Influence of Ascorbic Acid Supplementation on Type 2 Diabetes Mellitus in Observational and Randomized Controlled Trials; A Systematic Review with Meta-Analysis

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ABSTRACT - Purpose. There are controversial data regarding the beneficial effects of ascorbic acid (AA) supplementation in type 2 diabetes mellitus (T2DM). In this systematic review, we aimed to criticize the current relevant data from both observational and randomized controlled trials (RCTs). **Methods.** All observational and RCTs conducted to assess anti-hyperglycemic effects of AA in diabetics, published before January 2013, were included. To obtain all related studies Google Scholar, PubMed, Scopus, IranMedex, and Magiran web databases were searched. Exclusion criteria were animal studies, and studies conducted in Type 1 DM, children or pregnant women. Main outcome measures were fasting blood sugar (FBS), and glycated hemoglobin (HbA1c). According to degree of heterogeneity, fixed or random effect models were employed. Meta-analyses were done using Stats Direct software, version 3.0.97. The quality of included articles and publication bias were also assessed. **Results.** We selected 38 articles; 26 observational studies and 12 RCTs. Due to severe methodological heterogeneity in all observational studies and some of RCTs, we could pool data from only 5 RCTs in a meta-analysis. Single intake of AA versus placebo showed a significant effect on FBS; with the standardized mean difference (SMD): -20.59, 95% confidence intervals (95% CI): -40.77 to -0.4 (p= 0.04), but non-significant effect on HbA1c; SMD: -0.46, 95% CI: -1.75 to 0.84 (p= 0.4). Effect of other antioxidants with/without AA supplementation on FBSwere nonsignificant; SMD: -4.26 (p= 0.8), and SMD: -12.04 (p= 0.3), respectively. Also, their effect on HbA1c was non-significant; SMD: 0.53 (p= 0.11), and SMD: 0.28 (p= 0.34), respectively. **Conclusions.** Our study supports the positive effect of AA in reduction of FBS in diabetics, however, due to insufficient evidence ragarding long term safety of AA supplementation and limited number of RCTs, the long term use of this vitamin for its anti-diabetic properties cannot be strongly recommended.

INTRODUCTION

Diabetes mellitus (DM), as one of the most important worldwide health problems, shows an increasing prevalence. Currently, there are approximately 381 million diabetic patients, a figure that expects to rise to 592 million by 2035 (1). Various studies have established the key role of oxidative stress in the pathophysiology of diabetes and its complications (2-4). Oxidative stress reflects an imbalance between the formation of reactive oxygen species (ROS) and body's antioxidant defense system (2-4).

It has been shown that chronic diseases such as diabetes can diminish the antioxidative status of the body and increase the oxidative load (5). Under diabetic conditions, ROS are produced mainly through the glycation reaction. Oxidative stress can in turn promote glycation of hemoglobin (6) and impair the ability of β-cells of the pancreas for insulin secretion (7).

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On the other hand, several epidemiological studies have shown that individuals with low concentration of antioxidants are at increased risk of diabetes complications (8, 9).

Although the human body has its own antioxidant defense systems, this defense mechanism can be reinforced by the application of external source of antioxidants. Enzymatic or non-enzymatic antioxidants such as vitamins may have a role in oxidative stress (3, 10-12). The main source of majority of these antioxidants is the consumed food. Fruits, vegetables, and grains are among the richest sources of dietary antioxidants (5). Vitamins C and E are the well-known dietary antioxidants that may have beneficial effects against oxidative stress in diabetes. Several epidemiological studies have shown that individuals with low concentration of antioxidants are at increased risk of diabetes complications (8, 9). Recently, Xu et al (13) published the effect of vitamin E (VE) supplementation on diabetes improvement. Along with their work, we aimed to critically and systematically, assess the effect of ascorbic acid (AA) in diabetes.

Vitamin C or AA is a hydrophilic antioxidant that depends on the employed dosage could have either prooxidant - or antioxidant effects (14). At low concentration, AA shows pro-oxidant function and helps in ROS formation, whilst its antioxidant function is found at higher concentrations (15, 16). This vitamin as an essential micronutrient is acquired primarily through the consumption of fruits, and vegetables (17). However, AA is also readily available as an out of the counter drug that is usually consumed by healthy people. Data showed that 12.4% of the US adults take this vitamin as a dietary supplement (18).

While, high intake of AA might have a toxic effect (19), excess amounts of AA can be excreted through urine because of its water-soluble characteristic. Normally, consumption of doses up to 2000 mg/day is safe for the general population (20). The beneficial effect of AA consumption in diabetes is controversial. Some data support the idea that due to impairment of insulin secretion and ascorbate cycle in DM, AA is necessary to optimize the insulin secretory function of the islet cells (21). Another important function of AA is its ability to regenerate VE and some other antioxidants (22).

Overall, it has been hypothesized that the antioxidant effects of AA may improve the glycemic status of the DM, though not enough evidences exist in the literature to strongly support this idea. The present meta-analyses systematic review is a novel work, because we focused on anti-hyperglycemic effect of AA according to data, separately extracted from both observational and randomized controlled trials. Specifically, our main outcome measures were the assessment of association between AA and FBS, HbA1c or incidence of diabetes.

ABBRIVIATIONS

95% CI, 95% confidence intervals
AA, ascorbic acid
ADA, American Diabetes Association
DBP, diastolic blood pressure
FBS, fasting blood sugar
HbA1c, glycated hemoglobin
HDL-C, high density lipoprotein cholesterol
IFG, impaired fasting glucose
IGT, impaired glucose tolerance
LDL-C, low-density lipoprotein cholesterol
NGT, normal glucose tolerance
OGTT, oral glucose tolerance test
PL, placebo
PRISMA, preferred reporting items for systematic reviews and meta-analyses
RR, relative risk
RTC, randomized controlled trials
SBP, systolic blood pressure
SMD, standardized mean difference
STROBE, strengthening the reporting of observational studies in epidemiology
T2DM, type 2 diabetes mellitus
TC, total cholesterol
TG, triglycerides
VE, vitamin E
WHO, World Health Organization

METHODS

Search strategy

All relevant available observational studies, including cohort, case-control or cross-sectional studies as well as randomized controlled trials (RCTs) conducted to assess anti-hyperglycemic effect of AA in human and published before January 2013, were included. To obtain all related studies Google Scholar, PubMed, Scopus, IranMedex, and Magiran web databases were systematically searched. The used search terms were “antioxidant”, “diabetes”, “vitamin C”, “vit. C”, “ascorbic acid”, limited in human. In order to obtain the relevant information, we sent at least 3 e-

mails to corresponding authors, whenever the data was incomplete. In the next step, the title and then the abstract of papers were examined. The duplicated articles were excluded, and then potentially eligible studies were retrieved for perusal in full text.

Study selection

All observational studies or RCTs that met the following criteria were included: 1) observational studies or RCTs involving T2DM patients; 2) fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c), incidence of diabetes was estimated at baseline and at the end of a single intake of AA or its mixture with other antioxidants; 3) using oral glucose tolerance test (OGTT) or other accepted criteria for defining diabetes.

Studies that followed the above criteria, but conducted primarily in the healthy population, children, pregnant women, or patients with type 1 DM were excluded. Other exclusion criteria were defined as animal studies, *in vitro* studies, review articles, letters to the editor and thesis. No language restriction was set.

Data extraction and quality assessment

The following data were extracted: design of study, participants characteristics (age, sex), sample size in each groups (treatment or placebo), number of diabetic patients, number of participants received or not received AA, frequency and dosage of each supplementation (vitamin, placebo, or the mixture of vitamin with other antioxidants), concomitant therapy, duration of intervention, follow up, serum levels of AA, mean levels of FBS and HbA1c, case ascertainment, and endpoints. Primary outcome measures included the net changes in serum levels of FBS and HbA1c or incidence of diabetes after AA supplementation. The secondary outcomes were changes in serum concentrations of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and insulin as well as diastolic (DBP) and systolic blood pressure (SBP).

The methodological quality of each included observational/ cross-sectional studies, and RCTs was assessed using the STROBE (strengthening the reporting of observational studies in epidemiology) and Jadad scoring system (23, 24), respectively. Two authors independently assessed the quality of all studies. Five selected items from the recommended checklist of STROBE (24) was used for quality assessment. The items included: a) 1 point for confirmed DM diagnosis, according to the American Diabetes Association (ADA) or other accepted criteria; b) 1 point for providing the eligibility criteria; c) 1 point for presenting the key elements of study design; d) 1 point if dietary intake was estimated, using a valid questionnaire or tool, to measure the intake of AA and/or other antioxidants' nutrient; and e) 1 point for describing the characteristics of study participants. Studies that did not fulfill more than two criteria (≤ 3 points) were classified as low quality.

In Jadad scale, for each part addressed in the study, one point was considered, with possible scores ranged between 0 and 5 (randomized, double-blinding, description of withdrawals and dropouts, generation of random numbers and allocation concealment) (24). RCTs with score <3 were considered as low quality.

Data synthesis and analysis

As mentioned previously, our main outcome measures were the assessment of association between AA and FBS, HbA1c or incidence of diabetes. According to World Health Organization (WHO) definition, diabetes mellitus is described as having a FBS ≥ 126 mg/dl or 2-h OGTT with 75 g glucose ≥ 200 mg/dl (25). Those with 2-h plasma glucose of 140 to 199 mg/dl, or FBS 100 to 125 mg/dl are classified as impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), respectively (25). Subjects with FBS< 110 mg/dl are defined as normal glucose tolerance (NGT) (26). HbA1c, an index of average glucose control over the last 3 months, is considered as another marker of hyperglycemia when its value was greater than or equal to 6.5% (25).

Data from selected studies were extracted in the form of 2×2 tables by study characteristics. Included studies were weighted by effect size and then pooled. Data were analyzed using StatsDirect software version 3.0.97. Relative Risk (RR) and 95% confidence intervals (95% CI) were calculated using Mantel-Haenszel, Rothman-Boice (for fixed effects) and DerSimonian-Laird (for random effects) methods. The Cochran Q test was used to test heterogeneity. If the Q-statistic for heterogeneity was significant at the level of 0.1 or few included studies, random effects model was employed (27). In other cases the fixed effects model was used (28). Standardized effect size and 95% CI were calculated using Mulrow-Oxman (for

fixed effects) and DerSimonian-Laird (for random effects) methods. The degree of heterogeneity was quantified using I^2 statistic which is an estimate of the total variation across studies due to heterogeneity (29). Egger and Begg-Mazumdar tests were used to evaluate publication bias indicators in funnel plot (30). A p value ≤ 0.05 was considered as statistically significant.

RESULTS

This study is reported according to PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guideline (31).

Search results

A flow chart presenting the process of initiation and selection of the studies is shown (Figure-1). In the final step of selection, 38 articles were included to systematic review (32-57, 58-69), of which 5 articles were eligible to meta-analysis (58, 59, 61, 63, 65). The summary of eligible observational and RCT studies and their response to treatment are shown (Tables 1-4; a and b).

