# The Effect on Health of Some Cardiovascular Risk Factors

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*Arterial endothelium produces a large ramge of active factors which are indispensable for modulation of vasomotor tone and maintenance of vascular wall integrity. From these factors, nitric oxide (NO), wich is released by the endothelial cells as a response to acetylcholine or adenosine action on specific receptors, plays an important role.NO is the result of oxidation process of L-arginine into L-citrulline, under the action of endothelial nitric oxide synthase (NOSe), wich is activated by intracelluar $Ca^{2+}$ - calmodulin complex . Our study, performed in isolated organ bath, analyzed vascular reactivity of 12 guinea pigs' thoracic aorta rings. After phenylephrine -PHE $10^{-5}$ mol/L precontraction, the dose-effect curves for acetylcoline – ACH, adenosine 5' phosphate - 5'ADP and sodium nitroprusside – SNP were determined, before and after incubation of preparation, for 1 hour, with 5% hydrosoluble cigarettes smoke extract (CSE). Statistic analysis, performed with the use of t pair test and ANOVA parametric test, showed that incubation of vascular preparation with 5% CSE has increased the contractile response to PHE $10^{-5}$ mol/L ($p<0.05$), has reduced the endothelium-dependent relaxing response to ATP $10^{-5}$ mol/L ($p<0.001$) and 5'ADP $10^{-5}$ molo/L ($p<0.001$), but has not significantly modified the endothelium-independent relaxing response to SNP $10^{-5}$ mol/L ($p=0.05$). As a conclusion, vascular rings incubation with 5% CSE induced a decrease of endothelium NO synthesis under the action of AXH and 5'ADP, but did not change the smooth muscle fiber respomse in the presence of NO released by SNP.*

*Keywords: Arterial endothelium, acetylcholine, adenosine, L-arginine, L-citrulline, NOSe, phenylephrine*

Arterial endothelium is considered a mechanical and biological *barrier* between blood and vascular wall, and an *organ* that secretes active factors essential to modulation of vasomotor tonus and preservation of the integrity of vessel wall [1-3].

The most important of these factors is *nitric oxide* (NO) as results in the activation of *endothelial nitric oxide synthase* (NOSe) under the action of a variety of agonists, such as acetylcholine, adenosine, bradykinin, serotonin, etc.

Nose intracellular activation mechanism is based on the increase of $[Ca^{2+}]_{ic}$ to values between 70 and 100 nmol/L and formation of the $Ca^{2+}$ - calmodulin complex. The oxidation of L-arginine in L-citrulline under the action of NOSe requires the presence of NAD(P)H as an electron donor site and four enzyme cofactors: flavin-adenine dinucleotide - FAD, flavin mononucleotide - FMN, tetrahydrobiopterin – $BH_4$ and one hem group . NO diffuses into smooth muscle from endothelium, activates soluble guanylate cyclase (GCs) and causing relaxation of the muscle by increasing intracellular cGMP production [4-7].

Adenosine (5'ADP) acting on the endothelial $P_{2g}$ - purinergic receptors coupled to membrane phospholipase C by Gq protein that generates inositoltrifosfat ($IP_3$) and diacylglycerol (DAG). $IP_3$ stimulates the release of $Ca^{2+}$ from the endoplasmic reticulum, the increase in $[Ca^{2+}]_{ic}$, the $Ca^{2+}$-calmodulin complex formation and activation of NOSe. Vasodilatory effect is doubled by the opening of $K_{ATP}$ - dependent channels and membrane hyperpolarization.

Under the action of cardiovascular risk factors such as smoking, dyslipidemia, hypertension, diabetes etc. endothelial production of NO decreases defining *endothelial dysfunction* prior to development of atherosclerotic disease. *In vivo* and *in vitro* studies that follow the highlight of *endothelial dysfunction*, is based on two functional aspects: on the one hand decreased vasodilator response endothelial-dependent to acetylcholine or it responds paradoxically vasoconstrictor to acetylcholine, and on the other hand, the lack amending endothelium-independent vasodilator response to sodium nitroprusside (NPS) [8-11].

