Correlation of Flicker-Induced and Flow-Mediated Vasodilatation in Patients With Endothelial Dysfunction and Healthy Volunteers

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OBJECTIVE — Flicker-induced vasodilatation is reduced in patients with vascular-related diseases, which has at least partially been attributed to endothelial dysfunction of retinal vessels. Currently, the standard method to assess endothelial function in vivo is flow-mediated vasodilatation (FMD). Thus, the present study was performed to investigate whether a correlation exists between flicker-induced vasodilatation and FMD in patients with known endothelial dysfunction and healthy subjects.

RESEARCH DESIGN AND METHODS — In the present study, 20 patients with type 1 diabetes, 40 patients with systemic hypertension (systolic blood pressure 140–159 mmHg; diastolic blood pressure 90–99 mmHg) and/or serum cholesterol levels ≥0.65 mmol/l, and 20 healthy control subjects were included. The flicker response was measured using the Dynamic Retinal Vessel Analyzer. FMD was determined using a high-resolution ultrasound system, measuring brachial artery diameter reactivity during reperfusion after arterial occlusion.

RESULTS — The flicker response of both retinal arteries and veins was significantly reduced in the two patients groups. Likewise, FMD was significantly reduced in patients compared with healthy control subjects. However, only a weak correlation between flicker-induced vasodilatation and FMD was observed.

CONCLUSIONS — The study confirms that flicker responses and FMD are reduced in the selected patient groups. Whether the weak correlation between FMD and flicker is due to the different stimulation type, the different vascular beds measured, or other mechanisms has yet to be investigated.

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Stimulating the eye with diffuse flickering light is accompanied by an increase in retinal vessel diameters (1,2), retinal blood flow (3), and optic nerve head blood flow (4). Although this phenomenon has not yet been clarified in all details, there is general agreement that the increase of blood flow is caused by augmented neural activity in ganglion cells and the connected tissues. This, again, underlines the tight coupling between blood flow, neuronal function, and metabolism in the eye.

There is evidence that this adaptive regulation process is impaired under several pathological conditions. In particular, a couple of studies have shown that patients with diabetes (5,6), arterial hypertension (7), or glaucoma (8) show a reduced blood flow response to flicker-light stimulation. Given that all these diseases go hand in hand with endothelial dysfunction, it has been hypothesized that the reduced flicker response may be related to impaired endothelial vasodilatation of retinal vessels. This is supported by data showing that inhibition of nitric oxide (NO) synthase significantly reduces flicker-induced vasodilatation (9). Whether this impaired vascular function is just a local phenomenon or correlates with impaired endothelial function in other vascular beds, however, remains unclear.

Currently, measurement of flow-mediated vasodilatation (FMD) in the forearm is the most widely used technique for the assessment of endothelial function in vivo. First described in 1992, this method is based on reactive hyperemia after discontinuation of blood flow by inflating a cuff to suprasystolic values (10). The vasodilatation of the brachial artery is caused by shear stress-induced NO production after cuff deflation. It is known to be endothelium dependent and gives a reliable measure of endothelial function of peripheral arteries (10).

The current study tested the hypothesis that reduced flicker-induced retinal vasodilatation correlates with endothelial dysfunction in the brachial artery, as tested by means of FMD. FMD and flicker-induced vasodilatation were measured in healthy volunteers and in different groups of patients with known endothelial dysfunction, namely patients with type 1 diabetes and patients with mild systemic hypertension and/or hypercholesterolemia.

RESEARCH DESIGN AND METHODS

— The study protocol was approved by the ethics committee of the Medical University of Vienna and followed the guidelines set forth in the Declaration of Helsinki. All patients signed written informed consent prior to inclusion and passed a screening examination including physical and ophthalmological examination during the 10 days before the study day. A total of 80 individuals aged >18 years were included in this

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observer-blinded, controlled, parallel-group study.

Three groups were composed: group 1 included 20 patients with type 1 diabetes with no signs of diabetic retinopathy or mild nonproliferative diabetic retinopathy. The eyes were classified according to the Modified Arlie House Classification (11). A further inclusion criterion for type 1 diabetic patients was a serum cholesterol level <0.65 mmol/l.

Group 2 included 40 patients with mild essential systemic hypertension at rest and/or with serum cholesterol levels ≥0.65 mmol/l. Hypertension was defined as a blood pressure meeting the criterion of hypertension grade 1 of the World Health Organization blood pressure classification, with systolic blood pressure (SBP) from 140 to 159 mmHg and diastolic blood pressure (DBP) from 90 to 99 mmHg. Blood pressure was measured at two different occasions in a sitting position.

