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META-ANALYSIS

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Utility of Contrast-Enhanced Ultrasound for the Assessment of Skeletal Muscle Perfusion in Diabetes Mellitus: A Meta-Analysis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: This study evaluated the effectiveness of contrast-enhanced ultrasonography for the assessment of skeletal muscle perfusion in diabetes mellites.

Material/Methods: Electronic databases (Embase, Google Scholar, Ovid, and PubMed) were searched for required articles, and studies were selected by following pre-determined eligibility criteria. Meta-analyses of mean differences or standardized mean differences (SMD) were performed to evaluate the significance of difference in contrast-enhanced ultrasonography measured muscle perfusion indices between patients with diabetes and healthy individuals or between basal and final values of perfusion indices after insulin manipulation or physical exercise in patients with diabetes or healthy individuals.

Results: There were 15 studies included, with 279 patients with diabetes and 230 healthy individuals in total. The age of the study patients with diabetes mellitus was 55.8 years (95% CI: 49.6 years, 61.9 years) and these patients had disease for 11.4 years (95% CI: 7.7 years, 15.1 years). The percentage of males in group of patients with diabetes was 66% (95% CI: 49%, 84%), body mass index was 29.4 kg/m^2 (95% CI: 26.5 kg/m^2, 32.3 kg/m^2), hemoglobin A1c was 7.3% (95% CI: 6.7%, 7.9%), and fasting plasma glucose was 149 kg/m^2 (95% CI: 118 kg/m^2, 179 kg/m^2). Time to peak intensity after provocation was significantly higher in patients with diabetes than in healthy individuals (SMD 1.18 [95% CI: 0.60, 1.76]; $P<0.00001$). In patients with diabetes, insulin administration did not improve contrast-enhanced ultrasonography measured muscle perfusion indices but exercise improved muscle perfusion but at a level that was statistically non-significant (SMD between basal and post-exercise values (1.03 [95% CI: –0.14, 2.20]; $P=0.08$). In healthy individuals, lipids in addition to insulin administration was associated with significantly reduced blood volume and blood flow.

Conclusions: Our review showed that the use of contrast-enhanced ultrasonography showed that diabetes mellitus was associated with altered muscle perfusion in which insulin-mediated metabolic changes played an important role.

MeSH Keywords: **Microscopy, Acoustic • Ultrasonography • Ultrasonography, Doppler**

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Background

Contrast-enhanced ultrasound (CEU) is a noninvasive technique for quantitative imaging used for several purposes, including vascular perfusion. In this technique, a contrast agent containing inert gas-filled microbubbles (equivalent to red blood cells in size) is injected/infused into the blood circulation. Upon exposure to high-energy ultrasound in the region of interest (ROI), these microbubbles are destroyed. Microbubble replenishment starts from neighboring part of blood vessel and gradually the microbubble intensity is restored in the ROI. Kinetics of microbubbles in ROI are used to estimate perfusion indices, e.g., the concentration of microbubbles when fully replenished is proportional to the microvascular blood volume (MBV), and the rate at which the microbubbles replenish determines the microvascular flow velocity (MFV). MBV represents the total amount of capillaries participating in the microcirculation at a given moment whereas the blood flow is the product of blood volume and flow velocity [1–6].

CEU is used for the measurement of perfusion in skeletal muscles [7–9], cerebral perfusion [10], cranial surgery (11], renal blood flow [12,13], and vascular integrity of free-flap grafts [14,15]. CEU is also a promising method for noninvasive diagnosis of carotid atherosclerotic plaque neovascularization and for the prediction of cerebrovascular and cardiovascular events [16]. CEU may be the preferred method for the assessment of defective skeletal muscle blood flow responses to exercise and to investigate and quantify responses to therapy [17].

