

Gender Differences and Aging: Effects on the Human Heart

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Objectives. This study investigated the changes in myocyte size and number in the left and right ventricles that occur with aging in the female and male heart.

Background. Differences in life span between women and men may be related to a better preservation of myocardial structure in the female heart with aging. On this basis, the hypothesis was advanced that the aging process has a different impact on the integrity of the myocardium in the two genders.

Methods. Morphometric methodologies were applied to analyze the changes in number and size of ventricular myocytes in the hearts of 53 women and 53 men. The changes in mononucleated and binucleated myocytes with age were determined in enzymatically dissociated cells. The age interval examined varied from 17 to 95 years.

Results. Aging was associated with a preservation of ventricular myocardial mass, aggregate number of mononucleated and binucleated myocytes, average cell diameter and volume in the female heart. In contrast, nearly 1 g/year of myocardium was lost in the male heart, and this phenomenon accounted for the loss of ~64 million cells. This detrimental effect involved the left and right sides of the heart. In the remaining cells, myocyte cell volume increased at a rate of 158 $\mu\text{m}^3/\text{year}$ in the left and 167 $\mu\text{m}^3/\text{year}$ in the right ventricle.

Conclusions. Aging does not lead to myocyte cell loss and myocyte cellular reactive hypertrophy in women, indicating that gender differences may play a significant role in the detrimental effects of the aging process on the heart.

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Heart failure is the major cause of death in the elderly (1). From animal studies (1,2), the suggestion has been made that the growth reserve capacity of myocytes declines with aging, limiting the ability of the myocardium to respond to abnormal increases in ventricular loading or functional demand (3). Some observations in humans (4) have indicated that myocytes retain the capacity to hypertrophy and proliferate in the senescent heart; however, several reports (5,6) have demonstrated that the aging process of the heart is characterized by loss of ventricular myocytes and reactive hypertrophy of the remaining, viable cells. Although the mechanism responsible for myocyte cell death has not been identified, the possibility has been raised (7) that defects in the coronary vasculature and microvasculature may occur with aging, leading to ischemic myocytolytic necrosis. Reductions in the quantitative structural variables implicated in oxygen availability, diffusion and transport have been shown in the human heart (8). However, studies in animals and humans have not considered whether

gender differences influence the deleterious consequences of aging and whether the longer life span of women in the industrialized world is related, at least in part, to a better preservation of myocardial structure in the female heart later in life. This is a relevant issue because myocyte cell loss as a function of aging may potentiate other risk factors such as coronary artery disease and hypertension, which are common in the old population (1,9-11). Therefore, aging-induced myocyte cell loss may be critical for the development of heart failure in the elderly (5,6).

On this basis, the current investigation tested the hypothesis that the aging process may have a distinct impact on the integrity of myocardial structure in women and men. Because average heart weight in women and men is considered to be 280 and 320 g, respectively, (12), and pathologic hypertrophy involves hearts of ≥ 500 g (13-15), a 400-g and 450-g upper limit of heart weight for the inclusion in the study was arbitrarily established for women and men, respectively. This approach was introduced because body surface is nearly 10% greater in men than in women (16) and the possibility of an ~40% variation in maximal heart weight was introduced to compensate for the increase in epicardial fat with age in both genders (6). Heart weight is related to body weight and surface, and different criteria could have been used in the selection of the hearts. However, a maximal heart weight value was preferred for comparison with previous quantitative analyses of the myocardium (13-15).

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Methods

Patients. A total of 106 human hearts, 53 female and 53 male, were collected from 2,774 autopsies performed at the University Hospital of Parma Medical School from 1990 to 1994. All 106 hearts, which represented 3.8% of the total number of autopsies, were obtained within 24 h after death. According to clinical criteria and anatomic and histologic observations, these cases were considered to represent normal aging. A detailed description of the multiple variables involved in this selection has been published previously (6).

Briefly, preautopsy criteria for inclusion in the study were 1) sudden death associated with traumatic injury; 2) death within 5 days after hospital admission in which medical history excluded cardiovascular disease processes; 3) no previous medical record of hypertension, diabetes or ischemic heart disease; 4) body weight not >20% or <20% of the optimal weight for gender, height and age; and 5) absence of systemic disorders such as malignant neoplasia, connective tissue diseases and acquired immunodeficiency syndrome.

Autopsy criteria for exclusion from the study were 1) atherosclerosis of the major coronary arteries resulting in >30% reduction of vessel diameter; 2) severe atherosclerosis and aortic aneurysms; 3) valvular abnormalities including atherosclerotic lesions at the insertion of the cusps; 4) acute or healed myocardial infarction, or both; 5) heart weight >400 g and >450 g for women and men, respectively; 6) diffuse emphysema and chronic inflammatory processes of the respiratory system; and 7) presence of a malignant neoplasm with multiple metastatic localizations.

Histologic criteria for exclusion from the study were 1) neoplasms of the hematopoietic system; 2) amyloidosis, tuberculosis and sarcoidosis; 3) diffuse interstitial and perivascular fibrosis of the myocardium; 4) thickening and hyalinosis of the intermediate-sized vessels of the coronary tree; 5) multiple foci of replacement fibrosis >2 mm in diameter; 6) presence of inflammatory cells in the myocardial interstitium; and 7) sites of myocytolytic and contraction band necrosis.

Heart weight. After excision of the heart, great vessels were removed, the atria were dissected free along the atrial ventricular groove and the coronary arteries were cut perpendicular to their course to assess the degree of atherosclerosis. The epicardial fat was then carefully removed, the valves were cut free and the weight of the left ventricle inclusive of the septum and right ventricular free wall was determined. Ventricular mass volume was then computed by dividing ventricular weight by the specific gravity of muscle tissue, 1.06 g/ml (17). The two ventricles were sliced into 9 to 12 10-mm thick sections, perpendicular to the major axis of the heart from the apex to the base. Wall thickness was estimated by averaging 10 equally spaced measurements from each of the two middle tissue sections of each ventricle, which represented the portion of the ventricle halfway between the base and the apex of the heart. These determinations were restricted to the free wall of both ventricles. The trabeculae carneae and papillary muscles attached to the wall were not included in the assessment of wall

thickness. The two middle slices of the free wall of each ventricle were cut radially to obtain tissue fragments extending from the endocardial to the epicardial surface. These samples were fixed in 10% buffered formalin and embedded in glycol methacrylate. At the time of embedding, each specimen of the left ventricle was divided into two regions comprising the epimyocardium and endomyocardium. The fragments of the right ventricle were left intact. An additional sampling of two tissue blocks from each slice of each ventricle and two from the interventricular septum was obtained and embedded in paraffin for further estimation of the presence of myocardial fibrosis and tissue injury. The methacrylate-embedded material was used for quantitative analysis of the myocardium.

Tissue sampling for morphometric analysis. Twelve randomly chosen plastic-embedded tissue blocks, six from the endomyocardium and six from the epimyocardium of each left ventricle, were sectioned at a thickness of 1.0 μm using a JB 4 microtome (Du Pont) and stained with hematoxylin-eosin. Four blocks from the right ventricular free wall of each heart were sectioned and stained in an identical manner. The four blocks of tissue embedded in paraffin from each slice of each ventricle also were sectioned and stained with hematoxylin-eosin and trichrome. This sampling was assumed to provide an accurate evaluation of histologic damage in each heart.

