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The Neonatal Heart Has a Relatively High Content of Total Collagen and Type I Collagen, a Condition That May Explain the Less Compliant State

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Objectives. This study evaluated the extent of the collagen network in neonatal heart muscle and whether the type I/type III collagen ratio is the same as in the adult heart.

Background. The functional integrity and the stress–strain relation of heart muscle depends largely on the extracellular collagen matrix. The question therefore arises whether the altered compliance of the neonatal heart could relate to the developmental state of collagen and, in particular, the distribution of types I and III collagen. Type I collagen mainly provides rigidity and type III collagen elasticity.

Methods. Specimens from the left lateral wall of the left ventricle of human hearts (immature to full term, n = 23; 3 weeks to 12 years, n = 17) were used to determine the total collagen amount, using the hydroxyproline assay. Similar left ventricular specimens of human hearts (fetal to mature, n = 20; 2 months to 1.5 years, n = 6) were fixed in formalin, paraffin embedded and stained with Sirius red F3BA for total collagen. The ratio of total collagen to total protein was quantified spectrophotometrically. Frozen sections of left ventricular myocardium (immature to mature, n = 17; 4 months to 12 years, n = 10) were stained with antibodies raised against types I and III collagen. Antibody titration was done on human leiomyoma tissue with a known type I/type III collagen ratio. The endomysial collagen types were quantified using a spectrophotometer and expressed as a ratio. Adult human myocardium (n = 10) was used as reference.

Results. The study showed that the total amount of collagen increases with age. However, the ratio of total collagen to total protein and the ratio of type I to type III collagen were very high in hearts of the very young. During development, a gradual decrease occurred, with the total collagen/total protein ratio reaching normal levels at ~5 months after birth and the type I/type III collagen ratio stabilizing at a much later age.

Conclusions. These findings suggest that the relative high content of collagen, related to the myocytes, and the high ratio of type I to type III collagen provide the substrate for a rigid, less compliant heart in neonates.

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It is well known clinically that the neonatal heart has a limited reserve capacity to handle an increased volume load, particularly in immature and premature babies (1,2). A substantial shunt, for instance, in case of a widely patent arterial duct, readily causes left ventricular overload and failure. The reason for this phenomenon is not fully understood. On the basis of experiments in newborn lambs it has been suggested that the heart in the immediate newborn period is functioning at a very high level of performance and thus has a limited capacity to further increase its output (2,3). A comparative study of preterm and full-term newborn lambs before and after volume infusion revealed a similar left ventricular loading response but without further increase in contractility, also suggesting that the ventricle already functioned at its maximal level (4).

During the past decade, evidence has accumulated that mechanical behavior of the heart as a muscle pump also depends on the extracellular collagen matrix (5–7). Indeed, the arrangement of the collagen matrix in struts and weaves, interconnecting myocytes and enwrapping groups of myocytes in perimysial and epimysial sheaths (8–11) not only provides functional integrity of the heart muscle but also determines largely the stress–strain relation within ventricular myocardium during the diastolic and systolic phases (4,12). Moreover, the fibrillar collagen matrix of the myocardium consists primarily of types I and III collagen, which provide different functional characteristics (7,13). Type I collagen mainly provides rigidity, whereas type III collagen contributes to elasticity. On the basis of these observations one could hypothesize that the distribution and ratio of both collagen types within the myocardium could have an effect on the compliance of the heart. Hence, the question arises to what extent the collagen network of the neonatal heart muscle has already developed and whether the ratio of type

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I to type III collagen is the same as that in the adult heart. The present study was designed to evaluate these aspects.

Methods

The study is based on human fetal and neonatal hearts obtained at autopsy. Ten hearts from adults who died of noncardiovascular related disease served as reference. The hearts were sectioned perpendicular to the left ventricular long axis. Two adjacent slices at the level of the base of both papillary muscles were used for study. The tissue samples were either fixed in formalin or snap-frozen, except for one heart in which both techniques were used. The formalin-fixed samples were used for both hydroxyproline analysis and, with the exception of three samples, Sirius red staining. Tissue blocks were taken from the left lateral wall and processed further as necessary (see next section).

