THE IMPACT OF AGE ON VASCULAR SMOOTH MUSCLE FUNCTION IN HUMANS

Short title: Age and vascular smooth muscle function

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Conflicts of Interest

The authors report no conflicts of interest.

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Aim: Advanced age is associated with vascular endothelial dysfunction, characterized by reductions in endothelium-dependent vasodilation of conduit and resistance arteries, in part from decreased nitric oxide (NO) bioavailability. Although vascular smooth muscle function (SMF), assessed by responsiveness to an exogenous NO donor, is typically reported to be intact, many of these studies are limited by small sample size. Therefore, the purpose of this meta-analysis is to systematically review and determine whether vascular SMF is different between older *versus* young healthy subjects.

Design: We conducted a systematic search of MEDLINE, Cochrane and Scopus, since their inceptions until January 2014 for articles evaluating SMF in the brachial artery and/or resistance arteries (BASMF and RASMF, respectively), as assessed by the endothelium-independent vasodilator response to exogenous NO donors in older (≥60 years) and young (<30 years) groups of healthy subjects. Meta-analyses were performed to compare the mean difference (MD) in BASMF and the standardized mean difference (SMD) in RASMF between older and young groups. Subgroup analyses were performed to identify sources of heterogeneity.

Results: Fifteen studies assessing BASMF and 20 studies assessing RASMF were included, comprising 550 older and 516 young healthy subjects. After data pooling, BASMF and RASMF were lower in older compared with young groups (MD=-1.89 %, *P*=0.04; SMD=-0.46, *P*=0.0008, respectively). Significant heterogeneity was observed in the BASMF (I^2=74 %; *P*<0.00001) and RASMF (I^2=57 %; *P*=0.0008) meta-analyses. Subgroup analyses revealed that studies with (predominantly) males showed similar SMF responses between older and young groups.

Conclusions: Based on current published studies, vascular SMF is reduced in conduit and resistance arteries of otherwise healthy older subjects, particularly in women.

Keywords: advanced age; vascular smooth muscle function; conduit artery; resistance arteries.

INTRODUCTION

Older age is a primary risk factor for the development of cardiovascular diseases [1, 2]. The influence of advanced age on increased cardiovascular risk is, at least partly, mediated through its effects on the arterial vasculature [1]. In this regard, ageing is associated with vascular endothelial dysfunction in humans, characterized by reduced endothelium-dependent vasodilator function in conduit [3-13] and resistance [6, 9, 14-24] arteries. Alterations in the nitric oxide (NO) signaling pathway and/or decreased NO bioavailability have been proposed to contribute to the age-related decrease in endothelium-dependent vasodilator function [25, 26].

The ability of vessels to dilate is dependent on the function of the endothelium, but is also subordinated to vascular smooth muscle (vasodilator) function (SMF). Smooth muscle responsiveness represents the final, and frequently underappreciated, step of endothelium-dependent vasodilation. However, relatively little is known about the impact of advanced age on SMF. Some reports from animal studies indicate that advanced age is associated with reduced vascular SMF [27-29]; nonetheless, this finding is not universal [25]. In humans, studies assessing vascular SMF in healthy older and young subjects have reported variable results, possibly as a result of small sample sizes [3-6, 8-10, 12, 14-24, 30-43].

Therefore, the primary aim of this study was to perform a systematic review and meta-analyses of available studies comparing vascular SMF in older and young healthy subjects. We hypothesized that SMF would be reduced in conduit and resistance arteries of older compared with young healthy adults. We selected studies that assessed SMF, determined by the endothelium-independent vasodilator response to

exogenous NO donors, in the brachial artery (BASMF) and in resistance arteries (RASMF) of healthy young and healthy older subjects.

METHODS

The review is reported according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group guidelines [44].

Data sources and searches

Our systematic search included MEDLINE, Cochrane and Scopus, since their inceptions until January 2014. We used combinations of the subject headings "older", "vascular smooth muscle", "endothelium independent", "nitroglycerin", "sodium nitroprusside", "vascular function", "vasodilation" and "vascular reactivity"; the search strategy for MEDLINE is shown in Supplemental Figure S_1. We also performed hand searching in reference citations of articles included in meta-analysis and related citations in MEDLINE.

