

Indirect Assessment of Endothelial Function in Patients with Hypertension and Diabetes Mellitus

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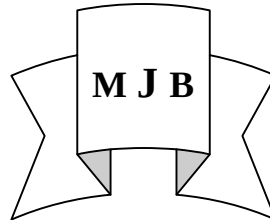
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Abstract

Thirty eight participants (27 men, 11 women) with age between 40-60 years were divided into 3 groups. Group I: Includes 13 healthy subjects. Group II: Includes 13 patients with essential hypertension. Group III: Includes 12 patients with type 2 diabetes mellitus.

Using high-resolution ultrasound, brachial artery diameters at rest, during reactive hyperaemia (endothelium-dependent dilation) and after sublingual glyceryl trinitrate (GTN) application (endothelium-independent dilation) were measured.

The percentage increase of brachial artery diameter after reactive hyperemia (flow-mediated dilatation% or FMD%) in control subjects was $8.85 \pm 0.25\%$ while it was found to decrease significantly ($P < 0.0001$) in hypertensive and diabetic patients by 60% and 67% respectively. Furthermore, the percentage increase of the brachial artery diameter after glyceryl trinitrate (glyceryl trinitrate-induced dilatation% or GTNID %) in control healthy subjects was $14.41 \pm 0.81\%$. The GTNID% was found to decrease significantly ($P < 0.006$) by 25% in hypertensive patients. No significant GTNID% change was found in diabetic patients.

The antioxidant-oxidant ratio index (RI) of the measured biochemical parameters was significantly lower in hypertensive patients (by 50%) and even more lower in diabetic patients (by 84%). The antioxidant-oxidant RI was found to be significantly and positively correlated with FMD% and GTNID% in diabetic patients only.

الخلاصة

38 مشارك، 27 ذكر و 11 انثى ، تراوحت اعمارهم بين 40 – 60 سنة، قسموا في ثلاثة مجاميع. المجموعة الاولى: تشمل 13 شخص سليم. المجموعة الثانية: تشمل 13 مريض بفرط الضغط الاول. المجموعة الثالثة: تتضمن 12 مريض بداء السكر الغير معتمد على الانسولين.

باستعمال ثبات عالي للامواج فوق الصوتية تم قياس قطر الشريان العضدي عند الراحة، وخلال تحفيز الاحتقان التفاعلي (التوسع المعتمد على الخلايا الطلائية)، وباستخدام كليسيريل تراينتريت تحت اللسان (التوسع الغير معتمد على الخلايا الطلائية)

تزداد نسبة قطر الشريان العضدي بعد تحفيز الاحتقان التفاعلي لدى الاشخاص السليمين بمقدار $8.85 \pm 0.25\%$ بينما وجدت منخفضة بشكل معنوي ($P < 0.0001$) لدى المرضى المصابين بفرط الضغط وداء السكري بنسبة 60 % و 67 % على التوالي. علاوة على ذلك، تزداد نسبة قطر الشريان العضدي بعد اخذ كليسيريل تراينتريت (التوسع المحفز من قبل كليسيريل تراينتريت) في

الأشخاص السليمين بمقدار $14.41 \pm 0.81\%$. ويقل هذا التوسع بشكل معنوي ($P < 0.006$) بنسبة 25% لدى مرضى فرط الضغط. ولا يوجد تغير معنوي لدى مرضى داء السكري. المعامل التناسبي للمادة المقاومة للأوكسدة الى المادة المؤكسدة للقيم الحياتية الكيميائية يقل بشكل هام لدى مرضى فرط الضغط بنسبة 50% ويقل بشكل كبير لدى مرضى داء السكري بنسبة 84%. وجد أن المعامل التناسبي للمادة المقاومة للأوكسدة الى المادة المؤكسدة له علاقة طردية هامة مع توسع الشريان العضدي المعتمد على الخلايا الطلائية والتوسع المحفز بواسطة كليسيريل تراينتريت لدى مرضى فرط الضغط.