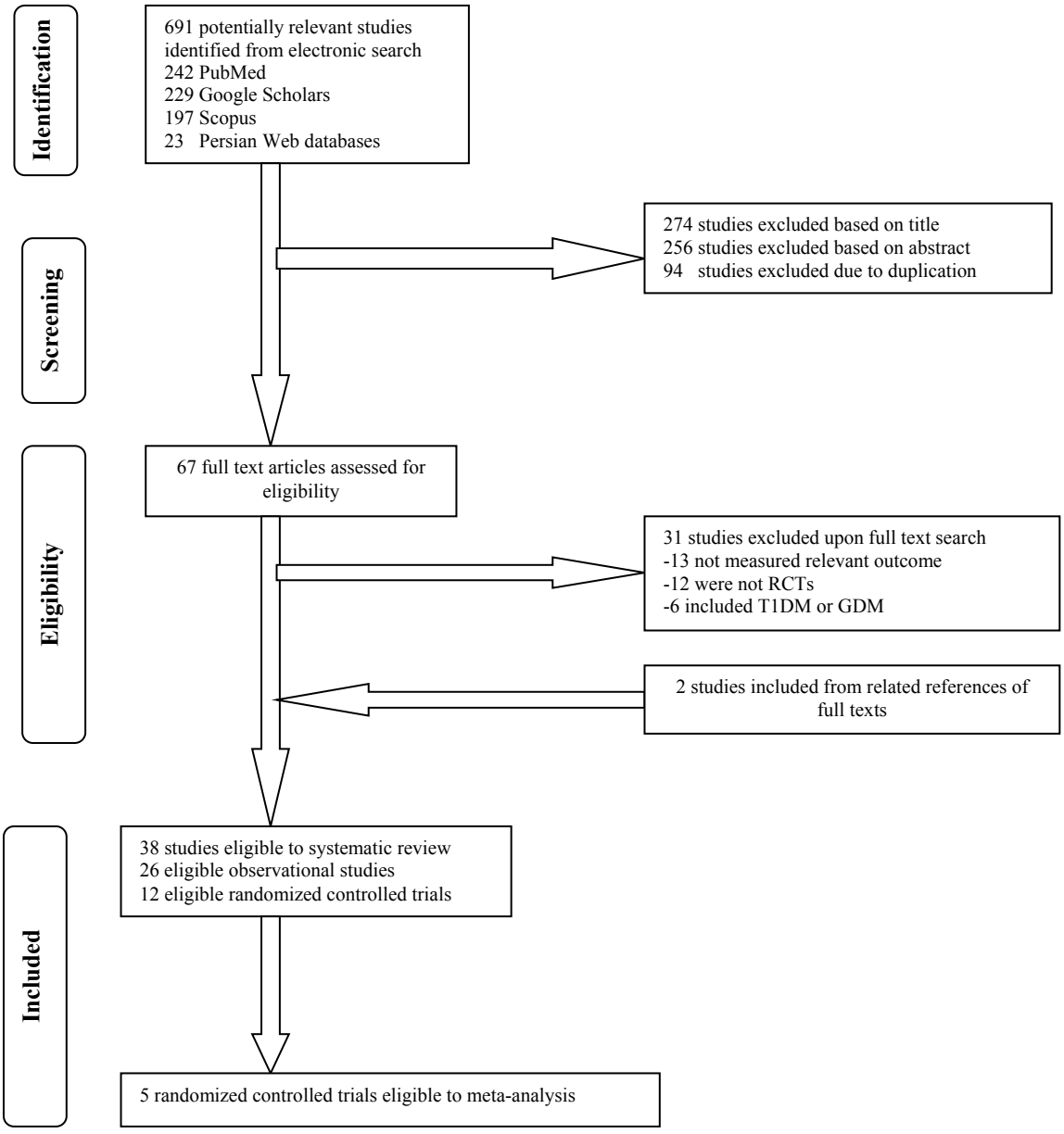


Figure 1. Flow diagram of the study selection process

Table 1-a. Observational studies of ascorbic acid intake and type 2 diabetes mellitus

Ref.	Sex	Age (yr)	Study design	No. of participants	No. of DM cases	Concomitant therapy	Duration of AA therapy	AA dosage	Follow up	Baseline serum AA (µmol/l)	Case ascertainment	End points	Quality score (0-5)
32	M/F	25-75	Co	2,115	93 (DM), 539 (IGT, IFG)	---------	During past 24h	NA	---------	39.3	OGTT, HbA1c measurement	Primary prevention of T2DM	5
33	M/F	>19	CS	3,816	NA	VA, VB6, Folate	During past 24h	NA	-------	53.9	Plasma level of glucose	Effect of orange juice consumption on risk factors of MetS	5
34	M/F	≥18	Co	7,697	418	VE components, Carotenoids, Retinol	During previous 30 days	NA	--------	55.1	HbA1c measurement (>6%) in persons self-reported non- DM	Primary prevention of DM	5
35	M/F	50-71	Co	14,109	NA	Iron, Zinc, Selenium, Folate, VA, VE, BC, Calcium	During previous year	NA	---------	NA	Physician diagnosed	Effect of vitamin intake on DM Risk	5
36	M/F	NA	CC	149	89	---------	NA	NA	-------	64.7	FBS measurement	Assessment of antioxidant status in T2DM	3
37	M/F	≥30	Nested CC	300	100	----------	During previous day	NA	---------	25	OGTT	Relationship between serum vit.C and DM	5
38	M/F	36	Co	1,065	46	Iron, BC, Riboflavin, Calcium, Folate, VB12, VK	During past 5 days	NA	17 yr	NA	HbA1c measurement	Prediction of raised HbA1c and risk of DM	4
39	M/F	40-75	Co	21,831	735	--------	During previous year	NA	12 yr	53.9	Self-reported DM, and HbA1c measurement	Relationship between fruit and vegetable intake, serum vit.C and DM risk	5
40	M/F	32-72	CC	88	46	----------	During previous year	NA	---------	21.6	FBS measurement	Comparison the level of vit.C in T2DM and non-DM	5
41	M/F	39-68	CS	77	77	VE, Carotenoids	During past 24 h	NA	--------	NA	Known DM, FBS measurement	Effect of diet on glucose and lipid profiles	4
42	M/F	≥65	CC	1,038	103	Iron, Zinc, Cu, Folate, VD, VE, VB6, Calcium, Thiamin, Niacin Riboflavin	During past 4 days	NA	------	35.6	Self-reported DM, HbA1c measurement	Effect of nutrient intake on DM	5
43	M/F	40-69	Co	4,304	383	VE components, Carotenoids	During previous year	NA	23 yr	NA	Social insurance reported, use of diabetic medication, OGTT at baseline	Dietary antioxidant intake and T2DM Risk	5
44	M/F	52-58	CC	52	42	VA, VE	Serum level of AA	NA	------	88.6	FBS and HbA1c measurement	Antioxidants status in DM	5
45	M/F	>60, Mean 70.4	Co	1,987	189	----------	Serum level of AA	NA	--------	35.3	Medical history of DM	Effect of plasma vit.C on age-related eye diseases	4

46	M/F	25-74	Co	9,573	1,010	VA, VB, VE, VD	During previous 30 days	NA	20 yr	NA	Self-reported DM	Vitamin use and DM risk	5
47	M/F	35-64	CS	1,773	1,178	VA, VB, VE, multivitamin	During previous year	NA	2 yr	NA	HbA1c measurement	Effect of diet and life styles on HbA1c	5
48	M/F	30-65	CC	95	62	----------	Serum level of AA	NA	---------	59.6	FBS measurement	Comparison vit.C level in plasma and mononuclear leukocytes of DM and non-DM	5
49	M/F	45-74	Co	6,458	250	----------	During previous year	NA	4 yr	50.9	Self-reported DM, HbA1c measurement	Relationship between serum vit.C, DM and HbA1c	5
50	M/F	40-74	CC	2,040	237	----------	During past 24 h	NA	---------	41.8	OGTT	Assessment of relationship between vit.C level and DM	5
51	M/F	Mean age: 63.5	CS	69	69	----------	Serum level of AA	NA	---------	33.0	Known DM, HbA1c measurement	Effect of glycemic control on antioxidant status	4
52	M/F	34-62	CC	647	467	VA, VE	Serum level of AA	NA	---------	47.3	Known DM, HbA1c measurement	Effect of lipid peroxidation and antioxidant status on DM	5
53	M	age at OGTT: 70-89	Co	338	97; (26 DM, 71 IGT)	----------	During 6-12 mo preceding the interview	NA	20 yr	NA	OGTT after 20 yr follow-up	Predict of glucose intolerance and DM by dietary factors	4
54	M/F	Mean age: 68.8	CC	40	20	----------	During past 4 days	NA	---------	49.6	Known DM, BG measurement	Level of vit.C in T2DM with adequate dietary vit.C	4
55	M/F	43-84	CC	2,141	167	VE, BC	During previous year	NA	---------	NA	Self-reported, serum HbA1c and non fasting BG measurement	Effect of antioxidant-nutrient intake on HbA1c	4
56	M/F	51-63	CC	66	33	---------	Serum level of AA	NA	---------	61.3	FBS and HbA1c measurement	Association between hyperglycemia and plasma or leukocyte level of vit.C	4
57	M/F	18-74	CC	308	134	-----	Serum level of AA	NA	-----	NA	Known DM	Metabolism of vit.C in DM	3

M: male; **F:** female; **Co:** cohort; **CS:** cross-sectional; **CC:** case-control; **NA:** non-application; **AA:** ascorbic acid; **VE:** vitamin E; **BC:** beta-carotene; **VA:** vitamin A; **VD:** vitamin D; **VK:** vitamin K; **VB6:** vitamin B6; **FBS:** fasting blood sugar; **OGTT:** oral glucose tolerance test; **HbA1c:** glycosylated hemoglobin; **BG:** blood glucose; **T2DM:** type 2 diabetes mellitus; **DM:** diabetes mellitus; **IGT:** impaired glucose tolerance; **IFG:** impaired fasting glucose; **Mets:** metabolic syndrome.

Table 1-b. Response to treatment with ascorbic acid in eligible observational studies

Ref.	Results	Ref.	Results
32	Significant inverse association between serum AA and HbA1c, FBS and 2hBG per 1 SD serum AA=21.8µmol/l) in adjusted and un-adjusted analysis	33	Non-significant difference in mean level of glucose, OR of high glucose, and OR of Mets vs. un-consumers of orange juice
34	Significant inverse association with HbA1c after adjusted for confounders (especially among 18-44 yr, females, or Mexican Americans subgroups)	35	Non-significant OR of DM risk by frequent use of multivitamins after adjustment, but significant low DM risk with individual use of AA
36	Significant inverse association between serum AA and FBS or DM duration	37	Significant inverse association between serum AA and FBS or 2hBG in subjects with inadequate status of AA
38	Non-significant effect of AA intake on raised HbA1c	39	Significant low DM risk or low HbA1c by increase serum AA in un- and adjusted analysis
40	Significant inverse assciation between serum AA and FBS	41	Significant inverse association between serum AA and FBS
42	Significant inverse association between serum AA and HbA1c in males by univariate linear regression analysis	43	Non-significant effect of AA intake on risk of T2DM
44	Non-significant difference in mean level of AA between DM and non-DM	45	Significant low AA level in diabetic women
46	Non-significant association between AA intake and DM incidence after adjustment	47	Significant inverse association between AA intake and HbA1c after adjustment
48	Non-significant association between serum and leukocytes' AA and FBS or DM duration, non-significant difference in mean level of AA between DM and non-DM, significant decrease in leukocytes' AA in FBS>250 mg/dl	49	Significant inverse association between serum AA and HbA1c or prevalent undiagnosed hyperglycemia (per 1 SD serum AA=20 µmol/l) in un- and adjusted analysis
50	Non-significant inverse association between serum AA and DM risk after adjustment for AA intake and other covariates, non-significant association between FBS and serum AA	51	Non-significant association between HbA1c and serum AA
52	Significant inverse association between serum AA and HbA1c	53	Significant inverse association between AA intake and development IGT and DM independently of changes in intake of fat and AA during 20 yr follow up
54	Significant low AA level in DM with adequate intake of AA	55	Non-significant association between AA intake and HbA1c in DM, but significantly negative association after age and sex adjusted in non-DM, remained significant after adding BG and smoking
56	Non-significant increase in serum and leukocyte level of AA in DM vs. non-DM, non-significant inverse association between serum AA and HbA1c, FBS or DM duration	57	Very low level of AA in maturity onset diabetes, Non-significant difference between mean leukocyte's AA in DM and non-DM, No association between serum AA and BG

OR: Odds Ratio; **Mets:** Metabolic syndrome; **FBS:** Fasting blood sugar; **2hBG:** after 2 hour post prandial blood glucose; **T2DM:** Type 2 diabetes mellitus; **AA:** ascorbic acid; **SD:** standard deviation; **IGT:** impaired glucose tolerance.