As a control, 20 healthy subjects with SBP <140 mmHg, DBP <90 mmHg, serum cholesterol levels <0.55 mmol/l, and normal ocular findings were included in group 3. Care was taken that the control group was comparable in age and sex distribution to the patient groups. Further exclusion criteria for all subjects were ametropia >3 dpt, other relevant ocular abnormalities, a clinically relevant illness prior to the study, pregnancy or lactation, and a patient or family history of epilepsy. Participants had to abstain from beverages containing alcohol or caffeine for 12 h before the study.

Retinal vessel analyzer

The diameters of one temporal retinal artery and vein between 1 and 2 disc diameters from the margin of the optic disc were continuously measured using the Dynamic Vessel Analyzer (DVA; IMEDOS, Jena, Germany). The DVA comprises a fundus camera (FF 450; Carl Zeiss Meditec, Jena, Germany), a digital video camera, and a personal computer with analyzing software for the determination of retinal vessel diameters that are analyzed from digitized images. The system provides excellent reproducibility and sensitivity (12). After selection of the measurement location, the DVA is able to follow the vessels during movements within the measurement window. Retinal vessel diameters were measured for 4 min. For the second minute, full-field flickering light with a frequency of 12.5 Hz was used for stimulation by square–wave pattern modulation of the fundus camera illumination at a contrast ratio of 25:1.

Table 1—*Baseline data of the three participating groups*

	Patients with type 1 diabetes	Patients with hypertension and/or hypercholesterolemia	Healthy subjects
Sex (*n*) (male/female)	7/13	17/23	7/13
Age (years)	37 ± 11	47 ± 11*	38 ± 12
MAP (mmHg)	82 ± 8	90 ± 11*	83 ± 8
Cholesterol (mmol/l)	0.50 ± 0.10	0.65 ± 0.10*	0.45 ± 0.05
A1C (%)	7.5 ± 1.3*	5.6 ± 0.4	5.2 ± 0.3
IOP (mmHg)	13 ± 2	14 ± 2	14 ± 3
Retinal artery diameter (μm)	130 ± 20*	118 ± 16	117 ± 14
Retinal vein diameter (μm)	156 ± 24	151 ± 21	153 ± 19

Data are means ± SD, unless otherwise indicated.*Significant differences ($P < 0.05$, ANOVA).

FMD

To measure FMD, each subject was in the supine position with the left arm supported on a foam block and a pneumatic cuff placed on the upper arm proximal to the measurement area. A high-resolution ultrasound system with a 7.0-MHz transducer (Vivid seven Pro; GE Vingmed Ultrasound, Horten, Norway) was used to measure the brachial artery diameter. The probe was fixed in an adjustable swivel arm to maintain an identical position during the experiments. The brachial artery was scanned in a longitudinal section proximal to its bifurcation, which was used as an anatomical marker. The end-diastolic diameter was measured. All measurements were performed by the same experienced operator. Baseline diameter of the brachial artery was assessed as the mean of 1 min of continuous measurement. Thereafter, the cuff on the upper arm was inflated to suprasystolic pressure (250 mmHg) for 4.5 min. FMD was then induced by sudden cuff deflation. The vessel diameter was measured for the following 2 min.

Experimental paradigm

All subjects were studied under dilated pupil after instillation with tropicamide (Mydriaticum "Agepha"-Gtt; Agepha, Vienna, Austria). After a 20-min resting period in a sitting position, baseline measurements of arterial blood pressure and pulse rate were performed. Thereafter, retinal vessel measurements including flicker stimulation were performed. Intraocular pressure (IOP) was measured after the flicker experiment. Finally, FMD was assessed as described above.

Measurement of IOP and systemic hemodynamics

IOP was measured with a slit-lamp–mounted Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland). Before each measurement, two drops of oxybuprocainhydrochloride combined with sodium fluorescein were instilled for local anesthesia. SBP, DBP, and mean arterial blood pressures (MAP) were measured on the upper arm by an automated oscillometric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA). Pulse rate was automatically recorded by the same unit from a finger-pulse oxymetric device.

