Brachial Artery Flow-Mediated Dilation in Healthy Men, Men with Risk Factors, and Men with Acute Myocardial Infarction. Importance of Occlusion-Cuff Position

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\textbf{Introduction and objectives.} Flow-mediated dilation (FMD) is endothelium-dependent and can be assessed by ultrasound in the brachial artery. We sought to determine the most suitable position for the occlusion cuff for the study of FMD in three groups of adult men.

\textbf{Subjects and methods.} We included 160 subjects, mean age 58.5 ± 7.8 years: 40 healthy subjects, 80 with cardiovascular risk factors, and 40 patients with AMI. In a subgroup of 60 subjects, the first 10, 30, and 20 of each group, respectively, FMD was evaluated twice, after upper arm occlusion and forearm occlusion to induce hyperemia.

\textbf{Results.} In the initial substudy, the FMD after upper arm occlusion was 7.6 ± 2.4% in healthy subjects, 5.1 ± 2.2% in men with risk factors (p < 0.0001), and 3.5 ± 2.2% in AMI patients (p < 0.041, with respect to the risk-factor group). FMD after forearm occlusion was 4.6 ± 1.5%, 2.3 ± 2.1% (p < 0.006), and 2.2 ± 1.9%, respectively, with no significant statistical differences between the risk-factor and AMI groups. Only upper arm occlusion was performed in the remaining participants, as planned, because it provided the most accurate information. Overall, the FMD was, respectively, 7.8 ± 3.1%, 5 ± 2.6% (p < 0.0001) and 3.3 ± 3% (p < 0.004, with respect to the risk-factor group). FMD was directly related to HDL cholesterol and inversely related to resting diameter and number of risk factors.

\textbf{Conclusion.} The best approach to studying FMD is proximal occlusion since it allows for a better stratification of men with endothelial dysfunction. With this technique, a worsening of endothelial function in acute myocardial infarction can be demonstrated.

\textbf{Key words:} Endothelium. Vasodilation. Risk factors. Ischemic heart disease.

Dilatación de la arteria humeral mediada por flujo en varones sanos, con factores de riesgo e infarto agudo de miocardio. Importancia de la posición del manguito oclusor

\textbf{Introducción y objetivos.} La dilatación mediada por flujo (DMF) es dependiente del endotelio y puede estudiarse con ultrasonidos en la arteria humeral. Quisiéramos conocer la localización idónea de la oclusión arterial para analizar adecuadamente la DMF en tres grupos de varones adultos.

\textbf{Sujetos y métodos.} Se incluyó a 160 sujetos, con edad media de 58.5 ± 7.8 años: 40 sujetos sanos, 80 con factores de riesgo cardiovascular y 40 pacientes con IAM. En un subgrupo de 60 sujetos –los primeros 10, 30 y 20 de cada grupo, respectivamente– se evaluó la DMF por duplicado, tras oclusión en el brazo y en el antebrazo para inducir la hiperemia.

\textbf{Resultados.} En el subestudio inicial, tras oclusión proximal, la DMF fue 7.6 ± 2.4% en sujetos sanos, 5.1 ± 2.2% en sujetos con factores de riesgo (p < 0.0001), y 3.5 ± 2.2% en pacientes con IAM (p < 0.041 respecto al grupo con factores de riesgo). La DMF tras compresión distal fue respectivamente: 4.6 ± 1.5%, 2.3 ± 2.1% (p < 0.006), y 2.2 ± 1.9%, sin diferencias estadísticamente significativas entre los grupos con factores de riesgo e IAM. En el resto se evaluó la DMF mediante compresión proximal por aportar datos más precisos, según estaba previsto. Globalmente, la DMF en los 160 sujetos estudiados fue 7.8 ± 3.1%, 5 ± 26% (p < 0.0001) y 3.3 ± 3% (p < 0.004, respecto al grupo con factores de riesgo), respectivamente. La DMF se relacionó directamente con el cHDL e inversamente con el diámetro basal y número de factores de riesgo.

