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Association between the level of circulating adiponectin and prediabetes: A meta-analysis

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ABSTRACT

Aims/Introduction: Adiponectin has been proposed to have an essential role in the regulation of insulin sensitivity and metabolism, but previous studies on levels of adiponectin in prediabetes remain inconsistent. The present study aimed to assess the differences of adiponectin levels between prediabetes patients and healthy controls by carrying out a meta-analysis.

Materials and Methods: We carried out a systematic literature search of PubMed, EMBASE, and other databases for case–control studies and cohort studies measuring adiponectin levels in serum or plasma from prediabetes patients and healthy controls. The pooled weighted mean difference (WMD) and 95% confidence interval (CI) were used to estimate the association between adiponectin levels and prediabetes.

Results: Three cohort studies and 15 case–control studies with a total of 41,841 participants were included in the meta-analysis. The results showed that circulating adiponectin levels in prediabetes patients were significantly lower than that of healthy controls (WMD −1.694 μg/mL; 95% CI −2.151, −1.237; $P < 0.001$). Subgroup analysis showed more significant differences between prediabetes patients and healthy controls when the ratio of the homeostatic model assessment of insulin resistance was >2.12 (WMD −2.95 μg/mL; 95% CI −4.103, −1.806; $P < 0.001$) and average age was >60 years (WMD −2.20 μg/mL; 95% CI −3.207, −1.201; $P < 0.001$). Additionally, WMD in adiponectin showed a trend of direct correlation in subgroups of homeostatic model assessment of insulin resistance ratio, body mass index and age.

Conclusions: The present meta-analysis supports adiponectin levels in prediabetes patients being lower than that of healthy controls,indicating that the level of circulating adiponectin decreases before the onset of diabetes.

INTRODUCTION

Type 2 diabetes is a complex metabolic disease, the prevalence of which has tripled in the past 30 years, and diabetes is predicted to cover more than 320 million people by 2025[1]. Before the occurrence of diabetes, there is an intermediate stage called prediabetes, which is generally defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or both. According to the report of the American Diabetes Association[2], IGT is defined as fasting plasma glucose <7.0 mmol/L and 2-h plasma glucose on the 75-g oral glucose tolerance test between 7.8 and 11.0 mmol/L, and impaired fasting glucose (IFG) is defined as plasma glucose concentration of between 6.1 and 6.9 mmol/L. There are currently 79 million people in the USA with prediabetes. Approximately 30% of those with prediabetes will progress to type 2 diabetes within a decade[3]. Type 2 diabetes is associated with increased mortality, mostly as a result of cardiovascular causes, compared with populations who have normal glucose tolerance[4]. Fortunately, large numbers of studies have shown that prediabetes can be reversed by changing lifestyle and pharmacological interventions[5]. Thus, it is of great importance to diagnose prediabetes at an early stage, and carry out effective interventions before cardiovascular events emerge.

Adiponectin, a 30-kDa complement C1-related protein, is the most abundant secreted protein expressed in adipose

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tissue, and plays a crucial role in the regulation of insulin sensitivity and glucose metabolism. Lower circulating adiponectin levels is associated with obesity and negatively correlated with insulin resistance[6]. In addition, it has been proposed that adiponectin exerts antidiabetic, anti-atherogenic and anti-inflammatory activities in metabolic diseases[7]. Therefore, circulating adiponectin levels might represent a significant clinical diagnostic biomarker for the future development of prediabetes. However, its role in the development of diabetes remains unclear[8].

Understanding the association between circulating levels of adiponectin and prediabetes could provide useful information on the disease, and might help impose a stricter follow up and possibly an early treatment initiation, thus preventing the progression to diabetes. In addition, given the fact that low adiponectin levels could serve as a risk factor for cardiovascular diseases in prediabetes, adiponectin levels in prediabetes might help monitor the prognosis of cardiovascular diseases. Furthermore, knowing that adiponectin exerts antidiabetic, anti-atherogenic and anti-inflammatory activities in metabolic diseases, pharmacological adiponectin treatments could be applied in prediabetes. However, currently, no study has systematically summarized the existing evidence to explore the certain association between the level of adiponectin and prediabetes.

To investigate adiponectin levels in patients with prediabetes, a systematic review of all studies reporting total adiponectin levels in patients with prediabetes and a meta-analysis of the best available evidence were carried out.

MATERIALS AND METHODS

Search Strategy

Three investigators identified articles through a comprehensive systematic electronic search of PubMed, EMBASE and other databases up to 30 April 2014 using the following MeSH terms: 'prediabetes,' 'impaired glucose tolerance,' 'impaired fasting glucose,' 'IGT,' 'IFG' and 'adiponectin.' Also, reference lists of relevant articles were screened for eligibility. In addition, we wrote to authors to ask for unpublished or more complete information. No language restriction was applied for searching. Any discrepancy was resolved by consultation to reach a consensus with a fourth investigator. Our meta-analysis was carried out according to the Meta-analysis of Observational Studies in Epidemiology guidelines[9].

Inclusion Criteria

All of the included studies were required to meet the following inclusion criteria:

1. Case–control studies or cohort studies design.
2. Studies should report serum or plasma adiponectin levels on prediabetes patients (diagnosed consistently by either American Diabetes Association [ADA] or World Health Organization [WHO] criteria) compared with healthy controls.
3. Data of total adiponectin mean and standard deviation (SD), or sufficient data to estimate adiponectin mean and SD should be provided.
4. No medications known to influence circulating adiponectin were used.

We excluded literature reviews, letters to the editor, cross-sectional studies, randomized controlled trials, studies of animals or cell lines, studies of genetic variation in adiponectin-related genes and studies of gestational diabetes. We also excluded studies on populations with diseases other than prediabetes. Studies of medication treatment and studies classifying prediabetes into diabetes were also excluded.

Data Extraction

A standard data extraction form was used by three investigators independently to collect the information from all suitable studies. Any disagreements were resolved by discussion during a consensus meeting with a fourth investigator. The following information were extracted from each eligible study: first author's name, year of publication, region of studies, type of study design, sample size, methods of adiponectin measurement, the type of blood sample, adiponectin levels of cases and controls (mean and SD), the number of males and females, the age of cases and controls (mean and SD), the body mass index (BMI) of cases and controls (mean and SD), homeostasis model assessment of insulin resistance ratio (HOMA-IR ratio) and predefined criteria (a modification of the Newcastle–Ottawa Scale [NOS]). To retrieve the missing data, we also contacted the authors of the primary studies.

Quality Evaluation of Literature

Quality evaluation of the studies was carried out independently by three viewers according to a modification of the NOS. The NOS tool contains nine items, and scores ranged from 0 to 9[10]. The main criteria include: (i) the selection of cases and controls; (ii) the comparability; and (iii) the exposure.