Statistical analysis

Changes in retinal vessel diameters were expressed as percent change over baseline values. Baseline values were calculated as an average of the last 20 s before start of the flicker stimulation. Flicker response was calculated as an average of the last 20 s of the stimulation period. Flow-mediated dilatation of the brachial artery was expressed as percentage change of diameter measured 60 s after cuff deflation compared with baseline. An ANOVA model was used for significance testing of the retinal vessel response to flicker stimulation and to FMD over time within the groups as well as between the three groups. Pearson product-moment correlation coefficient was calculated to assess correlation between the variables. To adjust for multiple testing, a modified Bonferroni procedure was applied (13). For all calculations, a P value <0.05 was considered as the level of significance.

RESULTS — Baseline characteristics of all three groups included are given in Table 1. IOP was comparable in all three groups. In the diabetic group, A1C was

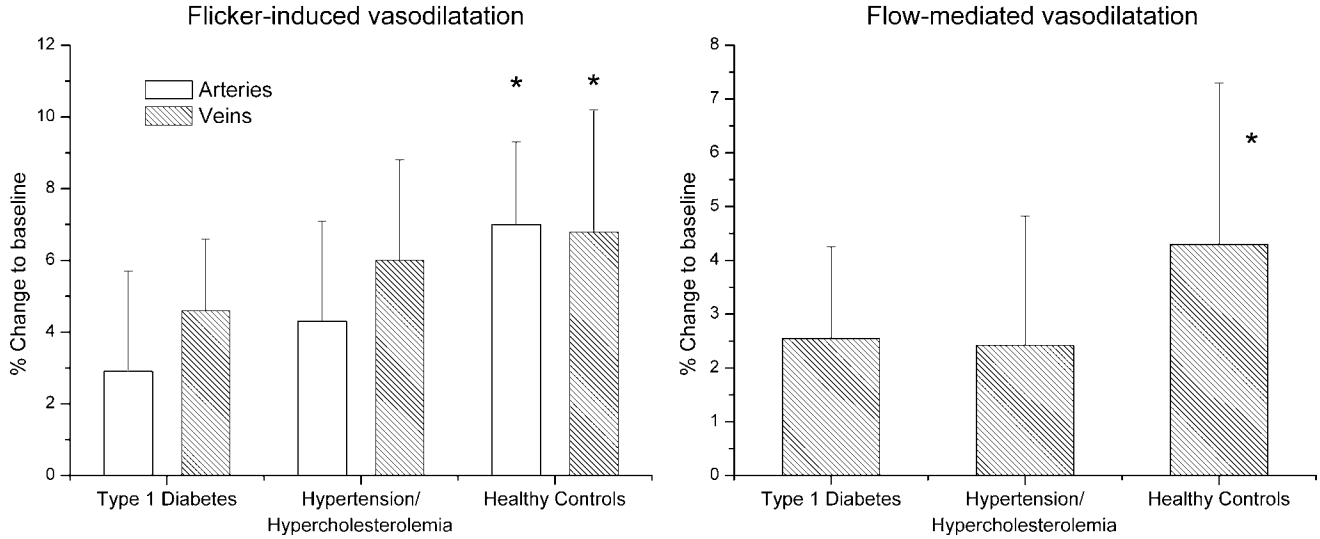

Figure 1—*Flicker-induced vasodilatation and FMD in the three different groups included. Group means ± SD. *Significant differences (P < 0.05, ANOVA).*

significantly increased compared with the other groups (ANOVA, $P < 0.001$) (Table 1). MAP and total cholesterol were increased in group 2 (ANOVA, $P < 0.003$). Blood glucose levels at the time of measurement were 8.4 ± 3.4 mmol/l in patients with diabetes. Average baseline vessel diameters were slightly increased in type 1 diabetes (ANOVA, $P = 0.018$). A detailed summary of concomitant medication can be found in online appendix Table A1 (available at http://care.diabetesjournals.org/cgi/content/full/dc08-2130/DC1). Subjects in the healthy control group were medication free. Flicker stimulation did not affect IOP or MAP in any of the groups.

Flicker-induced vasodilatation

In the healthy group, stimulation with flicker light induced a vasodilatation of 7.0 ± 2.3% in retinal arteries (ANOVA, time effect, $P < 0.001$) (Fig. 1) and a dilatation of 6.8 ± 3.4% ($P < 0.001$) in retinal veins. In patients with type 1 diabetes, retinal arterial diameters increased by 2.9 ± 2.8% ($P < 0.001$) and retinal veins by 4.6 ± 2.0% ($P < 0.001$). Patients with systemic hypertension and/or hypercholesterolemia showed a vasodilatation of 4.3 ± 2.8% ($P < 0.001$) in retinal arteries and a vasodilatation of 6.0 ± 2.4% ($P < 0.001$) in retinal veins. Thus, flicker-induced dilatation was reduced in patients with type 1 diabetes and patients with systemic hypertension and/or hypercholesterolemia compared with healthy control subjects. This effect was significant at a level of $P < 0.001$ for retinal arteries and at a level of $P = 0.045$ for retinal veins (ANOVA, effect between groups). Results between the two patients groups, however, were not significantly different.

