Regional myocardial blood flow and cardiac function in a naturally occurring congestive cardiomyopathy of turkeys

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SUMMARY  Round heart disease, a presumed viral myocarditis of turkeys, provides a unique opportunity for the study of congestive cardiomyopathy. Regional myocardial blood flow and cardiac output measurements were made in nine, 19 to 34 day old anaesthetised birds using 113Ce labelled microspheres (15 μm diameter). Atrial, right ventricular and weighted-average left ventricular myocardial blood flow values were similar in control (n = 5) and round heart disease (n = 4) turkeys. The left ventricular subendocardial/subepicardial blood flow ratio of 0.89 ± 0.02 (mean ± SE) in round heart disease birds was, however, reduced compared with the value of 1.19 ± 0.09 in the control birds (P < 0.05). Round heart disease turkeys also had lower systemic pressures and lower cardiac outputs when compared with control birds. M-mode echocardiograms were obtained in 42 unanaesthetised 17 to 37 day old turkeys, 34 control and eight with round heart disease. Echocardiographic evidence of left ventricular dysfunction characterised by left atrial and left ventricular dilation and a markedly reduced left ventricular shortening fraction was found in round heart disease turkeys. Paradoxical motion of the interventricular septum was present in two of eight round heart disease turkeys but in none of the control turkeys. The interventricular septum/left ventricular posterior wall ratio in control and round heart disease turkeys were similar. Although the body weight of control and round heart disease turkeys were similar, and the diastolic thickness of the left ventricular wall were not substantially different, the ventricular weight/body weight ratio in round heart disease turkeys was increased approximately 52%. The increased ventricular weight was not due to myocardial oedema, as myocardial water content was similar in control and round heart disease turkeys.

The features which characterise round heart disease in turkeys: left atrial and left ventricular dilatation, reduced left ventricular shortening fraction, systemic hypotension, low cardiac output, relative subendocardial underperfusion, and an increase in ventricular mass, make it a useful model for congestive cardiomyopathy.

Congestive cardiomyopathy, defined as a primary myocardial disorder associated with poor systolic ejection function, is secondary to multiple causes. Characteristic haemodynamic findings include low cardiac output, congestive heart failure, increased left ventricular end diastolic pressure and increased systolic and diastolic ventricular volumes with left ventricular dilatation.

Since patients with congestive cardiomyopathy often present the clinician with a spectrum of cardiac findings, only isolated information regarding the natural history of this complex disease can be obtained. Thus an animal model such as represented by round heart disease (RHD) in the domestic turkey provides the investigator with a unique opportunity for the study of the developmental components of congestive cardiomyopathy.

In the turkey, RHD is characterised by left ventricular dilatation, congestive heart failure and increased early mortality when compared with unaffected turkeys. This cardiomyopathy is presumed to be secondary to a viral disease since viral-like particles, morphologically similar to C-type particles of RNA tumour viruses, have been described within...
vesicles of the sarcoplasmic reticulum in cardiac muscles of all RHD birds. In addition, fine structural alterations seen in RHD are similar to those described in a variety of experimentally produced and naturally occurring viral myocardopathies. Isolated cases of this viral cardiomyopathy occur in all turkey flocks. However, inbreeding of turkeys with RHD has markedly increased the occurrence of cardiac dilatation and our inbred flock now show approximately 70% of birds with cardiac dilatation by one month of age. In these birds at 3 weeks of age a gradual deposition of fibroelastic tissue in the subendocardial region of the left ventricle is associated with beginning left ventricular dilatation. In the end stage, marked left ventricular endocardial fibroelastosis has been reported and is often associated with involvement of the mitral valve, chordae tendinae, and papillary muscles. Haemodynamic measurements in the chronic stage of this disease are similar to those in humans with congestive cardiomyopathies and include: 1) reduced cardiac output; 2) elevated right and left ventricular filling pressures; and 3) systemic hypotension.

The purpose of this report is to present changes in both regional myocardial blood flow and echocardiographic measurements in young turkeys in the early phase of their developing cardiomyopathy and to propose that relative underperfusion of the left ventricular subendocardial muscle may play a role in the development of endocardial fibroelastosis in this model.

