# Exercise training counteracts the abnormal release of plasma endothelin-1 in normal subjects at risk for hypertension

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*Key words:*
**Endothelin; Exercise; Hypertension.**

*Background.* **The hypothesis that in normotensive offspring of hypertensive parents exercise training could influence the systemic release of endothelin (ET)-1 during a provocative testing protocol was tested.**

*Methods.* **The provocative handgrip test was performed in four groups of healthy young age-matched males: offspring of hypertensive parents following a regular swimming exercise regimen (group A, n = 14); offspring of hypertensive parents and leading a sedentary lifestyle (group B, n = 11); normal volunteers with no family history of hypertension: sedentary (group C, n = 10), and following a regular swimming regimen (group D, n = 10). The plasma ET-1 was measured at baseline, after 4 min of handgrip exercise at 50% maximal capacity and following 2 (R2) and 10 (R10) min of recovery from handgrip.**

*Results.* **ET-1 plasma levels, within the normal range in all groups at baseline (group A 0.94 ± 0.32 pg/ml, group B 0.84 ± 0.26 pg/ml, group C 0.78 ± 0.35 pg/ml, group D 0.85 ± 0.26, p = NS) showed a progressive and significant increase in group B during and after handgrip exercise (peak handgrip 1.08 ± 0.5 pg/ml, p = NS; R2 1.35 ± 0.36 pg/ml, p < 0.05; R10 2.76 ± 0.75 pg/ml, p < 0.01). Significant differences were found at R2 and R10 when the ET-1 levels measured in group B were compared to those observed in group A, group C and group D. Multivariate analysis demonstrated that the serum levels of ET-1 significantly contributed to predict handgrip-induced changes when the diastolic blood pressure was the dependent variable.**

*Conclusions.* **Routine aerobic exercise appeared to counteract the handgrip-induced abnormal release of plasma ET-1 and may favorably affect the preclinical endothelial alterations seen in healthy offspring of hypertensive parents.**

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## Introduction

Endothelin (ET)-1, a 21 amino acid peptide, has been reported to have a wide spectrum of biological actions including: 1) the long-term regulation of the basal tone of blood vessels; 2) the potentiation of the activity of other vasoconstrictors and hormonal agents; 3) a mitogenic effect that may be involved in vascular wall remodeling[1,2]. Recent studies support the view that normotensive subjects, genetically prone to develop essential hypertension, may have a preclinical hypertensive condition and that various stimuli may elicit it. With regard to this, Noll et al.[3] reported an increase in ET-1 plasma levels during mental stress only in normotensive offspring of hypertensive parents. More recently, our group demonstrated that the isometric handgrip (HG) test can induce a progressive increase in ET-1 plasma levels during the recovery period of the test only in the offspring of hypertensive parents, suggesting that early dysfunctional changes of local vascular regulation can be activated in the preclinical state of hypertension[4]. Other data suggest that an excessive biohumoral response to stressful situations or an increased cardiovascular reactivity may be a risk factor for cardiovascular disease, particularly in susceptible pre-hypertensive individuals, and predict future blood pressure levels[5].

Epidemiological studies showed, on the other hand, that physical training is protective against cardiovascular disorders, including hypertension[6,7]. These favorable exercise effects have been attributed to: 1) systemic factors such as the modulation of sympathetic activity[8]; 2) local factors such as vascular functional adaptations linked to up-regulation of nitric oxide by endothelial cells[9-11]; 3) enhanced perfusion and vascular blood flow[11-13]. However, the possibility

that routine physical activity may modify the early impairment of the endothelial function or the abnormal release of ET-1 in normotensive young subjects from families in which one or two parents have primary hypertension was not previously investigated. We therefore selected, among physical activities, swimming, a dynamic exercise that is considered effective and safe for the prevention and treatment of hypertension[14]. Swimming exercises produce significant cardiovascular adaptations that include a greater capacity for vasodilation in skeletal muscle and an enhanced cardiac function[14].