Introduction

Endothelium is a single-cell lining covering the internal surface of blood vessels, cardiac valves and numerous body cavities. Therefore, Endothelial cells make up a large organ. Endothelial cells play an important regulatory role of the vascular tone to maintain vasodilation and nutritious blood flow in humans [1]. These cells secrete many growth factors and vasoactive substances and plays a key role in vasodilatation by releasing endothelium-derived relaxing factor (EDRF), a substance that is now known to be nitric oxide (NO) and endothelium-derived contracting factors that maintain vascular homeostasis [2]. Nitric oxide, synthesized by the vascular endothelium (from L-arginine by nitric oxide synthase), causes relaxation of vascular smooth muscle, inhibition of platelet aggregation and cell proliferation through the activation of soluble guanylate cyclase and the increase of intracellular cyclic guanosine monophosphate (GMPc) levels, which in turn lower intracellular calcium levels [1, 2] and activates protein kinase with consequent dephosphorylation of myosin light chains and muscle relaxation [4]. A dysfunction of the vascular endothelium has been implicated in the pathophysiology of several cardiovascular diseases [1]. Certain disease states may contribute to reduced nitric oxide synthesis, this

includes hypertension and diabetes [4]. There is evidence that the activation of nuclear enzyme poly (ADP-ribose) polymerase (PARP) in diabetic patients importantly contributes to the development of endothelial dysfunction. [5]. Hypertensive patients have both impaired endothelium-dependent vasodilatation related to decreased availability of NO, and increased activity of the endothelin (ET-1) system, which participate in their increased vascular tone and may predispose them to atherosclerosis [6].

The NO release can be evoked by stimulation of cholinergic (muscarinic) and other receptors on endothelium or by mechanical interaction between blood flowing in vessel and the vascular wall in response to an increase in flow and shear stress [3]. Therefore, the flow-induced dilation is due to local release of NO [2].

High resolution ultrasonography has been used to evaluate endothelial function noninvasively by measuring changes in the diameter of the brachial artery due to increased blood flow and shear stress induced by inflation or deflation of a pressure cuff (flow-mediated dilatation) [9]. The dilation of the vessel is due to the release of nitric oxide [1, 2].

Subjects and Methods

The present study was conducted at Department of Echo-Doppler in Marjan

Teaching Hospital / Hilla in cooperation with the Department of Physiology, Al-Mustansiriya College of Medicine, between Oct. 2002 to March 2003.

The subjects were recruited from outpatient clinics or inpatient ward. A medical history and physical examination were obtained and laboratory tests were performed in all subjects. Thirty-eight subjects (27 men and 11 women) were involved. Their ages were between 40-60 years. All subjects had no history of smoking or alcohol drinking. They were divided into 3 groups:

Group I: Includes 13 healthy subjects (10 men and 3 women), their mean age was 46.62 ± 1.91 years (mean $\pm$ SEM) and they are served as a control group. The control subjects include normal individuals who had no history of diabetes mellitus, hypertension or heart diseases. They had a normal physical examination.

Group II: Includes 13 patients with essential hypertension (9 men and 4 women). Their mean age was 51.15 ± 1.68 years. They had a systolic blood pressure (SBP) = or >160 mm Hg, and a diastolic blood pressure. (DBP) = or >95 mm Hg or both [7]. These patients had a normal fasting blood glucose level.

Group III: Includes 12 patients with type 2 diabetes mellitus (8 men and 4 women). Their mean age was 51.83 ± 1.69 years. They had a fasting plasma glucose level $> \text{or} = 126$ mg / 100 ml [8].

The technique used in the present study was based on that recorded by Hashimoto *et al*, (1999). The right brachial artery was scanned over a longitudinal section 3 to 5 cm above the right elbow. An ECG monitor integrated with the ultrasound machine was applied. A pneumatic cuff was placed around the forearm distal to the target

artery and was inflated to a pressure of 250 mm Hg. The inflation was held for 5 minutes. Increased flow was then induced by sudden cuff deflation. A second scan was performed for 120 seconds after cuff deflation. Then, 15 minutes later, another resting scan was recorded to confirm vessel recovery. Sublingual glyceryl trinitate (GTN) tablet (0.5 mg) in form powder was then administered and 5 minutes later the last scan was performed. The diameter of the brachial artery was measured from the anterior to the posterior interface between the media and adventitia (M-line) at a fixed distance, synchronized with the R-wave peaks on the ECG. All measurements were made at end diastole to avoid possible errors resulting from variable arterial compliance. Vasodilatation was observed after cuff release. The diameter change caused by flow-mediated dilatation (FMD) was expressed as the percent change relative to that at the initial resting scan (percent FMD). The diameter change caused by GTN was expressed in the same way, as the percent change relative to that at the recovery scan, glyceryl trinitate-induced dilatation (GTNID).