Table 2-a. Randomized controlled trials of ascorbic acid intake and type 2 diabetes mellitus

Ref.	Sex	Age (yr)	Trial design	Treatment duration	Daily dosage AA	Mean±SD baseline FBS (mg/dl) in study groups and groups size (n)						Mean±SD baseline HbA1c (%) in study groups and groups size (n)						OA	Baseline serum AA (µmol/l)
						AA	PL	OA	M	AA+ OA	AA+ OAM	AA	PL	OA	M	AA+ OA	AA+ OAM		
58	M/F	30-70	ORCT	3 mo	500 mg	201± 82.7 (30)	182± 46.2 (29)	-----	------	-------	-----	9.4± 1.7 (30)	8.7± 1.6 (29)	-----	------	------	------	------	NA
59	M	33-63	DRCT	8 w	200 mg	153.4± 44.9 (17)	155.6± 55.4 (17)	159.4± 50.7 (16)	-----	149.2± 30.2 (15)	-----	7.7± 1.4 (17)	8.1± 1.6 (17)	7.7± 0.9 (16)	------	8.05± 0.9 (15)	------	EPA	NA
60	F	≥ 40 yr	DRCT	~9 yr	500 mg	NA (816)	NA (822)	NA (2474)	----	NA (2462)	-----	-----	----	--------	-----	--------	-------	VE, BC	NA
61	M/F	33-69	DRCT	3mo	1,250 mg	176.7± 46.7 (68)	226± 87 (68)	------	----	------	-----	10.4± 2 (68)	10.2± 1.9 (68)	-----	----	-----	-----	-----	NA
62	M/F	33-75	PRCT	6 w	500 or 1000 mg	169.3± 34 By 1000 mg (43)	------	152.7± 34.5 By 500 mg AA (41)	----	-----	-----	8.8± 1.3 By 1000 mg (43)	----	8.4± 1.7 (By 500 mg AA) (41)	-----	----	-----	------	NA
63	M/F	30-69	DRCT	3 mo	200 mg	---	164± 51 (18)	-----	173± 51 (16)	198± 48 (18)	177± 41 (17)	-----	9.2± 2 (18)	----	10.4± 2.7 (16)	11.2± 3.4 (18)	9.3± 1.6 (17)	OA (VE), M (Mg,Zn)	61.9
64	M/F	35-60	RCT	~7.5 yr	120 mg	-----	101.5± 9 (1533)	------	----	-----	101.3± 9 (1613)	-----	-----	-----	----	------	-----	VE, BC, Se, Zn	51.1
65	M/F	29-75	RCT	4 w	500 mg	227± 89.7 (14)	210.7± 73.2 (14)	200± 70.2 (14)	-----	235.1± 73.2 (14)	-----	5.3± 1.3 (14)	5.5± 0.8 (13)	4.6± 0.8 (14)	----	4.6± 0.6 (14)	----	VE	76.6
65	M/F	29-75	RCT	9 w	500 mg	230.3± 92.5 (13)	202.2± 68.6 (13)	184.6± 63.1 (10)	-----	237.2± 75.8 (13)	-----	5.3± 1.3 (13)	5.6± 0.8 (13)	4.4± 0.6 (10)	----	4.6± 0.6 (13)	-----	VE	78.9
66	M/ F	57-72	RCT	2-4 mo	1000 mg	---------	----- (148)	-------	-----	------- (149)	-----	NA	NA	NA	NA	NA	NA	VE, BC, Zn, Se, Cu, Mn	NA
67	M/F	59-63	RCT-CO	90 d/ 4 w wash-out	2 g	181.8± 10.8 (27)	-----	------	181.8± 10.8 (27)	------	------	9.3± 0.3	-----	------	9.3± 0.3	------	------	Mg	NA
68	M/F	72± 0.5	RCT-CO	4 mo/ 30 d wash-out	0.5 g twice daily	158.4± 7.2 (40)	158.4± 7.2 (40)	-----	------	------	-------	8.1± 0.4	8.1± 0.4	-----	------	-----	-----	----	41.2
69	M/F	19-76	DRCT-CO	4 mo	500 mg	135± 63 (50)	135± 63 (50)	-----	-----	-----	------	9.8± 2	9.8± 2	------	------	------	------	----	41.1

ORCT: open label randomized; **DRCT:** double blind randomized; **PRCT:** parallel randomized; **CORCT:** cross over randomized; **CO:** cross over; **AA:** ascorbic acid;

PL: placebo; **OA:** other antioxidants; **M:** mineral; **FBS:** fasting blood sugar; **NA:** non applicable; **EPA:** Eicosapentaenoic acid; **BC:** beta carotene; **Se:** selenium; **Mg:** Magnesium.

Table 2-b. Response to treatment in eligible RCTs of ascorbic acid intake and type 2 diabetes mellitus

Ref.	serum AA±SD (µmol/l)		Mean±SD FBS (mg/dl) after intervention						Mean±SD HbA1c (%) after intervention						Case ascertainment	End points	Results
	Received AA	Not received AA	AA	PL	OA	M	AA+ OA	AA+ OAM	AA	PL	OA	M	AA+ OA	AA+ OAM			
58	-------	-------	189.8± 68.3	195± 52.6	----	----	-----	-----	9± 1.6	8.9± 1.6	-----	-----	------	------	FBS, HbA1c	Prevention of T2DM's complications	Not benefit
59	------	-------	132.6± 35.1	135.2± 12	130.4± 37	-----	136± 38	-----	7.05± 1.1	7.07± 0.9	7± 1.2	-----	7.09± 1.06	-----	FBS, HbA1c	Effect of EPA and AA on risk factor of CVD in DM	Benefit
60	107.9	73.8	NA	NA	NA	NA	NA	NA	------	------	-----	-----	------	------	Self-reported, hypoglycemic medications, BG screening	Primary prevention of T2DM	Non-significant effect on DM risk, significant reduction in DM risk in without history high cholesterol, non-significant 2 or 3-way interactions among the agents for DM risk
61	------	----------	148.1± 37.7	233.6± 78.6	-----	------	-----	------	7.9± 1.2	10.2± 1.7	-----	------	------	------	FBS, HbA1c	Metabolic control of DM	Benefit
62	------	----------	144.8± 33.4 By 1000 mg AA	------	159.3± 40.3 By 500 mg AA	-----	------	------	7.7± 1.3 By 1000 mg AA	------	8.4± 1.6 By 500 mg AA	------	------	------	FBS, HbA1c	Metabolic control of DM	Benefit by 1000 mg AA
63	76.6± 13.6 (AA+VE)	62.4± 18.7	-----	175± 49	----	176± 46	181± 42	165± 46	----	10± 2	----	10.6± 2.2	11± 2.2	9.4± 2.2	FBS, HbA1c	Effect of VE, AA and minerals on DM	Benefit in FBS by AA+OAM, not benefit in HbA1c in all groups
64	----------	---------	--------	90.1± 9	------	----	-----	90.2± 10.3	-----	------	------	-----	-----	-----	FBS	Primary prevention of DM	Significant inverse association between baseline AA level and FBS after multiple adjusted, non-significant inverse association between AA intake and FBS
65 (4 weeks)	---------	----------	207.4± 62.4	201.7± 77.4	195.8± 53.6	-----	204.2± 84.8	------	5.3± 1.1	5.4± 1.1	5.06± 1	-----	4.9± 0.7	-----	FBS, HbA1c	Metabolic control of DM by AA and VE	Benefit in FBS by AA+OA
65(9 weeks)	-------	---------	233.8± 84.3	207.8± 86.8	191.6± 59.8	-----	197.3± 77.6	------	5.8± 1.5	5.5± 1.5	5.02± 1.2	-----	5.1± 1.05	-----	FBS, HbA1c	Metabolic control of DM by AA and VE	Benefit in FBS by AA+OA
66	-------	----------	------	105.7± 33.8	-----	------	102.2± 26.3	------	NA	NA	NA	NA	NA	NA	FBS	Effect of antioxidants on traditional RF of CVD	Not benefit
67	-------	---------	163.8± 9	----	-----	174.6± 9	-----	----	8.5± 0.3	-----	-----	8.9± 0.3	-----	-----	FBS, HbA1c	Metabolic control of DM by AA and Mg	Benefit

68	75.6± 3.7	43.4± 2.8	154.8± 9	156.6± 12.6	-----	-----	------	-----	7.2± 0.3	8± 0.4	-----	-----	-------	-------	FBS, HbA1c	Metabolic control of DM	Non-significant effect on FBS, Significant improvement in whole body glucose disposal, and HbA1c, Non-significant correlation with whole body glucose disposal after adjusted for free radical levels
69	95.4± 28.4	39.7± 28.4	134.1± 63.9	142.2± 65.7	-----	----	------	------	9.7± 1.8	9.7± 1.7	-----	-----	------	------	FBS, HbA1c	Effect of AA on diabetic hyperlipidemia	Not benefit

AA: ascorbic acid; **PL:** placebo; **OA:** other antioxidants; **M:** mineral; **OAM:** other antioxidants and mineral; **FBS:** fasting blood sugar; **HbA1c:** glycosylated hemoglobin;
NA: non applicable; **EPA:** Eicosapentaenoic acid; T2DM: type 2 diabetes mellitus; **CVD:** cardiovascular disease; **DM:** diabetes mellitus**; Mg:** Magnesium.

Table 3-a. Secondary outcomes in eligible RCTs of ascorbic acid intake and type 2 diabetes mellitus

Ref.	Mean±SD baseline TC (mg/dl) in study groups and groups size (n)						Mean±SD baseline TG (mg/dl) in study groups and groups size (n)						Mean±SD baseline LDL-C (mg/dl) in study groups and groups size (n)					
	AA	PL	OA	M	AA+OA	AA+OAM	AA	PL	OA	M	AA+OA	AA+OAM	AA	PL	OA	M	AA+OA	AA+OAM
58	196.7± 35.5 (30)	191± 34.9 (29)	-----	-----	------	-------	170.5± 73 (30)	168.6± 53.2 (29)	-----	-----	-----	-----	113.7± 27.6 (30)	108.3± 31 (29)	-----	-----	-----	------
59	172.6± 25.5 (17)	154.6± 9.5 (17)	201± 38.1 (16)	-----	204.8± 44.4 (15)	-----	163.7± 56.4 (17)	134.1± 71.6 (17)	203± 53.1 (16)	-----	153.6± 45.3 (15)	-----	104.2± 28.2 (17)	97.3± 19 (17)	108.7± 27.4 (16)	-----	128.2± 30.5 (15)	-----
61	192.6± 54.9 (68)	256.3± 85.6 (68)	-----	-----	-----	-----	196± 66.2 (68)	276± 121.3 (68)	-----	-----	-----	-----	114.3± 23.9 (68)	115.9± 33.7 (68)	-----	-----	-----	------
62	198.3± 38.1 By 1000 mg AA (43)	------	191.8± 34.7 By 500 mg AA (41)	-----	-----	-----	210± 65.1 By 1000 mg AA (43)	-----	202.7± 55.1 By 500 mg AA (41)	----	-----	-----	130.9± 35.5 By 1000 mg AA (43)	-----	127.9± 40.1 By 500 mg AA (41)	-----	----	-----
65 (4 weeks)	232.2± 41.4 (13)	228.9± 49.6 (14)	232.4± 57 (14)	-----	231.6± 69 (14)	------	390.1± 269.8 (12)	345± 223.1 (14)	256.9± 135.8 (14)	-----	368.7± 330.3 (13)	------	148.8± 30.9 (8)	136.2± 50.6 (10)	153.4± 44.8 (11)	-----	141.7± 45.6 (8)	------
65 (9 weeks)	238.3± 36.6 (12)	230.5± 51.2 (13)	226± 57.5 (10)	-----	234.5± 70.9 (13)	------	372.5± 273.2 (12)	356± 228.3 (13)	217± 103.7 (10)	-----	369.9± 345 (12)	------	149.7± 29.6 (8)	145.2± 51 (8)	140.4± 37.7 (9)	-----	144± 43.7 (8)	------
66	-----	------ (148)	-----	------	----- (149)	-----	----	----	-----	-----	-----	-----	-----	---- (148)	-----	----	----- (149)	-----
67	239.7± 7.7 (27)	-----	-----	239.7± 7.7 (27)	----	------	221.4± 17.7 (27)	----	-----	221.4± 17.7 (27)	----	-----	-----	-----	-----	----	-----	-----
68	278.4± 11.6 (40)	278.4± 11.6 (40)	----	-----	-----	-----	230.3± 8 (40)	230.3± 8 (40)	-----	-----	-----	-----	220.4± 15.5 (40)	220.4± 15.5 (40)	-----	-----	-----	-----

69	270.7± 46.4 (50)	270.7± 46.4 (50)	-----	-----	-----	------	248± 132.9 (50)	248± 132.9 (50)	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

AA: ascorbic acid; **PL:** placebo; **OA:** other antioxidants; **M:** mineral; **OAM:** other antioxidants and mineral; **TC:** total cholesterol; **TG:** triglycerids; **LDL-C:** low density lipoprotein cholesterol; **NA:** non applicable.

