



Submitted: 25.03.2022
Accepted: 14.04.2022
Early publication date: 16.08.2022

Endokrynologia Polska
DOI: 10.5603/EPa2022.0067
ISSN 0423-104X, e-ISSN 2299-8306

Circulating irisin in nonalcoholic fatty liver disease: an updated meta-analysis

Shanhu Qiu^{1,2}, Qianqian Wang³, Xue Cai⁴, Zilin Sun³, Tongzhi Wu⁵

¹Department of Endocrinology, Shenzhen People's Hospital, The Second Clinical Medical College of Jinan University, The First Affiliated Hospital of Southern University of Science and Technology, Shenzhen, China

²Research and Education Centre of General Practice, Zhongda Hospital, Southeast University, Nanjing, China

³Department of Endocrinology, Zhongda Hospital; Institute of Diabetes, School of Medicine, Southeast University, Nanjing, China

⁴Department of Nursing Management, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, China

⁵Adelaide Medical School and Centre of Research Excellence (CRE) in Translating Nutritional Science to Good Health, The University of Adelaide, Adelaide, Australia

Abstract

Introduction: Exogenous administration of recombinant irisin may reverse hepatic steatosis and steatohepatitis. However, it remains controversial as to whether nonalcoholic fatty liver disease (NAFLD) shows reduced circulating (serum/plasma) irisin levels. A meta-analysis was conducted to address this issue.

Material and methods: A literature search of databases was performed up to June 2021. Observational studies that reported circulating irisin in NAFLD ascertained by any methods (e.g. ultrasonography or magnetic resonance) and compared with any controls were eligible for inclusion. Standardized mean differences (SMDs) and 95% confidence intervals (CIs) were obtained using a random-effects meta-analysis model.

Results: Eleven studies enrolling 1277 NAFLD cases and 944 non-NAFLD controls were included. The approaches used for NAFLD ascertainment included ultrasonography (4 studies), magnetic resonance (3 studies), and liver biopsy (5 studies). Meta-analysis showed that circulating irisin in NAFLD was comparable to any non-NAFLD controls (10 studies with 11 datasets; SMD -0.09, 95% CI: -0.48 to 0.29), including the body mass index (BMI)-matched and lean controls (both $p \geq 0.80$). Restricting studies to NAFLD ascertained by magnetic resonance or liver biopsy rather than ultrasonography showed that serum irisin was reduced in NAFLD (5 studies, SMD -0.63, 95% CI: -1.14 to -0.13). Meta-analysis also suggested that circulating irisin did not differ between mild and moderate-to-severe NAFLD (7 studies; SMD 0.02, 95% CI: -0.25 to 0.30), and this association was not significantly moderated by study location (Europe versus Asia).

Conclusions: Circulating irisin in NAFLD did not differ from any non-NAFLD controls and was unlikely to be affected by disease severity or racial-ethnic difference.

Key words: irisin; nonalcoholic fatty liver disease; meta-analysis; liver biopsy

Introduction

Nonalcoholic fatty liver disease (NAFLD) has emerged as a worldwide public health challenge, affecting approximately 25% of the global population [1]. It may progress from simple steatosis to nonalcoholic steatohepatitis, cirrhosis, and hepatocellular carcinoma, and is associated with increased risk of diabetes, cardiovascular disease, and all-cause mortality [1–4]. Although physical inactivity, abdominal obesity, insulin resistance, and inflammation are recognized as relevant drivers for the development of NAFLD [3–5], its pathogenesis and the mechanisms underlying its progression are not fully understood, limiting the therapeutic options for NAFLD [4, 6].

