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Systematic review with meta-analysis: risk factors for non-alcoholic fatty liver disease suggest a shared altered metabolic and cardiovascular profile between lean and obese patients

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Summary

Background: The pathogenesis of non-alcoholic fatty liver disease (NAFLD) is closely associated with the co-occurrence of multiple pathological conditions characterising the metabolic syndrome (MetS), obesity in particular. However, NAFLD also develops in lean subjects, whose risk factors remain poorly defined.

Methods: We performed a meta-analysis of 15 studies, along with the data pertaining to our own population (n=336 patients). Data from lean (n=1966) and obese (n=5938) patients with NAFLD were analysed; lean (n=9946) and obese (n=6027) subjects without NAFLD served as controls.

Results: Relative to the lean non-NAFLD controls, lean patients with NAFLD were older (3.79±0.72 years, $P=1.36\times10^{-6}$) and exhibited the entire spectrum of the MetS risk factors. Specifically, they had a significant ($P=10^{-10}$) increase in plasma glucose levels (6.44±1.12 mg/dL) and HOMA-IR (0.52±0.094-unit increment), blood lipids (triglycerides: 48.37±3.6, $P=10^{-10}$ and total cholesterol: 7.04±3.8, mg/dL, $P=4.2\times10^{-7}$), systolic (5.64±0.7) and diastolic (3.37±0.9) blood pressure (mm Hg), $P=10^{-10}$, and waist circumference (5.88±0.4 cm, $P=10^{-10}$); values denote difference in means±SE. Nevertheless, the overall alterations in the obese group were much more severe when compared to lean subjects, regardless of the presence of NAFLD. Meta-regression suggested that NAFLD is a modifier of the level of blood lipids.

Conclusion: Lean and obese patients with NAFLD share a common altered metabolic and cardiovascular profile. The former, while having normal body weight, showed excess of abdominal adipose tissue as well as other MetS features.

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as a major global health issue, the prevalence of which has increased dramatically over the last few decades.[1,2] In fact, non-alcoholic steatohepatitis (NASH)—the severe clinical form of NAFLD—has become one of the leading aetiologies requiring liver transplantation in adults.[1,3]

The pathogenesis of NAFLD is closely associated with the co-occurrence of multiple pathological conditions characterising the Metabolic Syndrome. In fact, the progression and even the long-term prognosis of NAFLD are strongly dependent on the presence of type 2 diabetes, obesity and/or cardiovascular disease.[1,4]

The classical phenotype of a patient with NAFLD is primarily an obese or overweight individual, exhibiting insulin resistance or type 2 diabetes and some degree of cardiovascular disease, most commonly arterial hypertension. A dose-dependent relationship between NAFLD and body mass index (BMI) has been established, whereby the risk of developing NAFLD increases by about ~1.2 per unit

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increment in the BMI, as shown in a recent meta-analysis.[5] Nevertheless, owing to the increased incidence of NAFLD in lean (non-obese) patients, research focus has recently shifted to this population. While this phenomenon was initially observed in Asian population,[6] it has since been recognised as a global health issue.[7] There are, however, some essential aspects of the pathogenesis of "lean-NAFLD" that remain poorly understood, including the pre-disposing risk factors of the disease.[8]

Lean-NAFLD has become a major clinical challenge due to the fact that when obesity—the primary risk factor that patients actually perceive—is not present, the diagnosis of the liver disease is delayed or even overlooked, resulting in compromised effectiveness or complete absence of the required treatment.

In the present study, we hypothesised that lean and obese patients with NAFLD share a common metabolic and cardiovascular profile. Hence, we performed a comprehensive meta-analysis of published epidemiological studies to gain insights into the pathogenesis of NAFLD in lean subjects, particularly its association with the Metabolic Syndrome. To provide a quantitative estimation of the relevance of each component of the cluster of Metabolic Syndrome risk factors, we adopted different strategies. First, we focused on the universe of lean (non-obese) subjects and stratified the population into groups having and not having NAFLD respectively. This approach allowed us to identify the risk factors that are more likely to pre-dispose lean subjects to NAFLD. In addition, we assessed the difference in the magnitude of the effect of each outcome (Metabolic Syndrome risk factors) in lean and obese patients, which were stratified according to the presence or absence of NAFLD, aiming to indirectly measure the impact of NAFLD on the observed effect sizes. All the estimations were performed in ethnically and culturally diverse populations that included adults of both sexes; hence, the impact of individual risk factors associated with lean-NAFLD was stratified according to ethnicity.

2 | METHODS

We followed the appropriate methods for conducting a meta-analysis of observational studies (MOOSE) (Table S1).

2.1 | Search strategy

To identify studies for inclusion in the meta-analysis, we searched for published studies on PubMed, Ovid-Medline and Google Scholar using the following keywords and terms: "lean non-alcoholic fatty liver disease" and "nonobese non-alcoholic fatty liver disease". In addition, the reference section of all retrieved articles was checked for additional literature sources, and the PubMed link "related articles" was used to identify potentially relevant papers; the search was also performed in The Cochrane Library. The literature search included all studies published before March 2017 and no country restrictions were imposed. The authors reviewed all abstracts independently to determine the alignment with the eligibility criteria, or to establish the appropriateness of the research topic. If these criteria were met, the article was retrieved and reviewed in its entirety. There were no discrepancies in this process; details on data search/collection, including Boolean search are summarised in Figures S1A, B and C.