Study selection

To be included in the analysis, an observational report had to assess BASMF and/or RASMF in an older group (mean age ≥60 years) and a young group (mean age <30 years) of healthy subjects. In the event of multiple publications pertaining to the same research, the first published or most comprehensive study was included. Inclusion of studies was not limited by publication status or language.

Data extraction and quality assessment

The following variables were extracted into a pre-formatted spreadsheet: authors, year of publication, characteristics of study participants (*n*, gender, age, height, weight, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), physical activity, maximal oxygen consumption (VO_{2max}), morbidities, risk factors to health, medication) and vascular variables (vascular region, vasodilation parameter, baseline vascular tone, NO donor, dosage, time of analysis after NO donor administration, wall-tracking system, BASMF, RASMF, endothelial function). A systematic appraisal of quality for observational research (SAQOR) [45] previously applied in meta-analysis of observational studies evaluating arterial function [46] was performed to provide assessment of study quality. The SAQOR was adjusted to assess 1) the older sample group, 2) the young sample group, 3) quality of vascular SMF measurement, 4) confounding variables and 5) data. Overall, the SAQOR was scored out of 16, quality deemed better with a greater score (Table S_1 and S_2).

Data synthesis and analysis

The meta-analyses and subgroup analyses were performed using Review Manager software (RevMan 5.2, Cochrane Collaboration, Oxford, UK). The primary outcomes were the mean difference (MD) in BASMF and the standardized mean difference (SMD) in RASMF between older and young groups. SMD summary statistic allowed us to standardize RASMF values expressed in different units into a uniform scale to complete this meta-analysis. Each MD and SMD was weighted according to the inverse variance method [47] and they were pooled with a random-effects model [48]. SMD of 0.2, 0.5, and 0.8 represents small, medium, and large effect sizes, respectively [49]. Heterogeneity between studies was assessed using the chi-squared test for heterogeneity and I^2 statistics. Potential moderating factors were evaluated by

subgroup analysis comparing studies grouped by dichotomous or continuous variables potentially influencing vascular SMF. Median values of continuous variables were used as cut-off values for grouping studies. Publication bias was evaluated by estimating the asymmetry of the Begg and Mazumdar's funnel plot [50]. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Study selection and characteristics

The flow diagram of the process of study selection is shown in Figure 1, which resulted in the inclusion of 33 articles. Thirteen of these articles assessed BASMF, 19 assessed RASMF and 1 assessed both BASMF and RASMF. One of the articles assessing BASMF presented two groups of older subjects, each of which had been independently compared with a location-matched young group [43]. Therefore, these data were evaluated as two individual studies. Table 1 shows the main clinical characteristics of the 15 BASMF studies and 20 RASMF studies, comprising a total of 550 subjects in the older group and 516 subjects in the young group. Older and young groups were gender-matched in all studies (omitting 1 study in which gender-related data was not available [22]). All subjects were free from co-morbidities and risk factors according to cut-off values, non-smokers (except for 1 study allowing < 5 cigarettes per day [23]) and not taking medications (other than oral contraceptives reported in 1 study [31]). The quality of the studies was moderate-to-high according to a previously validated scale [45, 51]. The mean score was 13.9±1.2 for studies assessing BASMF and 11.8±1.2 for studies assessing RVSMF, out of a possible 16 points (Table S_1 and S_2). As for the evaluation of potential bias, the Begg and Mazumdar's funnel plot for the MD in BASMF was moderately asymmetric, suggesting the presence of publication bias and/or other

biases (Figure S_2). The Begg and Mazumdar's funnel plot for the SMD in RVSMF was relatively symmetrical (Figure S_3).

Brachial artery smooth muscle function (BASMF)

All studies assessing BASMF evaluated the vasodilator response to 0.4 mg of nitroglycerin by means of high-resolution ultrasound (Table 2). Resting brachial diameter ranged from 3.0 to 4.4 mm, with older groups commonly presenting a larger resting brachial diameter than young groups. After data pooling, the meta-analysis revealed that BASMF was lower in older compared with young groups (15 studies, MD=-1.89%; P=0.04) (Figure 2). Significant heterogeneity was detected (I^2=74%; P<0.00001). In subgroup analyses, studies above the median in presence of females in the study group showed lower BASMF in older compared with young groups (8 studies, MD=-3.38%; P=0.01). In contrast, studies below the median in presence of females had similar BASMF in older and young groups (7 studies, MD=0.19%; P=0.80). Both sex subgroups were significantly different when compared with each other (P=0.02) (Table S_3). In addition, lower brachial artery endothelial function in older compared with young groups was related to lower BASMF (*P*=0.03, Table S_3). No other potential moderating factor (n, age, height, weight, BMI, SBP, DBP, VO_{2max}, vascular assessment, methodological quality, year of publication) significantly influenced the MD in BASMF between older and young groups in subgroup analyses (Table S_3).