Irisin, a myokine that is regulated by peroxisome proliferator-activated receptor γ coactivator-1 α and triggered by exercise stimuli [7–9], is proven to modulate systemic metabolism by stimulating browning and improving insulin resistance [7, 10, 11]. Moreover, recent animal studies have suggested that exogenous administration of recombinant irisin and overexpression of irisin-encoding gene (fibronectin type III domain-containing protein 5) may reverse hepatic steatosis and steatohepatitis partly via restoring autophagy impairment and fatty acid oxidation and preventing cytokine-mediated apoptosis of hepatocytes [12, 13]. These have underlined the therapeutic potential of irisin for metabolic disorders including NAFLD, and stimulated the investigation on potential changes



Zilin Sun, Department of Endocrinology, Zhongda Hospital; Institute of Diabetes, Southeast University, Nanjing, 210009, China, tel: (+86) 02583262813; e-mail: sunzilin1963@126.com

Shanhu Qiu, Shenzhen People's Hospital, The Second Clinical Medical College of Jinan University, Shenzhen, China; Research and Education Centre of General Practice, Zhongda Hospital, Southeast University, China, tel: (+86) 02583262815; e-mail: tigershanhu@126.com

of irisin secretion in patients with NAFLD. However, studies that explored circulating (serum/plasma) irisin levels in NAFLD showed highly contradictory results [13–23], with some noting lower levels in patients with NAFLD, and others showing comparable or even higher levels versus controls. The differences in the ascertainment approaches for NAFLD (liver biopsy versus ultrasonography), the selection of controls [body mass index (BMI)-matched versus unmatched], and the ELISA kits used for irisin measurement represent substantial concerns in interpreting the association between irisin and NAFLD [24]. A recent meta-analysis of 5 observational studies by Hu et al. showed that circulating irisin levels were higher in NAFLD than healthy controls and in the mild NAFLD group than in the moderate-severe group in Asians [25]. However, methodological concerns have been raised recently regarding its inclusion of a small number of studies and the ways of handling heterogeneity [24].

Given these, we conducted an updated meta-analysis to investigate circulating irisin levels in NAFLD versus controls, along with exploration of the sources of heterogeneity.

Material and methods

Search strategy

This meta-analysis was conducted based on the Meta-analysis of Observational Studies in Epidemiology reporting guidelines (Supplementary File — Tab. S1), and it adhered to a pre-designed but unpublished protocol with reference to the study by Cai et al. [26]. A systematic literature search was performed in databases including PubMed, Scopus, and Cochrane Library up to 1 Jan 2021, which was updated on 23 June 2021. The searching terms/words included “irisin, FNDC 5” and “nonalcoholic fatty liver disease, steatosis”. The detailed search strategy is provided in Table S2. We also manually checked reference lists from eligible studies for other relevant publications.

Inclusion criteria

Studies eligible for inclusion should meet the following criteria: (i) were cross-sectional, case-control, or cohort studies; (ii) NAFLD was confirmed by any methods (e.g. ultrasonography, computed tomography [CT], magnetic resonance [MR], or liver biopsy); (iii) compared with non-NAFLD controls or within different disease stages (of NAFLD); (iv) reported outcomes on circulating (serum/plasma) irisin measured by enzyme-linked immunosorbent assay (ELISA) kits; and (v) published in English-language. Studies were excluded if they: (i) were reviews, meta-analysis, meeting abstracts, or commentaries; (ii) employed ultrasonography to diagnose

NAFLD; or (iii) did not provide adequate information to abstract data on irisin.

Data extraction

The following information from eligible studies were collected using a pre-designed Excel form: first author, publication year, study origin, study design, proportion of males, sample sizes of NAFLD (cases) and control, blood samples and ELISA kits used for irisin measurement, irisin levels [means and standard deviations (SDs)], methods for NAFLD ascertainment, and the means of age, BMI, fasting blood glucose (FBG), fasting insulin, homeostasis model assessment for insulin resistance (HOMA-IR), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) between groups.

If the SDs of irisin were not provided, they were calculated or imputed based on the following approaches: (i) averaging the width of the interquartile ranges by 1.35; (ii) multiplying standard error by the square root of the corresponding sample size; or (iii) dividing the width across minimum and maximum data by 4 [26, 27]. Moreover, medians were considered equal to means. For studies that reported irisin outcomes in figures, data were collected using the Engauge Digitizer software (Version 10.10, Free Software Foundation, Boston, Massachusetts, USA) [28]. All the data were collected by one investigator (S.Q.) and checked by another one (W.Q.). Disagreements, if they occurred, were resolved by discussion with a third author (X.C.).