Total collagen. *Hydroxyproline analysis.* The heart specimens used were obtained from immature (16 to 27 weeks, n = 6), premature (27 to 35 weeks, n = 12) and mature (36 to 42 weeks, n = 5) babies and from 17 infants and children. Hydroxyproline analysis was performed on heart slices that had been fixed initially in 4% buffered formalin; subsequent blocks taken from the left lateral wall were snap-frozen in liquid nitrogen. Cryostat sections were cut at 20-μm thickness and collected in a cold Eppendorf test tube, and the exact wet weight of the tissue was determined. The total collagen concentration was determined by measuring the hydroxyproline levels according to the method of Laurent et al. (14).

Sirius red staining. The heart specimens used were obtained from fetuses (15 to 16 weeks' gestation, n = 2), immature (16 to 25 weeks, n = 6), premature (27 to 35 weeks, n = 8) and mature (36 to 42 weeks, n = 4) babies and from six infants (Table 1). The Sirius red staining was done on tissues fixed in 4% buffered formalin. The samples were embedded in paraffin, sectioned at 5-μm thickness and stained with Sirius red (0.1% Sirius red F3BA dissolved in saturated picric acid, pH 2.0). Sirius red stains all types of collagen.

The total amount of endomysial collagen and that of all remaining proteins was quantified spectrophotometrically, using a Vickers M85A scanning and integrating microdensitometer (15). The maximal absorption for Sirius red is at a wavelength of 557 nm, and that for total bound picric acid is at 434 nm. From each section 40 nonoverlapping, randomly selected areas were measured at 557 nm, and 10 fields were measured at 434 nm. Care was taken not to include perimysial collagen. For each section the mean of the 40 measurements at 557 nm was calculated and divided by the mean of the 10 readings at 434 nm and multiplied by 100 (16).

Types I and III collagen. These hearts were obtained from immature (16 to 27 weeks, n = 5), premature (27 to 36 weeks, n = 7) and mature babies (36 to 42 weeks, n = 5) and from 10 infants and children. Unfixed specimens were snap-frozen in isopentane, cooled in liquid nitrogen. Sections were cut at 6-μm thickness and mounted on organosilane-coated glass slides, dried overnight at room temperature and fixed in cold acetone (10 min, 4°C).

Types I and III collagen were immunostained with mouse anti-type I collagen and mouse anti-type III collagen (clone I-8H5, IgG2a and clone III-53, IgG1, respectively; both gifts of K. Iwata, MD, Fuji Chemical Industries Ltd.). The method applied was a two-step indirect immunoalkaline phosphatase technique. The end product was visualized with Fast Red and quantified with a microdensitophotometer at a wavelength of 500 nm (17). Types I and III collagen were measured, excluding collagens localized perivascularly. Leiomyoma tissue, known to have a ratio of type I to type III collagen of ~1 (18), was used to establish the appropriate dilutions of the anticollagen antibodies to achieve identical optical densities. Leiomyoma tissue sections served as a reference during every staining procedure. The results were expressed as a ratio of type I to type III collagen.

Statistical analysis. The results with regard to collagen values in relation to age were analyzed using the Spearman rank correlation test.

Table 1. Ratio of Total Collagen to Total Protein, Quantified Spectrophotometrically, Using the Sirius Red F3BA Stain in Each Heart

Age/Gender	Collagen/Protein
Prenatal (wk)	
Fetal	
15/F	24.1
16/F	24.3
Immature	
16/M	25.2
19/M	23.8
19/M	24.0
20/M	21.4
20/M	26.4
25/F	19.6
Premature	
27/F	19.1
28/M	19.1
30/F	20.4
30/F	19.3
30/F	17.0
30/F	19.6
35/F	17.5
35/F	15.7
Mature	
36/F	17.4
39/F	20.3
41/F	17.7
42/M	15.1
Postnatal	
2 mo/F	15.3
3 mo/F	14.2
5 mo/F	12.9
8.5 mo/M	10.2
1 yr/M	14.4
1.5 yr/F	11.5

F = female; M = male.