Our study was to assess the endothelial-dependent vasodilator response to adenosine in conditions of endothelial dysfunction induced by the direct action of water-soluble extract of cigarette smoke (EFT) on the arterial endothelium and evidenced by modification of vasodilator endothelial-dependent response to acetylcholine.

## Experimental part

### *Material and method*

### Study on thoracic aorta

We used a total of six guinea pigs, sex F, aged 6-8 weeks and weighing between 250 and 300 g, coming from Bucharest Cantacuzino Institute. From each preparation of thoracic aorta, collected after slaughter of the animals by intraperitoneal administration of sodium thiopental 50 mg/kg body weight, were obtained by 2 vascular rings, each with a width of 2-3 mm. They were placed in two organ baths containing 10 mL of Tyrode's solution with the following composition: NaCl 149.2 mmol/L, KCl 2.7 mmol/L, $NaHCO_3$ 11.9 mmol/L, $CaCl_2$ 1.8 mmol/L $MgCl_2$, 0.5 mmol/L, $NaH_2PO_4$ 0.4mmol/L and glucose 5.5 mmol/L. For balancing, vascular preparations were preloaded to a force 1.5cN for 60 min.

The cigarette smoke extract (EFT), representing the water-soluble component of cigarette smoke entirely introduced into Tyrode's solution has been obtained by adapting the methods described by literature. In brief, the smoke of 2 filter cigarette (0.8 mg tar and nicotine 0.6 mg/cigarette) was bubbled with a vacuum system, in 15 mL of Tyrode's solution preheated to $37^0$C, each cigarette being *smoked* in a period of 5 min.

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In the first step of the experiment was tested the control reactivity of the vascular rings to PHE, ACH, 5'ADP and NPS (Sigma Chemicals Co.) and then they were incubated for 1 hour with 5% EFT. After removing the incubation solution, the preparations were repeatedly washed with Tyrode's solution and vascular reactivity test was repeated.

For recording muscle tension variations was used an isometric force transducer model FORT 10 (World Precision Instruments, WPI Inc.). The results were graphically expressed by connecting the transducer to a data acquisition unit in computerized BIOPAC MP100 system and the data processing and rendering graphical was performed using the software *AQKNOWLEDGE* 3.72. [33-34]

PHE-induced contraction was expressed in absolute values (cN), and the relaxation induced by ACH or SNP as a relative value (%) of pre-contraction at PHE $10^{-5}$ mol/L. For statistical analysis of the effects induced by submaximal concentrations of reactants ($10^{-5}$ mol/L) was used *t* test pair and the parametric ANOVA test. The obtained values were considered significant for $p < 0.05$ [12].

Study on mammary artery

The study included 20 middle-aged patients: 58 ± 7 years of which 12 subjects were male and 8 subjects were female. All evaluated subjects (except the F (-) Control group) were smokers. The consumption of cigarettes has been appreciated in years. The value is calculated by multiplying the number of cigarette packets per day by the number of years of smoking. 1 packet per year is a pack of cigarettes (20 cigarettes) smoked daily for one year. The study excluded patients who smoked cigarettes from sheets (cigar, havana) or from the pipe.

The internal mammary artery was obtained during the coronary bypass surgery. After sampling, it was cleaned from adherent tissues, and then cut into circular rings. They were then suspended with two wires (one fixed and the other connected to a force transducer) in a Krebs-Henseleit organ bath (NaCl 118 mmol/L, KCl 4.7 mmol/L , $NaHCO_3$ 25 mmol/L, $CaCl_2$ 1.6 mmol/L, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L $KH_2PO_4$ and 11.1 mmol/L glucose, *p*H 7.4) at 37 °C. Using a micrometer, the vascular rings were preloaded at optimal tension.

Internal mammary artery fragments were cut into L = 2.5-3 mm rings and placed into two organ baths (V = 10 mL) (BIOPAC MP 100, System Inc, USA). With a FORT 10 isometric force transducer (World Precision Instruments Inc.), the isometric tension generated by the vascular rings (preloaded to 1.5 g of force and 1 hour calibration) was measured continuously, Krebs-Henseleit solution was changed over a period of 15 minutes ). The data obtained were processed using a BIOPAC AcqKnowledge software version 3.7.2 (BIOPAC System Inc. USA).