Diabetes mellitus is a chronic endocrine and metabolic disorder with increasing global prevalence. Complexity of its pathophysiological mechanisms and adverse effects on metabolic and vascular processes leading to neuropathies and cardiovascular complications forms the basis of high morbidity and mortality [18]. The global prevalence of diabetes in adults has increased from 4.7% in 1980 to 8.5% in 2014. In 2015, diabetes caused an estimated 1.6 million deaths [19]. Type 2 diabetes mellitus is associated with a high risk of macrovascular and microvascular complications for which insulin resistance and endothelial dysfunction play a major role. Insulin also plays important roles in the regulation of vascular tone and tissue perfusion by vasodilating the pre-capillary arterioles in muscle and hence increasing the microvascular perfusion and capillary exchange surface area in muscle [20]. Muscle perfusion changes according to the metabolic demand of skeletal muscle as changes in oxygen consumption and metabolite supply are large and quick. For this to be accurately detected, measuring the low skeletal muscle perfusion at rest and particularly capillary blood flow in a defined muscle volume is essential [4].

Several studies have used CEU for the assessment of the muscle perfusion in patients with diabetes as well as in healthy individuals to evaluate the effect of insulin modulation in muscle perfusion at rest or during exercise. However, there has been no synthesis of such studies so far. The present study was designed to conduct a literature survey of relevant studies and to perform a meta-analysis of CEU measured muscle perfusion indices in patients with diabetes and healthy individuals in order to evaluate the effectiveness of this diagnostic tool in measuring diabetic muscle perfusion.

Material and Methods

The present study was carried out by following Cochrane Collaboration guidelines and is reported in accordance with PRISMA statement.

Inclusion and exclusion criteria

Inclusion criteria were as follows: a study a) used CEU for skeletal muscle perfusion evaluations in diabetes mellitus patients and matched the outcomes in non-diabetic controls; b) evaluated the effect of insulin administration on muscle perfusion in diabetic patients; c) evaluated the effect of muscle exercise on muscle perfusion in diabetic patients; and d) evaluated the effect of insulin administration with or without lipid administration in healthy individuals. Exclusion criteria were as follows: a study a) used CEU in diabetic patients but did not use a control group for comparison; b) reported associational outcomes rather than values or changes of indices; c) used ultrasonography without contrast agents; and d) used CEU for related conditions such as peripheral artery disease or systemic sclerosis in non-diabetic patients.

Literature search

A comprehensive literature search was carried out in electronic databases (Embase, Google Scholar, Ovid, and PubMed) for the identification and acquisition of relevant research articles. Important keywords and MeSH terms used in logical combinations were: contrast enhanced ultrasound/sonography, CEU/CEUS, diabetes mellitus, insulin, resistance, exercise, glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), time to peak intensity (TTP), maximum time, occlusion, provocation, contrast agent, microbubbles, MBV, blood flow, flow velocity, diagnosis, diagnostic accuracy, calf muscles, forearm flexor, microvasculature and muscle reperfusion. The search encompassed research articles published before September 2018 in the English language. Additionally, research articles were manually searched from the references list of relevant original studies and review articles.

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Data and analyses

Data regarding the demographic, anthropometric, clinical, pathological characteristics of the patients and methodological, analytical, and outcome data of the included studies along with other relevant information were obtained from research articles retrieved from databases and were organized in datasheets. Quality of the included studies was assessed using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies of the United States National Institutes of Health [21].

Meta-analysis endpoints were: a) difference in "time to peak intensity" after a provocation between patients with diabetes and healthy individuals; b) difference in blood flow indices between patients with diabetes and healthy individuals; c) change in blood flow indices in patients with diabetes after insulin administration or muscle exercise; and d) effect of lipid addition to insulin administration on muscle perfusion indices in healthy individuals.

Meta-analyses of mean differences (MD) or standardized mean differences (SMD) were performed with RevMan software (version 5.3; Cochrane Collaboration) under random effects model. Overall effect size of each meta-analysis was an inverse variance weighted average of the individual studies outcome. I^2 index was used to estimate statistical heterogeneity between the studies. Data are presented as weighted average with 95% confidence interval (CI).

Results

Fifteen studies [22–36] were included in the meta-analysis (Figure 1). These observational studies recruited a total of 279 patients with diabetes and 230 healthy individuals to assess skeletal muscle perfusion. ROI in these studies was either in the deep forearm flexor muscle or in the calf muscle. Contrast agents consisted of either lipid microbubbles containing sulphur hexafluoride gas (Sonovue brand) or microbubbles containing lipid-shelled octafluoropropane (Definity brand). In these studies, the contrast agents were either given as bolus injection or infused at a rate of 1 mL to 1.5 mL per minute.