FMD

FMD of the brachial artery was 4.3 ± 3.0% in the healthy group (ANOVA, time effect, $P < 0.001$) (Fig. 1). In both patients with type 1 diabetes and patients with systemic hypertension and/or hypercholesterolemia, FMD was significantly attenuated to 2.6 ± 1.7% in group 1 (ANOVA; time effect: $P < 0.001$; effect between groups: $P = 0.045$) and to 2.4 ± 2.4% in group 2 (time effect: $P < 0.001$; effect between groups: $P = 0.045$). Again, FMD of the brachial artery was not significantly different between the two patients groups.

Correlation analysis

A correlation between FMD and flicker-induced vasodilatation in retinal arteries ($r = 0.3$, $P = 0.044$) was found (Fig. 2). No correlation, however, was observed between FMD and flicker response in retinal veins (data not shown). Flicker-induced vasodilatation was negatively correlated with plasma cholesterol levels ($r = -0.33$, $P = 0.044$) but not with age ($r = -0.33$, $P = 0.08$) (Fig. 3). There was also no significant correlation between FMD and age ($r = -0.35$, $P = 0.081$) or cholesterol ($r = -0.22$, $P = 0.090$) after P value adjustment. Given that patients with diabetes are known to have a reduced flicker response, the type 1 diabetic group has been excluded in the latter analyses.

CONCLUSIONS

— Given that impaired endothelial function has been observed to be an early feature in several systemic and ocular vascular-related diseases, much attention has been paid to the development of methods to noninvasively assess endothelial function in humans. As one of the most widely used techniques, the ultrasound-based FMD has been shown to give a reliable estimate of endothelium-dependent vasodilatation (10). FMD is based on the capacity of blood vessels to self-regulate vascular tone in response to changes of shear stress caused by changes in blood flow. This regulation is dependent on endothelium-derived NO (14) and can therefore be used as a marker for endothelial function.

Reduced FMD has been found in patients with mild systemic hypertension (15), hypercholesterolemia (16), and diabetes (17), indicating for an impaired endothelial function in these patient groups. Additionally, it has been shown that FMD can predict future cardiovascular events (18). However, the technique of FMD is hampered by the limited spatial resolution of the ultrasound systems currently available. In addition, measurement of FMD requires significant training and involves a subjective component when data are evaluated.

Flicker-induced vasodilatation may be another attractive noninvasive approach. It has been shown that flicker response is significantly diminished in

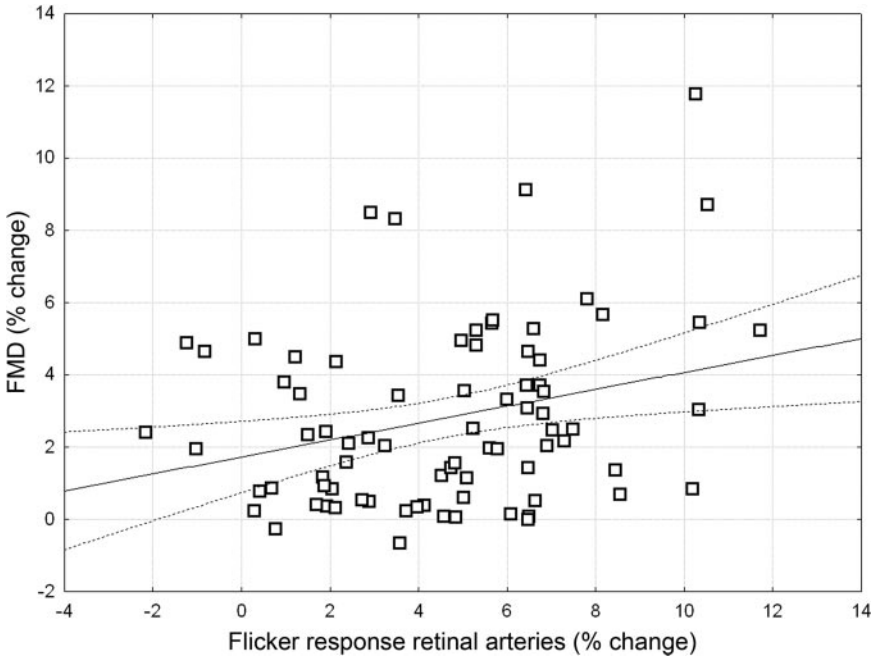

Figure 2—*Correlation analysis between FMD and flicker response of retinal arteries (*r *= 0.3,* P *= 0.044).*

