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CE-RESEARCH LETTER TO THE EDITOR

# Vascular smooth muscle cell dysfunction in patients with hemochromatosis

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Dear Sir,

Hereditary hemochromatosis is a condition characterized by iron overload, potentially responsible for serious complications, such as diabetes, heart failure, arthritis, and liver diseases [1, 2]. On the other hand, increased iron stores have been identified as an independent risk factor for cardiovascular disease (CVD) and atherosclerosis [3–5]. Consistently, in patients with hereditary hemochromatosis, increased iron load was significantly associated with endothelial dysfunction, a well-known, early marker of atherosclerosis, and increased intima–media thickness [6]. Nevertheless, there is no consensus on whether hemochromatosis associates with an increased risk for CVD [2, 7]. Therefore, how endothelial dysfunction can coexist with a normal risk profile for CVD in hemochromatosis patients remains unclear.

To clarify this issue, we studied vascular reactivity, in 11 male hemochromatosis patients—diagnosis made according to the criteria of the American Association for the Study of Liver Diseases [8] - and 12 age and BMI matched, healthy male controls, whose clinical characteristics are shown in Table 1 We evaluated the patient genetic variant HFE C282Y, H63D and S65C. At variance with the widely diffused association between C282Y homozygosis or C282Y/H63D compound heterozygosis and hereditary hemochromatosis, in southern Italy, such an association is present only in part of the patient population [9]. In the current study, two patients were homozygotes for C282Y, one for H63D, six were heterozygotes for both C282Y and H63D, one for H63D, and one for both H63D and S65C. Subjects with hypertension, diabetes, dyslipidemia, history of cardiovascular events, and cigarette smokers were excluded from the study. None of the patients studied was taking any medication. The patients with hemochromatosis were studied before the iron depletion procedure. We evaluated vascular function using the forearm perfusion technique associated with plethysmography. This approach is regarded as the gold standard to dissect the endothelial and smooth muscle cells (VSMC) components of vascular response. Written informed consent was obtained by all the subjects studied and the protocol, designed according the current guidelines of good clinical practice, was approved by the Federico II University Ethics Committee. As previously described [10], a plastic cannula was inserted into the brachial artery of the non-dominant arm and used for the step-wise infusions of acetylcholine (Ach) and sodium nitroprusside (NP). Forearm blood flow (FBF) response to Ach infusion represents the cumulative effect of the release of nitric oxide (NO) by endothelial cells and the relaxation of VSMC induced by NO. FBF response to the infusion of NP, an NO donor, is solely dependent on VSMC relaxation. FBF was measured by strain gauge plethysmography. Patient sample size of the current protocol was calculated based on the expected difference in the slope of the response to NP. Assuming a significance level of 5% and 90% study power, a sample of 11 patients was sufficient to demonstrate a difference of 25% between the two groups. Comparison between hemochromatosis and healthy controls was performed by two-way ANOVA for repeated measures (IBM SPSS, version 26) or Student's *t* test, as appropriate. Results are expressed as mean ± SEM.

Infusion of Ach elicited a progressive and marked vasodilatory response (Fig. 1A). In patients with hemochromatosis, this response was markedly lower than in controls, as shown by the markedly different slopes of the dose response curves ($0.32 \pm 0.06$ and $0.14 \pm 0.05$ ml $dL^{-1}$ $min^{-1}$ $\mu g^{-1}$

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**Table 1** Clinical characteristics of the population studied

| | Hemochromatosis ($n=11$) | Healthy controls ($n=12$) |
|---|---|---|
| Sex (M/F) | 11/0 | 12/0 |
| Age (yrs) | 45.0 ± 3.8* | 42.2 ± 4.5 |
| BMI (kg/m$^2$) | 26.3 ± 0.79 | 25.4 ± 0.83 |
| Body Weight (kg) | 78.8 ± 1.4 | 73.7 ± 2.7 |
| Fat Body Mass (kg) | 19.9 ± 0.9 | 19.4 ± 0.9 |
| Lean Body Mass (kg) | 59.0 ± 1.1 | 54.2 ± 2.5 |
| Blood glucose (mg/dl) | 85 ± 1.8 | 81.3 ± 2.2 |
| Serum insulin (μ/ml) | 12.6 ± 1.5 | 10.4 ± 1.3 |
| Total cholesterol (mg/dl) | 196.9 ± 1.8 | 196.3 ± 1.9 |
| HDL cholesterol (mg/dl) | 48.2 ± 1.4 | 51.4 ± 1.4 |
| LDL cholesterol (mg/dl) | 122.5 ± 1.8 | 120.5 ± 3.1 |
| Triglycerides (mg/dl) | 131.3 ± 4.1 | 122.2 ± 7.3 |
| Serum Ferritin (μg/l) | 988 ± 315 | 113 ± 17 |
| SBP (mmHg) | 126 ± 2 | 119 ± 4 |
| DBP (mmHg) | 77 ± 2 | 72 ± 2 |
| Heart Rate (bpm) | 69 ± 3 | 64 ± 2 |