Morphometric sampling at a magnification of $\times 1,000$ consisted of counting the total number of myocyte nuclear profiles, N_n , in a measured area, A , of tissue sections in which cardiac muscle fibers were sectioned transversely. A square tissue area equal to 10,036 μm^2 was delineated in the microscopic field by an ocular reticle (No. 105844, Wild Heerbrugg Instruments, Inc.) containing 42 sampling points. Sixty fields from the endomyocardium and 60 from the epimyocardium were evaluated in the left ventricle, and 40 fields from the endomyocardium and 40 from the epimyocardium were measured in the right ventricle of each heart to determine the mean number of nuclear profiles per unit area of myocytes, $N(n)_A$. Average nuclear length, \bar{D}_n , was determined from 50 measurements from each region of each ventricle, which were made at a magnification of $\times 1,250$ in longitudinally oriented myocytes (7,8,13). In addition, myocyte diameter in the region of the nucleus, \bar{d}_m , was measured in these myocytes. The volume percent of myocytes in the tissue, $V(m)_V$, was obtained by counting the fraction of points overlying the myocyte compartment in each of the 120 and 80 fields examined in the left and the right ventricle, respectively (17). Similarly, the volume fraction of the interstitium was evaluated from the number of points lying over this tissue component.

Morphometric analysis. The regional data of myocyte nuclear numeric density, $N(n)_A$, nuclear length, \bar{D}_n , and volume fraction of myocytes in the myocardium, $V(m)_V$, were combined to yield the average measurement of the number of myocyte nuclei per unit volume of myocytes, $N(n)_V$, in the ventricle using the equation (5,6,17)

$$N(n)_V = N(n)_A / \bar{D}_n. \quad [1]$$

The aggregate volume of myocytes in the ventricle, $V(m)_T$, was then derived from the ventricular volume measurement, V and $V(m)_V$:

$$V(m)_T = V \times V(m)_V \quad [2]$$

The total number of myocyte nuclei in the ventricle, $N(n)_T$, was computed from $N(n)_V$ and $V(m)_T$:

$$N(n)_T = N(n)_V \times V(m)_T \quad [3]$$

$V(m)_T$ divided by $N(n)_T$ yielded the average myocyte cell volume per nucleus, $V(m)_n$, in the ventricle in each heart:

$$V(m)_n = V(m)_T / N(n)_T \quad [4]$$

Preparation of isolated ventricular myocytes. Small fragments of the left and right ventricular free walls dissected from the epimyocardium and endomyocardium were placed in a solution containing 250 U/ml of collagenase type II (Sigma Chemical). The solutions were supplements of modified commercial MEM Eagle Joklik. HEPES-MEM containing (in mmol/liter⁻¹): sodium chloride 117, potassium chloride 5.7, sodium bicarbonate 4.4, potassium phosphate 1.5, magnesium chloride 17, HEPES 21.1 and glucose 11.7, with amino acids and vitamins, 2 mmol/liter L-glutamine, and 21 mU/ml insulin; pH was adjusted to 7.2 with sodium hydroxide. Osmolality of this solution is 292 mOsm. Tissue fragments in collagenase solution were shaken for ~1 h, in a temperature-controlled bath at 32°C. Subsequently, supernatant cell suspensions were shaken in resuspension medium containing creatine, collagenase and 0.3 mmol/liter calcium chloride. Intact cells were enriched by centrifugation and discarding the supernatant. This procedure was repeated four to five times in each preparation to remove nonmyocyte cells, cell debris and the residual collagenase. Each centrifugation was performed at 30 g for 3 min. Smears were made, and the cells were stained with propidium iodide. Twenty-six female and 21 male hearts were used for these preparations. The distribution of nuclei in myocytes involved the analysis of 500 cells/ventricle in each heart.

Sampling size. The magnitude of sampling utilized in this investigation was selected on the basis of previous work performed in our laboratory (17) and the principle of Poisson statistics (18). The latter can be used as a reasonable guideline for morphometric data collection, because it provides a more conservative estimate of necessary counts than more specific formulations derived from point counts and profile counts (17). By assuming that biologic variability among patients in a given group is at least 10%, counting errors in each subject should also be limited by the same order of magnitude for the least frequent structure (17). Specifically, in each left ventricle of each heart of the male group, the sampling of 120 fields resulted in the collection of a total number of nuclei ranging from a minimum of 160 to a maximum of 406. According to the Poisson statistics, this amount of sampling yielded a sampling error of 7.9% and 5.0%, respectively. Corresponding values for the right ventricle were 123 and 256 nuclei, which yielded sampling errors of 9.0% and 6.3%. The total number of nuclei

counted in the left and right ventricles of the 53 male hearts were 13,960 and 9,262, respectively, which implied sampling errors of 0.85% and 1.0%. In the 53 female hearts, the number of nuclei counted in the left ventricle varied from 110 to 360 and in the right ventricle from 108 to 320. Thus, sampling errors were 9.5%, 5.3%, 9.6% and 5.6%. Because a total of 13,862 and 11,238 nuclei were counted in the 53 left and right ventricles, sampling error was <1% in both cases. Significantly smaller sampling errors were obtained for volume fraction determinations and number of myocytes utilized for the estimation of the distribution of nuclei in the cells. The nested analysis of variance (19) performed after the code was broken demonstrated that the number of blocks sampled, the number of sampling points and profile counts were in excess of what would have been the minimum required for optimal efficiency.

Statistical analysis. All morphometric data were collected without knowledge of other data, and the code was broken at the end of the experiment. Correlation coefficients between each set of data and age were determined by linear regression analysis (18). Comparisons between slopes were made by the analysis of covariance (18) and values of $p < 0.05$ were considered significant.

Results

Patients. Hearts were obtained from a total of 106 patients, 53 women and 53 men. This population included 10 women and 12 men from an earlier study (8). More men ($n = 25$) than women ($n = 7$) died suddenly as a result of traumatic injury. Conversely, pulmonary thromboembolism was more frequently seen in women ($n = 22$) than in men ($n = 6$). Cerebral hemorrhage was a comparable cause of death in both women ($n = 10$) and men ($n = 12$). Other causes of death included pulmonary infection (women, $n = 5$; men, $n = 0$), liver cirrhosis (women, $n = 2$; men, $n = 4$), gastrointestinal bleeding (women, $n = 1$; men, $n = 1$), acute pancreatitis (women, $n = 0$; men, $n = 2$), infarction of the small intestine (women, $n = 1$; men, $n = 0$), acute cholecystitis (women, $n = 1$; men, $n = 0$), acute meningitis (women, $n = 1$; men, $n = 0$) and unknown causes (women, $n = 3$; men, $n = 3$). A complete medical history could not be obtained from the patients in whom trauma was the terminal event. Their suitability for investigation was based on the absence of a previous hospital record and on the lack of gross findings on histologic documentation of pathologic processes after a complete autopsy. A careful morphologic examination of the kidneys and brain was performed to exclude lesions of small arteries and arterioles consistent with systemic hypertension. In the majority of cases death occurred in association with a disease state. However, these subjects were included in the study because the pathologic conditions developed terminally and, at most, had little effect on the heart.