Statistical Analysis

The mean, SD or standard error (SE) on plasma or serum adiponectin levels were extracted in all included studies[11]. The meta-analysis was based on sufficient information directly providing the mean and SD. Weighted mean differences (WMDs) along with the corresponding 95% confidence intervals (CIs) in adiponectin levels of all suitable cases and controls were estimated using a fixed-effects model. If there was significant heterogeneity, we used a random effects model[12]. First, heterogeneity tests were carried out by means of Cochran's Q test and I^2 statistic to evaluate statistical heterogeneity among studies. Statistically significant heterogeneity was considered when the P-value was <0.1 and the I^2 value was more than 50%[13]. Subsequently, the following tests were carried out to identify the sources of heterogeneity between the results of different studies. Subgroup analysis was carried out to investigate influencing factors. Many

subgroups were analyzed according to geographic region, sample size, age, BMI, HOMA-IR ratio, blood sample, method, quality score and sex[14]. Restricted maximum likelihood-based random effects meta-regression analysis was carried out to evaluate the aforementioned potential heterogeneity factors. Univariate meta-regression analysis was carried out first, after which the variables that were significant at the 0.1 level were entered into the multivariable model. To identify potentially influential studies, sensitivity analysis was also carried out to examine whether the effect estimate was robust by repeating the random effect meta-analysis after omitting one study at a time. Furthermore, cumulative meta-analysis was carried out to evaluate the evolution of the combined estimates over time according to the ascending date of publication. Finally, the possibility of publication bias was assessed by Begg's funnel plots and Egger's tests[15].

All statistical analyses were carried out using STATA version 12.0 (StataCorp LP, College Station, TX, USA). A two-sided *P*-value <0.05 was considered statistically significant.

RESULTS

Literature Search Results

A flow chart shows our process of study selection (Figure 1). A total of 1,942 potentially relevant articles were identified in PUBMED, EMBASE and other databases, and 278 duplicates were removed. A total of 1,664 potentially relevant articles were

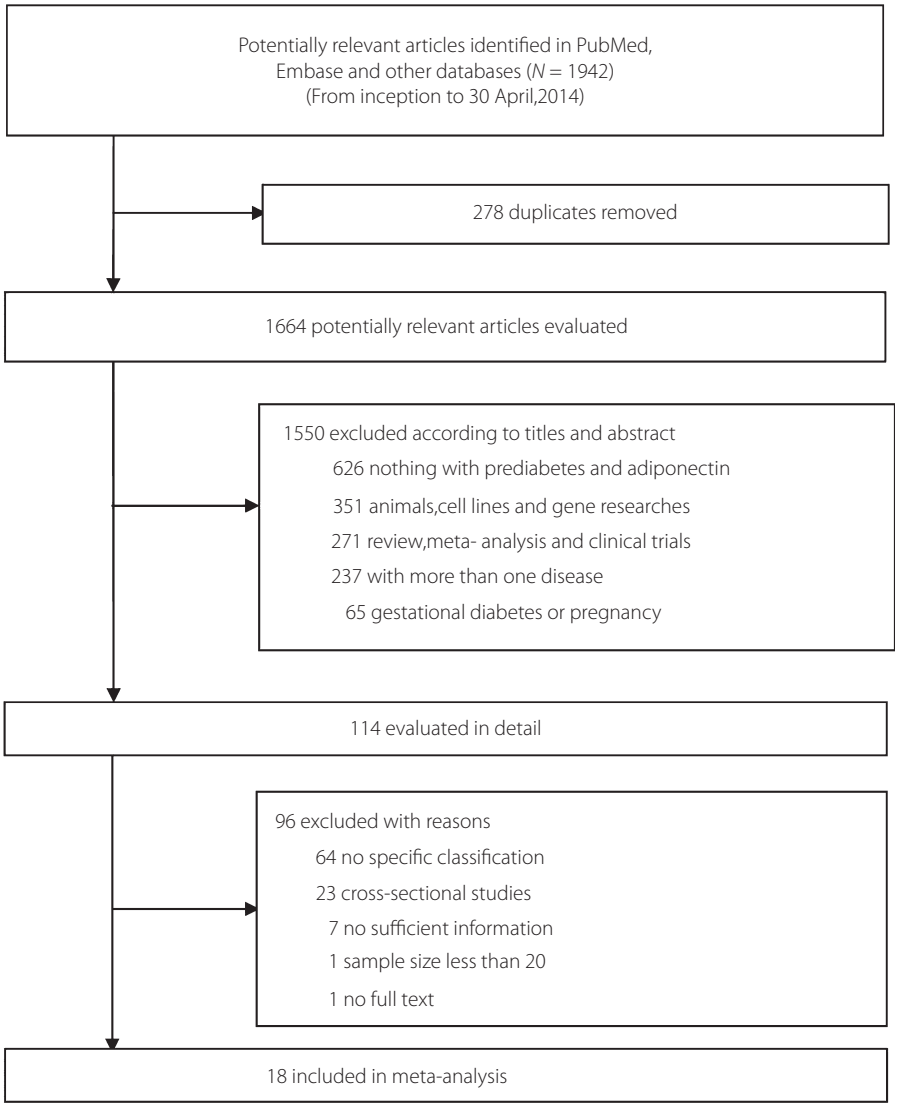

Figure 1 | Flow chart of study selection. After careful discussion among the investigators, a total of 18 studies were included to carry out the meta-analysis.

evaluated according to their titles and abstracts: 626 studies had no relationship with prediabetes or adiponectin levels; 351 studies were focused on animals, cell lines and genes; 271 studies belonged to reviews, meta-analyses and clinical trials; 237 studies discussed prediabetes along with another disease; and 65 studies specifically researched gestational diabetes. Subsequently, 114 articles were evaluated in detail: 64 studies had not referred to prediabetes and healthy controls specifically; 23 studies were cross-sectional studies; seven studies had no sufficient data to extract or calculate mean and SD; one study had no full text to extract useful data; and one study had <20 samples in all groups. Finally, 18 available studies were included in our meta-analysis.

