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Cardiovascular Risk by Gender in an Italian Pilot Study

Elena Matteucci* and Ottavio Giampietro

Department of Internal Medicine, University of Pisa, Italy

Abstract: Part of the international variation in the gender differences in total mortality remains unexplained. Although a continuous decrease in age-standardised death rates was observed in Italy, the difference between male and female rates became relatively small or even reversed (as the rate for ischaemic heart diseases).

We performed an extensive evaluation of cardiovascular risk factors - demographic and clinical characteristics, biochemical parameters, and oxidative biomarkers - in a sample of healthy subjects (94 women and 75 men) from Central Italy and analysed their relationship with the response to ergometer exercise.

In addition to a proportion of smokers similar to that observed in men, a clustering of peculiar female cardiovascular risk factors emerged. This included: higher platelet counts, low participation in leisure-time aerobic physical activities, hypertensive response to exercise (BMI-dependent, unlike in men), and low fraction of heart rate reserve. Physical inactivity and high serum IL-6 levels were independently predictive of having both chronotropic incompetence and abnormal heart rate recovery.

Keywords: Sex, risk factors, ergometer exercise, heart rate reserve, interleukin 6, lifestyle.

INTRODUCTION

Cardiovascular diseases largely contribute the higher mortality in men. Smoking is an important cause of the international variation in the gender differences in total mortality, yet a great part of this variation remains unexplained [1]. Fluctuations in the gender gap in life expectancy suggest the differences reflect environmental as well as genetic factors [1]. Mortality rates declined for ischaemic heart disease (IHD), stroke, and total cardiovascular diseases between 1970 and 2000 in Western Europe. However, the percentage annual change of the cause-specific mortality rates for the age group 45-74 was negative and significant for IHD, total cardiovascular diseases, and all-cause mortality (not for stroke) in Western European men, whereas only for IHD in women [2]. In Italy a continuous decrease in age-standardised death rates was observed (www.euro.who.int/eprise/main/WHO/informationSources/Data/20011017_1), but the difference between male and female rates became relatively small or even reversed. Indeed, in 2000 the rate for ischaemic heart diseases was 25% in women *vs* 23% in men.

In this study, we performed an extensive evaluation of cardiovascular risk factors in a sample of healthy subjects from Central Italy and analysed their relationship with the response to ergometer exercise.

METHODS

Healthy subjects (94F/75M) were recruited from the local community. They were taking no drugs, and had no clinical signs or symptoms of illness. All subjects gave their informed consent and the ethical committee of the hospital approved the study.

The medical examination included standardised personal and family health history, physical examination, blood chemistry analysis and exercise test.

Among the risk factors taken into consideration were: demographic and clinical characteristics (body size, blood pressure, cigarette smoking, lifestyle), biochemical parameters (fasting plasma glucose and insulin, HbA1c, blood cell count, lipids, C-reactive protein, fibrinogen, folate, total homocysteine, electrolytes, interleukin 6, urinary albumin and electrolyte excretion), and biomarkers of oxidative stress or antioxidant protection (plasma thiols, plasma and erythrocyte malondialdehyde, erythrocyte Na/H exchange, erythrocyte velocity of ferricyanide reduction) [3-5]. Erythrocyte Na/H exchange (RBC NHE) hyperactivity, once considered marker of essential hypertension, is now ascribed to the direct stimulatory effect of oxidative stress [4]. The erythrocyte velocity of ferricyanide reduction (RBC vfcy) is a plasma membrane oxido-reductase that transfers reducing equivalents from intracellular reductants to extracellular oxidants (such as ferricyanide) and thus enables the cell respond to changes in intra- and extra-cellular redox environments [5].

Data Collection

Demographic, socio-economic and lifestyle information was obtained by Lifestyle European Prospective Investigation of Cancer and Nutrition questionnaire (EPIC) [6]. Subjects reported the daily number of hours they engaged in housework and the weekly number of hours they engaged in physical activities during leisure and during work. Essential

*Address correspondence to this author at the Dipartimento di Medicina Interna, Via Roma 67, 56126 Pisa, Italy; Tel: 0039 050 993246; Fax: 0039 050 553414; E-mail: ematteuc@int.med.unipi.it

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hypertension was defined as current use of antihypertensive medication or an office blood pressure ≥140/90 mmHg. The body mass index (BMI) was calculated as kg/m^2; the body surface area (BSA) as $m^2 = kg^{0.425} * cm^{0.725} * 0.007184$.