Characteristics of the included studies are given in Table 1. Age of diabetes mellitus patients was 55.8 years (95% CI: 49.6 years, 61.9 years) and these patients had disease for 11.4 years (95% CI: 7.7 years, 15.1 years). Percentage of males in patients with diabetes was 66% (95% CI: 49%, 84%), body mass index (BMI) was 29.4 kg/m^2 (95% CI: 26.5 kg/m^2, 32.3 kg/m^2), HbA1c was 7.3% (95% CI: 6.7%, 7.9%) and FPG was 149 mg/dL (95% CI: 118 mg/dL, 179 mg/dL). Age of healthy individuals was 38.9 years (95% CI: 34.8 years, 43.0 years); and BMI was 24.9 kg/m^2 (95% CI: 22.4 kg/m^2, 27.5 kg/m^2). The percentage of

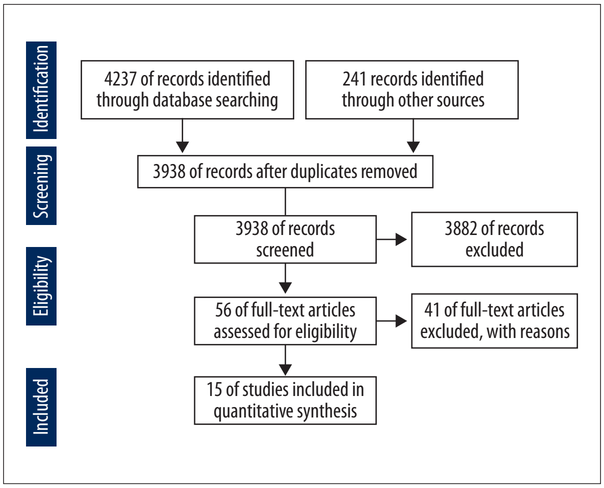

Figure 1. A flowchart of study screening and selection process.

males in the healthy individuals was 48% (95% CI: 40%, 56%). In general, the quality of the included studies was moderate. Outcomes of the quality assessment of the included studies are presented in Table 2.

TTP after provocation was significantly higher in patients with diabetes than in healthy individuals (SMD 1.18 [0.60, 1.76]; $P<0.00001$). The blood flow was also significantly less in patients with diabetes in comparison to healthy individuals (–0.49 [–0.94, –0.05]; $P=0.03$) (Figure 2).

In patients with diabetes, insulin administration did not significantly improve muscle perfusion (SMD between post-insulin and basal values was –0.16 (95% CI: –0.86, 0.54; $P=0.65$) (Figure 3). In obese non-diabetic individuals too, the insulin administration significantly improved muscle perfusion (SMD between post-insulin and basal values –1.56 (95% CI: –2.49, –0.64; $P=0.0009$). On the other hand, exercise improved muscle perfusion in patients with diabetes, but statistically non-significantly (SMD between post-exercise and basal values 1.03 [95% CI: –0.14, 2.20]; $P=0.08$).

In healthy individuals, insulin administration significantly increased skeletal muscle blood volume (MD final minus baseline values 0.76 [95% CI: 0.37, 1.15]; $P=0.0001$), and blood flow (MD 0.82 [95% CI: 0.28, 1.35]; $P=0.003$) but not blood flow velocity (MD –1.32 [95% CI: –3.42, 0.79]; $P=0.22$) after 120 minutes of infusion. In these healthy individuals, lipids in addition to insulin administration was associated with significantly reduced blood volume and blood flow (Figure 4). The SMD of the mean change between insulin-lipid and insulin-only administration was –0.54 (95% CI: –0.92, –0.16, $P=0.006$) for blood volume and –0.62 (95% CI: –1.17, –0.06, $P=0.03$) for blood flow. However, SMD of the mean change in blood flow velocity between insulin-lipid and insulin-only administration was not significantly different (1.07 [95% CI: –0.54, 2.67]; $P=0.19$).

