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EFFECTS OF CHANGED DIET TO FLOW MEDIATED DILATATION OF BRACHIAL ARTERY

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Abstract
Aim of the research was to detect and identify initial changes of elastic properties of magistral arteries (arterial stiffness). Flow mediated dilatation-(FMD) method was used to prove, i.e. to set hypothesis that the effects of strict dietary regime to endothelial function of brachial artery in patients with stable angina and atherosclerotic changes in coronary arteries less than 50% confirmed by coronarography will lead to significant increase of FMD values. Total 60 subjects aged 40-75 were enrolled in the study. Diet group consisted of 30 subjects with atherosclerotic changes in coronary arteries less than 50% confirmed by coronarography. Control group consisted of 30 subjects not receiving medical treatment. Following initial examination, follow-up was carried out after 3 and 6 months. As expected, the flow velocity following cuff release was higher at each measurement in both diet and control group compared to baseline values. Flow velocity following nitroglycerin administration in the diet group was insignificantly higher in both follow-ups, whereas it was lower in the control group in both second and third measurement. Analysis of variance results for relations within groups show no differences. On the basis of the obtained results, FMD measurements on brachial artery may contribute to early detection of atherosclerotic changes, especially in patients with multiple risk factors.

Key words: **effect, atherosclerosis, FMD, diet, color doppler**

Introduction

Atherosclerosis is a narrowing of arterial lumen, developed due to local thickening of intima called athera or plaque, whose most common cause is an injury of arterial wall (Vrhovac, Jakšić, Reiner, & Vucelić, 2008). It has been perceived for a long time as a simple depositing of lipids and calcium into the arterial walls, but in fact it represents a set of specific cellular and molecular processes with inflammation characteristics (Epstein & Ross, 1999). Common risk factor are involved in development and progression of atherosclerosis, such as hyperlipoproteinema, diabetes mellitus, arterial hypertension, obesity and smoking, but also newly discovered risk factors such as changes in coagulation and fibrinolytic process, inflammation parameters, homocysteine and especially endothelial dysfunction (Bonetti, Lerman, & Lerman, 2003). Blood vessel endothelium is a huge paracrine organ which secretes numerous factors responsible for regulation of vascular tone, cellular growth, platelet and leucocytes interaction, and thrombogenicity. Endothelial protective role in physiologic conditions is based on adhesion prevention of the circulating platelets and leucocytes, maintaining vascular system in vasodilatory state and migration and proliferation inhibition of blood vessel smooth muscle cells. Dysfunctional endothelium has no protective function enabling for expression of various adhesive molecules and binding of different form of leucocytes, thus forming the basis for arthrosclerosis development (Bonetti et al., 2003; Massaro et al., 2015; Papamichael et al., 2005). Celermajer and associates developed FMD technique in 1992 as a non-invasive method for measuring function of blood vessels endothelium. Ten years later, in 2002, Corretti and associates published initial guidelines for ultrasound assessment of FMD of brachial artery. Meta-analysis was carried out in 2005 with 250 studies that used FMD method in their research and it was discovered that technical details of FMD measurement (occlusion location and duration) may provide explanation for discrepancies in FMD among the studies (Bots, Westerink, Rabelink, & de Koning, 2005). Oral antioxidants intake (vitamin C, vitamin E, alpha-lipoic acid) – lead to reduction of circulatory free radicals. Intra-arterial administration of C vitamin leads to FMD increase. Therefore, intake of antioxidants is not recommended three days prior to FMD measurement. It has been documented that smoking impairs endothelial function (Bots et al., 2005). Caffeine is the most common cause of guanylate cyclase inhibition, which is one of the enzymes important for vessel dilatation (Papamichael et al., 2005). Exercise, that is, physical activity improves FMD with seemingly healthy adults, obese

men and postmenopausal women. It is recommended that no exercise should be done 12 hours prior to FMD. Food intake, especially rich in carbohydrates and fat, attenuates FMD with apparently healthy subjects. Fasting FMD should be performed. Aim of the research was to detect and identify initial changes of elastic properties of magistral arteries (arterial stiffness).