Exercise Testing Procedure

The examination program included measurements of resting heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure. A maximum, symptom-limited ECG-monitored exercise test was performed according to the Bruce protocol [7]. The maximal exercise-induced change in SBP and DBP relative to maximum exercise capacity was calculated (ΔExSBP/W and ΔExDBP/W) [7]. A hypertensive response to exercise (HRE) was defined as a peak SBP during exercise ≥210 mmHg in men and ≥190 mmHg in women [8]. Peak HR achieved, the percentage of age-predicted HR achieved, and the increment in HR from standing rest to peak exercise were measured. The chronotropic response index at peak exercise was calculated as the ratio of heart rate reserve (HRR) used to metabolic reserve used at peak stage [9]. Chronotropic incompetence was defined as a fraction of HRR at peak exercise < 0.8. An abnormal heart rate recovery was considered to be a decrease of <22 beats/min (bpm) at 2 min in recovery [10].

Laboratory Methods

Fasting venous blood samples and 24-h urine collections were obtained on a single occasion. Glucose, C-reactive protein (CRP), total/HDL cholesterol, triglycerides, and albumin were measured using a MODULAR ANALYTICS SWA (Roche Diagnostics, Italy). LDL cholesterol was calculated by the Friedewald formula. HbA1c was evaluated by Bio-Rad DIAMAT™ Analyzer System, plasma fibrinogen using an automated coagulation analyzer (Sysmex, Japan), plasma folate and total homocysteine by AxSYM System and IMX System, respectively (Abbott Diagnostics, Italy), insulin by radioimmunoassay (Medgenix Diagnostics, Belgium). Insulin resistance was estimated by homeostasis model assessment ($HOMA_{IR}$). Serum interleukin 6 (IL-6) was assayed by Quantikine HS (R&D Systems, UK).

Plasma thiols (P SH), erythrocyte glutathione (RBC GSH), plasma and erythrocyte membrane malondialdehyde (P and RBC MDA), RBC NHE activity, and RBC vfcy were measured as previously reported [3-7, 11].

Statistical Analyses

The results are expressed as mean±SD or geometric mean. Data not normally distributed were log-transformed before t-tests and ANOVA. The level of significance was set at $P < 0.05$. Student's t-test with Bonferroni corrections, Mann-Whitney U-test, ANOVA and Kruskal-Wallis rank test were used to determine differences between independent groups as appropriate. Comparison of categories was by chi-square test. We estimated the associations by Spearman rank correlation analysis, stepwise regression analysis, multiple linear and logistic regression. A Student-Newman-Keuls post hoc test was used to assess the significance of the difference between specific means.

RESULTS

Demographic and Clinical Characteristics

The distribution of age and education was similar for female and male respondents (Table **1**). Women had lower body size measures than man. The percentages of never-smokers and smokers were similar between women and men (58% and 24% *vs* 49% and 24%, respectively). Overall, 76% of respondents were currently employed; the percent unemployed was higher in women (35%) than in man (24%). A sedentary professional activity was most common (85% and 68% of female and male respondents, respectively). Higher proportions of respondents with a manual or heavy manual professional activity were observed in men (32%) than in women (15%). Almost no man performed household activities, whereas the mean duration of household activities was 2.8 h/day in women. Time dedicated to physical activity was higher in men because of a high participation in exercise, walking and cycling.

Among women, time spent in housework decreased with increasing educational level: 4 h/day in primary school, 3.4±1.1 in secondary school, 2.3±1.2 in high school, 2.0±1.1 in graduate ($p<0.0001$). Among women (not men), leisure time physical activities correspondingly increased: 0.7 h/day, 1.1±0.4, 1.5±0.5, and 1.5±0.4, respectively ($p<0.01$). Female (not male) SBP decreased with increasing educational level: 138 mmHg, 125±15, 114±18, 113±16, respectively ($p<0.01$). Employment status did not change the distribution of these variables in both groups. School level or employment status made no difference with regard to smoking.