Quality assessment

Methodological quality for each included observational study was assessed by 2 investigators (S.Q. and X.C.) with reference to the Newcastle-Ottawa Scale, which assigns a maximum of 4 stars for selection category, 2 for comparability category, and 3 for exposure category. In addition, methodological quality was also rated based on the details reported on irisin measurement, which majorly included the detection sensitivity, and the intra- and inter-assay variation of the ELISA kits used.

Statistical analysis

Because the ELISA kits used for irisin measurement varied and showed substantially different reference values, standardized mean differences (SMDs) and 95% confidence intervals (CIs) were chosen as the summary estimates [24], which were obtained using the random-effects meta-analysis model (which better accounts for heterogeneity than the fixed-effects meta-analysis model [27]).

To explore circulating irisin in NAFLD, we employed the following approaches. Firstly, meta-analyses were

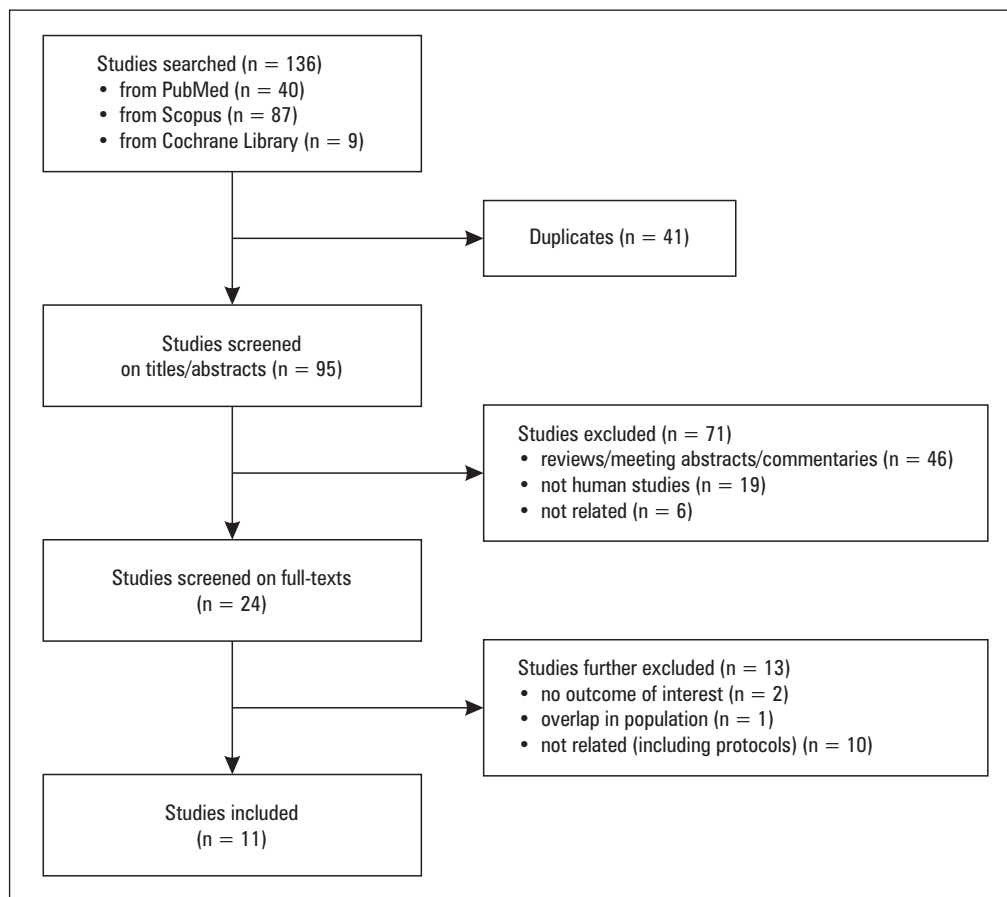


Figure 1. Search flowchart

performed to assess the differences of irisin in NAFLD versus controls, where we combined different categories of NAFLD or different controls into a single group to overcome the unit-of-analysis error [27], whenever possible. Secondly, analyses were restricted to studies using different controls, which included BMI-adjusted controls or lean controls. Finally, analyses were conducted to explore whether irisin differed between different NAFLD groups stratified by disease severity (termed as mild versus moderate-to-severe groups). Moreover, subgroup and meta-regression analyses based on study location (Europe *vs.* Asia), ascertainment approaches (ultrasonography *vs.* MR versus liver biopsy), blood samples (serum *vs.* plasma), sex difference (proportions of males), and clinical markers including the averages of BMI, FBG, fasting insulin, HOMA-IR (log-transformed), ALT, and AST, were performed to explore the sources of heterogeneity. Sensitivity analyses upon the removal of each study individually or the inclusion of only studies that employed CT, MR, or liver biopsy to ascertain NAFLD were conducted to assess the robustness of our outcomes.

In this meta-analysis heterogeneity was quantified using I^2 statistic, with its value higher than 50% indica-

tive of substantial heterogeneity [27]. Publication bias was assessed by Begg test and Egger tests, with $p < 0.10$ being considered significant [27, 29]. All analyses were conducted using Stata 14.0 (StataCorp LP, College Station, TX).

Results

Literature search

