EXERCISE METABOREFLEX ACTIVATION AND ENDOTHELIAL FUNCTION IMPAIRMENT
IN ATRIAL FIBRILLATION

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Short title: Metaboreflex in atrial fibrillation

No conflict of interest exists

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ABSTRACT

Exercising muscle hypoperfusion stimulates afferents (metaboreceptors) involved in the regulation of ventilation. Atrial fibrillation (AF), particularly when combined with diseases causing endothelial (ED) impairment, like hypertension (HP) and diabetes mellitus (DM), depresses the ED activity and enhances exercise hyperventilation. The relationship between these two functions, and the underlying mechanisms are unexplored.

In AF lone or associated with HP or DM (12 subjects in each cohort), we investigated the brachial artery flow-mediated dilatation (FMD) (ED function) and ventilation during the recovery phase of handgrip (metaboreflex, MTR), while on placebo or oral vitamin C (double-blind crossover), both before and after cardioversion (CV) to sinus rhythm. Baseline ED impairment was increasingly more severe and ergoreflex activity more pronounced in AF+HP and AF+DM compared with lone AF. Vitamin C and CV significantly improved both FMD and MTR activity in lone AF and AF+HP, and vitamin C did not produce any additive effect when administered after CV. In AF+DM, neither vitamin C nor CV were effective.

This study provides the following information: AF generates oxidative injury, which is less when the arrhythmia is lone and greater when associated with HP; in DM, the oxidative injury generated by AF is refractory to a rather weak antioxidant, like vitamin C, or the baseline damage is such as to prevent any additive influence of AF; in AF a cause-effect link exists between ED dysfunction and MTR activity; ventilatory advantages of CV seem to be inversely related with the extension of the underlying ED oxidative impairment.

Key words: Diabetes, Endothelium, Hypertension, Metaboreflex.
Inadequate perfusion of exercising muscles may cause local overproduction and accumulation of muscle metabolic by-products, which triggers stimulation of group III and IV neural afferents located in the skeletal muscle. These afferents, that are conventionally called ergoreceptors, have two subtypes: metaboreceptors (sensitive to metabolic products of muscle work) and mechanoreceptors (activated by deformatinal changes in limbs and joints). An overstimulation of these receptors affects patient’s symptoms and the ventilatory response to exercise (22,26). Endothelium, by modulating the exercise-induced neurogenic vasoconstriction and increasing arterial conductance, contributes to the adequacy of exercising muscle perfusion (17,13), suggesting the possibility of an interaction with ergoreceptors. Despite the physiological and clinical implications, this possibility has not been explored previously.

We reasoned that, for an endothelium-ergoreceptor link to be proven, it should be demonstrated that: a) the receptor stimulation is proportional to the degree of baseline endothelial dysfunction; b) interventions that improve endothelium also attenuate the ergoreflex; c) the response is paired independently of the type of intervention set into action.

We aimed at investigating these points and considered atrial fibrillation (AF) an ideal pathophysiological model, because the intrinsic endothelial dysfunction of this arrhythmia (27,3,9,10) is emphasized (10,11) when it is associated with comorbidities causing endothelial impairment, such as hypertension and diabetes mellitus, (15,20,23) and because endothelial activity can promptly be restored with sinus rhythm cardioversion (CV) (9,10).

We investigated the metaboreflex activity and the brachial artery flow-mediated dilatation (FMD) in patients with lone AF and in patients with Type 2 diabetes mellitus or high blood pressure, as comorbid diseases. CV was utilized as an intervention to improve endothelial responsiveness. Because increased metabolic burden developed in fibrillating myocytes suggests that augmented production of reactive oxygen species is likely in AF (21), we also used the antioxidant vitamin C as an alternative endothelium-protective method.
METHODS