Methods

Aim of the research is to detect and identify initial changes of elastic properties of magistral arteries (arterial stiffness). Flow mediated dilatation-(FMD) method is used to evaluate the elasticity, that is, the stiffness of the artery and thus indirectly point to present atherosclerotic changes. Ultrasound scan is used to evaluate extracranial and femoral artery scanning for morphologic and hemodynamic atherosclerotic changes and then compare findings of flow mediated dilatation and ultrasound scanning.

Subject

The study included 60 subjects age 40-75. Treatment group consisted of 30 subjects with atherosclerotic changes confirmed by coronarography that were put on strict diet. Control group consisted of 30 subjects age 40-75 not receiving medical treatment. Following initial examination, follow-up was carried out after 3 and 6 months.

Variable sample and measurements

We had defined FMD pathological findings (below 7%) and carotid IMT - CIMT (exceeding 0.9 mm). *Inclusion criteria:* Performed coronarography that indicated atherosclerotic changes of coronary arteries; age 40-75; baseline laboratory parameters and lipid profile, fibrinogen, CRP values. *Excluding criteria:* diabetes mellitus (preprandial blood glucose value >5.6 or with OGTT); cardiac decompensation–class III-IV according to NYHA functional classification; digestive tract disease that might cause poor medicine absorption; clinically presented hypothyroidism; serum creatinine >170 mmol/l, CK> 3x the upper limit of the normal range, AST and ALT > 1,5 the upper limit of the normal range; known absolute treatment contraindications for used medicines; age below 40 or above 75; non-compliance with the diet regime; refusal of a subject to cooperate in the trial. Numerous factors affect FMD reactivity, such as temperature, diet, medicines, sympathomimetic stimuli, just to name few (Yogo et al., 2014). If possible, all vasoactive medicines taken by a subject must be discontinued for duration of least 4 and 1/2 of half-life of such medicines. Subjects must not be under physical strain nor ingest substances that might affect FMD such as coffee, caloric food and C vitamin, or smoke at least 4-6 hours before trial. Investigator must be familiar with menstrual cycles as they affect FMD. Endothelial function is measured in the morning, preprandially. One investigator who was not aware of the subjects' characteristics performed all the measurements. Flow mediated dilatation (endothelium dependent) and nitroglycerine induced dilatation (endothelium independent) of the right brachial artery was analyzed by use of high resolution ultrasound system in B mode with 10-MHz linear transducer. Subjects were resting in supine position with right supine arm for at least 10 minutes before hemodynamic measuring. Right brachial artery was depicted in longitudinal projections 2-15 cm above elbow, so as to obtain the clearest image of anterior and posterior wall layers. Median artery diameter was measured at the end of diastole, which was identified by simultaneous ECG monitoring (at the beginning of QRS complex). At last three cardiac cycles were analyzed and average value calculated in each testing. Flow velocity was measured under fixed angle of 68º. Hyperemic flow increase was induced with tourniquet set around the forearm at 300 mmHg pressure for 4.5 minutes. Hyperemic flow (with increased flow that creates endothelium dependent stimuli for vasodilatation) was recorded for the first 15 seconds while diameter was measured 45-60 seconds following release of cuff pressure. Relative flow increase during reactive hyperemia was considered as the highest flow recorded during the first seconds following release of cuff pressure divided with the flow recorded in resting. Endothelium-dependent dilatation is expressed as percentage change of artery diameter following reactive hyperemia compared to baseline. Following 10 minute rest, a new imaging was performed at rest. Thereafter, the subject was sublingually given NTGL spray (endothelium independent vasodilator) and 4.5 minutes later, the final examination was performed. Endothelium-independent dilatation is expressed as percentage change of artery diameter following NTGL administration compared to baseline. Scanning of extracranial and femoral arteries was performed for each subject, searching for morphologic and hemodynamic changes. Type and extent of atherosclerotic changes were identified and IMT measurement carried out. The conducted trial was prospective, manipulative and comparative. The analysis was executed in Minitab 16.1 program (Minitab Inc, State College, Pennsylvania, USA). Descriptive statistical methods (graphic method, measures of central tendencies and variable measurement) were used for analysis, and for hypothesis testing the non-parametric Cohran Q test and variance analysis.