2.2 | Inclusion and exclusion criteria and data collection

The following meta-analysis inclusion criteria were considered when assessing the eligibility of the identified studies:

1. Observational studies (cross-sectional or longitudinal studies of which baseline data was retrieved) on the epidemiological risk factors of lean patients with NAFLD in which the authors provided body mass index (BMI) expressed in kg/m^2 and examined the presence of NAFLD as the main clinical endpoint.
2. Population-based or hospital-based studies.
3. A clear definition of NAFLD estimated by a valid imaging method to detect hepatic steatosis, such as liver ultrasound (US), computed tomography abdominal scan, proton-magnetic resonance spectroscopy or histological evaluation assessed by liver biopsy.
4. A clear exclusion of co-existing common chronic liver diseases and secondary causes of steatosis, including heavy alcohol consumption, total parenteral nutrition, hepatitis B and C virus infection, and the use of drugs known to precipitate steatosis.
5. A clear definition of lean and non-lean (overweight/obese) patients with NAFLD, expressed as a BMI cut-off, which allows identifying two groups of patients, thereby facilitating comparison across the studies. Four categories were evaluated in this meta-analysis, comprising of: (1) lean patients with NAFLD, defined as patients with a BMI $\leq$25; (2) lean subjects without NAFLD; (3) non-lean (overweight/obese) patients with NAFLD, defined as patients with BMI >25 and (4) overweight/obese subjects without NAFLD.
6. For each study, the following information had to be provided: demographic features of the subjects (age, sex, country of origin as a proxy of ethnicity), study design, method of assessment of fatty liver infiltration, anthropometric variables (waist circumference) and systolic and diastolic blood pressure.
7. Data on the following biochemical parameters were included in the analysis whenever available: homoeostatic model assessment-insulin resistance (HOMA-IR), fasting plasma glucose levels, serum liver enzymes (alanine-ALT and aspartate-AST aminotransferase and GGT—gamma-glutamyl-transferase), and blood lipids, including total cholesterol and triglycerides.
8. All quantitative variables had to be expressed as mean$\pm$standard deviation (SD); prior to the analysis, standard error (SE) or interquartile range were converted to SD while median was converted to mean.

Exclusion criteria: Studies pertaining to patients with NAFLD in which the authors failed to specify the BMI categories utilised, as

explained above (lean vs non-lean), duplicate publications, unpublished papers and papers that included data on NAFLD patients either using a non-standard definition of lean subjects or NAFLD defined by non-standard methods.

2.3 | Statistical analysis

A random effect model was adopted when summarising statistical synthesis; this model assumes that the treatment effect is not the same across all studies included in the analysis.

To specifically provide measures of the absolute difference between the mean values of each variable of interest calculated for any two groups (eg, lean-NAFLD vs lean non-NAFLD patients, or lean-NAFLD vs obese-NAFLD), we used the difference in means. This approach was justified, as we used outcome measurements on the same scale/unit. Results from studies that report laboratory data on SI units were converted to conventional units using appropriate conversion factors. For each analysis, a forest plot was generated to display results; as we hypothesised that ethnicity may provide an important source of variability, the estimate of the average effect of the studies was additionally stratified by ethnicity. Details regarding subgroup analyses, meta-regression and heterogeneity are fully disclosed in the Supporting information.

All calculations were performed using the Comprehensive Meta-Analysis computer program (Biostat, Englewood, NJ, USA).

2.4 | Assessment of study quality

The quality of the studies included in the meta-analysis was assessed using The Newcastle-Ottawa Scale (NOS) (Table S2).

3 | RESULTS

3.1 | Study selection

In addition to the published studies, we included in this meta-analysis epidemiological data from our own NAFLD study comprising of 336 Argentinean adults, who took part in an earlier case-control study (Table S3) that has been extensively described elsewhere.[9,10] For inclusion of our findings in the meta-analysis, we adopted the inclusion and exclusion criteria mentioned earlier.

All the investigations performed in our study were conducted in accordance with the guidelines of the 1975 Declaration of Helsinki. Written consent from all participating individuals was obtained in accordance with the procedures approved by Institutional Review Board-approved protocols (protocol number: 104/HGAZ/09, 89/100 and 1204/2012).

Following the previously described search strategy, 23 articles were initially identified as potentially relevant for the present investigation, based on the assessment of the titles and abstracts. Eight studies were subsequently excluded due to not meeting all the inclusion criteria: (1) in two cases, the authors used a non-conventional definition of a lean individual based on a non-standard BMI cut-off value (BMI<30);[11,12] (2) the authors of five studies did not report variables of interest according to the two groups adopted in the present study (lean vs non-lean);[13-17] and (3) in one case, multiple reports were made on the same cohort.[18]

Thus, the remaining 15 studies, along with our own population, were included in the meta-analysis,[6,7,15,19-29] which scored well in terms of the selection criteria, comparability of cases and controls on the basis of the design or analysis, and ascertainment of exposure (Table S2).

3.2 | Study characteristics

The study characteristics, including the cut-off BMI values used for the differentiation between lean and obese subjects, are shown in Table S4. All 16 studies included adults of both sexes, with the age ranging from 20 to 75 years.

In eleven studies, fatty liver was evaluated by liver ultrasound,[7,15,20-24,27-30] proton-magnetic resonance spectroscopy was used in one[6] and percutaneous liver biopsy was performed in four studies,[19,25,26] including our studied population.

Five studies—including ours—were based on Caucasian cohort,[7,19,23,26,27] while the remaining eleven studies included Asian population.[6,20-22,24-26,28-31] Complete details on the study design and sample size are fully disclosed in Table S4.

3.3 | Lean patients with NAFLD compared to lean non-NAFLD controls are older and exhibit the entire spectrum of the metabolic syndrome features, including abdominal (central) obesity

The results yielded by seven heterogeneous studies for which the authors provided raw data on the risk factors for the disease in lean non-NAFLD vs lean NAFLD patients[7,22-24,28,30,31]—including a total of 11 400 individuals—showed that subjects having NAFLD (n=1454) were 3.79±0.72 years older compared with the non-NAFLD cohort (n=9946), $P=1.36\times10^{-6}$, Figure S2. However, stratification of studies by country of origin showed a significant heterogeneity in the results pertaining to Japanese (I: 96.5, P=.0001) and Korean population (I: 90.5, P=.0001), which was not the case for China (I: 1, P=0). On the other hand, no significant heterogeneity was found in studies that included Caucasian population (I: 23.1, P=.25). The Begg and Mazumdar's rank correlation test (Kendall's tau 0.11, P=.38) confirmed absence of publication bias.