\textbf{Conclusión.} La oclusión proximal es el método óptimo para estudiar la DMF y conseguir una mejor estratificación de los sujetos con disfunción endotelial. Esta técnica permite demostrar que en el IAM se produce un empeoramiento de la función endotelial.

ABBREVIATIONS

FMD: Flow-mediated dilatation.
CRF: cardiac risk factors.
AMI: acute myocardial infarct.
C-HDL: high density lipoprotein cholesterol.
C-LDL: low density lipoprotein cholesterol.
ON: nitric oxide.

INTRODUCTION

Endothelial dysfunction entails less local availability of nitric oxide (NO). In addition to its vasodilator action, NO inhibits plaque aggregation, the proliferation of smooth muscle cells, and the interaction of leukocytes with the endothelium. In the initial phase of coronary atherosclerosis, paradoxical vasoconstriction has been observed in response to acetycholine, and an alteration of flow-mediated dilatation (FMD) following papaverin infusion; said alteration is dependent on the endothelium, as has been shown in dogs and in humans. In the peripheral arteries, such as the humeral artery, high-resolution echocardiography has permitted measurement of FMD in a simple and reproducible manner. The endothelial function in the humeral artery has been related to coronary endothelial function and the severity of the lesions. Changes in FMD have been described not only in the presence of classic risk factors, but also in children of 6 years of age with familiar hypercholesterolemia, second-hand smoke exposure, an increase in homocysteine, tetrahydrobiopterin deficiency, and even in healthy children with a family history of early ischemic heart disease.

Although it is a proven technique for non-invasive measurement of endothelial function, dilatation percentages vary considerably among published studies due to the variability inherent in the technique, the characteristics of the patients evaluated, and the method used to increase the flow, with a greater or lesser degree of hyperemia and ischemia in the area of measurement.

Our study was designed to establish the FMD numbers in middle-aged healthy men with cardiovascular risk factors (CRF) and in patients with AMI, and to determine the relationship between FMD and other variables. Given the probable influence of the location of the flow occlusion in the upper part of the arm or in the forearm on FMD, we included an initial sub-study limited to 60 subjects to find out what technique better differentiated the 3 groups and to be able to use this information for the rest of the participants, as well as in future studies of therapeutic intervention.

METHODS

Study groups

We included in our study 160 male subjects between the age of 45 and 70 years, divided into 3 groups. The group of healthy subjects was composed of 40 volunteers without cardiac risk factors, and the group with CRF was made up of 80 men, of which 40 had a single CRF (10 subjects with one of each of the CRF included: hypertension, hypercholesterolemia, smoking, and diabetes mellitus), and the other 40 had 2 or more CRF. There were 40 patients included in the group with AMI. The healthy subjects and the subjects with CRF were volunteers recruited from among hospital personnel, patient family members, and clinic patients. Before inclusion in the study, each study participant underwent an anamnesis, physical examination, ECG, and blood work that included a lipid profile and fasting glucose test. We ruled out ischemic heart disease in those subjects who presented with risk factors on treadmill stress test that was limited by symptoms. We excluded candidates taking angiotensin converting enzyme inhibitors (ACEI), nitrates, hypolipemians or antioxidants. Other vasodilatation medications were withdrawn 24 hours prior to the echocardiography study, with the exception of 6 patients with hypertension who were taking beta-blockers or calcium antagonists and 8 patients with AMI treated with angiotensin converting enzyme inhibitors (ACEI) or beta-blockers. Patients were not permitted to smoke 3 hours prior to the examination. The measurements of patients with AMI were made between the fourth and eighth day of evolution, after being asymptomatic for 48 hours. The study was approved by the hospital’s committee of ethics and clinical investigation, and all the participants signed an informed consent form.

Cardiovascular risk factors

We considered smokers those subjects who had a history of smoking 10 packs or more of cigarettes a year, and we defined as non-smokers those who had abstained from smoking for 10 years. Patients were considered to have hypercholesterolemia who had an C-LDL>150 mg/dL and were not receiving hypolipemiant treatment. We considered hypertensive those patients with an arterial pressure (AP) >140/90 mm Hg at the time of the study who had had a known history of hypertension. The criterion for diabetes mellitus was a fasting glucose of >126 mg/dL.