The literature search yielded a total of 136 citations (40 from PubMed, 87 from Scopus, and 9 from Cochrane Library). After excluding 41 duplicates and 84 citations based on title/abstract and/or full-text with the reasons listed in Figure 1, 11 studies (6 case-control and 5 cross-sectional) were included [13–23].

Study characteristics

The characteristics of included studies are summarized in Table 1. A total of 1277 NAFLD cases and 944 non-NAFLD controls were included. There were 4 studies ascertaining NAFLD by ultrasonography [20, 21, 23, 30], 3 by MR [16, 17, 23], and 5 by liver biopsy [13, 15, 18, 19, 22]. However, no studies used CT for NAFLD ascertainment. The mean age and BMI of enrolled par-

Table 1. Characteristics of included studies

Author (year), country	Groups	Sample size (n)	Age [years]	BMI [kg/m ²]	Male (%)	Blood sample	NAFLD confirm	Irisin ELISA kit used
Canivet (2020), France ^a	Lean control	3	44.0	21.0	0	Serum	Liver biopsy	Not specified
	Obese control	7	35.3	43.1	14.3			
	Steatosis	13	36.7	43.3	18.8			
	NASH	9	41.3	44.3	77.3			
Choi (2014), South Korea	Control	271	44.4	22.4	20.7	Serum	US	R&D, Minneapolis
	Mild NAFLD	47	47.1	24.9	38.3			
	Moderate-severe NAFLD	37	49.2	26.6	54.1			
Metwally (2019), Australia ^a	Steatosis: Grade 1	83	48.0	32.0	50.0	Serum	Liver biopsy	Phoenix Pharmaceuticals
	Steatosis: Grade 2-3	69	In total	In total	In total			
Montserrat-Mesquida (2020), Spain	Obese control: IFC 0	30	52.5	32.4	45.5	Plasma	MR	Cell Biolabs
	NAFLD: IFC 1	35	53.5	33.0	50.0			
	NAFLD: IFC 2–3	35	51.7	34.3	59.3			
Moreno-Perez (2018), Spain ^a	Control	24	42.0	24.8	100	Serum	MR	Phoenix Pharmaceuticals
	NAFLD	48	In total	In total	100			
Petta (2017), Italy [†]	Control	18	45.3	29.3	67.9	Serum	Liver biopsy	Phoenix Pharmaceuticals
	NASH	94	In total	In total	In total			
Polyzos (2014), Greece	Lean control	24	54.2	25.3	16.7	Serum	Liver biopsy	Phoenix Pharmaceuticals
	Obese control	28	52.6	30.9	28.6			
	Steatosis	15	53.9	31.9	33.3			
	NASH	16	53.9	34.1	18.8			
Rizk (2016), Egypt	Control	20	44.3	22.9	50.0	Serum	US	BioVendor, Bmo
	Obese control (with MS)	20	45.5	32.5	35.0			
	NAFLD (with MS)	20	47.9	35.9	45.0			
Shanaki (2017), Iran	Control	40	52.4	24.0	100	Plasma	US	BioVendor, Brno
	NAFLD	41	51.6	29.0	100			
	Control (with T2D)	41	57.1	26.9	100			
	NAFLD (with T2D)	40	53.9	29.3	100			
Waluga (2019), Poland	Control	25	42.0	22.2	44.0	Serum	Liver biopsy	BioVendor, Bmo
	NAFLD	25	31.0	31.1	48.0			
Zhang (2013), China	Control: IHTG Q1	74	53.1	27.1	34.0	Serum	MR, US	Aviscera Biosciences
	NAFLD: IHTG Q2	74	54.7	27.5	26.0			
	NAFLD: IHTG Q3	74	55.0	28.1	32.0			
	NAFLD: IHTG Q4	74	53.1	28.6	39.0			