Biochemical Parameters and Biomarkers of Redox Status

Compared with men, women had lower fasting plasma glucose (FPG), haematocrit, LDL cholesterol, triglycerides, plasma total homocysteine, plasma sodium and potassium, urinary sodium excretion corrected by BSA (Table **2**). In contrast, women had significantly higher platelet counts as well as higher levels of HDL cholesterol and plasma folate. $HOMA_{IR}$ (2.1±1.4 in women, 2.3±1.3 in men, p 0.2825) was significantly associated (|r| 0.53, $p<0.0001$) with BMI (coefficient 0.03, t value 7.64) and P MDA (0.16, 2.28).

Current smokers had the highest levels of IL-6 (3.3 pg/mL *vs* 2.1 never smokers and 2.4 ex smokers, p 0.0028), CRP (3.3 mg/L *vs* 2.4 and 2.3, p 0.0258), and RBC MDA (0.4±0.14 nmol/L *vs* 0.33±0.13 and 0.35±0.14, p 0.0199). Plasma folate and total homocysteine concentrations did not differ when subjects were divided according to cigarette use.

Concerning redox biomarkers, women differed from men only in the higher RBC GSH (0.81±0.13 mg/mL *vs* 0.75±0.12, p 0.008) that was associated (|r| 0.34, $p<0.0001$) with haematocrit (coefficient -0,01, t value -3.87), and resting SBP (-0.001, -2.18).

RBC NHE (5.6±2.1 mmol/L h *vs* 5.6±2.1), RBC vfcy (12.4±3.7 µmol/mL h *vs* 12.2±4.1), P SH (408±62 µmol/L *vs* 423±60), RBC MDA (349±136 nmol/L *vs* 350±142), and P MDA (483±224 nmol/L *vs* 498±257) did not differ between genders.

Table 1. Clinical Characteristics, Demographic and Lifestyle Information of the Study Participants by Gender

Characteristic	Women	Men	p
Age (years)	46±13	48±14	0.4734
BMI (kg/m^2)	25±4	26±3	0.0111
BSA (m^2)	1.69±0.16	1.96±0.16	<0.0001
Never smoker/smoker/ex-smoker	55/23/16	37/18/20	0.2922
School (primary/secondary/high/graduate)	1/44/29/20	0/32/24/19	0.7912
Work (full-time/part-time/non-worker)	43/18/33	50/7/18	0.0195
Type (sedentary/standing/manual/heavy)	28/24/7/2	25/14/9/9	0.0511
Housework (h/day)	2.8±1.3	0.01±0.1	<0.0001
Walking (h/week)	3.6±1.8	4.3±2.1	0.0060
Cycling (h/week)	1.9±1.3	2.5±1.7	0.0119
Exercise (h/week)	1.9±1.6	2.7±2.0	0.0110
Physical activity (h/day)	1.3±0.5	1.7±0.6	<0.0001

BMI = body mass index; BSA = body surface area.

Table 2. Biochemical Characteristics of the Study Participants by Gender

Characteristic	Women	Men	p
FPG (mmol/L)	4.9±0.5	5.3±0.7	<0.0001
HbA1c (%)	5.3±0.4	5.4±0.7	0.5590
FPI (µU/mL)	9.4±5.9	9.6±5.0	0.7870
Haematocrit (%)	41±3	45±3	<0.0001
Platelet count (x10^9/L)	266±62	240±49	0.0038
Total Cholesterol (mmol/L)	5.2±1.1	5.4±1.0	0.3575
HDL Cholesterol (mmol/L)	1.6±0.3	1.3±0.3	<0.0001
LDL cholesterol (mmol/L)	3.4±1.0	3.8±0.9	0.0166
Triglycerides (mmol/L)	[0.9, 0.3-1.8]	[1.2, 0.6-4.2]	0.0050
Folate (nmol/L)	12.2±5.8	9.7±3.7	0.0018
Total Homocysteine (µmol/L)	9.3±3.0	13.7±7.7	<0.0001
CRP (mg/L)	[2.6]	[2.6]	0.9278
IL-6 (pg/mL)	[2.1]	[2.2]	0.6328
Plasma Sodium (mEq/L)	138±2	139±2	0.0077
Plasma Potassium (mEq /L)	4.0±0.3	4.1±0.3	0.0286
Urinary Sodium/BSA (mEq 24h/m^2)	87±32	103±43	0.0049
UAER (µg/min)	[2.9]	[3.6]	0.1369

FPG = fasting plasma glucose; FPI = fasting plasma insulin; HDL = high density lipoprotein; LDL = low density lipoprotein; CRP = C-reactive protein; IL-6 = interleukin 6; BSA = body surface area; UAER = urinary albumin excretion rate.

