Original Article

Sympathetic Overactivation in Patients With Essential Hypertension and Hepatic Iron Overload

Gino Seravalle,* Raffaella Dell'Oro,* Fosca Quarti-Trevano, Domenico Spaziani, Silvio Bertoli, Flavio Airoldi, Giuseppe Mancia, Guido Grassi

Abstract—Iron overload has been recently shown to be associated with a hyperadrenergic state in genetic hemochromatosis. Whether this is also the case in essential hypertension, characterized by sympathetic activation and frequently by body iron overload, is unknown. In 17 healthy normotensive controls (age 52.3±3.2 years, mean±SE), in 21 age-matched patients with hypertension with iron overload (HT+), defined by serum ferritin levels, and in 28 hypertensives without this condition, we measured efferent postganglionic muscle sympathetic nerve traffic (microneurography), heart rate and blood pressure variability (power spectral analysis), serum ferritin, and metabolic variables. Muscle sympathetic nerve traffic was significantly (P<0.02 at least) greater in HT+ than in patients with hypertension without iron overload and normotensive subjects both when expressed as bursts incidence over time (41.8±1.4 versus 31.5±1.4 and 23.6±0.9 bursts/min) and as bursts corrected for heart rate (55.3±1.8 versus 42.3±1.2 and 31.7±1.2 bursts/100 heartbeats). In HT+, low-frequency systolic blood pressure variability was significantly reduced. In HT+, but not in the other 2 groups, muscle sympathetic nerve traffic was significantly related to serum ferritin (r=0.51, P<0.03), transferrin saturation (r=0.47, P<0.03), and hepatic iron load (r=0.76, P<0.0001, magnetic resonance imaging), as well as to homeostatic model assessment index values (r=0.46, P<0.05). These data provide the first evidence that in HT+ elevated serum ferritin is associated with a hyperadrenergic state of greater magnitude than the one seen in patients with hypertension without iron overload. They also show that the potentiation of the sympathetic activation detected in HT+ is related to elevated serum ferritin and to the associated metabolic alterations, possibly participating in the increased cardiovascular risk characterizing iron overload. **(*Hypertension*. 2020;76:00-00. DOI: 10.1161/HYPERTENSIONAHA.120.15511.)**

Key Words: blood pressure ■ ferritin ■ insulin ■ iron overload ■ risk factors

Our group has recently shown that patients with genetic hemochromatosis display, along with iron overload in organs throughout the body, a marked increase in sympathetic neural influences to the heart and the peripheral circulation which can be almost completely reversed by iron depletion therapy.[1] This finding suggests that iron exerts in this disease sympathoexcitatory effects. Whether and to what extent the adrenergic activation triggered by iron deposition in organs throughout the body is peculiar to genetic hemochromatosis or it can be detected in other clinical conditions also characterized by sympathetic overactivity and iron overload is unknown. The issue has not only pathophysiological but also clinical relevance, given the evidence that greater sympathetic activation is associated with a poorer prognosis in several cardiovascular and noncardiovascular disease.[2–5]

In the present study, we investigated this issue by recording muscle sympathetic nerve traffic via the microneurographic technique in patients with essential hypertension which are characterized by a sympathetic activation[6,7] and a high prevalence of increased iron load in organs throughout the body.[8] Data were compared to those obtained in a group of age-matched essential hypertensives not displaying the above-mentioned alterations in iron deposition in organs throughout the body and in healthy normotensive controls.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Population

The study population consisted of 66 patients of both sexes (58 males and 8 females) and an age range between 33 and 64 years recruited for this specific study between 2014 and 2019. Twenty-one of them were mild-to-moderate essential hypertensives and displayed hepatic iron overload (see below) together with elevated serum ferritin values, whereas the remaining 28 hypertensives displayed normal serum ferritin values (Table 1). The 39 patients with hypertension were selected from those regularly followed at the outpatient hypertension clinic of the Istituto a Carattere Scientifico Multimedica Hospital and Magenta Hospital. Criteria for selection were the presence of

Received May 11, 2020; first decision May 27, 2020; revision accepted August 23, 2020.

From the Clinica Medica, Department of Medicine and Surgery, University of Milano-Bicocca, Italy (G.S., R.D., F.Q.-T., G.G.); Cardiologia, Ospedale di Magenta, Milan, Italy (D.S.); Istituto a Carattere Scientifico Multimedica, Sesto San Giovanni, Milan, Italy (S.B., F.A.); and University Milano-Bicocca and Policlinico di Monza, Italy (G.M.).

*These authors contributed equally to this work.

This paper was sent to Karen A. Griffin, Guest Editor, for review by expert referees, editorial decision, and final disposition.

Correspondence to Guido Grassi, Clinica Medica, University of Milano-Bicocca, via Pergolesi 33, 20052 Monza (Milano), Italy. Email guido.grassi@unimib.it

Table 1. Anthropometric, Hemodynamic, Echocardiographic, and Metabolic Parameters in the 3 Groups

