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META-ANALYSIS

Vascular smooth muscle function in type 2 diabetes mellitus: a systematic review and meta-analysis

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Abstract
Aims/hypothesis In type 2 diabetes, in contrast to the well-documented endothelial dysfunction, studies assessing vascular smooth muscle (VSM) function have yielded discrepant results over the last two decades. We therefore sought to determine whether or not VSM function is impaired in individuals with type 2 diabetes.
Methods We conducted a systematic search of MEDLINE, Cochrane, Scopus and Web of Science databases, from their respective inceptions until December 2012, for articles evaluating VSM function in individuals with type 2 diabetes. A meta-analysis was performed to compare the standardised mean difference (SMD) in VSM function between individuals with type 2 diabetes and age-matched controls. Subgroup analyses and meta-regression were used to identify sources of heterogeneity.
Results Twenty-seven articles (1,042 individuals with type 2 diabetes and 601 control subjects) were included in this analysis. VSM function was significantly impaired in diabetic compared with control subjects (SMD −0.68, 95% CI −0.84, −0.52; $p<0.001$). Although moderate heterogeneity among studies was found ($I^2=52\%$), no significant publication bias was detected. Subgroup analyses showed a further decline in VSM function assessed in the microcirculation compared with the macrocirculation of individuals with type 2 diabetes ($p=0.009$). In meta-regression, VSM function in the microcirculation was inversely associated with BMI and triacylglycerols and was positively associated with HDL-cholesterol.
Conclusions/interpretation In addition to the endothelium, the VSM is a source of vascular dysfunction in type 2 diabetes. An exacerbation of VSM function in the microcirculation may be a distinctive feature in type 2 diabetes.

Keywords Meta-analysis · Type 2 diabetes mellitus · Vascular smooth muscle function

Abbreviations

DBP	Diastolic blood pressure
HOMA-IR	HOMA of insulin resistance
NMD	Nitrate-mediated dilation of brachial artery
SAQOR	Systematic appraisal of quality for observational research
SBP	Systolic blood pressure
SMD	Standardised mean difference
VSM	Vascular smooth muscle

Introduction

The incidence of type 2 diabetes is growing rapidly, in part because of the ageing population and sedentary lifestyle [1]. In 2010, an estimated 257 million people worldwide had type 2 diabetes [1], representing a major public health issue. Type 2 diabetes is independently related to increased risk for cardiovascular complications [2], mainly involving the accelerated development of atherosclerotic vascular changes [3].

In the study of vascular function, the aim of exogenous nitrate administration is to relax vascular smooth muscle (VSM) in an endothelium-independent manner, thus reflecting VSM function [4, 5]. Once inside the VSM cell,

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nitrates prompt smooth muscle relaxation via a cascade of events involving the bioconversion of nitrate to nitric oxide, activation of soluble guanylate cyclase, synthesis of cyclic guanosine monophosphate and decrease in cytosolic calcium levels [6]. Endothelial and VSM dysfunction are considered to be primary signs of the early stage of atherosclerotic disease [7], with a significant prognostic role in high-risk populations [8, 9]. Impaired endothelial function, which appears long before symptoms [10], has been consistently demonstrated in the macro- and microcirculation of individuals with type 2 diabetes (Table 1) in association with hypercholesterolaemia, hyperglycaemia, low-grade systemic inflammation and oxidative stress [11]. However, whether VSM function, usually preserved in obese individuals [12], is also impaired in those with type 2 diabetes has been debated in the last two decades, because of discrepant results in both the macro- and microcirculation (Table 1).

Given this uncertainty, we conducted a systematic review and meta-analysis of available studies comparing VSM function in individuals with type 2 diabetes and age-matched control subjects.

Methods

This review is reported according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) Group guidelines [13].

Data sources and searches Our systematic search included MEDLINE, Cochrane, Scopus and Web of Science databases, since their inceptions until December 2012. We used combinations of the subject headings: 'diabetes mellitus, type 2', 'vascular smooth muscle', 'endothelium independent', 'nitroglycerin', 'sodium nitroprusside', 'vascular function', 'vascular reactivity', 'vasodilation'. We also performed hand searching in reference citations of identified reviews and original articles selected for full-text retrieval.

Study selection To be included in this review, an observational report had to: (1) assess VSM function in individuals with type 2 diabetes; and (2) include a group of age-matched controls. In the event of multiple publications, only the most recent manuscript for a particular study population was included. Inclusion was not limited by publication status or language. Study selection was performed independently and in duplicate by two investigators (David Montero) and (Agnès Vinet). Discrepancies in inclusion/exclusion were solved by consensus or through consultation with a third reviewer (Guillaume Walther).

Data extraction and quality assessment The following variables were abstracted into a pre-formatted spreadsheet: authors, year of publication, characteristics of study participants (*n*, % women, age, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP) and duration of type 2 diabetes), metabolic variables (fasting insulin, fasting glucose, HbA_{1c}, total cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerols and NEFA) and vascular variables (region assessed, technique used, endothelial function and VSM function). The presence of concomitant cardiovascular disease and smoking was also determined. Additionally, if data were unclear or were not available in the published reports, we contacted the corresponding and/or first author by e-mail to request this information. A systematic appraisal of quality for observational research (SAQOR) [14], previously applied in meta-analysis of observational studies evaluating vascular function [15], was performed to provide assessment of study quality. The SAQOR was adjusted to assess: (1) the type 2 diabetic sample; (2) the control group; (3) quality of measurement; (4) confounding variables; and (5) data. Overall, the SAQOR was scored out of 16, quality deemed better with a greater score (Table 1). Data extraction and quality assessment were performed independently and in duplicate by two investigators (David Montero) and (Agnès Vinet). Discrepancies were solved by consensus or through consultation with a third reviewer (Guillaume Walther).