Exercise Testing

No patient presented basal ECG signs of left ventricular hypertrophy or exercise-induced angina. The maximum workload was significantly higher in men (Table **3**). The major absolute increments in SBP and HR were observed in men (Fig. (**1**)), who generally reached their target HR and had a higher chronotropic index. Women, who had lower baseline levels of SBP and DBP than men, showed higher increments relative to maximum exercise capacity. Indeed, thirty-one (33%) of women and 22 (29%) of men had a HRE although female exercise capacity was significantly lower.

Subjects with a HRE were older (53±13 years *vs* 44±13, p<0.0001) and had higher BMI (27±4 *vs* 25±4, p<0.001), resting SBP (134±15 mmHg *vs* 117±15, p<0.0001) and DBP (86±10 *vs* 76±10, p<0.0001), plasma sodium (139±2 mEq/L *vs* 138±2, p<0.05), plasma total cholesterol (5.6±1.0 mmol/L *vs* 5.2±1.0, p<0.05), blood platelet counts (270±68 x10^9/L *vs* 248±52, p<0.05) and serum IL-6 (2.8 pg/mL *vs* 2.3, p<0.05).

Fifty-one (54%) of women had a low chronotropic index, i.e. used up less than 80% of their HRR at the end of exercise, compared with 30 (40%) of men (Fig. (**1**)).

Subjects with a low chronotropic index were more likely to be smokers (33% *vs* 16%, p<0.05) and had higher plasma fibrinogen (8.1±1.7 µmol/L *vs* 7.8±1.4, p<0.05), serum IL-6 (2.9 pg/mL *vs* 2.0, p<0.0001), and RBC NHE activity (5.9±2.3 mmol/L h *vs* 5.2±1.8, p<0.05). They achieved higher ΔExSBP/W (0.60±0.24 *vs* 0.50±0.17, p<0.01). Moreover, they spent more time in household chores (1.8±1.8 h/day *vs* 1.3±1.5, p<0.05) but less time in regular exercise (1.8±1.4 h/week *vs* 2.7±2.1, p<0.01).

HR recovery patterns after exercise did not differ between women and men. Nineteen women and 12 men had an abnormal HR recovery (<22 bpm).

Exploratory Data Analysis

Baseline SBP was significantly associated (|r| 0.62, p<0.0001) with age (coefficient 0.53, t value 6.55), BSA (20.13, 3.86), HOMA $_{IR}$ (15.68, 3.66), plasma total cholesterol (2.83, 2.65), and plasma potassium (-10.07, -2.65). Baseline DBP was only associated (|r| 0.52, p<0.0001) with age (0.24, 4.61) and BMI (0.97, 5.31). Baseline HR correlated (|r| 0.37, p<0.0001) with age (-0.20, -2.35), haematocrit (0.91, 2.89), plasma folate (19.24, 3.29) and P MDA (12.33, 2.67).

ΔExSBP/W correlated (|r| 0.55, p<0.0001) with age (0.004, 3.81), BMI (0.01, 2.81), HbA1c (0.056, 2.02), time spent in housework (0.026, 3.02), time spent in cycling (-0.028, -3.06). In the multivariate analysis of covariance, remained predictive of the SBP increment both female gender (lambda 10.08, p 0.0018, power 0.903) and BMI (5.61, 0.0191, 0.651); the covariate-factor interaction was significant (13.191, 0.0004, 0.967) (Fig. (**2**), upper panel).

ΔExDBP/W correlated (|r| 0.40, p<0.0001) positively with time spent in housework (coefficient 0.01, t value 3.89), age (0.001, 2.36), and BMI (0.003, 2.19).