## Results and discussions

### *Thoracic aorta*

To study vascular reactivity was determined cumulative dose-response curves (concentrations ranging from $10^{-9}$ mol/L and $10^{-5}$ mol/L) to ACH, 5'ADP and NPS. The curves were obtained on the background precontraction with $10^{-5}$ mol/L PHE, with indomethacin $10^{-5}$ mol/L permanent presence in the bath.

*Vasoconstrictor response*

Incubation of the preparations with 5% EFT determined the increase of the contractile response to PHE $10^{-5}$ molL wich was for 3.59 ± 0.42 cN for batch incubated with 5% EFT compared to the control value of 2.96±0.36 cN ($p < 0.05$).

*Endothelial-dependent vasodilator response*

On the rings of guinea pigs incubated with 5% EFT and precontracted with $10^{-5}$ mol / L PHE, ACH led to reduced vasodilator response (fig. 1), and in the case of two of the preparations was obtained a paradoxical vasoconstrictor response. The reduction the vasodilator response was observed to all concentrations of ACH used (fig. 2A). The difference for submaximal concentration of $10^{-5}$ mol/L was statistically significant ($p < 0.001$).

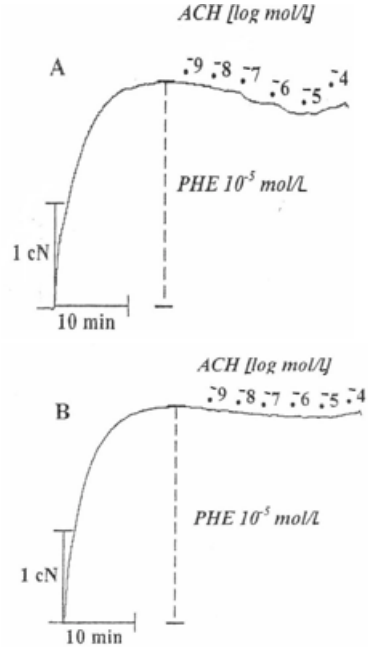


Fig. 1. Dose-effect curve to ACH ($10^{-9}$-$10^{-4}$ mol/L) on the background of precontraction with PHE $10^{-5}$ mol/L; A - control; B - after incubation with 5% EFT is observed the increasing of vasoconstrictor response to PHE and the decreasing of vasodilator response to ACH

In the presence of 5'ADP, the vasodilator response (fig. 2) was also decreased and the difference for submaximal concentration of $10^{-5}$ mol/L was statistically significant ($p < 0.001$)

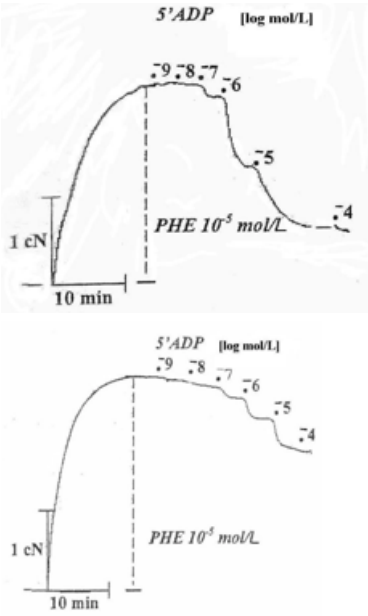


Fig. 2. Dose-effect curves to 5'ADP ($10^{-9}$-$10^{-4}$ mol/L) on the background of precontraction with PHE $10^{-5}$ mol/L; A - control; B - after incubation with 5% EFT is observed the increasing of vasoconstrictor response to PHE and the decreasing of vasodilator response to 5'ADP

Comparative analysis of vascular response at the submaximal doses of ACH and 5'ADP revealed the greater amplitude of the vasodilator response to 5'ADP at the control (64.61 ± 20.14%) and after incubation with EFT (24.15 ± 8.24%), compared to ACH (20.90 ± 9.45% at the control and 5.72 ± 3.80% after EFT incubation).

