



## Cardiovascular abnormalities in Klinefelter Syndrome <sup>☆</sup>

Daniela Pasquali <sup>a</sup>, Michele Arcopinto <sup>b</sup>, Andrea Renzullo <sup>a</sup>, Mario Rotondi <sup>c</sup>, Giacomo Accardo <sup>a</sup>, Andrea Salzano <sup>b</sup>, Daniela Esposito <sup>a</sup>, Lavinia Saldamarco <sup>b</sup>, Andrea M. Isidori <sup>e</sup>, Alberto M. Marra <sup>b</sup>, Antonio Ruvolo <sup>b</sup>, Raffaele Napoli <sup>b</sup>, Eduardo Bossone <sup>d</sup>, Andrea Lenzi <sup>e</sup>, Ragavendra R. Baliga <sup>f</sup>, Luigi Sacca <sup>b</sup>, Antonio Cittadini <sup>b,\*</sup>

<sup>a</sup> Department of Cardiothoracic and Respiratory Science, Endocrinology Unit, Second University of Naples, Italy

<sup>b</sup> Department of Internal Medicine, Cardiovascular Sciences and Clinical Immunology, University Federico II, Naples, Italy

<sup>c</sup> Unit of Internal Medicine and Endocrinology, "Fondazione Salvatore Maugeri" I.R.C.C.S., University of Pavia, Italy

<sup>d</sup> Department of Cardiac Surgery, I.R.C.S.S. Policlinico San Donato Milanese, Milan, Italy

<sup>e</sup> Department of Experimental Medicine, Sapienza University of Rome, Italy

<sup>f</sup> Division of Cardiovascular Medicine, The Ohio State University, Columbus, OH USA

### ARTICLE INFO

#### Article history:

Received 6 July 2012

Received in revised form 18 September 2012

Accepted 29 September 2012

Available online 23 October 2012

#### Keywords:

Klinefelter

Heart

Chronotropic incompetence

Cardiovascular abnormalities

### ABSTRACT

**Background:** Several epidemiological studies have demonstrated an increased mortality from cardiovascular causes in patients with Klinefelter Syndrome (KS). Little information is available about the nature of the underlying cardiovascular abnormalities. Aim of the study was to investigate exercise performance, left ventricular architecture and function, vascular reactivity, and carotid intima-media thickness in a group of patients with KS.

**Materials and methods:** Sixty-nine patients with KS and 48 age-matched controls participated in our population-controlled study. Forty-eight Klinefelter subjects were on testosterone treatment at the time of the investigation while 21 were naive and underwent a complete Doppler echocardiographic examination, a cardiopulmonary exercise test as well as a vascular study including measures of carotid intima-media thickness and endothelial function with flow-mediated dilation of the brachial artery. Patients with KS on testosterone therapy (n = 48) were also matched against a population of men with treated secondary hypogonadism (n = 21).

**Results:** Patients with KS exhibited a wide array of cardiovascular abnormalities including left ventricular diastolic dysfunction, reduced maximal oxygen consumption (p < 0.01), increased intima-media thickness (p < 0.05) (−34% and +42% vs. controls, respectively) and a high prevalence of chronotropic incompetence (55% of patients, p < 0.01). No significant difference was found between treated and untreated KS in variance with men treated for secondary hypogonadism.

**Conclusion:** Left ventricular diastolic dysfunction, impaired cardiopulmonary performance, chronotropic incompetence, and increased intima-media thickness suggest that cardiovascular abnormalities are a common finding in KS that is not reversed by testosterone replacement therapy and may represent the pathophysiological underpinnings of the increased risk of dying from heart disease.

© 2012 Elsevier Ireland Ltd. All rights reserved.

**Abbreviations:** KS, Klinefelter Syndrome; BP, Blood Pressure; BMI, Body Mass Index; ECG, 12-lead scalar Electrocardiogram; CPET, Cardio-pulmonary Exercise Test; FMD, Flow-mediated Dilation; HR, Heart Rate; HOMA, Homeostasis Model Assessment; TDI, Tissue Doppler Imaging; LV, Left Ventricle; IRT, Isovolumic Relaxation Time; IMT, Intima-Media Thickness; VO<sub>2</sub>, Oxygen Uptake; VE, Ventilation per Minute; VCO<sub>2</sub>, Carbon Dioxide Production; CI, Chronotropic Incompetence; HRR, Heart Recovery Rate.

<sup>☆</sup> Disclosure summary: The authors have nothing to disclose. The authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

\* Corresponding author at: Department of Internal Medicine, Cardiovascular Sciences and Clinical Immunology, Via Sergio Pansini, 5, 80131 Naples, Italy. Tel./fax: +39 081 7464375.

E-mail address: [antonio.cittadini@unina.it](mailto:antonio.cittadini@unina.it) (A. Cittadini).

## 1. Introduction

Klinefelter Syndrome (KS) was described for the first time in 1942 by the young clinician Harry F. Klinefelter and represents the most common genetic cause of human male infertility with a prevalence of 1 in 660 men [1,2]. KS is characterized by gynecomastia, azoospermia, hyalinized and small testes, elevated levels of FSH and hypogonadism, and is consequently a frequent cause of infertility. One of the paradoxes of the KS is that, despite its high prevalence, our knowledge about the disease is very limited. Several issues remain unresolved, including a systematic review of the cardiovascular abnormalities of KS, which may shed new light on the existence of a KS specific cardiomyopathy. This information appears particularly relevant in view of large registry-based studies from Denmark and UK which demonstrated elevated morbidity and reduced

life expectancy in KS, associated with a significant increase of cardiovascular mortality [3–6]. The cause of such elevated morbidity and mortality from cardiovascular diseases is still unclear, although several abnormalities described in KS such as hypogonadism, high frequency of metabolic syndrome [7,8] and mitral valve prolapse may increase overall cardiovascular risk [9,10]. The aim of the current study was to perform a systematic assessment of cardiovascular structure and function in KS subjects and to evaluate relationship with metabolic and hormonal status.