Variable	NT (n=17)	HT– (n=28)	HT+ (n=21)
Sex, M/F (n)	14/3	24/4	20/1
Age, y	52.3±3.2	52.7±2.3	50.8±2.5
BMI, kg/m^2	24.4±0.8	26.4±1.7	27.1±1.9
WHR, a.u.	0.76±0.02	0.77±0.01	0.78±0.01
Clinic BP, S/D, mm Hg	129.7±4.1/80.4±3.3	164.3±5.2*/98.4±4.5*	169.1±5.8*/100.7±4.9*
Clinic HR, bpm	71.4±2.2	73.8±2.5	74.3±2.7
24 h BP, S/D, mm Hg	125.3±3.4/75.2±3.0	158.8±3.7*/95.5±3.4*	161.9±4.0*/96.7±3.7*
24 h HR, bpm	67.1±1.9	67.9±2.1	68.5±2.0
LVEF, %	62.8±2.6	60.2±1.9	59.6±2.1
E/A ratio, a.u.	1.14±0.2	1.12±0.2	1.11±0.2
LVMI, g/m^2	90.8±3.9	107.5±5.6*	111.6±6.1†
Total cholesterol, mg/dL	188.6±12.5	204.8±12.3	229.4±13.7*
HDL cholesterol, mg/dL	48.4±5.6	53.8±4.8	57.2±5.1
Triglycerides, mg/dL	155.7±26.0	188.4±19.1*	206.0±22.1†
Plasma glucose, mg/dL	86.2±9.2	91.4±7.8	98.5±8.4
HOMA index, a.u.	1.59±0.3	1.67±0.2	2.23±0.4*‡
ALT, U/L	30.4±2.2	29.9±2.0	32.1±2.3
yGT, U/L	33.3±2.3	31.7±2.4	34.2±2.5
eGFR, mL/(min·1.73 m^2)	87.7±4.4	89.5±3.7	90.3±4.0
Hemoglobin, g/dL	14.5±0.4	14.8±0.3	14.7±0.3
Hematocrit, %	43.9±1.4	43.5±1.6	43.4±2.0
Respiration rate (breaths/min)	16.7±1.2	17.0±1.5	16.9±1.7
Hepatic steatosis, n (%)	5 (29%)	11 (39%)	21 (100%)
LIC, mmol/g	n.a.	n.a.	156.8±56.

Data are shown as means±SEM. a.u. indicates arbitrary units; ALT, alanine aminotransferase; BMI, body mass index; BP, blood pressure; D, diastolic; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; HR, heart rate; HT–, patients with hypertension without iron overload; HT+, patients with hypertension with iron overload; LIC, liver iron content; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; M/F, males/females; n.a., not assessed; NT, normotensive subjects; S, systolic; WHR, waist-to-hip ratio; and yGT, y-glutamyl transferase.

*P<0.05: refers to the statistical significance between HT+ and HT– vs NT.

†P<0.01: refers to the statistical significance between HT+ and HT– vs NT.

‡P<0.05: refers to the statistical significance between HT+ vs HT–.

an essential hypertensive state with or without the detection of iron overload. Seventeen subjects were normotensive healthy individuals with normal serum ferritin values recruited for this specific study among the administrative personnel of Istituto a Carattere Scientifico Multimedica Hospital and served as controls. Exclusion criteria were (1) congestive heart failure, as diagnosed by symptoms, signs, and alterations in echocardiographically determined left ventricular diameters and ejection fraction; (2) history of coronary or cerebrovascular disease; (3) atrial fibrillation or other major cardiac arrhythmias; (4) clinical, laboratory or instrumental evidence of valvular heart disease, left ventricular hypertrophy, or secondary hypertension; (5) true drug-resistant and apparent drug-resistant hypertension; (6) clinical conditions known to be associated with an iron overload, including genetic hemochromatosis (evaluated via assessment of hemochromatosis genotype homozygous for the C282Y mutation of the *HFE* gene) or a serum ferritin increase unrelated to body iron stores; (7) chronic liver disease and inflammatory disorders; (8) history of blood transfusions or parenteral administration of iron compounds; (9) history of cigarette smoking, excessive alcohol consumption (>60 g in men and >40 g in women) or use of antidepressant drugs; (10) use of antihypertensive drugs which were discontinued, if present, at least 1 week before the study; (11) respiratory diseases and sleep apnea; and (12) major concomitant diseases, such as renal insufficiency, diabetes mellitus, obesity, and other conditions know to affect sympathetic neural function.[6,7,9–12] All patients were evaluated on an outpatient basis. The study protocol was approved by the Ethics Committee of the Istituto a Carattere Scientifico Multimedica, Sesto San Giovanni (Milan, Italy). All patients gave written consent to the study after being informed of its nature and purpose.

Measurements

In all individuals, multiunit recordings of efferent postganglionic sympathetic nerve traffic to the skeletal muscle (MSNA) were obtained by a single operator through a tungsten microelectrode inserted into the right or left peroneal nerve, as previously described.[1,9–12] Other measurements included blood pressure (BP), which was obtained by a mercury sphygmomanometer, a finger photoplethysmographic device capable of providing accurate beat-to-beat systolic and diastolic values (Finapres, Ohmeda 2003, Englewood, FL)[1,10,11] and ambulatory BP recording obtained over the 24 hours by an oscillometric device (Spacelabs 90207, Spacelabs) with the recordings set

at 15-minute and 20-minute intervals during the daytime (from 07.00 to 23.00 hour) and the nighttime (from 23.00 to 07.00 hour) periods, respectively[1,10,11]; heart rate, which was monitored beat-to-beat by a tachograph triggered by the R wave of an EKG; respiration rate, which was measured by a strain gauge pneumograph positioned at midchest level; an echocardiographic evaluation of the end-diastolic and end-systolic left ventricular internal diameters, interventricular septum thickness, and posterior wall thickness. Left ventricular mass index was calculated by Devereux formulae and normalized to body surface area, whereas left ventricular ejection fraction was measured from the 4-chamber apical projection using the product area times length.[13,14] Color Doppler and pulse Doppler were used to measure mitral flow (early diastolic peak flow velocity [E wave] and late diastolic peak flow velocity [A wave]) and flow at the left ventricular outflow tract and to calculate E/A ratio[15]; body mass index (body mass index, body weight divided by squared height), and waist and hip circumferences, which were obtained with the patient standing and used to determine the waist-to-hip ratio; routine hematologic and blood chemistry (standard methods), including hepatic indices (AST [aspartate aminotransferase], ALT [alanine aminotransferase]), complete blood count, homeostatic model assessment (HOMA) index (calculated via the formulae: plasma glucose [mmol/L]×serum insulin [μU/mL]/22.5),[16] and serum iron profile (serum iron, transferrin, ferritin, transferrin saturation calculated via the formulae: serum iron/serum transferrin/1.42×100). As upper normal limits for serum ferritin, 350 μg/L for men and 200 μg/L for women were used based on the most frequent upper normal limits of ferritin present in the literature for iron overload screening.[17] An ultrasound examination of the liver to evaluate the presence of hepatic steatosis was also performed while magnetic resonance imaging (1.5 T) based on Gandon algorithm[18] was performed in patients with hypertension with iron overload to quantify liver iron concentration with reference values <36 μmol/g for physiological condition,<100 μmol/g for mild iron overload, between 100 and 300 μmol/g for moderate, and >300 μmol/g for severe iron overload.