For laboratory analyses, after an overnight fast, a venous fresh blood sample of 4 ml was obtained. Blood was centrifuged and the collected serum was investigated for fasting serum glucose, total serum cholesterol, serum uric acid, serum high-density lipoprotein (HDL) and serum triglyceride, by standard enzymatic methods. HDL cholesterol was measured with direct method[10]. Low-density lipoprotein (LDL) cholesterol was calculated by the use of the Friedewald formula:

Total cholesterol = HDL + LDL + VLDL

VLDL = Triglyceride / n, where n = 5.

Statistical analysis: All data were

expressed as mean $\pm$ SEM. The differences were assessed by paired or unpaired student's t- test. Correlations between variables were computed by Microsoft Excel program, which runs under Windows operating system. A value of $P < 0.05$ was considered to be statistically significant.

Results

Table 1 shows the general measured parameters of different groups. The percentage increase of the brachial artery diameter after reactive hyperemia (FMD%) in control subjects was $8.85 \pm 0.25\%$. FMD% was found to decrease significantly in hypertensive and diabetic patients by 60% and 67% respectively (Fig. 1). Furthermore, the percentage increase of the brachial artery diameter after GTN administration (GTNID%) in control subjects was $14.4 \pm 0.81\%$. The GTNID% was found to decrease significantly by 25% in hypertensive patients. In contrast, no significant GTNID% change was found in diabetic patients (Fig. 1).

In order to normalize the serum concentrations of various biochemical parameters, their means were expressed as 100% of the control subject's level. Figure 2 indicates that patients with hypertension have a significantly lower serum HDL concentration (by 28%) and a significantly higher serum LDL concentration (by 40%) relative to control subjects. In diabetic patients, there were a significant decrease in serum uric acid (by 28%) and serum HDL (by 26%) concentrations relative to their control counterpart levels. In addition, diabetic patients showed a significant increase in their serum fasting blood sugar (by 127%) and serum LDL (by 44%) concentrations in comparison to the control values.

To test the various measured biochemical parameters on the reactivity of the blood vessels, the blood biochemical parameters were classified as either oxidant type or antioxidant type of agents. Blood sugar [11] and LDL [12] were considered as oxidant agents. While uric acid [13] and HDL [14] were considered as antioxidant agents. Figure 3 shows that the antioxidant-oxidant ratio index (RI) ($RI = \text{sum of the multiplication of the concentrations of antioxidant agents} / \text{sum of the multiplication of the concentrations of oxidant agents}$) of the biochemical parameters was significantly lower in hypertensive (by 50%) and in diabetic patients (by 84%) relative to their counterpart value in control subjects.

Figures 4 and 5 show that there were a significant positive correlation between antioxidant-oxidant RI and the FMD% and GTNID% in diabetic patients only. Neither such correlation's could be obtained in healthy subjects or in hypertensive patients.

Discussion

The data of the present study demonstrate that, in comparison to the base level, the diameter of brachial artery of healthy subjects increases during reactive hyperemia (FMD, endothelium-dependent vessel dilatation). The increase in FMD% was impaired in hypertensive and diabetic patients. A conclusion is speaking of endothelium dysfunction in both conditions. This result is in agreement with previous published studies [15-17]. Who found that FMD% was impaired in hypertensive and diabetic patients. Higashi *et al*, (2001) suggested that endothelium function of patients with essential hypertension is impaired due to a decrease of nitric oxide production.

Endothelium dysfunction (ED) in diabetes may result from a decreased bioavailability of NO secondary to insulin resistance with exaggerated production of ET-1 stimulated by hyperglycemia. However, the present data indicate that GTNID% of the brachial artery was not impaired in diabetic patients, which is in parallel with those of other result[18]. In contrast, the present results have shown that GTNID% of the brachial artery was impaired in hypertensive patients, which is in complete agreement with other study[19].

The current results demonstrated that the antioxidant-oxidant ratio index (RI) was significant decrease in

hypertensive and more decrease in diabetic patients. This result is agreement with previous studies [20, 21]. The decrease in the antioxidant-oxidant ratio index in hypertensive patients recruited in the present study was due to a decrease in plasma HDL and an increase of plasma LDL that is in general in agreement with those of other results[22]. The current study shows a positive and a significant correlation between antioxidant-oxidant RI and both FMD% and GTNID% in diabetic patients. This result is in parallel with those of other results[23]. who found that there is evidence of reduction of vascular smooth muscle sensitivity to NO in diabetes.