## 2. Materials and methods

### 2.1. Patient population

Seventy-four consecutive young men with KS, seen at the Endocrine Units of the Second University of Naples and at Sapienza University of Rome, entered the study. Inclusion criteria were a verified KS karyotype (all subjects included were 47, XXY) and a signed informed consent [11]. Previous or current cardiovascular, respiratory and renal chronic diseases were considered exclusion criteria. One patient was excluded because of enalapril treatment for systemic hypertension as well as 4 patients initially enrolled did not complete the study. Then the analysis was made on 69 KS patients.

Testosterone replacement therapy was started in 48 subjects at a dose of 1000 mg long-lasting intramuscular testosterone every 3 months (Nebid, Bayer HealthCare) for a mean period of 36 months before the study. Hormonal replacement therapy was performed according to current guidelines [12] aiming at maintaining a low-normal serum total testosterone level. Twenty-one KS had a recent diagnosis and were still off replacement therapy. Matched controls were randomly selected among men attending the Endocrine outpatient clinics.

Forty-eight healthy male young adults comparable for age, physical activity and BMI participated in the study as controls. To improve the statistical meaning of the matching procedure, testosterone treated were analyzed separately from untreated naïve KS, and treated KS patients were also controlled against a group of 21 age- and BMI-matched men with a different form of hypogonadism (pituitary failure, secondary hypogonadisms) with low or low-normal gonadotropin levels on testosterone replacement therapy. Controls were defined as age, physical activity and BMI matched with the cases when the differences between them were less than 2 years, respectively. Written informed consent was obtained by all patients and controls, the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Second University of Naples. Serum testosterone, FSH, LH, SHBG, IGF-1, PRL, adiponectin, thyroid hormones, insulin and routine blood work were assessed in all study population at the study entry.

### 2.2. Study protocol

Anthropometric variables including height, weight, heart rate, and blood pressure (BP) were measured at the time of enrolment. The degree of adiposity was expressed as body mass index (BMI) and impedance test was also performed (TANITA bodyfat analyser, Model TBF-105).

Patients were evaluated with the following tests: 12-lead scalar electrocardiogram (ECG), Doppler echocardiography, cardiopulmonary exercise test (CPET), flow-mediated dilation (FMD) of the brachial artery. Heart rate (HR) was obtained from the ECG, and systolic and diastolic BP were measured by a cuff manometer after 15 min of supine rest. All tests and off line calculations were performed by investigators blinded as to the study protocol.

All echocardiographic and cardiopulmonary studies were reviewed by a central core laboratory under the supervision of E.B.

### 2.3. Assays

Plasma glucose levels were measured in duplicate immediately after sampling on a glucose analyzer (Beckman Instruments, Palo Alto, CA). Plasma lipids and triglycerides were measured using an automated commercially available system (Aeroset, Abbott Diagnostics). Serum insulin was determined by a commercial immunological kit (DAKO, Glostrup, Denmark). Insulin sensitivity was assessed by the homeostasis model assessment (HOMA) which is based on simultaneously sampled fasting levels of glucose and insulin [13].

Hormonal pituitary function was appropriately investigated in all subjects. In particular, serum levels of anterior pituitary hormones were evaluated in duplicates: GH, TSH, FSH, LH, and PRL were determined by IRMA, using commercial kits. Moreover, target organ hormones (free T4, free T3, testosterone, estradiol, SHBG, by RIA and IGF-1 by IRMA) were also evaluated using commercial kits as previously reported [14]. Free testosterone was calculated using the Vermeulen formula as previously reported [15]. Serum adiponectin was determined by a novel in-house time-resolved immunofluorometric assay (Human Total Adiponectin/Acrp30 Quantikine ELISA, R&D Padova Italy).

### 2.4. Echocardiography

An ultrasound system equipped with a 2.5 MHz multi-frequency transducer (Aplio, Toshiba, Japan) was used for complete M-mode, two-dimensional, Doppler and Tissue Doppler Imaging (TDI) echocardiographic analyses. M-mode and two-dimensional recordings were made with the patients in the lateral recumbent position, according to previously published methods [16]. Measures of left ventricular (LV) architecture and function were assessed according to standard formulae. The following parameters of diastolic function were measured as the mean of three to five consecutive beats: diastolic transmitral peak velocities, the E/A ratio, the isovolumic relaxation time (IRT), mitral deceleration time, and pulmonary vein velocities. Quantitative diastolic data were derived from TDI analysis. The sample volume (4 mm<sup>3</sup>) was placed in the LV basal portions of the septal and lateral walls [17].

### 2.5. Carotid ultrasonography

Real time high resolution echo-Doppler system equipped with a multi-frequency 7.5 MHz probe (Aplio, Toshiba, Japan) was employed to measure intima-media thickness (IMT), according to previously described techniques by a trained physician [18]. Briefly, ultrasonographic scanning was recorded with the subject in supine position, with a slide rotation of the neck. The probe was placed along the vessel axis and carotid arteries were explored with longitudinal (anterior, lateral, and posterior) and transverse scan. Multiple long axis and short axis sections were employed, paying attention to define the endothelial border from the origin of common artery beyond the carotid artery bifurcation. On each side, IMT was measured at the bifurcation and its proximal and distal segments, 1 cm before the bifurcation, on the echographic posterior wall of the common carotid artery, and 1 cm after the bifurcation, on the echographic posterior wall of the internal carotid artery always in the longitudinal scan.