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Table 1. Important characteristics of the included studies.

Study	n DM	n Hty	DD (years)	Age (years)	% Males	BMI (kg/m²)	Hb (%)	FPG	CEU brand	Contrast agent	Patient State for CEU	Infusion (mL/min)/ volume (mL)	ROI muscle	Vein
Chai 2011	0	11		21.7±0.4	72.7	23.2±0.7			SONOS 7500	Definity*			Forearm flexor	Antecubital
Chan 2009	9	0		42.6±8.3	89	23.1±10	7.9±1		SONOS 7500	Definity	Rest	1.5	Forearm flexor	
Chan 2011	17	0	20±12	40±8		26.3±3.3	8±2.3		SONOS 7500	Definity	Rest	1.5	Forearm flexor	
Clerk 2006	0	21		37±3					Phillips HDI 5000	Definity	Sitting		Forearm flexor	
Duerschmied 2008	10	10		69.9±12	60				LOGIQ 9 ultrasound	SonoVue	Rest	4.8 bolus	Calf (gastrocnemius)	Antecubital
Duerschmied 2009	52	58		68.7±11	71.2				LOGIQ 9 Ultrasound system	SonoVue	Rest	4.8 bolus	Calf	Antecubital
Emanuel 2018	12	0		55±6	66.7	33.1±2	6.5±0.5	144±36	Siemens-Acuson Sequoia 512	SonoVue	Supine	1.5/10	Vastus lateralis	
Irace 2017	25	12	17±9	38±13	76	24.6±3.1	7.6±0.7	148±64	Philips HD 11 XE	SonoVue	Rest	1.2/5	Forearm flexor	Antecubital
Keske 2009	0	8		41±3		33.7±1			Phillips HDI 5000	Definity	Rest	3/	Forearm flexor	Antecubital
Liu J 2011	0	22		22.5±1	45.5	22.5±0.5			SONOS 7505	Definity	Left Decubitus	1.5/30	Forearm flexor	Antecubital
Liu Z 2009	0	12		23.3±1.4	33.3	21.8±0.7			SONOS 7506	Definity	Supine	1/30	Forearm flexor	Antecubital
Lindner 2008	19	26		52±11	57.9	27.2±6	8.2±4.6		Phillips HDI-5000cv	Definity		0.2–0.27	Plantar flexor	
Russell 2017	17	0	7±1	52±2	64.7	31.2±1.1	7.7±0.3	180±14	5000cv	Definity	Rest/exercise		Forearm flexor	
Song 2014	58	30	6.2±6.8	70±9	58.6				My-lab90 scanner (Esaote)	SonoVue	Rest	2.4 bolus	Calf	Left |forearm vein
Womack 2009	22	20	2.5±4	53	14	34±6	6.9±2.2	117±60	Phillips HDI-5000cv	Definity	Rest/exercise	0.12–0.16	Forearm flexor (pollicus longus)	Antecubital

BMI – body mass index; CEU – contrast enhanced ultrasound; DD – disease duration; DM – diabetes mellitus; FPG – fasting plasma glucose; Hb – glycosylated hemoglobin; Hty – healthy; ROI – region of interest. * Definity (lipid perflutren microspheres containing octafluoropropane gas); Sonovue (lipid bubbles containing sulphur hexafluoride gas).

Discussion

CEU studies in patients with diabetes and healthy individuals have shown that muscle perfusion in diabetes is altered, in which insulin resistance plays an important role. Moreover, exercise may improve muscle perfusion in people with diabetes. CEU studies in healthy individuals have shown that insulin administration enhances muscle perfusion, but the addition of lipids to insulin administration attenuates this improvement, which supports the understanding that fats play a role in insulin resistance.

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Table 2. Quality assessment of the included studies.