patients with glaucoma or diabetes (5,8). Even more importantly, a reduced response has also been observed in patients with systemic hypertension, indicating a potential insight into vascular function in general (7), because an increase of blood pressure or IOP alone does not influence the flicker response (19,20). These results support the hypothesis that the observed changes reflect long-term alterations of the vasculature. The hypothesis that flicker-induced vasodilatation may at least partially reflect endothelial function has also been encouraged by the observation that flicker-induced vasodilatation is mainly dependent on an intact NO synthesis (9). Endothelial dysfunction due to abnormal release or action of NO is a well-recognized early feature of vascular damage, as it has been reported previously in vascular-related diseases like diabetes, hypercholesterolemia, systemic hypertension, and atherosclerosis (16,21,22).

Our findings of greater baseline vessel diameters in patients with diabetes are in good accordance with earlier studies (23). However, given that our measures were done only in one single artery and vein and not in all visible vessels, our data do not represent total cross-sectional retinal vessel diameters.

We observed a negative correlation between flicker-induced vasodilatation and blood cholesterol. This result is again a hint that endothelial dysfunction is involved in reduced flicker-induced vasodilatation, because cholesterol and oxidized LDL in particular are clearly associated with endothelial cell dysfunction (24) and reduced bioavailability of NO. Evidence has been provided that reduction of serum cholesterol increases FMD and may therefore be beneficial for endothelial functions (22,24). Whether this also holds true for flicker-induced vasodilatation has yet to be clarified. Correlations between FMD and age or plasma cholesterol, as observed earlier (16), and between flicker-induced vasodilatation and age failed to reach level of significance after adjustment for multiple testing. Our study was, however, not designed for these outcome analyses, and a larger sample size may be required to investigate these issues. The present study provides evidence that in patients with type 1 diabetes and in patients with systemic hypertension and/or hypercholesterolemia, both FMD and flicker-induced vasodilatation are reduced compared with healthy volunteers. However, our study failed to show a strong correlation between FMD and flicker-induced vasodilatation.

What could be the reason for the differing responses between the two vascular beds? First and most importantly, FMD and flicker-light–induced vasodilatation differ in the method of stimulation. Whereas the diameter increase in FMD is caused directly by the augmented shear stress in the endothelium and the connected tissue, flicker response is basically the vascular answer to increased neural activity in the retina. This may be of special importance in patients with diabetes or glaucoma, since it cannot be ruled out that in these patients decreased neural activity may partially account for the decreased flicker response.

Second, it has to be noted that the properties of the vascular beds investigated differ significantly. Whereas flicker stimulation investigates arteries in an order of 150–250 μm, FMD reflects endothelial function in significantly larger vessels with different vessel wall properties. Thus, the weak correlation between FMD and flicker may indicate that the stimulation answer in the conduit arteries and in the smaller retinal arteries do not carry the same information, although both are diminished in patients with endothelial dysfunction. This phenomenon is also known from other experiments showing that FMD and endothelium-dependent vasodilatation assessed with an invasive technique that mainly reflects the endothelial function of resistance arteries are both independently related to the risk of coronary heart disease (25). Flicker-induced vasodilatation may provide additional information to these techniques because of the smaller size of vessels assessed. This may particularly be interesting in diseases primarily affecting the microvasculature.

Flicker-induced vasodilatation offers a variety of significant advantages. On the one hand, it provides excellent reproducibility and sensitivity (12). On the other hand, it is easily performed and quick, although pupil dilatation is required with the fundus camera used in the present experiments. Most importantly, the system does not include a subjective component once an optimal fundus image is achieved.

As a limitation of the study, no information is available about blood nitrate concentration. Although none of the subjects under study was under nitrate medication, we cannot fully exclude that a nitrate-rich diet may influence FMD or flicker-induced vasodilatation.