The distribution of subjects in different decades of life (Table 1) shows that the number of hearts collected from young persons was limited in both genders. Larger groups were available at older ages. The values of left and right ventricular

Table 1. Patient Characteristics

Age Range (yr)	No. of Pts	Age (yr)	Body Weight (kg)	Height (cm)	Heart Weights (g)			
					Total Heart*	LV†	RV Free Wall	Myocardium
16-25								
Women	2	20 ± 0 (20-20)	59 ± 1 (58-59)	164 ± 1 (163-165)	285 ± 15 (270-300)	116 ± 4 (112-119)	38 ± 1 (37-38)	153 ± 3 (150-156)
Men	14	20 ± 2 (17-23)	71 ± 7 (62-86)	176 ± 6 (165-187)	341 ± 53 (260-420)	187 ± 24 (158-228)	56 ± 6 (47-65)	243 ± 27 (200-271)
26-35								
Women	2	26 ± 0 (26-26)	68 ± 5 (63-72)	163 ± 8 (155-170)	315 ± 35 (280-350)	125 ± 5 (120-130)	38 ± 8 (30-45)	163 ± 13 (150-175)
Men	3	30 ± 4 (26-35)	75 ± 9 (63-84)	191 ± 3 (189-195)	395 ± 28 (360-425)	197 ± 9 (186-207)	61 ± 2 (59-63)	258 ± 9 (249-266)
36-45								
Women	3	41 ± 2 (38-44)	66 ± 6 (58-73)	160 ± 4 (155-165)	310 ± 43 (250-350)	125 ± 29 (100-165)	44 ± 1 (43-45)	169 ± 28 (145-208)
Men	4	40 ± 2 (38-41)	81 ± 3 (76-85)	187 ± 4 (180-190)	375 ± 17 (370-390)	173 ± 7 (164-183)	53 ± 5 (46-58)	226 ± 8 (218-239)
46-55								
Women	6	53 ± 2 (49-55)	61 ± 7 (54-73)	161 ± 5 (153-170)	363 ± 37 (300-400)	141 ± 22 (114-176)	49 ± 5 (42-57)	190 ± 25 (156-230)
Men	6	51 ± 3 (46-55)	73 ± 6 (64-83)	174 ± 3 (170-178)	387 ± 44 (320-430)	169 ± 26 (130-212)	51 ± 5 (43-58)	220 ± 29 (174-265)
56-65								
Women	10	62 ± 2 (58-65)	62 ± 6 (51-71)	160 ± 5 (155-168)	337 ± 32 (290-400)	138 ± 27 (101-184)	41 ± 10 (22-58)	178 ± 31 (133-242)
Men	10	62 ± 3 (56-65)	72 ± 7 (58-79)	171 ± 4 (160-177)	384 ± 45 (300-450)	138 ± 21 (92-172)	50 ± 6 (40-57)	188 ± 25 (132-228)
66-75								
Women	10	70 ± 2 (68-74)	63 ± 6 (53-74)	163 ± 5 (155-173)	352 ± 39 (280-400)	138 ± 15 (116-171)	46 ± 7 (36-60)	184 ± 16 (155-209)
Men	7	69 ± 3 (66-75)	72 ± 6 (61-81)	174 ± 7 (164-185)	371 ± 20 (340-410)	165 ± 15 (139-181)	52 ± 5 (43-58)	217 ± 17 (182-239)
76-85								
Women	13	81 ± 2 (76-85)	62 ± 6 (53-70)	161 ± 4 (157-171)	366 ± 33 (280-400)	137 ± 17 (114-171)	47 ± 7 (30-55)	185 ± 23 (144-229)
Men	7	79 ± 3 (76-84)	65 ± 7 (58-74)	167 ± 6 (155-176)	339 ± 43 (280-430)	134 ± 30 (88-189)	45 ± 6 (39-58)	179 ± 35 (128-247)
86-95								
Women	7	89 ± 3 (86-95)	57 ± 5 (52-65)	156 ± 5 (150-161)	314 ± 41 (250-365)	111 ± 12 (99-136)	39 ± 8 (28-50)	150 ± 13 (130-164)
Men	2	88 ± 1 (87-89)	64 ± 9 (55-73)	168 ± 3 (165-170)	375 ± 15 (360-390)	160 ± 13 (147-172)	50 ± 1 (49-50)	209 ± 13 (196-222)

*Includes great vessels, atria and epicardial fat. †Includes interventricular septum. Unless otherwise indicated, data are expressed as mean ± SD (range). LV = left ventricle; RV = right ventricle.

weight presented in Table 1 were determined after a careful dissection of the epicardial fat. The interventricular septum was considered a portion of the left ventricle. With the exception of the age interval from 76 to 85 years, cardiac weights were consistently lower in women than in men. Although not included in this table, the amount of epicardial fat comprised ~20% of total heart weight in persons from 17 to 45 years. This variable progressively increased with age, accounting for as much as 50% of the heart weight in patients from 65 to 95 years. When regional values in the distribution of fat were examined, the amount in the right ventricle was higher in women and mostly in the late time intervals of life. In summary, the hearts of 106 subjects, 53 women and 53 men, were obtained and considered to be appropriate for the evaluation of the effects of aging and gender on the heart.

Gross anatomic characteristics. The relation between body weight and aging indicated that body weight decreased by 71 g/year in women and 68 g/year in men. However, these reductions were not statistically significant. Body surface remained essentially constant in women, but it decreased 30 cm²/year in men (data not shown). This latter change was statistically significant (p = 0.006). When the weights of the left ventricle, inclusive of the interventricular septum, right ventricle and entire myocardial mass were examined in the absence of epicardial fat, these variables were found to be preserved in women with age (Fig. 1). In contrast, 0.78 g (p = 0.0001), 0.16 g (p = 0.0001) and 0.94 g (p = 0.0001)/year of myocardium were

lost in the left ventricle, right ventricle and whole heart in men as a function of age (Fig. 1). The reduction in weight of the left ventricle was 4.9-fold greater than that of the right ventricle (p = 0.001). However, a comparison between the slopes of the two ventricles by analysis of covariance showed a lack of significant changes, indicating that the percent loss in muscle mass was similar in the left and right sides of the male heart. In addition, ventricular weights were lower in younger women, but the continuous decrease in muscle mass of the male heart with age progressively reduced this difference in the elderly heart.