Study Characteristics

The meta-analysis of 18 studies involved 41,841 participants: 5,879 individuals with prediabetes and 35,962 control subjects[16–33]. Among them, three studies presented two subgroups of prediabetes, each subgroup had been independently compared with a control group[17,23,29]. As a result, each of them was treated as an independent study. Therefore, a total of 21 studies were included in our final meta-analysis. The main characteristics of the 21 resulting studies were summarized in Table 1. The studies were published between 2001 and 2014, including three cohort studies and 15 case–control studies. Geographically, 14 studies were carried out in Asia, five in Europe

Table 1 | Characteristics of the included studies of circulating adiponectin and prediabetes

Study (year)	Region	Study design	Blood sample	Method	Sample size		Sex		Age (years)		BMI (kg/m^2)	Adiponectin (μg/mL)		HOMA-IR ratio	NOS
					Control	PD	Male	Female	Control	PD		Control	PD		
Christian (2001)[32]	Asia	Case–control	Plasma	ELISA	79	25	76	28	27 ± 6	31 ± 8	>30	7.5 ± 2.7	6.1 ± 2.0	NR	8
Nobert (2003)[28]	Asia	Case–control	Plasma	ELISA	94	33	93	34	28 ± 7	33 ± 8	NA	7.05 ± 2.70	5.44 ± 2.23	NR	7
Alice (2003)[26]	USA	Case–control	Plasma	RIA	108	18	0	126	46.7 ± 1.5	56.1 ± 1.8	25–30	6.18 ± 0.67	2.78 ± 0.78	NR	6
Chamukuttan (2003)[27]	Asia	Cohort	Plasma	RIA	50	32	73	68	45.7 ± 11.3	44.2 ± 5.3	25–30	14.9 ± 5.9	15.2 ± 7.5	NR	7
Kwame (2005)[22]	USA	Case–control	Serum	ELISA	19	8	4	23	49.1 ± 7.86	51.0 ± 9.3	>30	9.61 ± 5.09	10.42 ± 6.89	1.71	8
Munehide (2007)[21]	Asia	Case–control	Serum	Others	23	5	NR	NR	49.7 ± 10.2	43.2 ± 19.8	25–30	5.8 ± 2.2	6.8 ± 3.3	NR	6
Carl (2006)[16]	Europe	Case–control	Serum	ELISA	97	201	0	298	64	64	25–30	15.1 ± 6.3	12.9 ± 6.6	1.34	6
Sang (2007)[25]	Asia	Case–control	Plasma	RIA	36	49	35	50	47.5 ± 13.6	53.0 ± 9.7	<25	5.20 ± 2.87	4.00 ± 3.64	1.39	5
Noriyuki (2009)[23]	Asia	Case–control	Serum	ELISA											5
IFG					11	9	20	0	41.0 ± 12.0	49.3 ± 12.3	<25	9.2 ± 4.3	7.1 ± 2.2	2.25	
IGT					11	11	22	0	41.0 ± 12.0	45.9 ± 7.1	<25	9.2 ± 4.3	6.5 ± 1.5	1.46	
Kassi (2010)[18]	Europe	Case–control	Serum	ELISA	18	20	0	38	55 ± 9	61 ± 6	>30	11.9 ± 4.4	13 ± 5.8	1.58	6
Stefan (2010)[24]	Europe	Case–control	Plasma	ELISA	13	13	26	0	50.6 ± 10	50.0 ± 13	>30	5.2 ± 2.4	3.2 ± 0.9	1.78	6
Anke (2010)[29]	Europe	Case–control	Serum	ELISA											8
IFG					43	35	33	43	61.3 ± 9.3	61.9 ± 12.3	>30	8.8 ± 4.7	7.2 ± 4.7	1.90	
IGT					43	45	37	51	61.3 ± 9.3	63.3 ± 8.8	>30	8.8 ± 4.7	6.2 ± 3.2	2.59	
Ko (2010)[19]	Asia	Cohort	Serum	Others	224	52	360	0	40.3 ± 9.0	42.4 ± 9.4	25–30	5.72 ± 2.94	4.60 ± 2.10	NR	8
Wolfson (2011)[33]	Asia	Case–control	Plasma	ELISA	55	24	33	46	55.7 ± 9.5	58.8 ± 9.6	>30	12.60 ± 7.24	7.57 ± 4.19	2.57	6
Webb (2012)[31]	Asia	Case–control	Serum	Others	79	40	76	82	52.1 ± 9.8	55.1 ± 11.7	25–30	13.6 ± 3.23	12.40 ± 3.85	1.56	7
Sun (2013)[17]	Asia	Cohort	Serum	ELISA											7
Male					21,766	4,101	25,867	0	41.5 ± 9.1	45.2 ± 9.3	<25	6.6 ± 3.7	5.7 ± 3.3	NR	
Female					13,090	1,048	0	14,138	40.9 ± 10.0	47.7 ± 11.2	<25	10.5 ± 5.5	8.6 ± 5.0	NR	
Yiping (2014)[20]	Asia	Case–control	Plasma	RIA	22	61	NR	NR	49.8 ± 4.8	NR	25–30	11.20 ± 4.72	8.74 ± 3.49	2.84	6
Smitha (2014)[30]	Asia	Case–control	Serum	ELISA	81	49	64	66	46.53 ± 0.89	46.22 ± 1.06	<25	6.90 ± 0.45	5.57 ± 0.53	NR	7

Data presented as mean ± standard deviation. BMI, body mass index (calculated as weight in kg divided by height in m^2); ELISA, enzyme-linked immunosorbent assay; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-IR ratio, mean values of homeostatic model assessment of insulin resistance prediabetes patients to controls in a single study; NR, not reported; NOS, Newcastle–Ottawa Scale; PD, prediabetes; RIA, radioimmunoassay.

and two in the USA. All studies compared individuals with prediabetes with control subjects, ranging from 20 to 25,867 in total sample size. Among 21 studies, four studies[16–18,26] included only female participants, and five studies[17,19,23,24] included only male participants. The mean BMI of participants in all studies ranged from 22.1 to 40.16 kg/m^2, and the mean age ranged from 27 to 64 years. There were nine studies without HOMA-IR results, and the mean HOMA-IR ratio in 12 studies ranged from 1.06 to 6.96. Total and HWM adiponectin levels were measured by enzyme-linked immunosorbent assay in 14 studies, whereas four studies used radioimmunoassay and three used other methods. Additionally, 13 studies used serum specimens to measure the adiponectin level, while the remaining studies used the plasma. Furthermore, 13 studies elucidated that no participant took medications that could affect the adiponectin level, whereas eight studies did not mention the medication records. The overall quality score of the involved studies averaged 6.6 on a scale of 0 to 9.

Data Synthesis

The random effects meta-analysis results showed that the adiponectin levels in prediabetes patients were significantly lower than healthy controls (WMD –1.694 μg/mL; 95% CI –2.151, –1.237; $P < 0.001$). However, significant heterogeneity in this meta-analysis was present ($I^2 = 89.9\%$, $P < 0.001$; Figure 2). Therefore, subgroup analysis should be carried out to explore the possible reasons for this heterogeneity.