The aim of this study was to evaluate the potential of a regular swimming exercise regimen in influencing ET-1 release in the bloodstream of normotensive offspring of hypertensive parents. To this end, the ET-1 plasma levels at baseline, at peak effect and during the recovery period of the isometric HG test were assessed both in conditioned and sedentary subjects at high risk for hypertension and compared with the ET-1 plasma levels of normal subjects without a family history of hypertension.

## Methods

**Patients.** Forty-five healthy young age-matched male volunteers divided into four groups participated in the study. All were normotensive and free from cardiovascular diseases, as revealed by clinical examination and laboratory findings. Smokers and overweight individuals (weight > 10% than ideal) were excluded. The first group included 14 subjects (mean age 25.2 ± 1.5 years) with a family history of hypertension who participated in a leisure time regular exercise consisting of swimming (group A). The second group (group B) consisted of 11 sedentary normotensive subjects, offspring of hypertensive parents (mean age 25.5 ± 1.2 years). The control groups consisted of 10 healthy subjects (mean age 26.1 ± 1.1 years) without a family history of hypertension who led a sedentary lifestyle (group C) and 10 healthy subjects (mean age 26 ± 1.1 years) following a regular swimming regimen (group D). The family history of hypertension consisted of a hypertensive status (diastolic blood pressure-DBP ≥ 105 mmHg) of both parents detected at < 55 years of age. The standardized exercise program for both groups A and D consisted of four 60-min swimming sessions per week over a 10-week training period. The last session of each week was supervised by the project staff in order to control and modify the intensity of the workload on the basis of the heart rate response. Subjects of both groups with a heart rate exceeding 80% of the maximum heart rate achieved at the pretreatment graded exercise test were assigned to the "low intensity" level of exercise.

The study protocol was performed with the approval of the local Medical Ethics Committee, and informed consent was obtained from each subject.

**Exercise testing.** All subjects underwent maximal exercise testing on a bicycle 1 week before and 24 hours after the training program. The initial workload was 25 W and was increased by 25 W every 2 min. The first 2 min of recovery were against a workload of 20 W and thereafter against 0 W. The heart rate, systolic blood pressure (SBP) and DBP, and a 12-lead ECG were recorded every minute of each stage.

**Study protocol.** All subjects were studied in a laboratory (room temperature 22° to 24°C) during the morning hours (9.00 to 12.00 a.m.) after an overnight fast. They were in the supine position for at least 1 hour before the start of the HG exercise and were instructed to avoid Valsalva-like maneuvers. HG was performed with a calibrated HG dynamometer (Vigorimeter Martin, Berlin, Germany) starting 48 hours after the last bout of exercise to avoid any artifact due to the short-term effects of exercise. All subjects were instructed to exert the maximal compressive force with the dominant arm 3 times, at 3 min intervals. After 30 min of rest, 50% of the average maximal voluntary contraction was performed for 4 min by each subject. The heart rate, SBP and DBP were recorded, using an ECG and a cuff sphygmomanometer, at baseline, at peak HG, at 2 min of the recovery time (R2), and then every 2 min throughout an average recovery period of 10 min (R10).

A short 18G polyethylene cannula was inserted into an antecubital vein of the non-dominant arm 30 min before the beginning of the study, and kept patent by slow infusion of 5% dextrose. Blood samples for ET-1 and catecholamine determination were drawn immediately before the beginning of the test (basal), at peak HG and at R2 and R10.

**Biochemical assays.** Blood samples were collected in pre-chilled tubes (Becton Dickinson Vacutainer System, Madrid, Spain) containing EDTA-K3 (15%) and aprotinin (1000 kIU/ml of blood) and promptly centrifuged at 1600 rpm for 15 min at 4°C. Using a pipette, the plasma was then collected in polypropylene tubes and stored at -80°C until assayed. At the time of analysis, the plasma samples were acidified with an equal volume of 0.1% trifluoroacetic acid and centrifuged at 2500 rpm for 30 min at 4°C to eliminate any proteolytic activity. The plasma compounds tested were first concentrated by extraction through C18 Sep-Pak cartridges (Millipore Corporation, Bedford, MA, USA).