The chronotropic index correlated (|r| 0.40, p<0.0001) with housework (-0.040, -3.590), regular exercise (0.020, 2.358), BSA (-0.183, -2.115), and IL-6 (-0.086, -1.594). Having chronotropic incompetence was associated with cigarette use in the simple logistic regression model (likelihood

Table 3. Blood Pressure and Heart Rate Response to Exercise Testing of the Study Participants

Characteristic	Women	Men	p
Basal SBP (mmHg)	124±20	130±12	0.0360
Basal DBP (mmHg)	75±18	79±19	0.0085
Basal HR (bpm)	82±14	81±17	0.9032
Max workload (W)	103±27	155±40	<0.0001
ΔEx SBP (mmHg)	57±20	75±22	<0.0001
ΔEx DBP (mmHg)	5±8	2±7	0.0239
ΔEx SBP/W	0.57±0.23	0.51±0.17	0.0486
ΔEx DBP/W	0.06±0.09	0.02±0.05	0.0006
HR increment (bpm)	68±23	75±21	0.0516
Peak HR (bpm)	150±23	156±19	0.0515
Predicted HR achieved (%)	86±11	91±9	0.0024
Chronotropic index	0.73±0.20	0.82±0.19	0.0056
HR recovery	35± 17	36±17	0.8077

SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; ΔEx SBP = maximal exercise-induced change in SBP; ΔEx DBP = maximal exercise-induced change in DBP; ΔEx SBP/W = maximal exercise-induced change in SBP relative to maximum exercise capacity; ΔEx DBP/W = maximal exercise-induced change in DBP relative to maximum exercise capacity.