Results

The study included total 60 subjects age 40-75. Treatment group consisted of 30 subjects with atherosclerotic changes confirmed by coronarography that were put on strict diet. Control group consisted of 30 subjects age 40-75 not receiving medical treatment. Gender and age structure of subjects are depicted in Tables 1 and 2. Average age in the diet group was 55.5 and in the control group 53.8. Around 50% of subjects in both study groups are of

51 to 60 years of age, thus leading to conclusion that the groups are almost identical in terms of age structure, with averagely slightly younger subject in the control group, but still belonging to the same biological category as the diet group. As for the gender structure, the groups were identical, with equal number of male and female subjects.

Table 1. Gender structure and average age of the subjects

	Gender			Age		
Group	Male	Female	Total	Male	Female	Total
Diet	15	15	30	55.2	55.9	55.5
Control group	15	15	30	51.6	56.4	53.8

Table 2. Age structure of the subjects

	Age structure of subjects in diet group			Age structure of subjects in control group		
Age	Male	Female	Total	Male	Female	Total
41 – 50	2	4	6	2	5	7
51 – 60	8	9	17	7	6	13
61 +	5	2	7	10	6	4
Total	15	15	30	15	15	30

Since the subject sample is balanced in terms of gender and age structure, and certain subject groups almost identical in these parameters, age and gender influence to study result has been avoided. Out of total 60 included subjects, changes in carotid and femoral arteries were found in 36 of them, whereas 24 of subjects had no atherosclerotic changes in examined arteries.

Table 3. Artery status in subjects

Group	Number		%	
	NAD	Plaque	NAD	Plaque
Diet group	11	19	*36.7%*	*63.3%*
Control group	13	17	*43.3%*	*56.7%*

Cochran Q test established no statistically significant differences between subject groups in terms of found arterial plaques sizes ($Q = 0.333$, broj stepeni slobode $= 2$, $p = 0.846$). In the diet group, no statistically significant differences were found in terms of initial diameter during three measurements ($F = 0.06$, $p = 0.946$). No differences were found in control group either ($F = 0.25$, $p = 0.780$). Following cuff release in the diet group, no statistically significant differences were found in the diameter between three measurements ($F = 0.02$, $p = 0.981$), nor in control group ($F = 0.91$, $p = 0.407$). Difference in diameter measuring following 10 minutes rest in the diet group had no statistical significance ($F = 0.19$, $p = 0.824$), and neither in control group ($F = 0.02$, $p = 0.983$). There was no statistically significant difference in the arterial diameter following nitroglycerin administration throughout time in the diet group ($F = 0.21$, $p = 0.812$), and neither in control group ($F = 0.26$, $p = 0.775$). As expected, after blood pressure meter cuff release, the flow velocity was higher in both diet and control group during each measuring compared to baseline. After nitroglycerin administration, the flow velocity was slightly higher in the diet group during both follow-ups, but it was lower in the control group during second and the third follow-up. Results of variance analysis for relations within groups show no differences (table 4).

A number of pathological endothelium- dependent FMD in subjects was separately analyzed and it showed that it was increased in both control and diet group, which is depicted in the Table 5.

A number of pathological endothelium- independent FMD was analyzed and it showed that it was decreased in the diet group during the first and second follow-up, while it was increased in the control group, which is depicted in the Table 6.

Table 4. Results of variance analysis for relations within groups For flow velocity parameter in the first, second and third measuring

Subject group	Flow velocity	F	p	Conclusion
Diet group	Initial velocity	0.35	0.708	No difference
	Following cuff release	0.63	0.536	No difference
	Following rest	0.46	0.632	No difference
	Following NTGL administration	0.86	0.428	No difference
Control group	Initial velocity	0.81	0.450	No difference
	Following cuff release	1.33	0.270	No difference
	Following rest	1.31	0.275	No difference
	Following NTGL administration	2.30	0.107	No difference

Table 5. Average endothelium-dependant FMD in subjects

Subject group	Initial measurements		Following 3 months		Following 6 months	
	FMD	Number of pathologic FMD	FMD	Number of pathologic FMD	FMD	Number of pathologic FMD
Diet group	2.36%	23	4.52%	20	3.04%	24
Control group	2.34%	22	4.41%	23	2.28%	24

Table 6. Average endothelium-independent FMD in subjects

Subject group	Initial measurements		Following 3 months		Following 6 months	
	FMD	Number of pathologic FMD	FMD	Number of pathologic FMD	FMD	Number of pathologic FMD
Diet group	12.44%	8	12.55%	5	15.26%	3
Control group	14.03%	3	13.44%	3	16.26%	4