The changes in ratio of left and right ventricular weight to body surface with aging in both men and women are illustrated in Figure 2. These ratios did not vary in the female heart from age 20 to 95 years. Although ventricular weight and body surface changed in a similar fashion with age in the male heart, the ratios of these variables continued to decrease from age 17 to 90 years. The reduction in the left side of the heart (-0.28 g/m² per year, p = 0.0001) was greater than that in the right side (-0.04 g/m² per year, p = 0.0001). The 7.0-fold difference between the ventricles was statistically significant (p = 0.0001). The effects of aging on the thickness of the left and right ventricular free walls were also examined. In both instances, wall thickness remained substantially constant up to 95 years of age. Throughout the life interval studied, wall thickness measured 13.2 ± 1.3 mm in the left ventricle and 5.04 ± 0.44 mm in the right ventricle of women. Corresponding

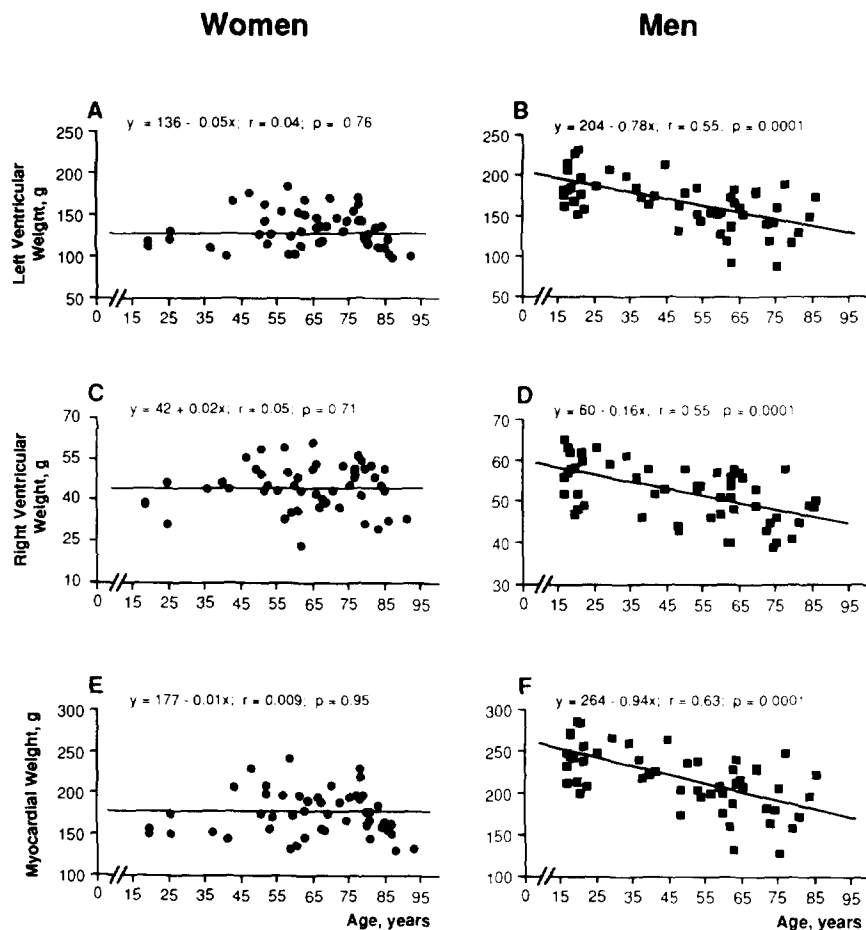


Figure 1. Effects of aging on cardiac weight in women and men.

values in men were 13.6 ± 1.36 mm and 4.90 ± 0.50 mm. No statistically significant differences were detected between these variables in the two genders. In summary, aging was associated with a preservation of cardiac muscle mass in women and a reduction in left and right ventricular weights in men.

Morphometric analysis of number of myocyte nuclei in myocardium. Light microscopic examination of tissue sections showed that small foci of replacement fibrosis <2 mm in diameter were occasionally seen in the left and right ventricular myocardium of both men and women (data not shown). Moreover, a few areas of interstitial fibrosis were encountered in the different layers of the ventricular wall. These two forms of collagen accumulation in the myocardium were more frequently observed in the subendocardial region of the left ventricle of female and male hearts. With the exception of young persons up to 40 years of age, these types of alterations were present in all hearts of both men and women and appeared to increase in older subjects. The volume percent of myocytes and other interstitial structures in the myocardium was determined in areas of the ventricles in which morphologic alterations were not apparent. The small foci of replacement and interstitial fibrosis just described were not included in this evaluation because they did not represent a quantifiable amount of tissue.

The fraction of myocardium occupied by muscle cells remained essentially constant with age in both the left and the right ventricle of women. Similarly, the percent of interstitium did not vary with age. In contrast, the volume fraction of myocytes increased with aging by 0.12%/year ($p = 0.0001$) in the left and 0.10%/year ($p = 0.0001$) in the right ventricle of men (data not shown). Consequently, a statistically significant decrease was detected in the volume percent of interstitium as a function of age in the male heart. According to equation 1 in the Methods section, the primary determinations required for the estimations of the changes in myocyte size and number involved assessment of the number of myocyte nuclear profiles per unit area of myocytes and the evaluation of the longitudinal diameter of myocyte nuclei. Subsequently, the total number of myocyte nuclei in each ventricle in each case was computed in accordance to equation 3 and the values were plotted as a function of age (Fig. 3). Aging was accompanied by lack of changes in the aggregate number of myocyte nuclei in the left and right sides of the female heart. In contrast, the progression of life in men was characterized by a loss of 43 million myocyte nuclei/year ($p = 0.0001$) in the left ventricle and 14 million myocyte nuclei/year ($p = 0.0001$) in the right ventricle. Since the left and right ventricles in young men up to 45 years of age possessed $\sim 5.9 \pm 1.4 \times 10^9$ myocyte nuclei and

Women

Men

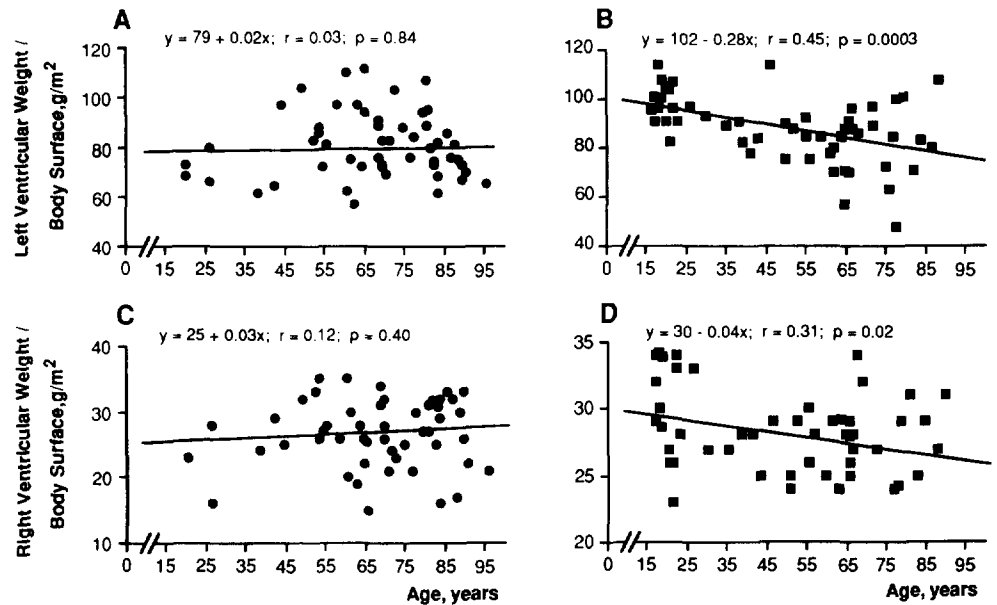


Figure 2. Effects of aging on the relations between cardiac weight and body surface in women and men.