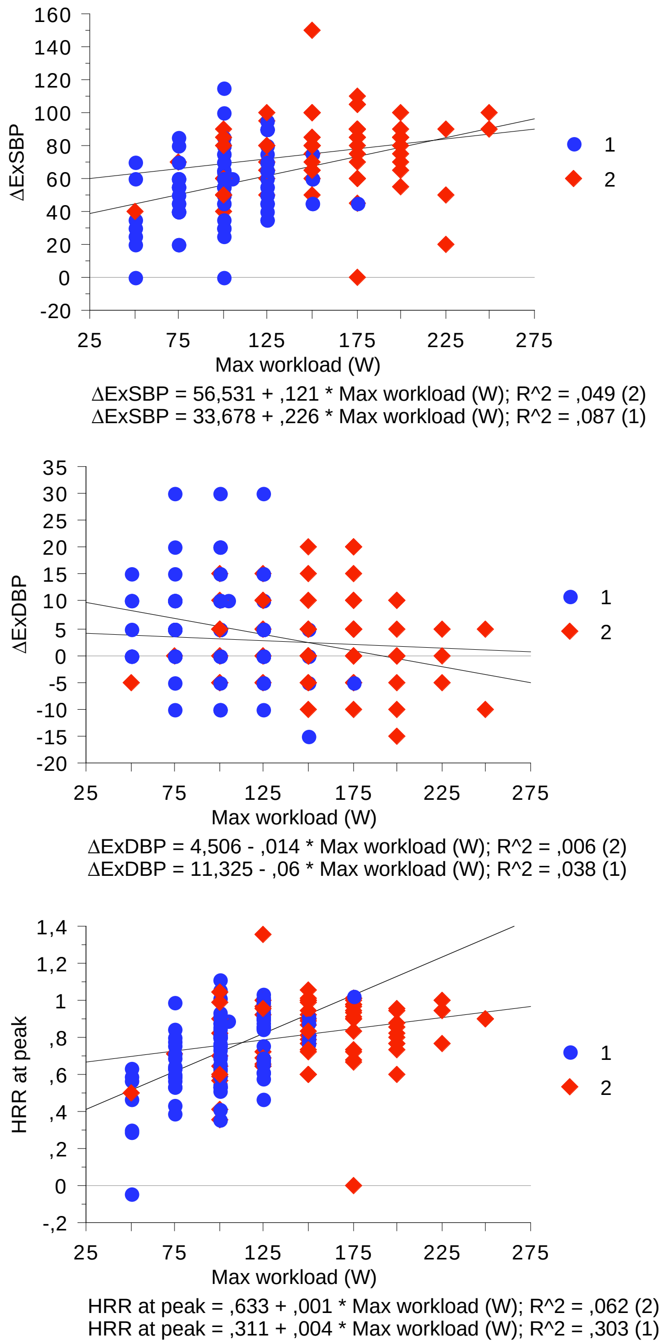

Fig. (1). Regression lines of the maximal increase in systolic blood pressure (ΔExSBP), diastolic blood pressure (ΔExDBP) and the fraction of heart rate response (HRR) *vs* exercise workload (W) for women (1, blue circles) and men (2, red rhomboids). Respective regression equations are set below.

ratio test: p = 0.0076, Chi-square 7.14). In the multiple logistic regression (including gender, cigarette use, exercise, and IL-6), the relationship between smoking and low chronotropic index in men and women was explained away on the basis of serum concentration of IL-6 (likelihood ratio test: p value 0.0017, Chi-square 9.89) (Fig. (**2**), lower panel). Having chronotropic incompetence and/or abnormal heart rate recovery was associated with both serum concentrations of IL-6 (positively) and time engaged in regular exercise (negatively): the likelihood ratio test yielded a p of 0.0019 (Chi-square 9.69) and 0.014 (6.00), respectively (Fig. (**3**)).

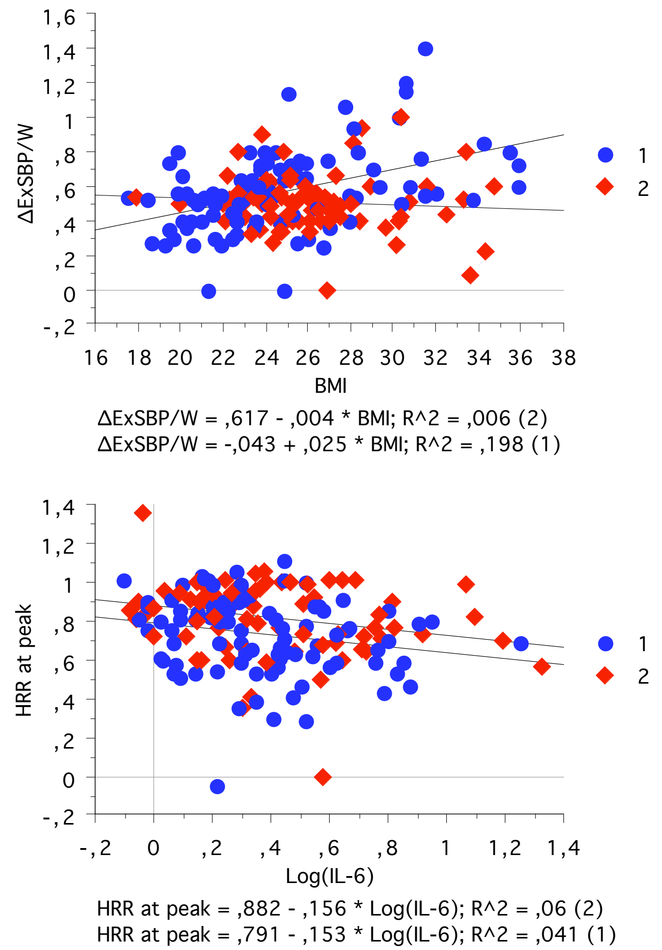

Fig. (2). In the upper panel, the relationship between BMI and the SBP increment relative to maximum exercise capacity (ΔExSBP/W) for women (1, blue circles) and men (2, red rhomboids). In the lower panel, the relationship between chronotropic index (HRR at peak) and serum concentration of interleukin 6 in women (1, blue circles) and men (2, red rhomboids).