Methods

MYOCARDIAL BLOOD FLOW

Control (Nicolas) turkeys used in this study were obtained from a commercial hatchery. Turkeys with RHD were obtained from an inbred flock with a high incidence of cardiomyopathy, which is maintained at the University of Minnesota School of Veterinary Medicine.

Radionuclide labelled microspheres of 15 - 5 μm in diameter have been used successfully for blood flow measurements in the avian species. Cardiac output and regional blood flow measurements were made using the arterial reference sample technique 10 in nine turkeys ranging in age from 19 to 34 days and ranging in weight from 171 to 589 g. The five turkeys which served as controls were compared with four inbred turkeys with cardiomyopathy. All birds were screened for the presence of cardiac dilatation by ECG and/or echocardiographic examination. The birds were anaesthetised intramuscularly with 50 mg·kg⁻¹ ketamine hydrochloride (Ketalar, Parke-Davis) and 0.5 mg·kg⁻¹ acepromazine (Ayerst, Ayerst Lab. Inc.). An endotracheal tube was secured in place. Supplemental small doses of anaesthesia were administered as required. Body temperature was maintained with a heating lamp. The interclavicular air sacs were opened and the lungs were artificially unidirectionally ventilated. A gas mixture of 97% O₂ and 3% CO₂ was passed into the trachea at a rate of approximately 3.6 litre·min⁻¹·kg⁻¹. The proximal portions of the right and left carotid arteries were looped. A No. 20 gauge 3.8 cm teflon catheter was advanced retrograde across the aortic valve and positioned in the left ventricle. A second, No. 20 gauge 3.8 cm teflon catheter was positioned in the aortic arch. The aortic and left ventricular pressures, measured using Bell and Howell (Type 4-327-0111) pressure transducers and a lead 11 electrocardiogram were recorded on a Beckman RM 8-channel Dynograph Recorder.

After sonification for at least 10 min, approximately 150000 15 μm ¹¹Ce labelled microspheres (3M Co., St. Paul, Minnesota) were infused into the left ventricle over 5 to 8 s with 1 cm³ of 0.9% saline for each flow measurement. The reference blood sample was withdrawn through the catheter positioned in the aorta at a constant rate of 0.5 cm³·min⁻¹ for 2½ to 3 min using a Harvard syringe pump. The withdrawal was begun approximately 5 s before sphere embozlon. The dead space of our reference catheter system, consisting of the teflon catheter, adaptors, and sufficient PE 50 tubing to reach the syringe pump, measured 0.2 cm³. Sphere embolisation did not change heart rate or left ventricular pressure from pre-injection values. Arterial blood pH, PCO₂ and PO₂ was measured, immediately after withdrawal, on an Instrumentation Laboratory pH/gas Analyser (Model 113) calibrated for the birds body temperature. Arterial haematocrit was measured. At the completion of the study, the birds were killed and the heart, lungs, kidneys, liver, spleen, and small intestine were removed.

For regional myocardial blood flow measurements, both atria were separated from the ventricles, weighed to the nearest milligram on a Sartorius Digital Electronic balance (Model 3705), and placed in a counting vial. The right ventricular free wall was removed, weighed, and placed in a counting vial. The remaining interventricular septum and left ventricular free wall were separated and each was divided equally into subendocardial and subepicardial portions, weighed, and placed in counting vials. Buffered formalin was then added to the tissue samples and all tissue samples and the reference blood sample were counted in a Packard Autogamma Scintillation Spectrometer (Model 5912/9771). The tissue samples were then saved for...
histological examination. All counts per minute were corrected for background. The blood reference and myocardial samples were counted at a window setting selected to correspond to a peak energy of $^{141}$Ce.

The combined weight of the right and left ventricles ranged from 1,165 to 3,660 g. Before weighing, the hearts were trimmed of superficial fat and great vessels. All left ventricular tissue samples and six of nine atrial samples contained at least 400 microspheres. Samples, 0.3 to 3.0 g, were taken from the remaining organs and processed as the heart for blood flow determinations. All samples contained at least 400 microspheres.