In addition, the analysis of risk factors revealed significant differences in the magnitude of the effect of all the Metabolic Syndrome components in the group comprising of lean patients with NAFLD. Specifically, when compared to lean non-NAFLD subjects, a significant increment in the following parameters was noted for lean patients with NAFLD: (1) fasting plasma glucose levels (6.43±1.12 mg/dL, $P=10^{-10}$) (Figure 1) and HOMA-IR (0.52±0.094-unit increment, $P=10^{-10}$) (Figure S3); (2) blood lipids (total cholesterol: 7.04±3.80, $P=4.2\times10^{-7}$ and triglycerides: 48.37±3.66, $P=10^{-10}$, mg/dL) (Figures 2/Figure S10 and 3/Figure S11, respectively), (3) blood pressure

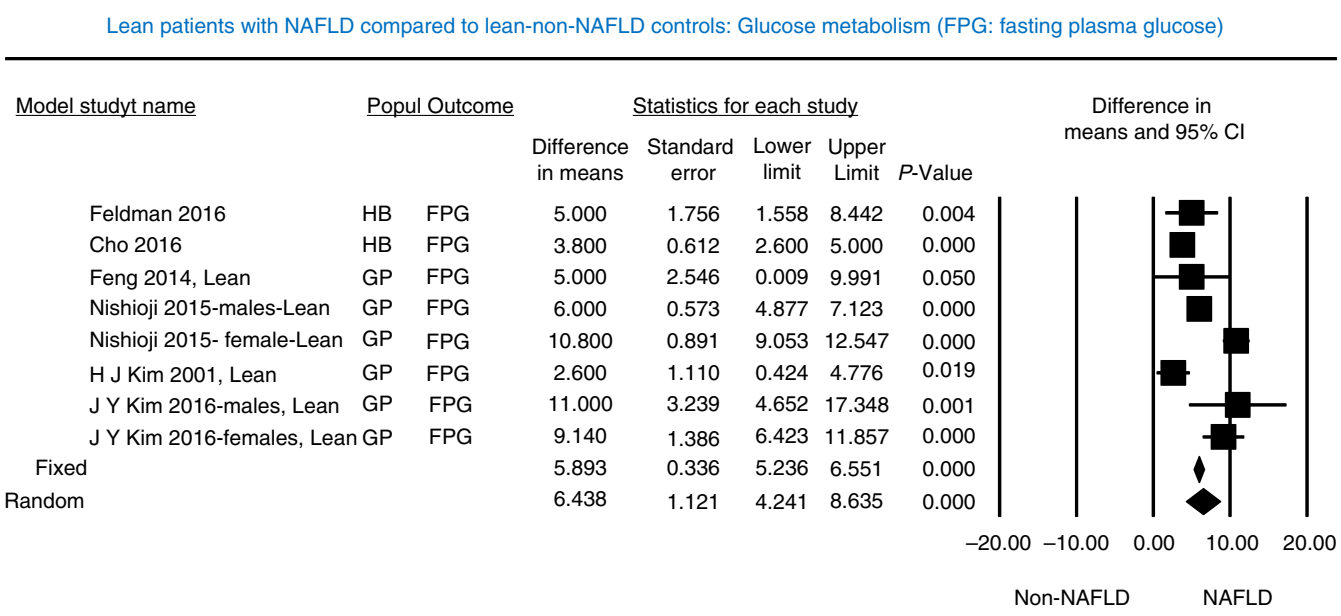

FIGURE 1 Association analysis of fasting plasma glucose (fPG) in lean (non-obese) patients with non-alcoholic fatty liver disease (NAFLD) vs lean non-NAFLD controls. The effect indicates the difference in means, standard error (SE) and the corresponding lower and upper limits, according to the presence or absence of NAFLD. The first author of the study and the year of publication are shown under the sub-heading: "study name". Popul: indicates design features, GP, general population, HB, hospital-based. In the graph, the filled squares denote the effect of individual studies, and filled diamonds express combined fixed and random effects

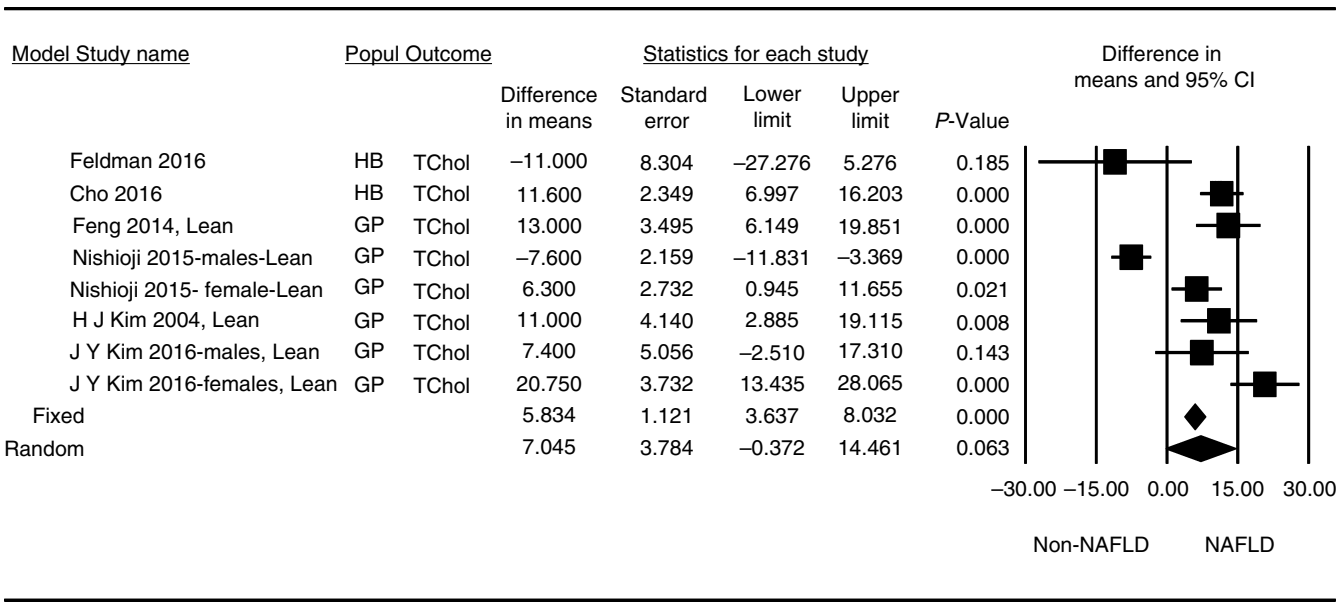

FIGURE 2 Association analysis of plasma total cholesterol—TChol in lean (non-obese) patients with non-alcoholic fatty liver disease (NAFLD) vs lean non-NAFLD controls. The effect indicates the difference in means, standard error (SE) and the corresponding lower and upper limits, according to the presence or absence of NAFLD. The first author of the study and the year of publication are shown under the sub-heading: "study name". Popul: indicates design features, GP, general population, HB, hospital-based. In the graph, the filled squares denote the effect of individual studies, and filled diamonds express combined fixed and random effects