Subgroup Analysis

Subgroup analysis was carried out to explore the sources of heterogeneity. Potential sources of heterogeneity were evaluated, including geographic region, sample size, age, HOMA-IR ratio, BMI, quality score, assay methods (Figure S1), the type of blood sample (Figure S2) and sex (Table 2). Almost all results of subgroup analysis showed that adiponectin levels in prediabetes patients were significantly lower than healthy controls, except in geographic region and sample size. As for geographic region, a significant decrease of adiponectin levels was observed between prediabetes patients and healthy controls in the included studies carried out in Asia (WMD –1.412 μg/mL; 95% CI –1.770, –1.053; $P < 0.001$) and Europe (WMD –1.937 μg/mL; 95% CI –2.745, –1.128; $P < 0.001$). However, it was not significantly different in adiponectin levels in the included studies carried out in the USA (WMD –2.157 μg/mL; 95% CI –5.921, 1.607; $P = 0.261$; Figure 3). For sample size, there was no significant difference in adiponectin levels between prediabetes patients and healthy controls in studies with sample sizes <50 (WMD –1.144 μg/mL; 95% CI –2.475, 0.187;

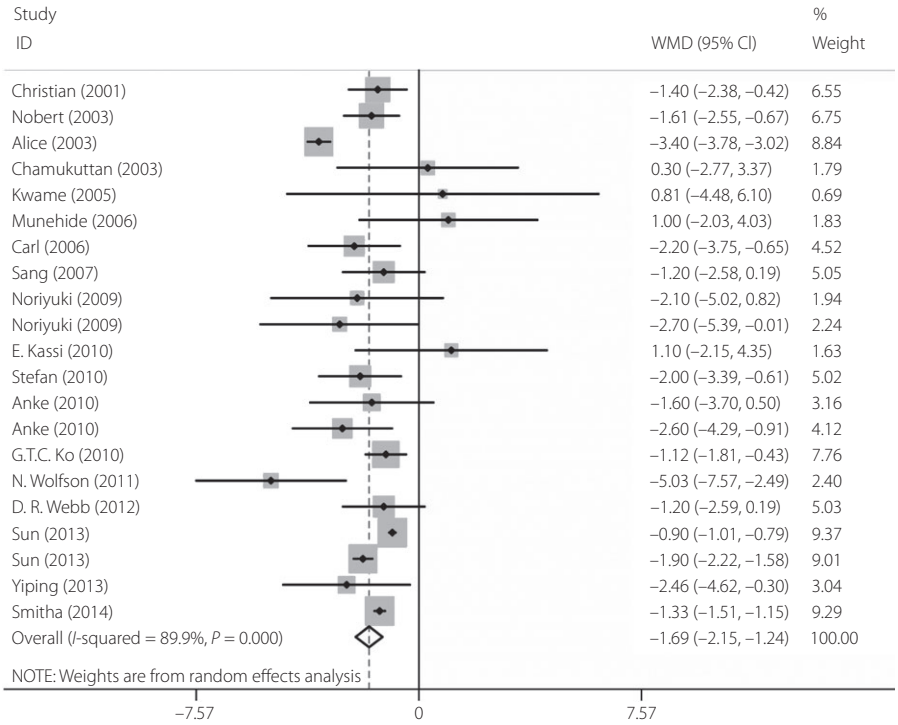

Figure 2 | Forest plot for adiponectin levels in prediabetes patients and healthy controls in included studies. Calculation based on random effects model. Results are expressed as weighted mean difference (WMD) and 95% confidence intervals (95% CI). The result showed that the adiponectin levels in prediabetes patients were significantly lower than healthy controls (WMD –1.694 μg/mL; 95% CI –2.151, –1.237; $P < 0.001$).

Table 2 | Subgroup analysis of the included studies of circulating adiponectin and prediabetes

Characteristic	No. participants	No. participants	Random effects WMD (95% CI)	*P*-value	Heterogeneity	
					I^2 (%)	*P*-value
All studies	41,841	18	–1.694 (–2.151, –1.237)	<0.001	89.9	<0.001
Region						
Asia	41,160	14	–1.412 (–1.770, –1.053)	<0.001	78.2	<0.001
Europe	528	5	–1.937 (–2.745, –1.128)	<0.001	3.7	0.385
USA	153	2	–2.157 (–5.921, 1.607)	0.261	58.6	0.120
Sample size						
<50	161	6	–1.144 (–2.475, 0.187)	0.092	30.3	0.208
50–100	495	6	–2.103 (–3.266, –0.941)	<0.001	48.4	0.084
>100	41,185	9	–1.679 (–2.235, –1.122)	<0.001	95.5	<0.001
Age (years)						
<50	40,947	12	–1.571 (–2.135, –1.007)	<0.001	93.9	<0.001
50–60	347	5	–1.715 (–3.016, –0.414)	0.010	62.2	0.032
>60	464	3	–2.204 (–3.207, –1.201)	<0.001	0.0	0.767
NR	83	1	–2.461 (–4.619, –0.303)	0.025		
HOMA-IR ratio						
<1.36	298	1	–2.200 (–3.751, –0.649)	0.005		
1.36–1.7	203	4	–1.189 (–2.102, –0.276)	0.011	3.6	0.375
1.71–2.12	131	3	–1.754 (–2.888, –0.621)	0.002	0.0	0.594
>2.12	270	4	–2.955 (–4.103, –1.806)	<0.001	7.8	0.354
NR	40,878	9	–1.539 (–2.128, –0.951)	<0.001	95.5	<0.001
BMI						
<25	40,262	6	–1.394 (–1.846, –0.943)	<0.001	88.9	<0.001
25–30	1,012	7	–1.587 (–2.834, –0.340)	0.013	87.4	<0.001
>30	440	7	–1.894 (–2.932, –0.857)	<0.001	49.1	0.067
NR	127	1	–1.610 (–2.546, –0.674)	0.001		
Quality score						
<7	805	10	–2.129 (–3.099, –1.158)	<0.001	69.6	0.001
≥7	41,036	11	–1.365 (–1.716, –1.015)	<0.001	79.6	<0.001
Method						
ELISA	41,042	14	–1.595 (–1.989, –1.202)	<0.001	79.8	<0.001
RIA	376	4	–2.001 (–3.622, –0.381)	0.015	79.3	0.002
Others	423	3	–1.051 (–1.655, –0.446)	0.001	0.0	0.398
Blood sample						
Serum	41,129	13	–1.374 (–1.754, –0.994)	<0.001	77.9	<0.001
Plasma	712	8	–2.130 (–3.103, –1.158)	<0.001	81.0	<0.001
Sex						
Male	26,211	5	–1.071 (–1.444, –0.698)	<0.001	20.9	0.281
Female	14,600	4	–2.178 (–3.384, –0.971)	<0.001	92.5	<0.001

BMI, body mass index; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; HOMA-IR ratio, mean values of homeostatic model assessment of insulin resistance in prediabetes patients to controls in a single study; NR, not reported; RIA, radioimmunoassay; WMD, weight mean difference.

$P = 0.092$; Figure S3). All subgroup analysis still showed significant heterogeneity. Furthermore, for HOMA-IR ratio group (Figure 4), WMD in adiponectin showed a trend of a direct correlation except HOMA-IR ratio <1.36. Additionally, WMDs in adiponectin showed a trend of direct correlation in subgroups of BMI and age (Figures 5 and 6). Furthermore, as for sex, the decrease of adiponectin levels between prediabetes patients and healthy controls in female participants (WMD –2.178 μg/mL; 95% CI –3.384, –0.971; $P < 0.001$) was more significant than that in male participants (WMD –1.071 μg/m:;95% CI –1.444, –0.698; $P < 0.001$; Figure 7).