^aInformation on participants were imputed using all the data available. BMI — body mass index; NAFLD — nonalcoholic fatty liver disease; US — ultrasonography; NASH — nonalcoholic steatohepatitis; MR — magnetic resonance; MS — metabolic syndrome; T2D — type 2 diabetes; IFC — intrahepatic fat content; IHTG — intrahepatic triglyceride

Participants ranged from 31.0 to 57.1 years and from 21.0 to 44.3 kg/m², respectively. Participants with NAFLD were generally overweight or obese, with their mean BMI over 25 kg/m².

About one half of the included studies (6 in 11 studies) were conducted in European countries, and most of them used fasting serum samples (9 in 11 studies) for irisin measurement by ELISA kits according to the protocols from the manufactures. The ELISA kits

used in the included studies were mainly purchased from the Phoenix Pharmaceuticals and BioVendor, and their intra- and inter-assay variations for these kits were provided in general. All studies provided adequate information for the diagnosis of NAFLD and the definition of controls. However, most of them neither reported whether NAFLD cases were consecutively selected or representatively chosen, nor provided the non-response rates for cases or controls.

Circulating irisin in NAFLD vs. non-NAFLD controls

Ten studies with 11 datasets compared circulating irisin in NAFLD ($n = 1125$) versus any non-NAFLD controls ($n = 944$) [13, 14, 16–23] (Fig. 2A). Meta-analysis showed that circulating irisin was comparable between NAFLD and controls (SMD -0.09 , 95% CI: -0.48 to 0.29 , $I^2 = 92\%$). Subgroup and meta-regression analyses revealed that circulating irisin in NAFLD versus controls could not be significantly moderated by study location, type of blood sample, ascertainment approach, sex difference,

or clinical variables including BMI, FBG, fasting insulin, HOMA-IR, ALT, and AST (all $p > 0.12$). Further analyses suggested that circulating irisin was comparable in NAFLD versus BMI-matched [13, 16, 19, 21, 23] or lean controls [13, 14, 19–22], with the SMD being 0.04 (95% CI: -0.25 to 0.32 , Fig. 2B) and -0.12 (95% CI: -1.36 to 1.12 , Fig. 2C), respectively. Sensitivity analysis upon the removal of each individual study or by restricting studies to those ascertained NAFLD by only MR or liver biopsy (6 studies; SMD -0.44 , 95% CI: -0.96 to 0.09 , $I^2 = 87\%$; Supplementary File — Fig. S1) showed