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Table 1 The measured parameters of different groups. *P1* and *P2*: The probability between the hypertensive and diabetic patients respectively relative to control subjects. BMI: Body mass index. SV: Stroke volume. FBS: Fasting blood sugar. T: Total. TG: Triglycerid. HDL: High density lipoprotein. LDL and VLDL: Low and very low density lipoprotein. NS: Not significant ($P > 0.05$).

	Healthy subjects	Hypertensive patients		Diabetes mellitus patients	
n =	13	13	P1	12	P2
Age (years)	46.62 ± 1.91	51.15±1.68	NS	51.83 ± 1.69	NS
BMI (kg/m²)	25.52 ± 0.49	28.32 ± 0.97	< 0.05	27.00 ± 0.91	NS
Systolic BP (mm Hg)	127.00 ± 2.87	166.46 ± 4.14	< 0.0001	123.00 ± 2.33	NS
Diastolic BP (mm Hg)	78.85 ± 1.55	100.92 ± 1.43	< 0.0001	75.08 ± 1.55	NS
Stroke volume (ml)	83.92 ± 4.94	70.23 ± 4.05	< 0.05	73.88 ± 3.44	NS
Cardiac index (L/min/m²)	3.08 ± 0.13	2.55 ± 0.08	< 0.01	2.88 ± 1.11	NS
Serum uric acid (mg/dl)	6.23 ± 0.21	6.71 ± 0.51	NS	4.46 ± 0.23	< 0.001
FBS (mg/dl)	89.31 ± 4.8	95.62 ± 4.44	NS	202.33 ± 12.74	< 0.001
Serum T. cholesterol (mg/dl)	190.88 ± 7.41	221.08 ± 11.79	< 0.05	241.33 ± 5.02	< 0.001
Serum TG (mg/dl)	141.31 ± 8.74	136. 76 ± 11.93	NS	163.83 ± 14.33	NS
Serum HDL (mg/dl)	50.20 ± 1.50	35.98 ± 1.21	< 0.001	37.13 ± 1.38	< 0.001
Serum LDL (mg/dl)	113.8 ± 5.77	159 ± 10.42	< 0.002	164.42 ± 6.03	< 0.001
Serum VLDL (mg/dl)	28.42 ± 1.74	27.37 ± 2.41	NS	32.82 ± 2.84	NS