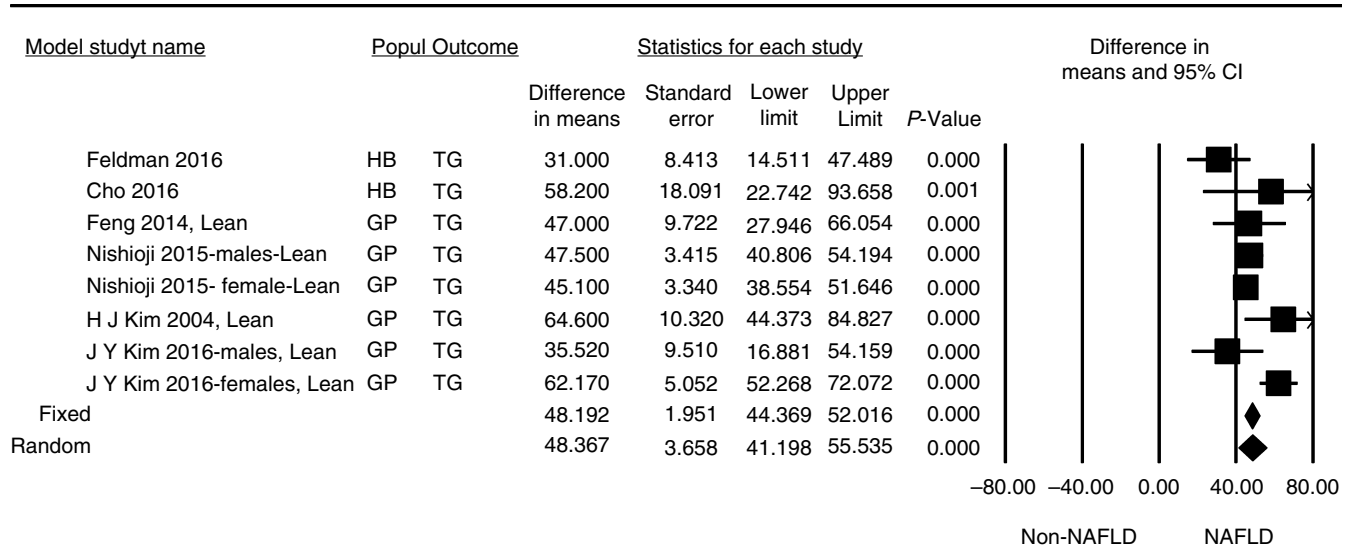

FIGURE 3 Association analysis of plasma triglycerides— triglycerides (TG) in lean (non-obese) patients with nonalcoholic fatty liver disease (NAFLD) vs lean non-NAFLD controls. The effect indicates the difference in means, standard error (SE) and the corresponding lower and upper limits, according to the presence or absence of NAFLD. The first author of the study and the year of publication are shown under the sub-heading: "study name". Popul: indicates design features, GP, general population, HB, hospital-based. In the graph, the filled squares denote the effect of individual studies, and filled diamonds express combined fixed and random effects

(systolic blood pressure: 5.64±0.67, $P=10^{-10}$ and diastolic blood pressure: 3.37±0.90, $P=10^{-10}$, mm Hg) (Figure 4 and Figure S4, respectively), (4) anthropometric features (BMI: 1.24±0.26-unit increment and waist circumference: 5.88±0.40-cm increment, $P=10^{-10}$) (Figure S5 and Figure 5, respectively), and (5) the level of liver enzymes (ALT: 6.09±0.78 IU/L, $P=10^{-10}$, AST: 3.03±0.81 IU/L, $P=4.1\times10^{-7}$ and GGT: 9.23±0.10 IU/L, $P=10^{-10}$, Figures S6-S8, respectively). All values indicate a difference in means±standard error (SE); decimals show two rounded values.

Of note, while the results reported above could not be explained by the difference in BMI (data not shown), meta-regression analysis showed that the difference in waist circumference explained the observed effects in fasting plasma glucose levels (slope: 1.55, $P=.014$), HOMA-IR (slope: 0.13, $P=.023$), systolic blood pressure (slope: 1.31, $P=.018$) and as expected BMI (slope: 0.19, $P=1\times 10^{-6}$). A note of caution should be added as the number of studies included in meta-regression might represent a potential limitation.

Consistency in the direction of the large majority of the observed effects across studies is also noteworthy. Nevertheless, with the exception of waist circumference, we found significant heterogeneity in the findings pertaining to all evaluated outcomes, which was mostly explained by the inclusion of data reported in studies conducted in Japan[28] and Korea[22,24,31] (Table S5); the estimate of the average effect for each of the studies stratified by ethnicity is shown in Figures S3-S13. On the other hand, no publication bias was noted (Table S5).