Table 3-b. Response to secondary outcomes in eligible RCTs of ascorbic acid intake and type 2 diabetes mellitus

Ref.	Mean±SD TC (mg/dl) after intervention						Mean±SD TG (mg/dl) after intervention						Mean±SD LDL-C (mg/dl) after intervention					
	AA	PL	OA	M	AA+OA	AA+OAM	AA	PL	OA	M	AA+OA	AA+OAM	AA	PL	OA	M	AA+OA	AA+OAM
58	178.2± 26.6	197.9± 35.2	-----	-----	------	-------	160.5± 53.3	171.4± 50.2	-----	-----	-----	-----	96.4± 27.8	115.4± 30.7	-----	-----	------	-----
59	165± 27.6	159.2± 23.6	163.6± 34.9	-----	168.4± 32	-----	139.5± 46.9	115.5± 73.8	171.8± 45.4	-----	128.1± 46.9	-----	172.6± 25.5	154.6± 9.5	201± 38.1	-----	204.8± 44.4	-----
61	178.9± 53.3	263.5± 86.4	---	-----	-----	------	166.2± 50	285.8± 109.1	-----	-----	-----	-----	95.2± 29	117.2± 32.2	-----	-----	-----	-----
62	187.7± 31.2 By 1000 mg AA	------	190± 36.8 By 500 mg AA	-----	-----	-----	186.6± 54 By 1000 mg AA	-----	201.7± 51.4 By 500 mg AA	----	-----	-----	125.9± 33.8 By 1000 mg AA	-----	124.8± 39.1 By 500 mg AA	-----	----	-----
65 (4weeks)	223.7± 64.2	219.2± 42.9	237.5± 50.6	-----	236.1± 49.4	------	320.3± 181.4	307.9± 144.9	246.2± 104.8	-----	380.8± 207.9	------	127.2± 50.6	138.6± 41.9	150.6± 42.7	-----	150.4± 33.8	------
65 (9weeks)	228.8± 32.8	214± 42.3	216.8± 46.6	-----	223.3± 61.6	------	306.2± 152.9	323.2± 217.9	194.8± 50.5	-----	261.5± 112.5	------	149.8± 27.4	137.7± 52.4	141.4± 41.3	-----	139.9± 36.7	------
66	------	226.9± 38.4	-----	-----	230± 48.2	----	----	-----	-----	-----	-----	------	-----	148.6± 36.6	-----	-----	153± 37.9	----
67	228.1± 7.7	----	----	239.7± 7.7	-----	-----	194.9± 17.7	---	----	230.3± 17.7	----	-----	----	-----	-----	-----	-----	-----
68	224.3± 15.5	282.3± 19.3	----	-----	-----	-----	230.3± 6.2	186± 3.5	-----	-----	-----	-----	216.5± 23.2	158.5± 11.6	-----	-----	-----	-----
69	255.2± 50.3	255.2± 50.3	-----	-----	-----	------	274.6± 168.3	292.3± 203.7	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

AA: ascorbic acid; **PL:** placebo; **OA:** other antioxidants; **M:** mineral; **OAM:** other antioxidants and mineral; **TC:** total cholesterol; **TG:** triglycerids; **LDL-C:** low density lipoprotein cholesterol.

Table 4-a. Secondary outcomes in eligible RCTs of ascorbic acid intake and type 2 diabetes mellitus

Ref	Mean±SD baseline HDL-C (mg/dl) in study groups and groups size (n)						Mean±SD baseline SBP (mmHg) in study groups and groups size (n)						Mean±SD baseline DBP (mmHg) in study groups and groups size (n)					
	AA	PL	OA	M	AA+ OA	AA+ OAM	AA	PL	OA	M	AA+ OA	AA+ OAM	AA	PL	OA	M	AA+ OA	AA+ OAM
58	48.9 ± 8 (30)	49 ± 7 (29)	-----	----	-----	-----	139.7 ± 16.5 (30)	134.5± 14.6 (29)	----	----	-----	-----	81.1 ± 7.6 (30)	78.6± 10.9 (29)	-----	----	-----	-----
59	38.1 ± 10.4 (17)	42.2 ± 15.8 (17)	42.6 ± 5.2 (16)	----	41.5 ± 6.1 (15)	-----	139 ± 11 (17)	140 ± 12 (17)	135± 13 (16)	----	138 ± 9 (15)	-----	-----	-----	------	----	-----	-----
61	51.1 ± 21.8 (68)	49.4 ± 14.8 (68)	------	----	-----	-----	-----	-----	----	----	----	---	----	----	----	----	----	----
62	45.9 ± 11.1 By 1000 mg AA (43)	----	36.8 ± 9.1 By 500 mg AA (41)	----	----	----	-----	-----	-----	------	-----	-------	------	----	------	----	-----	----
65(4 w)	32.3 ± 7.3 (13)	32.8 ± 9.5 (14)	36.6 ± 12.5 (14)	----	32.5 ± 8.4 (14)	-----	136.8 ± 24.8 (14)	145.7± 19.9 (14)	141.1± 26.4 (14)	------	141.8± 17.4 (14)	-----	76.1 ± 13.9 (14)	82.5 ± 10.9 (14)	79.3 ± 10.9 (14)	----	83.2 ± 9.9 (14)	------
65 (9 w)	32.4 ± 7.7 (12)	32.3 ± 9.7 (13)	37.2 ± 14.5 (10)	----	33.4 ± 7.9 (13)	-----	135 ± 24.9 (13)	147.3± 19.7 (13)	144± 25.6 (10)	-----	144.2± 15.4 (13)	-----	75 ± 13.8 (13)	82.7 ± 11.3 (13)	80.5 ± 12.6 (10)	----	83.5 ± 10.3 (13)	-----
66	-----	---- (148)	----	----	---- (149)	-----	----	----- (148)	-----	----	---- (149)	-----	-----	---- (148)	-----	----	---- (149)	----
67	46.4 ± 2.3 (27)	----	----	46.4 ± 2.3 (27)	----	----	149 ± 3 (27)	-----	----	149 ± 3 (27)	----	----	87 ± 2 (27)	----	-----	87 ± 2 (27)	-----	----
68	42.5 ± 3.9 (40)	42.5 ± 3.9 (40)	----	----	----	-----	-----	------	-----	------	-----	------	-----	----	-----	----	-----	-----

AA: ascorbic acid; **PL:** placebo; **OA:** other antioxidants; **M:** mineral; **OAM:** other antioxidants and mineral; **HDL-C:** high density lipoprotein cholesterol; **SBP:** systolic blood pressure; **DBP:** diastolic blood pressure.

Table 4-b. Response to secondary outcomes in eligible RCTs of ascorbic acid intake and type 2 diabetes mellitus

Ref	Mean±SD baseline HDL-C (mg/dl) after intervention						Mean±SD baseline SBP (mmHg) after intervention						Mean±SD baseline DBP (mmHg) after intervention)					
	AA	PL	OA	M	AA+ OA	AA+ OAM	AA	PL	OA	M	AA+ OA	AA+ OAM	AA	PL	OA	M	AA+ OA	AA+ OAM
58	51.9 ± 8.3	49 ± 9	-----	----	-----	-----	136.9 ± 16.7	142.3± 13	----	----	-----	-----	80.5 ± 6.2	85.7± 9.1	-----	----	-----	-----
59	40.8 ± 9.1	44.6 ± 13.3	44.6 ± 4.2	----	44.2 ± 3.8	-----	134 ± 8	138 ± 7	132± 7	----	133 ± 6	-----	-----	-----	------	----	-----	-----
61	67 ± 12.9	49.7 ± 15.6	------	----	-----	-----	-----	-----	----	----	----	---	----	----	----	----	----	----
62	47.8 ± 10.6 By 1000 mg AA	----	38.4 ± 8.8 By 500 mg AA	----	----	----	-----	-----	-----	------	-----	-------	------	----	------	----	-----	----
65(4w)	30.8 ± 7.3	29.5 ± 6.5	34.8 ± 10.6	----	31 ± 8	-----	132.5 ± 29.6	145.7± 26.1	141.1± 24.8	------	137.1± 19.7	-----	75.4 ± 12.2	83.2 ± 12.6	79.3 ± 6.7	----	84.3 ± 12.8	------
65 (9 w)	30.4 ± 7.4	31.4 ± 6.7	35.2 ± 10.7	----	33.6 ± 9.9	-----	130 ± 23.2	140 ± 21.5	139.5 ± 19.6	-----	138.8± 17.9	-----	73.8 ± 10.8	77.7 ± 8.1	79 ± 7.4	----	80 ± 11.4	-----
66	-----	47.5 ± 16.6	----	----	48.4 ± 17	-----	----	132.5 ± 19.9	-----	----	133.1 ± 18.7	-----	-----	75.8 ± 8.9	-----	----	76.4 ± 9.7	----
67	42.5 ± 1.9	----	----	42.5 ± 2.3	----	----	155 ± 3	-----	----	151 ± 4	----	----	88 ± 2	----	-----	86 ± 2	-----	----
68	38.7 ± 7.7	42.5 ± 11.6	----	----	----	-----	-----	------	-----	------	-----	------	-----	----	-----	----	-----	-----

AA: ascorbic acid; **PL:** placebo; **OA:** other antioxidants; **M:** mineral; **OAM:** other antioxidants and mineral; **HDL-C:** high density lipoprotein cholesterol; **SBP:** systolic blood pressure; **DBP:** diastolic blood pressure.

Table 5-a. Secondary outcomes in eligible RCTs of ascorbic acid intake and type 2 diabetes mellitus

Ref.	Mean±SD baseline serum AA(µmol/l) in study groups and groups size (n)						Mean±SD baseline insulin (µu/ml) in study groups and groups size (n)					
	AA	PL	OA	M	AA+OA	AA+OAM	AA	PL	OA	M	AA+OA	AA+OAM
58	NA (30)	NA (29)	-----	-----	----	-----	-----	-----	-----	-----	----	-----
59	NA (17)	NA (17)	NA (16)	-----	NA (15)	----	-----	-----	----	------	-------	-----
60	NA (816)	NA (822)	NA (2474)	------	NA (2462)	------	--------	-------	--------	-------	----------	------
61	NA (68)	NA (68)	------	------	-----	-----	--------	---------	-------	---------	----------	-------
62	NA 1000 mg AA (43)	----	NA 500 mg AA (41)	-----	------	-----	16.9± 3.1 By 1000 mg AA (43)	------	10.4± 2.4 By 500 mg AA (41)	----------	----------	-------
63	-----	56.8± 17 (18)	-----	62.4± 17 (16)	62.4± 17 (18)	62.4± 22.7 (17)	------	7.2± 2.8 (18)	----	7.2± 3.6 (16)	7.3± 4.2 (18)	7.4± 3.6 (17)
64	-----	52 (1533)	----	-----	-----	51.5 (1613)	-------	----------	------	--------	----------	-----
65 (4weeks)	75± 13 (14)	80± 13 (14)	81.2± 19.3 (14)	-----	71± 8.5 (14)	------	----------	--------	----------	---------	--------	---------
65 (9weeks)	76.1± 13 (13)	81.2± 12.5 (13)	77.8± 21 (10)	------	71± 9.1 (13)	--------	---------	-------	----------	----------	----------	---------
66	-----	NA	------	------	NA	-------	---------	-------	---------	-----	---------	------
67	NA	------	------	NA	--------	--------	-------	----------	------	---------	--------	---------
68	41.2± 5.4 (40)	41.2± 5.4 (40)	-----	-------	-------	-------	12.7± 0.7 (40)	12.7± 0.7 (40)	------	--------	--------	----------
69	41.2± 26.1 (50)	41.2± 26.1 (50)	------	----------	----------	---------	----------	--------	----------	----------	----------	----------

AA: ascorbic acid; **PL:** placebo; **OA:** other antioxidants; **M:** mineral; **OAM:** other antioxidants and mineral; **NA:** non applicable.