### 2.6. Flow-mediated vasodilation

An Aplio XG imaging system (Toshiba, Tokyo, Japan) equipped with a 7.5-MHz multifrequency linear-array probe, appropriately reset, was used to measure FMD of the brachial artery, according to validated methods [19]. Briefly, endothelium-dependent vasodilation was assessed by measuring the maximum increase in brachial artery diameter during reactive hyperemia created by the inflation of the cuff (250 mm Hg for 5 min) placed on the right arm. After sudden cuff deflation, brachial diameter was measured at least four times during the next 90 s.

### 2.7. Cardiopulmonary exercise test

All patients underwent an incremental symptom-limited CPET on a bicycle ergometer. After a 1-min warm-up period at 20 W workload, a ramp protocol of 15 to 20 W/min was started and continued until limiting symptoms for exercise termination appeared, according to methods previously described in detail [20]. Specifically, respiratory gas exchange measurements were obtained breath by breath by a commercially available system (Vmax 29C; Sensormedics, Yorba Linda, CA). Peak oxygen uptake (VO<sub>2</sub>) was recorded as the mean value of VO<sub>2</sub> during the last 20 s of the test. The ventilatory anaerobic threshold was detected by use of the V-slope method. The ventilation per minute (VE) vs. carbon dioxide production (VCO<sub>2</sub>) relationship was measured by plotting ventilation against VCO<sub>2</sub> obtained every 10 s of exercise (VE/VCO<sub>2</sub> slope). The VE/VCO<sub>2</sub> slope was calculated as a linear regression function, excluding the nonlinear part of the relationship after the onset of acidotic drive to ventilation.

Chronotropic incompetence (CI) was defined as a diminished heart rate response to exercise and was diagnosed when at least one of the following criteria was fulfilled: 1) HR failed to reach 85% of the age-predicted maximal HR; 2) HR increase below 80% of adjusted (percent) HR reserve, determined from the change in HR from the rest to peak exercise divided by the difference of the resting HR and age-predicted maximal HR [21]. We also assessed the heart recovery rate (HRR) as the decrease in HR from peak exercise to HR after 2 min of “active” unloaded cycling [22].

### 2.8. Statistics

Data were handled, stored, and analyzed using the SPSS package. The *t*-test for unpaired data and the chi-square test were used when appropriate. Bivariate correlations were calculated between hormonal and cardiovascular parameters. Data are reported as mean ± SEM.

## 3. Results

The baseline characteristics of controls and KS subjects on testosterone therapy are shown in Table 1. We found no difference in any anthropometric measures between the two groups, although BMI, total body fat, and fat body mass tended to be higher in KS. There were no differences in baseline blood pressure, heart rate and ECG findings. As expected, LH and FSH were significantly increased in KS patients whereas no significant differences were found as to testosterone, free testosterone, and adiponectin levels between the two

**Table 1**  
Anthropometric, biochemical and hormonal characteristics of controls and Klinefelter patients.

	Controls (n = 48)	Klinefelter on therapy (n = 48)	Klinefelter total (n = 69)	p <sup>a</sup>
Age (years)	29 ± 2	30 ± 3	30.5 ± 3	ns
BMI (kg/m <sup>2</sup> )	26 ± 1	27 ± 2	27.5 ± 2	ns
Total body fat (%)	21 ± 4	25 ± 2	27 ± 2	ns
Fat body mass (kg)	19 ± 4	25 ± 4	26 ± 2	ns
Lean body mass (kg)	65 ± 3	69 ± 2	68 ± 3	ns
LH (U/L)	5 ± 4	10 ± 2	12 ± 3	<.01
FSH (U/L)	5 ± 4	18 ± 3	20 ± 2	<.01
PRL (ng/mL)	8.6 ± 1	9.0 ± 1	8.9 ± 1	ns
GH (ng/mL)	1.2 ± 2	1.4 ± 4	1.3 ± 2	ns
IGF-1 (ng/mL)	140 ± 16	168 ± 15	164 ± 17	ns
Testosterone (ng/dL)	550 ± 15	469 ± 75	402 ± 98	ns
Testosterone free (ng/dL)	15 ± 6	13.5 ± 3	11.9 ± 7	ns
Estradiol (pg/mL)	33 ± 4	28 ± 3	29 ± 4	ns
TSH (μU/mL)	1.9 ± 1	2.6 ± 3	2.5 ± 2	ns
FT3 (pg/mL)	3.6 ± 2	3.9 ± 1	3.8 ± 1	ns
FT4 (pg/mL)	12 ± 1	11 ± 1	11 ± 9	ns
Total cholesterol (mg/dL)	169 ± 6	179 ± 11	177 ± 16	ns
LDL cholesterol (mg/dL)	104 ± 15	112 ± 14	111 ± 11	ns
HDL cholesterol (mg/dL)	41 ± 4	41 ± 3	41 ± 4	ns
Triglycerides (mg/dL)	102 ± 5	112 ± 14	98 ± 9	ns
Glycemia (mg/dL)	89 ± 7	99 ± 9	98 ± 11	ns
Insulinemia (μU/mL)	13.5 ± 5	17.5 ± 5	16 ± 7	<.01
HOMA index	2.2 ± 1	3.9 ± 1	3.6 ± 1	<.05
Adiponectin (mg/dL)	7 ± 1	9 ± 4	9 ± 4	ns
Metabolic Syndrome (n)	5/48	24/48	30/69	<.01

Data expressed as mean ± SEM; ns, not significant.

BMI: body mass index; HOMA: homeostasis model assessment.

Baseline characteristics of the two populations are shown. We found no difference in any anthropometric measures between the groups. As expected, LH and FSH were significantly increased in KS patients. Fasting insulinemia and HOMA index were significantly higher in KS compared with controls. Employing the NCEP/ATP III criteria, 24 out of the 48 KS patients (50%) and 5 out of 48 control subject (10.48%) had metabolic syndrome.