For catecholamine assay we used a previously described method[15]. For ET-1 assay, the Sep-Pak columns were first activated with 0.1% trifluoroacetic acid buffer. The retained material was eluted with 3 ml of a buffer containing acetonitril (60%) in 0.1% trifluoroacetic acid and dried under vacuum by a centrifugal evaporator system (Gyrovap, Howe and Co., London, UK). The radioimmunoassay of the reconstituted pellet was performed using a commercially available ET-1 ra-

dioimmunoassay kit (Peninsula Laboratories, Washington, DC, USA). Each measurement was performed twice, and generally the average value of the two measurements was considered, although differences between the two measurements were < 5%. In 2 cases that had one of the baseline measurements far above the normal ranges, only the other measurement was taken into account. The cross-reactivity of the system for ET-1 was 100%; according to the supplier it was < 7% for both ET-2 and ET-3. The intraassay and interassay variations in our laboratory were < 10%. The recovery was 80%. Plasma ET-1 levels are expressed as pg/ml.

**Statistical analysis.** The data in the tables, figures and text are expressed as mean ± SD. BMDP statistical software was used[16]. Analysis of variance and Student's t-tests were performed with Bonferroni's correction (BMDP-7D). Since data represent time (protocol)-related repetitions, a repeated measures model with structured covariance matrices (BMDP-5V: type = compound symmetry) was used for multivariate comparison of the studied groups[17]. Models were set to explain the dependent variables [either the diastolic or the mean blood pressure (from baseline to peak HG, R2 and R10)] on the basis of both the time-constant (weight and height) and time-varying (respectively from baseline to peak HG, R2 and R10: heart rate, ET-1, and norepinephrine) covariates while weighing for grouping (G: sedentary versus conditioned individuals with a family history of hypertension), time (T) and the G*T interaction. To construct the dependent variable mean blood pressure, the formula DBP + (SBP-DBP)/3 was used. The Akaike information criterion (AIC) was used to select the most successful model. A p value of $< 0.05$ was considered statistically significant.

## Results

All of the subjects enrolled in this protocol were successfully studied at all time points (Table I).

**Hemodynamics.** The heart rate increased from basal values with HG exercise in all four groups, and this increase was similar among groups. After a 10 min recovery period from HG, the heart rate returned to baseline values in all four groups. The systemic blood pressure, both systolic and diastolic, increased with HG in all four groups and returned to baseline values following a 10-min recovery period, with no differences observed among groups.

**Neurohormonal profiles.** The changes in plasma ET-1 levels with HG and during the recovery period are shown in figure 1. In normal subjects and in offspring of hypertensive parents in whom a routine exercise regimen was maintained, no changes in the ET-1 plasma profiles during or after HG were observed. In marked contrast, sedentary offspring of hypertensive parents displayed a marked increase in plasma ET-1 levels during the recovery period from HG. For example, the plasma ET-1 values were 2-fold higher than baseline