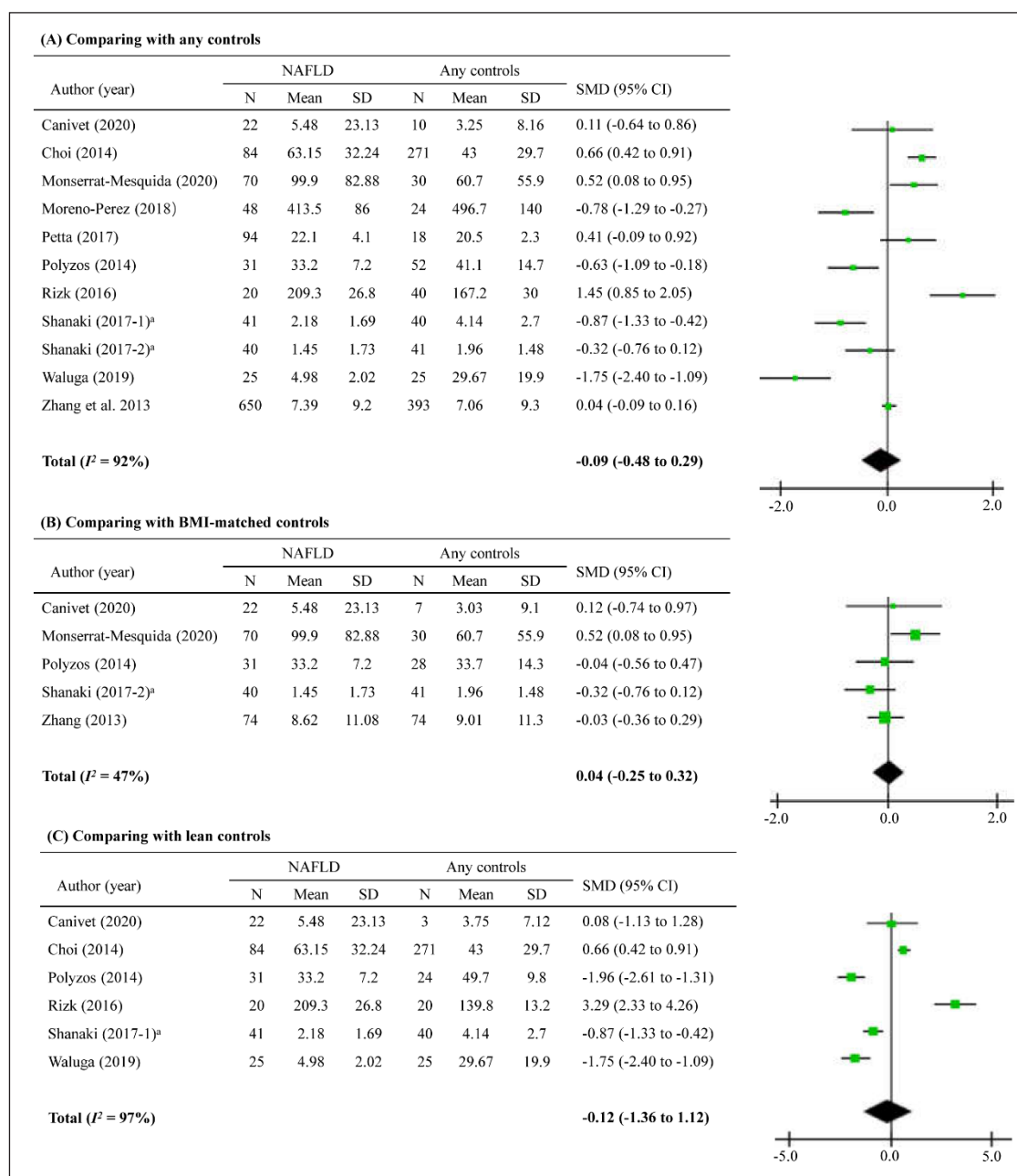


Figure 2. Circulating irisin in nonalcoholic fatty liver disease versus controls. **A.** Comparing with any controls; **B.** Comparing with BMI-matched controls; **C.** Comparing with lean controls. ^aIt had 2 individual datasets, with “-1” compared with healthy controls and “-2” with type 2 diabetes. NAFLD — nonalcoholic fatty liver disease; SMD — standardized mean difference; CI — confidence interval; SD, standard deviation; BMI — body mass index