$2.1 \pm 0.40 \times 10^9$ myocyte nuclei, aging was associated with a loss of 0.7% myocyte nuclei/year in both ventricles. The analysis of covariance documented the lack of difference between the two slopes. In summary, aging did not affect the number of myocyte nuclei in the female heart, but it resulted in a significant loss of myocyte nuclei in the male heart.

Quantitative analysis of number of ventricular myocytes. The documentation that the total number of myocyte nuclei remained constant in the female heart and decreased in the male heart can be directly equated with the behavior of the number of muscle cells in the ventricles only under the condition that the distribution of nuclei in myocytes does not

Women

Men

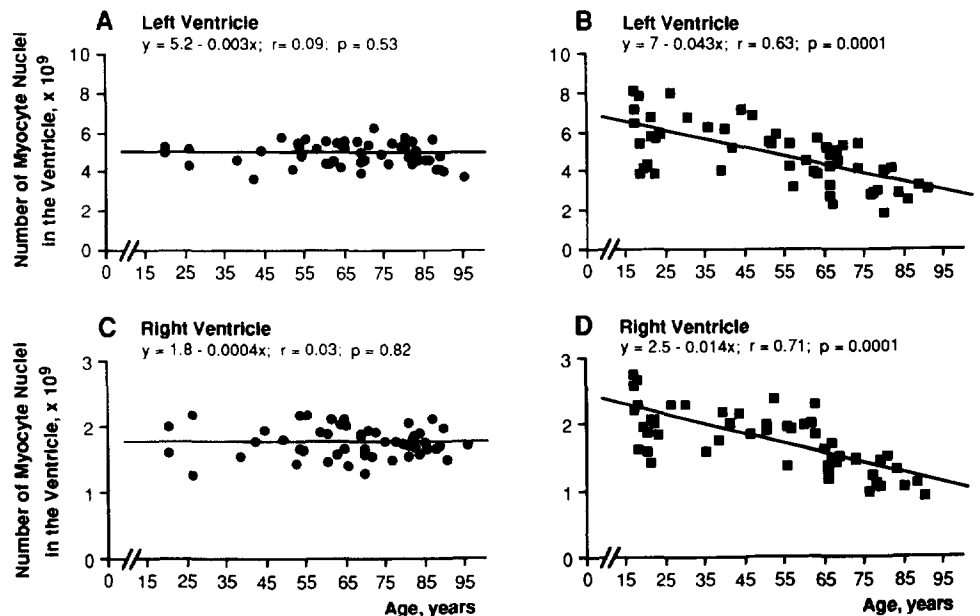


Figure 3. Effects of aging on the total number of ventricular myocyte nuclei in women and men.

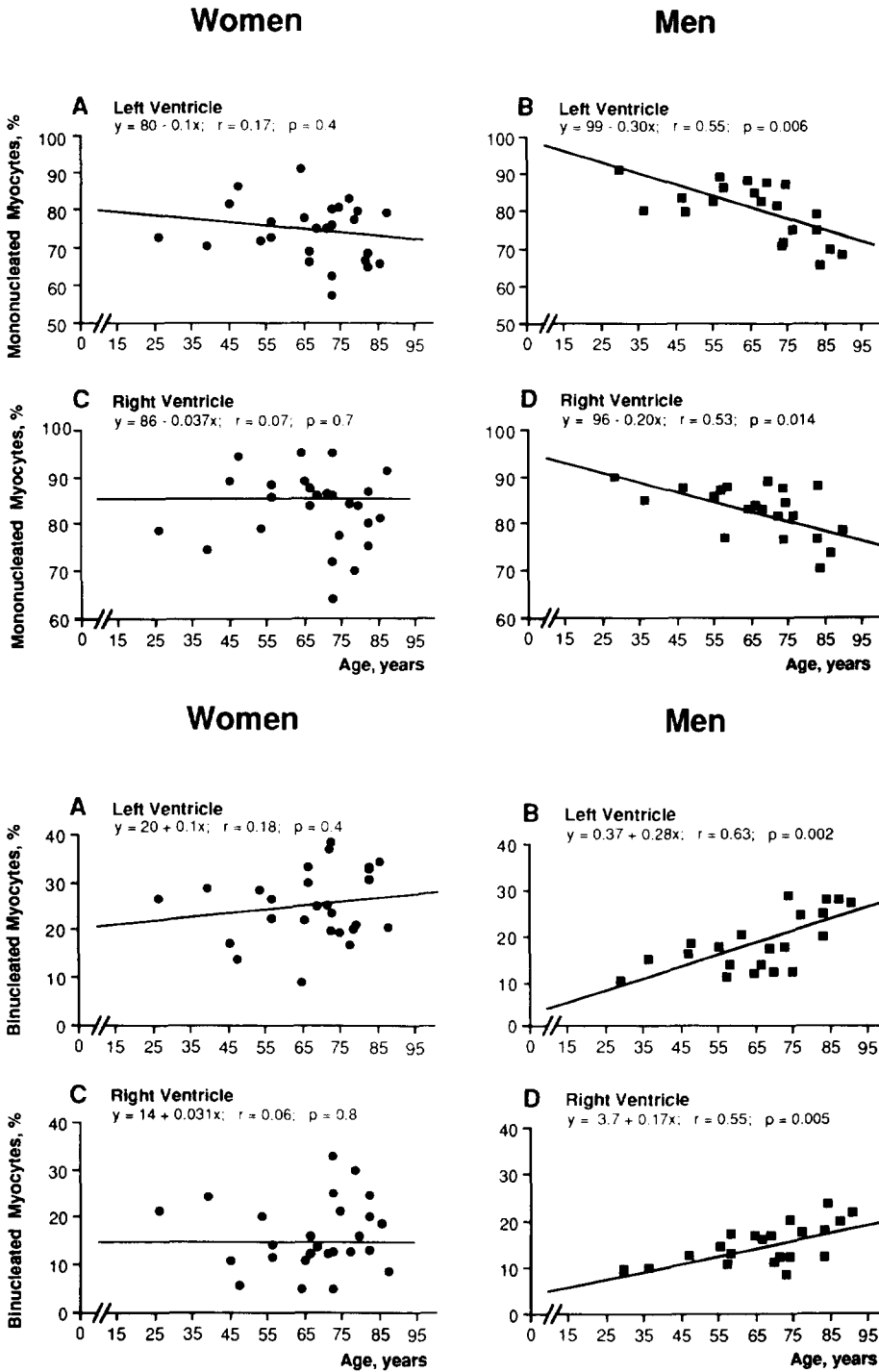


Figure 4. Effects of aging on the relative proportion of mononucleated (upper panels) and binucleated (lower panels) ventricular myocytes in women and men.