Simultaneous MSNA, beat-to-beat heart rate, and beat-to-beat BP recordings were digitized with a sampling frequency of 1000 Hz (PowerLab Recording System Model ML870 8/30; AD Instruments, Bella Vista, New South Wales, Australia). MSNA was quantified over a 30-minute period either as bursts incidence over time (bursts/min) or as bursts incidence corrected for heart rate values (bursts per 100 heartbeats).[1,9–12] Heart rate and systolic BP variability were evaluated via the spectral analysis technique.[19,20] Briefly, time series of BP and pulse pressure interval values were analyzed by a parametric spectral estimation method based on an autoregressive model. Baroreflex control of MSNA was determined by a method similar to that described by Kienbaum et al,[21] that is, by relating each spontaneous sympathetic neural burst to the diastolic BP and the cardiac interval during which the burst was generated. The method allows to obtain an estimate of spontaneous MSNA-baroreflex sensitivity almost superimposable to the one obtained via the classic vasoactive drugs infusion technique.[22]

Protocol and Data Analysis

Patients were selected initially evaluated for iron metabolism screening and then selected to undergo the measurements listed above. As far as the microneurographic evaluation, all subjects came to the laboratory in the morning, after a light breakfast and an overnight abstinence from alcohol and coffee consumption. They were put in supine position and fitted with the various measuring devices, except the microelectrode for sympathetic nerve traffic recording. BP was measured 3× with a mercury sphygmomanometer, and a microelectrode was inserted into the peroneal nerve to obtain MSNA. Sympathetic nerve traffic was recorded together with finger BP, heart rate, and respiration rate during a 30-minute period in a quiet semidark room kept at the constant temperature of 22°C to 24°C and analyzed by a single investigator unaware of the belonging of the subjects to the different groups. Ambulatory BP monitoring was performed the day following the microneurographic study. Comparisons between groups were made by 2-way ANOVA. The 2-tailed *t* test for unpaired observations was used to locate between-group differences. The Bonferroni correction was used for multiple comparisons. The Pearson correlation coefficient was used to determine the relationships between MSNA, serum ferritin, transferrin saturation, hepatic iron content, hepatic steatosis, HOMA index, plasma triglycerides, total cholesterol, and spontaneous baroreflex sensitivity. Data are expressed as means±SEM. A $P<0.05$ was taken as the minimal level of statistical significance. All analyses were performed with SAS software version 9.3 (SAS Institute Inc, Cary, NC).

Results

The main characteristics of the 3 groups of subjects are shown in Table 1. Age and sex distribution were superimposable in the different groups, whereas body mass index and waist-to-hip ratio showed a tendency, although not significant, to be greater in the 2 hypertensive groups as compared to the normotensive one. Echographic evidence of hepatic steatosis was found in 37 individuals, 32 of them being hypertensives. All the hypertensives patients with elevated serum ferritin showed at magnetic resonance imaging evaluation a liver iron overload of moderate degree. As expected, both clinic and 24-hour ambulatory BP values were significantly higher in the 2 hypertensive groups than in the normotensive one, whereas the corresponding heart rate values were similar. The degree of the BP elevation was slightly more pronounced, although not significantly, in the patients with hypertension with elevated than in those with normal serum ferritin values. Left ventricular ejection fraction and E/A ratio values were superimposable in all the 3 groups, whereas left ventricular mass index was significantly increased in the patients with hypertension as compared to normotensive individuals. Patients with hypertension without iron overload showed plasma cholesterol, HOMA index, and glucose levels similar to the ones detected in the normotensive individuals but significantly greater serum triglycerides levels. In the patients with hypertension with serum iron overload HOMA index, serum triglycerides and total cholesterol were significantly greater than in the normotensive subjects. In the case of HOMA index, a statistical significance was also detected between patients with hypertension with elevated than in those with normal serum iron load. Hematologic and renal variables were in the normal range and similar in the 3 groups.

The behavior of individual serum ferritin and serum transferrin and their mean values in the different groups of subjects are illustrated in the upper parts of Figure 1. Serum ferritin and transferrin levels were, as expected, significantly and markedly greater in the patients with hypertension with iron overload as compared to the values detected in the normotensive individuals and in the patients with hypertension without this condition. As shown in the lower parts of Figure 1, MSNA, expressed as bursts incidence over time (left lower) and as bursts incidence corrected for heart rate (right lower), was significantly greater in patients with hypertension with iron overload as compared to the other 2 groups. This was accompanied by the finding that low-frequency systolic BP variability was significantly increased in the 2 groups of patients with hypertension as compared to normotensive controls. This was the case also when patients with hypertension with iron overload were compared with those without this condition, whereas high-frequency heart rate variability was similarly reduced in the 2 groups of patients with hypertension when compared to the normotensive healthy controls (Table 2). Spontaneous baroreflex modulation of MSNA was