Criteria	Reference number of the study														
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
Was the research question or objective in this paper clearly stated and appropriate?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Was the study population clearly specified and defined?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Did the authors include a sample size justification?	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)?	NA	N	NA	NA	Y	Y	N	N	Y	NA	NA	Y	NA	Y	Y
Were the definitions, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Were the cases clearly defined and differentiated from controls?	NA	N	NA	NA	Y	Y	N		Y	NA	NA	Y	NA	Y	Y
If less than 100 percent of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible?	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Was there use of concurrent controls?	NA	N	NA	NA	Y	Y	N	Y	Y	NA	NA	Y	NA	Y	Y
Were the investigators able to confirm that the exposure/risk occurred prior to the development of the condition or event that defined a participant as a case?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Were the measures of exposure/risk clearly defined, valid, reliable, and implemented consistently (including the same time period) across all study participants?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Were the assessors of exposure/risk blinded to the case or control status of participants?	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N – no; NA – not applicable; Y – yes.

Muscle microvasculature forms an interface between the circulatory system and muscle interstitium for the exchange of nutrients and byproducts. Several factors including exercise, mixed meal, and pharmacological agents act to recruit muscle microvasculature, which may increase insulin delivery to cause. Thus, insulin resistance leads to altered muscle microvasculature recruitment. Inflammatory cytokines, free fatty acids, and angiotensin II type 1 receptor activation affect microvascular recruitment by affecting insulin resistance [20].

Amarteifio et al. [37] found a moderate inverse correlation between glycated hemoglobin, HbA1c, and the maximum CEU signal following the transient arterial occlusion (r=–0.53) in individuals with type 2 diabetes which indicated that elevated HbA1c might be directly associated with a decrease in local muscle micro-perfusion. Using ultrasound B-mode, color Doppler, and pulse wave Doppler imaging for foot arteries in 73 individuals with diabetes and non-diabetic individuals, Leoniuk et al. [38] found a positive correlation between HbA1c and flow resistance index in patients with type 2 diabetes. In the present study, a trend was seen between the%HbA1c and SMD of CEU measures of muscle perfusion between patients with diabetes and non-diabetic individuals (meta-regression coefficient 2.92 (CI 95%: –0.40, 6.23; *P*=0.073), although there was limited available data to study such a relationship collectively.

Other CEU studies have also reported important correlational data in this regard. In their regression analyses, Russel et al. [34] found that changes in muscle MBV response after resistance training significantly correlated with reductions in FPG and HbA1c after adjusting for age, sex,% body fat, and% lean mass. In the study of Lindner et al. [31], the combined angiographic severity score correlated with exercise blood flow (r=0.70, *P*=0.003) and flow reserve (r=0.56, *P*=0.047) in non-diabetic individuals with peripheral artery disease but not in diabetic peripheral artery disease patients. Chan et al. [24] found that diabetic microvascular complications were significantly correlating with the effect of supraphysiological insulin levels on capillary recruitment or de-recruitment in patients with type 1 diabetes. Thus, supraphysiological insulin levels increased

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Study or subgroup	Diabetes Mean	Diabetes SD	Diabetes Total	Healthy Mean	Healthy SD	Healthy Total	Weight	Std. mean difference IV, random, 95% CI
3.2.1 Time to peak intensity after provocation								
Duerschmid 2008	25.5	6	10	16	4	10	14.2%	1.78 [0.71, 2.86]
Duerschmid 2009	36.9	12	52	18.9	7	58	23.5%	1.84 [1.40, 2.29]
Irace 2017 LV	89.1	28.7	25	81.2	25.6	12	19.7%	0.28 [−0.41, 0.97]
Irace 2017 SV	5.6	2.2	25	4	1.7	12	19.4%	0.76 [0.05, 1.47]
Song 2014	81	37	58	38	27	30	23.1%	1.26 [0.78, 1.74]
Subtotal (95% CI)			**170**			**122**	**100.0%**	**1.18 [0.60, 1.76]**
Heterogeneity: Tau²=0.32, Chi²=17.03, df=4 (P=0.002); I²=77%								
Test for overall effect: Z=3.98 (P<0.0001)								
3.2.2 Blood flow								
Irace 2017 BF	133	62	25	121	91	12	15.9%	0.16 [−0.53, 0.85]
Irace 2017 TAPV	35	14	25	34	17	12	15.9%	0.07 [−0.62, 0.75]
Lindner 2008 BF	7.9	5.9	19	20	9.5	26	16.3%	−1.45[−2.12, −0.78]
Lindner 2008 FR	7.4	5	19	13.1	11.2	26	17.4%	−0.61 [−1.22, −0.01]
Wommack 2009 BF Exer	312	113	22	390	157	20	17.2%	−0.56 [−1.18, 0.05]
Wommack 2009 BF Rest	150	84.4	22	190	67.1	20	17.2%	−0.51 [−1.13, 0.10]
Subtotal (95% CI)			**132**			**116**	**100.0%**	**−0.49 [−0.94, −0.05]**
Heterogeneity: Tau²=0.20, Chi²=14.04, df=5 (P=0.002); I²=64%								
Test for overall effect: Z=2.18 (P=0.03)								