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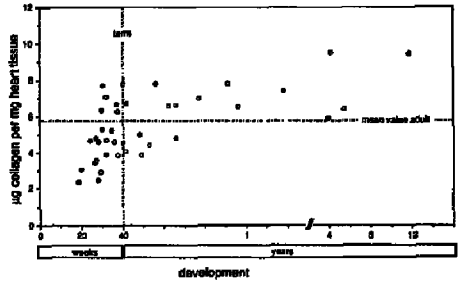


Figure 1. Graphic display of the hydroxyproline analysis of total collagen, expressed as micrograms (μg) of collagen per milligram (mg) of heart tissue, and related to age.

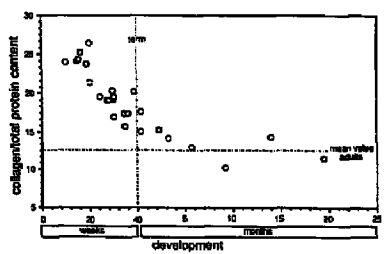
Results

Total collagen. *Hydroxyproline analysis.* Adult reference hearts had a mean (±SD) value of collagen in the left ventricle of 5.7 ± 0.89 mg/g wet weight of tissue. There was no correlation with age and gender of the patients (data not shown). In neonates, infants and children, on the other hand, there was considerable fluctuation with regard to the collagen concentration per milligram of heart tissue of left ventricular myocardium (Fig. 1). The Spearman rank correlation test showed a value of 0.6 and a significance of 6.6×10^{-6}.

Sirius red F3BA staining. Sirius red staining resulted in bright red staining of both collagen types, which contrasted well with the yellow staining of the heart muscle cells. From the earliest developmental stage, collagen was identified in both endomysial and perimysial locations, forming a fine meshwork around myocytes. The endomysial collagen content related to total protein is shown in Table 1 and Figure 2. The mean value obtained for adult hearts was 13.2 ± 2.1. In the youngest hearts, the ratio of total collagen to total protein was high compared with the mean value of the adult endomysial collagen content. During intrauterine development, there was a gradual decrease in this ratio, which stabilized at ~5 months of postnatal development. The Spearman rank correlation test was −0.9, with a significance of 0.00.

Types I and III collagen. The results of quantification of types I and III collagen are summarized in Table 2 and Figure 3. In adult human hearts the mean value of the ratio of type I to type III collagen was 0.5 ± 0.08. In preterm babies, the ratio of type I to type III collagen is high compared with that in adult hearts. During gestational development, the ratio of type I to type III collagen decreased. The one 12-year old heart had a ratio similar to that of the mean value in adult hearts; up to 6 years of age the values obtained remained high, averaging at 0.8. The Spearman rank correlation test when applied to the type I/type III collagen ratio revealed a value of −0.6, with a significance of 0.02.

Figure 2. Graphic display of the ratio of total collagen to the total protein content, using the Sirius red F3BA stain and quantified with the microdensitophotometer, related to age.



Discussion

This study shows that the neonatal heart contains a relatively high amount of endomysial collagen and, furthermore, has a high amount of type I collagen. These findings should be evaluated in light of the intricate architecture and characteristics of the connective tissue within the myocardium, considered to play a role in maintaining the functional integrity of the heart muscle (6,9,12,19,20). Moreover, it has been suggested that a relation exists between the presence of collagen and the compliance of the heart (21).