Data synthesis and analysis The meta-analysis and statistical analyses were performed using Review Manager software (RevMan 5.2; Cochrane Collaboration, Oxford, UK) and Comprehensive Meta-analysis software (version 2; Biostat, Englewood, NJ, USA). The primary outcome was the standardised mean difference (SMD) in VSM function between individuals with type 2 diabetes and control subjects. SMD summary statistic allowed us to standardise macro- and microcirculation studies into a uniform scale to complete the meta-analysis. According to Cohen guidelines [16], an SMD of 0.2, 0.5 and 0.8 represents small, medium and large effect sizes, respectively. Negative SMD corresponded to impaired vascular function in individuals with type 2 diabetes compared with control subjects.

Heterogeneity between studies was assessed using I^2 statistics; $I^2<50\%$ was considered to represent low heterogeneity while $I^2\geq50\%$ was considered to represent high heterogeneity. Random-effects models were used to calculate SMD when $I^2\geq50\%$ [17]. Publication bias was evaluated by estimating Begg and Mazumdar's funnel plot asymmetry and Egger's weighted regression test [18].

Potential moderating factors were explored by subgroup analyses comparing the summary results of studies grouped by sample size, age, sex, duration of type 2 diabetes, differences in variable risk factors (BMI, SBP, DBP, fasting insulin, fasting glucose, HOMA of insulin resistance (HOMA-IR), HbA_{1c}, total cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerols) between diabetic and control subjects,

Table 1 Main characteristics of studies included in the meta-analysis

Study, year of publication	*n*		Women (%)		Age of T2DM group (years)	Duration of T2DM (years)	Medication	Quality score (0–16)	Vascular region assessed	Vascular endothelial function	VSM function
	C	T2DM	C	T2DM							
Bruno et al [25], 2012	82	84	42.68	41.67	56.7 (7.6)	8 (5.93)	66 on OHG	14	Macro	↓FMD	↓NMD
Beer et al A [23], 2008	38	27	34.21	40.74	54.4 (7.7)	6.4 (6.2)	19 on OHG, 13 on Insulin	13	Micro	↓ACh	↓SNP
Beer et al B [23], 2008	38	27	34.21	18.52	57.9 (7)	10.8 (7.8)	15 on OHG, 19 on Insulin	13	Micro	↓ACh	↓SNP
Brooks et al [24], 2008	20	40	75	42.5	57.4 (7.8)	9.98 (7)	18 on OHG, 17 on Insulin	12	Micro	↓ACh	↓SNP
Karabag et al [31], 2007	16	59	50	50.85	53.4 (8.6)	8.1 (6.2)	59 on OHG	15	Macro	↓FMD	↔NMD
Sivitz et al [39], 2007	24	28	58.33	50	56.9 (13.23)	N/A	No medication within 2–4 weeks before evaluation	12	Micro	↓ACh	↓SNP
Sokolnicki et al [40], 2006	11	9	63.64	33.33	55 (9)	7 (3)	No medication within 2 weeks before evaluation	12	Micro	N/A	↓SNP
Woodman et al [46], 2006	15	47	60	17.02	55 (6.86)	N/A	6 on Metf, 7 on SU	14	Micro	↓ACh	↓SNP
Woodman et al [47], 2005	10	43	30	11.63	56 (6.56)	3.67 (3.25)	6 on SU	13	Micro	↓Ach	↓SNP
Vehkavaara et al [44], 2004	16	11	25	18.18	59 (6.63)	>3	11 on Metf	12	Micro	↓ACh	↔SNP
Ifrim et al [29], 2004	10	10	N/A	N/A	55.6 (11.57)	5.65 (7.4)	N/A	11	Macro	↓FMD	↔NMD
van Etten et al [43], 2002	21	23	66.67	65.22	58 (8)	N/A	8 on OHG, 11 on insulin, 4 on both OHG and insulin	13	Micro	↓5HT	↓SNP
Woodman et al [48], 2002	17	29	29.41	20.69	62 (9)	N/A	17 on Metf, 14 on SU, 1 on α-2 GInh	15	Macro	↓FMD	↓NMD
Tan et al [41], 2002	83	170	49	49	54.5 (8.8)	9.8 (6.8)	129 on OHG, 41 on insulin	14	Macro	↓FMD	↓NMD
Ihlemann et al [30], 2002	23	23	43.48	43.48	47.4 (8.15)	4.1 (3.36)	17 on OHG (7 on SU)	16	Macro	↓FMD	↓NMD
Matsumoto et al [37], 2002	25	124	48	45.16	58 (12.25)	11.6 (10.1)	N/A	11	Macro	N/A	↓NMD[a]
van de Ree et al [42], 2001	10	17	0	0	51 (7.01)	8.4 (6.89)	6 on Metf, 7 on SU, 9 on insulin	14	Micro	↓5HT	↔SNP
Kimura et al [32], 2001	12	15	66.67	46.67	70 (9)	7 (1.8)	SU	12	Macro	↓FMD	↔NMD
Heitzer et al [28], 2001	11	39	27.27	28.21	56 (24.98)	4.2 (3.75)	29 on Metf and/or SU	13	Micro	↓ACh	↔SNP
Ma et al A [35], 2001	20	17	40	41.18	58.4 (7.6)	N/A	N/A	10	Macro	↓FMD	↔NMD
Ma et al B [35], 2001	20	17	40	41.18	59.8 (7.47)	N/A	N/A	10	Macro	↓FMD	↓NMD
Anderson et al [22], 2001	12	12	41.67	58.33	47.3 (N/A)	N/A	OHG	12	Macro	↓FMD	↔NMD
Lim et al A [34], 1999	20	45	50	68.89	54 (10)	5.1 (5.8)	N/A	14	Micro	↓ACh	↓SNP
Lim et al B [34], 1999	20	14	50	64.29	55 (9)	5.4 (3.7)	N/A	14	Micro	↓ACh	↓SNP
Lim et al [33], 1999	28	16	100	100	43 (6)	4.8 (4.3)	N/A	15	Micro	↓ACh	↓SNP
Makimattila et al [36], 1999	12	30	0	0	51 (5.48)	3.5 (3.83)	12 on Metf or SU	14	Micro	↓ACh	↔SNP
Enderle et al [27], 1998	25	25	44	44	57.2 (7.4)	7.4 (6)	No medication within 4 weeks before evaluation	11	Macro	↓FMD	↔NMD
Pitei et al A [38], 1997	10	8	40	37.5	52.3 (13.7)	13.7 (5)	N/A	10	Micro	↓ACh	↓SNP
Pitei et al B [38], 1997	10	7	40	42.86	58.1 (13.1)	8.9 (1.2)	N/A	10	Micro	↓ACh	↔SNP
Cipolla et al [26], 1996	7	5	N/A	N/A	N/A[b]	N/A	3 on insulin	11	Micro	↑ACh	↔SNP
Williams et al [45], 1996	23	21	26.09	33.33	44 (9.17)	4 (N/A)	No medication within 12 h before evaluation	14	Micro	↓MCh	↓SNP