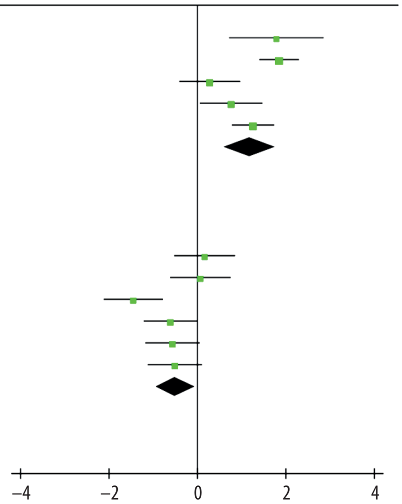

Figure 2. A forest graph showing the outcomes of a meta-analysis of standardized mean difference between patients with diabetes and healthy individuals in CEU measured muscle perfusion indices. In study identities abbreviations are as follows. BF – blood flow; FR – flow reserve; Exer – exercise; LV – large vessel; SV – small vessel; TAPV – time average peak velocity.

Study or subgroup	Post Mean	Post SD	Post Total	Basal Mean	Basal SD	Basal Total	Weight	Std. mean difference IV, random, 95% CI
3.3.1 Effect if insulin administration								
Chan 2009 MBF HSI	0.54	0.15	9	0.59	0.13	9	16.5%	−0.34 [−1.27, 0.59]
Chan 2009 MBF LSI	0.37	0.4	9	0.33	0.5	9	16.5%	0.08 [−0.84, 1.01]
Chan 2009 MBF HSI	3.65	0.5	9	3.15	0.4	9	15.7%	1.05 [0.05, 2.05]
Chan 2009 MBF LSI	2.55	0.3	9	3.45	0.4	9	13.0%	−2.42 [−3.71, −1.14]
Chan 2011 MBV	3.17	1.03	17	3.29	0.94	17	19.1%	−0.12 [−0.79, 0.55]
Emanuel 2018 MFV	0.07	0.09	17	0.03	0.18	17	19.1%	0.27 [−0.40, 0.95]
Subtotal (95% CI)			**70**			**70**	**100.0%**	**−0.16 [−0.86, 0.54]**
Heterogeneity: Tau²=0.55, Chi²=19.09, df=5 (P=0.002); I²=74%								
Test for overall effect: Z=0.45 (P=0.65)								
3.3.2 Effect of exercise								
Russell 2017 MBF	1.28	3.5	17	0.18	0.12	17	50.1%	0.43 [−0.25, 1.11]
Wommack BF	312	113	22	147	84.4	22	49.9%	1.62 [0.93, 2.31]
Subtotal (95% CI)			**39**			**39**	**100.0%**	**1.03 [−0.14, 2.20]**
Heterogeneity: Tau²=0.59, Chi²=5.80, df=1 (P=0.02); I²=83%								
Test for overall effect: Z=1.73 (P=0.08)								

