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Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis

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ABSTRACT

Hypoadiponectinemia might represent a risk factor for nonalcoholic fatty liver disease (NAFLD). We performed a systematic review and meta-analysis to evaluate the serum total adiponectin levels in patients with simple nonalcoholic fatty liver (NAFL), those with nonalcoholic steatohepatitis (NASH), and controls. Data were extracted from PubMed, EMBASE, and Cochrane Central Register of Controlled Trials electronic databases (up to December 2009). The main outcome was the weighted mean differences (WMDs) in adiponectin between comparison groups. Twenty-eight studies were included in the systematic review. A meta-analysis of 27 studies that reported data on 2243 subjects (698 controls and 1545 patients with NAFLD) was performed. Controls had higher serum adiponectin compared with NAFL patients (12 studies, random-effects WMD [95% confidence interval {CI}] = 3.00 [1.57-4.43], I^2 = 80.4%) or NASH patients (19 studies, random-effects WMD [95% CI] = 4.75 [3.71-5.78], I^2 = 84.1%). The NASH patients demonstrated lower adiponectin compared with NAFL patients (19 studies, random-effects WMD [95% CI] = 1.81 [1.09-2.53], I^2 = 71.7%). By performing a meta-regression analysis, body mass index, age, sex, and type 2 diabetes mellitus failed to account for heterogeneity. However, the performance of liver biopsy on controls had significant effect on the outcome and accounted for 76.7%, 85.5%, and 22.8% of the between-study variance for comparisons between controls vs NAFLD, NAFL, and NASH patients, respectively. Based on liver histology, serum adiponectin levels are similar in NAFL patients and controls, but hypoadiponectinemia may play an important pathophysiological role in the progression from NAFL to NASH.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease, affecting nearly 30% of the general population in the Western world. Its incidence in both adults and children is rising, in conjunction with the growing epidemics of obesity and type 2 diabetes mellitus (T2DM) [1,2]. The histologic spectrum of NAFLD encompasses a wide spectrum of liver damage ranging from simple nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) and NASH-related cirrhosis with its complications [3]. Nonalcoholic fatty liver and NASH cannot currently be differentiated by clinical or laboratory tests, and liver biopsy continues to be the criterion standard. The natural history of NAFLD depends on the histologic subtype. Nonalcoholic fatty liver has a generally benign long-term prognosis. Only a minority of patients with NAFL develops advanced liver disease (progression to cirrhosis in 3%), but this condition is causing increasing alarm because of its marked prevalence. In contrast, NASH is a progressive fibrotic disease and a leading cause of cryptogenic cirrhosis. Nonalcoholic steatohepatitis–related cirrhosis may have a similar prognosis to cirrhosis of other causes, leading to liver failure and hepatocellular carcinoma and may recur after transplantation. Cirrhosis and liver-related death in NASH patients occur in 20% and 12%, respectively, over a 10-year period. In addition to higher liver-related morbidity and fatality, patients with NAFLD appear to have a higher all-cause mortality [1,2].

Although the pathogenesis of NAFLD remains elusive, insulin resistance (IR) seems to play a key role, so that NAFLD is considered the hepatic component of the metabolic syndrome [4]. The prevailing theory of the pathogenesis of NAFLD is the "multihit hypothesis." The first hepatic insult (hit) is the dysregulation of fatty acid metabolism, which leads to simple steatosis (NAFL). The initial hit renders hepatocytes susceptible to secondary insults that follow ("multiple hits"), which finally lead from NAFL to NASH or even cirrhosis [5].

Adipose tissue has recently emerged as an endocrine gland by producing multiple proteins, collectively referred to as *adipocytokines*. Apart from adipocytokines, typical cytokines like tumor necrosis factor (TNF)–α, interleukin (IL)-6, IL-1, IL-8, and IL-18 are secreted by inflammatory cells infiltrating adipose tissue [6]. Adipocytokines are considered to play an important role in the pathogenesis of the metabolic syndrome, including NAFLD, because they alter insulin sensitivity in insulin-targeted organs, such as the skeletal muscle and the liver. Adipocytokines' alterations are involved in the "multiple-hit" process, and their imbalance plays an important role in both the development NAFL and the progression from NAFL to NASH [5].

In this setting, there is increasing evidence for the role of adiponectin in NAFLD. Adiponectin is the most abundant and adipose-specific adipocytokine. Contrary to other adipocytokines, adiponectin is paradoxically increased with decreasing fat mass [7]. Two different adiponectin receptor isoforms, AdipoR1 and AdipoR2, have been described to date [8]. Adiponectin has been proposed to play a role in linking nutrition and insulin sensitivity with the immune system and inflammation [9]; it exhibits anti-inflammatory, antiatherogenic, and antidiabetic properties. Circulating concentrations of adiponectin are determined primarily by genetic factors, nutrition, exercise, and abdominal adiposity. Adiponectin circulates in multimers, including high–molecular weight (HMW), medium–molecular weight (MMW), and low–molecular weight (LMW) adiponectin. The HMW adiponectin has been proposed to be the biologically more active form of adiponectin and to have a stronger association with IR and cardiovascular disease [10]. Adiponectin is linked to other adipocytokines and cytokines in a complicated communication network, which is only partially elucidated [5]. For example, the roles of adiponectin and TNF-α are antagonistic; adiponectin inhibits the expression, secretion, and action of TNF-α, thereby improving insulin sensitivity, whereas TNF-α suppresses adiponectin transcription, secretion, and action, thereby aggravating IR [11]. Another example is the adiponectin–IL-18 cross talk; adiponectin reverses IL-18–mediated endothelial cell death, thereby possibly diminishing IL-18–dependent vascular injury and inflammation [12]. These are only simple examples of the continuous and complicated relationship among the different beneficial and detrimental adipocytokines/cytokines, which reflect in IR and NAFLD [5].

In the liver, adiponectin is considered to have insulin-sensitizing, antifibrogenic, and anti-inflammatory properties by acting on hepatocytes, hepatic stellate cells, and hepatic macrophages (Kupffer cells), respectively [7]. It acts through the activation of 5-adenosine monophosphate–activated protein kinase and peroxisome proliferator-activated receptor–α pathways and inhibition of toll-like receptor-4–mediated signaling [7,10]. As a result, modifications of genes expression occur, which subsequently lead to decreased gluconeogenesis, decreased free fatty acid influx into the liver, increased free fatty acid oxidation, and decreased de novo lipogenesis. Apart from the metabolic consequences, adiponectin has antifibrotic action in the liver, mainly through down-regulating the expression of aldehyde oxidase, transforming growth factor and connective tissue growth factor, and anti-inflammatory action by suppressing TNF-α and other proinflammatory cytokines and by inducing anti-inflammatory cytokines, such as IL-10 [7].

In clinical terms, hypoadiponectinemia might represent an independent risk factor for NAFLD and has been associated with IR and NAFLD [13]. Lower serum adiponectin is generally believed to be associated with more advanced histologic subtypes of NAFLD and has been proposed by some authors as a noninvasive marker of NAFLD [14,15]. However, controversy exists in the literature; some authors have reported similar serum adiponectin levels between NAFL and NASH [16-24], between NAFL and controls [15,16,21,25,26], or even between NASH and controls [21,27,28].

The objective of this systematic review was to evaluate serum total adiponectin levels in patients with NAFL and NASH and to summarize the results in a meta-analysis. A definite answer to whether serum adiponectin decreases with advancing NAFLD severity could provide useful information for the pathogenesis and prognosis of NAFLD. Furthermore, it could be the basis for experimental and clinical trials investigating the use of recombinant adiponectin [29] or treatment approaches that up-regulate adiponectin in NASH [7].