Resistance artery smooth muscle function (RASMF)

RASMF was determined by evaluating the absolute peak [6, 14, 15, 18, 23, 24, 30-33, 35], percent increase from baseline [8, 9, 16, 17, 19-21, 34] or slope [22] in vasodilation in response to sodium nitroprusside (Table 3). After data pooling, the

RASMF was lower in older compared with young groups (20 studies, SMD=-0.46; P=0.0008) (Figure 3). Significant heterogeneity was detected (I^2=57%; P=0.0008). In subgroup analyses, studies above the median in presence of females showed lower RASMF in older compared with young groups (10 studies, SMD=-0.77; P=0.001). In contrast, studies below the median in presence of females presented similar RASMF in older and young groups (9 studies, SMD=-0.17; P=0.20). Both sex subgroups were significantly different when compared with each other (*P*=0.02) (Table S_3). No other potential moderating factor (n, age, height, weight, BMI, SBP, DBP, VO_{2max}, vascular assessment, methodological quality, year of publication) significantly influenced the SMD in RASMF between older and young groups (Table S_3).

DISCUSSION

In this systematic review and meta-analysis, we pooled and analyzed data from 33 articles comparing vascular SMF, determined by the vasodilator response to exogenous NO donors, in 550 older and 516 young healthy subjects. The main finding of this meta-analysis is that vascular SMF is significantly lower in older compared to young individuals, a finding that is similarly present in conduit and resistance arteries (Figure 2 and 3). In subgroup analyses, we found that sex altered the impact of age on vascular SMF. More specifically, studies including females only or a higher ratio of females to males displayed a significant and marked impact of age on conduit and resistance artery SMF, whilst studies involving males only or a lower ratio of females to males reported no effect of age on SMF (Table S_3).

The impact of age on vascular SMF had not been explored in detail in most previous individual studies (Table 2 and 3), possibly because of low statistical power and

presence of confounding factors inherent in cross-sectional comparisons. Results from this meta-analysis indicate that, in addition to endothelial dysfunction, older age is associated with smooth muscle dysfunction in conduit and resistance arteries, as represented by reduced smooth muscle sensitivity to a NO donor. Consistent with this finding, studies performed in older rats demonstrated reduced vasodilator response to NO [27-29] and decreased vascular smooth muscle expression of soluble guanylyl cyclase (sGC) [27, 52] (i.e., the principal intracellular receptor of NO and mediates vasodilation via formation of cyclic guanosine monophosphate (cGMP) [53]). In humans, decreased expression and activity of sGC in brain tissue have been related to advanced age and Alzheimer's disease, respectively, although no specific vascular measures were provided [54, 55]. Whether an age-related reduction in expression and activity of sGC in vascular smooth muscle contributes to a blunted vascular responsiveness to NO needs further investigation.

A consistent finding from previous studies is that brachial artery flow-mediated dilation, as well as resistance artery responses to acetylcholine, are impaired in older healthy humans. As most of these previous studies found no difference in the endothelium-independent vasodilation to exogenous NO donors, the general consensus was that the lower flow-mediated dilation and resistance artery responses to acetylcholine supported the presence of a dysfunctional endothelium. Our results, however, suggest the presence of a small-to-moderate but significant age-related impairment in SMF that is present across the vascular tree. Moreover, our finding also suggests that a portion of the impaired NO-dependent responses in conduit and resistance arteries in older healthy subjects may be in part due to reduced smooth muscle sensitivity to NO. This important observation should be taken into

consideration when interpreting the lower NO-dependent responses in healthy older humans.