Table 5-b. Response to secondary outcomes in eligible RCTs of ascorbic acid intake and type 2 diabetes mellitus

Ref.	Mean±SD serum AA (µmol/l) after intervention						Mean±SD insulin (µu/ml) after intervention					
	AA	PL	OA	M	AA+OA	AA+OAM	AA	PL	OA	M	AA+OA	AA+OAM
58	NA	NA	-----	-----	----	-----	-----	-----	-----	-----	----	-----
59	NA	NA	NA	-----	NA	----	-----	-----	----	------	-------	-----
60	NA	NA	NA	------	NA	------	--------	-------	--------	-------	----------	------
61	NA	NA	------	------	-----	-----	--------	---------	-------	---------	----------	-------
62	NA 1000 mg AA	----	NA 500 mg AA	-----	------	-----	8.8± 1.3 By 1000 mg AA	------	11± 2.4 By 500 mg AA	---------	----------	-------
63	-----	62.4± 17	-----	56.8± 17	73.8± 11.3	79.5± 11.3	------	7.6± 3.5	----	8± 4.4	7± 3.9	7.2± 3.1
64	-----	NA	----	----	----	NA	----------	------	----------	------	--------	------
65 (4weeks)	100.5± 13	79.5± 18.2	81.2± 17	-----	92.5± 9.1	------	----------	------	----------	---------	--------	------
65 (9weeks)	98.2± 12.5	77.8± 12.5	75.5± 17.6	------	93.1± 13	--------	---------	------	----------	----------	----------	------
66	-----	NA	------	------	NA	-------	---------	------	---------	-----	---------	------
67	NA	------	------	NA	--------	--------	-------	----------	------	---------	--------	------
68	75.6± 3.7	43.4± 2.8	-----	-------	-------	-------	10.5± 0.9	12.9± 0.6	------	--------	--------	------
69	92.5± 29.2	44.6± 21.9	-------	-------	---------	--------	---------	------	--------	-------	------	------

AA: ascorbic acid; **PL:** placebo; **OA:** other antioxidants; **M:** mineral; **OAM:** other antioxidants and mineral; **NA:** non applicable.

Studies' characteristics

All included studies were conducted in adult patients involving 92,945 subjects. Among them, 26 studies (32-57) were observational studies involving 82,176 participants and the remaining 12 articles were RCTs (58-69) involving 10,769 subjects. Separate details of included articles, according to study design, are provided below.

Association between diabetes and AA intake in observational studies

Except one study that only male subjects were enrolled in (51), the rest of the studies were conducted in both genders. The age of participants ranged from 18 to 89 years (Tables1a-1b). Study design of the majority of articles (12 studies) were classified as case-control (36, 37, 40, 42, 44, 48, 50, 52, 54-57), however 10 cohorts (32, 34, 35, 38, 39, 43, 45, 46, 49, 51), and 4 cross-sectional (33, 41, 47, 51) studies were also presented. Number of the participants varied from 40 to 21,831. In 7 studies, participants were followed for a varied amount of time between 2-23 years, (38, 39, 43, 46, 47, 49, 53). In 12 studies (33-35, 38, 41-44, 46, 47, 52, 55), AA intake was concomitant with the consumption of other antioxidant vitamins such as A, E, carotenoids, B6, B12, D, or minerals like iron, zinc, calcium, selenium, copper, magnesium, and chromium. The most used diagnostic criteria for DM was FBS and HbA1c measurements.

The nonsignificant association between AA and diabetes was reported by 12 studies, from which 6 studies assessed this association with FBS (33, 44, 48, 50-52), 4 studies with HbA1c levels (38, 51, 55, 56), and 4 studies with DM risk (35, 43, 46, 50). Remained eligible studies showed a significant inverse association between AA and diabetes from which 8 studies evaluated this relation with FBS (32, 36, 37, 40, 41, 45, 53, 54), 7 studies with HbA1c levels (32, 34, 39, 42, 47, 49, 52), and 1 study with DM risk (39).

Due to using the 24 hours recall food intake tool, the net amount of AA intake was unclear. Moreover, because of the methodological heterogeneity, performing the meta-analysis to assess the effect of AA intake on glycemic improvement was not possible. The quality score of the included observational studies varied from 3 to 5. Most of the studies classified as high quality with scores ≥3.

Association between FBS and AA intake

Within 11,471 participants in 14 observational studies (32, 33, 34, 37, 40, 41, 44, 45, 48, 50, 53, 54, 56, 57) conducted to assess correlation between FBS and AA intake, 1,148 subjects were diabetics and 610 case were IGT (Impaired glucose tolerance) or IFG (impaired fasting glucose). According to these studies, DM or susceptibility to it presented in 15.32% of participants. There was a significant inverse association between manifestation of either DM or IGT/IFG and AA intake in 1,250 cases (10.90% of total participants). Interestingly, in the majority of cases (1,173 cases, 10.22% of total participants), sole intake of AA was used.

Association between HbA1c and AA intake

Overall, 11 observational studies assessed the association between HbA1c levels and AA intake (32, 34, 38, 39, 42, 47, 49, 51, 52, 55, 56). Among 44,900 participants that were enrolled in these studies 3,466 and 632 subjects became diabetics or IGT/ IFG, respectively. In the other word, only in 9.13% of total participants in these studies, diabetes or susceptibility to diabetes development was found. A significant inverse association was found between HbA1c levels and antioxidants intake in 3,783 of the cases (8.42% of total participants), of which 1,686 cases (3.75% of total participants) used AA alone.

Association between DM risk and AA intake

From 51,857 participants in 5 eligible observational studies (35, 39, 43, 46, 50) which assessed the association between DM risk and AA intake, only 2,365 (4.56%) diabetics were defined. Between onset of diabetes and antioxidants' intake, a significant inverse association was found in 735 cases (1.42% of total participants). In all the participants, sole intake of AA was considered.

AA supplementation and diabetes in RCTs

Among selected studies that shown in Tables-2a, 2b, 3a, 3b, 4a and 4b, only two studies (59, 60) conducted just in males or females, and the rest was conducted in both genders. The types of RCTs among the 7 eligible studies were double-blind (59-61, 63, 67-69), in 3 studies (64, 65, 66) non-blind, 1 open-label (58), and 1 parallel (62). A number of the participants varied from 54 to 6,574 with treatment duration from 4wk to 9 years. In 7 studies (59, 60, 63-67) AA intake was compared to nutrient

antioxidants with/without AA that later included vitamins E, β-carotene, zinc, selenium, copper, magnesium, and eicosapentaenoic acid. AA dosage varied from 120 mg up to 2 g/day. In most of these studies, the net change of FBS and HbA1c after consumption of AA was reported and shown a significant decrease in FBS or HbA1c levels after consumption (59, 61-65, 67).

Qualitative analysis

The quality of these 38 eligible studies was checked by STROBE or Jadad scale according to the design of the study (Table-1a and Table-6).

Meta-analysis

Main outcome measures included net changes in FBS, HbA1c or incidence of DM by single or mixture consumption of AA with other antioxidants. Most of the trials were too heterogeneous to perform a meta-analysis except 5 RCTs (58, 59, 61, 63, 65) that details of the findings are described below.

Effect of AA in comparison to placebo on FBS levels in diabetic patients

The summary for the standardized effect size of mean differences of FBS in diabetic patients "ΔFBS" for AA therapy, in five trials, compared to placebo, in four studies (58, 59,61, 65), was -20.59 with 95% CI: -40.77 to -0.4 (p= 0.04, Figure 2-a). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.69) and could be combined, thus the fixed effects for individual and summary of effect size for standardized mean was applied. In the evaluation of publication bias, Egger regression test on the normalized effect vs. precision for all included studies of "ΔFBS" among AA vs. placebo therapy in diabetic patients was 0.84 (95% CI: -2.22 to 3.91, p= 0.45) and Begg-Mazumdar Kendall's test on the standardized effect vs. variance indicated tau= 0.4, p= 0.48 (Figure 3-a).

Effect of antioxidants in comparison to placebo therapy on FBS level in diabetic patients

The summary for standardized effect size of mean differences of FBS in diabetic patients "ΔFBS" for antioxidants therapy, in three included trials, compared to placebo, in two studies (59, 65), was -4.26 with 95% CI: -36.85 to 28.32 that was greater than null (p= 0.8, Figure 2-b). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.94) and therefore could be combined. However, because of low number of included studies, the random effects for individual and summary of effect size for standardized mean was applied. Publication bias for "ΔFBS" in diabetic patients among antioxidants vs. placebo therapy could not be evaluated because of too few strata.

Table 6. Quality assessment of RCTs included in the meta-analysis

Ref.	Randomization	Allocation concealment	Random sequence generation	Blinding	Reporting of withdrawals	Jaded score
58	Y	Y	Y	U	Y	4
59	Y	Y	U	Y	U	3
60	Y	U	Y	Y	U	3
61	Y	Y	U	Y	U	3
62	Y	Y	U	U	U	2
63	Y	Y	U	Y	Y	4
64	Y	Y	U	Y	U	3
65	Y	Y	Y	Y	U	4
66	Y	Y	Y	U	U	3
67	Y	U	U	Y	Y	3
68	Y	U	Y	Y	U	3
69	Y	Y	U	Y	Y	4

Y: yes; **U:** unclear.

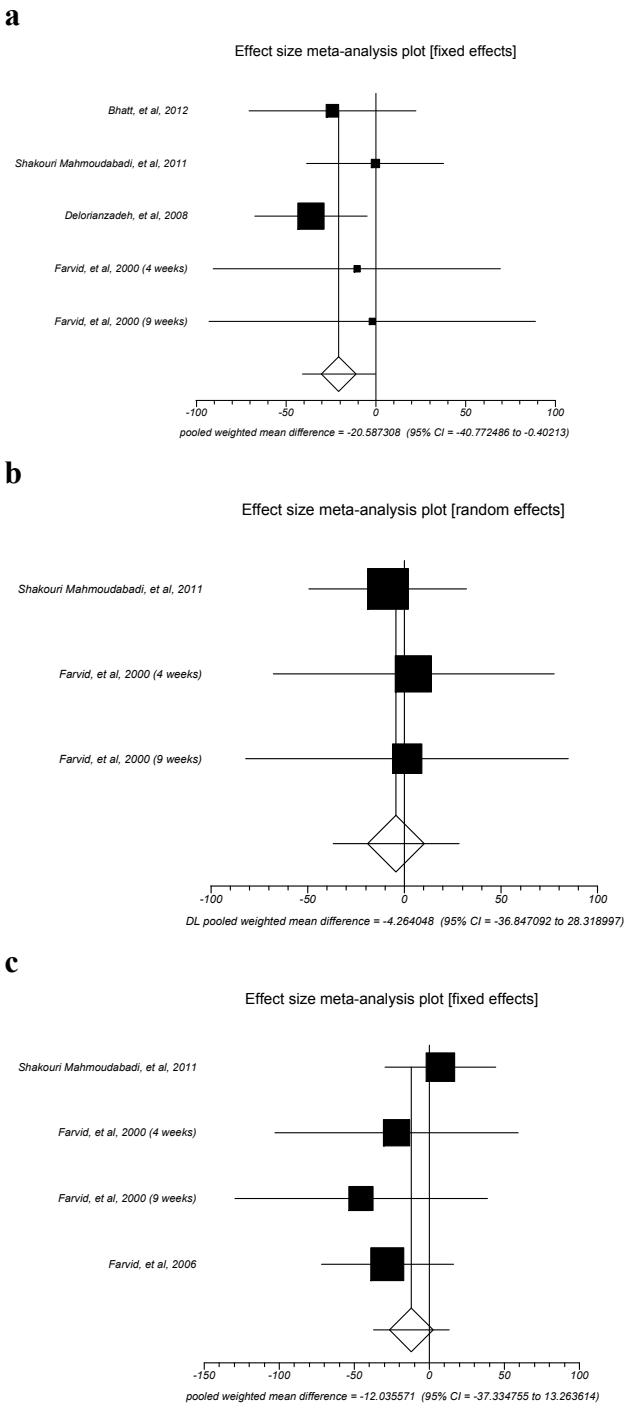


Figure 2. Individual and pooled relative risk for the outcome of "changing in fasting blood sugar" in the studies in diabetic patients; a- AA comparing to placebo therapy, b- antioxidants comparing to placebo therapy, c- AA plus antioxidants comparing to placebo therapy