2. Methods

2.1. Search strategy

We performed a computerized literature search in PubMed and EMBASE electronic databases and Cochrane Central Register of Controlled Trials. Search was not limited by publication time and not restricted to English literature. Medical Subject Headings (MeSH) database was used as a terminological search filter. From the combination of terminological (MeSH terms) and methodological search filters ("PubMed clinical queries"), relevant journal articles were retrieved [30]. More specifically, after a preliminary search of terms, we formatted our query, which was "(adiponectin [MeSH] OR apM1 OR AdipoQ OR Acrp30 OR GBP28 OR GBP-28) AND ((fatty liver) [MeSH] OR hepatitis [MeSH] OR (liver cirrhosis) [MeSH] OR steatosis OR steatohepatitis OR (non-alcoholic fatty liver) OR (nonalcoholic fatty liver) OR (non-alcoholic steatohepatitis) OR (nonalcoholic steatohepatitis) OR NAFLD OR NASH OR (fatty liver disease) OR FLD OR (steatorrhoeic hepatosis))" limited to "Humans" (last update July 31, 2009).

The bibliographic search was extended to the "Related Articles" link next to each selected article in PubMed and its references. Finally, automatic alerts (up to December 31, 2009) [query 1: "adiponectin [MeSH] OR apM1 OR AdipoQ OR Acrp30 OR GBP28 OR GBP-28"; query 2: "(fatty liver) [MeSH] OR hepatitis [MeSH] OR (liver cirrhosis) [MeSH] OR steatosis OR steatohepatitis OR (non-alcoholic fatty liver) OR (nonalcoholic fatty liver) OR (non-alcoholic steatohepatitis) OR (nonalcoholic steatohepatitis) OR NAFLD OR NASH OR (fatty liver disease) OR FLD OR (steatorrhoeic hepatosis)"] were activated in PubMed ("My NCBI") to add relevant articles published after the initial search.

Two reviewers (SAP and KAT) independently assessed the extracted data (titles, abstracts, references, and full-text articles). Chance-adjusted interrater agreement was calculated using Cohen *k* statistics and found to be satisfactory. Any discrepancy was solved by consultation of a third reviewer not involved in the initial procedure (JK).

2.2. Eligibility of relevant studies

Studies of any design reporting serum total adiponectin levels in biopsy-proven adults with NAFLD were suitable for the systematic review and meta-analysis. Further classification in NAFL or NASH were mainly based on Brunt et al [31] or NAFLD activity score (NAS) [32] systems. Studies enrolling a percentage of patients with NASH-related cirrhosis within the NASH group were not excluded; however, when possible, data without cirrhotic patients were retrieved. Liver biopsy on healthy individuals, who served as controls, was not an inclusion criterion because it may meet ethical considerations. Controls were chosen on the basis of normal liver ultrasound results and serum aminotransferases levels, in most studies.

Studies were excluded from the systematic review and the meta-analysis if (1) enrolled patients had a disease other than NAFLD) (ie, alcoholic fatty liver disease, viral or autoimmune hepatitis), were exclusively cirrhotic, or were not subjected to liver biopsy; (2) there were patient overlaps; (3) additional data were needed but corresponding authors did not provide them; (4) studies had serious methodological flaws; (5) studies were interventional, and there were not different comparison groups at baseline (ie, control vs NAFL, NAFL vs NASH); or (6) studies were reviews, editorials, case reports, letters to the editor, hypotheses, studies on animals or cell lines, abstracts from conferences, or unpublished studies.

2.3. Outcomes

Comparison groups were (1) controls vs NAFLD patients, (2) controls vs NAFL patients, (3) controls vs NASH patients, and (4) NAFL patients vs NASH patients. The main outcome of the meta-analysis was the weighted mean difference (WMD) in adiponectin across comparison groups. The investigation of the effect of differences between comparison groups in age, BMI, percentage of male subjects, and percentage of patients with T2DM served as secondary outcomes.

2.4. Statistical analysis

Adiponectin levels across comparison groups were extracted as mean difference ± standard deviation (SD). When not reported, missing mean differences and SDs were estimated on the basis of reported confidence interval (CI), where available and confirmation was sought through communication with the authors. Standard error of the mean was transformed into SD. When arithmetic mean and corresponding SD were not available for NAFLD group, SD (pooled variance) was calculated by means and sample sizes from NAFL and NASH groups and SD by the formula $SD^2_{NAFLD} = [(n_{NAFL} - 1)\ sd^2_{NAFL} + (n_{NASH} - 1)\ sd^2_{NASH}]/(n_{NAFL} + n_{NASH} - 2)$, where n is the sample size and sd is the standard deviation.

Weighted mean differences (WMD) in adiponectin were calculated for all eligible studies for the meta-analysis and combined random-effects model [33]. Heterogeneity between the results of different studies was examined by I^2 test (I^2 >50%, significant heterogeneity; I^2 = 25%-50%, moderate heterogeneity; I^2 <25%, insignificant heterogeneity), which can be interpreted as the percentage of total variation across several studies due to heterogeneity [34]. To assess the extent of publication bias, Egger test was used [35].

A priori hypotheses to explain potential heterogeneity across studies included possible effect modifiers, such as BMI, age, and percentages of male subjects and T2DM patients, chosen on the basis of biological plausibility. Difference in study design regarding the definition of controls (ascertainment of nonexposure), performed either by interventional (liver biopsy) or noninterventional methods (liver ultrasound), was also considered as a potential source of heterogeneity. To obtain study-level measures to reflect the relative difference in BMI, age, and percentages of male subjects and patients without T2DM between comparison groups, ratios of mean value in the first group (control or NAFL) to that in the second (NAFLD, NAFL, or NASH) were calculated for continuous variables (BMI, age); and ratios of the percentage in the first group to that in the second were calculated for male subjects

and patients without T2DM. The percentage of patients without T2DM was preferred over that of those with T2DM to avoid null values because many studies used T2DM mellitus as an exclusion criterion. No calculation was needed for the performance of liver biopsy in controls.

The effect of study-level characteristics on adiponectin levels was checked through restricted maximum likelihood (REML)–based random-effects meta-regression analysis. To stabilize variance, all values of study-level characteristics were treated with logarithmic transformation. Univariate meta-regression analyses were performed first. Variables significant at the .1 level were entered into the multivariable model. Nonsignificant covariates ($P > .05$) in the multivariable model were deleted. To gauge an approximate estimation of the exploratory capacity of the model, the percentage of between-study variance explained by the model (τ_{exp}) was estimated, as elsewhere described [36].

Meta-analysis was conducted using Stata/SE 10.0 for Windows (StataCorp, College Station, TX). The report of the study was complemented in adherence with the Meta-analysis Of Observational Studies in Epidemiology group standards for reporting meta-analysis of observational studies [37].

3. Results

3.1. Search results

We initially identified 655 potentially relevant articles in the database search. A flowchart summarizing search results and identification of eligible studies is provided in Fig. 1. Twenty-one of them were initially included in the systematic review. Furthermore, automatic alert up to December 31, 2009, provided 7 more articles eligible for the systematic review (total number of eligible studies = 28). One article was excluded from the meta-analysis [38] because the methodology used for serum adiponectin measurement was largely different from that used in other studies.

3.2. Systematic review

The 28 included studies were published between 2004 and 2009 and reported data on 2309 subjects (709 controls and 1600 NAFLD patients) [14-28,38-50]. The main characteristics of included studies are summarized in Table 1. Eleven studies were held in Europe, 9 in Asia, 7 in America, and 1 in Australia. Twenty-six were cross-sectional studies, and 2 were randomized controlled trials (RCTs). Only baseline characteristics (before intervention) from both the control and NASH groups were used from the RCTs. Histologic classification was based on the classification proposed by Brunt et al [31] in 20 studies, NAS in 4 studies [32], and Younossi et al in 3 studies [15] and was not available in 1 study. Biopsy on controls was performed on 5 studies, and data were not available in 6 studies. Serum adiponectin was measured by enzyme-linked immunosorbent assay (ELISA) in 15 studies, radioimmunoassay (RIA) in 12 studies, and chemiluminescence in 1 study.