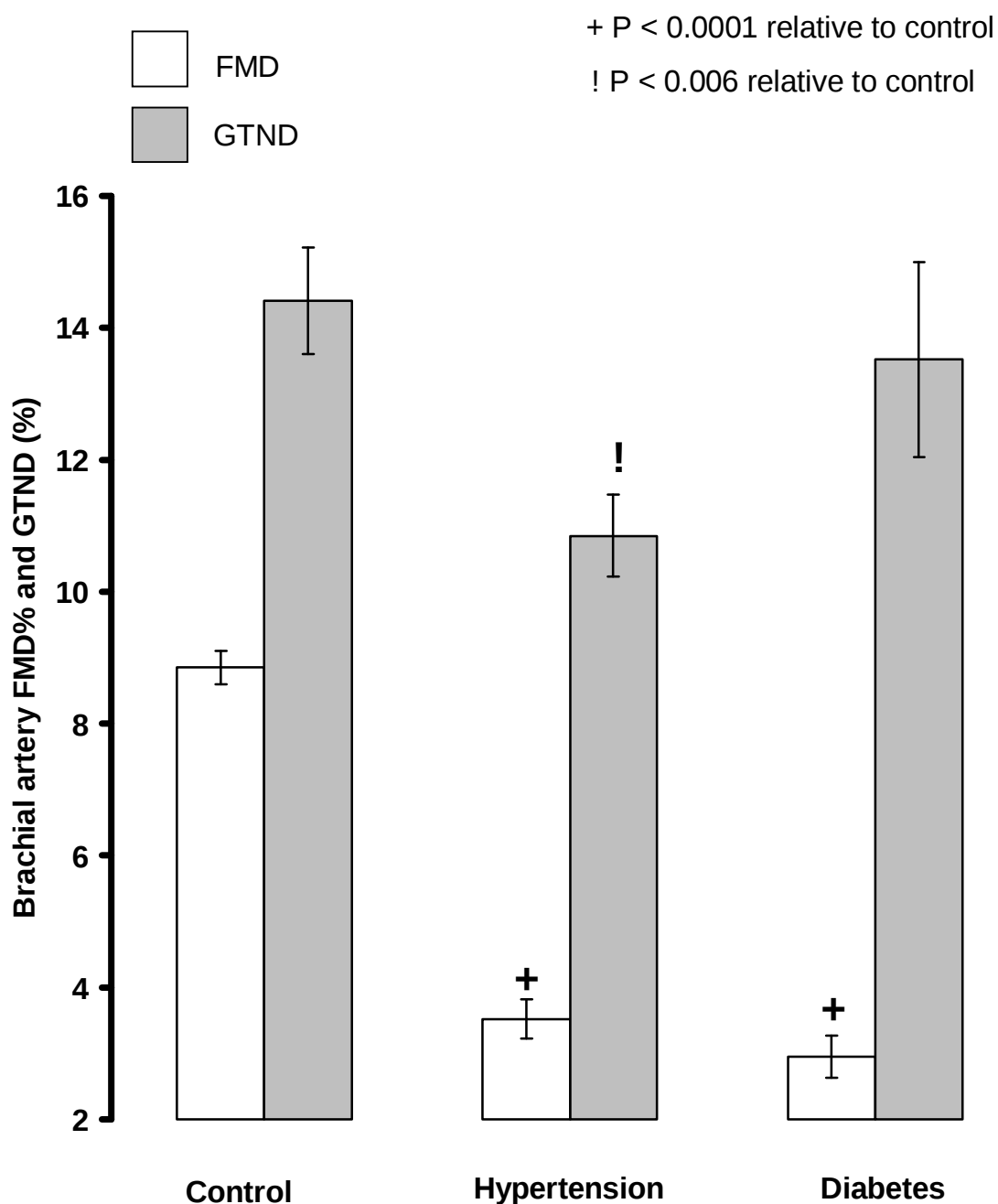


Figure 1:FMD% and GTND% in healthy subjects, hypertensive, and diabetic patients. Data are expressed as a mean $\pm$ SEM of 12-13 observations.