vary with age (5,6). Therefore, myocytes were enzymatically dissociated in a group of 26 female and 21 male hearts obtained from persons of different age, and the percent of mononucleated, binucleated, trinucleated and tetranucleated myocytes was determined. Figure 4 illustrates that the fraction of mononucleated and binucleated myocytes remained constant in the female heart with aging. In contrast, in men, mononucleated myocytes decreased by 0.3%/year ($p = 0.006$) in the left and 0.2%/year ($p = 0.014$) in the right ventricle. The

percent of binucleated myocytes in the male heart increased by 0.3%/year ($p = 0.002$) in the left and 0.2%/year ($p = 0.005$) in the right ventricle. Trinucleated and tetranucleated myocytes were only occasionally observed, constituting a negligible component of the human heart in both genders. In the 26 female hearts, 18 trinucleated cells were detected in the 13,000 myocytes examined in the left ventricle, whereas 6 trinucleated cells were found in the right ventricle; corresponding values for tetranucleated cells were 2 in the left ventricle and 3 in the

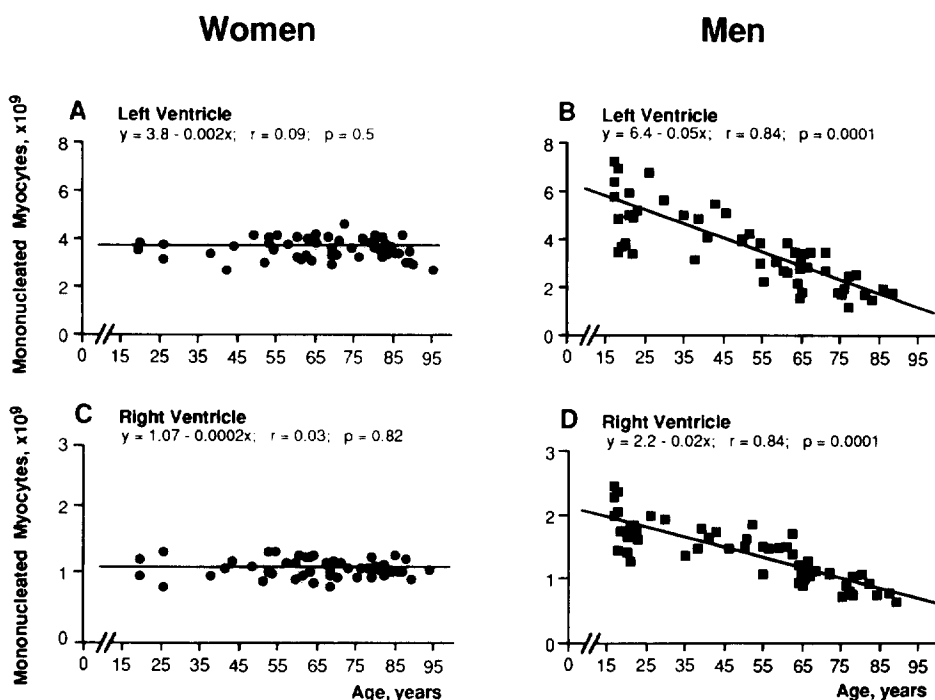
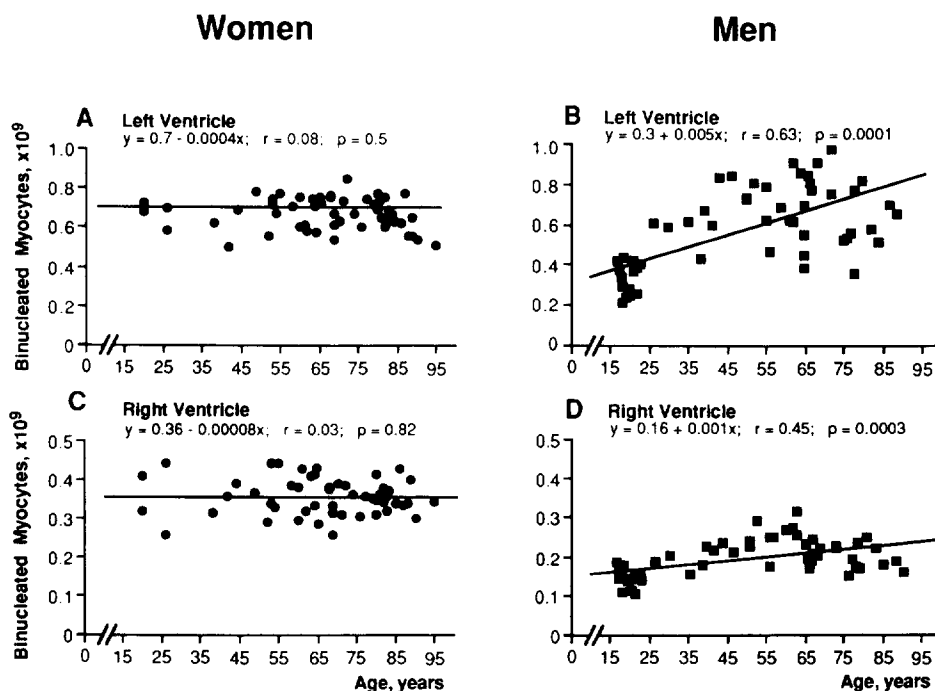


Figure 5. Effects of aging on the total number of mononucleated (upper panels) and binucleated (lower panels) ventricular myocytes in women and men.



right ventricle. In the 21 male hearts, 13 trinucleated and 3 tetranucleated cells were observed in the left ventricle; in the right ventricle, only 5 trinucleated myocytes were encountered.

The distribution of nuclei in myocytes (Fig. 4), in combination with the aggregate number of ventricular myocyte nuclei (Fig. 3), allowed computation of the number of cells in each ventricle. This variable was obtained by calculating in each case, in each ventricle, the number of mononucleated and binucleated myocytes, utilizing the proportions of these two cell populations depicted by the slopes in the upper and lower

panels of Figure 4. The female heart contained an average $3.6 \pm 0.43 \times 10^9$ mononucleated myocytes and $0.67 \pm 0.08 \times 10^9$ binucleated myocytes in the left ventricle, and $1.05 \pm 0.14 \times 10^9$ mononucleated and $0.36 \pm 0.05 \times 10^9$ binucleated myocytes in the right ventricle. The total number of myocytes was calculated to be $4.31 \pm 0.52 \times 10^9$ and $1.42 \pm 0.18 \times 10^9$ in the left and the right ventricle, respectively. Aging did not affect the aggregate number of myocytes in either ventricle (Fig. 5).