Meta-Regression

To further investigate the impact of the aforementioned characteristics on WMD in adiponectin, restricted maximum likelihood-based random effects meta-regression analyses were carried out (Table 3). WMD was used as the dependent variable. Geographic region, sample size, age, HOMA-IR ratio and BMI were

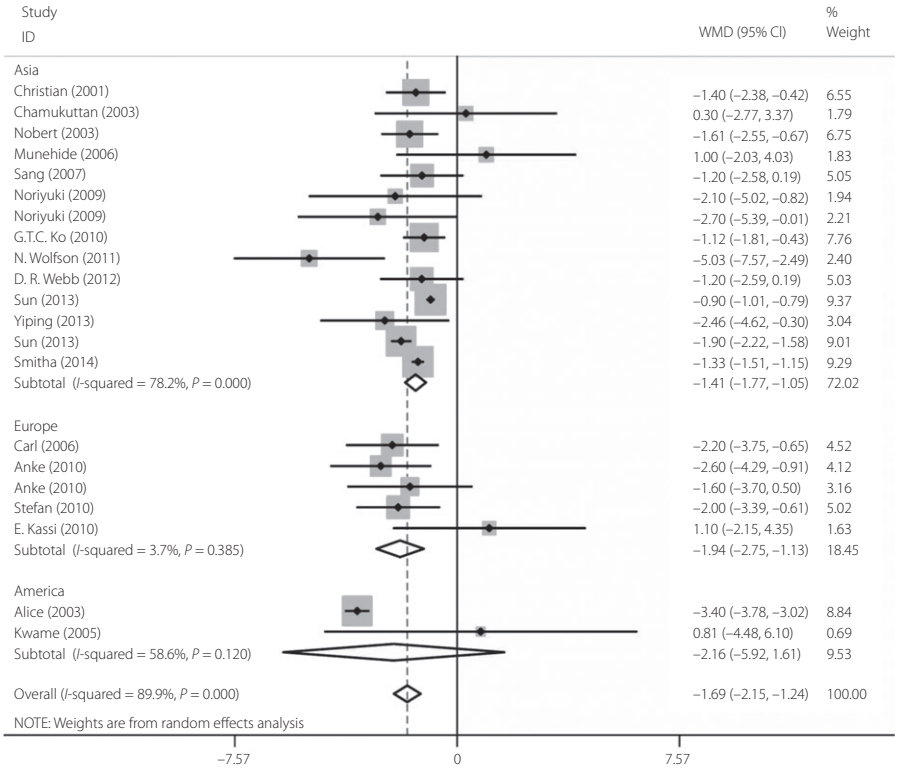

Figure 3 | Subgroup meta-analysis for adiponectin levels in prediabetes patients and healthy controls by geographic region. Calculation based on random effects model. Results are expressed as weighted mean difference (WMD) and 95% confidence intervals (95% CI). Significant decrease of adiponectin levels was observed between prediabetes patients and healthy controls in the included studies carried out in Asia (WMD –1.412 μg/mL; 95% CI –1.770, –1.053; $P < 0.001$) and Europe (WMD –1.937 μg/mL; 95% CI –2.745, –1.128; $P < 0.001$).

used as explanatory covariates. The result of univariate meta-regression analysis showed that geographic region could contribute significantly to the heterogeneity (Asia: 14 studies, $P = 0.001$; Europe: 5 studies, $P = 0.053$). Additionally, sample size (21 studies, $P = 0.398$), age (20 studies, $P = 0.393$), HOMA-IR ratio (12 studies, $P = 0.074$) and BMI (19 studies, $P = 0.391$) cannot account for heterogeneity of the analysis.

Cumulative Meta-Analysis

The result of cumulative meta-analysis from the year 2001 by Christian *et al.*[32] showed that the random effects pooled WMD was instable. However, a statistically significant effect was observed in the study by Sang *et al.*[25] in 2007, and it changed little after that study, showing the stability of the result in the present meta-analysis.

Sensitivity Analysis and Publication Bias

A sensitivity analysis was carried out by omitting one study at a time. We used random effects to estimate and calculate the WMD for the remaining studies. The result showed that none of the individual studies dramatically influenced the effect of the meta-analysis when any one of the studies was excluded, showing that the results of the meta-analysis were stable and reliable (Figure S4). Publication bias was evaluated by Begg's funnel plots and Egger's tests ($t = –1.42$, $P = 0.173$; Figure 8). No publication bias was observed in the present meta-analysis.

DISCUSSION

The present meta-analysis of relevant studies suggested that adiponectin levels were significantly lower in patients with prediabetes compared with healthy controls (random-effects WMD −1.96; 95% CI −2.15, −1.24; $I^2 = 89.9\%$). Subgroup analysis showed more significant differences between prediabetes patients and healthy controls when the HOMA-IR ratio was >2.12 (WMD −2.95 μg/mL; 95% CI –4.103, –1.806; $P < 0.001$) and mean age >60 years (WMD–2.20 μg/mL; 95% CI –3.207, –1.201; $P < 0.001$).

Many studies have been shown to uncover the relationship between adiponectin and prediabetes. A meta-analysis published in *Journal of the American Medical Association* in 2009 with a total of 14,598 participants and 2,623 incident cases showed that lower adiponectin levels were associated with a higher incidence of insulin resistance and type 2 diabetes in