DISCUSSION

Using the Framingham Heart Study dataset, the effect of midlife cardiovascular risk factors on late-age survival has been estimated [12]. Physiological indices measured earlier in life can predict the residual life span distribution [13]. Thus, changing these respective indices could affect life-span distributions, as observed recently in the Italian population. In this study, we evaluated several risk factors in conjunction with the haemodynamic responses during exercise in a group of healthy women and men. In addition to a proportion of smokers similar to that observed in men, our study identified a clustering of specific female cardiovascular risk factors: higher platelet counts, low participation in leisure-time aerobic physical activities, hypertensive response to exercise (BMI-dependent, unlike in men), and low fraction of heart rate reserve. Physical inactivity and high serum IL-6 levels (rather than cigarette use) were independently and significantly predictive of having both chronotropic incompetence and abnormal heart rate recovery.

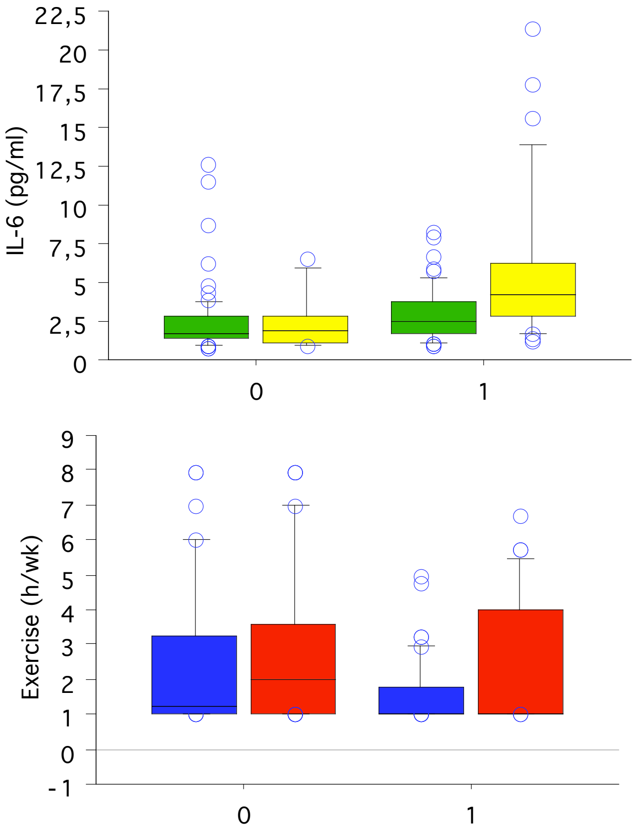

Fig. (3). In the upper panel, serum inteleukin 6 concentrations according to chronotropic index (normal in group 0, or < 0.8 in group 1) in non smokers (green boxes) and smokers (yellow boxes). In the lower panel, time spent in regular exercise (hours/week) according to chronotropic index (normal in group 0, or < 0.8 in group 1) in women (blue boxes) and men (red boxes).

At first glance, women showed a cardio-protective combination of *clinical indices*. Indeed, compared with men, they were leaner and had lower resting blood pressure. Gender-associated differences in blood pressure have been observed in humans and attributed to various potential mechanisms [14]. An increased salt dietary intake has been documented in our male sample.

Regarding *demographic characteristics*, women reported similar smoking prevalence than men and significantly lower participation in standard aerobic physical activities, with the exception of household chores that seemed to be an exclusive prerogative of female gender in Italy. The American Heart Association classified physical inactivity as a major cardiovascular risk factor [15]. In a population of Dutch women who participated in the EPIC in 1993-1995, blood pressure was most clearly associated with time spent in sports. Leisure-time activity was inversely related to cardiovascular disease risk indicators, but work activity and housework were not [16]. Italian women reported low levels of regular participation in sports. Educational levels were associated with lower household and higher leisure-time physical activity; in turn, regular physical activity influenced physical work capacity at the bicycle ergometer.

Additional peculiar female *haematological features* were the lower level of haematocrit and the higher platelet counts. Sample size did not allow a close evaluation of the relation between female hormonal state and platelet count. Differences in platelet counts by sex have been reported [17]. Mild platelet count elevation is a risk factor for venous thrombosis; its contribution to the rates of cardiovascular events is still to be explored despite its possible correlation with inflammatory markers and long-term mortality in unstable angina [18].