3.4 | Lean vs obese patients with and without NAFLD: meta-analysis of risk factors

Although compared to lean controls, lean patients with NAFLD were more insulin resistant, had higher levels of blood lipids as well as elevated blood pressure and increased waist circumference, the overall changes in the Metabolic Syndrome risk factor levels were much more severe in the obese group. Specifically, when compared to lean patients with NAFLD, a significant increase in the following parameters was noted for obese patients with NAFLD: (1) fasting plasma glucose levels (3.16±0.73 mg/dL, $P=.000017$), HOMA-IR (0.98±0.20-unit increment, $P=2.3\times10^{-5}$; and (2) and blood pressure (systolic blood pressure 4.41±0.73, $P=2\times10^{-8}$ and diastolic blood pressure 3.20±0, $P=5.1\times10^{-7}$, mm Hg); a trend in triglycerides was observed (plasma triglycerides levels 8.58±4.82 mg/dL, $P=.075$), Table 1. As expected, 5.93±0.40-unit increment in the BMI and 11.58±0.83-cm increase in waist circumference was noted for obese patients with NAFLD relative to the lean NAFLD group. There were also significant differences in the liver enzyme levels—specifically ALT (3.40±1.01 IU/L, $P=.0007$), AST (2.10±0.67, $P=.001$) and GGT (4.27±1.59, $P=.007$)—between these two groups. Complete details, including the sample size of each group, are fully disclosed in Table S4.

The magnitude of the difference in the value of each individual Metabolic Syndrome component noted for lean and obese individuals, however, barely reached statistical significance when subjects were grouped according to the presence or absence of NAFLD

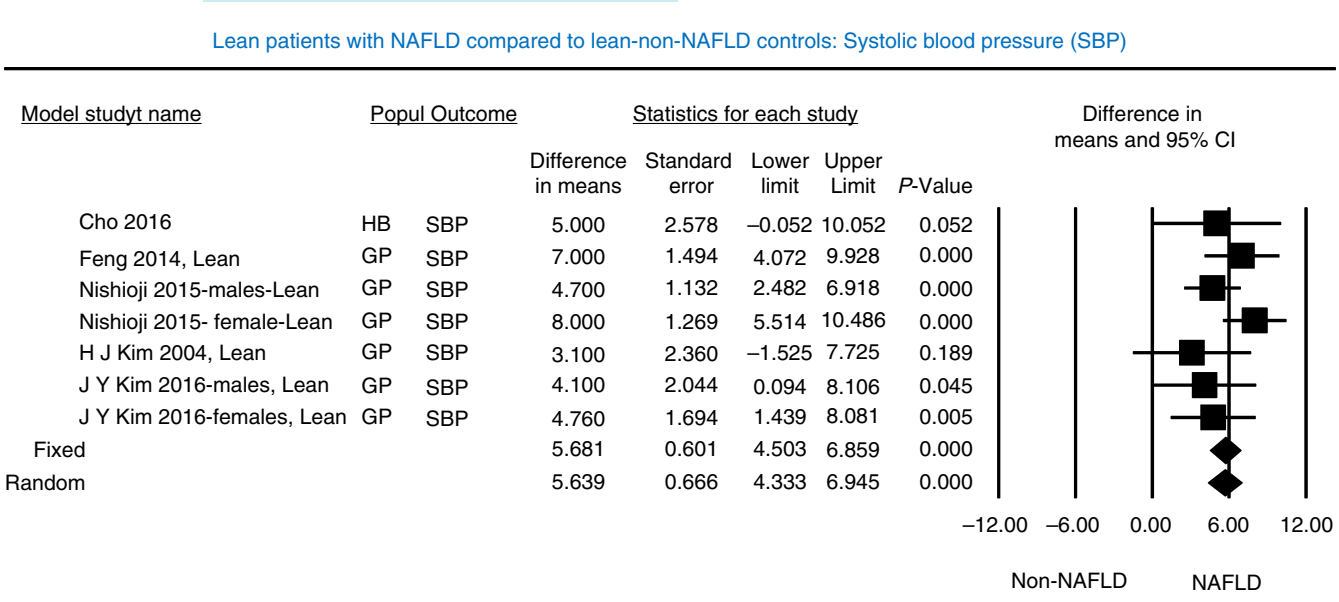

FIGURE 4 Association analysis of systolic blood pressure—SBP in lean (non-obese) patients with non-alcoholic fatty liver disease(NAFLD) vs lean non-NAFLD controls. The effect indicates the difference in means, standard error (SE) and the corresponding lower and upper limits, according to the presence or absence of NAFLD. The first author of the study and the year of publication are shown under the sub-heading: "study name". Popul: indicates design features, GP: general population, HB: hospital-based. In the graph, the filled squares denote the effect of individual studies, and filled diamonds express combined fixed and random effects