Study population. It consisted of 12 consecutive patients with lone AF (group 1) who satisfied the inclusion criteria, and of an equal number of patients with AF and high blood pressure (group 2), or Type 2 non-insulin dependent diabetes mellitus (group 3) as comorbidities, who were matched to the 12 patients with lone AF according to the matching criteria reported below. Five patients in group 1 (41%), 4 in group 2 (34%) and 5 in group 3 (41%) had hypercholesterolemia or hypertriglyceridemia as defined by a value of total cholesterol > 6.2 mmol/L and a value of tryglycerides > 2.3 mmol/L. According to the referring physicians’ reports, these patients had stable AF for 3 to 5 months. Stability was confirmed by ambulatory ECG evidence of fibrillation in the last two follow-up visits, separated by an interval of a month. Patients were enrolled if they had not had previous myocardial infarction, had no significant valvular heart disease, did not suffer from angina pectoris, or lung disease; their New York Heart Association functional class did not exceed class II; their age was sixty or less, in order to minimize the influence of ageing on endothelial function (1,12). Type 2 diabetes mellitus was diagnosed according to the criteria elaborated by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (28). In these patients diabetes was known for 5.2±1.1 years. They had no history of cardiovascular disease but AF, had stable weight and fasting glucose levels over a minimum of 5 weeks prior to enrolment; control of diabetes was achieved with diet alone (3 patients), or diet plus biguanide or sulfonnylurea preparations (9 patients); they had diabetes without known coronary or vascular disease, known diabetic retinopathy, clinically evident distal neuropathy, autonomic insufficiency measured by variations in RR interval with cycled breathing and by the presence of > 20 mmHg decrease of upright blood pressure without a change in heart rate; glycosylated hemoglobin levels were < 7 % with therapy. Two patients in group 3 reported to have been taking ACE-inhibitors for a few months until 6 months before enrolment. They were normotensive at the time of recruitment. Hypertensive patients had a blood pressure
reading (the average of 3 measurements, each performed in 3 separate days) $\geq 150/90$ and $\leq 170/105$. Clinical sitting blood pressure was measured after 15 min of rest; diastolic pressure was read as phase V of Korotkoff sounds. All these patients had no evidence of secondary hypertension, according to tests including plasma renin activity (PRA), serum potassium, plasma aldosterone and catecholamine concentrations, ultrasonic duplex scanning of the renal arteries (14). The duration of hypertension could be determined in all cases and averaged $5.9 \pm 2.6$ years. Among them, 5 patients were untreated and 7 had received one or more antihypertensive agents for at least 2.8 years; current drug therapy consisted of diuretics in 3 cases, ACE-inhibitors in 3 cases, and both drugs in 1 case. Treated patients were requested to discontinue medications 2 weeks before studies and during that period blood pressure was closely monitored for any evidence of accelerated hypertension (increase of diastolic pressure $> 10$ mmHg). If temporary treatment withholding was judged risky (mostly because of the poor response to the current therapy), that case was excluded from the study.

Participants were not involved in any regular physical training program and were not receiving lipid lowering agents, antioxidant vitamins, or aspirin; they never smoked or were ex-smokers of at least 8 months, with a pack-years index of smoking of less than 10. No participants were receiving $\beta$-blockers; 57% were on digoxin and 22% were taking verapamil. Cardioactive preparations were withheld for at least five half-lives prior to vascular studies. Anticoagulation therapy was such as to maintain in all patients prothrombin time within a target of 2.0-2.5 x control for at least 4 weeks before external CV. The procedure was clinically-indicated in any instance and was guided by the findings of transesophageal echocardiography, which in our hospital is a current procedure prior to CV in patients with AF. All subjects gave written informed consent before enrolment; the protocol was approved by the local Ethics Committee.
**Matching.** The group with lone AF was the reference group. Five variables were used to match 12 patients with hypertension and AF, and 12 patients with type 2 diabetes and AF, to the 12 reference subjects: 1) sex (women all were post-menopausal ones, not taking estrogen replacement therapy), 2) age, 3) smoking habit (never smoker, former smoker), 4) total cholesterol plasma concentration; 5) body mass index. Matching was exact for variables 1,2 and 3 and was made to the nearest available subject with comorbidity for variables 4 and 5. The nearest available matching was performed with the use of a multivariate linear discriminating function (18). None of the patients recruited for this study had been involved in previous studies in our laboratory.