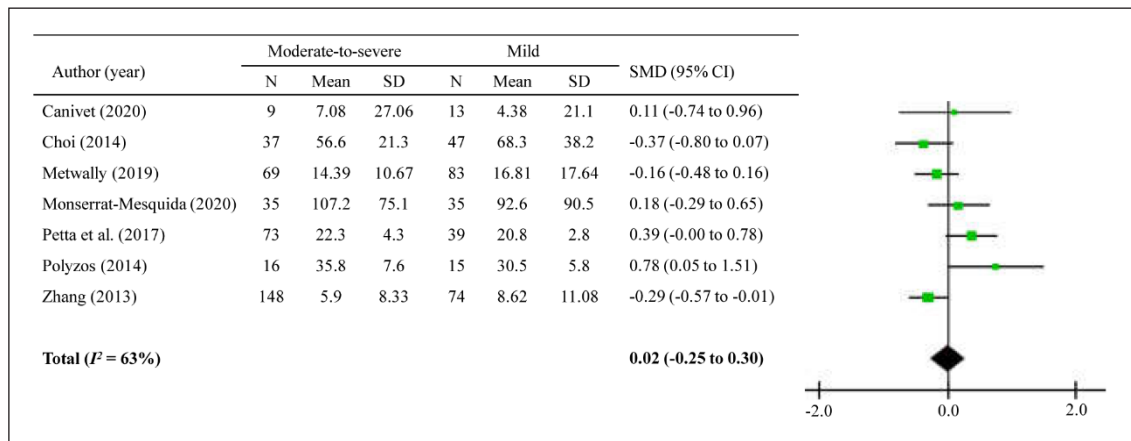


Figure 3. Circulating irisin in nonalcoholic fatty liver disease at different stages. SMD — standardized mean difference; CI — confidence interval; SD — standard deviation

comparable results to the primary ones. However, this reduction became significant when irisin was measured in serum (5 studies, SMD -0.63 , 95% CI: -1.14 to -0.13 , Fig. S2). No evidence of publication bias was detected by Egger's test ($p = 0.51$) or Begg's test ($p = 0.35$).

Circulating irisin by different stages of NAFLD

Seven studies reported circulating irisin in different stages of NAFLD ($n = 693$) [13–16, 18, 19, 23], with most of them suggesting a null difference. Pooled results showed that circulating irisin did not differ between mild and moderate-to-severe NAFLD (SMD: 0.02 , 95% CI: -0.25 to 0.30 , $I^2 = 63%$, Fig. 3), and this association was unaffected by study location or ascertainment approaches (both $p \geq 0.33$). Sensitivity analysis after removing each study individually showed that the results remained minorly changed.

Discussion

Main findings

Our meta-analysis, which comprised 11 observational studies with more than 1200 patients with NAFLD, showed that circulating irisin in NAFLD was comparable to non-NAFLD controls, regardless of the ascertainment approaches for NAFLD or the differences in study location (indicative of racial-ethnic difference). Moreover, disease severity of NAFLD had little impact on circulating irisin.

Interpretations

A previous meta-analysis conducted in 2020, which included 5 individual studies of about 430 participants with NAFLD, showed that circulating irisin in NAFLD was comparable to controls with a weighted mean difference of 7.51 (95% CI: -12.53 to 27.56) ng/mL, but became higher when restricting studies to Asians [25].