Data are mean, mean (SD), or *n*. Some studies presented two subgroups of type 2 diabetes (T2DM), each of which had been independently compared with a single control (C) group, thus they were evaluated as individual studies (distinguished by A or B)

[a]NMD measured in interlobar arteries

[b]C and T2DM groups were age-matched according to Cipolla et al 1996 [26]

↓, Significant decrease of vascular function in T2DM compared with control; ↔, no significant difference in vascular function between T2DM and control; ACh, acetylcholine-mediated blood flow; FMD, flow-mediated dilatation of brachial artery; MCh, metacholine-mediated blood flow; Metf, metformin; N/A, data not available; OHG, oral hypoglycaemic agent; SNP, sodium nitroprusside-mediated blood flow; SU, sulfonylureas; 5HT, serotonin-mediated blood flow; α-2 GInh, α-2 glucosidase inhibitors

vascular region assessed, vascular technique and endothelial function. Median values of continuous variables were used as cut-off values for grouping studies. Univariate meta-regression analysis was performed to further identify the possible sources of heterogeneity, using the aforementioned continuous variables.

Results

Study selection and characteristics The flow diagram of the process of study selection is shown in Fig. 1. Our search of MEDLINE, Cochrane, Scopus and Web of Science databases and manual review of articles cited in the identified and related publications initially retrieved 3,613 articles. Of these, 3,553 were excluded because they were not related to our present meta-analysis. We obtained and reviewed the full text of the remaining 60 articles, and excluded 33 for the following reasons: no VSM function data available (n=18), no age-matched control subjects (n=12), no VSM function data reported in control subjects (n=2) [19, 20] or duplicate data (n=1) [21]. Finally, 27 observational reports were included in the meta-analysis.

The 27 articles included in the meta-analysis encompassed 1,042 individuals with type 2 diabetes and 601 control subjects [22–48]. Four of these articles presented two subgroups of type 2 diabetes, each of which had been independently compared with a single control group, thus they were evaluated as individual studies [23, 34, 35, 38]. The characteristics of the resulting 31 studies are shown in Table 1. All of the studies compared individuals with type 2 diabetes with age-matched control subjects, ranging from 12 to 253 in total sample size. The mean clinical characteristics of all subjects in the included studies ranged from 41 to 70 years for age, 21.70 to 37 kg/m^2 for BMI, 112 to 156 mmHg for SBP and 67 to 95 mmHg for DBP. The mean fasting insulin, fasting glucose, HOMA-IR and HbA_{1c} ranged from 34.7 to 277.8 pmol/l, from 4.5 to 12.6 mmol/l, from 1.1 to 19.2 and from 4.4 to 11% (25 to 97 mmol/mol), respectively. The mean total cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerols ranged from 4.4 to 6.0 mmol/l, from 0.95 to 1.78 mmol/l, from 2.0 to 4.1 mmol/l and from 0.9 to 2.9 mmol/l, respectively. The mean duration of type 2 diabetes ranged from 3.5 to 13.7 years. With respect to the vascular region assessed, 12 out of 31 studies evaluated the macrocirculation and 19 out of 31 evaluated the microcirculation. Impaired endothelial function was reported in 28 (11 in the macrocirculation, 17 in the microcirculation) out of 29 studies in which it was assessed and impaired VSM function was reported in 19 (6 in the macrocirculation, 13 in the microcirculation) out of 31 studies.