**Vascular Studies.** We performed imaging studies of the brachial artery with a high resolution ultrasound Philips 11 MHz linear-array transducer (Philips Medical System, DA Best, The Netherlands). After the clearest view of the artery was found, anatomic landmarks were noted, the skin was marked, and the transducer was held in a constant position by a stereotactic clamp. Images were obtained by the same investigator throughout the study. Vasodilation was assessed by measurement of the maximal change in diameter of the brachial artery during reactive hyperemia created by an inflated cuff (50 mmHg above systolic pressure for 5 min) on the forearm. Arterial diameter was measured in millimetres, coincident with the R waves on the ECG, for 6 cardiac cycles, with these 6 measurements averaged. Evaluations of the vasodilator response from repeated studies were performed by an individual who was blinded to the sequence; images were stored on a video format and were then analyzed with an image analysis software. Flow velocity was assessed by pulsed Doppler with the range gate (1.5 mm) in the center of the artery. The cuff was inflated for 5 min and then rapidly deflated. A 90 sec scan was taken immediately after deflation. Blood flow was calculated multiplying the velocity-time integral of the Doppler flow signal by the cross sectional area of the vessel, and heart rate. The FMD was calculated as the absolute and percent (reactive hyperemia-baseline/baseline x 100) maximal increase in diameter during reactive hyperemia compared with baseline.
**Antioxidant enzyme assessments.** Allantoin, as a marker of increased oxidative stress (19) was measured in 5 male subjects in each group, by gas chromatography-mass spectrometry after anion exchange extraction (5). By this method, the normal value for healthy male subjects 40 to 70 years old, are 14.9 µmol/L (16). Glutathione peroxidase 1 activity (U/g of hemoglobin), as an index of enzymatic inactivation of reactive oxygen species (2), was determined in triplicate from venous blood samples collected on 3 consecutive days (1) in 4, 3 and 4 male patients in group 1,2 and 3, respectively. Blood samples were obtained while in AF before starting the study protocol and while in sinus rhythm after protocol completion. The value reported at either step is the mean of 3 samples. Glutathione peroxidase 1 was measured as previously described (2), in washed red cells, obtained immediately after sampling, from whole blood hemolyzed by adding ice-cold demineralized ultrapure water to yield a 50% hemolysate. Hemolysates were frozen at –80°C for later analysis. All samples at each step from each individual were analyzed in the same analytical run.

**Metaboreflex Evaluation.** A maximal voluntary handgrip test was measured as the greatest of the peak forces produced by three brief maximal handgrip contractions preliminarily performed before the metaboreflex test. Metaboreceptor stimulation consisted of a 3 minute ventilation recording during test, followed by a handgrip session that was performed twice, (4 hour interval) in a random order, according to the following protocol: a) a 5-minute session of rhythmic handgrip achieved by squeezing the balloon of a sphygmomanometer (30 squeezes/per min) at 50% of the predetermined maximal capacity, followed by a 3 minute control recovery; and b) the same protocol was followed soon after interruption of exercise by 3 minutes of blood flow stasis on the exercise arm by inflating an upper arm biceps tourniquet to 30 mmHg above systolic blood pressure at the beginning of recovery (24). The metaboreflex contribution to ventilation was computed as the difference in ventilation between the value at the second and third minute recovery with and that without post-handgrip circulatory occlusion (22).
**Echocardiography.** Two dimensional and Doppler cardiac ultrasounds were carried out by standard methods. Systolic pulmonary arterial pressure, left atrial dimension, left ventricular end-systolic and end-diastolic chamber dimensions and volumes, by the area-length method (to evaluate ejection fraction), were measured by current methods.

**Cardioversion Procedures.** External CV was performed under light anesthesia with thiopental sodium. Synchronized CV was carried out with a 200 J shock. A 300 J shock was administered if the former one was unsuccessful. Continuous electrocardiographic monitoring was performed and ventilation was assisted.

**Study Protocol.** The scheme of the study protocol is depicted in Fig. 1. Before CV, patients in each group were randomly assigned to receive placebo or extended release vitamin C (2 gr/day) (4), with crossover to the other treatment after a week. Following CV, patients were maintained on the same regimen as immediately before the procedure, with crossover to the other regimen after a week. At the end of each of these periods, we determined, in all patients, plasma renin activity, serum aldosterone and catecholamine concentrations, and performed vascular and ergoreflex studies. On all occasions, studies were carried out twice, at 3 and 5 hours after the last dosing, respectively, and averages of the results were taken as representative values.

The study was double-blind relative to the drug regimen, and investigators who read the results were blind to the study design and purposes.