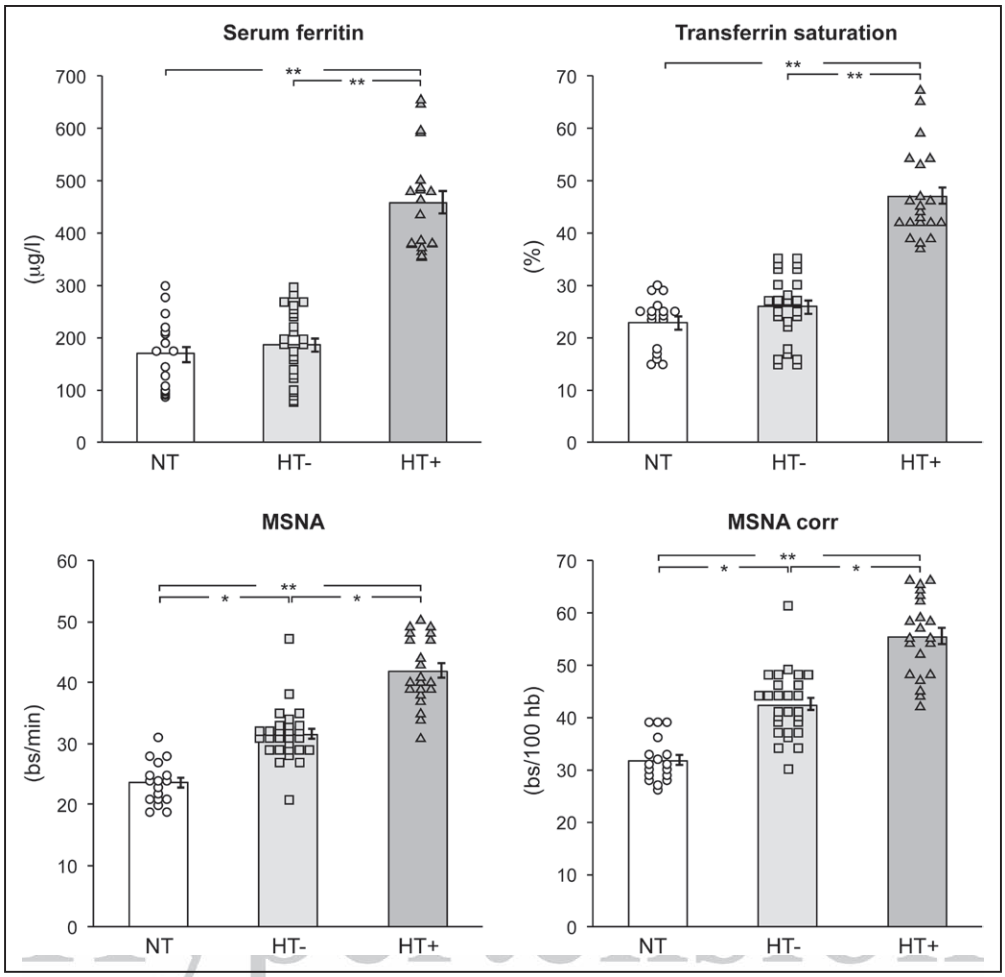

Figure 1. Individual and average values (±SEM) of serum ferritin, transferrin saturation, and muscle sympathetic nerve traffic (MSNA), expressed as bursts incidence over time (MSNA) and as bursts incidence corrected for heart rate (MSNA corr) in normotensive subjects (NT) and in patients with hypertension without iron overload (HT–) and with iron overload (HT+). bs/100hb indicates bursts/100 heartbeats; and Bs/min, bursts/min. **P*<0.02 and ***P*<0.001 refer to the statistical significance between groups.

slightly, although not significantly, greater in the normotensive group as compared to the hypertensive ones without and with iron overload (Table 2).

Figure 2 shows the relationships between different parameters of iron metabolism and sympathetic activity. Upper parts show that in the whole study population there was a highly significant direct relationship between serum ferritin, transferrin saturation, and MSNA. The relationships between serum ferritin, transferrin saturation, and hepatic iron load were seen in patients with hypertension with iron overload (Figure 2, lower) but not in the normotensive control subjects and in patients with hypertension without iron overload. No significant relationship was also found in the 3 groups of subjects of the present study between MSNA values and baroreflex MSNA modulation (data not shown).

In the study population as a whole, a weak but significant relationship was found between MSNA and hepatic steatosis (r=0.33, P<0.05). The presence of hepatic steatosis correlated in the patients with hypertension with serum iron overload with HOMA index (r=0.56, P<0.01), plasma triglycerides (r=0.47, P<0.05), and total plasma cholesterol (r=0.49, P<0.05). Furthermore, in the patients with hypertension with iron overload, but not in the other 2 groups of individuals of the present study, MSNA displayed a significant and direct relationship with HOMA value, both when expressed as bursts incidence over time and as bursts incidence corrected for heart rate (r=0.46 and r=0.49, P<0.05 for both). Similarly in the group of patients with iron overload, but not in the other 2, transferrin saturation values significantly and directly correlated with HOMA index values (r=0.53, P<0.05).