An identical analysis in the male heart demonstrated that

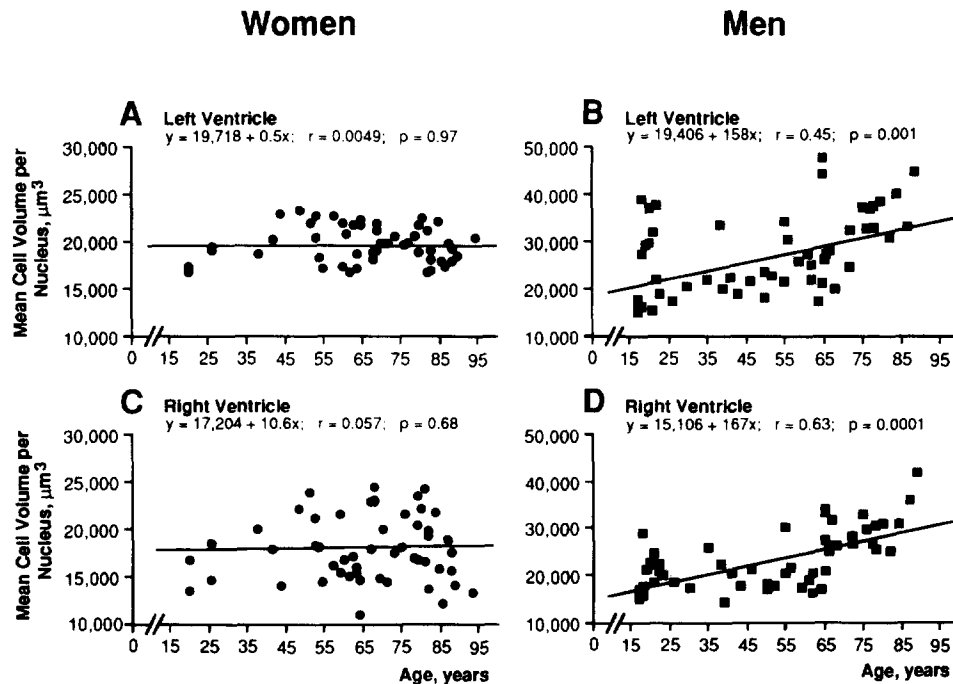


Figure 6. Effects of aging on the changes in myocyte cell volume per nucleus in the left and right ventricles of women and men.

the absolute number of mononucleated myocytes decreased by 50 million/year ($p = 0.0001$) in the left ventricle and by 20 million/year ($p = 0.0001$) in the right ventricle. In contrast, binucleated myocytes increased by 5.0 million/year ($p = 0.001$) and 1.0 million/year ($p = 0.0003$) in the left and the right ventricle, respectively (Fig. 5). These reductions resulted in a total loss of 45 million myocytes/year ($p = 0.001$) in the left and 19 million myocytes/year ($p = 0.001$) in the right ventricle of the male heart. The continuous loss of myocytes with age did not permit the calculation of an average number of left and right ventricular myocytes throughout the life span of the male subjects. In summary, the number of myocytes did not change with age in the female heart, but aging led to a loss of 64 million myocytes/year in the male heart.

Morphometric analysis of myocyte size and shape. Figure 6 shows the changes in myocyte cell volume with aging in female and male hearts. Cell size per nucleus was computed on the basis of equation 4 in Methods. Myocyte cell volume per nucleus was not altered by the aging process in women, whereas, in men, this variable increased by $158 \mu\text{m}^3/\text{year}$ ($p = 0.001$) and $167 \mu\text{m}^3/\text{year}$ ($p = 0.0001$) in the left and the right ventricle, respectively. The small difference in the extent of myocyte hypertrophy with age between the two ventricles was not statistically significant. The analysis of myocyte diameter, measured at the level of the nucleus, demonstrated that this dimensional property of the cell did not change with age in the female heart (Fig. 7). In contrast, myocyte diameter increased as a function of age in the left and right ventricles of men. In summary, aging did not alter the dimensional properties of myocytes in the female heart, but it led to cellular hypertrophy of the male heart.

Discussion

Our results indicate that the aging process did not alter the number, size, shape and proportion of mononucleated and binucleated myocytes of the female heart from age 20 to 95 years. In contrast, the male heart lost from age 17 to 89 years 3.24×10^9 myocytes in the left ventricle and 1.37×10^9 in the right ventricle. These aggregate values were the consequences of a loss of 45 million myocytes/year in the left and 19 million myocytes/year in the right ventricle of men. Myocyte cell loss was accompanied by a progressive increase in average myocyte cell volume and this reactive hypertrophic response was capable of compensating, at least in part, for the destruction in muscle mass, preserving left and right ventricular wall thickness with age. However, ventricular weights declined as a function of age. Conversely, the integrity of myocardial structure in the female heart resulted in the lack of alterations in ventricular myocardial volume with the evolution of life in women.

Gender, aging and heart weight. Congestive heart failure is the leading cause of death in the elderly, particularly in men (20-22). However, the etiologic mechanism responsible for the greater detrimental impact of aging on men is unknown. Women are less frequently affected by cardiovascular events (20-22) and have a greater capacity to sustain a hemodynamic overload with age (23). This contention also has been supported by experimental results showing that genetically determined hypertension impairs cardiac performance more in male than in female rats as a function of age (24,25). Of relevance, total peripheral resistance is lower and cardiac output is higher in women than men of similar age and arterial pressure (23).

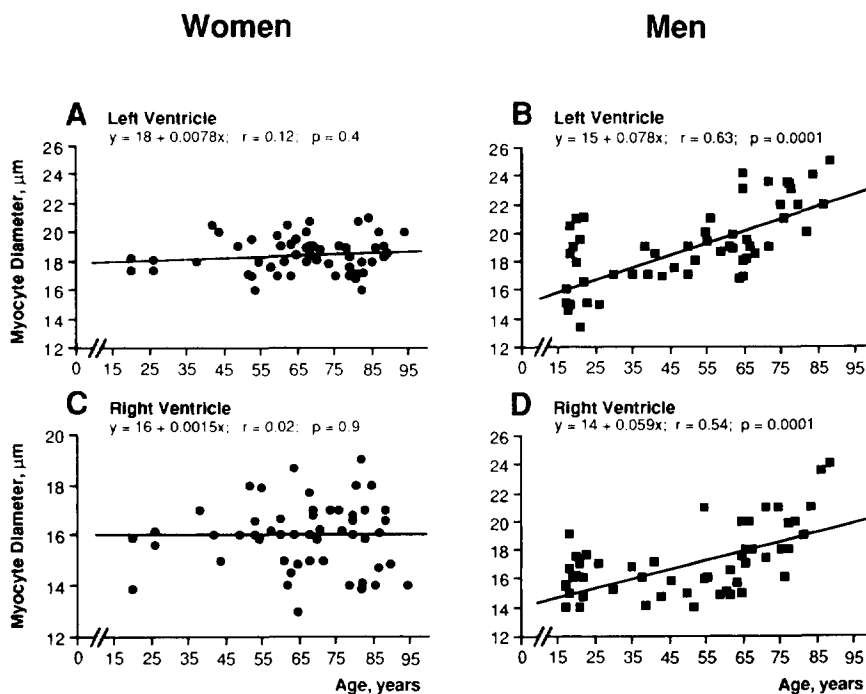


Figure 7. Effects of aging on the changes in diameter of left and right ventricular myocytes in women and men.

Systemic arterial blood pressure tends to be higher in men (23,26,27). These hemodynamic characteristics may be implicated, at least in part, in the larger cardiac muscle mass and higher myocardial mass/body surface ratio observed in the current study in men with respect to women. Such a difference was found to persist for most of the life span of men and women and to become undetectable only in old persons. Thus, it is possible that the work load of the male heart is greater throughout life, leading to an attenuation of the growth reserve of the myocardium with age in men. Such unfavorable conditions may have worsened progressively with time in view of the continuous loss of muscle mass with aging in the male heart. This phenomenon was not apparent in women.