*Endothelial-independent vasodilator response*

Between NPS induced relaxation before and after incubation with 5% EFT, no significant difference ($p = 0.05$) was found, this being for the submaximal dose (NPS $10^{-5}$ mol/L) of 98.91 ± 17.20% for control and 87.50 ± 9.13% after incubation.

*Mammary artery*

The reactivity of the internal mammary artery vascular ring was evaluated in the organ bath. Breast artery fragments were obtained from aortocoronarian bay-pass surgery in patients with coronary vascular disease diagnosed by coronary artery (n = 12). The data obtained by measuring the reactivity of the artery rings in the F (-) CI (+) and F (+) CI (+) lots are listed in table 1 and table 2.

By statistical processing of the obtained data, we noticed that changes in the vasoconstrictor response to PHE $10^{-5}$M were not observed between the two batches (table 1). Moreover, neither the endothelial-independent vasodilator-independent response to NPS was significantly altered when comparing the two batches (table 1). In contrast, the endothelial-dependent vasodilatory response of 5'-ADP was significantly altered, the values obtained being lower in the group F (+) CI (+) compared to the group F (-) CI (+) (fig. 4).

By processing the data presented in table 2 we observed a decrease in the vasodilator response to 5'ADD for all doses used, except for the $10^{-9}$M dose, in the F (+) CI (+) vs. F (-) Cl (+). Statistical analysis of data demonstrated a significant decrease in endothelial - dependent vasodilator

| Hill Parameters | F(-)CI(+) (n = 6) | F(+)CI(+) (n = 6) |
|---|---|---|
| **Precontraction induce by PHE $10^{-5}$ M** | | |
| $E_{max}$ (g force) | 1.29 ± 0.09 | 0.43 ± 0.21 |
| $EC_{50}$ (-Log[M]) | 6.16 ± 0.55 | 5.94 ± 0.20 |
| **Endothelial-dependent relaxation induce by 5'ADP (%)** | | |
| $E_{max}$ (%) | 69.33 ± 3.14 | 40.02 ± 13.72 |
| $EC_{50}$ (-Log[M]) | 6.67 ± 0.81 | 4.04 ± 0.61 |
| **Endothelial-independent relaxation induce by NPS (%)** | | |
| $E_{max}$ (%) | 97.61 ± 2.97 | 95.45 ± 1.74 |
| $EC_{50}$ (-Log[M]) | 8.70 ± 0.26 | 8.05 ± 0.13 |

**Table 1**
PARAMETERS OF VASCULAR REACTIVITY

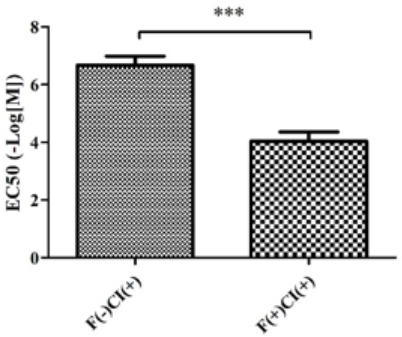


Fig.3.

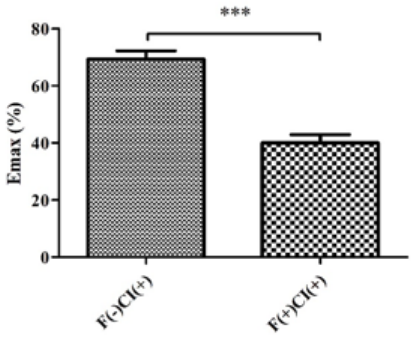


Fig.4. . Endothelial-dependent vasodilator response to 5 'ADP (***: $p < 0.001$)