Discussion

There are 3 novel findings in the present study. First, patients with essential hypertension with documented iron overload independent of genetic hemochromatosis display a sympathetic activation significantly greater in magnitude than the one detected in age-matched patients with hypertension characterized by a high BP state of similar clinical severity but without alterations in iron deposition in organs throughout the body. Second, the magnitude of the adrenergic overdrive in this group of patients is directly related to serum ferritin levels, serum transferrin saturation, hepatic iron content, and detection of hepatic steatosis. Third, the potentiation of the sympathetic activation detected in patients with hypertension with iron overload occurs without being associated with a further increase in the BP values. This finding may suggest that a sympathetic overdrive does not

Table 2. Low- and High-Frequency HRV, Low-Frequency SBP Variability, and BxMSNA Modulation in Healthy NT and in HT– and With HT+ Iron Overload

Variable	NT (n=17)	HT– (n=28)	HT+ (n=21)
HRV, LF, ms^2	475.1±98	533.5±76	579.9±82
HRV, HF, ms^2	396.4±49	185.6±42*	190.7±48*
SBP, LF, mmHg^2	4.8±1.4	7.2±1.1†	8.8±1.4*‡
BxMSNA, a.u.	–3.44±0.3	–3.25±0.2	–3.01±0.3

Data are shown as means±SEM. a.u. indicates arbitrary units; BxMSNA, baroreflex-muscle sympathetic nerve activity; HF, high frequency; HRV, heart rate variability; HT–, patients with hypertension without iron overload; HT+, patients with hypertension with iron overload; LF, low frequency; NT, normotensive subjects; and SBP, systolic blood pressure.

*P<0.01: refers to the statistical significance between HT+ and HT– vs NT.

†P<0.05: refers to the statistical significance between HT+ and HT– vs NT.

‡P<0.05: refers to the statistical significance between HT+ vs HT–.

necessarily result in a pressor effect, as already reported in other conditions characterized by sympathetic overactivity, such as in the normotensive obese state,[23] in the normotensive diabetic condition,[24] or finally in the congestive heart failure state with normal BP values.[9,25,26] As discussed below, the absence of a BP effect, however, does not mean that the sympathetic activation characterizing iron overload is exempt from producing adverse cardiovascular effects.[6,7] Taken together these findings support the conclusion that a potentiation of the sympathetic activation characterizing essential hypertension is detectable when a condition of iron overload, which is quite common in the high BP state, accompanies the BP elevation. Previous studies have indeed shown that this condition can be detected in 21% of the patients with hypertension regularly followed in an outpatient clinic.[8] The study results also suggest that the sympathetic abnormality reflects the iron overload state and correlates with the presence of hepatic steatosis.

Two other findings of the present study merit a brief discussion. First, in patients with hypertension with iron overload, the marked elevation in MSNA was accompanied by an increase in the low-frequency oscillations of systolic BP variability more pronounced than the one detected in patients with hypertension without iron overload. Although this finding may suggest that the augmented sympathetic neural drive detected in patients with hypertension complicated by iron overload extends to cardiovascular districts other than the muscle one, given the well-known limitations of the time-domain approach,[7,20] further studies specifically investigating sympathetic activity in the different regional districts (including the cardiac one) are needed to support this hypothesis. Second, in our patients with hypertension with iron overload, we found that the sympathetic activation was coupled with a reduction in the high-frequency component of heart rate variability of similar magnitude to the one seen in the group of patients with hypertension without this condition. This suggests that in essential hypertension the iron overload state affects the sympathetic but not the parasympathetic component of cardiovascular autonomic function, at variance from what we previously reported in genetic hemochromatosis in which both autonomic components were markedly altered.[1] Third, the potentiation of the sympathetic activation seen in patients with hypertension with iron overload may have adverse consequences on different organs. For example, at the level of the heart, it may favor the development and progression of left ventricular hypertrophy, given the evidence that a chronic increase in plasma norepinephrine favor