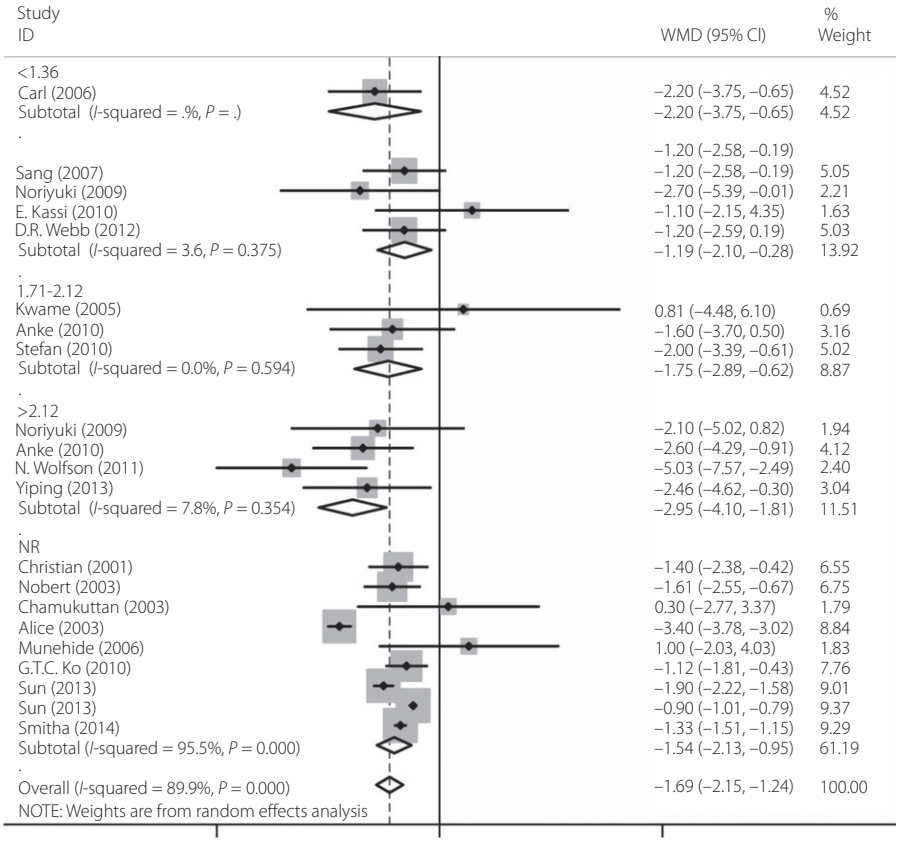

Figure 4 | Subgroup meta-analysis for adiponectin levels in prediabetes patients and healthy controls by homeostasis model assessment of insulin resistance ratio (HOMA-IR) ratio. Calculation based on random effects model. Results are expressed as weighted mean difference (WMD) and 95% confidence intervals (95% CI). The total WMD in the included studies with homeostasis model assessment of insulin resistance ratio is significant and it showed a trend of a direct correlation except homeostasis model assessment of insulin resistance ratio <1.36.

humans[34]. A cross-sectional, genetic epidemiology study in 2009 with 1,599 American Samoan adults suggested that adiponectin is an independent risk factor of type 2 diabetes, and might help distinguish those at higher risk of developing this disease[35]. Furthermore, a most recent and up-to-date cohort study in 2014 carried out by Yamamoto Sin Japan suggested that higher levels of circulating adiponectin are associated with a lower risk of type 2 diabetes, and that adiponectin could confer a benefit in both persons with and without prediabetes[36]. The same results were shown in other studies[37–39]. In addition, several case–control studies by Pauer *et al.*[40] reported that prediabetes are associated with lower circulating adiponectin concentrations in patients with insulin resistance and type 2 diabetes[41–43], as well as in patients with prediabetic conditions[25,44–46].

However, inconsistent results regarding this have been reported in another two studies[8,47–49]. Using the adiponectin gene summary statistics genetic risk scores, Mente *et al.*[42,47] found no evidence of an association between adiponectin-lowering alleles and insulin sensitivity, which do not support a causal role for reduced circulating adiponectin levels in insulin resistance and type 2 diabetes. In addition, Hammana *et al.*[7] found no alterations in adiponectin levels despite insulin resistance, glucose intolerance and subclinical chronic inflammation in cystic fibrosis patients. Thus, the relationship between adiponectin values and insulin resistance or inflammation is unclear as a result of other confounding diseases[8].

The insulin-sensitizing effect of adiponectin was summarized by three independent routes[50]. First, *in vitro* studies have suggested that both isoforms of adiponectin receptor (AdipoR1 and AdipoR2) can increase adenosine monophosphate-activated protein kinase phosphorylation and peroxisome proliferator-activated receptor-α activity by adiponectin binding, thus increasing fatty acid oxidation and glucose uptake[51]. The mechanism is related to phosphorylation of acetyl coenzyme A carboxylase, fatty-acid oxidation, glucose uptake and lactate production in myocytes, and reducing gluconeogenesis in the liver[52]. Second, in skeletal muscle, adiponectin activates the expression of involved molecules in fatty-acid transport, such as uncoupling protein 2 required

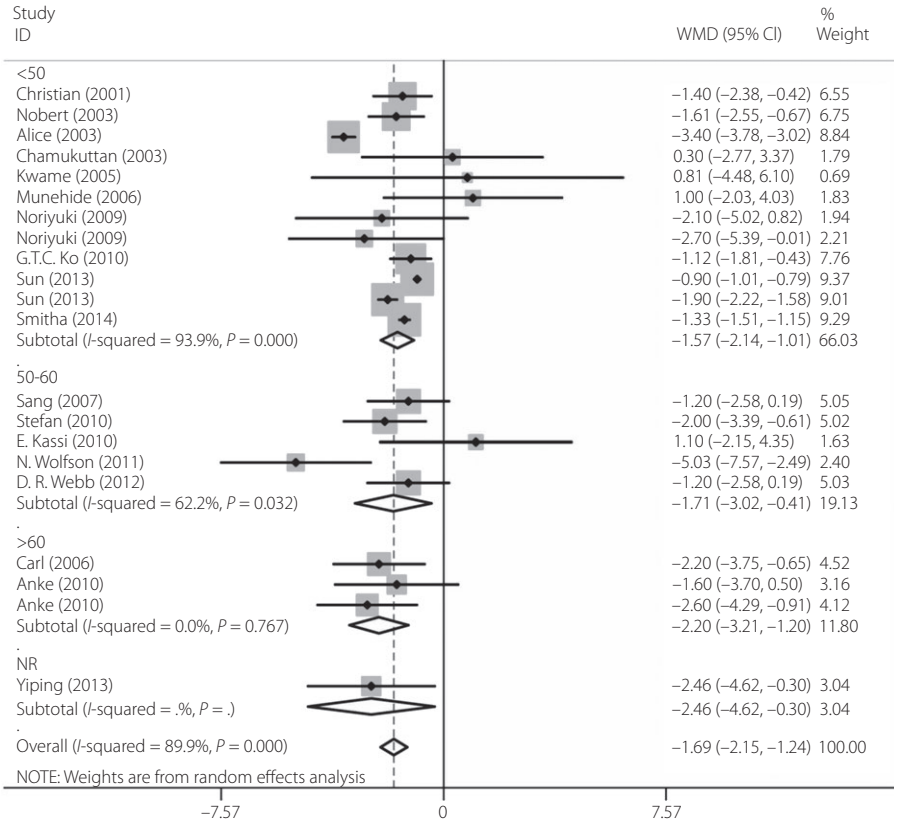

Figure 5 | Subgroup meta-analysis for adiponectin levels in prediabetes patients and healthy controls by age. Calculation based on random effects model. Results are expressed as weighted mean difference (WMD) and 95% confidence intervals (95% CI). The total WMD in the included studies with age is significant and it showed a trend of a direct correlation with age.

during energy dissipation and CD36, acyl-coenzyme A oxidase involved in combustion of fatty acid[53]. These changes result in decreased triglyceride content in skeletal muscle. Third, adiponectin activates fatty-acid combustion and energy consumption through peroxisome proliferator-activated receptor-α activation[54], which leads to decreased triglyceride content in the liver and skeletal muscle, and thus increased insulin sensitivity. An animal study carried out by Maeda *et al.*[55] showed that adiponectin/ACRP30-knockout mice delayed clearance of free fatty acid in plasma, lower levels of fatty-acid transport protein 1 messenger ribonucleic acid in muscle, higher levels of tumor necrosis factor-alpha messenger ribonucleic acid in adipose tissue and high plasma tumor necrosis factor-alpha concentrations, resulting in severe diet-induced insulin resistance. Iwabu *et al.*[56] found that decreased levels of adiponectin and AdipoR1 in obesity could have causal roles in mitochondrial dysfunction and insulin resistance seen in Muscle-R1KO mice. Furthermore, Okada-Iwabu *et al.*[57] found that AdipoR agonist ameliorated diabetes of obese rodent model *db*/*db* mice, and concluded that orally active AdipoR agonists are a promising therapeutic approach for the treatment of insulin resistance and type 2 diabetes.