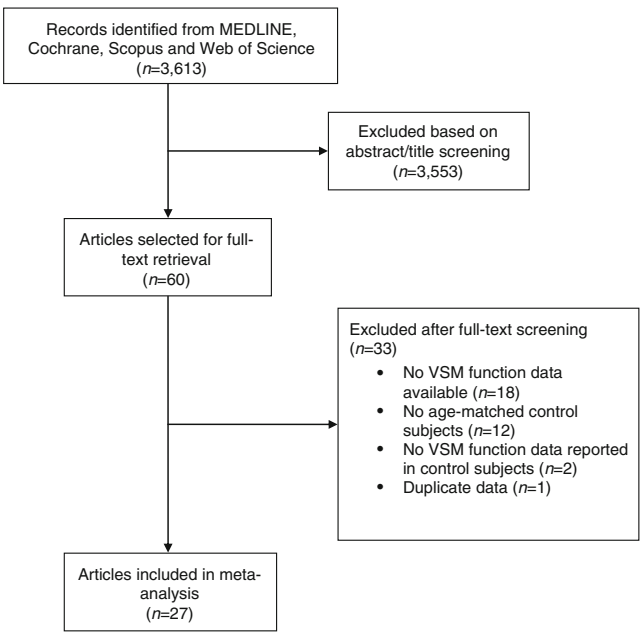

Fig. 1 Flow diagram of the process of study selection

VSM function VSM function was determined in all of the included studies by evaluating the response to known endothelium-independent vasodilator substances related to either the macro- or the microcirculation (Table 1). The SMD in VSM function between individuals with type 2 diabetes and control subjects was used as the primary outcome. After data pooling, the meta-analysis was performed. The meta-analysis revealed that VSM function was significantly impaired in diabetic compared with control subjects (31 studies, 1,643 subjects; SMD −0.68; 95% CI −0.84, −0.52; p<0.001) (Fig. 2). Significant heterogeneity was found in this analysis (I^2=52%; p<0.001). VSM function was also found to be significantly impaired in diabetic compared with control subjects when considering only studies assessing either the macrocirculation (12 macrovascular studies, 910 subjects; SMD −0.46; 95% CI −0.67, −0.25; p<0.001) or the microcirculation (19 microvascular studies, 733 subjects; SMD −0.85; 95% CI −1.06, −0.65; p<0.001).

Subgroup and meta-regression analyses Because we found some evidence of study heterogeneity according to potential moderating factors of each study, subgroup analyses were conducted to determine the sources of heterogeneity (Table 2). Heterogeneity was reduced below 50% of I^2 in both subgroups after dividing the studies by the difference (Δ) between diabetic and control subjects in DBP, fasting insulin, HDL-cholesterol, triacylglycerols, vascular region assessed and microvascular technique. In the remaining subgroup analyses, except for the Δ between diabetic and control subjects in HbA_{1c}, heterogeneity was also reduced below 50% of I^2 in one of the complementary subgroups. The SMD

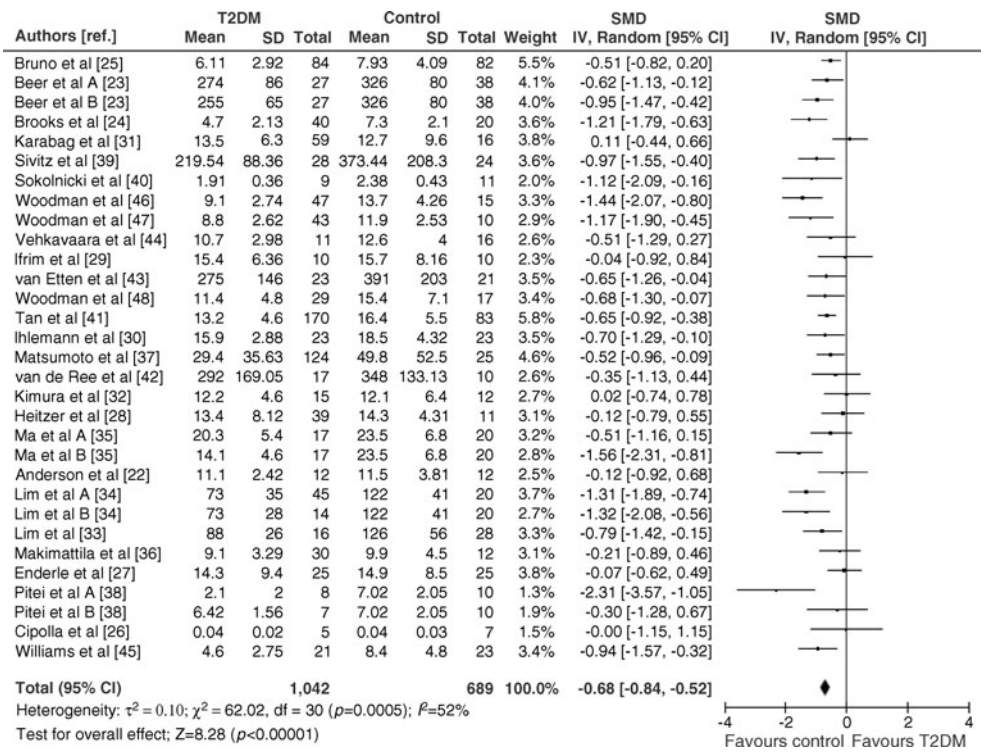

Fig. 2 Forest plot of SMD in VSM function between diabetic and control subjects. IV, inverse variance; T2DM, type 2 diabetes mellitus. Squares represent the SMD in VSM function for each study. The diamond represents the pooled SMD in VSM function. Some studies presented two subgroups of type 2 diabetes (T2DM), each of which had been independently compared with a single control group, thus they were evaluated as individual studies (distinguished by A or B)

in VSM function between diabetic and control subjects was further reduced in subgroups with Δ between diabetic and control subjects in SBP >11.00 mmHg (p=0.007), in DBP >3.00 mmHg (p=0.007) and in HDL-cholesterol ≤−0.21 mmol/l (p=0.03) compared with their complementary subgroups. Moreover, the SMD in VSM function between diabetic and control subjects was decreased in studies assessing the microcirculation compared with macrovascular studies (p=0.009). When considering only macrovascular studies, the SMD in VSM function between diabetic and control subjects did not reach significance between any of the subgroup analyses (see electronic supplementary materials [ESM] Table 1). In microvascular studies, the SMD in VSM function between diabetic and control subjects was significantly reduced in subgroups with Δ between diabetic and control subjects in LDL-cholesterol >0 mol/l (p=0.04) and in triacylglycerols >1.02 mmol/l (p=0.02), compared with their complementary subgroups (ESM Table 2). As regards the technique used for VSM function assessment, there was no significant difference between the two main microvascular techniques (iontophoresis vs plethysmography, p=0.17) (Table 2) or between the main macrovascular technique (brachial nitrate-mediated dilation [NMD]) and plethysmography (p=0.15). In contrast, a significant difference was found between NMD and iontophoresis (p=0.003).