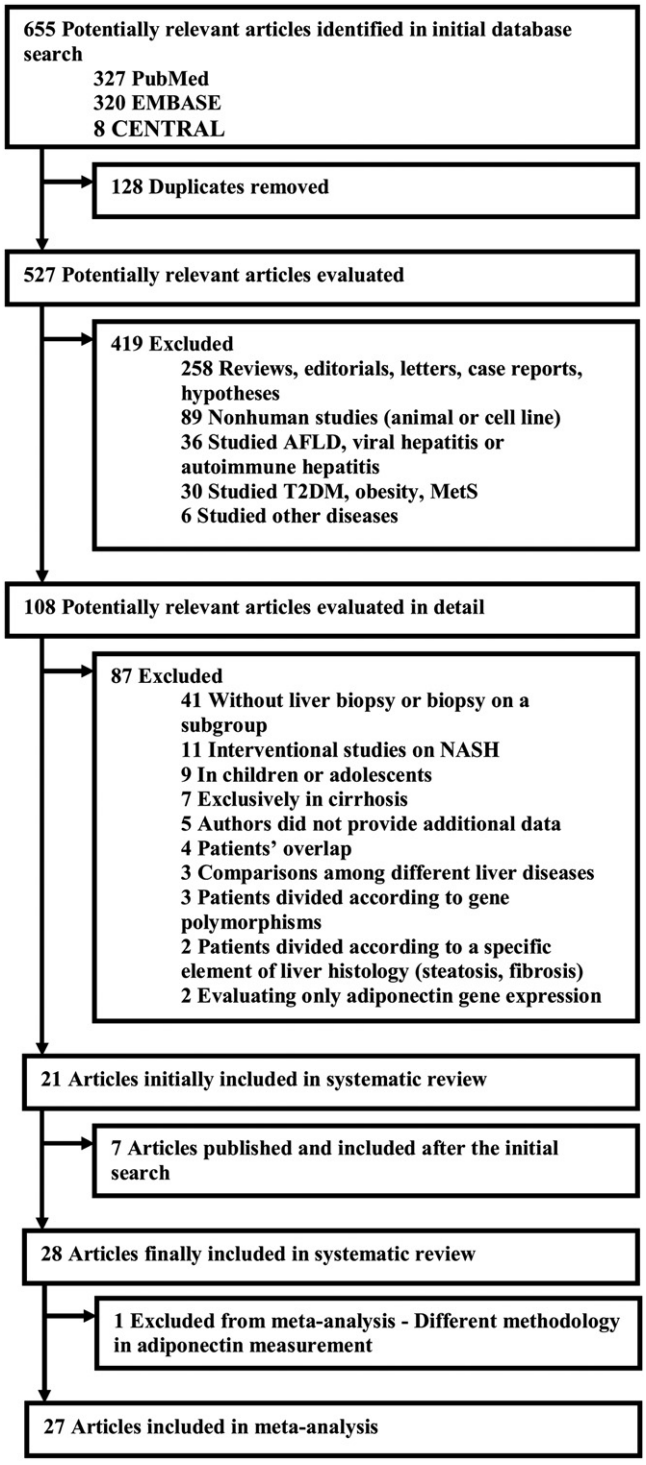

Fig. 1 – Identification of eligible studies.

Table 2 shows the main clinical and biochemical data per group of each study included in the systematic review. Data for NAFLD were omitted when they did not provide additional information, that is, when there was no control or NAFL or NASH group. Apart from serum adiponectin, serum levels of aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transpeptidase, as well as the homeostatic model assessment of insulin resistance (HOMA-IR) index, are summarized.

Table 1 – Main characteristics of studies included in the systematic review

First author, year, origin [a]	Study design	Method of a diponectin measurement	Histologic definition	Biopsy on controls	Patients with cirrhosis (n)	Additional information
Abdelmalek, 2009, USA [38]	RCT	Chemiluminescence	NAS	No	12	
Argentou, 2009, Greece [16]	Cross-sectional	RIA	NAS	Yes	0	All patients were subjected to bariatric surgery.
Arvaniti, 2009, Greece [40]	Cross-sectional	RIA	NAS	NA	NA	A group of 38 patients with NAFLD without biopsy was excluded.
Aygun, 2006, Turkey [41]	Cross-sectional	ELISA	Brunt	No	0	A group of 20 patients with NAFLD without biopsy was excluded.
Bugianesi, 2005, Italy [17]	Cross-sectional	RIA	Brunt	No	0	A group of 56 patients with NAFLD without biopsy was excluded; 3 patients with NASH-related cirrhosis were excluded.
Gastaldelli, 2009, USA [42]	RCT	RIA	NAS	No	0	
Haukeland, 2006, Norway [43]	Cross-sectional	RIA	Brunt	No	1	
Huang, 2007, Taiwan [18]	Cross-sectional	RIA	Brunt	NA	0	All patients were subjected to bariatric surgery.
Hui, 2004, Australia [19]	Cross-sectional	RIA	Brunt	No	0	16 patients with NASH-related cirrhosis were excluded.
Hyogo, 2007, Japan [44]	Cross-sectional	RIA	Brunt	No	0	
Jarrar, 2008, USA [25]	Cross-sectional	ELISA	Younossi	Yes	NA	Another group of 12 nonobese without NAFLD (not biopsy-proven), used as second control group, was excluded.
Kaser, 2005, Austria [20]	Cross-sectional	RIA	Brunt	NA	0	All patients were subjected to bariatric surgery.
Kitade, 2009, Japan [45]	Cross-sectional	ELISA	NA	NA	NA	
Lemoine, 2009, France [21]	Cross-sectional	ELISA	Brunt	Yes	2	Controls were subjected to surgery for benign liver tumors.
Lesmana, 2009, Indonesia [27]	Cross-sectional	ELISA	Brunt	No	2	
Munoz, 2009, Mexico [28]	Cross-sectional	ELISA	Brunt	No	10	
Musso, 2005, Italy [39]	Cross-sectional	ELISA	Brunt	No	0	
Nannipieri, 2009, Italy [26]	Cross-sectional	ELISA	Brunt	Yes	0	All patients were subjected to bariatric surgery or minor elective surgery.
Pagano, 2005, Italy [22]	Cross-sectional	RIA	Brunt	No	NA	
Palekar, 2006, USA [23]	Cross-sectional	RIA	Brunt	NA	0	
Shimada, 2007, Japan [14]	Cross-sectional	ELISA	Brunt	NA	0	Patients with fibrosis stage 3-4 were excluded.
Sun, 2008, China [46]	Cross-sectional	ELISA	Brunt	No	NA	
Targher, 2006, Italy [47]	Cross-sectional	ELISA	Brunt	No	0	
Targher, 2008, Italy [48]	Cross-sectional	ELISA	Brunt	No	0	Another group of 45 nonobese without NAFLD (not biopsy-proven), used as second control group, was excluded.
Vuppalanchi, 2005, USA [49]	Cross-sectional	RIA	Younossi	No	0	
Wong, 2006, China [24]	Cross-sectional	ELISA	Brunt	No	1	
Yalniz, 2006, Turkey [50]	Cross-sectional	ELISA	Brunt	No	0	
Younossi, 2008, USA [15]	Cross-sectional	ELISA	Younossi	Yes	NA	Another group of 32 patients with NAFLD, used as validation cohort, was initially blinded and was excluded. Data from the ongoing EPI-NAFLD study

NA indicates not available.
[a] References are presented in first-author order.