*Mean ± SEM

in controls and patients, respectively; $P<0.05$), although ANOVA for repeated measures did not show significant interaction between hemochromatosis and Ach response ($p=0.08$). As compared with controls, patients with hemochromatosis showed a much lower response to NP. The slopes of the dose–response curves were $1.0 \pm 0.2$ and $2.0 \pm 0.2$ ml dL$^{-1}$ min$^{-1}$ μg$^{-1}$ in patients and controls, respectively; $P<0.005$). Indeed, ANOVA for repeated measures demonstrated a significant interaction between hemochromatosis and NP response ($p=0.01$, Fig. 1B). These data prima facie would be compatible with a combined, endothelial and VSMC defect. However, if the slopes of the Ach curves are normalized by the NP slopes, they become were similar in patients with hemochromatosis and controls ($0.12 \pm 0.02$ and $0.15 \pm 0.02$, respectively, $P=0.3$), suggesting that the impairment of VSMC response to NO might be the cause of vascular dysfunction in patients with hemochromatosis.

An isolated defect in endothelial function was previously reported in hemochromatosis using the flow mediated dilation (FMD) technique [6]. The function of VSMC was explored by sublingual glyceryl trinitrate and was found unchanged. When this approach is used, many variables can play a role (i.e., amount and time of glyceryl trinitrate absorption, its plasma concentration, blood pressure changes evoked by glyceryl trinitrate, etc.) and interfere with VSMC response to NO. We show that VSMC function is severely impaired in patients with hemochromatosis when direct intra-arterial infusion of graded doses of NP is used as a stimulus. The intact Ach/NP slope ratio in patients with hemochromatosis, seen in the current study, suggests that endothelial function is not affected by iron overload, at least in this disease. On the other hand, hemochromatosis patients carrying the C282Y mutation of the HFE gene did not show CIMT difference vs the healthy population [11] or higher risk of restenosis after coronary stent implantation [12]. Finally, pulse wave velocity and other markers of endothelial function did not change after a program of venesection, suggesting that iron depletion in patients with hemochromatosis does not affect endothelial activity [13]. Recently, a study in mouse has demonstrated that VSMC function can be altered by iron overload, suggesting a potential mechanism for the defect we observe in the current work [14].

Due to the characteristics of the current study and the limited number of subjects studied, we could not explore

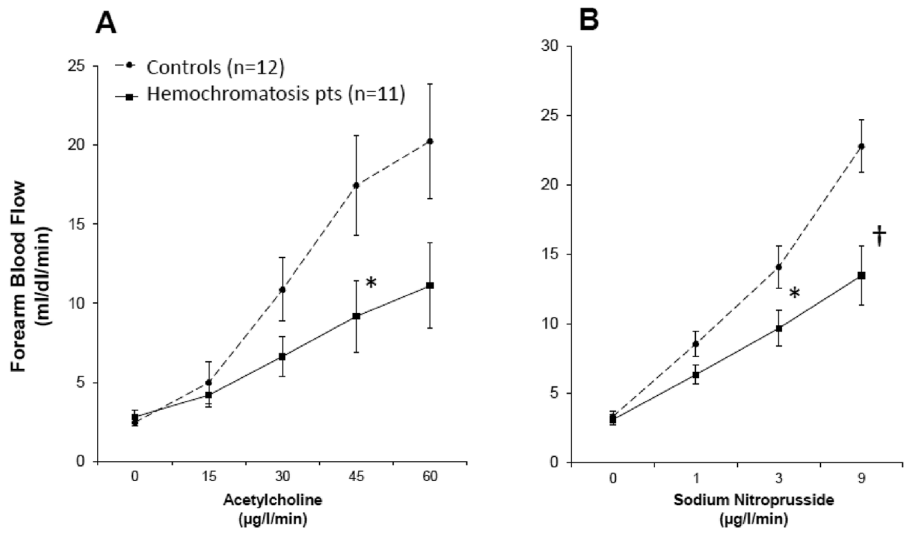


**Fig. 1** Forearm blood flow response to infusion of Ach (panel A) or sodium nitroprusside (panel B) into the brachial artery in patients with hemochromatosis (continuous line) and control subjects (dotted line). Data (mean ± SEM) were analyzed by ANOVA for repeated measures. $P=0.08$ for the interaction between hemochromatosis and Ach. $P=0.01$ for the interaction between hemochromatosis and nitroprusside. Student's t test comparisons: *$p<0.05$, †$p>0.005$ vs controls

differences among different hemochromatosis genotypes with regard VSMC function. However, data available in literature indicate that no differences in FMD of the brachial artery or carotid intima–media thickness among carriers of different HFE genotypes were detectable [15].

In conclusion, the response of VSMC to NP is altered in patients with hemochromatosis. Therefore, the defective response to Ach cannot be simply attributed to endothelial dysfunction, but rather to a defective response of VSMC to the NO released by the endothelial cells, as documented by the unchanged Ach slopes when normalized by the NP slopes. The observed lack of association between hemochromatosis and CVD risk [7], appears to be in line with the current finding that endothelial function is unaltered in patients with hemochromatosis.

## Declarations

**Conflict of interest** The authors declare that they do not have any conflict of interest related to the current manuscript.

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