Meta-regression analysis was also used to evaluate the relationship between the SMD in VSM function between diabetic and control subjects and study moderating factors. The results of this analysis are shown in Fig. 3. The Δ between diabetic and control subjects in BMI (B=−0.04, p=0.02), in SBP (B=−0.02, p=0.004), in DBP (B=−0.03, p=0.003), in HDL-cholesterol (B=1.19, p<0.001), in triacylglycerols (B=−0.40, p=0.01) and SMD of endothelial function (B=0.36, p=0.02), were significantly associated with SMD in VSM function between diabetic and control subjects. No significant association between moderating factors and SMD in VSM function between diabetic and control subjects was detected when considering only macrovascular studies. In microvascular studies, the Δ between diabetic and control subjects in BMI (B=−0.06, p=0.048), in HDL-cholesterol (B=1.03, p=0.003) and in triacylglycerols (B=−0.50, p=0.03), were significantly associated with the SMD in VSM function between diabetic and control subjects.

Quality assessment and potential bias Based on the quality assessment criteria, 23 out of 31 studies included in the meta-analysis (quality score ≥12) were considered to have a low-bias risk, and the remaining eight studies were considered to be of moderate-bias risk (quality score of 10 or 11) (Tables 1 and 3). The funnel plot for SMD in VSM function of studies included in the meta-analysis was notably symmetrical, suggesting the absence of a significant publication bias (Fig. 4). Also, no significant funnel plot asymmetry was detected by Begg and Mazumdar's rank correlation test (p=0.97) or Egger's test (p=0.52).

Table 2 Subgroup analyses of the SMD in VSM function between subjects with type 2 diabetes and control subjects

	Studies		VSM function		
Group	Number[a]	References	SMD (95% CI)	$I^2_{\text{Heterogeneity}}$	$p_{\text{Difference}}$
n					
≤44	16	[22, 26, 29, 32–36, 38, 40, 42–45]	−0.64 (−0.91, −0.38)	46	0.75
>44	15	[23–25, 27, 28, 30, 31, 34, 37, 39, 41, 46–48]	−0.68 (−0.90, −0.49)	59	
Mean age					
≤54.90 years	15	[22, 29–31, 33, 34, 36, 38, 40–42, 45, 46]	−0.72 (−1.00, −0.45)	61	0.70
>54.90 years	15	[23–25, 27, 28, 32, 35, 37, 39, 43, 44, 47, 48]	−0.66 (−0.85, −0.46)	43	
Sex					
≤41.67% women	14	[23, 28, 35, 36, 38, 42, 44–48]	−0.79 (−1.05, −0.53)	50	0.38
>41.67% women	15	[22, 24, 25, 27, 30–34, 37, 39–41, 43]	−0.64 (−0.85, −0.43)	55	
Mean duration of T2DM[b]					
≤7 years	12	[23, 28–30, 32–34, 36, 40, 45, 47]	−0.70 (−0.97, −0.43)	46	0.54
>7 years	10	[23–25, 27, 31, 37, 38, 41, 42]	−0.58 (−0.85, −0.31)	63	
Difference in BMI					
≤3.05 kg/m^2	12	[28–31, 35–37, 40, 41, 43, 48]	−0.54 (−0.76, −0.32)	42	0.07
>3.05 kg/m^2	14	[22–25, 27, 33, 34, 39, 42, 45–47]	−0.83 (−1.06, −0.61)	52	
Difference in SBP					
≤11.00 mmHg	11	[27, 30, 33, 35–37, 41–43, 47, 48]	−0.58 (−0.74, −0.42)	0	0.007
>11.00 mmHg	8	[23–25, 34, 35, 46]	−1.05 (−1.35, −0.74)	59	
Difference in DBP					
≤3.00 mmHg	10	[24, 25, 27, 30, 35, 37, 41–43, 48]	−0.59 (−0.73, −0.44)	0	0.007
>3.00 mmHg	9	[23, 33–36, 46, 47]	−1.02 (−1.29, −0.74)	42	
Difference in fasting insulin					
≤79.17 pmol/l	6	[33, 36, 44, 46–48]	−0.81 (−1.17, −0.45)	41	0.44
>79.17 pmol/l	6	[23, 34, 39, 45]	−0.98 (−1.21, −0.74)	0	
Difference in fasting glucose					
≤3.80 mmol/l	12	[23, 25, 27, 34, 36, 41–43, 46–48]	−0.70 (−0.91, −0.49)	48	0.96
>3.80 mmol/l	11	[22, 28, 31, 33–35, 39, 45]	−0.69 (−1.01, −0.37)	57	
Difference in HOMA-IR					
≤5.26	6	[23, 34, 36, 46–48]	−0.90 (−1.27, −0.53)	54	0.94
>5.26	6	[23, 33, 34, 39, 44, 45]	−0.92 (−1.18, −0.66)	0	
Difference in HbA_{1c}					
≤2.50% (≤4 mmol/mol)	11	[25, 28, 31, 33, 34, 36, 39–41, 46, 47]	−0.71 (−0.98, −0.44)	63	0.84
>2.50% (>4 mmol/mol)	9	[27, 30, 32, 34, 38, 43–45]	−0.67 (−1.03, −0.30)	56	
Difference in total cholesterol					
≤0.20 mmol/l	12	[22, 25, 27, 28, 30, 35, 36, 40, 41, 47, 48]	−0.58 (−0.80, −0.37)	41	0.47
>0.20 mmol/l	9	[31–34, 42, 43, 45, 46]	−0.74 (−1.13, −0.36)	68	
Difference in HDL-cholesterol					
≤−0.21 mmol/l	12	[22, 23, 25, 30, 34, 35, 39, 43, 44, 46, 47]	−0.86 (−1.09, −0.63)	45	0.03
>−0.21 mmol/l	11	[28, 31–36, 41, 42, 45, 48]	−0.50 (−0.74, −0.26)	42	
Difference in LDL-cholesterol					
≤0.00 mmol/l	12	[23, 25, 28, 30, 35, 36, 39, 42, 47, 48]	−0.68 (−0.88, −0.48)	30	0.85
>0.00 mmol/l	10	[22, 31–34, 41, 43, 45, 46]	−0.71 (−1.03, −0.40)	66	
Difference in triacylglycerols					
≤0.92 mmol/l	11	[25, 28, 31, 32, 35, 40, 43–45, 48]	−0.55 (−0.82, −0.29)	49	0.10
>0.92 mmol/l	11	[23, 29, 33, 34, 36, 39, 42, 46, 47]	−0.86 (−1.12, −0.61)	42	
Difference in baseline brachial diameter					
≤0.10 mm	6	[22, 27, 30, 31, 35, 41]	−0.36 (−0.66, −0.07)	46	0.45