Some studies, however, have not found an association between adiponectin levels and prediabetes[47,49]. Some studies have not found lower adiponectin levels in prediabetes compared with healthy controls[21,22,27]. Furthermore, adiponectin is expressed in different multimer complexes, and the high-molecular weight (HMW) multimer is the most potent biological form, which is decreased in patients with prediabetes compared with normal controls[17,23].

The present results showed significant heterogeneity among the studies (I^2 = 89.9%, $P < 0.001$; Figure 2). There are two sources of heterogeneity: one is within-study variability, which means a difference within a study of estimating the same effect size; the other is between-study variability, which means differences among studies in estimating effect size. In the present study, the meta-analysis showed that there was large heterogeneity among studies. Subsequent subgroup analysis stratified by eight potential sources was carried out (Table 2). We found significant differences in circulating adiponectin levels between prediabetes patients and healthy controls in the subgroup analysis stratified by HOMA-IR ratio, age, sample size, blood sample and quality score. No significant difference was observed in circulating adiponectin levels between prediabetes

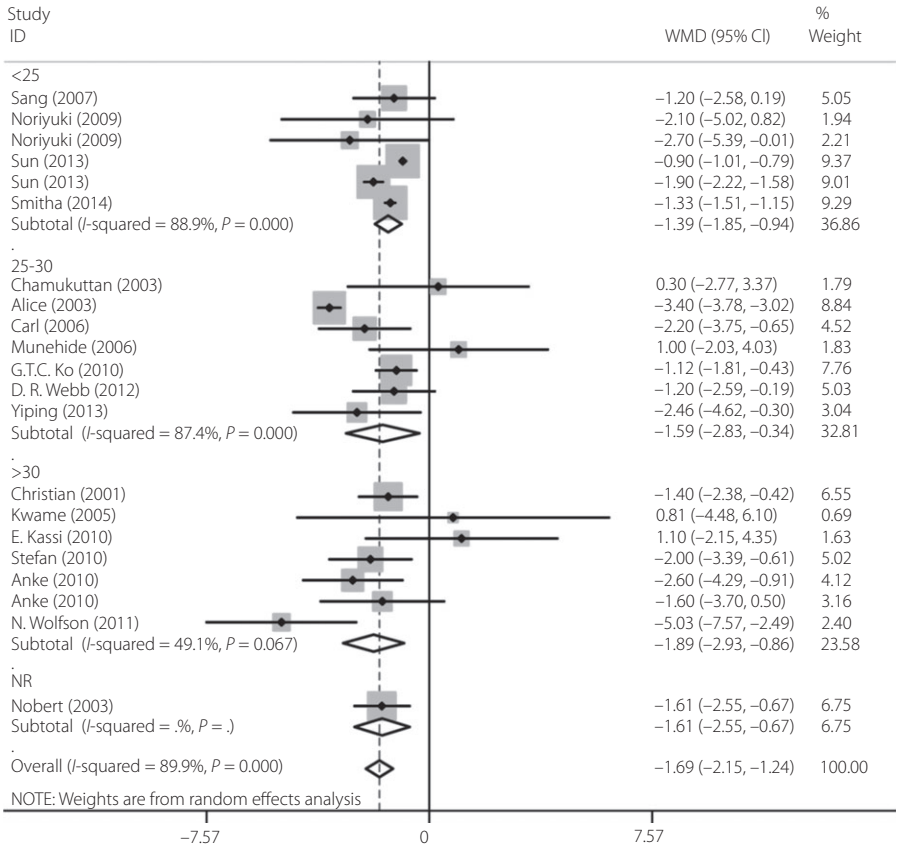

Figure 6 | Subgroup meta-analysis for adiponectin levels in prediabetes patients and healthy controls by body mass index. Calculation based on random effects model. Results are expressed as weighted mean difference (WMD) and 95% confidence intervals (95% CI). The total WMD in the included studies with body mass index is significant and it showed a trend of a direct correlation with body mass index.

patients and healthy controls only in the USA. In addition, when HOMA-IR ratio and age were used in the subgroup analysis, it showed the accepted fact that HOMA-IR ratio and age are directly related to the level of adiponectin. To further investigate the source of heterogeneity, we carried out a meta-regression, and found that geographic region might contribute to the overall heterogeneity (Asia $P = 0.001$). However, no significant contribution was found in HOMA-IR ratio, age, BMI and sample size. To conclude, the geographic region might be the main source of heterogeneity.

To the best of our knowledge, this is the most comprehensive meta-analysis to estimate the association between adiponectin levels and prediabetes. Adequate numbers of cases and controls were included from all available publications concerned with circulating adiponectin levels and prediabetes, which greatly increased the statistical power of the analysis and provided enough evidence for us to make a correct conclusion. Furthermore, participants in 13 included studies were mentioned without treating medications that could affect the level of circulating adiponectin, whereas the records of drug usage were not mentioned for the other participants in eight included studies. It is known to all that prediabetes patients can be cured by exercise and healthy diet, so there is no need to take medications. Thus, medication had little impact on the adiponectin level, and it strengthened the reliability of the present results. Furthermore, in order to eliminate the influence of sex, subgroup analysis of sex was carried out, which showed that the decrease of adiponectin levels between prediabetes patients and healthy controls in female participants was more significant than that in male participants. The results of mean adiponectin levels in female and male participants, respectively, were also consistent with the fact that serum adiponectin is higher in females than males. In addition, sensitivity analysis showed that no single study affected the pooled WMD qualitatively. Furthermore, cumulative meta-analysis showed that no substantive change had occurred in pooled WMD after the study was published in 2007, suggesting the stability of the association between low adiponectin levels and prediabetes patients. Furthermore, no publication bias was detected in the present meta-analysis, which showed that the pooled results of our study should be reliable. To summarize, these results confirm the strengths of our meta-analysis.