The observation in the current study that cardiac hypertrophy did not develop with age in either women or men is in contrast to previously published work (28-30). The claim has been made (28) that heart weight increases by 1.5 g/year in women and 1 g/year in men. However, these early investigations did not exclude from the analysis patients affected by disease states of the myocardium. Moreover, the contribution of epicardial fat to the evaluation of heart weight was not determined. These two variables may have significantly influenced heart weight measurements, because accumulation of fat on the surface of the left and right ventricles may account for as much as 50% of heart weight (6). Variations in body surface were also not carefully considered, and those in combination with loading abnormalities and adipose tissue infiltration may have resulted in an increase in heart weight, independently from normal aging. A similar conclusion was reached from *in vivo* echocardiographic measurements (31). Our results are consistent with this contention and tend to challenge the common belief that cardiac hypertrophy devel-

ops with age (1,3). In addition, gender differences seemed to exist in the adaptation of the heart with age: Ventricular myocardial weight remained essentially constant in women and decreased in men. However, gross anatomic variables do not necessarily reflect responses at the cellular level (4,6,13-15,32).

Gender, aging and myocyte cell loss. In recent years, several investigations (6,32,33) have documented that loss of myocytes is a consistent alteration of the myocardium in the presence of ventricular dysfunction and failure. Myocyte cell loss can occur as a result of ischemic necrosis (33) or through the activation of the suicide program (34,35). This latter condition may be hypothesized to be a component of the aging process of the heart (35), but no demonstration has been provided as yet. Apoptosis, which is used interchangeably with programmed cell death, has been shown experimentally in myocytes and interstitial cells during early postnatal development of the heart (35) and after ischemic-reperfusion injury of the myocardium (34). In the current study, the examination of human specimens obtained at autopsy did not permit analysis of whether myocyte necrosis and apoptosis were present in the female and male hearts as a function of age. However, the total number of myocytes in the left and right ventricles remained essentially constant in women from age 20 to 95 years. In contrast, a loss of nearly 1% of myocytes/year was demonstrated in both ventricles of men. A man at 20 years of age has $\sim 5.8 \times 10^9$ (mononucleated 5.4×10^9 ; binucleated 0.4×10^9) and 2.0×10^9 (mononucleated 1.8×10^9 ; binucleated: 0.2×10^9) myocytes in the left and the right ventricle, respectively. The same man at 70 years of age is expected to have 3.6×10^9 (mononucleated 2.9×10^9 ; binucleated 0.7×10^9) and 1.0×10^9 (mononucleated 0.8×10^9 ; binucleated: 0.2×10^9) myocytes in the two ventricles. Such a change is the consequence of

a 38% and 50% overall loss in the aggregate number of myocytes in the left and right sides of the heart in a 50-year interval.

The bases for the differential effect of aging on ventricular myocytes in men and women is currently unknown. Although the incidence of cardiovascular events in women increases after menopause (26), sex hormones do not appear to influence myocyte cell death in the myocardium with aging. The number of myocytes in the left and right ventricles showed no tendency to decrease in women from 55 to 95 years. In contrast, comparable losses of myocytes were observed in both ventricles of men from young to old age. This phenomenon suggests that a common mechanism may be involved in the activation of myocyte cell death in the left and right ventricular myocardium in men.

Capillary numerical density and the volume percent of capillary lumen in the tissue decrease as a function of age in the human heart and this alteration is coupled with an increase in capillary spacing and the diffusion distance for oxygen (8). However, this information is limited to the left ventricle in men, and gender differences were not investigated. Experimental work has demonstrated that the intramural branches of the coronary vasculature and the capillary properties implicated in oxygen availability and transport become progressively impaired with age in both ventricles of the male rat heart (7,8). Unfortunately, there are no reports on the quantitative structural characteristics of the coronary vasculature and microvasculature of the female heart in different species. Thus, the hypothesis may be raised that the phenomenon of myocyte loss in the male heart is mediated by local ischemia and tissue injury. Whether the preservation of the number of myocytes in the female heart with aging documented in the current study is due to the integrity of the coronary circulation is an important unanswered question. The inability to obtain myocardial samples from human hearts fixed by coronary perfusion interfered with a detailed analysis of the coronary vascular tree in both genders.

Gender, aging and myocyte cellular properties. The adult mammalian heart is constituted by mononucleated and binucleated myocytes, whereas trinucleated and tetranucleated myocytes are negligible components of the myocardium (36,37). Limited observations in humans (36,37) indicate that mononucleated and binucleated cells represent ~85% and 14%, respectively, of the whole myocyte population of the left ventricle. Binucleated myocytes have been suggested (36) to represent a smaller proportion, 7%, of the right ventricular myocardium. However, under pathologic conditions, the fraction of binucleated cells has been claimed (36) to increase to nearly 25% and 11% in the left and the right ventricle, respectively. Our current results confirm these early findings, but they also demonstrate that the proportion of mononucleated and binucleated cells was affected by aging in men. In the male heart, mononucleated cells progressively decreased with age, whereas binucleated cells continuously increased in the left and right ventricles. A similar phenomenon did not occur in the female heart. The changes in the relative contribution of

mononucleated and binucleated cells in the aging male heart may have been the consequence of a prevailing death of mononucleated cells with time, nuclear mitotic division in the absence of cytokinesis of mononucleated cells, fusion of mononucleated cells or a combination of these.

An additional difference in the structural properties of myocytes as a function of age in men and women concerned the absence of myocyte cellular hypertrophy in the female heart and an increase of $163 \mu\text{m}^3/\text{year}$ in average cell volume in the male heart. Myocyte cellular hypertrophy involved both ventricles. The mechanism of this hypertrophic reaction is difficult to identify. However, myocyte cell loss (8) can be expected to result in a greater stress on the remaining myocytes, which undergo reactive hypertrophy in response to the increased load (33). Experimental observations (38,39) have documented that alterations in myocyte performance develop with age in different strains of male rats and this phenomenon increases the magnitude of work load of the heart. The mechanical behavior of the myocardium is not altered in the female rat heart (40). Although it is unknown whether a similar condition is present in the female human heart, it is tempting to speculate that the preservation of the aggregate number of ventricular myocytes with intact intrinsic mechanical properties may provide a basis for the absence of muscle cell hypertrophy in women as a function of age.

Study limitations. Several limitations in the current investigation must be acknowledged. The number of hearts in some of the age intervals examined was very small, and this factor may have influenced the collected results. Medical history was not available for several patients and the existence of a disease state was excluded only on the basis of the anatomic and histologic examination. The life styles of women and men may differ, particularly at younger ages, and men may attain a larger muscle mass early in adulthood, a phenomenon that may affect the subsequent reduction in ventricular weight with age in the male heart. Conversely, women may have more uniform life styles throughout their lives. Finally, a critical heart weight measured at autopsy was established as an essential criterion for inclusion in the study. This variable was only subsequently normalized for body weight and body surface, and this approach has to be considered in the interpretation of the collected results.

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