Table 2 – Main clinical and biochemical characteristics per group of studies included in the systematic review

First author[a]	Group	n (male)	Overt diabetes mellitus (n)	Age (y)	BMI (kg/m^2)	WC (cm)	AST (IU/L)	ALT (IU/L)	GGT (IU/L)	HOMA-IR	Serum total adiponectin (μg/mL)
Abdelmalek [38]	Control	11 (7)	0	44.3 ± 11.4	24.9 ± 4.9	NA	20.8 ± 3.3	14.0 ± 8.6	NA	NA	54.9 ± 50.4
	NAFL[b]										
	NASH	55 (20)	18	47.1 ± 11.5	33.6 ± 5.9	NA	67.7 ± 34.2	96.0 ± 50.3	NA	NA	25.0 ± 25.2[*]
	NAFLD[b]										
Argentou [16]	Control	9 (2)	2	37.1 ± 9.8	55.2 ± 8.6	136 ± 8	16.7 ± 6.4	17.1 ± 8.2	20.1 ± 6.2	4.3 ± 2.2	5.70 ± 1.31
	NAFL	31 (9)	5	38.1 ± 9.2	56.3 ± 8.5	152 ± 16[*]	24.1 ± 8.1	33.1 ± 15.6	44.7 ± 76.1	5.5 ± 3.0	4.65 ± 2.08
	NASH	10 (6)	5	41.4 ± 9.1	58.0 ± 7.0	160 ± 15[*]	30.1 ± 17.0[*]	52.3 ± 26.6[*,†]	94.9 ± 104.7	9.8 ± 6.2[*,†]	3.47 ± 2.59
	NAFLD	41 (15)	10	38.9 ± 9.2	56.7 ± 8.1	154 ± 16[*]	25.6 ± 11.0[*]	37.7 ± 20.3[*]	57.0 ± 85.4[*]	6.6 ± 4.4[*]	4.36 ± 2.24[*]
Arvaniti [40]	Control[b]										
	NAFL	18 (9)	NA	49.2 ± 12.6	29.9 ± 3.8	99 ± 12	44.0 ± 21.9	61.6 ± 37.3	84.0 ± 52.4	3.6 ± 3.5	10.7 ± 4.8
	NASH	25 (13)	NA	45.7 ± 15.6	29.5 ± 3.8	99 ± 11	38.2 ± 13.8	55.0 ± 24.8	68,0 ± 64.7	4.7 ± 3.5	6.6 ± 4.7[*]
	NAFLD[b]										
Aygun [41]	Control	20 (10)	0	45.8 ± 59.9	28.3 ± 21.5	NA	17.5 ± 19.2	19.9 ± 32.6	15.6 ± 20.6	1.8 ± 0.9	7.74 ± 19.7
	NAFL[b]										
	NASH[b]										
	NAFLD	29 (15)	4	46.4 ± 55.5	30.6 ± 18.3	NA	44.9 ± 149.7[*]	71.9 ± 162.1[*]	66.4 ± 248.8[*]	7.1 ± 6.5[*]	4.99 ± 11.3[*]
Bugianesi [17]	Control	42 (34)	2	43 ± 11	27.8 ± 3.5	94 ± 9	19 ± 5	19 ± 6	22 ± 16	2.3 ± 1.2	10.70 ± 5.89
	NAFL	44 (42)	0	38 ± 10	27.0 ± 2.3	NA	36 ± 14[*]	74 ± 32[*]	36 ± 14[*]	3.4 ± 1.8[*]	5.44 ± 2.12[*]
	NASH	69 (64)	7[†]	43 ± 11[†]	27.6 ± 3.8	NA	42 ± 18[*]	89 ± 42[*,†]	42 ± 18[*]	4.6 ± 3.2[*,†]	5.60 ± 2.33[*]
	NAFLD	113 (106)	7	41 ± 11	27.3 ± 3.3	100 ± 7[*]	39 ± 16[*]	81 ± 38[*]	40 ± 17[*]	4.0 ± 2.6[*]	5.52 ± 2.22[*]
Gastaldelli [42]	Control	20 (6)	0	46 ± 13.4	28.6 ± 4.9	NA	19.6 ± 3.5	17.6 ± 3.2	NA	1.2 ± 0.6	13.5 ± 6.3
	NAFL[b]										
	NASH	47 (21)	22[*]	51 ± 6.9[*]	33.4 ± 4.8[*]	NA	45.1 ± 15.4	64.5 ± 29.2	65.3 ± 69.7	4.8 ± 3.9	6.5 ± 3.4[*]
	NAFLD[b]										
Haukeland [43]	Control	30 (16)	0	41.8 ± 9.1	23.1 ± 2.2	NA	NA	25.0 ± 11.2	NA	NA	12.0 ± 5.3
	NAFL	22 (9)	5[*]	48.4 ± 11.9	28.9 ± 3.7[*]	NA	46.6 ± 23.6	90.0 ± 52.8[*]	235.4 ± 471.9	2.16 ± 0.89	8.4 ± 4.6[*]
	NASH	25 (15)	9[*]	45.3 ± 10.7	31.6 ± 5.0[*]	NA	64.0 ± 27.6[†]	113.8 ± 65.8[*]	111.4 ± 77.5	2.58 ± 1.34	5.6 ± 2.9[*,†]
	NAFLD	47 (24)	14[*]	46.7 ± 11.3	30.4 ± 4.6[*]	NA	55.9 ± 27.0	102.4 ± 60.7[*]	169.5 ± 329.7	2.37 ± 1.14	6.9 ± 4.0[*]
Huang [18]	Control[b]										
	NAFL	23 (8)	2	28.2 ± 8.8	44.9 ± 4.7	125 ± 15	29.7 ± 19.6	49.1 ± 45.6	27.7 ± 14.1	4.27 ± 2.35	5.19 ± 1.78
	NASH	88 (25)	19	29.6 ± 8.6	45.5 ± 5.9	125 ± 14	34.2 ± 20.5	50.7 ± 38.7	40.1 ± 31.2	4.51 ± 3.91	4.88 ± 1.65
	NAFLD[b]										
Hui [19]	Control[d]	82 (53)	7	48.7 ± 11.7	29.8 ± 3.9	97 ± 13	NA	20.3 ± 11.6	28.1 ± 13.5	2.5 ± 1.6	12.5 ± 7.6
	NAFL[d]	29 (20)	3	41.7 ± 12.3	29.9 ± 3.9	100 ± 11	NA	69.3 ± 35.0	88.2 ± 83.3	3.4 ± 1.8	8.3 ± 4.8
	NASH[d]	64 (42)	18	48.2 ± 12.6	30.9 ± 4.4	105 ± 15	NA	89.1 ± 47.3	106.2 ± 107.0	5.2 ± 2.3	6.0 ± 4.4
	NAFLD[d]	93 (62)	21	46.2 ± 12.8	30.6 ± 4.2	102 ± 12	NA	82.9 ± 44.6	100.7 ± 100.2	4.6 ± 2.3	6.7 ± 4.6
Hyogo [44]	Control	30 (16)	0	48.4 ± 11.9	22.7 ± 2.2	NA	22.0 ± 6.0	24.0 ± 8.0	37.0 ± 39.9	NA	NA
	NAFL	10 (8)	1	43.6 ± 11.4	26.3 ± 1.1	NA	31.5 ± 17.9	60.4 ± 47.2	76.4 ± 28.0	1.81 ± 0.79	7.72 ± 3.81
	NASH	66 (46)	20	50.1 ± 12.4	27.2 ± 3.7	NA	52.8 ± 32.3	93.3 ± 67.9	89.4 ± 100.7	3.3 ± 2.16	4.97 ± 1.96[*]
	NAFLD	76 (54)	21	49.3 ± 12.4	27.1 ± 3.5	NA	49.9 ± 31.5	89.0 ± 66.3	87.7 ± 94.3	3.1 ± 2.1	5.29 ± 2.27
Jarrar [25]	Control	38 (5)	NA	40 ± 10	47.5 ± 9.4	NA	18.7 ± 3.9	21.7 ± 7.5	NA	NA	10.2 ± 7.4
	NAFL	19 (2)	NA	37 ± 9	47.2 ± 7.5	NA	20.6 ± 8.1	24.4 ± 14.6	NA	NA	12.2 ± 7.6
	NASH	26 (11)	NA	44 ± 11	47.5 ± 8.3	NA	35.1 ± 25.3	46.0 ± 30.4	NA	NA	6.7 ± 6.5[†]
	NAFLD[c,d]	45 (13)		41 ± 10	47.4 ± 8.0		29.0 ± 18.1	36.9 ± 23.8			9.3 ± 7.5