**Table I.** Average values of evaluated variables.

| | HR (b/min) | SBP (mmHg) | DBP (mmHg) | MBP (mmHg) | ET-1 (pg/ml) | NE (pg/ml) |
|---|---|---|---|---|---|---|
| Group A | | | | | | |
| Basal | 65 ± 5.8 | 121.3 ± 11.9 | 76.3 ± 9.2 | 91.9 ± 10.5 | 0.94 ± 0.32 | 187.4 ± 77.2 |
| Peak HG | 85 ± 10.9* | 147.3 ± 10.3* | 93.2 ± 7.3* | 109.8 ± 6.8 | 0.92 ± 0.34 | 224.9 ± 77.1** |
| R2 | 66 ± 5.1 | 123.6 ± 11.8 | 75.9 ± 6.9 | 75.3 ± 7.5 | 0.97 ± 0.29 | 189.1 ± 70.1 |
| R10 | 64 ± 4.5 | 120 ± 7.9 | 77.3 ± 7.8 | 90.6 ± 7.1 | 0.92 ± 0.39 | 168.2 ± 54.1 |
| Group B | | | | | | |
| Basal | 64 ± 5.8 | 120 ± 9.2 | 77 ± 6.7 | 90.8 ± 6.6 | 0.84 ± 0.26 | 192.1 ± 101.5 |
| Peak HG | 96 ± 11.3* | 165.9 ± 9.7* | 98 ± 5.3* | 109.6 ± 5.9* | 1.08 ± 0.5 | 247.1 ± 125.8** |
| R2 | 76 ± 2.4 | 128 ± 10.1 | 80 ± 6.4 | 89 ± 6.7 | 1.35 ± 0.36** | 201.3 ± 89.8 |
| R10 | 71 ± 4.1 | 124 ± 4.6 | 77.2 ± 10.5 | 86.3 ± 6.6 | 2.76 ± 0.75*** | 161.5 ± 81.3 |
| Group C | | | | | | |
| Basal | 68 ± 4.4 | 121 ± 11.8 | 76.4 ± 9.3 | 92.1 ± 10.3 | 0.78 ± 0.35 | 192.1 ± 155.3 |
| Peak HG | 91 ± 7.4* | 160 ± 10.5* | 101 ± 8.4* | 109.9 ± 7.6* | 0.96 ± 0.58 | 250.1 ± 96.6** |
| R2 | 78 ± 3.4 | 122.4 ± 8.5 | 79 ± 8.7 | 89.1 ± 8.2 | 0.87 ± 0.31 | 164.7 ± 53.5 |
| R10 | 68 ± 4.4 | 116 ± 8.9 | 75.8 ± 8.7 | 86.4 ± 6.4 | 0.76 ± 0.35 | 122.9 ± 39.7 |
| Group D | | | | | | |
| Basal | 64 ± 4.2 | 120 ± 11 | 76.1 ± 9.2 | 90.7 ± 9.3 | 0.85 ± 0.26 | 182.1 ± 77.3 |
| Peak HG | 84 ± 7.0* | 150 ± 10.3* | 91 ± 6.4* | 110.1 ± 6.6* | 0.94 ± 0.32 | 240.1 ± 86.6** |
| R2 | 66 ± 3.4 | 122 ± 7.5 | 76 ± 7.7 | 90.1 ± 8 | 0.87 ± 0.31 | 164.0 ± 50.5 |
| R10 | 66 ± 4.4 | 116 ± 7.9 | 75.8 ± 8.7 | 89.4 ± 5.4 | 0.78 ± 0.35 | 162.9 ± 49.7 |

Data are expressed as mean ± SD. DBP = diastolic blood pressure; ET-1 = endothelin-1; HG = handgrip; HR = heart rate; MBP = mean blood pressure; NE = norepinephrine; R2 and R10 = 2 and 10 min of recovery; SBP = systolic blood pressure. * $p < 0.001$, ** $p < 0.05$, *** $p < 0.01$ vs basal values.