| Parameters | F(-)CI(+) (n = 6) | F(+)CI(+) (n = 6) |
|---|---|---|
| **Precontraction induce by PHE $10^{-5}$ M** | | |
| g forţă | 3.91 ± 1.09 | 3,78 ± 1,45 |
| **Endothelial-dependent relaxation induce by 5'ADP (%)** | | |
| $10^{-9}$M | 0 | 0 |
| $10^{-8}$M | 2.13 ± 0.63 | 1.34 ± 0.59 |
| $10^{-7}$M | 6.06 ± 1.98 | 4.14± 1.59 |
| $10^{-6}$M | 16.43 ± 4.81 | 10.14 ± 2.94 |
| $10^{-5}$M | 28.37 ± 7.81 | 14.93± 1.75 |
| $10^{-4}$M | 39.72 ± 5.23 | 22.18 ± 3.04 |
| **Endothelial-independent relaxation induce by NPS (%)** | | |
| $10^{-9}$M | 4.31 ± 2.53 | 1.11 ± 1.24 |
| $10^{-8}$M | 28.93 ± 12.58 | 23.45 ± 8.45 |
| $10^{-7}$M | 49.24 ± 16.51 | 37.08 ± 5.24 |
| $10^{-6}$M | 70.05 ± 11.82 | 59.02 ± 7.94 |
| $10^{-5}$M | 86.29 ± 13.74 | 75.58 ± 10.32 |
| $10^{-4}$M | 100 | 90.14 ± 4.95 |

**Table 2**
CHANGES IN VASCULAR REACTIVITY IN PREPARATIONS OBTAINED FROM GROUP F (-) CL (+) AND F (+) CL (+)

response and endothelial - independent (NPS - induced) vasodilator response for F (+) CI (+) vs. F (+) preparations. F (-) CI (+) ($p \leq 0.01$) (fig. 5,6).

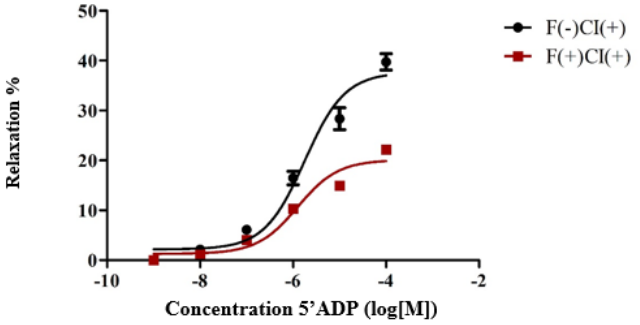


Fig. 5. Dose-effect curve for 5'ADP for vascular rings from F (-) CI (+) and F (+) CI (+) groups

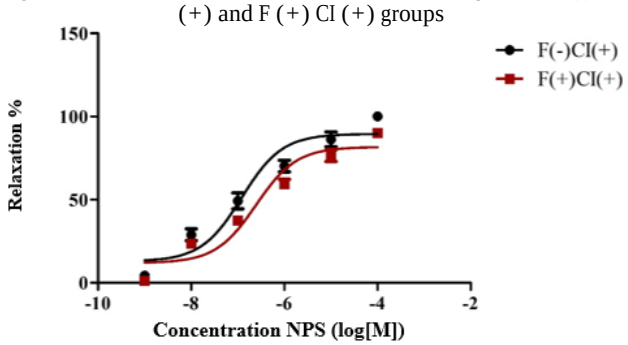


Fig. 6. Dose-effect curve for NSP for vascular rings from F (-) CI (+) and F (+) CI (+) groups

*Assessment of the effects of smoking on flux-mediated vasodilation*

All subjects under study were smokers except the F (-) Control and F (-) CI (+) groups. The consumption of cigarettes was appreciated in packages - years (1 packet - year = 20 cigarettes / day for 1 year).The values obtained from the study are presented in table 3.

Following the analysis of the data obtained, a significantly higher consumption of cigarettes in the F (+) CI (+) group compared to the F (+) Control group ($p \leq 0.05$) was observed.

In this study we determined FMD as a marker of endothelial dysfunction in the studied groups to observe the influence of smoking on this parameter. Based on the statistical analysis of the data obtained, we observed a significant decrease in the FMD value in the F (+) CI (+) vs. F group. Lots F (-) Control and F (+) Control (* #: $p \leq 0.001$). In group F (-) CI (+) we noticed a significant decrease in FMD vs. F (-) Control (*: $p \leq 0.001$), instead of comparing the values with those of the F (+) Control group, we found no significant changes (ns). Moreover, FMD values for the F (+) Control group were significantly lower compared to the F (-) Control group (*: $p \leq 0.001$) (fig. 7).