Kaser [20]	Control[b]										
	NAFL	9 (2)	0	34 ± 12.9	47.9 ± 8.1	NA	21.4 ± 5.6	23.4 ± 4.8	27.6 ± 32.5	2.34 ± 2.61	6.9 ± 3.5
	NASH	13 (4)	0	44 ± 11.2	45.6 ± 4.9	NA	26.8 ± 16.1	41.2 ± 31.3	49.8 ± 27.8	2.32 ± 2.20	5.4 ± 3.3
	NAFLD[b]										
Kitade [45]	Control[b]										
	NAFL	11 (7)	NA	43.6 ± 14.1	25.8 ± 1.2	NA	37.5 ± 10.0	69.8 ± 31.2	64.2 ± 47.8	2.26 ± 0.96	8.25 ± 0.95
	NASH	28 (17)	NA	45.4 ± 14.7	28.5 ± 6.9[†]	NA	63.4 ± 27.0[†]	105.5 ± 65.7[†]	68.4 ± 37.1	5.79 ± 4.15[†]	5.06 ± 1.90[†]
	NAFLD[b]										
Lemoine [21]	Control	10 (8)	0	51 ± 11	23.7 ± 2.3	NA	NA	24 ± 15	NA	0.5 ± 0.4	11.0 ± 5.3
	NAFL	17 (10)	3	47 ± 12	27.1 ± 4.2[*]	NA	NA	54 ± 28[*]	NA	2.9 ± 2.5	9.9 ± 5.8
	NASH	57 (28)	25[*,†]	51 ± 12	29.3 ± 4.5[*,†]	NA	NA	84 ± 48[*,†]	NA	6.1 ± 5.1[*,†]	8.1 ± 5.1
	NAFLD	74 (38)	28[*]								
Lesmana [27]	Control	30 (14)	0	31.6 ± 7.4	22.2 ± 2.4	NA	19.1 ± 4.3	18.0 ± 8.4	NA	1.5 ± 0.49	5.85 ± 3.8
	NAFL[b]										
	NASH	30 (19)	5	42.5 ± 12.4[*]	27.2 ± 5.4[*]	NA	54.9 ± 35.8[*]	80.4 ± 62.2[*]	NA	3.69 ± 6.5[*]	4.43 ± 3.88[*]
	NAFLD[b]										
Munoz [28]	Control	49 (25)	0	38.5 ± 13.5	22.5 ± 1.8	NA	21.1 ± 7.3	26.1 ± 14.2	33.5 ± 27.2	NA	10.3 ± 6.5
	NAFL[b]										
	NASH	52 (33)	14[*]	40.6 ± 14.2	31.3 ± 5.7	NA	70.3 ± 72.8[*]	103.8 ± 83.2[*]	144.7 ± 150.7[*]	4.9 ± 4.6	8.7 ± 9.6[*]
	NAFLD[b]										
Musso [39]	Control	25 (23)	0	38 ± 10	25.2 ± 3.0	87 ± 10	26 ± 15	30 ± 20	41 ± 20	NA	11.55 ± 4.20
	NAFL[b]										
	NASH	25 (23)	0	37 ± 10	25.3 ± 1.0	90 ± 10	41 ± 15[*]	87 ± 35[*]	89 ± 90[*]	NA	5.48 ± 1.70[*]
	NAFLD[b]										
Nannipieri [26]	Control	17 (5)	0	41 ± 11	33.6 ± 10.7	NA	18 ± 8	21 ± 13	28 ± 17	2.6 ± 1.8	6.3 ± 3.7
	NAFL	52 (7)	11[*]	43 ± 11	46.3 ± 8.0[*]	NA	21 ± 7	23 ± 7	34 ± 58	2.5 ± 1.7	5.9 ± 4.3
	NASH	34 (13)	13[*]	40 ± 10	49.2 ± 6.4[*]	NA	39 ± 28[*]	52 ± 25[*]	50 ± 32[*]	4.3 ± 2.1[*,†]	3.9 ± 1.6[*]
	NAFLD[c,d]	86 (20)	24[*]	42 ± 11	47.4 ± 7.4	NA	28 ± 15	34 ± 14	40 ± 48	3.2 ± 1.9	5.1 ± 3.2
Pagano [22]	Control	20 (17)	0	42 ± 17.9	26.6 ± 4.5	NA	20 ± 4.5	21 ± 4.5	17 ± 18	1.95 ± 1.16	15.7 ± 7.2
	NAFL	9 (NA)	0	NA	NA	NA	34 ± 12.0[*]	71 ± 42.0[*]	99 ± 114[*]	3.30 ± 1.20[*]	6.2 ± 2.4[*]
	NASH	8 (NA)	0	NA	NA	NA	35 ± 5.7[*]	64 ± 19.8[*]	84 ± 93[*]	3.75 ± 1.70[*]	5.7 ± 1.4[*]
	NAFLD	17 (15)	0	44 ± 11.6	27.4 ± 3.1	NA	35 ± 11.6[*]	68 ± 31.0[*]	94 ± 101[*]	3.61 ± 2.13[*]	5.9 ± 1.7[*]
Palekar [23]	Control[b]										
	NAFL	39 (26)	12	47.4 ± 10.1	30.5 ± 4.0	NA	44.2 ± 22.3	67.3 ± 38.8	NA	NA	10.4 ± 5.1
	NASH	41 (16)[†]	15	54.2 ± 10.0[†]	34.2 ± 6.5[†]	NA	59.6 ± 36.9[†]	70.2 ± 30.1	NA	NA	10.7 ± 6.5
	NAFLD[b]										
Shimada [14]	Control[b]										
	NAFL	19 (10)	2	46.4 ± 15.6	25.9 ± 3.8	NA	48 ± 32	76 ± 62	78 ± 66	1.9 ± 1.9	6.0 ± 3.8
	NASH	66 (40)	35	49.1 ± 16.2	27.1 ± 3.6	NA	60 ± 34	99 ± 73	63 ± 61	3.4 ± 2.0[†]	3.6 ± 2.2[†]
	NAFLD[b]										
Sun [46]	Control	54 (NA)	NA	NA	21.6 ± 2.7	NA	NA	16.2 ± 4.5	NA	NA	10.2 ± 2.8
	NAFL	58 (NA)	NA	NA	31.1 ± 2.4[*]	NA	NA	26.2 ± 6.4[*]	NA	NA	6.7 ± 3.5[*]
	NASH	52 (NA)	NA	NA	32.8 ± 5.2[*]	NA	NA	87.7 ± 12.5[*,†]	NA	NA	4.3 ± 2.3[*,†]
	NAFLD[c,d]	110			31.9 ± 3.7			55.3 ± 9.3			5.6 ± 2.9
Targher [47]	Control	60 (40)	0	48 ± 3	26.0 ± 2.0	91 ± 5	21 ± 2	22 ± 3	20 ± 5	1.68 ± 1.0	13.8 ± 3.6
	NAFL[d]	10 (NA)	2	46 ± 2.5	25.8 ± 3.0	NA	31 ± 8	37 ± 10	40 ± 12	3.06 ± 1.8	8.0 ± 3.1
	NASH[d]	50 (NA)	9	48 ± 3.1	27.2 ± 2.1	NA	52 ± 28	122 ± 49	70 ± 31	5.18 ± 2.6	5.1 ± 2.5
	NAFLD	60 (40)	11[*]	47 ± 3	26.3 ± 2.0	95 ± 8[*]	46 ± 22[*]	105 ± 42[*]	54 ± 24[*]	4.11 ± 2.2[*]	6.1 ± 2.8[*]