Another important finding of this meta-analysis was the influence of sex on the difference in vascular SMF between young and older humans. Studies that involved (predominantly) males, which is a common observation in studies in the field of cardiovascular physiology, found similar BASMF and RASMF between older and young groups. However, studies with females only or studies with a relatively high ratio of females to males reported a significantly lower BASMF and RASMF in the older group compared with the young group. One potential explanation for this finding may be related to menopausal status in women. Indeed, reduced vasodilation to exogenous NO donors has been reported in conduit and resistance arteries of healthy older women but not in those on hormonal replacement therapy. This suggests that hormone replacement therapy in postmenopausal women may preserve the sensitivity of vascular smooth muscle to NO [56-58]. In this regard, it is important to emphasize that none of the studies included in our meta-analyses involved women on hormonal therapy. Consequently, based on the assumption that the majority of older women were post-menopausal, the lower vascular SMF in older groups could be a result of the loss of vascular protection from circulating estrogens associated with post-menopause. Nevertheless, removal of estrogen with ovariectomy in rodents does not appear to alter vasodilator responses to sodium nitroprusside [59, 60], suggesting that loss of estrogen after menopause may not be the sole factor contributing to reduced vascular SMF in older women. Alternatively, estrogen-independent alterations in the NO signaling pathway could underlie the altered vascular SMF in older women in view of the decreased NO-stimulated vascular cGMP formation in middle-aged and older

women (with no report of menopause) compared with men [61]. Whilst speculative, the sex-related reduced vascular sensitivity to NO might partly explain why women remain more symptomatic and present lower cardiovascular status compared with men after long-term nitrate therapy for heart failure [62].

A potential explanation for a lower NO-mediated dilation of the brachial artery in older subjects may relate to the larger baseline diameter. As demonstrated in several previous studies, the brachial artery dilator response to ischemia or glyceryl trinitrate is inversely related to the baseline diameter [63-67]. Nonetheless, in the present study, the difference in baseline brachial diameter between older and young groups did not modify the MD in BASMF according to subgroup analysis (Table S_3). Moreover, the magnitude of the difference in baseline brachial diameter between older men and young men (4 studies) was not different than the difference between older women and young women (3 studies) (P=0.24). Furthermore, the impact of sex on BASMF is not explained by artery diameter [68]. Taken together, we believe differences in brachial artery diameter between older and young groups do not entirely explain the age-related decline in BASMF observed in the present meta-analysis.

As for the prognostic value of vascular SMF, adverse cardiovascular events were associated with reduced smooth muscle sensitivity to NO in high-risk populations when assessed in conduit [69] and resistance arteries [70] by means of ultrasound and plethysmography, respectively. It should be noted that blood flow responses in the microcirculation assessed via plethysmography and laser Doppler techniques —such as those used in the studies included in the RASMF meta-analysis— may be, to a certain degree, dependent on microvascular structure, which in turn is strongly associated

with cardiovascular events [71]. Conversely, NO-dependent responses determined by micromyography did not predict cardiovascular events in high-risk patients, suggesting that resistance artery function per se has low clinical relevance [72]. Similarly, it cannot be discarded that the age-related reduction in RASMF is an observation dependent on differences in microvascular structure. Further research is warranted to determine the extent to which vascular structural changes with ageing influence the non-invasive estimates of smooth muscle responsiveness to NO and their clinical significance.

There are some limitations in this meta-analysis. First, cross-sectional comparisons may be misleading when addressing the question of the impact of advanced age [73]. Second, we were unable to accurately determine the level of physical activity or fitness in the included studies. Since physical activity or exercise training has well-established effects on endothelial function [74], we were unable to identify the potential impact of this factor on our results. Likewise, body composition, fat distribution and blood lipids could be suggested as potential moderating factors, albeit not investigated in this meta-analysis. Third, although single-dose (0.4 mg of nitroglycerin) is the common procedure to evaluate BASMF (Table 2), there are physiological and methodological variables that may affect the reproducibility of BASMF [75, 76]. In addition, the administration of 0.4 mg of nitroglycerin is considered to induce a maximal NO-mediated vasodilator response [75], which could be dissociated from vascular smooth muscle submaximal responses to lower or step-wise doses of NO. In this respect, dose-response studies might have higher sensitivity to detect small differences in BASMF between older and young groups [75].

In conclusion, the current meta-analysis provides evidence that advanced age is associated with a relatively small, but significant impairment in conduit and resistance artery SMF in healthy humans. The magnitude of vascular smooth muscle dysfunction seems more prevalent in women than in men, potentially related to the loss of circulating estrogens in women after menopause. A potential implication of our finding is that the impairment in conduit and resistance artery endothelial function with advanced age may, at least in part, be attributed to changes in vascular SMF in healthy adults. Further studies are needed to determine sex-related mechanisms that may influence the ageing-associated alterations in vascular SMF in humans.