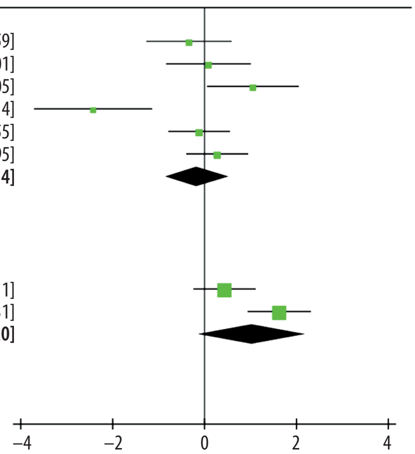

Figure 3. A forest graph showing the outcomes of a meta-analysis of standardized mean difference between basal and post intervention values of muscle perfusion indices in patients with diabetes. In study identities abbreviations are as follows. BABF – brachial artery blood flow; MBF – microvascular blood flow; MBV – microvascular blood volume; MFV – microvascular flow velocity.

MBV in individuals with low insulin sensitivity and increased microvascular complications but decreased MBV in individuals with high insulin sensitivity and increased microvascular complications [24].

However, Emanuel et al. [29] found no correlation of peripheral insulin sensitivity with skeletal muscle MBV at baseline, or with MBV during hyperinsulinemia or with the percent change in MBV. Peripheral insulin sensitivity also did not significantly correlate with MBV during hyperinsulinemia and combined vasodilator (iloprost) infusion, or with the MBV percentage change

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Study or subgroup	Lipid-insulin change Mean	SD	Total	Saline-insulin change Mean	SD	Total	Weight	Std. mean difference IV, random, 95% CI
3.1.1 Blood volume								
Chai 2011	−0.01	2.33	10	1.4	3.7	11	19.5%	−0.43 [−1.30, 0.44]
Liu J 2011	−0.2	1.68	22	0.9	1.34	22	39.4%	−0.71 [−1.32, −0.10]
Liu Z 2009	−0.1	1.29	22	0.35	0.67	22	41.1%	−0.43 [−1.03, 0.17]
Subtotal (95% CI)			**54**			**55**	**100.0%**	**−0.54 [−0.92, −0.16]**
Heterogeneity: Tau^2=0.00, Chi^2=0.49, df=2 (P=0.78); I^2=0%								
Test for overall effect: Z=2.77 (P=0.0006)								
3.1.2 Blood flow								
Chai 2011	0.05	1.32	11	0.55	1.39	11	26.4%	−0.35 [−1.20, 0.49]
Liu J 2011	−0.05	0.34	22	0.4	0.43	22	35.5%	−1.14 [−1.78, −0.50]
Liu Z 2009	−0.05	1.17	22	0.25	0.65	22	38.1%	−0.31 [−0.91, 0.28]
Subtotal (95% CI)			**55**			**55**	**100.0%**	**−0.62 [−1.17, −0.06]**
Heterogeneity: Tau^2=0.12, Chi^2=3.94, df=2 (P=0.14); I^2=49%								
Test for overall effect: Z=2.18 (P=0.03)								
3.1.3 Blood flow velocity								
Chai 2011	0.01	0.067	11	0	0.071	11	32.9%	−0.35 [−1.20, 0.49]
Liu J 2011	0.01	0.07	22	−0.01	0.073	22	34.4%	−1.14 [−1.78, −0.50]
Liu Z 2009	0.05	0.053	22	−0.1	0.051	22	32.7%	−0.31 [−0.91, 0.28]
Subtotal (95% CI)			**55**			**55**	**100.0%**	**−0.62 [−1.17, −0.06]**
Heterogeneity: Tau^2=1.85, Chi^2=26.94, df=2 (P<0.00001); I^2=93%								
Test for overall effect: Z=1.30 (P=0.19)								

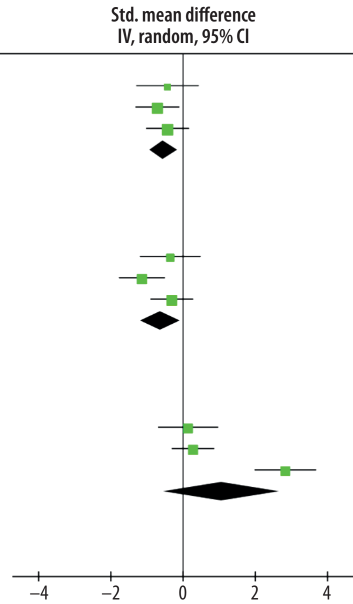

Figure 4. A forest graph showing the outcomes of a meta-analysis of standardized mean difference in mean changes in muscle perfusion indices between insulin and insulin-lipid infusion in healthy individuals. In insulin-lipid group, insulin administration was followed by lipids administration to observe changes in muscle perfusion whereas in insulin-saline group changes were observed after insulin administration only.

induced by combined insulin and iloprost infusion in patients with type 2 diabetes.