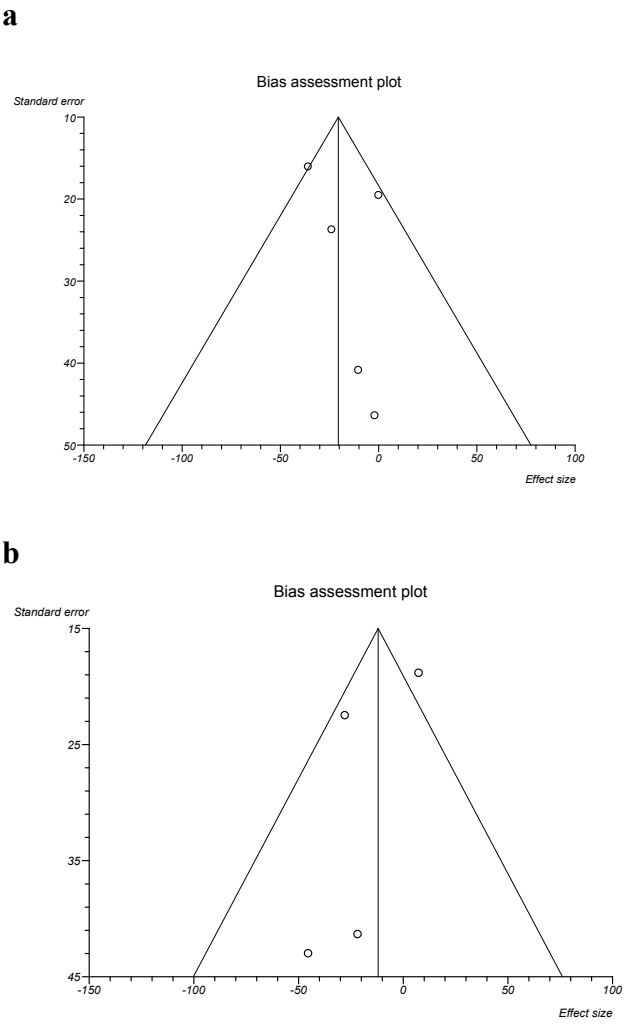


Figure 3. Publication bias indicators for the outcome of "changing in fasting blood sugar" in diabetic patients; a- AA comparing to placebo therapy, b- AA plus antioxidants comparing to placebo therapy

Effect of AA plus antioxidants in comparison to placebo therapy on FBS levels in diabetic patients

The summary for standardized effect size of mean differences of FBS in diabetic patients "ΔFBS" for AA plus antioxidants therapy, in four included trials, compared to placebo, in three studies (59, 63, 65), was -12.04 with 95% CI: -37.34 to 13.26 that was greater than null (p= 0.3, Figure 2-c). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.52) and could be combined, thus the fixed effects for individual and summary of effect size for standardized mean was applied. In the evaluation of publication bias, Egger regression test on normalized effect vs.

precision for all included studies was -1.5 (95% CI: -6.46 to 3.45, p= 0.32) and Begg-Mazumdar Kendall's test on standardized effect vs. variance indicated tau= -0.33, p= 0.33 (Figure 3-b).

Effect of AA in comparison to placebo therapy on HbA1c in diabetic patients

The summary for the standardized effect size of mean differences of HbA1c in diabetic patients "ΔHbA1c" for AA therapy, in five included trials, compared to placebo, in four studies (58,59, 61, 65), was -0.46 with 95% CI: -1.75 to 0.84 that was greater than null (p= 0.4, Figure 4-a). The Cochrane Q test for heterogeneity indicated that the studies are heterogeneous (p < 0.0001) and could not be combined, thus the random effects for individual and summary of effect size for standardized mean was applied. In the evaluation of publication bias, Egger regression of normalized effect vs. precision for all included studies of "ΔHbA1c" among AA vs. placebo therapy in diabetic patients was 11.52 (95% CI: 5.5 to 17.54, p= 0.01) and Begg-Mazumdar Kendall's test on the standardized effect vs. variance indicated tau= 0.6, p= 0.48 (Figure 5-a).

Effect of antioxidants in comparison to placebo therapy on HbA1c in diabetic patients

The summary for the standardized effect size of mean differences of HbA1c in diabetic patients "ΔHbA1c" for antioxidants therapy, in three included trials, compared to placebo, in two studies (59, 65), was 0.53 with 95% CI: -0.11 to 1.17 that was greater than null (p= 0.11, Figure 4-b). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.9) and could be combined, but because of the low number of included studies the random effects for individual and summary of effect size for standardized mean was applied. Publication bias for included studies for "ΔHbA1c" in diabetic patients among antioxidants vs. placebo therapy could not be evaluated because of too few strata.

Effect of AA plus antioxidants in comparison to placebo therapy on HbA1c in diabetic patients

The summary for standardized effect size of mean differences of HbA1c in diabetic patients "ΔHbA1c" for AA plus antioxidants therapy, in four included trials, compared to placebo, in three studies (59, 63, 65), was 0.28, (95% CI: -0.3 to 0.85 greater than null, p= 0.34, Figure 4-c). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.63) and could be combined, thus the fixed effects for individual and summary of effect size for standardized mean was applied.

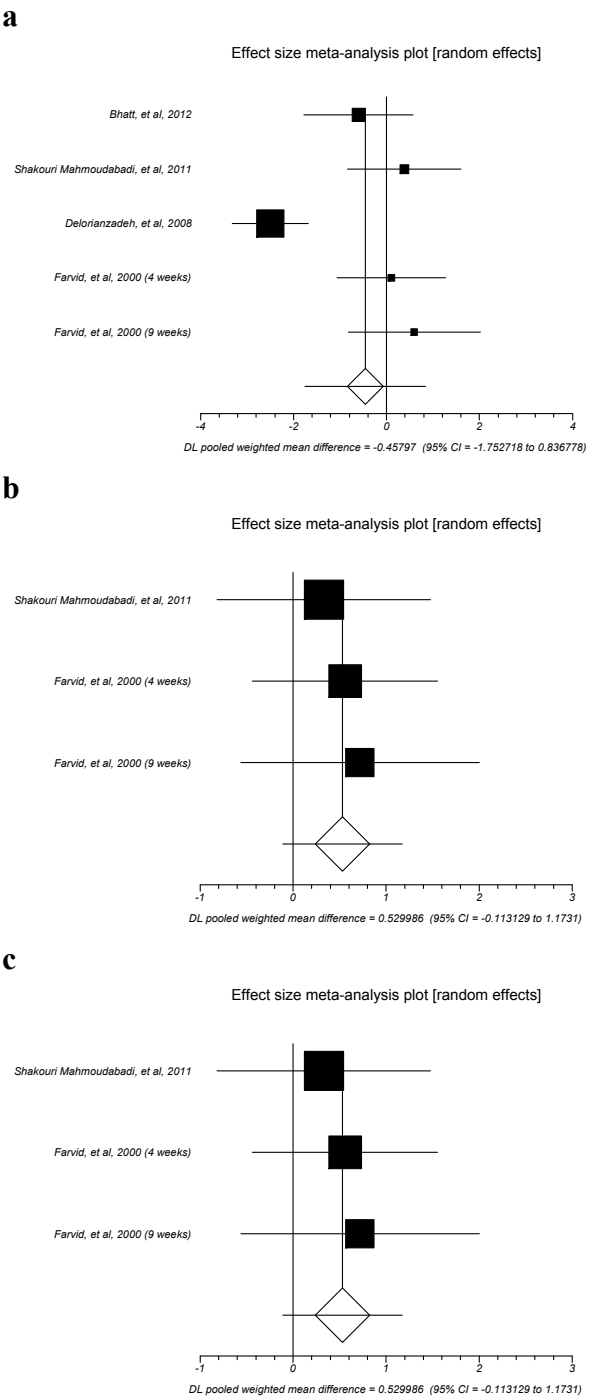


Figure 4. Individual and pooled relative risk for the outcome of "ΔHbA1c" in diabetic patients; a- AA comparing to placebo therapy, b- antioxidants comparing to placebo therapy, c- AA plus antioxidants comparing to placebo therapy**.**

In evaluation of publication bias, Egger regression on normalized effect vs. precision for all included studies of "ΔHbA1c" among AA plus antioxidants vs. placebo therapy in diabetic patients was -1.87 (95% CI: -5.64 to 1.89, p= 0.17) and Begg-Mazumdar Kendall's test on standardized effect vs. variance indicated tau= -0.67, p= 0.08 (Figure 5-b).

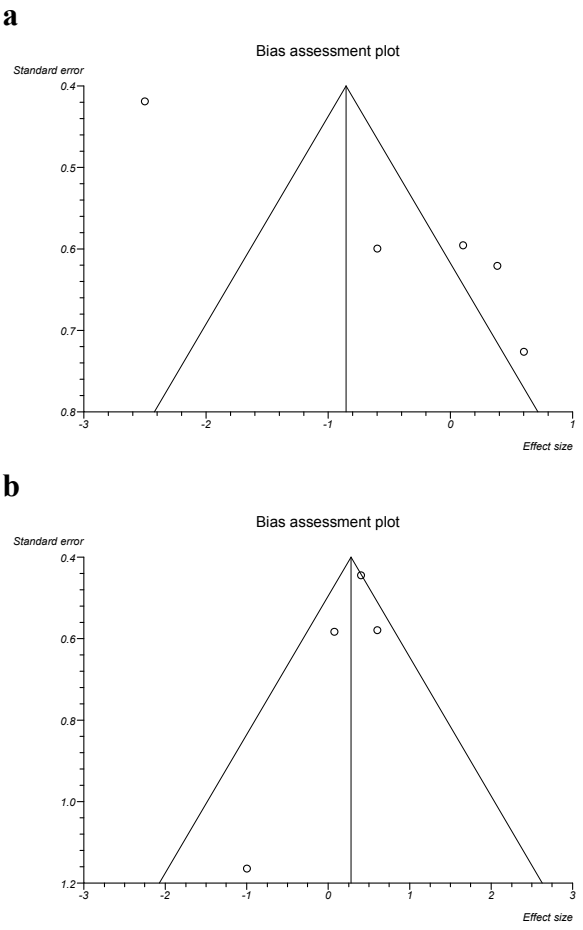


Figure 5. Publication bias indicators for the outcome of "ΔHbA1c" in diabetic patients; a- AA comparing to placebo therapy, b- AA plus antioxidants comparing to placebo therapy.

Effect of AA in comparison to placebo therapy on TC in diabetic patients

The summary for standardized effect size of mean differences of TC in diabetic patients "ΔTC" for AA therapy, in five included trials compared to placebo, in four studies (58, 59, 61, 65), was= -15.16 with 95% CI: -28.57 to -1.75 (p= 0.03, Figure 6-a). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.71) and could be combined, thus the fixed effects for individual and summary of effect size for standardized mean was applied.

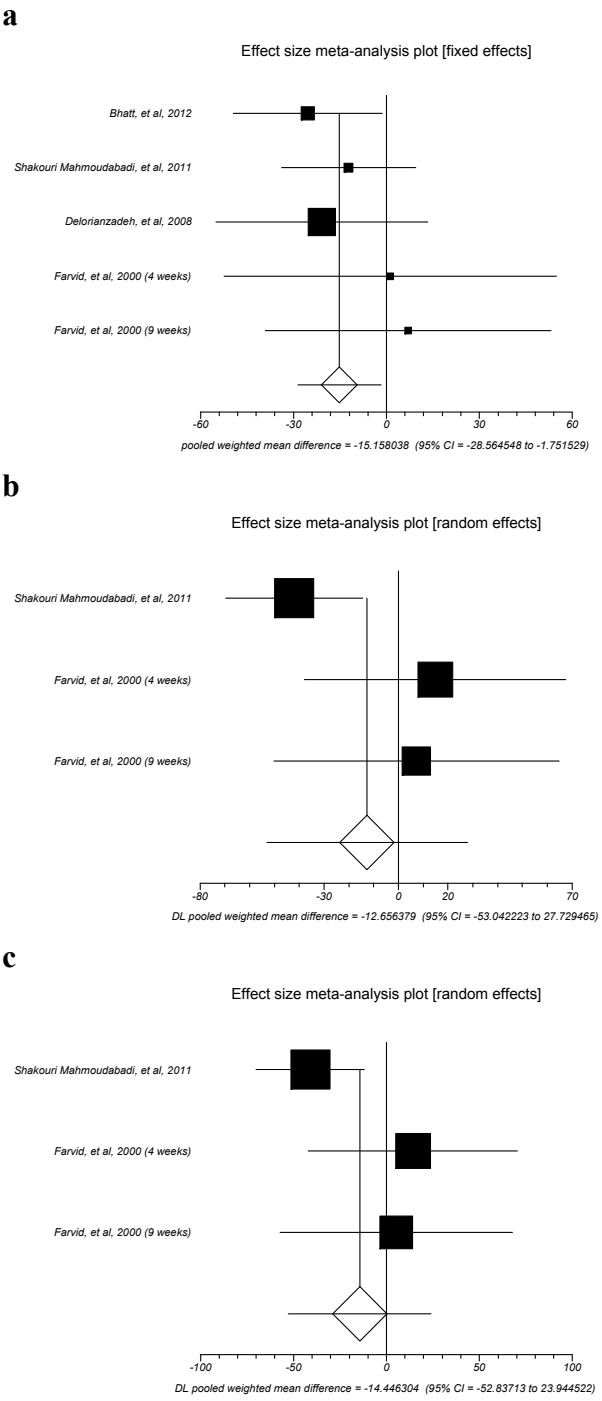
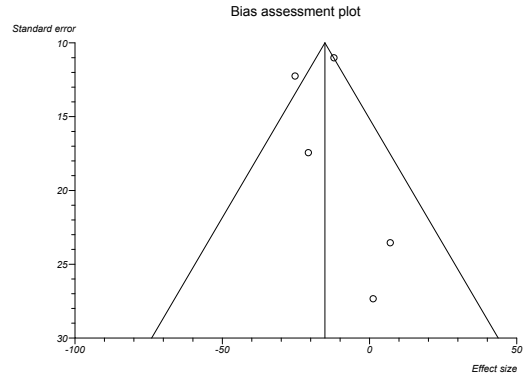