After evaluating the FMD values of the studied groups vs. Control, we also followed the comparison of the groups with patients with ischemic cardiopathy, smokers vs. Non-smokers, to see if there are significant differences between values, differences arising from smoking. Thus, we noticed a more pronounced (significant) decrease in FMD values for the F (+) CI (+) group compared to the group F (-) CI (+) ($p \leq 0.05$) (fig.8).

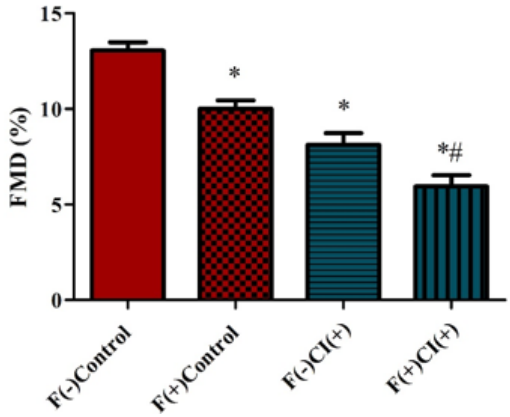


Fig. 7. Comparison of FMD values in studied lots, *: $p \leq 0.001$ vs. F (-) Control and #: $p \leq 0.001$ vs. F (+) Control

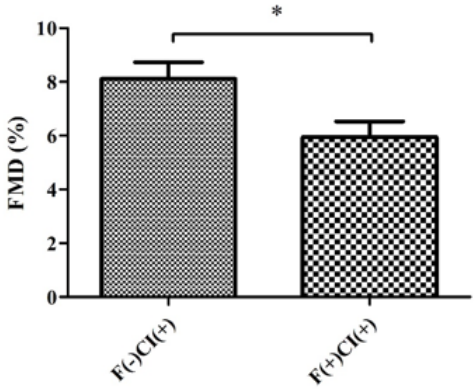


Fig.8. Comparison of FMD values in group F (-) CI (+) vs. F (+) Cl (+), *: p & lt; 0.05

Moreover, we have also followed the correlation between the number of years and the FMD in the F (+) CI (+) group to see if smoking is responsible for FMD modification (fig. 9).

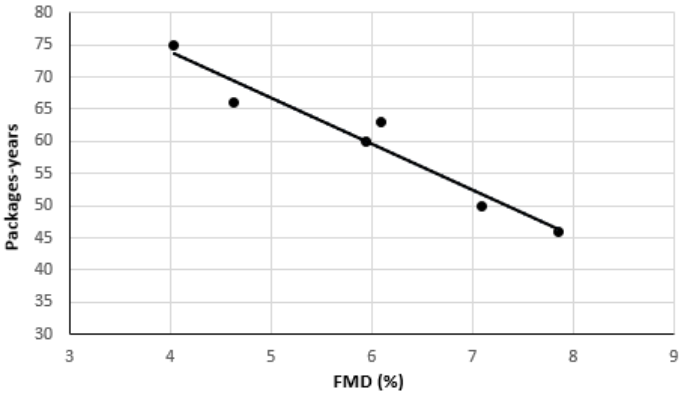


Fig. 9. Correlation between cigarette consumption and FMD in group F (+) CI (+)

*Thoracic aorta*

Our results on guinea-pig vascular rings showed that endothelial-dependent relaxation mediated by NO release was diminished as a result of EFT incubation. The permanent presence in the bath of indomethacin, a potent inhibitor of prostacyclin production ($PGI_2$) via the cyclooxygenase pathway, excluded the possibility that EFT induces endothelial dysfunction of another potent endothelial-dependent vasodilator factor.

| Parameters | F(-)Control (n = 4) | F(+)Control (n = 4) | F(-)CI(+) (n = 6) | F(+)CI(+) (n = 6) |
|---|---|---|---|---|
| Packages - years | - | 45 ± 8.00 | - | 60 ± 15 |
| FMD (%) | 13 ± 1.03 | 10 ± 0.93 | 8.11 ± 2.02 | 5.95 ± 1.91 |
| IMC (kg/m²) | 24 ± 4.12 | 23 ± 2.95 | 21.8 ± 3.09 | 22 ± 3.56 |