Ultrasound studies have found that the intima-media thickness (IMT) correlates with age (r=0.6; *P*<0.05) as well as with the duration of diabetes (r=0.35; *P*<0.05) [39]. IMT values were significantly higher in children with insulin-dependent diabetes than in healthy children [40]. In children with diabetes, IMT values increased with age and were higher in boys in both diabetic and non-diabetic children. However, in children with diabetes, IMT did not correlate with disease duration [40].

In one study, a negative correlation was observed between the change in MBV and BMI (r=10.482, *P*=0.027) in response to insulin administration in obese individuals [26]. Age and ankle-brachial index are not found to correlate significantly with the TTP [28]. In the present study we also found that there was no significant relationship between age and SMD in CEU measured muscle perfusion indices between diabetic and non-diabetic individuals (meta-regression coefficient 0.053 (95% CI: –0.109, 0.215); *P*=0.487). In muscle, glucose metabolism is associated strongly with interstitial insulin levels in comparison with plasma insulin levels, and transendothelial transport acts as rate limiting factor for the action of insulin on muscle glucose metabolism. It has been shown that in patients with diabetes, exercise can improve impaired vasodilation in response to insulin infusion [41] and exercise increases arterial blood flow after glucose ingestion in patients with well-controlled type 2 diabetes [42]. In healthy individuals too, exercise has been found to be associated with significantly increased MBV acutely after exercise [43], whereas hemodynamic effects of insulin were blunted in patients with type 2 diabetes [44,45]. Using CEU in healthy individuals, studies have shown that insulin increases glucose uptake to increase capillary recruitment and consequently blood flow [7,46–48].

CEU is one of the useful methods to assess defective skeletal muscle blood flow responses to exercise and to quantify responses of a prescribed therapy. Weber et al. [3] found CEU feasible for skeletal muscle perfusion quantification when they used CEU for measuring skeletal muscle perfusion and compared it with microvascular density in muscle biopsies. They found that CEU measured local blood volume significantly related to fiber-adjacent capillarization reflective of physiologic capillary recruitment. Based on comparability of the coefficient of variation of MBV with previous findings [49,50], Mertz et al. 2011 [42], found CEU was as effective as contemporary methods for MBV assessment. However, technical issues need careful consideration to achieve reliable outcomes, e.g., selection of the ROI is a tedious and time-consuming process which needs the presence of a trained analyst. Availability of more systematic ROI selection methods can further improve the reliability of CEU-based outcomes [25].

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The present study had several limitations. First, there was limited data available regarding CEU muscle perfusion for patients with diabetes, especially with regards to different indices such as TTP, MBV, MBF, and MFV, and conditions such as non-insulin dependent diabetes mellitus and type 1 diabetes mellitus. This necessitated us to use SMD-based meta-analyses, and therefore we could not estimate the quantum of difference. This may have also contributed to higher statistical heterogeneity. Because all of the included studies were observational in design, the qualitatively generated data may only be ranked at a moderate level. Some relevant studies could not be included because these studies lacked a control and still other studies reported associational outcomes rather than indices or their changes.

Conclusions

Contrast-enhanced perfusing imaging of muscle perfusion indices such as TTP, MBV, MBF, and MFV have indicated that diabetes mellitus is associated with altered muscle perfusion in which insulin mediated metabolic changes play an important role as insulin administration to healthy but not diabetic individuals was associated with increased muscle perfusion. Moreover, the administration of lipids with insulin attenuated muscle perfusion in healthy individuals. Nevertheless, limited data were available for individual muscle perfusion indices in individuals with diabetes, which necessitates further studies for refinement of these outcomes.

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