**Statistical Analysis.** Values are expressed as mean ± SD. Patient characteristics at baseline were compared using an unpaired t test or the Fisher exact test. Differences in FMD dilatation of the brachial artery and ergoreflex contribution to ventilation between placebo and active vitamin C and between AF and sinus rhythm states were analyzed by paired t test. A repeated measure ANOVA test and Newman-Keuls multiple comparison procedure were used for testing differences among groups and between pre- and post-cardioversion
evaluations. A p value < 0.05 was considered significant. Statistical analyses were performed by means of Stata 7.0 package.

RESULTS

Table 1 summarizes the clinical and echocardiographic characteristics of the study patients. Higher levels of systemic blood pressure in group 2 (values in the table were those detected after withdrawal of the antihypertensive treatment) were the only statistically significant difference among groups. Aldosterone and norepinephrine concentrations and PRA were within normal limits in each group (Table 2). Antioxidant enzyme activity was determined in a limited number of patients in each group and showed a trend towards an increase of allantoin, and towards a decrease of glutathione peroxidase, from group 1 to group 2 to group 3. A trend of the activity of both enzymes towards improvement after CV was also seen in groups 1 and 2 and not in group 3.

Vascular Studies. Results of vascular studies are reported in Table 3. It did not matter if vitamin C was given preceding or following placebo, and data were pooled together independently of the administration sequence. The baseline brachial artery lumen diameter was similar among groups and in no cases varied significantly with CV while on placebo and on vitamin C. Absolute and percent FMD before CV while on placebo, were significantly lower in groups 2 and 3 than in group 1. In patients in group 1, and to a lesser extent in group 2, while on placebo, FMD and the ratio of changes in the artery lumen to changes in flow significantly improved with restoration of sinus rhythm. Compared to placebo, the antioxidant vitamin C produced results qualitatively similar to these when given before CV. Vitamin C did not cause any further improvement after CV, in addition to that produced by restoration of sinus rhythm. In group 3, neither CV nor vitamin C did affect FMD. As shown in Table 2, PRA, aldosterone and norepinephrine remained steady at the various study steps.

Metaboreflex Evaluation. Fig. 2 illustrates the patterns of ventilation, in groups 1,2 and 3 in the presence of AF, at rest, during handgrip, and recovery, without and with blood flow stasis
by cuff inflation while on placebo and on vitamin C. Fig. 3 illustrates the patterns of ventilation with the same methods while on placebo, before and after restoration of sinus rhythm. A significant difference in ventilation between the recovery with post-handgrip circulatory occlusion and the recovery without, reflects the metaboreflex component of the ventilatory response to exercise. In group 2 and 3 the difference was greater than in group 1, and in groups 1 and 2 it was lost with vitamin C and with CV. Group 3 was refractory to either remedy.

**DISCUSSION**

Vascular tests performed on placebo before CV, confirm that FMD further worsens when AF is associated with hypertension and diabetes, diseases that cause endothelial dysfunction (7,9). This report provides the new information that the more severe the baseline endothelium impairment the larger the exercise metaboreceptors’ stimulation; the ergoreflex, in fact, was significantly more activated in the comorbidity groups compared with the lone AF one.

**FMD and CV.** As already reported, CV was able to improve FMD in AF alone (5,8) or associated with high blood, and not when combined with diabetes mellitus (7). This study shows, for the first time, that the ergoreflex is attenuated when FMD is augmented with CV (groups 1 and 2) and is unchanged when FMD remains unvaried (group 3). Before inferring that there is a link between the two variables it should be critically discussed whether in this experimental setting FMD actually identifies with endothelial activity; and the mechanisms of its improvement should be analyzed.