(continued on next page)

Table 2 *(continued)*

First author[a]	Group	n (male)	Overt diabetes mellitus (n)	Age (y)	BMI (kg/m^2)	WC (cm)	AST (IU/L)	ALT (IU/L)	GGT (IU/L)	HOMA-IR	Serum total adiponectin (μg/mL)
Targher [48]	Control NAFL[b]	45 (45)	5	47 ± 2	26.7 ± 2	96 ± 3	22 ± 3	26 ± 3	29 ± 3	3.8 ± 2.1	11.0 ± 2.6
	NASH NAFLD[b]	45 (45)	6	47 ± 2	26.5 ± 2	96 ± 3	47 ± 24 *	100 ± 38 *	58 ± 20 *	4.9 ± 2.5 *	6.8 ± 2.4
Vuppalanchi [49]	Control NAFL[b]	19 (9)	0	43 ± 14	31 ± 4	NA	26 ± 12	27 ± 8	NA	3.2 ± 3.0	7.3 ± 3.5
	NASH NAFLD[b]	21 (10)	0	41 ± 13	33 ± 4.5	NA	75 ± 56 *	99 ± 77 *	NA	7.0 ± 5.4 *	4.9 ± 2.7 *
Wong [24]	Control	41 (17)	1	42 ± 10	24.1 ± 6.8	83 ± 9	24 ± 4	24 ± 9	26 ± 16	1.0 ± 0.7	7.9 ± 4.5
	NAFL	28 (19)	12	44 ± 9	27.1 ± 4.0	93 ± 10 *	37 ± 25 *	62 ± 47 *	94 ± 140 *	2.8 ± 2.9 *	4.5 ± 2.1 *
	NASH	52 (33)	34	45 ± 9	30.0 ± 5.0 *,†	98 ± 10 *,†	31 ± 18 *	38 ± 40 *,†	47 ± 43 *	3.0 ± 1.9 *	4.2 ± 2.6 *
	NAFLD	80 (52) *	46	45 ± 9	29.0 ± 4.8 *	95 ± 10 *	33 ± 20 *	47 ± 44 *	33 ± 20 *	2.9 ± 2.3 *	4.3 ± 2.4 *
Yalniz [50]	Control NAFL[b]	25 (10)	0	37.1 ± 7.8	24.1 ± 1.9	NA	19.6 ± 6.2	19.4 ± 4.5	NA	1.8 ± 0.7	17.3 ± 2.8
	NASH NAFLD[b]	37 (25)	5	40.1 ± 8.1	28.7 ± 3.3 *	NA	60.6 ± 34.6 *	114.9 ± 87.9 *	NA	7.0 ± 9.2 *	11.1 ± 2.1 *
Younossi [15]	Control	32 (13)	NA	39.3 ± 9.8	47.0 ± 9.1	NA	18.0 ± 3.7	21.9 ± 8.1	NA	2.1 ± 2.2	9.3 ± 6.3
	NAFL	15 (1)	NA	37.4 ± 8.3	45.7 ± 4.8	NA	19.9 ± 6.8	22.1 ± 12.2	NA	3.4 ± 2.3	12.1 ± 8.4
	NASH	22 (9)	NA	42.5 ± 10.4	48.2 ± 8.7	NA	35.9 ± 27.1 *,†	47.9 ± 32.1 *,†	NA	3.2 ± 7.0	6.1 ± 5.2 *,†
	NAFLD[c,d]	37 (10)	NA	40.4 ± 9.6	47.2 ± 7.1	NA	29.4 ± 19.0	37.4 ± 24.1	NA	3.3 ± 5.1	8.5 ± 6.5

Data are presented in numbers or mean ± SD. ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; NA, not available; WC, waist circumference.

[a] References are presented in first-author order.

[b] Group not existing in the study or without logical sense, that is, NAFLD data if there is no control group or if there is no NAFL or NASH group.

[c] Computed by formula.

[d] Statistical significance not reported in the study.

* $P < .05$ compared with control group.

† $P < .05$ between NAFL and NASH.

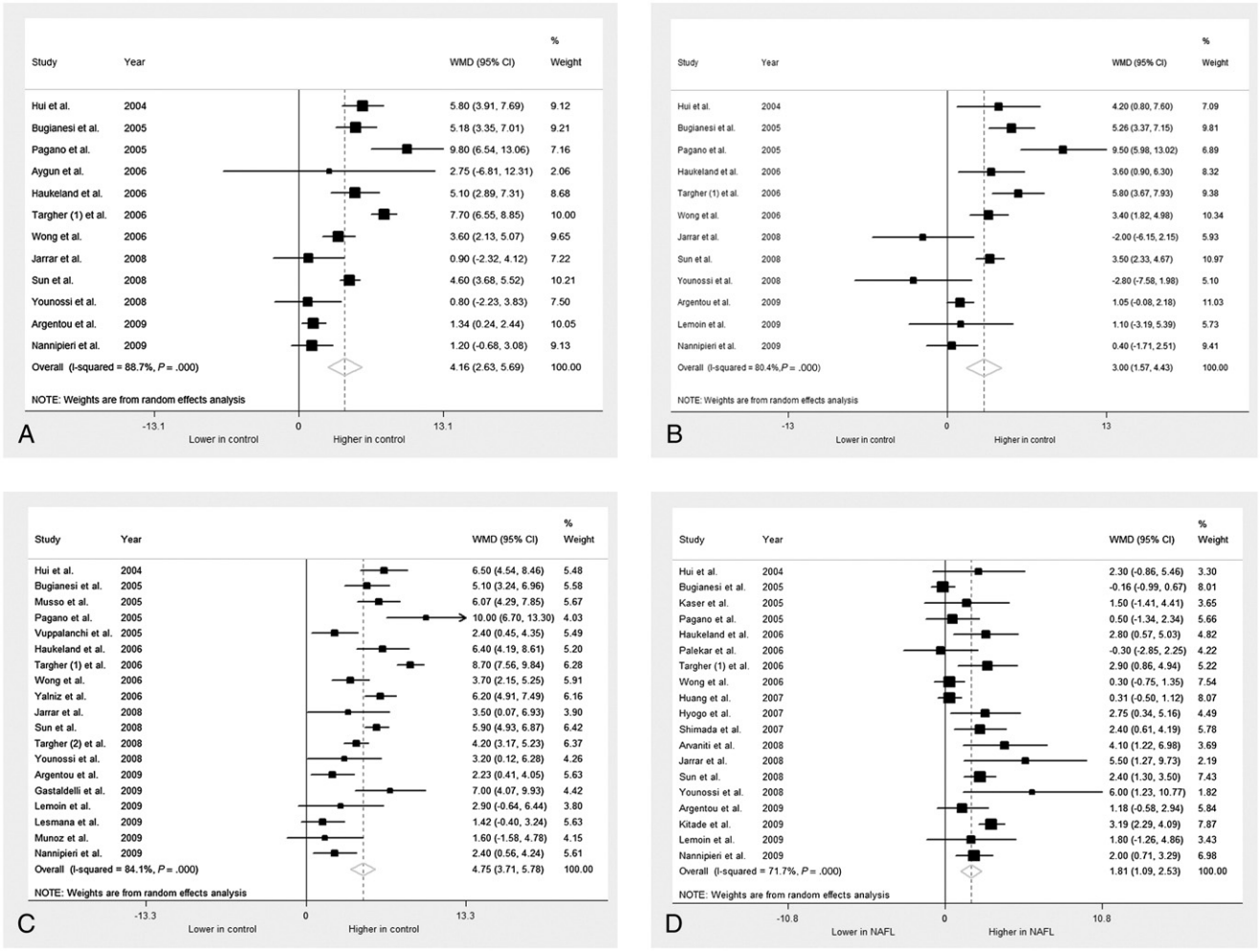

Fig. 2 – Quantitative synthesis of adiponectin levels between (A) controls and NAFLD patients, (B) controls and NAFL patients, (C) controls and NASH patients, and (D) NAFL and NASH patients.