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FIGURE LEGENDS

FIGURE 1 Flow diagram of the process of study selection

FIGURE 2 Forest plot of the mean difference (MD) in brachial artery smooth muscle function (BASMF) between older and young groups. BASMF was expressed as the percentage increase in brachial artery diameter from baseline to maximal vasodilation. Squares represent the MD in BASMF for each study. The diamond represents the pooled MD in BASMF across studies. CI, confidence interval; IV, inverse variance; SD, standard deviation

Figure 3. Forest plot of the standardized mean difference (SMD) in resistance artery smooth muscle function (RASMF) between older and young groups. Squares represent the SMD in RASMF for each study. The diamond represents the pooled SMD in RASMF across studies. CI, confidence interval; IV, inverse variance; SD, standard deviation

TABLE 1. Main clinical characteristics of studies included in the meta-analyses

	n		Females (%)		Age (years)		BMI (m^2/kg)		SBP (mm Hg)		DBP (mm Hg)		
Study, year of publication	**YNG**	**OLD**	**YNG**	**OLD**	**YNG**	**OLD**	**YNG**	**OLD**	**YNG**	**OLD**	**YNG**	**OLD**	**Morbidities**
Studies assessing brachial artery SMF													
DeVan et al [4], 2013	21	25	12	17	24±5	62±6*	23±5	26±6*	111±11	121±12*	60±5	75±6*	none
Pierce et al [42], 2011	16	18	0	0	25±4	63±5*	26±4	27±3	118±13	124±11	69±9	78±5*	none
Black et al [3], 2009	9	8	100	100	26±3	60±6*	23±3	31±6*	101±12	122±14*	56±9	67±8*	none
Donato et al [6], 2009	27	23	0	0	22±5	62±5*	25±5	26±5*	117±11	124±10*	63±11	78±10*	none
Walker et al [12], 2009	26	15	0	0	23±10	66±4*	24±5	26±4	116±10	124±12*	61±5	74±8*	none
Donato et al [5], 2007	51	44	0	0	23±7	63±7*	24±7	26±7*	114±7	121±13*	62±7	75±7*	none
Gates et al [39], 2007	10	12	50	50	21±3	60±7*	22±3	25±3*	105±6	114±17	60±6	70±10*	none
Parker et al [41], 2006	8	8	100	100	22±4	70±7*	22±3	25±3*	109±15	131±11*	68±7	78±11*	none
Eskurza et al [36], 2006	9	9	11	11	26±3	64±6*	24±4	26±2	110±6	108±9	60±9	71±9*	none
Eskurza et al [38], 2005	10	9	0	0	22±3	62±6*	23±3	26±3	110±9	111±12	58±6	69±3*	none
Heiss et al [40], 2005	20	20	50	65	25±4	61±9*	22±4	24±4	117±9	122±13	77±9	81±9	none
Eskurza et al [37], 2004	11	9	0	0	25±3	64±6*	24±3	26±3	113±7	116±12	63±7	73±12*	none
McCrohon et al [10], 2000	10	10	100	100	28±5	61±3*	23±2	24±2	106±13	124±9*	75±10	79±12	none
Woo et al A [43], 1997	19	19	68	68	29±5	63±5*	24±8	23±4	115±11	129±14*	76±10	83±8	none
Woo et al B [43], 1997	19	19	68	68	28±6	63±4*	23±3	25±3	108±9	127±13*	75±7	79±11	none
Mean	**18**	**17**	**37**	**39**	**25**	**63**	**23**	**26**	**111**	**121**	**66**	**75**	—
Studies assessing resistance artery SMF													