From the *biochemical viewpoint*, female gender seemed favoured by nature. In women, blood levels of glucose, triglycerides, and homocysteine were lower, whereas those of HDL-cholesterol and folate were higher. RBC GSH, i.e. cellular antioxidant protection, was heightened. It has been consistently reported that women have lower FPG levels than men and display a more rapid fall in plasma glucose during prolonged fasting [19]. FPG has been found independently associated with resting and exercise blood pressures and development of hypertension after 7-years follow-up in healthy men [20]. Interestingly, in our study, insulin resistance by HOMA $_{IR}$ was significantly associated not only with body mass, as expected, but also with a marker of oxidative damage (P MDA). In turn, resting *systolic blood pressure* increased with increasing age, BSA, HOMA $_{IR}$, plasma total cholesterol, whereas it showed an inverse relationship with plasma potassium. Sex differences in sodium, potassium, and water regulation have been suggested involved in the lowering of cardiovascular disease risk among women [21, 22]. Resting *diastolic blood pressure* was positively related with age and body mass. The resting *heart rate* declined with age, while it showed a positive correlation with haematocrit, plasma folate, and P MDA. Heart rate positive correlation with folate deserves attention since pulse rate has been found to decline both with ageing [12] and regular exercise. Exercise has been implicated in the up-regulation of neuronal nitric oxide synthase, which results in vagal bradycardia *in vitro* and in animals. The molecular mechanisms responsible may involve interplay between haemodynamic elements and oxidative stress [23]. Increased radical production (P MDA) could stimulate nitric oxide formation that critically depends on the availability of folic acid.

Oxidative stress is thought to be involved in age-associated diseases. GSH content decreases with age in many tissues, whereas it is unclear whether it differs between genders. So far, no gender specific difference in RBC GSH content was found, although 1) estrogen is one of the hormones that regulate GSH homeostasis, and 2) age- and-gender dependent alterations in the activities of glutathione-related enzymes have been observed [24, 25]. Our finding of a gender-dependency in RBC GSH content is partially at variance with previous data in mice where no difference was found. The result could depend on the human population studied or on the analytical procedure that was performed immediately after blood withdrawal without prolonged sample manipulation.

Exercise ECG is the most commonly used form of stress testing, frequently evaluated in women even though ECG changes during exercise are less sensitive and specific in women as compared with men [26]. Gender differences exist in the ventilatory response to progressive exercise [27] and the progression of metabolic responses [28]. Contrary to the other component of cardiac power output, however, mean arterial pressure has been reported to be independent of body size [29].

Examined women had lower baseline blood pressure than men. Nonetheless, their response to exercise was characterised by major increases in maximal SBP and DBP relative to maximal achieved workloads. The enhanced SBP response to graded exercise was associated positively with age, BMI, HbA1c, and time spent in household chores, while negatively with regular cycling. The enhanced DBP response was influenced by time dedicated to housework more than by age and body mass. A HRE has been reported to predict the future development of hypertension, whereas its prognostic significance with regard to likelihood of myocardial perfusion abnormalities and mortality rate is unclear [8]. It has been associated with a high prevalence of transient ischaemic dilation of the left ventricle in patients without other significant perfusion defects [30]. Intriguingly, only women were particularly susceptible to the potentiating effects of body mass on the SBP response to exercise. An abnormal blood pressure response was also associated with HbA1c levels and lack of physical fitness (more time spent in housework than in exercise). Indeed, protein glycosylation may affect artery compliance in healthy subjects [7].

Chronotropic incompetence is predictive of increased all-cause mortality and coronary heart disease incidence; moreover, considering both chronotropic incompetence and abnormal heart rate recovery most powerfully stratified risk [31]. Physical inactivity and high serum IL-6 levels were independently predictive of having both these pathological responses. Compared with men, a large proportion of women showed an impaired HR reserve. Chronotropic incompetence was more frequent in smokers who had abnormal inflammatory (IL-6 and fibrinogen) and oxidative (RBC NHE) biomarkers. IL-6 belongs to the class of systemic inflammatory markers; its circulating levels have been associated with coronary artery disease risk [32].

In summary, our study identified a set of gender-specific cardiovascular risk factors potentially capable of predicting survival characteristics, which could be used as indicators for health surveillance programmes in Italy (and elsewhere). An analysis of longitudinal data containing regular measurements of these physiological indices in numerous individuals could help us understand their predictive ability.

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