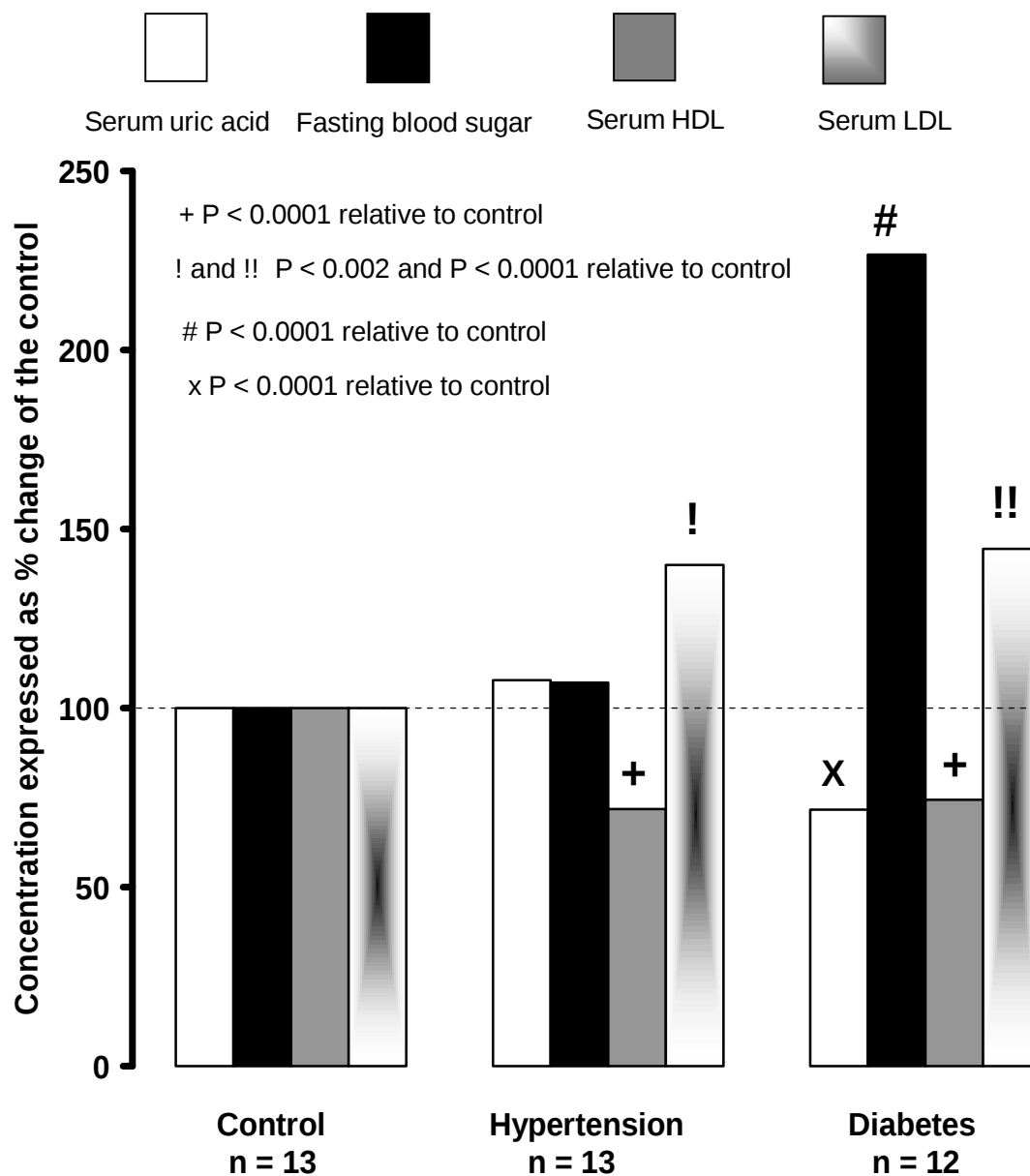


Figure 2: Percent changes of serum uric acid, fasting blood sugar, HDL, and LDL concentrations in healthy subjects, hypertensive, and diabetic patients.