<sup>a</sup> t-Test performed between treated KS and controls.

study groups. Total and HDL cholesterol, glycemia, and triglycerides did not show significant differences between the two study groups while fasting insulin and HOMA index were significantly higher in KS compared with controls. By using the NCEP/ATP III criteria [23], 24 out of the 48 KS patients (50%) and 5 out of 48 control subject (10%) had the metabolic syndrome ( $p < 0.01$ ). Specifically, 18 KS patients had increased waist circumference, 16 had impaired fasting glucose, 3 had diabetes mellitus, 12 had elevated triglycerides, 13 had decreased HDL cholesterol, and 4 had high diastolic blood pressure.

LV architecture and systolic function were similar in the two study groups. With regard to diastolic function, KS showed a significant prolongation of both isovolumic relaxation time and mitral deceleration time, a decreased E/A ratio and a normal E/E' ratio and pulmonary vein velocities consistent with mild diastolic dysfunction without increased filling pressures. Exercise capacity and cardiopulmonary performance were significantly impaired in patients with KS compared with normal subjects, as documented by the markedly reduced  $VO_2$  uptake and workload both at peak exercise ( $-34\%$  vs. controls) and at anaerobic threshold ( $-24\%$  vs. controls). Stress test was symptom-limited and mean respiratory exchange ratio obtained from expired respiratory gas analysis at peak exertion was above 1.05 in both groups, indicating intense, maximal effort. A lower proportion of predicted maximum HR (78% vs. 91%) and a lower increase in HR from baseline (74 bpm vs. 91 bpm) was achieved in Klinefelter patients compared with controls. Consequently, KS displayed a remarkably increased prevalence of CI: 25 out of 48 (52%) in KS vs. no subjects in controls. HRR at 2 min was significantly higher in KS than in controls (Table 2).

The vascular studies displayed a significant increase of mean internal carotid IMT in KS patients compared with controls ( $+45\%$ ,  $p < 0.05$ ) whereas FMD was similar in the two groups (Table 2).

Bivariate correlates between hormonal and cardiovascular parameters showed a significant positive correlation between IGF-1 and FMD, LH and HRR and a negative correlation between testosterone and lean mass (not shown). Possible explanations for this apparently surprising finding include the genetic defect underlying KS rather than the hypogonadism per se, the age of our patient population and the methodology employed to measure lean mass [24–26]. No other correlations were found between cardiovascular parameters and hormonal measures.

To gain further insight into the cardiovascular consequences of testosterone replacement therapy in KS, we also enrolled 21 newly diagnosed KS subjects off therapy and 21 patients affected by hypogonadotropic hypogonadism (HH) treated with long-lasting intramuscular testosterone therapy. Interestingly, no differences were found as to the cardiovascular parameters between the two KS groups, suggesting failure of adequate hormone therapy to normalizing the observed abnormalities. To further support a major role of the chromosomal abnormalities rather than hypogonadism per se, the patients with secondary hypogonadism under testosterone therapy displayed normal cardiovascular parameters that were significantly different from KS patients (Table 3).

#### 4. Discussion

The current study demonstrates that patients affected by KS exhibit a wide range of cardiovascular abnormalities, some of them herein reported for the first time. Specifically, we found evidence for LV diastolic

**Table 2**  
Ultrasound and CPET characteristics of controls and Klinefelter patients.

	Controls (n = 48)	Klinefelter on therapy (n = 48)	P
LV End-diastolic volume index (mL/m <sup>2</sup> )	67 ± 4	62 ± 4	ns
LV End-systolic volume index (mL/m <sup>2</sup> )	25 ± 2	25 ± 2	ns
LV mass index (g/m <sup>2</sup> )	70 ± 4	73 ± 2	ns
Relative wall thickness	0.34 ± .01	0.35 ± .01	ns
Ejection fraction (%)	61 ± 1	62 ± 3	ns
Deceleration time (ms)	149 ± 5	185 ± 11	<.05
Isovolumic relaxation time (ms)	81 ± 4	109 ± 5	<.001
E/A	2.0 ± .4	1.4 ± .3	<.05
E'/A'	2.1 ± .3	1.5 ± .3	ns
E/E'	5.4 ± .2	6.2 ± .4	ns
adur-A dur (ms)	15 ± 2	16 ± 5	ns
PVa (m/s)	0.30 ± .04	0.31 ± .03	ns
Resting systolic arterial pressure (mm Hg)	123 ± 4	116 ± 4	ns
Resting HR (bpm)	83 ± 6	73 ± 4	ns
$VO_2$ at the AT (mL/kg/min)	17 ± 1	12 ± 1	<.05
Workload at the AT (watt)	88 ± 7	68 ± 6	<.05
HR at the AT (bpm)	124 ± 7	107 ± 6	ns
Peak oxygen consumption (mL/kg/min)	36 ± 4	24 ± 2	<.001
Peak workload (watt)	189 ± 10	148 ± 9	<.001
Peak systolic arterial pressure (mm Hg)	170 ± 12	160 ± 7	ns
VE-VCO <sub>2</sub> slope	26 ± 1	29 ± 1	<.05
Peak HR (bpm)	174 ± 5	147 ± 7	<.01
HR reserve (% bpm)	85 ± 4	65 ± 4	<.01
HR at 2-min recovery (bpm)	35 ± 2	41 ± 5	<.001
Increase in HR (% bpm)	91 ± 6	74 ± 4	<.05
Chronotropic incompetence (n/tot)	0/48	25/48	<.01
Mean internal carotid IMT (mm)	0.31 ± .03	0.45 ± .03	<.05
Flow-Mediated Vasodilation (%)	14 ± 1	14 ± 1	ns

Data expressed as mean ± SEM. ns, not significant.