3.3. *Meta-analysis*

A meta-analysis of 27 studies, which reported data on 2243 subjects (698 controls and 1545 patients with NAFLD), was performed [14-28,39-50]. Patients with NAFLD demonstrated significantly lower adiponectin values than controls, yet significant heterogeneity was present (12 studies, random-effects WMD [95% CI] = 4.16 [2.63-5.69], I^2 = 88.7%; Fig. 2A). No sign of publication bias was detected (Egger test, P = .863). Controls were found with significantly higher adiponectin levels when compared with patients with NAFL (12 studies, random-effects WMD [95% CI] = 3.00 [1.57-4.43], I^2 = 80.4%; Fig. 2B) or patients with NASH (19 studies, random-effects WMD [95% CI] = 4.75 [3.71-5.78], I^2 = 84.1%; Fig. 2C). Neither comparisons were found to be implicated with publication bias (Egger test P = .881 and .268, respectively).

Patients with NASH demonstrated significantly lower adiponectin values compared with patients with NAFL, also with significant heterogeneity across studies (19 studies, random-effects WMD [95% CI] = 1.81 [1.09-2.53], I^2 = 71.7%; Fig. 2D). Egger test failed to detect significant amount of publication bias (P = .062).

3.4. *Meta-regression*

To further investigate the impact of the predefined study-level characteristics on WMD in adiponectin, REML-based random-effects meta-regression analyses were performed for all planned comparisons. Weighted mean difference was used as the dependent variable. Performance of liver biopsy in controls and the ratios used to reflect the relative differences between comparison groups (in BMI, age, and percentages of male subjects and patients without T2DM) were entered as explanatory covariates.

These ratios failed to account for heterogeneity in any of the preplanned comparisons. However, the performance of liver biopsy in controls was found to have significant effect on the outcome (in those comparisons that involved controls) (P = .001, P = .02, and P = .042 for comparisons between controls vs patients with NAFLD, NAFL, and NASH, respectively; Table 3). Furthermore, it was found to account for the 76.74%, 85.54%, and 22.80% of the between-study variance, respectively. Subgroup analysis using the peformance of liver biopsy on controls as the subgroup factor is depicted in Fig. 3.

To visualize the direction and magnitude of effect of the significant covariate in the meta-regression model, a post hoc

Table 3 – Meta-regression model with the performance of liver biopsy on controls as the explanatory variable

Effect size	Coefficient	Standard error	t	P >| t |	95% CI
Comparison between controls and patients with NAFLD (12 studies)					
Biopsy on controls	4.53	1.00	4.51	.001	2.29-6.76
Constant	–3.39	1.75	–1.94	.081	–7.29 to 0.50
Fit of model without heterogeneity		Q (10 *df*)		31.50	
		Probability > Q		<.001	
Proportion of variation due to heterogeneity		I^2		0.683	
REML estimate of between-studies variance		τ^2		1.494	
Comparison between controls and patients with NAFL (12 studies)					
Biopsy on controls	4.40	1.02	4.30	.002	2.12-6.67
Constant	–4.17	1.75	–2.37	.039	–8.08 to 0.26
Fit of model without heterogeneity		Q (10 *df*)		18.72	
		Probability > Q		.044	
Proportion of variation due to heterogeneity		I^2		0.466	
REML estimate of between studies variance		τ^2		1.01	
Comparison between controls and patients with NASH (19 studies)					
Biopsy on controls	2.58	1.17	2.19	.042	0.10-5.06
Constant	0.18	2.14	0.09	.933	–4.33 to 4.70
Fit of model without heterogeneity		Q (17 *df*)		88.85	
		Probability > Q		<.001	
Proportion of variation due to heterogeneity		I^2		0.809	
REML estimate of between studies variance		τ^2		3.34	

analysis was performed, using this covariate as the subgroup factor. In those studies in which liver biopsy had been performed on controls, differences in adiponectin levels seem to be attenuated, whereas heterogeneity across them was absent.

4. Discussion

Our systematic review and meta-analysis of relevant studies showed that serum total adiponectin was higher in controls compared with NAFL or NASH patients and higher in NAFL compared with NASH patients independently of BMI, age, sex, and the presence of T2DM. However, adiponectin was similar between controls and NAFL patients when controls were subjected to liver biopsy.

The performance or not of liver biopsy on controls explained most of heterogeneity across studies in the comparisons between controls and NAFLD or NAFL patients, but it seems to have a smaller impact in the comparison between controls and NASH. However, the results of the subgroup analysis (Fig. 3) were unexpected. Defining "apparently healthy" controls by normal levels of serum aminotransferases or absence of fatty liver on ultrasound is subjected to selection bias because some individuals with normal levels of serum aminotransferases and absence of fatty liver on ultrasound may have NAFLD [51,52]. The logical impact of this bias is that the mean differences between NAFL or NASH and controls would be reduced in the subgroup where liver biopsy was not performed; on the contrary, our subgroup analysis indicated the opposite result. We have no solid explanation for this paradox; however, by reviewing the control groups of included studies, we considered the following: (1) Three [16,21,26] of 6 studies with liver biopsy on controls included patients subjected to bariatric or minor elective surgery or surgery for benign liver tumors, thereby committing a different selection bias. There are 2 more studies [18,20] whose controls were subjected to bariatric surgery, but it is unidentified if they were simultaneously subjected to liver biopsy. (2) Studies with liver biopsy on controls are relatively newer, especially in the comparisons between control and NAFLD or NAFL groups (Fig. 2). Initial studies usually overestimate statistical significances until newer studies—and usually better methodologically—give a less striking result.

Importantly, the statistically significant outcomes of this meta-analysis should cautiously be interpreted in terms of clinical practice. The estimated difference of 3 to 5 μg/mL in adiponectin between controls and NAFL or NASH or 2 μg/mL between NASH and NAFL (Fig. 2) might be clinically significant, considering that the range of serum adiponectin (measured by RIA or ELISA) was found to be about 5 to 10 μg/mL (Table 2). This means that higher adiponectin is associated with no or milder liver injury and vice versa. Based on the aforementioned finding, we can speculate that hypoadiponectinemia may play a pathophysiological role in the progression from NAFL to NASH (secondary hit), whereas its role in the development of NAFL (first hit), if any, is yet to be defined. There are increasing data supporting the antifibrogenic [53,54], anti-inflammatory [55], and antiapoptotic [56,57] effect of adiponectin in the liver, mediated mainly via activation of 5-adenosine monophosphate–activated protein