Table 2 (continued)

Group	Studies		VSM function		
	Number[a]	References	SMD (95% CI)	$I^2_{\text{Heterogeneity}}$	$p_{\text{Difference}}$
>0.10 mm	3	[25, 32, 35]	−0.67 (−1.41, 0.07)	78	
Vascular region assessed					
Macrocirculation	12	[22, 25, 27, 29–32, 35, 37, 41, 48]	−0.46 (−0.67, −0.25)	46	0.009
Microcirculation	19	[23, 24, 26, 28, 33, 34, 36, 38–40, 42–47]	−0.85 (−1.06, −0.65)	40	
Microvascular technique					
Iontophoresis	8	[23, 24, 33, 34, 38]	−1.02 (−1.31, −0.73)	36	0.17
Plethysmography	9	[28, 36, 39, 42–47]	−0.72 (−1.02, −0.43)	43	
Vascular endothelial function					
≤−0.89 SMD	13	[22, 25, 28, 32, 33, 35, 36, 41–43, 46, 47]	−0.62 (−0.85, −0.40)	49	0.71
>−0.89 SMD	12	[23, 24, 27, 29–31, 34, 44, 45, 48]	−0.69 (−0.97, −0.41)	59	

Median values of continuous variables were used as cut-off values for grouping studies. Difference of each variable risk factor was calculated as type 2 diabetes mellitus (T2DM) group value minus control group value

[a]Certain enrolled studies were not included because the value used for subgroup analysis was not reported therein

[b]Mean time (years) since diagnosis of T2DM

Discussion

In this systematic review and meta-analysis, we pooled and analysed data from 31 studies comparing VSM function in subjects with type 2 diabetes and age-matched controls. The results of our analysis revealed a moderate-to-large impairment of VSM function in diabetic subjects (SMD −0.68; 95% CI −0.84, −0.52). We also performed subgroup and meta-regression analyses because of significant heterogeneity. Subgroup analysis showed that there was a stronger decrease in VSM function in diabetic subjects in microcirculation studies compared with macrocirculation studies. Furthermore, VSM function was negatively associated with BMI, SBP, DBP and triacylglycerols and was positively associated with HDL-cholesterol and endothelial function (Fig. 3).

In type 2 diabetes, in contrast to the well-documented endothelial dysfunction, studies assessing the status of VSM function have provided controversial findings