Blood chemistry

All blood samples were collected during a period of fasting. For patients with AMI the sample was drawn during the first 24 hours after admission. The parameters tested were glucose, total cholesterol, high density cholesterol, C-HDL, C-LDL, triglycerides, and fasting glucose.
TABLE 1. Characteristics of the study groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Subjects without CRF (n=40)</th>
<th>Subjects with CRF (n=80)</th>
<th>Subjects with AMI (n=40)</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>59±7</td>
<td>58±8</td>
<td>57±8</td>
<td>.98</td>
<td>1.00</td>
<td>.67</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>196±28</td>
<td>229±42</td>
<td>210±41</td>
<td>&lt;.001</td>
<td>&lt;.037</td>
<td>.34</td>
</tr>
<tr>
<td>C-LDL, mg/dL</td>
<td>115±24</td>
<td>148±37</td>
<td>136±37</td>
<td>&lt;.001</td>
<td>.24</td>
<td>&lt;.023</td>
</tr>
<tr>
<td>C-HDL, mg/dL</td>
<td>54±12</td>
<td>52±14</td>
<td>41±9</td>
<td>.94</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>132±66</td>
<td>168±138</td>
<td>162±55</td>
<td>.25</td>
<td>1.00</td>
<td>.64</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>95±12</td>
<td>119±39</td>
<td>104±19</td>
<td>&lt;.001</td>
<td>.12</td>
<td>.62</td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>4.6±0.6</td>
<td>5.1±0.7</td>
<td>4.9±0.7</td>
<td>&lt;.003</td>
<td>&lt;.004</td>
<td>1.00</td>
</tr>
<tr>
<td>Arm circumference, cm</td>
<td>27±3</td>
<td>28±2</td>
<td>28±3</td>
<td>.31</td>
<td>1.00</td>
<td>.91</td>
</tr>
<tr>
<td>FMD, %</td>
<td>7.8±3.1</td>
<td>5±2.6</td>
<td>3.3±2.9</td>
<td>&lt;.001</td>
<td>&lt;.004</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Data expressed as mean±standard deviation. p1 indicates comparison of subjects with and without risk factors; p2, comparison of subjects with risk factors and patients with AMI; p3, comparison of subjects without risk factors and patients with AMI; CRF, cardiovascular risk factors; FMD, flow-mediated dilatation.

Study protocol

Ultrasound is a widely accepted technique for the measurement of FMD of the humeral artery. The images were obtained with a Hewlett-Packard Sonos 2500 unit and a 7.5 MHz transducer. After optimizing gain and depth to identify the arterial lumen-wall interface, the image underwent zoom magnification. These parameters were kept constant during the study. We visualized the right humeral artery longitudinally between 2 and 8 cm above the antecubital flap. Measurements were made to coincide with the ECG R-wave, between the anterior and posterior mid-adventitia, averaging 3 consecutive cardiac cycles. After obtaining the best definition of the arterial wall, we marked off the exact area with adhesive strips on the skin and took a baseline measurement. The circumference of the arm was also measured at the same point.

Reactive hyperemia was provoked by inflating a pneumatic sleeve to 220 mm Hg for 4 and a half minutes and then deflating it. After deflation, we measured the arterial diameter at 60 seconds and 90 seconds to determine the maximum post-hyperemia diameter. FMD was expressed as the percentage of change in the diameter from the baseline measurement.

In the first 60 individuals (10 healthy subjects, 30 subjects with CRF, and 20 subjects with AMI) we took 2 measurements 30 minutes apart randomly in half of the subjects in each group, after proximal occlusion of the upper arm and then after distal occlusion of the forearm, or vice versa. We also calculated the resting blood flow in these subjects 15 seconds after deflation, multiplying the area of the arterial segment by the flow integral.

Reactive hyperemia was considered to be present when a high rate of increase in the flow was observed with respect to baseline.

Images were recorded on super-VHS tape by a researcher and the measurements were made by 2 observers (who did not know the subjects’ individual characteristics) on the same recorded image and during the same cardiac cycles, and then both measurements were averaged. The studies were performed between 17 hours and 19 hours after a post-ingestion period of more than 2 and half hours.