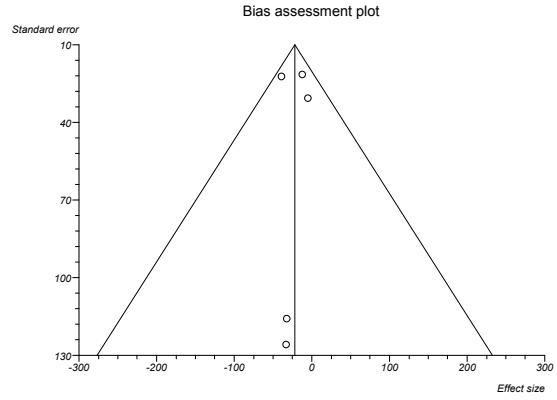
Figure 6. Individual and pooled relative risk for the outcome of "ΔTC" in diabetic patients; a- AA comparing to placebo therapy, b- antioxidants comparing to placebo therapy, c- AA plus antioxidants comparing to placebo therapy.

In the evaluation of publication bias, Egger regression of normalized effect vs. precision for all included studies of "ΔTC" among AA vs. placebo therapy in diabetic patients was 1.24 (95% CI: -1.65 to 4.13, p= 0.27) and Begg-Mazumdar Kendall's test on the standardized effect vs. variance indicated tau= 0.4, p= 0.48 (Figure 7-a).

a



b



c

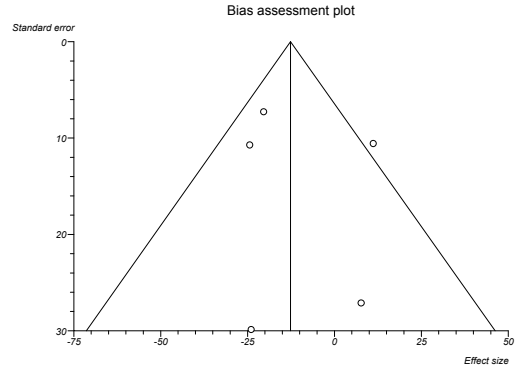


Figure 7. Publication bias indicators in diabetic patients; a- for the outcome of "ΔTC" in studies with AA comparing to placebo therapy, b- for the outcome of "ΔTg" in studies with AA comparing to placebo therapy, c- for the outcome of "ΔLDL-C" in studies with AA comparing to placebo therapy.

Effect of antioxidants in comparison to placebo therapy on TC level in diabetic patients

The summary for the standardized effect size of mean differences of TC in diabetic patients "ΔTC" for antioxidants therapy, in three included trials compared to placebo in two studies (59, 65), was -12.66 with 95% CI: -53.04 to 27.73 that was greater than null (p= 0.54, Figure 6-b). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.09) and could be combined however, because of the low number of included studies, the random effects for individual and summary of effect size for standardized mean was applied. Publication bias for included studies for "ΔTC" in diabetic patients among antioxidants vs. placebo therapy could not be evaluated because of too few strata.

Effect of AA plus antioxidants in comparison to placebo therapy on TC level in diabetic patients

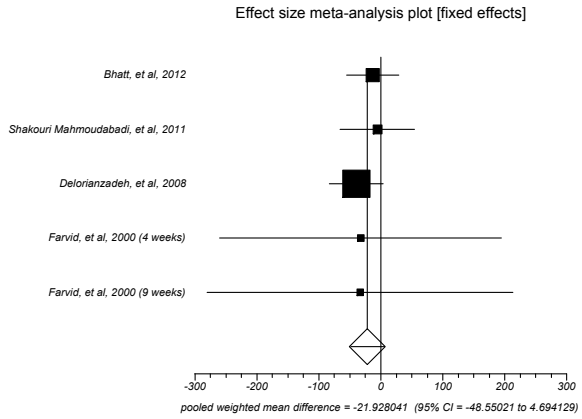
The summary for the standardized effect size of mean differences of TC in diabetic patients "ΔTC" for AA plus antioxidants therapy, in three included trials compared to placebo in two studies (59, 65) was -14.45 with 95% CI: -52.84 to 23.95 that was greater than null (p= 0.46, Figure 6-c). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.15) and could be combined however, because of few low number of included studies the random effects for individual and summary of effect size for standardized mean was applied. Publication bias for included studies for "ΔTC" in diabetic patients among AA plus antioxidants vs. placebo therapy could not be evaluated because of too few strata.

Effect of AA in comparison to placebo therapy in Tg in diabetic patients

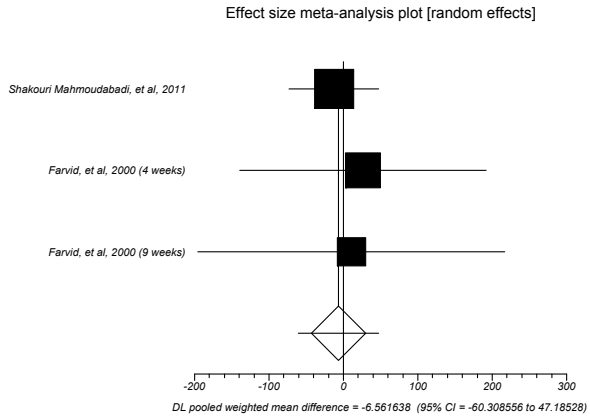
The summary for the standardized effect size of mean differences of Tg in diabetic patients "ΔTg" for AA therapy, in five included trials compared to placebo in four studies (58, 59, 61, 65), was= -21.93 with 95% CI: -48.55 to 4.69 that was greater than null (p= 0.11, Figure 8-a). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.89) and could be combined, thus the fixed effects for individual and summary of effect size for standardized mean was applied. In evaluation of publication bias, Egger regression on normalized effect vs. precision for all included studies of "ΔTg" among AA vs. placebo therapy in diabetic patients was -0.01 (95% CI: -1.7 to 1.67,

p= 0.98) and Begg-Mazumdar Kendall's test on the standardized effect vs. variance indicated tau= 0, p= 0.82 (Figure 7-b).

a



b



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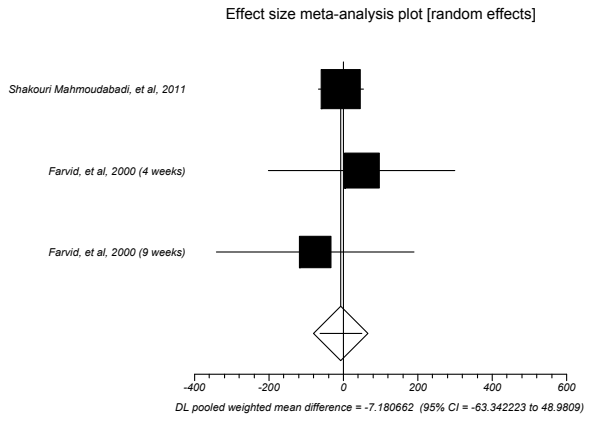


Figure 8. Individual and pooled relative risk for the outcome of "ΔTg" in diabetic patients; a- AA comparing to placebo therapy, b- antioxidants comparing to placebo therapy, c- AA plus antioxidants comparing to placebo therapy**.**

Effect of antioxidants in comparison to placebo therapy on Tg level in diabetic patients

The summary for standardized effect size of mean differences of Tg in diabetic patients "ΔTg" for antioxidants therapy, in three included trials compared to placebo in two studies (59, 65), was -6.56 with 95% CI: -60.31 to 47.19 that was greater than null (p= 0.8, Figure 8-b). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.89) and could be combined, but because of low number of included studies the random effects for individual and summary of effect size for standardized mean was applied. Publication bias for included studies for "ΔTg" in diabetic patients among antioxidants vs. placebo therapy could not be evaluated because of too few strata.

Effect of AA plus antioxidants in comparison to placebo therapy on Tg level in diabetic patients

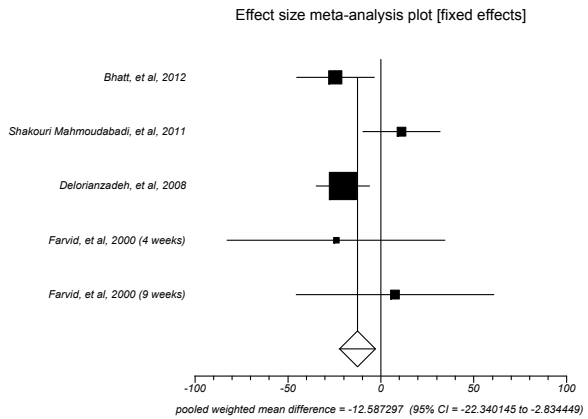
The summary for standardized effect size of mean differences of Tg in diabetic patients "ΔTg" for AA plus antioxidants therapy, in three included trials compared to placebo in two studies (59, 65), was -7.18 with 95% CI: -63.34 to 48.98 that was greater than null (p= 0.8, Figure 8-c). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.8) and could be combined, but because of low number of included studies the random effects for individual and summary of effect size for standardized mean was applied. Publication bias for included studies for "ΔTg" in diabetic patients among AA plus antioxidants vs. placebo therapy could not be evaluated because of too few strata.

Effect of AA in comparison to placebo therapy in LDL-C in diabetic patients

The summary for the standardized effect size of mean differences of LDL-C in diabetic patients "ΔLDL-C" for AA therapy, in five included trials compared to placebo in four studies (58, 59, 63, 65), was -12.59 with 95% CI: -22.34 to -2.84 (p= 0.01, Figure 9-a). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.07) and could be combined, thus the fixed effects for individual and summary of effect size for standardized mean was applied. In the evaluation of publication bias, Egger regression of normalized effect vs. precision for all included studies of "ΔLDL-C" among AA vs. placebo therapy in diabetic patients was 0.69 (95% CI: -4.43 to 5.78, p= 0.7) and Begg-Mazumdar Kendall's test on the

standardized effect vs. variance indicated tau= 0.2, p= 0.82 (Figure 7-c).

a



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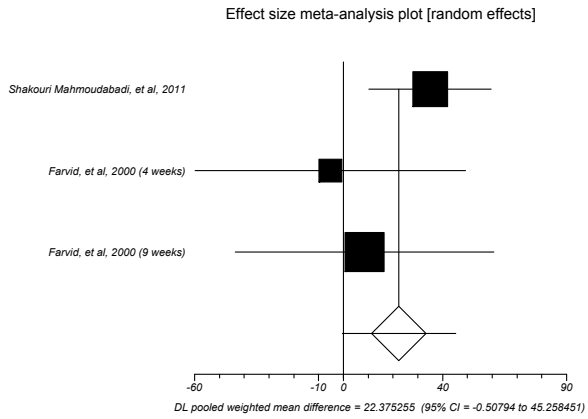

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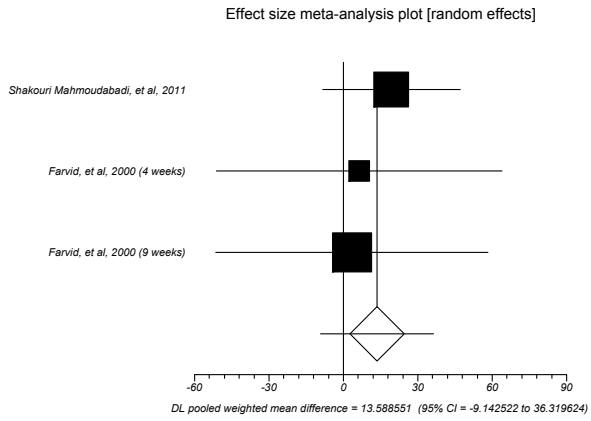

Figure 9. Individual and pooled relative risk for the outcome of "ΔLDL-C" in diabetic patients; a- AA comparing to placebo therapy, b- antioxidants comparing to placebo therapy, c- AA plus antioxidants comparing to placebo therapy.