Millet et al [30], 2012	20	42	85	86	24±3	69±7*	22±3	23±4	116±8	128±10*	67±7	71±7*	none
Donato et al [8], 2011	16	22	25	32	25±4	64±5*	24±3	28±5*	111±12	126±14*	71±8	78±9*	none
Westby et al [24], 2011	14	14	0	0	25±4	61±7*	24±2	26±2*	114±11	125±11*	66±7	78±7*	none
Kirby et al [16], 2010	13	13	38	38	21±4	66±11*	22±2	24±3*	N/A	N/A	N/A	N/A	none
Tew et al [19], 2010	15	14	20	29	27±2	65±6*	25	27	119±12	124±12	75±9	79±7	none
Donato et al [6], 2009	15	18	0	0	21±4	62±4*	25±4	26±4*	117±8	124±8*	63±8	78±8*	none
Nicholson et al [31], 2009	10	10	40	40	29±9	68±4*	24±3	25±2	116±16	122±13	62±8	69±12	none
Kirby et al [17], 2009	14	14	36	36	22±4	65±7*	25±3	24±3	N/A	N/A	N/A	N/A	none
Donato et al [32], 2008	11	14	18	29	23±7	64±4*	24±2	26±3	110±10	123±11*	71±7	78±7*	none
Galetta et al [9], 2006	16	16	50	31	27±2	65±4*	23±2	24±2	118±7	122±6	75±3	80±2*	none
Al-Shaer et al [21], 2006	15	17	47	24	22±4	62±12*	25±4	28±4*	116±12	126±12*	67±12	78±8*	none
Newcomer et al [33], 2005	8	8	0	0	24±6	67±6*	25±3	26±3	113±11	129±8*	74±11	81±8	none
Weverling-Rijnsburger et al [34], 2004	7	8	0	0	22	80*	N/A	N/A	N/A	N/A	N/A	N/A	none
Ahlers et al [14], 2004	14	10	0	0	23±3	62±7*	N/A	N/A	118±11	120±19	74±11	72±13	none
Smith et al [35], 2003	10	20	0	0	28±3	62±4*	25±5	29±3	115±9	124±9	64±9	81±9*	none
Minson et al [18], 2002	10	10	50	50	22±6	77±16*	24±3	24±3	120±13	137±19*	71±9	79±13	none
DeSouza et al [15], 2002	22	41	0	0	28±5	61±6*	23±6	27±4*	114±14	122±13*	65±9	76±13*	none
Wang et al [20], 2002	12	10	0	0	24±3	67±3*	22±5	23±4	127±17	139±22	77±10	73±16	none
Taddei et al [23], 2000	12	12	33	33	27±2	63±6*	23±4	24±4	119±5	119±6	77±3	78±3	none
Gerhard et al [22], 1996	11	7	N/A	N/A	20-29	60-69*	N/A	N/A	N/A	N/A	N/A	N/A	none
Mean	**13**	**16**	**23**	**23**	**24**	**66**	**24**	**26**	**116**	**126**	**70**	**77**	—

Data are *n*, % of females, mean or mean ± SD. One study presented two groups of older subjects, each of which had been independently compared with a young group [43], thus they were evaluated as individual studies (distinguished by A and B). * significantly different from young group at $P<0.05$
BMI, body mass index; DBP, diastolic blood pressure; OLD, older group; SBP, systolic blood pressure; SMF, smooth muscle function; YNG, young group

TABLE 2. Brachial artery smooth muscle function (SMF) assessment of studies included in the meta-analysis

Study, year of publication	Artery	Wall-tracking system	Resting diameter (mm)		SMF			EF
			YNG	OLD	Stimulus	Dose (mg)	OLD vs. YNG	OLD vs. YNG
DeVan et al [4], 2013	brachial	computerized	4.0±0.4	4.0±0.5	NTG sublingual	0.4	↔	↓
Pierce et al [42], 2011	brachial	computerized	4.0±0.4	4.1±0.5	NTG sublingual	0.4	↔	↓
Black et al [3], 2009	brachial	computerized	3.3±0.1	3.6±0.6	NTG sublingual	0.4	↔	↓
Donato et al [6], 2009	brachial	computerized	N/A	N/A	NTG sublingual	0.4	↔	↓
Walker et al [12], 2009	brachial	computerized	4.1±0.4	4.4±0.5*	NTG sublingual	0.4	↔	↓
Donato et al [5], 2007	brachial	computerized	N/A	N/A	NTG sublingual	0.4	↔	↓
Gates et al [39], 2007	brachial	computerized	3.7±0.4	4.0±0.8	NTG sublingual	0.4	↔	↓
Parker et al [41], 2006	brachial	computerized	3.0±0.5	3.1±0.4	NTG sublingual	0.4	↓	↓
Eskurza et al [36], 2006	brachial	computerized	4.0±0.6	4.3±0.7	NTG sublingual	0.4	↔	↓
Eskurza et al [38], 2005	brachial	computerized	4.1±0.4	4.4±0.5	NTG sublingual	0.4	↔	↓
Heiss et al [40], 2005	brachial	computerized	3.7±0.9	4.2±0.5*	NTG sublingual	0.4	↔	↓
Eskurza et al [37], 2004	brachial	manual	4.1±0.3	4.3±0.3	NTG sublingual	0.4	↔	↓
McCrohon et al [10], 2000	brachial	manual	3.1±0.3	3.6±0.4*	NTG sublingual	0.4	↓	↓
Woo et al A [43], 1997	brachial	manual	3.5±0.5	3.6±0.6	NTG sublingual	0.4	↔	↔
Woo et al B [43], 1997	brachial	manual	3.4±0.6	3.7±0.5	NTG sublingual	0.4	↔	↓