CPET: cardiopulmonary exercise testing; LV: left ventricle; HR: heart rate;  $VO_2$ : oxygen uptake; AT: anaerobic threshold; VE: ventilation per minute; VCO<sub>2</sub>: carbon dioxide production; IMT: intima-media thickness.

LV architecture and systolic function were similar in the two study groups.

Patients with KS displayed a diastolic filling pattern characterized by reduction of the E/A ratio, with a prolongation of IRT and normal E/E' ratio and pulmonary vein velocities.

We found evidence for chronotropic incompetence in KS patients; these patients exhibited a decrease not only of exercise duration and maximal  $VO_2$  but also of anaerobic threshold.

Patients with Klinefelter Syndrome also displayed a significant increase of carotid IMT.

dysfunction, impaired exercise performance, CI, and increased IMT. Moreover, replacement testosterone therapy does not appear to normalize the impaired cardiovascular parameters.

Patients with KS displayed a diastolic filling pattern characterized by reduction of the E/A ratio, with a prolongation of IRT and no significant changes of tissue Doppler and pulmonary vein indexes. This pattern is consistent with a mild diastolic dysfunction, more specifically with an abnormal relaxation pattern without elevated resting diastolic filling pressures. An impairment of diastolic function is a common finding in many cardiac diseases, and it often precedes and causes systolic dysfunction. It has been documented that 30–40% of heart failure syndromes are secondary to impaired diastolic function [27]. Therefore, the diastolic dysfunction observed in the current study

could be the prelude to more serious limitations of cardiac function and physical performance. Abnormal relaxation indeed results in persistent pressure generation at end diastole and may thus lead to reduced LV distensibility, which in turn is known to contribute to exercise intolerance. Exercise intolerance is largely dependent on reduced stroke volume during exercise caused by the limited increase in the LV end-diastolic volume despite normal ejection fraction and the increased LV filling pressure and left atrial pressure during exercise [28]. However, although the mild diastolic dysfunction observed in the Klinefelter patients may partly account for the diminished peak oxygen consumption through the mechanisms outlined above, the magnitude of reduction (–31%) raises the suspicion that other factors may have been at play.

In this respect, we found for the first time evidence for CI in KS patients. CI is a common finding in patients with cardiovascular disease, produces exercise intolerance that potentially impairs quality of life, and more importantly, is an independent predictor of major adverse cardiovascular events and overall mortality in asymptomatic populations [21,29]. The pivotal role of heart rate as determinant of exercise performance is underlined by the recognition that maximal aerobic exercise in healthy humans is achieved by a 2.2-fold increase in HR, a 1.5-fold increase in arteriovenous oxygen difference, and only a 0.3-fold increase in stroke volume [21,30]. Therefore, the increase in HR is the strongest contributor to the ability to perform sustained aerobic exercise and, consequently, recognition of CI is of the outmost importance [21,27–32].

With regard to the underlying mechanisms of CI, they are not completely understood but seem to result from a disruption of the delicate balance between the sympathetic and parasympathetic divisions of the autonomic nervous system. Interestingly, heart recovery in Klinefelter patients, which describes another relevant feature of CI, was similar or even slightly improved compared with controls. HRR, the physiological drop of HR after physical exercise, appears directly related to the parasympathetic tone. Thus, the perturbation of the autonomic nervous system observed in Klinefelter patients appears dependent upon increased sympathetic drive commonly associated with reduced  $\beta$ -receptor density and sensitivity rather than upon impaired parasympathetic withdrawal [32].

KS patients exhibited a decrease not only of exercise duration and maximal  $\text{VO}_2$  but also of anaerobic threshold. Exercise duration and maximal  $\text{VO}_2$  are indexes of work capacity that are not entirely objective because they may be limited by symptoms or patient motivations. The anaerobic threshold is the exercise level above which aerobic energy production is supplemented by anaerobic mechanism and is therefore more objective [33]. Despite the fact that exercise capacity was impaired, KS patients did not experience significant difference in daily activities, compared with healthy controls. This finding might be explained by the fact that most daily activities occur at relatively low levels of oxygen consumption.

Patients with Klinefelter Syndrome also displayed a significant increase of carotid IMT, a surrogate marker of atherosclerotic disease. This alteration was present for each side on carotid axis in patients with KS and caused a diffuse and homogeneous increase of the arterial wall thickness in both the common and internal carotid arteries. It is widely accepted that ultrasonographic findings of increased carotid artery IMT represent subclinical marker of early atherosclerosis and are associated with unmodifiable and modifiable risk factors, with the occurrence of new carotid plaques and with the subsequent risk of new or recurrent stroke and myocardial infarction [34–39]. Evaluation of atherosclerosis is relevant in a population like KS since low levels of testosterone have been associated with coronary artery disease in men [40]. Furthermore, testosterone administration showed beneficial effects in acute coronary syndrome [41]. The clinical relevance of increased IMT has been recently outlined by Polak and colleagues, who demonstrated in the Framingham cohort that the maximum IMT of the internal carotid artery improves the classification of risk of cardiovascular disease [42].

**Table 3**

Anthropometric, hormonal, echo and CPET characteristics according to testosterone therapy in Klinefelter patients (KS) and in patients with secondary hypogonadism (HH).