In interpreting the factors underlying enhancement of conduit artery FMD, an important but often overlooked issue is that any process that reduces the arterial lumen could affect the measured responses to flow, without true impact on endothelial function, due to the inverse relationship between the baseline diameter and its flow-mediated increase (29). Such a mechanism can confidently be ruled out in this study, because baseline brachial artery variations at any step were insignificant in all patients’ cohort. Sinus rhythm decreases the
myocardial metabolic requirement, prolongs the time for ventricular filling, restores the booster pump properties of the atria so that cardiac performance, peripheral blood flow distribution and endothelium-mediated vasodilation may be improved. Presumably, these changes occurred in all patient groups; group 3, however, failed to benefit from CV. Yet, similar responses to nitroglycerine (data not shown) preceding and following CV document that the endothelium independent vasorelaxation is comparable. An irregular ventricular activity due to AF, increases the neural adrenergic discharge and may cause a neural imbalance (30). CV would attenuate the adrenergic traffic and restore the endothelial counterregulatory function (31). This mechanism may be consonant with results with CV in groups 1 and 2, but not with those in group 3. A role for PRA, aldosterone or norepinephrine is unlikely, because these factors did not change according to FMD with CV. On the other hand, previous studies have shown that in AF acetylcholine increases forearm blood flow, NO availability, plasma concentration of stable NO products like nitrite and nitrate, endocardial NOS expression (27,3). Finally, and even more significantly, results with the antioxidant vitamin C strongly support an involvement of oxidative injury in endothelial dysfunction in human AF. Under this respect, it is remarkable that at the time of the study any drug treatment potentially interacting with the antioxidant endothelium protective activity of vitamin C, had been withheld. From all these considerations we draw the following inferences. a) Changes in FMD with CV actually reflect endothelial activity. b) Variations in the flow pattern (21) and/or oxidative stress (8) (see also the antioxidant enzyme activities in Table 3) with reversion to sinus rhythm, modulate the vascular endothelial function and promote its improvement. c) Restoration of an organized atrial contraction that avoids atrial production of superoxide (7) and oxidative injury (16), could play a contributory role. d) A link does exist between endothelial function and exercise ergoreflex activation.

**CV and Vitamin C in Comorbidities.** Vitamin C, a rather weak antioxidant, could restore FMD most in lone AF, less in AF with hypertension and not at all in AF with diabetes. Consistently, even if reversion to sinus rhythm invariably restores conduit artery regular
pulsatile blood flow, FMD following CV increased less in the hypertension group than in the lone AF one, and did not improve at all in the diabetes group. These results are consonant with the pattern shown by the antioxidant enzyme activity, and may suggest that the oxidative injury is lower in hypertension than in diabetes, AF exerts an additive endothelial depressive influence in the former and not in the latter comorbidity, in which the restrain of the background NO activity is probably such to impede any additive or synergistic effect.

The endothelium-ergoreflex link. In a previous study (9) that was performed in similar categories of patients, we found that the ventilatory efficiency (steep slope of the ventilation to carbon dioxide output relationship) was invariably compromised, the arterial carbon dioxide pressure was reduced, and both were brought back toward normal by CV in the lone AF and the hypertension groups. Because lung function and arterial oxygen saturation were within normal limits and were not affected by CV, we deduced that in the presence of AF, exercise was associated with an early intervention of extrapulmonary factors increasing the ventilatory response. This mechanism was abolished in patients whose endothelial activity benefited from CV. The present study indicates metaboreceptors as the extrapulmonary factors that impair ventilatory efficiency when endothelial dysfunction causes exercising muscle underperfusion. An impaired endothelial responsiveness to vascular shear stress and lack of a physiological vasodilation, that maintains elevated the impedance to left ventricular ejection and prevents an adequate increase of stroke volume, are suggested as possible reasons of exercising muscle perfusion inadequacy in AF.

Clinical perspectives. In chronic heart failure, group IV efferents from skeletal muscles have been hypothesized to play a significant role in the generation of breathlessness and in the progress of the disease (11,24,25). The present study demonstrates that the metaboreceptor contribution to exercise ventilation is significantly enhanced in patients with AF (8) and provides the information that CV modulates the ergoreflex in AF alone or when it is associated with high blood pressure. This may offer an explanation for the impairment in similar patients in ventilatory efficiency, and for the ability of CV to correct these
inconveniences in the former two conditions, and not in patients with diabetes as a comorbid
disease (7).

REFERENCES


Grants

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Legends for Figures

Figure 1. Schematic presentation of the study design.

Figure 2. Mean values of ventilation (VE) in groups 1, 2 and 3, at rest, during handgrip (5 min) and recovery (3 min) of metaboreflex test, before cardioversion (CV) to sinus rhythm, while on placebo.

* = p < 0.01 vs no occlusion
§ = p < 0.01 vs corresponding value in group 1

Figure 3. Mean values of ventilation (VE) in groups 1, 2 and 3, at rest, during handgrip (5 min) and recovery (3 min) of metaboreflex test before CV while on placebo and after vitamin C.