Discussion

Flow mediated vasodilatation values in subjects with atherosclerotic changes in coronary arteries confirmed by coronarography that were under the strict diet were 2.36%, 4.52% and 3.04% at initial measuring, following 3 months and 6 months, respectively. Flow mediated vasodilatation values in control group were 2.34%, 4.41% and 2.28% at initial measuring, following 3 months and 6 months, respectively . There were 23 subjects with pathologic FMD values (<7%) in the diet group and 22 subjects in the control group. Following 3 months, the number of pathologic FMD values decreased in the diet group from 23 to 20, whereas it increased from 22 to 23 in the control group. Following 6 months, the number of pathologic FMD values increased in the both diet and control group to 24. FMD is a validated technique for quantifying endothelial function and has proven prognostic values for any future cardiovascular events.

A number of vasoactive substances are being released from the endothelium (including prostacyclin, endothelin, endothelial growth factor, interleukins, plasminogen inhibitor and nitric oxide). NO is possibly the main vasodilatation mediator and has been intensively studied as of its discovery in 1980 (Harrison, 2014). Flow mediated dilatation is taking place in the artery that supplies blood to an organ when the arterial blood flow is increased. Vasodilatation of peripheral vascular bed does not cause dilatation contrary to blood flow direction (upstream) if the flow increase has been prevented and FMD is fully blocked by nitric oxide synthase (NOS), and it is very likely that the stimuli is the increased shear stress between the blood and adjacent layer of arterial wall, glycocalyx (Matsuzawa, Kwon, Lennon, Lerman, & Lerman, 2015). Finally, the change in the cytoskeleton of endothelial cells is the event that most probably lead to NOS activation, and such activation cannot occur without functional glycocalyx (Cassuto et al., 2014; Matsuzawa et al., 2015). Endothelial function plays a pivotal role in disturbed vascular biology during atherosclerotic development. Evidence suggests that endothelial dysfunction is one of the causes for development and destabilization of atherosclerotic plaques. Flow dependent dilatation enables noninvasive, cheap and repeated measuring of endothelial function. It is widely used in pathophysiologic researches and large studies pertaining to epidemiology of vascular diseases. Improvement in methodology and analysis standardization makes it a very attractive method for assessment of performed intervention arterial procedures. In medicinal products development programs, FMD improvement may immediately provide early signals for mutual benefit, as an absence of vascular toxicity and additional assessment for structural arterial disease and cardiovascular end-point results, while not adding to the costs of those products and avoiding long-term trials (Charakida, Masi, Lüscher, Kastelein, & Deanfield, 2010; Orlandi et al., 2014). During FMD testing, vasodilatation occurs after acute blood flow increase, emerging following removal of artificially developed obstacle to blood flow in the extremity (cuff of the blood pressure meter) during certain time period (Corretti et al., 2002; Widlansky, Gokce, Keaney, & Vita, 2003). This hyperemia increases laminar shear forces parallel to the long axis of the vessel which is transduced via luminal mechanoreceptors to the endothelial cell. This event increases G-protein expression of phosphokinase A, signaling an increase of endothelial nitric oxide synthase (eNOS) activity which catalyzes the conversion of L-arginine to NO (Münzel, Daiber, Ullrich, & Mülsch, 2005; Sessa, 2004). NO then diffuses into the tunica media where it activates guanylate cyclase which converts guanosine triphospate into guanosine monophospate to induce relaxation of the smooth muscle and subsequent vasodilatation. (Hafezi-Moghadam et al., 2002).

Conclusion

According to the foregoing, we can conclude that FMD measurements performed on brachial artery may contribute to early detection of atherosclerotic changes, especially in subjects with multiple risk factors. Positive FMD findings may serve in clinical practice as a predictor of atherosclerosis, i.e. as indication for undertaking proper measures to reduce atherosclerosis. FMD values may confirm positive treatment response in terms of improvements, that is, confirm border or negative response in terms of persistence of atherosclerotic process. FMD measurement may be a good prognostic tool in preventive cardiology; it can predict short-term postoperative events in high risk patients and assess long-term cardiovascular risk with less risk patients.

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