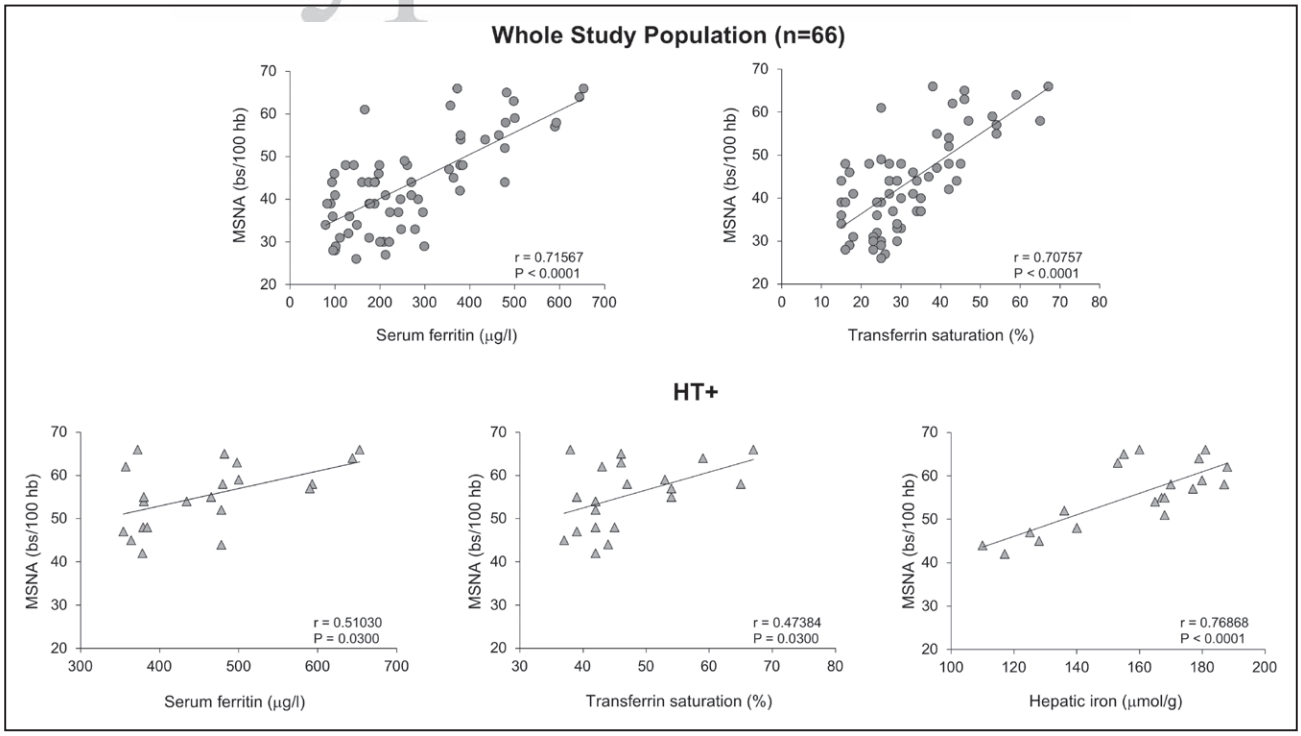

Figure 2. Relationships between different parameters of iron metabolism and symapthetic activity. **Upper**, Regressing muscle sympathetic nerve traffic (MSNA), expressed as bursts incidence corrected for heart rate (bursts/100 heartbeats [bs/100hb]), on serum ferritin, transferrin saturation in the whole study population. Correlation coefficients (r) and P values are shown. **Lower**, Regressing MSNA, expressed as bursts incidence corrected for heart rate (bs/100hb), on serum ferritin, transferrin saturation, and hepatic iron content in patients with hypertension with iron overload (HT+). Correlation coefficients (r) and P values are shown.

in experimental animals an increase in cardiac mass and cardiac wall thickness even when BP remains within the normotensive range.[27] At the level of the liver, it may reduce hepatic ketogenesis and constrict the portal vein system favoring the development of portal hypertension.[28] At kidney level, it may reduce renal blood flow and directly enhance tubular reabsorption, thereby favoring fluid retention.[29]

Our study also provides insights on the possible mechanisms responsible for the different sympathetic profile of the hypertensive state with versus the hypertensive condition without iron overload. We can rule out that this was due to a different clinical severity of the hypertensive state because (1) the magnitude of the BP increase was slightly but not significantly different between the hypertensive states without and with iron overload and (2) the increase in left ventricular mass, taken as marker of target organ damage, was also not significantly different in the 2 groups. We can also rule out that the difference in the sympathetic activation detected in our patients with hypertension with and without iron overload might be ascribed to a different baroreceptor modulation of MSNA in the 2 conditions because baroreflex MSNA sensitivity was similar in the 2 groups. We can, on the other hand, suggest that the differences in the sympathetic activation might depend on 3 nonmutually exclusive mechanisms. First, we can speculate that they depend on the greater HOMA index, and thus on the increased circulating plasma insulin values, reported when iron overload is detected in essential hypertension because insulin has been shown to exert profound central sympathoexcitatory effects.[7,8,30,31] We can also speculate that the metabolic abnormalities detected in patients with hypertension with iron overload might be responsible for these differences, given the evidence that these alterations potentiate, particularly when combined together such as in the metabolic syndrome, cardiovascular sympathetic drive.[32] We can finally advance the hypothesis that the observed sympathetic differences might depend on the iron overload per se because iron (1) impairs the ability of reflexogenic areas anatomically located in the cardiac chambers as well as in the pulmonary circulation (so-called cardiopulmonary volume receptors) to inhibit central adrenergic neural outflow,[6,33] thereby contributing to the development of the sympathetic overdrive and (2) favors, as free iron, the occurrence of oxidative stress reactions, nitric oxide crowding, and thus throughout different mechanisms (which may include the inflammatory process and the immune reaction[34]) triggers sympathetic activation.[35] This latter mechanism appears to be supported by the finding that nitric oxide affects central sympathetic neural outflow both directly and indirectly, that is, via a vasodilatatory-induced reflex activation.[36]

Perspectives

Our study has some limitations and a clinical implication. One limitation is that our data did not allow us to clarify the so-called chicken-and-egg question, that is, whether the insulin resistance state and the concomitant metabolic alterations were the cause or rather the consequence of the sympathetic activation.[37,38] A further limitation refers to the fact that we could not assess indices of oxidative stress reaction activity of iron to confirm a pathogenetic hypothesis. The clinical implication is that the activation of the sympathetic cardiovascular influences that occur in hypertension complicated by iron alterations may represent one of the pathophysiological mechanisms responsible for the increased cardiovascular risk reported in the iron overload states.[39,40]

Sources of Funding

None.

Disclosures

None.