	KS No therapy (n=21)	KS On therapy (n=48)	HH On therapy (n=21)	p UKS vs HH	p TKS vs HH
Age (years)	31 ± 5	30 ± 3	35 ± 6	ns	ns
Therapy duration (months)	–	36 ± 8	52 ± 11	ns	ns
BMI (kg/mq)	28 ± 2	27 ± 2	26 ± 4	ns	ns
Total body fat (%)	29 ± 5	25 ± 2	22 ± 4	ns	ns
Fat body mass (kg)	31 ± 9	25 ± 4	19 ± 4	ns	ns
Lean body mass (kg)	69 ± 2	69 ± 2	65 ± 3	ns	ns
LH (U/L)	15 ± 2	10 ± 2	0.8 ± 3	<.01	<.01
FSH (U/L)	22 ± 3	18 ± 3	1.0 ± 4	<.01	<.01
Testosterone (ng/dL)	390 ± 102	476 ± 76	450 ± 88	ns	ns
Total Cholesterol (mg/dL)	176 ± 21	179 ± 11	189 ± 9	ns	ns
Triglycerides (mg/dL)	89 ± 14	112 ± 14	123 ± 21	ns	ns
Glycemia (mg/dL)	97 ± 17	99 ± 9	89 ± 8	ns	ns
Insulinemia ( $\mu\text{U}/\text{mL}$ )	14 ± 2	17.5 ± 5	13 ± 7	ns	ns
HOMA index	3.5 ± .9	3.9 ± 1	2.3 ± 1	<.05	<.05
Metabolic Syndrome (n/tot)	10/21	24/48	6/21	ns	ns
LV End-diastolic volume index ( $\text{mL}/\text{m}^2$ )	58 ± 5	61 ± 4	66 ± 4	ns	ns
LV End-systolic volume index ( $\text{mL}/\text{m}^2$ )	22 ± 2	25 ± 2	25 ± 4	ns	ns
Ejection fraction (%)	62 ± 4	62 ± 3	62 ± 1	ns	ns
Deceleration Time (ms)	170 ± 8	185 ± 11	145 ± 8	<.05	<.05
Isovolumic Relaxation Time (ms)	104 ± 6	109 ± 5	79 ± 4	<.01	<.01
E/A	1.6 ± .2	1.4 ± .3	2.0 ± .6	<.05	<.05
E/E'	6.1 ± .6	6.2 ± .4	5.5 ± .7	ns	ns
adur-A dur (ms)	15.4 ± .6	16 ± .5	14 ± .8	ns	ns
PVA (m/s)	0.30 ± .04	0.31 ± .03	0.30 ± .02	ns	ns
$\text{VO}_2$ at the AT ( $\text{mL}/\text{kg}/\text{min}$ )	14 ± 2	13 ± 1	16 ± .1	<.05	<.05
Workload at the AT (watt)	65 ± 18	68 ± 6	89 ± 6	<.05	<.05
Peak oxygen consumption ( $\text{mL}/\text{kg}/\text{min}$ )	21 ± 6	24 ± 2	36 ± 2	<.01	<.01
Peak workload (watt)	141 ± 25	148 ± 9	198 ± 9	<.001	<.001
VE- $\text{VCO}_2$ slope	28 ± 5	29 ± 1	25 ± 1	<.05	<.05
Peak HR (bpm)	146 ± 4	147 ± 7	177 ± 6	<.001	<.001
HR reserve (%bpm)	68 ± 2	65 ± 4	86 ± 4	<.01	<.01
HR at 2 min recovery (bpm)	39 ± 7	41 ± 5	34 ± 2	<.05	<.05
Increase in HR (bpm)	75 ± 6	74 ± 4	92 ± 6	<.05	<.05
Chronotropic incompetence (n/tot)	10/21	25/48	0/21	<.01	<.01
Mean internal carotid IMT (mm)	0.42 ± .02	0.44 ± .03	0.30 ± .03	<.05	<.05
Flow-Mediated Vasodilation (%)	13 ± 2	14 ± 1	14 ± 1	ns	ns

Data expressed as mean ± SEM; ns, not significant.

UKS: untreated KS; TKS: treated KS.

CPET: cardiopulmonary exercise testing; BMI: body mass index; HOMA: homeostasis model assessment; LV: left ventricle; HR: heart rate;  $\text{VO}_2$ : oxygen uptake; AT: anaerobic threshold; VE: ventilation per minute;  $\text{VCO}_2$ : carbon dioxide production; IMT: intima-media thickness.

No differences were found as to cardiovascular parameters, suggesting inefficacy of hormone therapy at normalizing the observed abnormalities.

KS patients also presented with a high prevalence of metabolic syndrome, with half of the patient population displaying the typical features. In particular, fasting glucose, triglycerides, and waist circumference were the variables that mostly accounted for higher number of KS patients meeting the ATP III criteria, while HDL cholesterol and blood pressure were similar in the study groups. Interestingly, adiponectin was not decreased in KS subjects but comparable to the control values despite the higher body mass.

Taken together, the abnormalities found in KS include both pre-clinical alterations that may prelude to future cardiovascular events such as increased IMT and metabolic syndrome, and reduced exercise capacity, partly due to chronotropic incompetence and diastolic dysfunction. As mentioned above, some of these alterations are recognized as independent predictors of long term poor outcome.

Previous work from Bojesen's group showed a high prevalence of metabolic syndrome in KS, as well as reduction of maximal exercise performance and subclinical changes of LV function [2,7,8]. In these studies, the authors found significant correlations between hormonal parameters, in particular between testosterone levels and cardiac alterations, leading to the speculation that the hypogonadism secondary to KS may significantly reverberate on body composition by increasing truncal fat and decreasing muscle mass, in turn reducing exercise performance through sarcopenia. Fricke and colleagues in two consecutive papers found a markedly increased prevalence of mitral valve prolapse in patients with KS, confirming a known relation between such valve disease and sexual karyotype alterations, e.g. Turner's syndrome [9,10]. Notwithstanding an accurate echocardiographic examination of the mitral valve, we could not confirm increased prevalence of mitral valve prolapse in our study population.

The findings of the current study appear dependent upon the Klinefelter syndrome per se rather than upon the patient hormonal status. This hypothesis is supported by the lack of correlations between altered cardiovascular indexes and hormonal parameters and by the presence of CI that is neither correlated with testosterone levels nor is known to be associated with hypogonadism in the general population. More importantly, the observation that KS patients treated with replacement therapy still present with cardiovascular abnormalities similar to naïve patients supports the concept of KS specific cardiovascular disease.