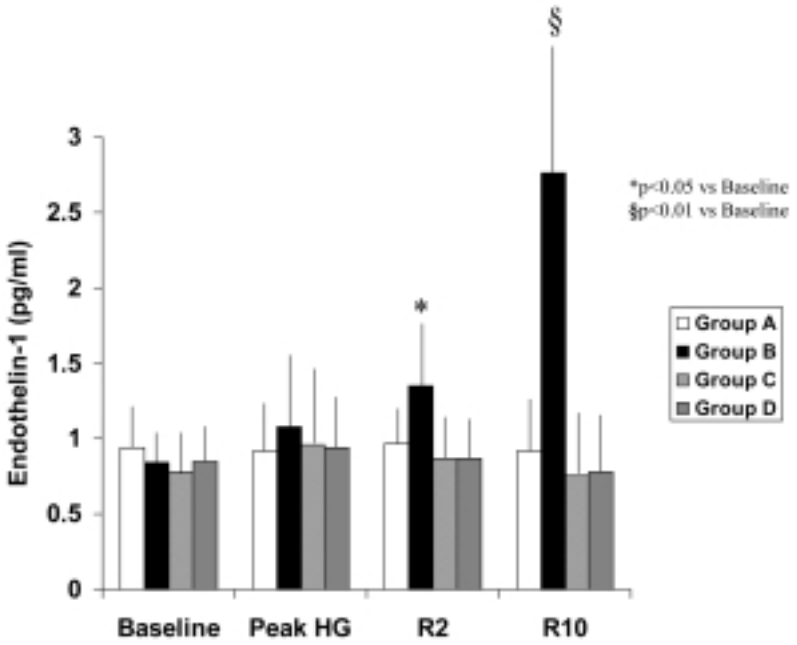


**Figure 1.** *Endothelin-1 plasma levels throughout the handgrip (HG) exercise test in the four study groups. A robust and persistent increase in the plasma levels of endothelin-1 was observed in group B. R2 and R10 = 2 and 10 min of recovery.*

values in this group of sedentary subjects as well as compared to normal control values. Plasma norepinephrine increased in all four groups with HG exercise, and returned to baseline values during the recovery interval. No differences were observed in norepinephrine values between the four study groups.

**Multivariate comparison between groups.** The highest AIC (-341.35) was found in the model where the mean blood pressure response to HG (dependent variable) did not include weight among the explanatory covariates. Height was not contributory ($\chi^2 = 0.008$, p = NS). Among time-varying covariates, the heart rate did not significantly contribute to explain the dependent variable mean blood pressure ($\chi^2 = 0.57$, p = NS), whereas the contribution of norepinephrine was only marginally significant ($\chi^2 = 2.76$, p = 0.09). In contrast, ET-1, although not a significant time-varying covariate ($\chi^2 = 1.91$) in this latter model, provided a slight contribution (p = 0.17). The grouping covariate [sedentary or conditioned offspring of hypertensive parents ($\chi^2 = 0.70$, p = NS)] and the interaction term G*T ($\chi^2 = 2.46$, p = NS) were found not to be significant at multivariate analysis. When the model was run to explain the dependent covariate mean blood pressure with weight, the ET-1 contribution was marginally significant ($\chi^2 = 2.75$, p = 0.09), the other covariates staying the same as without weight, with a very similar AIC (-339.62). Interestingly, when the model was run to explain the dependent covariate DBP with or without weight, the ET-1 contribution was always significant ($\chi^2 > 4.42$, p < 0.036) and the contribution provided by the remaining covariates was essentially similar to that they had in the other models, with AICs in the same range (-337.75 and -339.89). On the other hand, when the model was run to explain SBP as the dependent covariate, with or without weight, the ET-1 contribution was always statistically not significant and the contribution provided by the remaining covariates was essentially similar to that they had in the other models.

## Discussion

Exercise training is protective against the risk of developing hypertension in subjects with a family history of hypertension[6-8]. In the present study, a robust and persistent increase in the systemic levels of the potent vasoactive peptide ET-1 was observed following HG-induced exercise in sedentary young male offspring of hypertensive parents. In marked contrast, in age-matched offspring of hypertensive parents in whom an aerobic exercise regimen was followed for an extended period of time (6 months) no change in systemic ET-1 levels was found following the HG exercise challenge. This differential response to HG exercise in sedentary vs active subjects with a familial risk of developing hypertension was not an expression of a global neurohormonal response since the degree of sympathetic system activation was similar for both groups. Taken together, the new and unique findings of the present study demonstrated that ET-1 synthesis and release are altered in currently normotensive subjects at risk of developing hypertension and that this abnormality in ET-1 release was not present in subjects performing a regular aerobic exercise program. A potential contributory

mechanism of the favorable effects of exercise in patients at risk of developing hypertension may thus be the normalization of ET-1 synthesis and release.