* = p < 0.01 vs no occlusion
§ = p < 0.01 vs corresponding value in group 1
Table 1. Clinical and echocardiographic data of the study patients (Means±SD).

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N°</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Age, years</td>
<td>64±2</td>
<td>61±3</td>
<td>63±4</td>
</tr>
<tr>
<td>Gender, men / women</td>
<td>9/3</td>
<td>9/3</td>
<td>9/3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79±5</td>
<td>78±3</td>
<td>80±4</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27±3</td>
<td>26±2</td>
<td>26±3</td>
</tr>
<tr>
<td>New York Heart Association functional class, I-II</td>
<td>10/2</td>
<td>9/3</td>
<td>9/3</td>
</tr>
<tr>
<td>Duration of atrial fibrillation, months</td>
<td>2.7±1.5</td>
<td>2.9±1.1</td>
<td>3.1±1.2</td>
</tr>
<tr>
<td>Cholesterolemia, mmol . l⁻¹</td>
<td>6.0±1.2</td>
<td>6.1±1.3</td>
<td>5.9±1.1</td>
</tr>
<tr>
<td>Triglyceridemia, mmol . l⁻¹</td>
<td>2.3±0.4</td>
<td>2.2±0.6</td>
<td>2.3±0.5</td>
</tr>
<tr>
<td>Systemic arterial pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>123±4</td>
<td>162±6 *</td>
<td>122±2</td>
</tr>
<tr>
<td>Diastolic</td>
<td>81±3</td>
<td>101±4 *</td>
<td>79±3</td>
</tr>
</tbody>
</table>

**Echocardiography**

|                        |             |             |             |
| Left ventricular ejection fraction, % | 63±3        | 64±2        | 64±3        |
| Left atrial diameter, mm         | 39±2        | 40±4        | 38±2        |
| Systolic pulmonary artery pressure, mmHg | 22±3        | 21±2        | 20±2        |

*: p<0.01 vs the corresponding value in group 1
Table 2. Humoral data in the three cohorts at the various study steps (Means±SD).

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before CV</td>
<td>Before CV</td>
<td>Before CV</td>
</tr>
<tr>
<td>Placebo</td>
<td>Vitamin C</td>
<td>Placebo</td>
</tr>
</tbody>
</table>

Plasma renin activity, ng. ml⁻¹. h⁻¹

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before CV</td>
<td>2.2±2.0</td>
<td>2.3±2.2</td>
<td>2.4±2.1</td>
</tr>
<tr>
<td>After CV</td>
<td>2.4±2.5</td>
<td>2.3±2.3</td>
<td>2.3±2.4</td>
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</table>

Serum norepinephrine concentration, pg. ml⁻¹

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<th></th>
<th>Group 1</th>
<th>Group 2</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Before CV</td>
<td>158±78</td>
<td>162±64</td>
<td>166±54</td>
</tr>
<tr>
<td>After CV</td>
<td>164±65</td>
<td>157±60</td>
<td>159±57</td>
</tr>
</tbody>
</table>

Serum aldosterone concentration, pg. ml⁻¹

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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<tbody>
<tr>
<td>Before CV</td>
<td>218±22</td>
<td>219±18</td>
<td>222±17</td>
</tr>
<tr>
<td>After CV</td>
<td>209±24</td>
<td>214±21</td>
<td>211±16</td>
</tr>
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</table>
Table 3. Antioxidant enzyme activity (Means±SD).

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
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<th>Group 3</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before CV</td>
<td>After CV</td>
<td>Before CV</td>
<td>After CV</td>
<td>Before CV</td>
<td>After CV</td>
</tr>
<tr>
<td>Glutathione peroxidase 1, U/g of hemoglobin</td>
<td>38.2±8.5</td>
<td>49.5±7.2 **</td>
<td>34.3±10.1</td>
<td>40.1±7.8</td>
<td>26.5±8.4 *</td>
<td>28.2±7.8 *</td>
</tr>
<tr>
<td>N= 5</td>
<td>N= 5</td>
<td>N= 5</td>
<td>N= 5</td>
<td>N= 5</td>
<td>N= 5</td>
<td>N= 5</td>
</tr>
<tr>
<td>Allantoin, µmol. L⁻¹</td>
<td>22.1±1.2</td>
<td>17.2±1.3 **</td>
<td>25.8±1.3 *</td>
<td>22.4±1.1 *</td>
<td>34.1±1.1 *</td>
<td>32.2±1.2 *</td>
</tr>
</tbody>
</table>

N= number of patients, * p<0.05 vs corresponding value in group 1, ** p<0.05 vs before CV.
Table 4. Results of the vascular assessments (Means±SD).