Blood flow (cm$^3$·min$^{-1}$) of each tissue sample was calculated by solving the following proportion for $F_i$:

$$\frac{A_i}{F_i} = \frac{A_R}{F_R},$$

where $A_i$ - tissue sample count rate (cpm); $A_R$ - reference sample count rate (cpm); $F_i$ - tissue sample flow rate (cm$^3$·min$^{-1}$); $F_R$ - reference sample flow rate (cm$^3$·min$^{-1}$). Tissue sample blood flow was divided by sample weight to express flow as cm$^3$·min$^{-1}$ per gram of tissue. The cardiac output was calculated from the activity of the total number of microspheres that was injected and substituting this for the activity of the tissue sample ($A_i$) in the equation shown above.

Computations were performed on a Hewlett-Packard 9831A computer.

**Microscopic Examination**

Tissue from the left ventricle was fixed in 10% buffered formalin and embedded in paraffin. Histologic sections were stained with hematoxylin and eosin and Masson trichrome for light microscopic examination.

**Echocardiographic Examination**

M-mode echocardiograms were obtained in 42 unanaesthetised, 17 to 37 day old turkeys ranging in weight from 223 to 731 g. Thirty-four turkeys served as controls and eight turkeys had cardiac dilatation. Included in this study were four of the five control turkeys and three of the four RHD turkeys in which blood flow measurements were subsequently obtained (that is, 1 to 4 days later). Nonfocused transducers producing frequencies between 3.5 to 7.5 MHz with a repetition rate of 1000 impulses per second were used. Echocardiograms were recorded at paper speeds of 100 to 200 mm·s$^{-1}$ on a Honeywell Strip Chart Fiberoptic Recorder interfaced with a Smith-Kline Echoline 20-A Ultrasonomoscope. The transducers were placed 1 to 2 cm to the right of the midsternal line, perpendicular to the chest wall and one centimeter below the suprasternal notch. The turkeys were either supine or placed in a slight left lateral position. In a preliminary study, chamber localisation was verified by observing the echocardiographic “contrast” effect.

FIG 1 Cardiac chamber localisation using injections of indocyanine green (ICG) and isotonic saline in a 350 g, 33 day old turkey.

A) Injection of ICG into the venous circulation (SVC-superior vena cava) produces a “cloud” of echoes in an anterior right ventricle (RV).*

B) Injection of ICG into the cervical carotid artery produces a “cloud” of echoes in a posterior great vessel (i.e. aorta [Ao]).

C) Echocardiographic scan from the aorta to the left ventricle (LV) demonstrating echocardiographic continuity of the anterior aortic wall with the interventricular septum (IVS) and of the posterior aortic wall with the anterior mitral valve leaflet (MV).

LA left atrium

*The right ventricular side of the interventricular septum is only seen in the right hand end of the tracing in the top panel.
**FIG 2** Upper: left ventricular endocardium from a 28 day old turkey with RHD is thickened by increased fibrous tissue. Masson trichrome stain (Original magnification × 350).

**FIG 2** Lower: Normal left ventricular endocardium from a 24 day old control turkey. Masson trichrome stain (Original magnification × 350).
and subepicardial portions of the left ventricular myocardium were determined. In addition, the atrial weight to body weight ratio, the left ventricular (free wall plus interventricular septum) to body weight ratio, the right ventricular to body weight ratio, the interventricular septum (1VS) to body weight ratio, and the left ventricular to right ventricular weight ratio were determined in these 23 birds.

**Statistical Analysis**

Statistical analysis of data from animals within a group employed Student's two-tailed t test for paired samples. Differences between groups were evaluated by the two-tailed Student's t test for unpaired samples, except when sample variability was disparate; in those cases the Welch-Aspin test was employed. The criterion for statistical significance was P < 0.05. Results are expressed as mean ± standard error unless otherwise indicated.