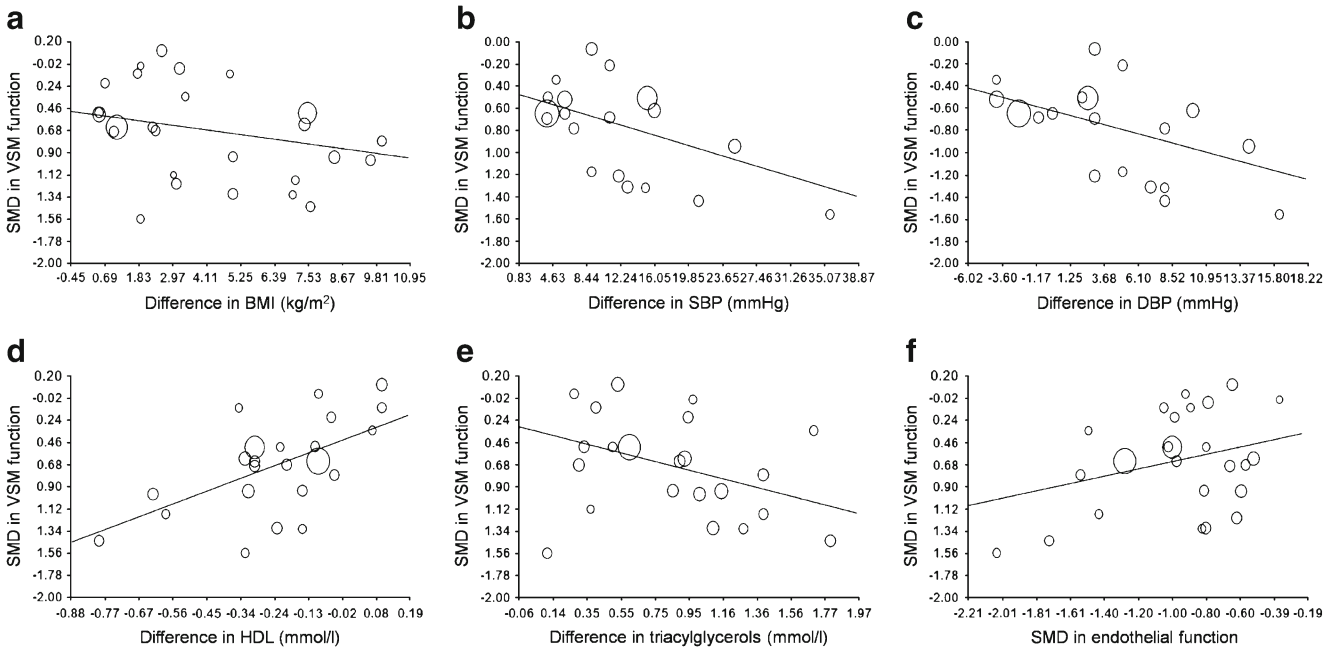

Fig. 3 Meta-regression plots of SMD in VSM function according to the difference in BMI (B=−0.04, p=0.02) (**a**), in SBP (B=–0.02, p=0.004) (**b**), in DBP (B=−0.03, p=0.003) (**c**), in HDL-cholesterol (B=1.19, p<0.001) (**d**), in triacylglycerols (B=−0.40, p=0.01) (**e**) and the SMD in endothelial function (B=0.36, p=0.02) (**f**). The size of each circle is proportional to the study's weight. The difference in each variable risk factor was calculated as type 2 diabetes mellitus group value minus control group value

Table 3 Quality assessment of studies included in the meta-analysis[a]

Study, year of publication	T2DM group (0–5)	Control group (0–5)	Quality of measurement (0, 1)	Confounding variables (0–3)	Data (0–2)	Total quality score
Bruno et al [25], 2012	5	5	1	2	1	14
Beer et al A [23], 2008	4	5	1	2	1	13
Beer et al B [23], 2008	4	5	1	2	1	13
Brooks et al [24], 2008	5	4	1	1	1	12
Karabag et al [31], 2007	5	5	1	3	1	15
Sivitz et al [39], 2007	4	4	1	2	1	12
Sokolnicki et al [40], 2006	3	5	1	2	1	12
Woodman et al [46], 2006	5	5	1	2	1	14
Woodman et al [47], 2005	4	5	1	2	1	13
Vehkavaara et al [44], 2004	4	4	1	2	1	12
Ifrim et al [29], 2004	4	3	1	2	1	11
van Etten et al [43], 2002	4	4	1	2	2	13
Woodman et al [48], 2002	5	5	1	3	1	15
Tan et al [41], 2002	5	5	1	2	1	14
Ihlemann et al [30], 2002	5	5	1	3	2	16
Matsumoto et al [37], 2002	4	4	0	2	1	11
van de Ree et al [42], 2001	4	5	1	3	1	14
Kimura et al [32], 2001	4	4	1	2	1	12
Heitzer et al [28], 2001	5	4	1	2	1	13
Ma et al A [35], 2001	3	3	1	2	1	10
Ma et al B [35], 2001	3	3	1	2	1	10
Anderson et al [22], 2001	3	4	1	3	1	12
Lim et al A [34], 1999	5	4	1	3	1	14
Lim et al B [34], 1999	5	4	1	3	1	14
Lim et al [33], 1999	5	5	1	3	1	15
Makimattila et al [36], 1999	5	4	1	3	1	14
Enderle et al [27], 1998	4	5	0	1	1	11
Pitei et al A [38], 1997	3	3	1	2	1	10
Pitei et al B [38], 1997	3	3	1	2	1	10
Cipolla et al [26], 1996	3	3	1	2	2	11
Williams et al [45], 1996	5	5	1	2	1	14

Some studies presented two subgroups of type 2 diabetes (T2DM), each of which had been independently compared with a single control group, thus they were evaluated as individual studies (distinguished by A or B)

[a]Adapted from the SAQOR [14]

(Table 1). Previous speculations on these inconsistent results, such as sample size and duration of type 2 diabetes, did not account for the heterogeneity in VSM function when quantitatively assessed in the meta-analysis (Table 2). The heterogeneity in VSM function could, however, be explained in part by differences in BMI, arterial pressure, and dyslipidaemia among diabetic and control subjects in the aforementioned studies (Fig. 3). The main finding of this meta-analysis was, therefore, to confirm the impairment of VSM function, in parallel with endothelial dysfunction, in type 2 diabetes. It is interesting to note that endothelial dysfunction in persons with type 2 diabetes should then be asserted with caution, given that endothelial-dependent vasodilatation depends, at least in part, on VSM function. This study suggested a novel mechanism of cardiovascular disease in persons with type 2 diabetes. VSM dysfunction was previously reported in asymptomatic adults with risk factors for atherosclerosis [49], and it was clearly demonstrated to predict future cardiovascular events even better than endothelial function in such individuals [9, 50]. Moreover, the association of VSM function with cardiovascular risk factors differed from that of endothelial function, and was more strongly related to vascular disease than endothelial function in individuals with type 2 diabetes [51]. Consequently, the prognostic significance of VSM function may be worth reconsidering in order to improve the accuracy of risk

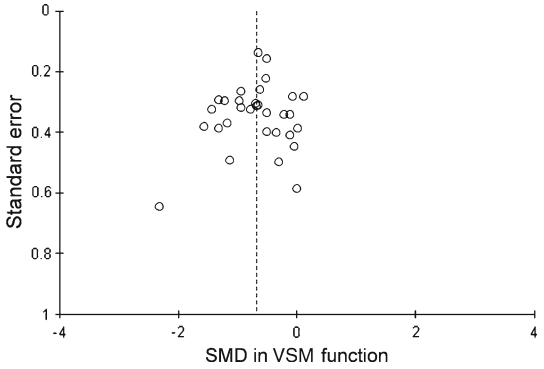

Fig. 4 Funnel plot of studies included in the meta-analysis. Funnel plot asymmetry: $p=0.97$ and $p=0.52$ according to Begg and Mazumdar's rank correlation test and Egger's test, respectively [18]

assessment and prevent cardiovascular complications in individuals with type 2 diabetes.