Statistical analysis

Data was expressed as mean±standard deviation (SD). The comparison of the measurements from each group was determined by variance analysis (ANOVA) and the post hoc Bonferroni test. We performed linear regression analysis for univariate correlations and multiple regression analysis in which FMD was the dependent variable, and we considered the following as independent variables: glucose, total cholesterol, C-HDL, C-LDL, triglycerides, baseline diameter, arm circumference, and the number of risk factors present. A difference of $P<.05$ was considered significant.

RESULTS

The characteristics of the participants are shown in Table 1. In the group with AMI, the number of risk factors was 1.6±0.9, of which 22% were hypertensive; 72%, smokers; 20%, diabetic; and 42%, dyslipemic. In the group with risk factors the number was similar: 1.7±0.8. The age of the subjects was the same in all 3 groups. The total cholesterol and the C-LDL were higher in the group with CRF than in the other 2 groups as this was a pre-established criterion for inclusion in the group. The C-HDL was lower in the group with AMI. There was no difference in the triglyceride values. The largest baseline diameter was found in subjects with FRC. The baseline diameter was directly related to arm circumference ($r=0.47; P<.0001$) and...
inversely related to the DMF ($r=0.38; \ P<.0001$).

After twice measuring the 20 fixed images of 20 arterial segments, intra-observer variability was $1.4\% \pm 1.1\%$, while inter-observer variability was $1.6\% \pm 1.4\%$, expressed as a percentage of variation in arterial diameter, with a correlation coefficient between both measurements of 0.98. The intra-observer variability for repeated measurements of the baseline diameter, including the 60 subjects evaluated on 2 occasions at a 30-minute interval, was $1.36\% \pm 1.4\%$, data similar to that found in other studies.$^5$

### Vasodilatation according to the location of flow occlusion

In the first part of the study, with proximal occlusion around the arm, vasodilatation was $7.6\% \pm 2.4\%$ in the group of healthy subjects, while in the group of subjects with CRF it was $5.1\% \pm 2.2\% \ (P<.01)$, and $3.5\% \pm 2.2\%$ in the group of subjects with AMI ($P<.041$ with respect to the group of subjects with CRF). After occlusion of the forearm, vasodilatation was, respectively, $4.6\% \pm 1.5\%$, $2.3\% \pm 2.1\%$, and $2.2\% \pm 1.9\%$. There was only a significant difference between the group of healthy subjects and the group of subjects with CRF ($P<.006$) (Figure 1). In the healthy subjects, the greatest increase in flow after proximal and distal occlusion was, respectively, $487\% \pm 186\%$ and $334\% \pm 112\% \ (P<.0001)$; in the subjects with CRF it was $529\% \pm 215\%$ and $330\% \pm 90\% \ (P<.001)$, and in the group with AMI it was $488\% \pm 273\%$ and $338\% \pm 82\% \ (P<.003)$. Vasodilatation after proximal compression, as compared to distal compression, increased by 3% in the healthy subjects, 2.8% in the subjects with CRF, and 1.3% in patients with AMI; that is to say, we observed increases in vasodilatation proportionate to the theoretical endothelial function. In the rest of the participants, we used only the proximal technique because of its superiority, as was specified in the study objectives and design.

In the overall group, the FMD was $7.8\% \pm 3.1\%$ in the healthy subjects, $5\% \pm 2.6\%$ in the subjects with CRF, and $3.3\% \pm 3\%$ in the patients with AMI. The differences between the 3 groups were significant (Figure 2). If the subjects with CRF were divided into 2 subgroups according to the existence of only 1 CRF or the existence of 2 or more CRF, we did not find a difference between the 2 sub-groups, or between subjects with 2 or more CRF and subjects with AMI. We also did not find differences among the various CRF in the sub-group with only one CRF (data not shown).