**Results**

**Microscopic Examination**

By light microscopy examination two of the four birds with RHD showed slight to moderate fibrosis which was restricted to the endocardial surface of the left ventricle. Two of the five control birds demonstrated a very minimal increase in subendocardial fibrous tissue (fig 2). Mononuclear interstitial infiltrates were seen only in the youngest bird with RHD, 19 days of age. The cellular infiltrates involved both the subendocardial and subepicardial portions of the left ventricle. No necrosis of muscle cells was present in association with the cellular infiltrates. Neither RHD birds nor control birds showed areas of interstitial fibrosis within the left ventricular myocardium. All intramyocardial vessels appeared to be normal; there was no evidence of occlusive vascular disease or lymphatic obstruction.

**Blood Gases**

The cloacal temperature of the RHD turkeys was similar to the temperature in the control turkeys of 38.3 ± 0.8°C (P > 0.05). Table 1 contains values for the arterial pH, PaO₂, PaCO₂, and hematocrit of the five control and four RHD turkeys in which blood flow measurements were obtained. There were no significant differences between control and RHD turkeys in any of these measurements. Although the arterial PaO₂'s are high, our values for pH are similar to values reported for unaesthetised, unrestrained birds.

**Haemodynamic Measurements**

The heart rate, aortic pressure, left ventricular end systolic pressure, and cardiac index values in the control and RHD turkeys are presented in table 2. The heart rate of control and RHD turkeys was similar. However, the aortic systolic and diastolic pressures and cardiac index in control turkeys were significantly greater than values in RHD turkeys. Although the mean value for the left ventricular end-diastolic pressure in the RHD turkeys tended to be higher than the mean value in the control birds, it was not statistically significantly different, probably due to the small number of observations. It is possible that the wide range of values obtained (that
TABLE 1  Arterial blood gas and haematocrit measurements

<table>
<thead>
<tr>
<th></th>
<th>pHa (units)</th>
<th>PaCO₂ (kPa)</th>
<th>PaO₂ (kPa)</th>
<th>Haematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=5)</td>
<td>7.62 ± 0.10</td>
<td>3.72 ± 0.66</td>
<td>22.21 ± 4.92</td>
<td>25.4 ± 1.4</td>
</tr>
<tr>
<td>RHD (n=4)</td>
<td>7.54 ± 0.05</td>
<td>4.52 ± 0.66</td>
<td>40.56 ± 6.38</td>
<td>25.0 ± 2.0</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

n= number of turkeys
Values given are mean ± SE
RHD = round heart disease
NS = not significant
Conversion: 1 kPa = 7.52 mmHg

TABLE 2  Haemodynamic measurements

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats min⁻¹)</th>
<th>Aortic pressure (kPa)</th>
<th>LVEDP (kPa)</th>
<th>Cardiac index (cm³ min⁻¹ kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
<td></td>
</tr>
<tr>
<td>Control (n=5)</td>
<td>274 ± 39</td>
<td>11.97 ± 0.78</td>
<td>8.64 ± 0.93</td>
<td>0.8 ± 0.27</td>
</tr>
<tr>
<td>RHD (n=4)</td>
<td>252 ± 25</td>
<td>7.71 ± 0.40</td>
<td>5.19 ± 0.66</td>
<td>2.13 ± 0.80</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

n= number of turkeys
Values given are mean ± SE
LVEDP = left ventricular end diastolic pressure
RHD = round heart disease
NS = not significant
Conversion: 1 kPa = 7.52 mmHg

TABLE 3  Non-cardiac tissue blood flows

<table>
<thead>
<tr>
<th></th>
<th>Control n=5</th>
<th>Round heart disease n=4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>3.10 ± 0.74</td>
<td>2.26 ± 0.83</td>
</tr>
<tr>
<td>Hepatic</td>
<td>1.46 ± 0.21</td>
<td>1.00 ± 0.74</td>
</tr>
<tr>
<td>Spleen</td>
<td>5.61 ± 2.23</td>
<td>8.35 ± 3.17</td>
</tr>
<tr>
<td>Duodenum</td>
<td>3.36 ± 0.91</td>
<td>1.87 ± 0.54</td>
</tr>
</tbody>
</table>

Flow is given as cm³ min⁻¹ g⁻¹
n= number of measurements

is 0.66 to 3.99 kPa (5 to 30 mmHg) also reflects the spectrum of ventricular dilatation observed in affected birds.

