Random, 95% CI
Allard JP 2008	49.6	1.7	29	52.4	1.8	18	26.7%	-2.80 [-3.84, -1.76]
Azevedo V 2021	42.8	7.8	44	41.6	8.4	64	23.2%	1.20 [-1.89, 4.29]
Kang H 2006	50	11	31	46	11	60	19.3%	4.00 [-0.77, 8.77]
Toshimitsu K 2007	58.28	10.04	28	49.56	12.08	18	15.0%	8.72 [2.01, 15.43]
Vilar L 2007	44.3	9.2	16	49.3	6.9	9	15.7%	-5.00 [-11.38, 1.38]
Total (95% CI)			**148**			**169**	**100.0%**	**0.83 [-3.06, 4.72]**

Heterogeneity: Tau² = 14.46; Chi² = 23.16, df = 4 (P = 0.0001); I² = 83%

Test for overall effect: Z = 0.42 (P = 0.68)



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Supporting documents

Supporting File. PRISMA_Consort checklist .docx