References

1. Seravalle G, Piperno A, Mariani R, Pelloni I, Facchetti R, Dell'Oro R, Cuspidi C, Mancia G, Grassi G. Alterations in sympathetic nerve traffic in genetic haemochromatosis before and after iron depletion therapy: a microneurographic study. *Eur Heart J.* 2016;37:988–995. doi: 10.1093/eurheartj/ehv696
2. Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med.* 1984;311:819–823. doi: 10.1056/NEJM198409273111303
3. Brunner-La Rocca HP, Esler MD, Jennings GL, Kaye DM. Effect of cardiac sympathetic nervous activity on mode of death in congestive heart failure. *Eur Heart J.* 2001;22:1136–1143. doi: 10.1053/euhj.2000.2407
4. Sander D, Winbeck K, Klingelhöfer J, Etgen T, Conrad B. Prognostic relevance of pathological sympathetic activation after acute thromboembolic stroke. *Neurology.* 2001;57:833–838. doi: 10.1212/wnl.57.5.833
5. Zoccali C, Mallamaci F, Parlongo S, Cutrupi S, Benedetto FA, Tripepi G, Bonanno G, Rapisarda F, Fatuzzo P, Seminara G, et al. Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease. *Circulation.* 2002;105:1354–1359. doi: 10.1161/hc1102.105261
6. Grassi G. Assessment of sympathetic cardiovascular drive in human hypertension: achievements and perspectives. *Hypertension.* 2009;54:690–697. doi: 10.1161/HYPERTENSIONAHA.108.119883
7. Grassi G, Mark A, Esler M. The sympathetic nervous system alterations in human hypertension. *Circ Res.* 2015;116:976–990. doi: 10.1161/CIRCRESAHA.116.303604
8. Piperno A, Trombini P, Gelosa M, Mauri V, Pecci V, Vergani A, Salvioni A, Mariani R, Mancia G. Increased serum ferritin is common in men with essential hypertension. *J Hypertens.* 2002;20:1513–1518. doi: 10.1097/00004872-200208000-00013
9. Leimbach WN Jr, Wallin BG, Victor RG, Aylward PE, Sundlöf G, Mark AL. Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. *Circulation.* 1986;73:913–919. doi: 10.1161/01.cir.73.5.913
10. Grassi G, Quarti-Trevano F, Seravalle G, Arenare F, Volpe M, Furiani S, Dell'Oro R, Mancia G. Early sympathetic activation in the initial clinical stages of chronic renal failure. *Hypertension.* 2011;57:846–851. doi: 10.1161/HYPERTENSIONAHA.110.164780
11. Grassi G, Seravalle G, Quarti-Trevano F, Scopelliti F, Dell'Oro R, Bolla G, Mancia G. Excessive sympathetic activation in heart failure with obesity and metabolic syndrome: characteristics and mechanisms. *Hypertension.* 2007;49:535–541. doi: 10.1161/01.HYP.0000255983.32896.b9
12. Narkiewicz K, van de Borne PJ, Cooley RL, Dyken ME, Somers VK. Sympathetic activity in obese subjects with and without obstructive sleep apnea. *Circulation.* 1998;98:772–776. doi: 10.1161/01.cir.98.8.772
13. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American society of echocardiography committee on standards, subcommittee on quantitation of two-dimensional echocardiograms. *J Am Soc Echocardiogr.* 1989;2:358–367. doi: 10.1016/s0894-7317(89)80014-8
14. Park SH, Shub C, Nobrega TP, Bailey KR, Seward JB. Two-dimensional echocardiographic calculation of left ventricular mass as recommended by the American Society of echocardiography: correlation with autopsy and M-mode echocardiography. *J Am Soc Echocardiogr.* 1996;9:119–128. doi: 10.1016/s0894-7317(96)90019-x
15. Paulus WJ, Tschöpe C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, Marino P, Smiseth OA, De Keulenaer G, Leite-Moreira AF, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection

fraction by the heart failure and echocardiography associations of the European society of cardiology. *Eur Heart J.* 2007;28:2539–2550. doi: 10.1093/eurheartj/ehm037
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28:412–419. doi: 10.1007/BF00280883
17. Worwood M, May AM, Bain BJ. Iron deficiency anaemia and iron overload. In: Bain B, Bates I, Laffan MA, eds. *Dacie and Lewis Practical Haematology*. 12th ed. Amsterdam, the Netherlands: Elsevier; 2017:165–186.
18. Gandon Y, Olivié D, Guyader D, Aubé C, Oberti F, Sebille V, Deugnier Y. Non-invasive assessment of hepatic iron stores by MRI. *Lancet.* 2004;363:357–362. doi: 10.1016/S0140-6736(04)15436-6
19. Sassi R, Cerutti S, Lombardi F, Malik M, Huikuri HV, Peng CK, Schmidt G, Yamamoto Y. Advances in heart rate variability signal analysis: joint position statement by the e-cardiology ESC working group and the European Heart Rhythm association co-endorsed by the Asia pacific heart rhythm society. *Europace.* 2015;17:1341–1353. doi: 10.1093/europace/euv015
20. Baselli G, Cerutti S, Civardi S, Liberati D, Lombardi F, Malliani A, Pagani M. Spectral and cross-spectral analysis of heart rate and arterial blood pressure variability signals. *Comput Biomed Res.* 1986;19:520–534. doi: 10.1016/0010-4809(86)90026-1
21. Kienbaum P, Karlssonn T, Sverrisdottir YB, Elam M, Wallin BG. Two sites for modulation of human sympathetic activity by arterial baroreceptors? *J Physiol.* 2001;531(pt 3):861–869. doi: 10.1111/j.1469-7793.2001.0861h.x
22. Grassi G, Seravalle G, Brambilla G, Trabattoni D, Cuspidi C, Corso R, Pieruzzi F, Genovesi S, Stella A, Facchetti R, et al. Blood pressure responses to renal denervation precede and are independent of the sympathetic and baroreflex effects. *Hypertension.* 2015;65:1209–1216. doi: 10.1161/HYPERTENSIONAHA.114.04823
23. Grassi G, Seravalle G, Cattaneo BM, Bolla GB, Lanfranchi A, Colombo M, Giannattasio C, Brunani A, Cavagnini F, Mancia G. Sympathetic activation in obese normotensive subjects. *Hypertension.* 1995;25(4 Pt 1):560–563. doi: 10.1161/01.hyp.25.4.560
24. Grassi G, Biffi A, Dell'Oro R, Quarti Trevano F, Seravalle G, Corrao G, Perseghin G, Mancia G. Sympathetic neural abnormalities in type 1 and type 2 diabetes: a systematic review and metanalysis. *J Hypertens*. 2020;38:1436–1442. doi: 10.1097/HJH.0000000000002431
25. Kaye DM, Lambert GW, Lefkovits J, Morris M, Jennings G, Esler MD. Neurochemical evidence of cardiac sympathetic activation and increased central nervous system norepinephrine turnover in severe congestive heart failure. *J Am Coll Cardiol.* 1994;23:570–578. doi: 10.1016/0735-1097(94)90738-2
26. Grassi G, Seravalle G, Cattaneo BM, Lanfranchi A, Vailati S, Giannattasio C, Del Bo A, Sala C, Bolla GB, Pozzi M. Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. *Circulation.* 1995;92:3206–3211. doi: 10.1161/01.cir.92.11.3206
27. Patel MB, Stewart JM, Loud AV, Anversa P, Wang J, Fiegel L, Hintze TH. Altered function and structure of the heart in dogs with chronic elevation in plasma norepinephrine. *Circulation.* 1991;84:2091–2100. doi: 10.1161/01.cir.84.5.2091
28. Bosh J, Groszmann J, Shah VH. Evolution in the understanding of the pathophysiological basis of portal hypertension: how changes in paradigm are leading to succeful new treatments. *J Hepatol.* 2015:62(suppl 1):S121–S130. doi: 10.1016/j.jhep.2015.01.003
29. Barret CJ. Renal sympathetic nerves – what have they got to do with cardiovascular disease? *Exp Physiol.* 2015;100:359–365. doi: 10.1113/expphysiol.2014.080176
30. Scherrer U, Sartori C. Insulin as a vascular and sympathoexcitatory hormone: implications for blood pressure regulation, insulin sensitivity, and cardiovascular morbidity. *Circulation.* 1997;96:4104–4113. doi: 10.1161/01.cir.96.11.4104
31. Landsberg L. Insulin-mediated sympathetic stimulation: role in the pathogenesis of obesity-related hypertension (or, how insulin affects blood pressure, and why). *J Hypertens.* 2001;19(3 Pt 2):523–528. doi: 10.1097/00004872-200103001-00001
32. Quarti Trevano F, Dell'Oro R, Biffi A, Seravalle G, Corrao G, Mancia G, Grassi G. Sympathetic overdrive in the metabolic syndrome: meta-analysis of published studies. *J Hypertens.* 2020;38:565–572. doi: 10.1097/HJH.0000000000002288
33. Cardoso LM, Fernandes LG, Alves AM, Pedrosa ML, Silva ME, Colombari E, Moraes MF, Chianca Júnior DA. Cardiopulmonary reflex is attenuated in iron overload conscious rats. *Nutr Neurosci.* 2007;10:121–128. doi: 10.1080/10284150701413964
34. Abboud FM, Harwani SC, Chapleau MW. Autonomic neural regulation of the immune system: implications for hypertension and cardiovascular disease. *Hypertension.* 2012;59:755–762. doi: 10.1161/HYPERTENSIONAHA.111.186833
35. Le Lan C, Loréal O, Cohen T, Ropert M, Glickstein H, Lainé F, Pouchard M, Deugnier Y, Le Treut A, Breuer W, et al. Redox active plasma iron in C282Y/C282Y hemochromatosis. *Blood.* 2005;105:4527–4531. doi: 10.1182/blood-2004-09-3468
36. Lepori M, Sartori C, Trueb L, Owlya R, Nicod P, Scherrer U. Haemodynamic and sympathetic effects of inhibition of nitric oxide synthase by systemic infusion of N(G)-monomethyl-L-arginine into humans are dose dependent. *J Hypertens.* 1998;16:519–523. doi: 10.1097/00004872-199816040-00013
37. Julius S, Jamerson K. Sympathetics, insulin resistance and coronary risk in hypertension: the 'chicken-and-egg' question. *J Hypertens.* 1994;12:495–502.
38. Masuo K, Lambert GW. Letter to the editor: the article "autonomic imbalance as a predictor of metabolic risks, cardiovascular disease, diabetes, and mortality autonomic imbalance predicts CVD, DM, mortality" by Wulsin et al could resolve the "chicken-and-egg" question on the role of sympathetic overactivity and insulin resistance in hypertension. *J Clin Endocrinol Metab.* 2015;100:L68–L69. doi: 10.1210/jc.2015-2426
39. Salonen JT. The role of iron as a cardiovascular risk factor. *Curr Opin Lipidol*. 1993;4:277–282.
40. Kiechl S, Aichner F, Gerstenbrand F, Egger G, Mair A, Rungger G, Spögler F, Jarosch E, Oberhollenzer F, Willeit J. Body iron stores and presence of carotid atherosclerosis. Results from the Bruneck study. *Arterioscler Thromb.* 1994;14:1625–1630. doi: 10.1161/01.atv.14.10.1625

Novelty and Significance

What Is New?

- The article reports the results of the first study ever done examining whether and to what extent the sympathetic overactivity characterizing essential hypertension is affected by an iron overload condition, which frequently is found in the high blood pressure state, particularly when associated with metabolic abnormalities.
- Muscle sympathetic nerve traffic, heart rate and blood pressure variability, spontaneous baroreflex sensitivity, different markers of iron metabolism, and metabolic variables were examined in patients with essential hypertension without and with iron overload.

What Is Relevant?

- Data show that patients with hypertension with iron overload display muscle sympathetic nerve traffic values (1) significantly greater than those detected in hypertensives without this condition and (2) significantly related to serum ferritin, transferrin saturation, and hepatic iron load.
- The greater levels of muscle sympathetic nerve traffic are accompanied by increased low-frequency oscillations of systolic blood pressure variability.
- The sympathetic activation detected in patients with hypertension with iron overload is significantly and directly related to homeostatic model assessment index.

Summary

Patients with hypertension with iron overload display a potentiation of the sympathetic activation characterizing high blood pressure which appears to be related to iron load and to the metabolic alterations frequently accompanying this condition. The adrenergic activation may participate in determining the increased cardiovascular risk associated with iron overload.