In summary, our data indicate that in both patient groups with endothelial dysfunction as assessed with FMD, flicker responses are diminished. The reason why no major correlation was found between FMD and flicker-induced vasodilatation needs to be the subject of further studies. Furthermore, whether flicker stimulation may also serve as a predictor for risk of

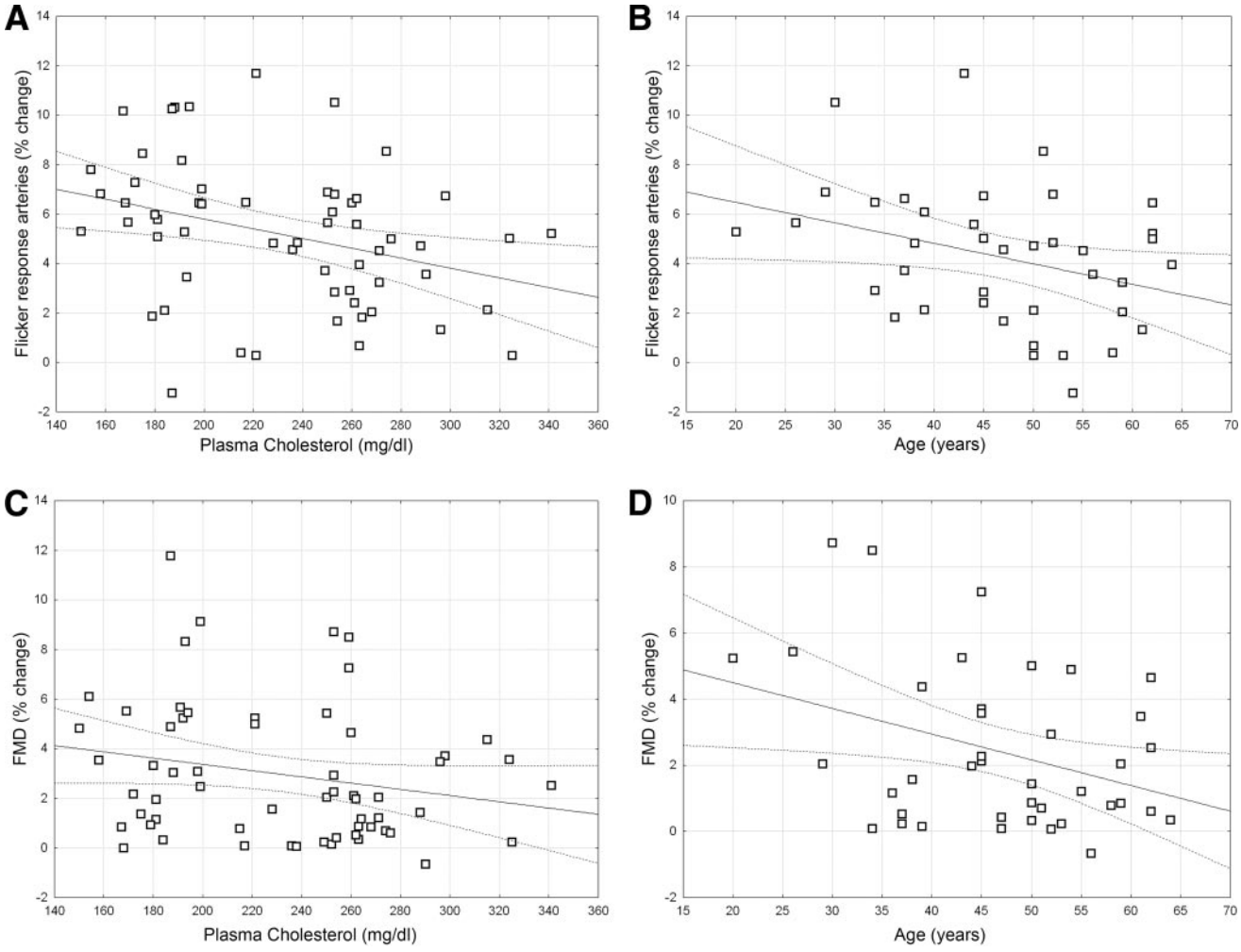

Figure 3—*Correlation analysis between flicker-induced vasodilatation and plasma cholesterol levels* (r = *−0.33,* P = *0.044)* (A)*, between FMD and age* (r = *−0.35,* P = *0.081)* (D)*, and vice versa* (r = *−0.33,* P = *0.08)* (B) (r = *−0.22,* P = *0.090)* (C).

systemic diseases, as it has been shown for FMD, has yet to be investigated in longitudinal studies. The system is, however, a candidate for assessing endothelial function in clinical routine because it induces minimum discomfort to the subject, provides good reproducibility and sensitivity, and does not include a subjective component.

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