### Relationship between FMD and other variables

On univariate analysis, the FMD was related to the c-HDL ($r=0.32; \ P<.0001$) (Figure 3), the baseline diameter ($r=-0.39; \ P<.0001$), brachial circumference ($r=-0.17; \ P=0.27$), C-LDL levels ($r=-0.25; \ P<.001$), hypertension ($r=-0.28; \ P<.0001$), smoking ($r=-0.31; \ P<.0001$), and the number of risk factors ($r=-0.4; \ P<.0001$). Multivariate analysis was performed via multiple linear regression analysis with the stepwise method to construct with regression equation. Upon inclusion in the model of the continuous variables studied, the FMD was significantly associated with the...
...number of risk factors ($P<.0001$), the baseline diameter ($P<.0001$), and C-HDL ($P<.0001$). The level of linear association of these values with the FMD was 0.6. The glucose, C-LDL, total cholesterol, triglycerides, age, and brachial circumference values did not meet the criteria for inclusion in the model. The regression equation was: $\text{FMD} = -0.98$, number of risk factors $= -1.34$, baseline diameter $= +0.07$, and C-HDL $= +0.98$.

**DISCUSSION**

The current study shows that only the FMD after proximal occlusion differentiates male patients with AMI from with subjects with the greatest risk of suffering from it. The vasodilatation observed after proximal occlusion is better related to the theoretical endothelial function.

The early detection of atherosclerosis and the search for prognostic markers for the appearance of coronary episodes constitutes a very active research area. High-resolution echocardiography allows the detection of anatomical changes, such as arterial thickening or functional changes such as FMD. Although FMD is considered an expression of endothelial function, it explains 1 part of this function, and may not be related to other biochemical changes.

**Physiological foundations**

The physiological foundation for this technique is the activation of endothelial receptors that are sensitive to friction and synthesis and the subsequent liberation of NO caused by vasodilatation, which can be abolished with a prior infusion of NG-nomonomethyl-L-arginine (LNMMA). Nevertheless, there are still unresolved questions, such as the half-life of NO is considered to be from 5 to 30 seconds in biological fluids, but according to our observations and those of other authors, the artery can take more than 20 minutes to recuperate its baseline diameter after 4 and a half minutes of ischemia. We cannot discount the participation of other vasodilator molecules such as adenosine or the prostaglandins.

**Endothelial function in the study groups**

It has been shown previously that FMD is significantly less in subjects with CRF and in patients with heart disease.

Unlike other studies, we considered various reportable aspects: homogeneity with respect to sex and age, the inclusion of patients with AMI, and different evaluation timetables. We included only men in the study to reduce variability, as deterioration of the endothelial function is delayed about 10 years in women, there is modulation of FMD as a function of the menstrual cycle, and a smaller baseline could overestimate the percentage of dilatation with regard to men. The mean age was the same in the 3 groups, as age is a potent predictor of endothelial function. We included patients with AMI, but not stable heart patients due to the scant information available during the first week of the AMI and the fact that many of these patients receive statins or ECAI, which modifies FMD. The studies were performed in the afternoon, basically because of the availability of equipment, although it is also worth mentioning that FMD could be greater at that time then in the first hours of the day.

The FMD was approximately 8% in the group of healthy subjects, 5% in the group with CRF and 3.5% in the group with AMI, with significant differences. As in previous studies, FMD worsened in direct relation to the number of risk factors present. With this data we are able state that endothelial dysfunction exists in middle-aged men, with FMD of less than 5%, but this number must be considered as an orientation number only as it could be dependent on the sample studied and the methodology applied.

With unstable angina and in the acute phase of an AMI, as shown in this study, there is less dilatation dependent on the endothelium. This worsening of endothelial function can be explained for the most part by oxidative stress, circulating pro-inflammatory molecules, and plaque activity. Our study data prove that FMD is not only a marker for coronary risk, but a probable reactant in the acute phase of these episodes.