To provide further insights into the relative role of hypogonadism of chromosomal abnormalities in determining the cardiovascular phenotype, we also studied another group of patients affected with secondary hypogonadism under adequate testosterone replacement therapy. This group of normal karyotype hypogonadal patients displayed cardiovascular parameters similar to those observed in the control group and, consequently, also significantly different from KS under adequate testosterone replacement therapy. Therefore, testosterone therapy does not normalize cardiovascular abnormalities in KS patients despite normal testosterone levels at variance with patients with secondary hypogonadism, suggesting that the chromosomal abnormality plays a major role in inducing cardiovascular phenotype of KS patients.

More research is warranted to clarify the relationship between testosterone administration and heart, in the look of recent evidence that suggests a role of testosterone in some cardiovascular disease [43–45].

#### 4.1. Clinical implications

Several epidemiological studies have unequivocally shown that patients with KS have raised mortality by 50% with a median loss of 2 years from several specific causes, including cancer, cardiovascular, nervous system and respiratory diseases [3–5]. A Danish register study on morbidity in KS demonstrated that hypothyroidism, diabetes, obesity, ischemic heart disease, thrombosis of the deep veins, pulmonary embolism, and intestinal thrombosis were all more frequent among KS subjects, leading to a 70% increase of hospital admissions [6], while in the UK registry mortality from ischemic heart disease

was decreased [4]. Given this disparate evidence in literature, our findings suggested that there is a generalized increase of cardiovascular risk factors that are known to predispose to future cardiovascular accidents and are independent predictor of poor outcome such as CI and impaired exercise capacity. Whether this broad range of cardiovascular abnormalities represent the pathophysiological underpinnings for the raised mortality consistently observed in Klinefelter Syndrome is currently unknown and needs further research, in particular longitudinal investigations from large international registries [46].

#### 4.2. Study limitations

Notwithstanding the novelty of the findings herein reported, the current study was not specifically designed to provide mechanistic insights into the pathophysiology of the abnormalities found in KS. Further studies are currently ongoing in our laboratory to address these issues.

#### 4.3. Conclusions

Left ventricular diastolic dysfunction, impaired cardiopulmonary performance, chronotropic incompetence, and increased intima-media thickness are a common finding in KS and are not reversed by replacement testosterone therapy.

#### Acknowledgment

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

#### References

- [1] Klinefelter Jr HF, Reifenstein Jr EC, Albright F. Syndrome characterized by gynaecomastia, aspermatogenesis without A-Leydigism, and increased excretion of follicle stimulating hormone. *J Clin Endocrinol Metab* 1942;2:615–27.
- [2] Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter Syndrome: a national registry study. *J Clin Endocrinol Metab* 2003;88:622–6.
- [3] Bojesen A, Juul S, Birkebaek N, Gravholt CH. Increased mortality in Klinefelter syndrome. *J Clin Endocrinol Metab* 2004;89:3830–4.
- [4] Swerdlow AJ, Higgins CD, Schoemaker MJ, Wright AF, Jacobs PA, United Kingdom Clinical Cytogenetics Group. Mortality in patients with Klinefelter Syndrome in Britain: a cohort study. *J Clin Endocrinol Metab* 2005;90:6516–22.
- [5] Bojesen A, Gravholt CH. Morbidity and mortality in Klinefelter syndrome (47, XXY). *Acta Paediatr* 2011;100:807–13.
- [6] Bojesen A, Juul S, Birkebaek NH, Gravholt CH. Morbidity in Klinefelter syndrome: a Danish register study based on hospital discharge diagnoses. *J Clin Endocrinol Metab* 2006;91:1254–60.
- [7] Bojesen A, Kristensen K, Birkebaek NH, et al. The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. *Diabetes Care* 2006;29:1591–8.
- [8] Andersen NH, Bojesen A, Kristensen K, et al. Left ventricular dysfunction in Klinefelter syndrome is associated to insulin resistance, abdominal adiposity and hypogonadism. *Clin Endocrinol* 2008;69:785–91.
- [9] Fricke GR, Mattern HJ, Schweikert HU, Schwanitz G. Klinefelter's syndrome and mitral valve prolapse. An echocardiographic study in twenty-two patients. *Biomed Pharmacother* 1984;38:88–97.
- [10] Fricke GR, Mattern HJ, Schweikert HU. Mitral valve prolapse in Klinefelter syndrome. *Lancet* 1981;26(2):1414.
- [11] Radicioni AF, De Marco E, Gianfrilli D, et al. Strategies and advantages of early diagnosis in Klinefelter's Syndrome. *Mol Hum Reprod* 2010;16:434–40.
- [12] Radicioni AF, Ferlin A, Balercia G, et al. Consensus statement on diagnosis and clinical management of Klinefelter Syndrome. *J Endocrinol Invest* 2010;33:839–50.
- [13] Cittadini A, Napoli R, Monti MG, et al. Metformin prevents the development of chronic heart failure in the SHHF rat model. *Diabetes* 2012;61:944–53.
- [14] De Bellis A, Colao A, Savoia A, et al. Effect of long-term cabergoline therapy on the immunological pattern and pituitary function of patients with idiopathic hyperprolactinaemia positive for antipituitary antibodies. *Clin Endocrinol* 2008;69:285–91.
- [15] Basciani S, Watanabe M, Mariani S, et al. Hypogonadism in a patient with two novel mutations of the luteinizing hormone  $\beta$ -subunit gene expressed in a compound heterozygous form. *J Clin Endocrinol Metab* 2012;97:3031–8.
- [16] Cittadini A, Ines Comi L, Longobardi S, et al. A preliminary randomized study of growth hormone administration in Becker and Duchenne muscular dystrophies. *Eur Heart J* 2003;24:664–72.