**Table 3**
MEDIAN VALUES OF FLUX-MEDIATED VASODILATION (FMD) IN THE STUDIED GROUPS

From the perspective of numerous literature data [13-17] on the mechanism of development of endothelial dysfunction following acute and chronic exposure to cigarette smoke we can consider that the decrease in the NO-dependent vasodilator response was the result of the action of at least two factors: endothelial absorption of some components of the smoke such as nicotine, and the action on the endothelium of a complex mixture of 4700 chemical compounds, such as reactive oxygen species, as well as other organic and inorganic oxidation compounds that can easily affect an unstable molecule such as NO [18-20].

Increasing the contractile response to PHE may be the expression of a decrease in NO production because stimulation of $\alpha$1-adrenergic receptors is followed by activation of NOSe by a $Ca^{2+}$-dependent mechanism. Appropriate NO production is within a *vasomotor balance*, according to which a vasoconstrictor factor stimulates the compensatory release of a vasodilator factor such as NO or adenosine.

Also within this *vasomotor balance*, the paradoxal vasoconstrictor effect of ACH at the maximum dose of $10^{-4}$ mol/L (fig. 1) can be discussed, but should be differentiated from that at any dose in endothelial dysfunction.

Both effects can be explained by the predominant distribution of M1-type cholinergic receptors in the vascular wall. They are coupled by the Gq11 protein with PLC which causes the growth of [$Ca^{2+}$] in the inositol phosphate (IP3) pathway. ACH-dependent vasomotor balance is provided on the one hand by M1 receptors expressed by the endothelial cell whose stimulation has a predominant vasodilator effect by activating NOS and endothelial NO production and, on the other hand, M1 receptors expressed by smooth muscle fiber Whose vascular stimulation results in a direct vasoconstrictor effect. ACH - dependent vasomotor disbalance is established when the endothelial component is depleted (maximum dose) or deficient (endothelial dysfunction) [21-25].

In contrast with ACH, on thoracic guinea pig aorta, 5'ADP did not result in a paradoxical vasoconstrictor response at the maximum dose or in endothelial dysfunction preparations.

From the experimental point of view, namely the study of vascular reactivity in the isolated organ bath, the obtaining of a vasodilatory response to 5'ADP as well as the lack of vasoconstrictor response, are advantageous aspects for evaluation of endothelial dysfunction. Thus, in our opinion, evaluation of endothelial dysfunction based on the vasodilator response of adenosine has the same practical significance as that of classical vascular response to ACH [26-29].

*Mammary artery*

In the present study we assessed the vascular reactivity of breast artery preparations in two groups: F (-) CI (+) - non-smokers with ischemic cardiopathy and F (+) CI (+) - smokers with ischemic cardiopathy to observe the differences between And assess the impact of smoking on this disease. Individuals with ischemic heart disease have an endothelial dysfunction manifested by decreased vascular reactivity.

However, in the evaluation of dependent and independent edotelic relaxation and phenylephine-induced contraction, we observed significantly lower values in the F (+) CI (+) group compared to the F (-) Cl (+) group. These results indicate a worsening of endothelial-dependent and independent response in CI smokers. Non-smoking with CI. There is little data available that clearly shows endothelium-dependent vasomotor responses to smokers, the existing data being contradictory [30-33]

Clearly, there is a complex relationship between them, other than the mere appearance of a direct lesion [34]. A possible explanation could be the "upregulation" of the antioxidant capacity of vessels exposed to different toxins, such as those in cigarette smoke, which can result in a higher response in the organ bath. It has been found in aortic rodent models that smoking may promote vascular endothelial re-uptake, however, on human cells, a decrease in cigarette smoke exposure has been found. Other studies demonstrate a dose-dependent association between smoking and the potentially reversible dysfunction of endothelium-dependent vasodilation, an effect responsible for endothelial dysfunction [18]. It has been found that smoking causes a decrease in endothelium-dependent relaxation of the brachial artery [18]. Other contradictory studies claim that smoking increases endothelium-dependent relaxation but does not have an effect on endothelium-independent relaxation.