**Vasodilatation as related to proximal and distal occlusion**

In order to find the method that best defined the study groups, in the first part of our study we performed 2 measurements on each subject, with the occlusion sleeve around the arm and around the forearm. After statistically analyzing the results and observing a
higher percentage of dilatation and better separation between the groups, the remaining participants were only evaluated via the proximal occlusion technique as had been pre-established. Mannion et al\(^\text{33}\) showed in normal subjects greater vasodilatation after compression of the upper arm with regard to compression of the forearm. Recently, Berry et al\(^\text{34}\) reproduced these findings and also observed different temporal dilatation behavior. Vogel et al\(^\text{35}\) studied FMD in 2 groups of 10 normal subjects and a group of subjects with CRF; they only observed a significant difference between the 2 groups after proximal occlusion, not following distal occlusion. In our study occlusion of the forearm also differentiated these groups when the sample size was increased.

Recently, Silber et al\(^\text{36}\) showed that the magnitude of flow-mediated dilatation had a direct relationship to stress of the systolic wall that, in part, depends on the speed of the flow.

The results of this study confirm that the greater ischemia and hyperflow following proximal occlusion determines greater vasodilatation and better separation of the 3 study groups, especially among subjects with CRF and patients with AMI, an observation that had not been described previously. In the guidelines recently published by the International Brachial Artery Reactivity Task Force it is noted that proximal compression as it is related to distal compression increases vasodilatation but makes the acquisition of images difficult, without indicating a preference for either one of them.\(^\text{37}\) With the data obtained, we can affirm that proximal compression is a better study method because it differentiates better between those subjects with lower vasodilatation values, a significant action when it comes time to establish the prognostic value of this technique or in studies of therapeutic intervention.

Relationship between C-HDL and endothelial function

The variables with an independent predictive value for FMD were baseline diameter, number of risk factors, and C-HDL. The inverse relationship between the baseline diameter and FMD is a constant in previous studies, and constitutes an interference factor to be taken into account; in fact, a weak inverse relationship has been observed between FMD and arm circumference. The direct relationship between C-HDL and FMD has already been described. Leeson et al\(^\text{38}\) observed a relationship between FMD and the variables of C-HDL and low birth weight in 333 children between the ages of 9 and 11 years. In other studies, the FMD in 2 groups of 10 young men was studied according to the existence of low or high C-HDL maintained over time; the FMD was related directly to C-HDL and inversely with oxidized C-LDL.\(^\text{39}\) In the coronary bed elevated C-HDL is related to better vascular reactivity following acetylcholine infusion.\(^\text{40}\)

With respect to C-LDL, some authors have found a relationship with FMD,\(^\text{39}\) while others have not,\(^\text{10}\) as was the case in our study.

Prognostic value of the technique

In spite of the relationship between FMD and the prognostic variables in ischemic heart disease, it is still not known with certainty whether FMD provides additional information to other methods relative to the diagnosis and follow-up of patients with ischemic heart disease.\(^\text{41}\) Recently, Neunteufl et al\(^\text{35}\) studied 73 patients with chest pain who underwent coronary angiography. Using proximal occlusion methodology, and after 5 years of follow-up, they observed that 50% of subjects with FMD<10% had coronary episodes (coronary angioplasty [ACTP], coronary bypass, or non-fatal AMI) compared to 15% of subjects with FMD>10%.

STUDY LIMITATIONS

According to Berry et al, maximum vasodilatation is observed 50 seconds after compression of the forearm and 75 seconds after compression of the arm.\(^\text{34}\) In this study, measurements were carried out at 60 seconds and 90 seconds after deflation, considering the maximum value. The window of time used continues to appear appropriate given these considerations. We did not undertake a study of vasodilatation independent of the endothelium with sublingual nitroglycerine because this would excessively prolong the testing, with the participants made uncomfortable as a result and, on the other hand, because this also has not been done in similar studies.\(^\text{27-29}\)

CONCLUSIONS

Flow-mediated dilatation following proximal occlusion of the arm, as opposed to that observed after distal occlusion of the forearm, was significantly different, not only between subjects with and without CRF, but also between subjects with AMI and subjects with CRF. This better separation of subjects with a diminished vasodilatation capacity is a result of the greater stimulus for vasodilatation caused by greater hyperflow and ischemia in the zone being measured. FMD obtained after proximal occlusion is directly related to C-HDL and inversely related to the number of risk factors and the baseline arterial diameter.

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