ET-1 induces potent and long-acting vasoconstrictive effects within a number of circulatory systems and has also been implicated as a factor which influences those growth characteristics which would promote vascular remodeling[2,18]. An important source of ET-1 is the endothelial cells of blood vessels[1]. The peptide is localized within intracellular constitutive vesicles of endothelial cells and is released rapidly, with the formation and transit time of a vesicle from the trans-Golgi network to the plasma membrane taking approximately 10 min[19]. Eighty percent of ET-1 secretion is polarized towards the basolateral portion of the endothelial cells, thus suggesting that it acts locally in an autocrine and paracrine way[20]. So, very low plasma levels of ET-1 are the result of spillover into the bloodstream and only partly reflect the local synthesis and secretion of the peptide occurring in tissues[20,21].

Shear stress has a key role in increasing ET-1 gene expression and production as well as in promoting the release of the mature peptide[2,10]. So far, low levels of wall shear stress stimulate the synthesis and secretion of ET-1; in contrast, high shear stress downregulates the ET-1 system. Physical conditioning is well known to enhance intravascular shearing forces by increasing the arterial blood flow to the exercising muscles[11]. Linked to the shear effect, conditioning is also associated with an increased endothelium-derived nitric oxide biosynthesis and with an increased vasodilator capacity of the skeletal muscle vasculature[12,21-23]. Nevertheless, even the vasoconstrictor reactivity has been found to be modified by conditioning[8]. It has been shown that the vasculature develops a supersensitivity to catecholamines during the initial period of an exercise program, a phase which is followed by vascular hyporesponsiveness and by inhibition of the adrenergic neuronal release of norepinephrine over the long term. These functional effects occur in all muscular type arteries, independent of the exercising muscle groups[11,23].

Intense physical training (> 8-12 weeks) is an appropriate means of inducing changes in vascular tone and peripheral vascular resistance[24,25]. In addition, it seems that a training program of 30 min of moderate exercise 3 times weekly is sufficient for most of the beneficial hemodynamic and metabolic effects to occur[8]. However, the adaptations are reversible and disappear within 6-10 weeks after completion of muscular training[26]. In a recent study aimed at investigating the relationship between ET-1 and exercise in male athletes it was observed that: 1) ET-1 plasma levels increased soon after the cycle exercise test only when the exercise intensity was higher (130%) than their individual ventilatory threshold; 2) ET-1 levels were correlated with the intensity of exercise[27]. It was also found that ET increased only in the circulation of the non-working leg[28]. In addition, animal experiments demonstrated that the production of ET-1 in the heart is increased by running[29]. On the basis of these results, it was suggested that endothelial and myocardial ET-1 may contribute to the changes induced by dynamic exercise, increasing the blood flow to the working muscles and participating in the regulation of cardiac activity.

Recently, we demonstrated that even isometric exercise by HG may induce an increase in the peripheral release of ET-1[4]. It is well known that intense muscle activation by isometric exercise triggers a pressure reflex associated with an intense and rapidly occurring increase in heart rate and arterial pressure. The systemic response is neurally-mediated through a reduction in the vagal activity to the heart and blood vessels[30]. With regard to local adjustment, it has been shown that: 1) during the period of contralateral isometric exercise, the forearm blood flow increases and the forearm resistance decreases[31]; 2) soon after the end of the exercise, in the resting arm the forearm blood flow decreases and the forearm resistance temporarily rises above control levels[32,33]. However, the rise in hemodynamics is but one of several mechanisms that are activated in order to increase muscle perfusion and oxygen supply to the working arm[13]. As confirmed by the data of this study, HG was found to be associated with an increase, differing between subjects at low risk and sedentary and trained offspring of hypertensive parents, in the plasma ET-1 concentrations. The possible mechanism of this behavior is unclear and requires further investigations. However, it is interesting to speculate that the sustained increase in ET-1 levels during the recovery period may be due to an early endothelial impairment in subjects at risk and that training may represent a non-pharmacological treatment for the restoration of endothelial function.

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