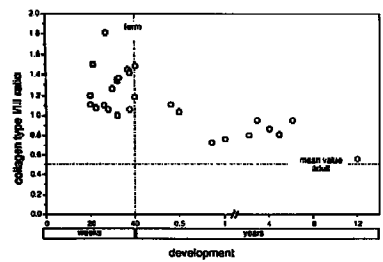
The extracellular matrix of the ventricular myocardium is organized in a three-dimensional network that enwraps muscle fibers and blood vessels. The endomysium surrounds individual myocytes and is composed of a meshwork of collagen fibers that are connected by struts to the basal lamina of the cells. The endomysial collagen is anchored in the perimysium, which connects groups of myocytes. This

Table 2. Ratio of Type I to Type III Collagen, Quantified Spectrophotometrically, Mean Value for Types I and III Collagen in Each Section and Ratio of Type I to Type III Collagen in Leiomyoma Tissue, Which Served as Reference

Age/Gender	Type I/Type III Collagen Ratio	Type I Collagen (mean value)	Type III Collagen (mean value)	Reference Type I/Type III Collagen Ratio
Prenatal (wk)				
Immature				
20/F	1.11	29.20	21.60	1.22
20/F	1. 5	26.60	18.10	1.22
21/F	1.50	23.40	12.80	1.22
23/M	1.08	26.80	20.40	1.22
26/M	1.10	40.96	37.18	1.00
Premature				
27/M	1.81	56.50	27.40	1.14
28/F	1.06	38.91	36.80	1.00
30/F	1.26	48.02	38.10	1.00
32/F	1.41	65.60	40.80	1.14
32/F	1.00	48.00	48.02	1.00
32/M	1.34	73.60	48.20	1.14
33/M	1.37	54.80	35.20	1.14
Mature				
37/M	1.45	78.90	47.70	1.14
38/M	1.42	91.00	56.20	1.14
38/F	1.06	41.19	38.68	1.00
40/F	1.18	43.74	30.27	1.22
40/F	1.48	62.95	33.57	1.27
Postnatal				
4 mo/M	1.11	59.10	46.80	1.14
6 mo/M	1.04	52.10	39.50	1.27
11 mo/M	0.73	30.29	34.22	1.22
1 yr/F	0.76	35.15	46.49	1.00
2 yr/M	0.80	32.83	41.20	1.00
3 yr/M	0.94	45.40	38.00	1.27
4 yr/F	0.87	50.61	45.91	1.27
4.5 yr/F	0.79	47.00	46.60	1.27
6 yr/M	0.93	55.23	46.55	1.27
12 yr/M	0.55	34.82	63.52	1.00

F = female; M = male.

Figure 3. Graphic display of the ratio of type I to type III collagen, quantified with the microdensitopholometer, related to age.



intricate arrangement is considered of functional importance in preventing overstretching during its phase of end-diastole.

The two types of collagen that dominate in human myocardium are types I and III. They coexist in the extracellular matrix and have a distribution in the endomysial meshwork similar to that in the struts (22,23). These two types of collagen have different physical characteristics. Collagen type I mainly provides rigidity, whereas collagen type III mainly provides elasticity. In the normal heart there is more type III than type I collagen, the ratio of type I to type III collagen being ~0.5.

On the basis of our observations one may thus hypothesize that the high endomysial collagen content, once related to the total mass of myocardial cells, could cause rigidity of the neonatal heart. With age there is a statistically significant gradual decrease in the relative amount of collagen, although the overall collagen content of the left ventricle shows an increase. This phenomenon almost certainly relates to the increase in number and volume of the myocytes. However, despite this gradual decrease the relatively high amount of collagen should render the neonatal heart less compliant than in fully maturated hearts.

This hypothesis is further supported by our observation that the ratio of type I to type III collagen is high in neonates compared with that in adults. The ratio showed a statistically significant gradual decrease with age, but the level of adult hearts is reached only late in infancy. We accept that only a few patients could be studied in these age groups; nevertheless, the trend for a gradual but late decrease is distinct. Thus, the neonatal heart contains a high amount of collagen, once related to the total volume of myocytes, and a high ratio of type I to type III collagen. This combination may provide the background for a rigid, less compliant ventricle in immature and premature babies. Once confronted with an excessive volume load, the heart may soon reach its limits of distensibility, and, hence, heart failure may rapidly ensue.

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