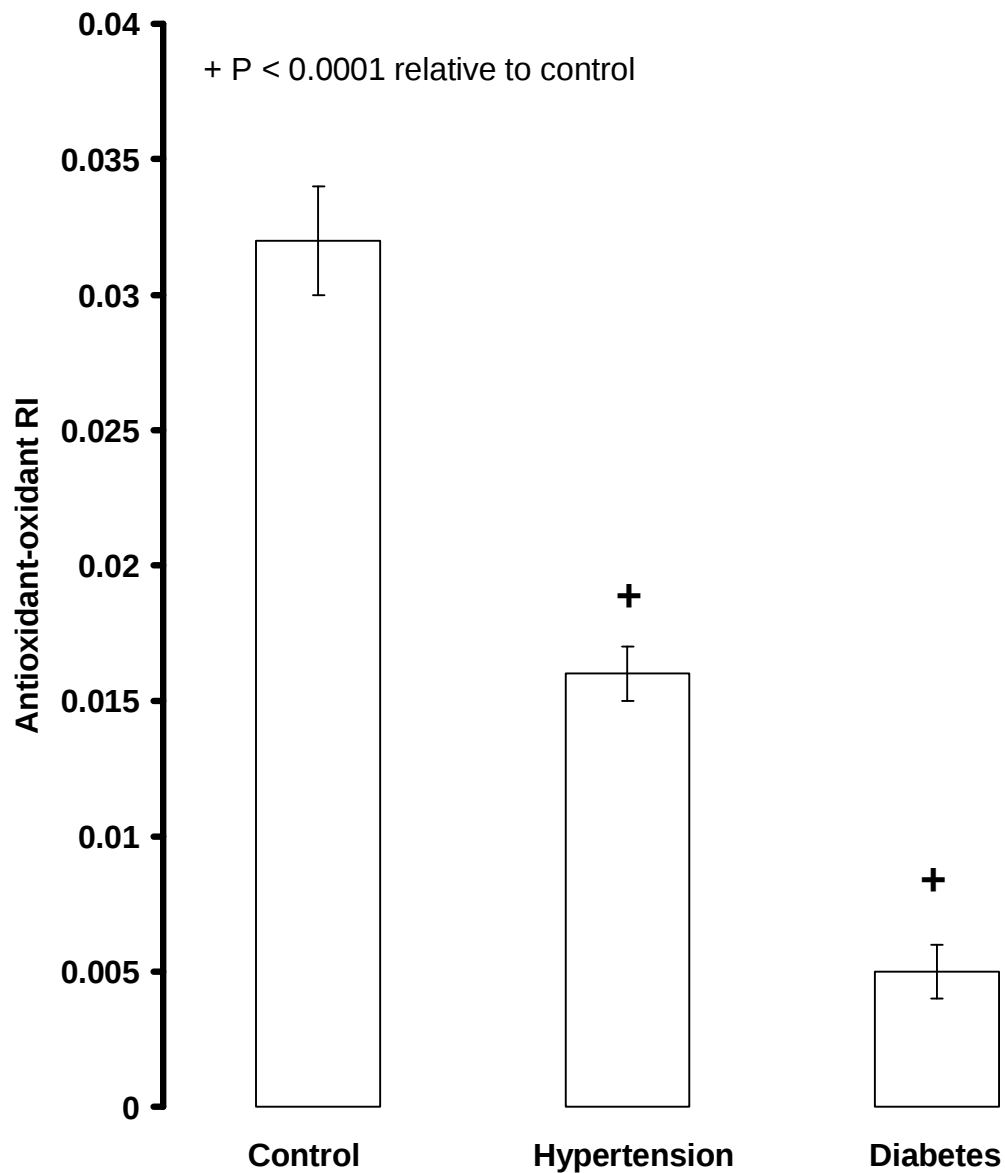


Figure 3: The antioxidant-oxidant RI level in healthy subjects, hypertensive, and diabetic patients. Data are expressed as a mean $\pm$ SEM of 12-13 observations.

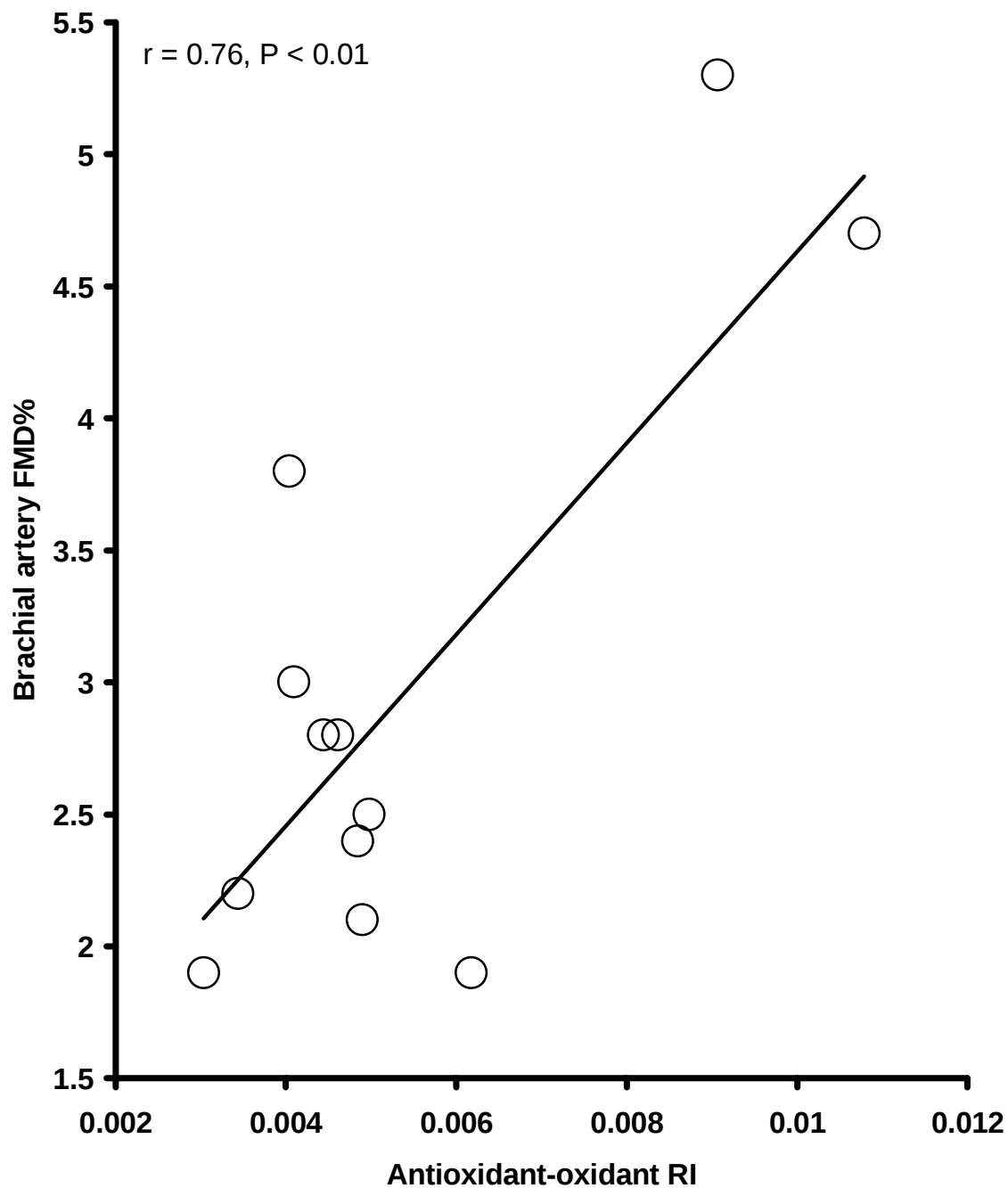


Figure 4: Correlation between antioxidant-oxidant RI and brachial artery FMD% in diabetic patients (n = 12).