However, that study only targeted healthy or lean controls, resulting in a very small number of eligible studies and increased risk of selection bias. Moreover, the authors did not take into consideration the variabilities in the reference ranges of the ELISA kits used for irisin measurement, raising the concern of whether the methodological approach was appropriate to obtain the accurate summary effect size [24]. As an attempt to address these issues, we conducted this updated meta-analysis, which enrolled 6 more studies and approximately 800 more cases with NAFLD, and employed SMDs as the summary effect size to account for the variations in the ELISA kits and the differences in the ascertainment approaches for NAFLD.

Our updated meta-analysis partly confirms the results reported in the previous meta-analysis that circulating irisin was comparable between NAFLD and controls [25]. However, as demonstrated by our subgroup and meta-regression analyses, we did not find any strong evidence that study location (e.g. Europe versus Asia) may affect irisin levels in NAFLD versus non-NAFLD controls, indicative of no racial-ethnic difference of irisin in NAFLD. Moreover, in line with most of the included individual studies [13, 15, 16, 23], we also did not detect any significant difference in irisin levels between different disease stages of NAFLD. This contrasts with the outcome that irisin was higher in mild NAFLD than in moderate-severe NAFLD in Asians [25], and therefore does not support the concept of “irisin resistance” — irisin may increase in a compensatory manner to counteract metabolic disturbances at the early stage [31, 32], or “irisin failure” — irisin may decrease due to the failure of compensation at the late stage [19].

Although ultrasonography is deemed to be an acceptable diagnostic technique for NAFLD [33], its accuracy was challenged due to its subjective interpretation of examination outcome and low sensitivities

(< 70%) when compared with CT, MR, or liver biopsy [30, 34]. However, our meta-regression and sensitivity analyses suggested that such differences were unlikely to affect irisin levels in NAFLD versus controls. Yet interestingly, we found that serum irisin was significantly reduced in NAFLD ascertained by MR or liver biopsy only, compared with controls. This implies that the choice of serum or plasma for irisin measurement is a confounding factor in interpreting irisin outcomes in NAFLD. Furthermore, in addition to the ascertainment approaches, our meta-analysis also showed that irisin levels in NAFLD versus controls could not be significantly moderated by clinical variables such as BMI, FBG, or HOMA-IR, which are suggested to be associated with irisin levels [31, 32, 35].

Strengths and limitations

The strengths of our meta-analysis include a large sample size and the use of a series of analyses to explore the sources of heterogeneity. However, our study should be interpreted with some caution. First, our study cannot prove the causality, and the observed heterogeneity could not be explained by a single factor like age, HOMA-IR, or liver enzymes. Second, there is evidence that obesity and adipose tissue may participate in the regulation of circulating irisin in addition to skeletal muscle [7, 19]. It remains unclear whether it is NAFLD itself or its comorbidities such as obesity or diabetes that affects circulating irisin, while most of the included studies failed to control for such factors when making comparisons. Moreover, data on lifestyle factors such as physical activity and nutrient intake were not well reported, which may also influence the comparison of irisin levels in NAFLD versus controls [8, 36, 37]. Third, although irisin has been proven to exist [38], the accuracy of the ELISA kits used for its detection remains unclear and needs to be validated against mass spectrometry [39, 40]. Finally, we did not search for grey literature such as doctoral dissertation, which may introduce the risk of publication bias. Moreover, the inclusion of only studies written in English language may incur selection bias.

Conclusions

In conclusion, our updated meta-analysis did not provide adequate evidence that circulating irisin in NAFLD differed from any non-NAFLD controls, including the lean ones, nor was it affected by disease severity or racial-ethnic differences. Well-designed prospective cohort studies documenting factors affecting circulating irisin, such as physical activity, nutrient intake, and obesity, are required to confirm our findings.

Acknowledgements

This work was partly supported by the Guangdong Basic and Applied Basic Research Foundation (Grant No. 2020A1515111021) and the National Natural Science Foundation of China (Grant No. 61844024). Shanhu Qiu has been supported by the "Best Young Scholars" Fellowship from Southeast University. The funders played no role in the design of the study nor in the collection, analysis, and interpretation of data or in writing the manuscript.

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