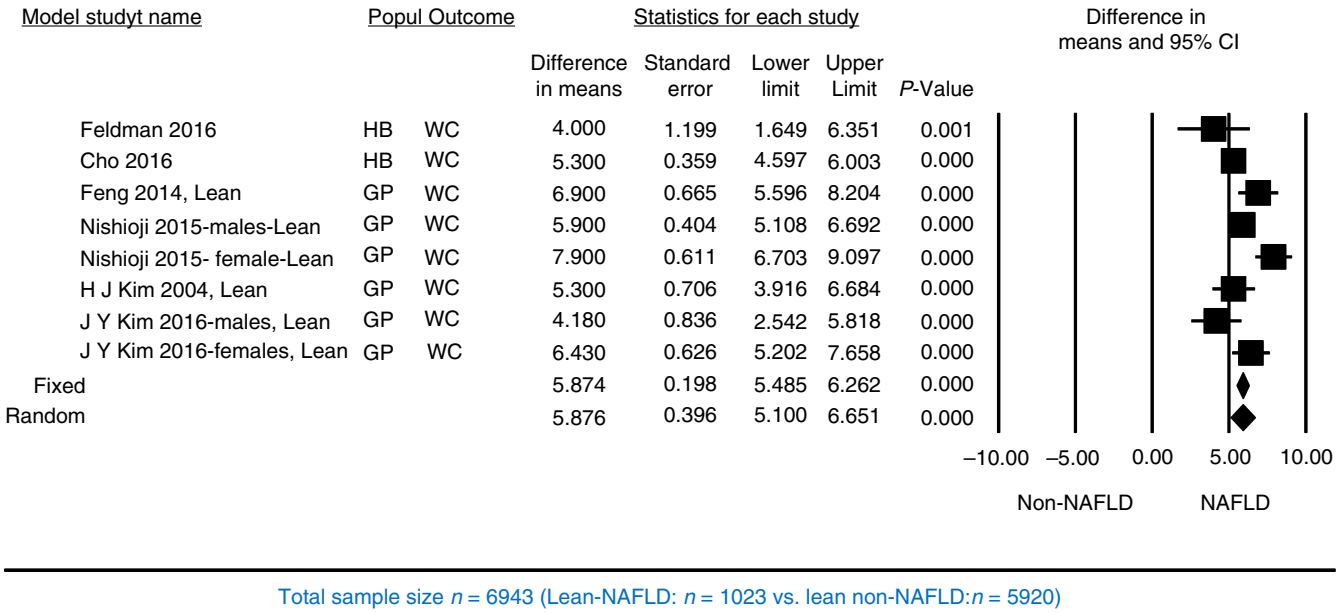

FIGURE 5 Association analysis of waist circumference—WC in lean (non-obese) patients with nonalcoholic fatty liver disease (NAFLD) vs lean non-NAFLD controls. The effect indicates the difference in means, standard error (SE) and the corresponding lower and upper limits, according to the presence or absence of NAFLD. The first author of the study and the year of publication are shown under the sub-heading: "study name". Popul: indicates design features, GP: general population, HB: hospital-based. In the graph, the filled squares denote the effect of individual studies, and filled diamonds express combined fixed and random effects

(Table 1). Interestingly, although the magnitude of the effects was similar in obese vs lean subjects without NAFLD, meta-regression analysis suggested that NAFLD exerts a significant effect on the level of blood lipids, including triglycerides and total cholesterol (Table 1). Specifically, the difference in means of blood lipid levels related to the lean and obese individuals was significantly higher in

TABLE 1 Meta-regression analysis to examine the impact of non-alcoholic fatty liver disease (NAFLD) on the risk factors of the metabolic syndrome

	NAFLD lean vs obese				Non-NAFLD Lean vs obese				Meta-regression analysis	
Outcome	Difference in means ±SE	*P* value	Sample size	Number of sub-studies	Difference in means ±SE	*P* value	Sample size	Number of sub-studies	Slope [a]	*P* value
Demographic features										
Age	0.59±0.69	NS	1966/5938	18	2.36±0.82	.004	8377/6027	7	−1.78	NS
Anthropometric features										
BMI	5.93±0.40	1×10^{-11}	1535/3867	16	5.19±0.50	1×10^{-11}	4351/932	7	0.70	NS
Waist circumference	11.58±0.83	1×10^{-11}	1535/3867	17	10.97±0.76	1×10^{-11}	4351/932	6	0.50	NS
Glucose metabolism										
Fasting plasma glucose	3.16±0.73	.000017	1535/3867	17	2.68±0.623	.000016	4351/932	6	0.43	NS
HOMA	0.98±0.20	2.3×10^{-5}	1464/5166	14	0.55±0.17	.0009	6092/5846	5	0.30	NS
Lipid metabolism										
Triglycerides	8.58±4.82	.075	1535/3867	17	25.67±4.38	5×10^{-8}	4351/932	6	**−17.42**	**.009**
Total cholesterol	0.79±1.23	NS	1535/3867	17	5.51±1.86	.003	4351/932	6	**−4.85**	**.007**
Cardiovascular outcomes (blood pressure)										
Systolic blood pressure	4.41±0.73	2×10^{-8}	1404/3402	13	6.47±1.13	3×10^{-8}	1438/89	6	−2.40	.06
Diastolic blood pressure	3.20±0.58	5.1×10^{-7}	1404/3402	13	3.45±0.58	3×10^{-8}	1438/89	6	−0.22	NS
Liver enzymes										
ALT	3.4±1.01	.0007	1939/5787	17	2.48±0.51	1.3×10^{-5}	8377/6027	7	0.78	NS
AST	2.10±0.67	.001	1939/5787	14	0.18±0.38	NS	8377/6027	5	**1.92**	**.011**
GGT	4.27±1.59	.007	1160/2702	11	5.26±1.42	.00022	3965/730	5	−1.26	NS

HOMA, homoeostatic model assessment-insulin resistance; BMI, body mass index; ALT and AST, alanine and aspartate aminotransferase; GGT, gamma-glutamyltransferase. Meta-regression analysis includes data extracted from 15 studies,[6,7,15,19-29] along with our own population. Meta-regression was used to examine the impact of the moderator variable (NAFLD) on effect's sizes using regression-based techniques.

Bold values correspond to significant slope and then differences for the comparison between lean and obese subjects according to the presence or absence of NAFLD by meta-regression analysis.

[a]To determine the slope we used meta-regression (methods of moments).

the group comprising of non-NAFLD patients in comparison to the group of patients with NAFLD (Table 1). Finally, meta-regression suggested that NAFLD is an important determinant of the magnitude of increase in AST only, as it was unrelated to the ALT or GGT levels (Table 1). These findings indicate that the difference in the AST increase between lean and obese patients is significantly higher only when NAFLD is present.

4 | DISCUSSION

4.1 | Summary of main findings

Based upon the results yielded by a comprehensive analysis of the results reported by 15 published studies, as well as our own clinical data, we have presented robust evidence on the pre-disposing risk factors of NAFLD in lean subjects. Specifically, our findings demonstrated that lean and obese patients with NAFLD share an altered metabolic and cardiovascular profile. However, as expected, the effects in lean patients with NAFLD were of a lesser magnitude relative to those noted in the obese group. Results yielded by the meta-regression analysis indicated that the presence of NAFLD buffers the extent of specific metabolic abnormalities, such as the circulating levels of lipids. For instance, NAFLD seems to be the key determinant of the level of both plasma triglycerides and total cholesterol. More specifically, smaller differences were observed between lean and obese NAFLD patients relative to those noted in the comparison of lean vs obese subjects without NAFLD. Taken together, these results suggest that lipid overload originating from either diet or associated with defects of free fatty acid metabolism cannot be properly stored in the adipose tissue; consequently, lipids accumulate