- [17] Yu CM, Sanderson JE, Marwick TH, Oh JK. Tissue Doppler imaging: a new prognosticator for cardiovascular diseases. *J Am Coll Cardiol* 2007;49:1903-14.
- [18] Oliviero U, Cittadini A, Bosso G, et al. Effects of long-term L-thyroxine treatment on endothelial function and arterial distensibility in young adults with congenital hypothyroidism. *Eur J Endocrinol* 2010;162:289-94.
- [19] Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the international brachial artery reactivity task force. *J Am Coll Cardiol* 2002;39:257-65.
- [20] Cittadini A, Saldamarco L, Marra AM, et al. Growth hormone deficiency in patients with chronic heart failure and beneficial effects of its correction. *J Clin Endocrinol Metab* 2009;94:3329-36.
- [21] Brubaker PH, Kitzman DW. Chronotropic incompetence: causes, consequences, and management. *Circulation* 2011;123:1010-20.
- [22] Cole CR, Foody JM, Blackstone EH, Lauer MS. Heart rate recovery after submaximal exercise testing as a predictor of mortality in a cardiovascularly healthy cohort. *Ann Intern Med* 2000;132:552-5.
- [23] Grundy SM, Brewer Jr HB, Cleeman Jr JL, et al. Definition of metabolic syndrome: Report of the National Heart, Lung and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004;109:433-8.
- [24] Mammi C, Calanchini M, Antelmi A, et al. Androgens and adipose tissue in males: a complex and reciprocal interplay. *Int J Endocrinol* 2012;2012:789653.
- [25] Carson III CC, Rosano G. Exogenous testosterone, cardiovascular events, and cardiovascular risk factors in elderly men: a review of trial data. *J Sex Med* 2012;9:54-67.
- [26] Vitale C, Mendelsohn ME, Rosano GM. Gender differences in the cardiovascular effect of sex hormones. *Nat Rev Cardiol* 2009;6:532-42.
- [27] Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. *Eur Heart J* 2011;32:670-9.
- [28] Fukuta H, Little WC. Contribution of systolic and diastolic abnormalities to heart failure with a normal and reduced ejection fraction. *Progr Cardiovasc Dis* 2007;49:229-40.
- [29] Lauer MS, Francis GS, Okin PM, Pashkow FJ, Snader CE, Marwick TH. Impaired chronotropic response to exercise stress testing as a predictor of mortality. *JAMA* 1999;281:524-9.
- [30] Higginbotham MB, Morris KG, Williams RS, McHale PA, Coleman RD, Cobb FR. Regulation of stroke volume during submaximal and maximal upright exercise in normal man. *Circ Res* 1986;58:281-91.
- [31] Higginbotham MB, Morris KG, Williams RS, Coleman RE, Cobb FR. Physiologic basis for the age-related decline in aerobic work capacity. *Am J Cardiol* 1986;57:1374-9.
- [32] Lauer MS. Autonomic function and prognosis. *Cleve Clin J Med* 2009;76:S18-22.
- [33] Ingle L. Theoretical rationale and practical recommendations for cardiopulmonary exercise testing in patients with chronic heart failure. *Heart Fail Rev* 2007;12:12-22.
- [34] Chambless LE, Heiss G, Folsom AR, et al. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. *Am J Epidemiol* 1997;146:483-94.
- [35] Ebrahim S, Papacosta O, Whincup P, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. *Stroke* 1999;30:841-50.
- [36] Zureik M, Touboul PJ, Bonithon-Kopp C, et al. Cross-sectional and 4-year longitudinal associations between brachial pulse pressure and common carotid intima-media thickness in a general population. The EVA study. *Stroke* 1999;30:550-5.
- [37] Prati P, Vanuzzo D, Casaroli M, et al. Determinants of carotid plaque occurrence. A long-term prospective population study: the San Daniele Project. *Cerebrovasc Dis* 2006;22:416-22.
- [38] Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 1997;96:1432-7.
- [39] Tsigoulis G, Vemmos K, Papamichael C, et al. Common carotid artery intima-media thickness and the risk of stroke recurrence. *Stroke* 2006;37:1913-6.
- [40] Rosano GM, Sheiban I, Massaro R, et al. Low testosterone levels are associated with coronary artery disease in male patients with angina. *Int J Impot Res* 2007;19:176-82.
- [41] Rosano GM, Leonardo F, Pagnotta P, et al. Acute anti-ischemic effect of testosterone in men with coronary artery disease. *Circulation* 1999;99:1666-70 [Erratum in: *Circulation* 2000; 10:584].
- [42] Polak JF, Pencina MJ, Pencina KM, O'Donnell CJ, Wolf PA, D'Agostino Sr RB. Carotid-wall intima-media thickness and cardiovascular events. *N Engl J Med* 2011;365:213-21.
- [43] Caminiti G, Volterrani M, Iellamo F, et al. Effect of long-lasting testosterone treatment on functional exercise capacity, skeletal muscle performance, insulin resistance and baroreflex sensitivity in elderly patients with chronic heart failure: a double-blind, placebo-controlled, randomized study. *J Am Coll Cardiol* 2009;54:919-27.
- [44] Cornoldi A, Caminiti G, Marrazzi G, et al. Effects of chronic testosterone administration on myocardial ischemia, lipid metabolism and insulin resistance in elderly male diabetic patients with coronary artery disease. *Int J Cardiol* 2010;142:50-5.
- [45] Kaushik M, Sontineni SP, Hunter C. Cardiovascular disease and androgens: a review. *Int J Cardiol* 2010;142:8-14.
- [46] Bossone E, Limongelli G, Malizia G, et al. The T.O.S.C.A. Project: research, education and care. *Monaldi Arch Chest Dis* 2011;76:198-203.