<table>
<thead>
<tr>
<th></th>
<th>BEFORE CV</th>
<th></th>
<th>AFTER CV</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Vitamin C</td>
<td>Placebo</td>
<td>Vitamin C</td>
</tr>
<tr>
<td>BAD, mm</td>
<td>3.73±0.61</td>
<td>3.74±0.54</td>
<td>3.81±0.72</td>
<td>3.79±0.64</td>
</tr>
<tr>
<td>FMD, mm</td>
<td>0.24±0.02</td>
<td>0.32±0.01*</td>
<td>0.38±0.01</td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>FMD, %</td>
<td>7.8±0.7</td>
<td>11.8±0.8*</td>
<td>12.6±0.8</td>
<td>13.1±0.6</td>
</tr>
<tr>
<td>Reactive hyperemia mL. min⁻¹</td>
<td>233±13.6</td>
<td>256±15.9</td>
<td>288±14.7</td>
<td>291±15.4</td>
</tr>
<tr>
<td>Δ FMD /Δ flow mm.mL⁻¹.min⁻¹.1000</td>
<td>1.03±0.20</td>
<td>1.25±0.3*</td>
<td>1.33±0.2</td>
<td>1.37±0.3*</td>
</tr>
</tbody>
</table>

Group 2

|                  | BEFORE CV |                | AFTER CV |                |
|                  |           |                |          |                |
| BAD, mm          | 3.78±0.59 | 3.76±0.63      | 3.79±0.70| 3.81±0.55      |
| FMD, mm          | 0.21±0.02 | 0.27±0.01*     | 0.32±0.01| 0.30±0.02      |
| FMD, %           | 5.9±0.6   | 9.2±0.4*       | 10.2±0.5 | 9.9±0.4       |
| Reactive hyperemia mL. min⁻¹ | 249±17.3 | 264±15.6      | 270±27.1 | 277±25.5       |
| Δ FMD /Δ mm.mL⁻¹.min⁻¹.1000 | 0.85±0.1  | 1.02±0.1*     | 1.08±0.2 | 1.07±0.1      |

Group 3

|                  | BEFORE CV |                | AFTER CV |                |
|                  |           |                |          |                |
| BAD, mm          | 3.75±0.68 | 3.77±0.62      | 3.77±0.63| 3.74±0.01      |
| FMD, mm          | 0.27±0.01 | 0.26±0.01†     | 0.26±0.02| 0.28±0.02†     |
| FMD, %           | 7.2±0.7   | 6.8±0.5†       | 6.9±0.6† | 7.3±0.5†      |
| Reactive hyperemia mL. min⁻¹ | 236±18.4 | 241±12.6      | 258±16.5 | 264±19.4       |
| Δ FMD /Δ flow mm.mL⁻¹.min⁻¹.1000 | 1.14±0.2  | 1.07±0.1     | 1.00±0.1 | 1.06±0.2      |

* = p < 0.01 vs placebo  
# = p < 0.01 vs corresponding value before CV  
† = p < 0.01 vs corresponding value in Group 1
STUDY PROTOCOL

Placebo
Vitamin C

Placebo
Vitamin C

Placebo
Vitamin C

Placebo
Vitamin C

CARDIOVERSION

days

Vascular Study
Metaboreflex
PRA
Aldosterone
Catecholamine

Copyright Information
GROUP 1

VE (L/min) Placebo Pre-CV

GROUP 2

VE (L/min) Placebo Pre-CV

GROUP 3

VE (L/min) Placebo Pre-CV

VE (L/min)

Placebo Pre-CV

Vitamin C Pre-CV

Vitamin C Pre-CV

Vitamin C Pre-CV

Rest Exercise Recovery

Rest Exercise Recovery

Rest Exercise Recovery

No Occlusion Occlusion

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