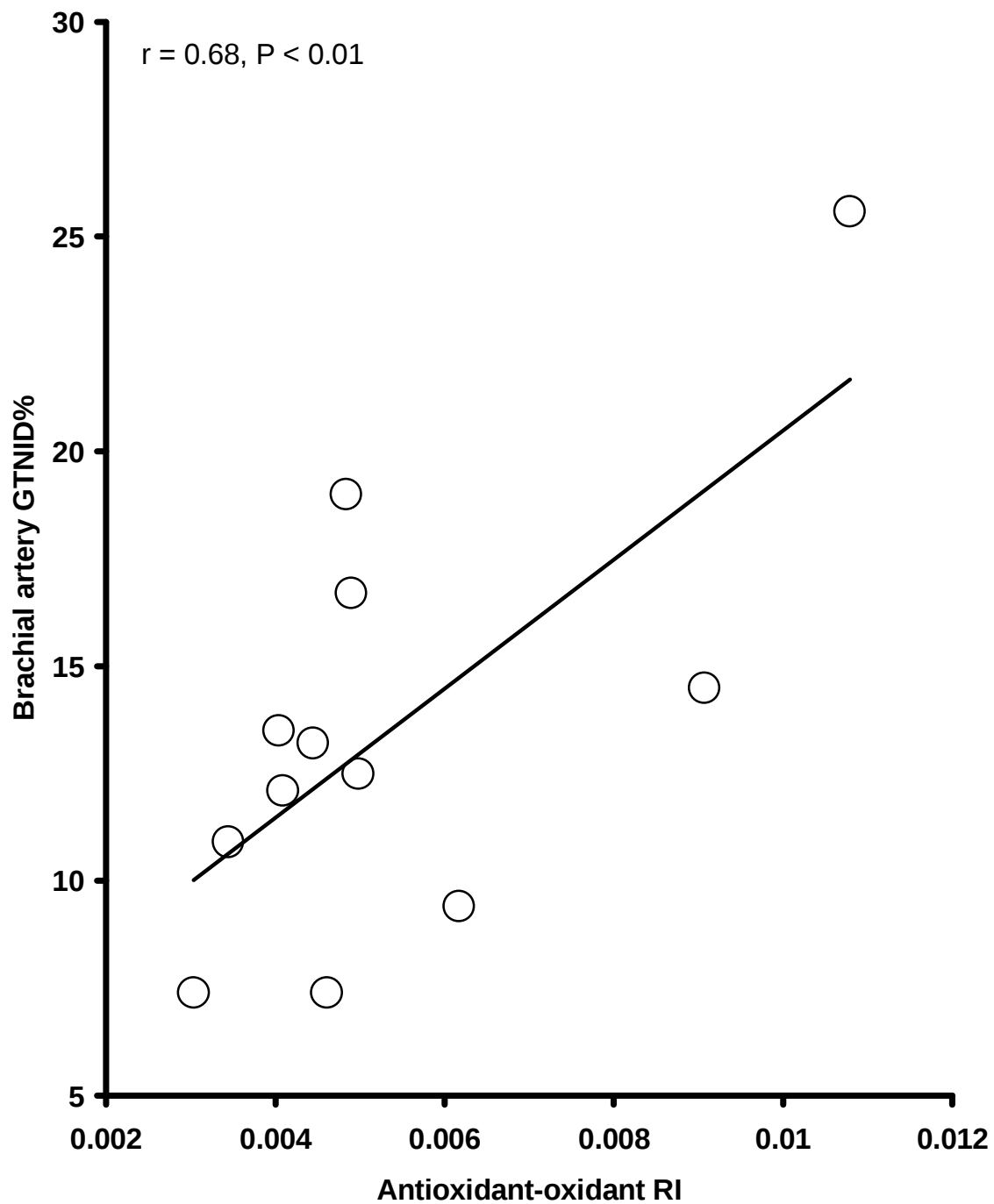


Figure 5: Correlation between antioxidant-oxidant RI and brachial artery GTNID% in diabetic patients (n=12).