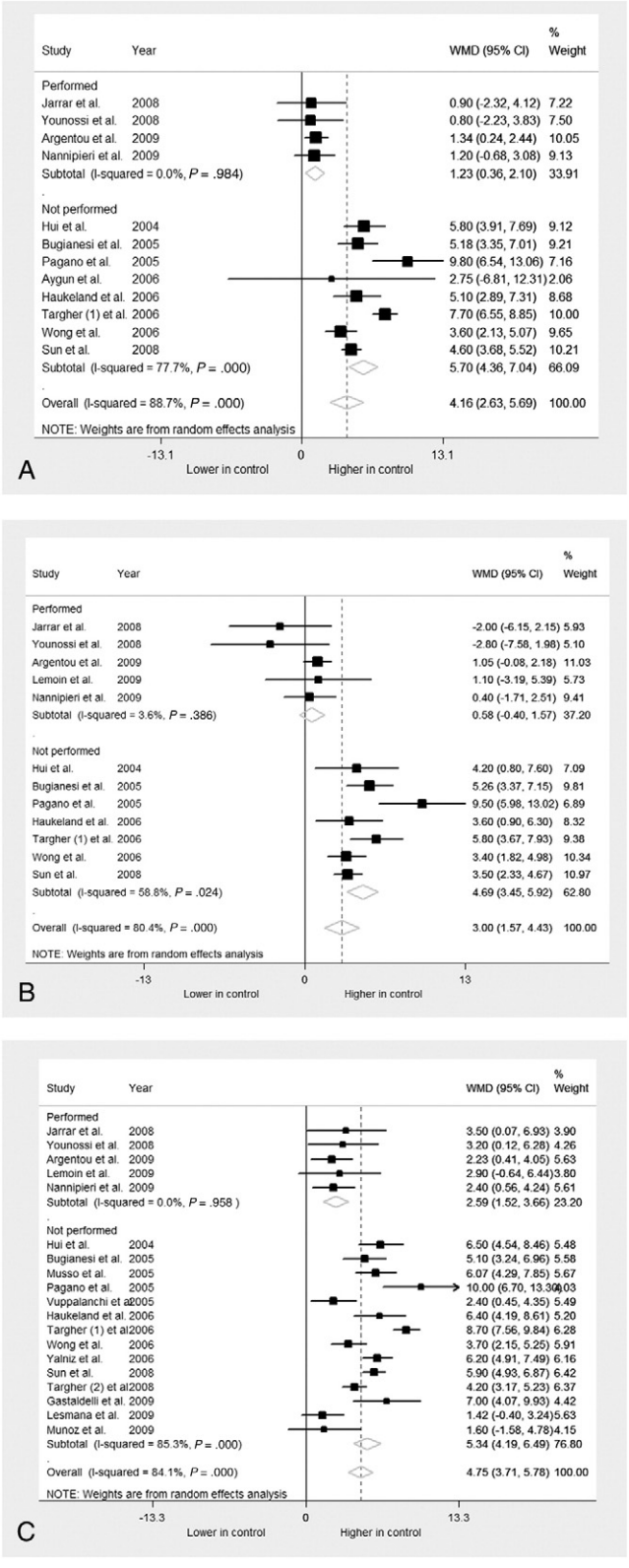

Fig. 3 – Quantitative synthesis using the peformance of liver biopsy on controls as the subgroup factor. Comparison between (A) controls and NAFLD patients, (B) controls and NAFL patients, and (C) controls and NASH patients.

kinase and peroxisome proliferator-activated receptor–α pathways [7,58]. Although adiponectin also decreases gluconeogenesis, de novo lipogenesis, and free fatty acid influx in the liver and increases free fatty acids β-oxidation [59], and thus hypoadiponectinemia has a potential role in the hepatic fatty acid metabolism dysregulation, it plays a secondary role in NAFLD pathogenesis, as it could be partially assumed from its total levels in the serum.

Although liver biopsy is considered the criterion standard for the assessment of liver fat, inflammation, and fibrosis, there is a need for less invasive diagnostic techniques to avoid the morbidities related to liver biopsy [52]. This meta-analysis cannot answer the question of whether serum total adiponectin could be used as a noninvasive marker of NAFLD, so as to submit only individuals with low serum adiponectin to liver biopsy; nor can it provide specific cutoff values. However, this study strengthens the potential role of adiponectin in the pathogenesis of NAFLD and provides claims for future studies evaluating the predictive and therapeutic potential of adiponectin in the NAFLD spectrum.

Serum HMW adiponectin rather than total adiponectin is strongly associated with IR, IR syndrome, and cardiovascular disease [10]; but the additional predictive value provided by HMW adiponectin in humans seems to be minimal, at least in patients with T2DM and impaired glucose tolerance [60]. Interestingly, HMW adiponectin was reported to inhibit in vitro the proliferation of hepatic stellate cells, thereby playing a potential role in inhibition of liver fibrosis [53]. Data regarding HMW adiponectin or its other multimers in NAFLD patients are limited. To our knowledge, there is only one cross-sectional study investigating serum levels of adiponectin multimers in biopsy-proven NAFLD patients [61]. Total serum adiponectin was significantly lower in NAFLD patients than BMI-matched controls (5.3 ± 1.9 vs 9.1 ± 3.5 μg/mL, respectively), but similar between NAFL and NASH patients. The HMW, MMW, and LMW adiponectin multimers were also lower in NAFLD patients (2.0 ± 1.2, 2.5 ± 0.6, and 0.8 ± 0.2 μg/mL, respectively) than controls (4.5 ± 2.5, 3.0 ± 0.7, and 1.6 ± 0.4 μg/mL, respectively), but similar between NAFL and NASH patients. The relative distribution of HMW and MMW multimers was also significantly different between NAFLD patients and controls (34%, 50%, and 16% vs 47%, 34%, and 19%, respectively) [61]. However, this study was underpowered to detect small differences in adiponectin multimers. There are also some other relevant studies strengthening the possible role of HMW adiponectin in liver steatosis, but their participants did not undergo liver biopsy. Both total adiponectin and HMW adiponectin were reported to decrease with increasing hepatic steatosis in obese adolescents (hepatic fat was quantified by magnetic resonance imaging) [62]. The HMW rather than MMW and LMW multimers was associated with fat in the liver in apparently healthy individuals (hepatic fat was quantified by ^{1}H magnetic resonance spectroscopy) [63]. The HMW rather than total adiponectin was associated with liver steatosis before and after pronounced weight loss induced by bariatric surgery (hepatic fat was quantified by ultrasound) [64]. Further studies in biopsy-proven NAFLD patients are needed to elucidate whether adiponectin multimers play a distinct role in the pathogenesis of NAFLD.

This study has certain limitations: (*a*) All included studies are currently cross-sectional (only baseline assessment was used from included RCTs), thereby not being able to prove a cause-effect relationship. No study to date has prospectively followed up adiponectin levels in NAFLD patients, and it is unknown what happens when NAFL progresses to NASH in the same individuals. However, this would be a time- and resource-consuming work and probably needs a multicentered basis to be performed. (*b*) The significant heterogeneity observed across study results reduces the reliability of the results, suggesting different outcomes across studies with different designs, especially regarding the performance or not of biopsy on controls. (*c*) Although waist circumference reflects visceral fat better than BMI and visceral fat is involved in the pathogenesis of NAFLD, we used BMI rather than waist circumference in the meta-regression because of insufficient data for waist circumference reported in the selected studies (Table 2). (*d*) Patients with NASH-related cirrhosis did not comprise a different category in this systematic review. There is limited evidence for serum adiponectin in NASH-related cirrhosis; most studies classify these patients, if any, along with NASH patients. However, it seems that adiponectin in NASH-related cirrhosis depends on the clinical stage, with higher levels observed in patients with more advanced disease [65-67].

In conclusion, this meta-analysis showed higher serum total adiponectin in controls compared with NASH patients and in NAFL compared with NASH patients. The initial finding of higher adiponectin in controls compared with NAFL patients, however, was subsequently abolished in the subgroup, where controls were subjected to liver biopsy. Based on these findings, we can speculate that hypoadiponectinemia may play a pathophysiological role in the progression from NAFL to NASH (secondary hit), whereas its role in the development of NAFL (first hit) is yet unclear. This study strengthens the role of adiponectin in the pathogenesis of NAFLD and provides claims for future studies evaluating the predictive and therapeutic potential of adiponectin in NAFLD spectrum.

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