Another major finding of this meta-analysis was that deterioration of VSM function was more advanced in the microcirculation than in the macrocirculation of diabetic subjects. Moreover, when considering distinct vascular techniques, we detected further decrease in VSM function in studies assessing the microcirculation by the iontophoresis technique, but not by plethysmography, compared with those assessing it by NMD in the macrocirculation. This result could be explained by the fact that forearm plethysmography, besides assessing the microcirculation (vessels <150 μm in diameter, primarily assessed by iontophoresis), also evaluates in part the response of larger vessels such as small resistance arteries. Interestingly, experimental studies reported that induced insulin resistance blocked VSM cell activation in the microcirculation without altering macrovascular blood flow in mice [52]. In humans, locally administered insulin causes a specific increase in VSM activity in the microcirculation of healthy adults [53]. Hence, VSM cells in the pre-capillary arterioles are proposed to be the primary vascular target for insulin, leading to a higher capillary recruitment, and thus play a decisive role in the availability of insulin and glucose to body tissues [54]. Accordingly, the aggravation of VSM dysfunction recognised in the microcirculation of individuals with type 2 diabetes may be a distinctive feature of this metabolic disorder associated with vascular insulin resistance.

VSM function was inversely related with traditional cardiovascular risk factors such as arterial pressure, BMI and triacylglycerols. These results are consistent with the previously reported association between hypertension and VSM dysfunction in individuals with type 2 diabetes [35, 50, 55]. A novel finding arising from this meta-analysis is that both BMI and triacylglycerols were related to VSM dysfunction in the microcirculation, but not in the macrocirculation, of diabetic subjects. Thus, the well-known adverse impact of adiposity and elevated triacylglycerol levels on vascular function might be crucial in the microcirculation of individuals with type 2 diabetes, as recently suggested [56]. Likewise, HDL-cholesterol did not predict VSM function in the macrocirculation, while it showed a strong positive association with VSM function in the microcirculation of diabetic subjects. Consequently, the specific influence of HDL-cholesterol in the microcirculation could explain, in part, its prognostic value in type 2 diabetes [57, 58]. In vitro studies indicate that HDL-cholesterol may have numerous direct actions in VSM cells [59]. One such action is the enhancement of VSM relaxation through the upregulation of cyclooxygenase type 2 expression and increased prostacyclin synthesis [60]. Additionally, sphingosine 1-phosphate, a lysosphingolipid component of HDL-cholesterol [61], might play a significant role in preserving nitric oxide bioavailability by inhibiting the generation of reactive oxygen species in VSM cells [62], thence favouring VSM function. Otherwise, the reasons underlying the lack of significant associations between BMI, triacylglycerols and HDL-cholesterol and VSM function in the macrocirculation of individuals with type 2 diabetes are unclear and need further investigation. Taking together, traditional risk factors have an adverse impact on VSM function and explain, to some extent, the aforementioned aggravation of VSM dysfunction in the microcirculation of individuals with type 2 diabetes.

Unexpectedly, VSM function was not related to markers of glucose homeostasis. This result may be partially explained by the effects of oral hypoglycaemic agents and insulin treatments (Table 1). Moreover, fewer studies reported markers of glucose homeostasis than other variable risk factors studied in this meta-analysis (Table 2), meaning that these markers possessed a reduced statistical power. On the other hand, although use of antihypertensive and/or lipid-lowering drugs in diabetic subjects was also reported [23, 25, 26, 31, 35, 37, 43, 48], most studies included in the meta-analyses either excluded those subjects [22, 33, 34, 36, 42, 45–47] or interrupted treatment before examination [31, 35, 37, 43]. This may account for the detected associations between VSM function and risk factors related to arterial pressure and dyslipidaemia (Fig. 3).

There are some limitations and strengths in the present meta-analysis. Significant heterogeneity was observed among the enrolled studies. However, a comprehensive evaluation of the sources of heterogeneity was performed through subgroup analyses. Additionally, considering that our primary outcome was an SMD in VSM function between diabetic and control subjects, we use the difference in study variables between diabetic and control subjects instead of baseline value of diabetic study variables, to search for moderating factors with accuracy. We also noted that estimates of statistical heterogeneity were moderate by current convention [63]. Individual patient data were not needed in our analysis, since the required aggregate data and standard

errors could be fully obtained from the published articles themselves [64]. Nonetheless, some potentially relevant studies were excluded from the analysis because control subjects were not age-matched with diabetic subjects. Finally, the quality of studies was evaluated by specific tools for the quality assessment of observational research [14, 15]. A low bias risk was estimated for study quality, and no risk of publication and reporting biases was detected.

In conclusion, this meta-analysis demonstrated the impairment of VSM function in individuals with type 2 diabetes. Therefore, in addition to the endothelium, the VSM needs to be considered as a potential cause of vascular dysfunction in type 2 diabetes. Moreover, individuals with type 2 diabetes exhibited an aggravation of VSM dysfunction in the microcirculation compared with the macrocirculation. This may be a distinctive feature in type 2 diabetes, associated with vascular insulin resistance, reported previously only in animals. We believe that the present study provides further evidence that risk factors such as adiposity, arterial pressure, triacylglycerols and especially HDL-cholesterol should be tightly controlled in individuals with type 2 diabetes in order to reduce the cardiovascular risk in this population.

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Contribution statement DM was responsible for the concept and design of the study. Acquisition of data was carried out by DM and AV. DM, GW, APM, NVS, ER and AV analysed and interpreted the data. Drafting of the manuscript was carried out by DM. DM, GW, APM, NVS, ER and AV critically revised the manuscript for important intellectual content. Statistical expertise was provided by DM and administrative, technical or material support was provided by DM, NVS and ER. All authors approved the final version of the manuscript.

DM had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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