in the liver. This specific result reinforces the findings of previous genetic association studies revealing that the non-synonymous rs58542926 C/T (p.Glu167Lys) variant in *TM6SF2* (transmembrane 6 superfamily member 2) gene is associated with a dual and opposite role in conferring risk for NAFLD and protecting against cardiovascular disease by reducing blood lipid levels.[10,32-34] A possible involvement of rs738409 C/G (p.Ile148Met), a non-synonymous variant in *PNPLA3* that is strongly associated with NAFLD and the disease severity,[35,36] should be also highlighted. Nevertheless, it remains to be established whether this variant contributes differently to the risk of NAFLD that lean and obese individuals are exposed to, and whether it affects their respective histological disease spectra, though the interaction of rs738409 with BMI is at best very modest.[36] In fact, few studies have evaluated the differential role of rs738409 in lean vs obese patients and patients with and without Metabolic Syndrome. Wei et al. observed in a Chinese community cohort a greater proportion of non-obese NAFLD patients carrying the *PNPLA3* G allele.[6] Conversely, results of the same authors but from a hospital-based study of patients with NAFLD assessed by liver biopsy showed that there was no significant difference in the frequency of the G-allele between non-obese and obese patients.[26]

In addition, meta-regression results indicated that serum levels of AST, but not ALT and GGT, are markedly modulated by the presence of NAFLD, whereas no differences in the AST serum levels were noted between lean and obese non-NAFLD subjects. In line with this finding, we previously observed that, in the context of abnormal accumulation of fat in the liver (NAFLD), there is a greater synthesis and release of aminotransferases, including the mitochondrial isoform of AST (also known as GOT2), which is indeed a compensatory mechanism in response to increased energetic demands.[37]

Finally, while NAFLD can be seen in children, age differences between lean NAFLD and lean non-NAFLD groups suggest that the onset of the disease occurs—at least in this special group of patients—at an older age.

A summary of the main findings is presented in Figure 6. Overall, the meta-regression analysis results reported here lend support to the conclusion that major metabolic and CV abnormalities are not qualitatively different in lean and obese patients with NAFLD. This observation, however, does not counter the hypothesised genetic influence on lean-NAFLD. Equally interesting explanations should be also considered, including a unique—albeit presently unknown—characteristic lean NAFLD lipidomic profile.[38] Regardless of the underlying mechanism/s of this disease, it is important to emphasise that lean NAFLD patients present low degree of Metabolic Syndrome-associated co-morbidities that definitively deserve clinical attention.

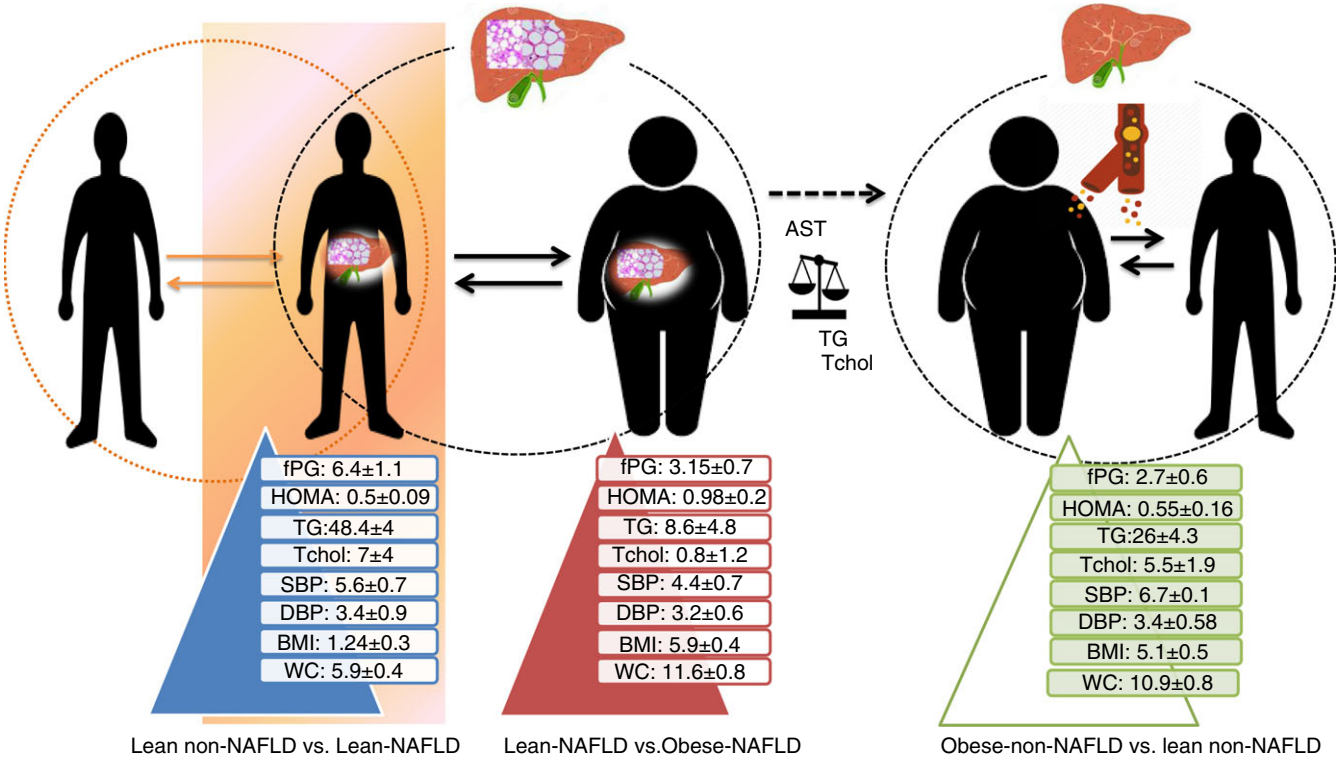

FIGURE 6 Summary of main findings: The lean—non-alcoholic fatty liver disease (NAFLD) paradox. The image illustrates the results yielded by the meta-analysis of risk factors associated with the Metabolic Syndrome in lean vs obese patients classified by the presence of NAFLD; comparisons with the corresponding non-NAFLD controls are also depicted. Values are expressed as differences in means ±SE; arrows indicate group comparisons. Dashed arrow indicates meta-regression analysis using NAFLD as the moderator variable. fPG, fasting plasma glucose; HOMA, homoeostatic model assessment-insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; Tchol, total cholesterol; BMI, body mass index; WC, waist circumference; ALT and AST, alanine and aspartate aminotransferase; GGT, gamma-glutamyltransferase