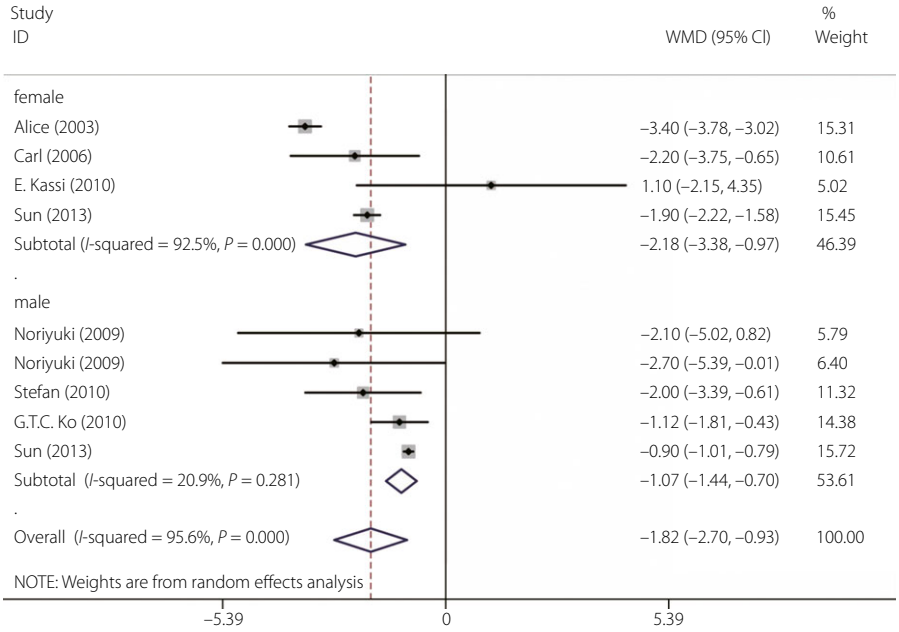

Figure 7 | Subgroup meta-analysis for adiponectin levels in prediabetes patients and healthy controls by sex. Calculation based on random effects model. Results are expressed as weighted mean difference (WMD) and 95% confidence intervals (95% CI). The total WMD in the included studies with sex is significant, and it showed that the decrease of adiponectin levels between prediabetes patients and healthy controls in female participants is more significant than that in male participants.

Table 3 | Univariate meta-regression of the included studies of adiponectin and prediabetes

Covariates	No. studies	Coefficient	Standard error	*t*	*P*	95% Confidence interval
Region						
Asia	14	1.912	0.490	3.90	0.001	0.881, 2.942
Europe	5	1.392	0.672	2.07	0.053	−0.020, 2.803
America	2			Drop because of collinearity		
Sample size	21	0.000025	0.000029	0.86	0.398	−0.000003, 0.00008
Age	20	−0.022	0.026	−0.87	0.393	−0.076, 0.031
HOMA-IR ratio	12	−1.145	0.573	−2.00	0.074	−2.421, 0.131
BMI	19	−0.066	0.075	−0.88	0.391	−0.225, 0.093

BMI, body mass index; HOMA-IR ratio, mean values of homeostatic model assessment of insulin resistance in prediabetes subjects to controls in a single study.

The possible limitations of the present study should also be considered. First, 15 case–control studies and three cohort studies, but no randomized controlled trial included in the meta-analysis, might substantially weaken the quality of this study. Second, our results were concluded without adjusting the confounding factors, such as smoking status, alcoholic consumption, environmental factors and other diet lifestyle factors. Third, this meta-analysis included small sample size studies and the backgrounds of patients varied, which would result in low statistical power and inconsistent results among studies. Finally, insufficient data were available. The influence of visceral adiposity could not be evaluated, as waist circumference or waist-to-hip ratio was not available in the majority of studies. Insufficient data of HMW adiponectin limited the estimate of the association between HMW adiponectin levels and prediabetes. Despite these limitations, the present findings could provide useful information on the diseases, and might help impose a stricter follow up and possibly an early treatment initiation, thus preventing the progression to diabetes. Furthermore, our findings might motivate more randomized controlled trials to be carried out to obtain better understanding of causal relationships between the level of adiponectin and prediabetes.

In conclusion, based on the findings of existing studies, adiponectin levels in prediabetes patients are lower than that of

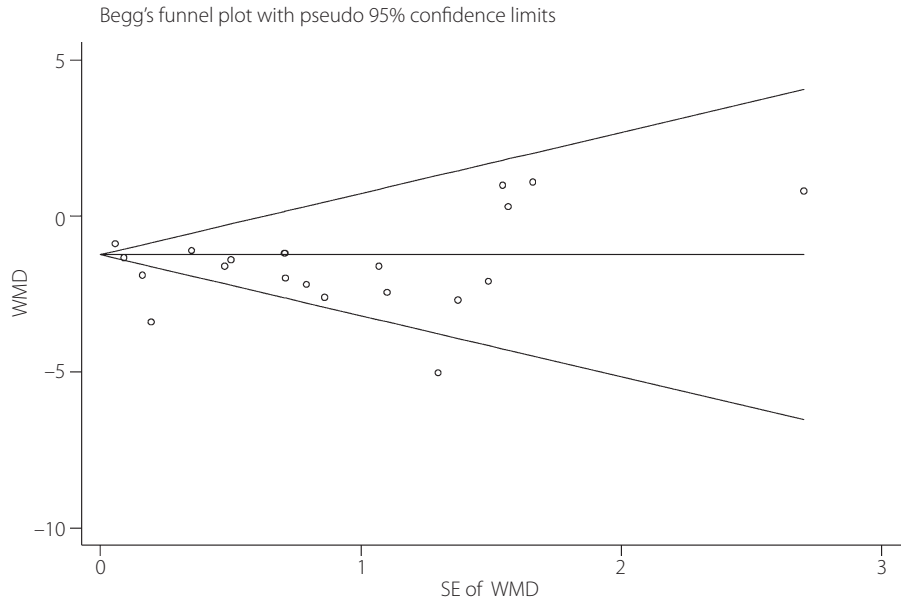

Figure 8 | Publication bias for adiponectin levels in prediabetes patients and healthy controls in the included studies. No publication bias was observed in Begg's funnel plots and Egger's tests ($t = -1.42$, $P = 0.173$). SE, standard error.

healthy controls, showing that adiponectin decreases before the onset of diabetes. This result should be taken with caution because of the substantial heterogeneity among existing studies. There is a need for more well-designed, high-quality studies to clarify the possible causal relationship between adiponectin levels and prediabetes patients. In addition, further investigation is required to clarify whether HMW adiponectin levels are also suppressed in prediabetes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 | Subgroup meta-analysis for adiponectin levels in prediabetes patients and healthy controls by measurement method for adiponectin.
Figure S2 | Subgroup meta-analysis for adiponectin levels in prediabetes patients and healthy controls by blood sample.
Figure S3 | Subgroup meta-analysis for adiponectin levels in prediabetes patients and healthy controls by sample size.
Figure S4 | Sensitivity analysis for adiponectin levels in prediabetes patients and healthy controls in included studies.