Effect of antioxidants in comparison to placebo therapy on LDL-C level in diabetic patients

The summary for the standardized effect size of mean differences of LDL-C in diabetic patients "ΔLDL-C" for antioxidants therapy in three included trials compared to placebo in two studies (59, 65), was 22.38 with 95% CI: -0.51 to 45.26, greater than null (p= 0.06, Figure 9-b). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.32) and could be combined, but because of the low number of included studies, the random effects for individual and summary of effect size for standardized mean was applied. Publication bias for included studies for "ΔLDL-C" in diabetic patients among antioxidants vs. placebo therapy could not be evaluated because of too few strata.

Effect of AA plus antioxidants in comparison to placebo therapy on LDL-C level in diabetic patients

The summary for the standardized effect size of mean differences of LDL-C in diabetic patients "ΔLDL-C" for AA plus antioxidants therapy, in three included trials compared to placebo in two studies (59, 65), was 13.59 with 95% CI: -9.14 to 36.32, greater than null (p= 0.2, Figure 9-c). The Cochrane Q test for heterogeneity indicated that the studies are not heterogeneous (p= 0.82) and could be combined, but because of low number of included studies the random effects for individual and summary of effect size for standardized mean was applied. Publication bias for included studies for "ΔLDL-C" in diabetic patients among AA plus antioxidants vs. placebo therapy could not be evaluated because of too few strata.

Secondary outcome measures

Due to few available data for HDL-C, insulin, SBP, and DBP in eligible RCTs, heterogeneity assessment and pooling data were impossible. However, detail of these data are shown in tables 4(a, b) and 5(a, b).

DISCUSSION

Overall, observational studies have shown an inverse association between AA status or self-reported intake of AA with/without antioxidants and development of T2DM that was significant in 20.74% of the total participants. However, due to methodological heterogeneity, meta-analysis of the

reported data was impossible. The meta-analysis of 5 eligible RCTs involving 385 subjects revealed that, even though a significant decrease in FBS levels following AA consumption in diabetic subjects might be seen, the changes were not significant when it comes to measuring the HbA1c levels after intervention with AA and/or other antioxidants. Although reduction in FBS was observed in two trials (59, 61), the greatest reduction was found in a the trials where AA was administered for at least 3 months and with a minimum dose of 1,250 mg per day (61). However, the meta-analysis of 2 RCTs that compared the effect of other antioxidants vs. placebo and also meta-analysis of 3 other RCTs that evaluated the mixed mode consumption of antioxidants and AA vs. placebo on FBS were not significant.

Many data have established the key role of oxidative stress in the glycation of hemoglobin, peroxidation of cell membrane lipids, and finally tissue damage (3, 70). A further evidence for the biological plausibility of these findings has been provided by recent studies in which the effects of AA in glucose metabolism have been assessed. AA has various functions against the oxidative process. This vitamin can scavenge ROS, inhibit the launch of chain reactions that lead to protein glycation, and protect against lipid peroxidation (71-73). Ascorbyl radical and dehydroascorbic acid are oxidation products of ascorbic acid that can be reduced back to AA by glutathione. In the meantime, the AA can support recycling back of VE and glutathione from their oxidized forms (71, 74).

A variety of epidemiological and observational studies have been conducted to assess the effects of AA on oxidative stress conditions in diabetics and have reported conflicting results. Observational prospective cohorts have indicated that low levels of serum AA are associated with a reduced risk of diabetes (52, 54, 75). A large, 12-year population-based study involving 21,831 participants in the European Prospective Investigation of Cancer-Norfolk Prospective Study identified 735 incident cases of diabetes and revealed a significantly lower DM risk by elevation of serum AA (odds ratio=0.38, CI 95%: 0.28-0.52) (39). In contrast, some RCTs have demonstrated no association between AA supplementation and the risk of T2DM. One randomized, open label, double-blind intervention trial reported no improvement in blood pressure, FBS, HbA1c, TG and HDL-C, after intervention with 500 mg/day of AA for 3 months in a group of 30 T2DM subjects, compared to baseline and also placebo group (58). The sole significant change was observed in the present study, was a remarkable improvement in TC and LDL-C levels. Some researchers suggested an improvement in glycemic control after AA supplementation (68, 69). For instance, study of Delvarianzadeh et al. (61) showed a link between AA intake and HbA1c whereas Shoff et al. (55) reported non-significant difference in mean HbA1c among the highest vs. lowest quintiles of AA supplementation in 2,141 subjects. Accordingly, our meta-analysis showed no significant association between HbA1c and AA intake vs. placebo.

Several possible reasons may account for the observed controversial results in observational studies and RCTs. Under different physiological conditions, AA can have preoxidant or antioxidant effects (76). However, required serum concentration and doses that are needed to induce the oxidative stress are different from those that are required for induction of other effects. This feature of AA is not usually considered when epidemiological studies are performed (76). Normal or high physiological level of AA (60-100 μmol/l) can attenuate the oxidative damage (77-79), while its prooxidant function that occurs in the presence of some metals such as copper and iron, can promote the oxidative damage (80). A level of 200 mg AA that is usually obtained from vitamin C-rich foods produces an average serum concentration of 90 μmol (81). In normal conditions, physiological availability of AA is low, which mostly is due to the instability of this vitamin, poor intestinal absorption, and easy excretion (75). High levels of glucose in the blood can induce intracellular AA deficiency, which is caused by competition of glucose with this vitamin for tissue uptake (82-84). Moreover, bioavailability of AA also depends on amounts of transporting proteins and their binding affinity (85) which is impaired in chronic conditions such as diabetes. It is known that cellular uptake of AA is orchestrated by blood levels of both glucose and insulin (86, 87). Therefore, the presence of hyperglycemia in diabetic subjects could increase the urinary loss of this vitamin and subsequently results in lower levels of AA in diabetics (88). Taking it as a whole, it is clear that diabetic subjects require higher doses of AA than recommended dietary allowance (RDA). Among healthy men and women, the daily RDA of AA is 90 and 75 mg, respectively. In hyperglycemic subjects, this measure should be increased by 35

mg/day (89). AA intake in all RCTs included in our meta-analysis was at least 200 mg daily from 8 weeks to 3 months. Two RCTs that investigated AA supplementation of 200 and 1,250 mg daily, showed a significant effect on FBS versus placebo over 8-12 weeks (59, 61). However, by daily supplementation of 200 (63) or 500 mg daily (58) for 12 weeks, or 500 mg daily for 4-8 weeks (65) the FBS was not improved versus placebo. After pooling data, effect size was increased and showed the significant beneficial effect of AA intake on FBS versus placebo. Due to few RCTs, we could not perform subgroup analysis to determine type of AA supplemet, effective dosage and treatment duration.

Although, observed benefits in some of our observational studies were related to co-administration of AA and antioxidants (34, 41, 42, 47,52), in majority of studies, single AA was taken (32, 36, 37, 39, 40, 45, 49, 53, 54). It is thought that the benefits reported in the epidemiological studies in which AA and/or antioxidants are co-administrated, might be related to intake of higher amounts of fruits and vegetables, as a complex mixture of micronutrients or synergistic interaction between natural antioxidants (56). It is expected that co-administration of two or more vitamins and antioxidants is more effective than single supplementation (90), and selecting the kind of antioxidants to combine together has a crucial importance. The benefits of mixture consumption of a hydrophilic (AA) with a hydrophobic (VE) antioxidant have been reported (91) as this benefit was shown in our included studies (34, 41, 42, 47, 52). However, as we mentioned previously, observed benefitial effects in our RCTs and most of observational studies were related to sole intake of AA. Steinberg et al (92) suggested that since many of pathological changes seen in diabetes have been developed few years before clinical presentation, it may take more than 5 years for antioxidant therapy to reverse the pathological changes. It is clear that beneficial effect of AA supplementation after a few weeks cannot be documented based on HbA1c measurement, because this measure reflects a mean glucose level over the last three months. We have to mention that total daily dosages of AA in included studies had a wide variation (93). In some included studies in our systematic review, small dose of AA and/or other antioxidants was employed while in others, high dose of antioxidants was administered. It should be noted that large intake of AA does not necessarily guaranty the full absorption. In fact, plasma AA concentration is tightly controlled by three mechanisms: intestinal absorption, tissue transport, and renal reabsorption. In addition, in response to sudden high oral intake of AA, exess AA is largely excreted in the urine (94-96). AA is generally considered safe in normal individuals, but in special conditions such as renal stones, hyperoxaluria, dialysis, renal failure or kidney transplantation, administration of high amounts of AA could be harmful due to oxalate formation (97). One of the diabetes complication is diabetic nephropathy that happens few years after onset of diabetes. Therefore, high dose of AA therapy should be avoided in these conditions. AA administration is also contraindicated in patients with systemic iron overload due to increased iron absorbtion, and in angioplastic patients due to increased risk of cardiovascular diseases (95, 97). Respectively, Lee et al (95) found that high dose of AA could increase the rate of cardiovascular complications in a 15 year prospective study.

Analysis of our secondary outcomes revealed a significant improvement in TC, and LDL-C levels in the AA group compared to placebo or antioxidants treated subjects, a result that was consistent with the findings of Ginter et al (98). As it is shown in human and animal studies, long term AA intake leads to elevation of ascorbate concentration in the liver, which subsequently could result in an enhanced rate of cholesterol transformation to bile acids (99, 100).

We could not see the beneficial effects of other antioxidants; eicosapentaenoic acid (59), or VE (63, 65) with/without AA on FBS, this finding was similar to results obtained by previous meta-analysis in diabetics (13, 101).

Our study supports the role of AA in reduction of FBS in diabetics, however, due to lack of evidences on long term safety of the vitamin supplementation and insufficient available RCTs involved in our meta-analysis, we cannot strongly recommend the long term use of this vitamin for its anti-diabetic properties. Although American Diabetes Associationhas recommended 8-10 daily servings of fruits and vegetables as a source of AA for diabetic patients (102), we think, according to our findings regarding the positive effect of AA on FBS, serving the only natural source of AA is not enough and AA supplements should be considered in diabetics.

Our study has some strengths and of course some limitations. Firstly, this meta-analysis, for the first time, assessed the effects of AA supplementation on plasma concentrations of FBS and HbA1c. Moreover, the trials included in this meta-analysis were all RCTs, which allow reliable inferences about causality. Among our limitations, we should mention that we needed to include small trials with limited subjects and varied dosage of AA. This variation limited our ability to performe subgroup analysis and definie dosage and duration of AA recommendations in T2DM. Our second limitation was considerable trial heterogeneity. Our third limitation was the wide variation in quality of RCTs included in this meta-analysis. Of 12 trials, only 4 trials had score equal to 4 (high-quality studies) and the others were catergorized as low quality studies. Moreover only for few studies, we were able to pool the data and perform the meta-analysis. These conditions could affect the confidence of this meta-analysis. Publication bias could be a potential limitation in this study. However, we tried to explore the possibility of this bias by using funnel plot and Egger's test and found that publication bias did not have significant effect on the results of AA and/or other antioxidants supplementation on FBS and lipid profiles. Finally, except in study performed by Lee et al. (95), no study has yet assessed the long-term safety and efficacy of AA intake on other tissues and organs.

We concluded that our systematic review of observational studies and meta-analysis of RCTs identified a significant correlation between AA and improvements in FBS level in diabetics. However, yet large-scale randomized trials are needed to investigate the effect of AA supplementation on FBS and HbA1c. Taken together, it should be appropriate to suggest that diabetic subjects without contraindication of AA intake might benefit more from taking a combination of antioxidants, from either natural sources and/ or fortified foods, and AA supplementation.

ACKNOWLEDGEMENT

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