Data are dose or mean ± SD. One study presented two groups of older subjects, each of which had been independently compared with a location-matched young group [43], thus they were evaluated as individual studies (distinguished by A and B). * significantly different from young group at $P<0.05$

EF, endothelial function; N/A, data not available; NMD, nitrate-mediated dilation; NTG, nitroglycerin; OLD, older group; SMF, smooth muscle function; YNG, young group; ↔, no significant difference between older and young groups; ↓, significant decrease in older compared with young groups

TABLE 3. Resistance artery smooth muscle function (SMF) assessment in studies included in the meta-analysis

			SMF		EF	
Study, year of publication	**Vascular region**	**Technique**	**Stimulus**	**OLD vs. YNG**	**Stimulus**	**OLD vs. YNG**
Millet et al [30], 2012	supramedial malleolar (skin)	laser Doppler	SNP iontophoresis	↔	local heating	↔
Donato et al [8], 2011	forearm	plethysmography	SNP infusion	↔	ACh infusion	↓
Westby et al [24], 2011	forearm	plethysmography	SNP infusion	↔	ACh infusion	↓
Kirby et al [16], 2010	forearm	plethysmography	SNP infusion	↔	ACh infusion	↓
Tew et al [19], 2010	forearm (skin)	laser Doppler	SNP iontophoresis	↓	ACh iontophoresis	↓
Donato et al [6], 2009	forearm	plethysmography	SNP infusion	↔	ACh infusion	↓
Nicholson et al [31], 2009	forearm	plethysmography	SNP infusion	↔	N/A	N/A
Kirby et al [17], 2009	forearm	plethysmography	SNP infusion	↓	ACh infusion	↓
Donato et al [32], 2008	forearm	plethysmography	SNP infusion	↔	ACh infusion	↓
Galetta et al [9], 2006	forearm	plethysmography	SNP infusion	↔	ACh infusion	↓
Al-Shaer et al [21], 2006	forearm	plethysmography	SNP infusion	↓	ACh infusion	↓
Newcomer et al [33], 2005	leg[a]	ultrasound	SNP infusion	↔	ACh infusion	↓
Weverling-Rijnsburger et al [34], 2004	forearm	plethysmography	SNP infusion	↔	ACh infusion	↔
Ahlers et al [14], 2004	forearm	plethysmography	SNP infusion	↔	ACh infusion	↓
Smith et al [35], 2003	forearm	plethysmography	SNP infusion	↔	Bk infusion	↔
Minson et al [18], 2002	forearm (skin)	laser Doppler	SNP microdialysis	↓	local heating	↓
DeSouza et al [15], 2002	forearm	plethysmography	SNP infusion	↔	ACh infusion	↓

Wang et al [20], 2002	lower leg (skin)	laser Doppler	SNP iontophoresis	↔	ACh iontophoresis	↓
Taddei et al [23], 2000	forearm	plethysmography	SNP infusion	↓	ACh infusion	↓
Gerhard et al [22], 1996	forearm	plethysmography	SNP infusion	↔	ACh infusion	↓

[a] Leg and forearm resistance artery SMF were reported. Leg resistance artery SMF was used in meta-analysis because of lower (as compared with forearm) coefficient of variation

ACh, acetylcholine; Bk, bradykinin; EF, endothelial function; N/A, data not available; OLD, older group; SMF, smooth muscle function; SNP, sodium nitroprusside; YNG, young group; ↔, no significant difference between older and young groups; ↓, significant decrease in older compared with young groups