4.2 | Limitations and strengths at study, outcome, and review level

Some limitations to our study should be noted, which are implicit in the studies included in the meta-analysis. Specifically, there was a significant heterogeneity in the overall results, primarily stemming from the data pertaining to studies conducted in Japan and Korea. The observed heterogeneity might be attributed to several factors, including heterogeneity in the main outcome of interest (hepatic steatosis) that might account for a certain level of clinical diversity. Furthermore, characteristics of the studies (eg, methodological differences in the study design), or even differences at the population level (such as dietary and environmental factors), are certainly highly important variables that may explain heterogeneity of the dataset as a whole.

Specifically, the analysis that combines hospital-based and population-based studies could be potentially problematic as they are inherently heterogeneous. In fact, the former include patients with more severe NAFLD and higher metabolic burden. We addressed this issue by stratifying the analysis by study design; however, heterogeneity remains in the large majority of the studied outcomes (Figures S14-S18).

Unfortunately, as the authors of a large majority of studies included in the review did not report the findings for male and female patients separately, we were unable to perform stratification of the results by sex. Consequently, potential presence of sexual dimorphism could not be explored.

Finally, when conducting database searches, we were unable to have access to Embase, which is accessed by subscribed users only. Nevertheless, we would not expect Embase retrieve additional studies not already covered by PubMed/Medline in this specific and relatively novel subject.

The main strength of this study, however, stems from the large sample size that was subjected to the analyses (7904 patients with NAFLD, 1966 of whom were lean and 5938 were obese, who were compared to 15 973 non-NAFLD controls, comprising of 9946 lean and 6027 obese subjects). Likewise, we were able to integrate the data related to the risk factors noted in ethnically diverse populations, specifically Asians and Caucasians. We observed a remarkable consistency in the magnitude and direction of the effects of all the components of the Metabolic Syndrome, in particular waist circumference (Figure 5), which suggests that clinical characteristics of lean NAFLD patients are not necessarily different in Asians from other ethnic groups.

In addition, the comparison between lean patients with NAFLD and lean controls allowed us to perform a unique analysis of the magnitude of the estimated risks participants of low or normal weight are exposed to. This point is of particular relevance, as performing a comparison of risk factors related to lean and obese patients with NAFLD, while interesting, would have resulted in failing to recognise the paradox that lean patients with NAFLD are, in fact, neither that "lean" nor necessarily metabolically healthy.

Nonetheless, the comparative analysis of lean vs obese patients with NAFLD allowed us to estimate the strength of the risk effects, which were significantly higher in the latter group. Interestingly, although these differences were similar between lean and obese subjects without NAFLD, NAFLD seems to be a modifier of differences in the circulating lipid levels. However, we were unable to ascertain whether the overall differences noted between lean and obese patients affect the natural history of the disease. As shown in the elegant and well-designed cohort study conducted by Leung et al., non-obese NAFLD patients tend to have a less severe form of the disease and may thus have a better prognosis relative to obese patients.[26] Nonetheless, there is some evidence that lean patients with NAFLD might have more liver inflammation and shorter survival than their overweight or obese counterparts,[39] though complete details of this study have not yet been published. Large-scale long-term observations of representative cohorts are, however, needed to convincingly prove or refute these assumptions.

4.3 | Implications for clinical practice and future research

The results of this meta-analysis showed that lean patients with NAFLD, while classified as non-obese according to the accepted BMI cut-off values, did show a significant 1.24±0.26-unit increment in the BMI when compared to lean non-NAFLD controls. More importantly, lean patients with NAFLD had a significantly higher (5.88±0.40 cm) waist circumference than that observed in the lean non-NAFLD group. Together, these findings suggest that, in concert with abnormal glucose control, abdominal (central) obesity could be the key mediator of the pathogenesis of NAFLD in lean subjects, as ~ 6 cm difference in the waist circumference may cause an adverse impact on the clinical phenotype. This conclusion is not surprising, as there is substantial evidence showing not only a highly predictive value of abdominal obesity in the risk of metabolic disorders and cardiovascular disease, but also supporting the cause-and-effect link between abdominal obesity and insulin resistance.[40]

The idea that lean patients with NAFLD preferentially store fat in the liver rather than adipose tissue is interesting and should be pursued in future research. In fact, fat accumulation in non-adipose tissues, such as the liver, has been regarded as a key feature distinguishing metabolically healthy from metabolically abnormal subjects. What factors exactly determine the "fat" trafficking from adipose tissue to the liver, however, are not entirely known. Some hypotheses support the notion of impaired adipose tissue expandability, which could be associated with genetic factors.[41] A future research direction would be also studying NAFLD patients that do not have features of Metabolic Syndrome.[42]

Finally, the findings yielded by this meta-analysis have some highly relevant clinical implications. First, they indicate that lean subjects should be screened early to detect NAFLD,[38] particularly if they present with abdominal obesity. Second, the lean-NAFLD paradox should not prevent patients from pursuing proper lifestyle management, including weight control and physical activity. The close association among lean-NAFLD, abdominal obesity, insulin resistance and cardiovascular disease also suggests that normal body weight

should not prevent doctors from early pharmacological intervention, if needed.

In conclusion, our study shows that lean-NAFLD is not much different from the traditional Metabolic Syndrome-associated NAFLD; most of these cases met indeed the criteria of the Metabolic Syndrome albeit being lean.

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AUTHORSHIP

Guarantor of the article: SS.

Author contribution: SS and CJP designed the study, performed the statistical analysis, analysed and interpreted the data, and prepared and wrote the manuscript. Both authors have read and approved the final manuscript.

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SUPPORTING INFORMATION

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