The present study demonstrates the decrease in both endothelium dependent and independent endothelial relaxation, suggesting the presence of endothelial dysfunction in smokers with ischemic cardiomyopathy compared to patients with non-ischemic cardiomyopathy, which strengthens the role of inducer of endothelial dysfunction in smoking people.

In the present study, we aimed to determine the flux-mediated vasodilatation (FMD) in people with smoker and non-smoker ischemic cardiopathy as compared to the number of patients and clinically healthy non-smokers in order to assess the implication of smoking in decreasing this parameter and implicitly in the occurrence of endothelial dysfunction. Based on the statistical interpretation of the values obtained, we noticed a decrease in the FMD value in the groups F (-) CI (+), F (+) CI (+) and F (+) Control vs. Lot F (-) Control. These data demonstrate the alteration of endothelial function in smokers and non-smokers with ischemic heart disease compared to healthy non-smokers. The results obtained are not surprising as ischemic heart disease itself has occurred due to endothelial dysfunction. However, when comparing control groups (smokers and non-smokers), we noticed a decrease in FMD value associated with early endothelial dysfunction in the smoker group compared to the non-smoker group, which suggests the direct involvement of smoking in the development of endothelial dysfunction, And in the absence of other pathologies. These results are supported by other studies demonstrating that smoking is associated with a decrease in FMD [35].

Since endothelial dysfunction occurs itself in patients with ischemic cardiopathy, we compared FMD values in smokers and non-smokers with ischemic cardiopathy to observe only the effect of smoking on FMD values. We have achieved a significant decrease in these values in people with ischemic cardiopathy smokers vs. Non-smoking, a result that once again suggests the potent impact of smoking on endothelial function.

**Conclusions**

Endothelial dysfunction is a complex pathogenic mechanism that involves a large number of incompletely elucidated aspects, but which is the common mechanism by which various cardiovascular aggression factors such as systemic action, such as smoking, determine the early development of atherosclerotic disease.

The study of the vasomotor function of guinea-pig aortae endothelium rings represents an experimentally

reproducible model for in vitro investigation of mechanisms involved in endothelial dysfunction induced by acute exposure to cigarette smoke.

The use of endothelial vasodilator response to adenosine has been shown a method reverse shuttle, viable and advantageous to identify the endothelial dysfunction induced by cigarette smoke. It also creates the premises for the development of new experimental models of endothelial function study, which may for example involve the role of adenosine and -dependent K + channels in the membrane of endothelial dysfunction induced by other cardiovascular risk factors or ischemic preconditioning.

Among the results obtained, we observed a decrease in the endothelium-dependent and endothelium-independent vasodilator response in smokers with ischemic cardiopathy compared to non-smokers with ischemic cardiopathy, suggesting that smoking is responsible for exacerbating endothelial dysfunction in these patients.We also followed the assessment of flux-mediated vasodilation (an early marker of endothelial dysfunction) in smokers vs. patients. Non-smokers and smokers with ischemic cardiopathy. Non-smokers with ischemic heart disease. After interpreting the results we noticed the installation of endothelial dysfunction in smokers and non-smokers with ischemic heart disease. Non-smoking (clinically healthy) outcome predictable. However, we noticed a significant decrease in FMD in control smokers compared to non-smokers control, which directly demonstrates the alteration of endothelial function by smoking. We also followed the correlation of FMD with the number of years (cigarettes smoked in one year) in patients with ischemic cardiopathy and we noticed a strongly negative correlation of these, thus demonstrating that a decrease in the number of cigarettes improved the endothelial function . Moreover, we also noticed a sharp decrease in FMD in smokers with ischemic cardiopathy compared to non-smokers with ischemic cardiopathy, which suggests the important effect of smoking on the increase of endothelial dysfunction in patients with ischemic cardiopathy.

Also, in some future studies, we will investigate bioactive compounds used in cardiovascular pathologies, according to protocols peviously published [36-42].

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