REGIONAL BLOOD FLOW
One of the assumptions of the microsphere technique for measurement of tissue blood flow is that there be no appreciable shunting of spheres across the microcirculation of the tissue in which flow is to be measured. The turkey erythrocyte is an oval nucleated cell measuring approximately 7 µm by 12 µm. Preliminary studies utilising isolated heart preparations showed that only 0.6% of 15 µm spheres passed into the coronary sinus drainage. Total shunting of spheres across the systemic circulation determined by lung uptake was found to be approximately 8% for 15 µm spheres in a previous study of 1 to 2 year old turkeys. Since total shunting of spheres across the systemic circulation averaged 7.3 ± 3.1% in these nine young turkeys, 15 µm
TABLE 4  Regional myocardial blood flow

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Round heart disease</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n mean</td>
<td>SE</td>
<td>n mean</td>
<td>SE</td>
</tr>
<tr>
<td>Atrial</td>
<td>5 2.29</td>
<td>0.93</td>
<td>4 2.20</td>
<td>0.58</td>
</tr>
<tr>
<td>Right Ventricle</td>
<td>5 3.18</td>
<td>0.26</td>
<td>4 2.43</td>
<td>0.36</td>
</tr>
<tr>
<td>Left Ventricle (total)</td>
<td>5 3.46</td>
<td>0.53</td>
<td>4 2.05</td>
<td>0.39</td>
</tr>
<tr>
<td>LV Endo</td>
<td>5 3.77</td>
<td>0.67</td>
<td>4 1.91</td>
<td>0.36</td>
</tr>
<tr>
<td>LV Epi</td>
<td>5 3.17</td>
<td>0.49</td>
<td>4 2.14</td>
<td>0.38</td>
</tr>
<tr>
<td>LV Endo/Epi</td>
<td>5 1.19</td>
<td>0.09</td>
<td>4 0.89*</td>
<td>0.02</td>
</tr>
</tbody>
</table>

n= number of measurements
Flow is given as cm³min⁻¹g⁻¹
LV Endo= left ventricular subendocardial blood flow
LV Epi= left ventricular subepicardial blood flow
LV Endo/Epi= left ventricular subendocardial/subepicardial blood flow ratio
*P<0.05 compared with value in control turkeys

TABLE 5  Echocardiographic measurements

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Round heart disease</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n mean</td>
<td>SD</td>
<td>n mean</td>
<td>SD</td>
</tr>
<tr>
<td>Left atrial dimension (mm) (LA)</td>
<td>24 6.85</td>
<td>1.56</td>
<td>8 11.53*</td>
<td>3.20</td>
</tr>
<tr>
<td>Aortic root dimension (mm) (Ao)</td>
<td>24 7.21</td>
<td>1.00</td>
<td>7 6.86</td>
<td>0.38</td>
</tr>
<tr>
<td>LA/Ao ratio</td>
<td>24 0.95</td>
<td>0.14</td>
<td>7 1.58*</td>
<td>0.39</td>
</tr>
<tr>
<td>Diastolic left ventricular internal dimension (mm) (LVIDd)</td>
<td>32 7.47</td>
<td>1.52</td>
<td>8 14.94*</td>
<td>4.59</td>
</tr>
<tr>
<td>Systolic left ventricular internal dimension (mm) (LVIDs)</td>
<td>32 7.77</td>
<td>1.31</td>
<td>8 13.38*</td>
<td>4.75</td>
</tr>
<tr>
<td>Interventricular septum (diastolic thickness) (mm) (IVS)</td>
<td>31 2.61</td>
<td>0.62</td>
<td>8 1.94</td>
<td>0.56</td>
</tr>
<tr>
<td>Interventricular septum (systolic thickness) (mm) (IVS)</td>
<td>31 3.84</td>
<td>0.97</td>
<td>8 2.06</td>
<td>0.50</td>
</tr>
<tr>
<td>Left ventricular posterior wall (diastolic thickness) (mm) (LVPW)</td>
<td>31 2.73</td>
<td>0.71</td>
<td>8 2.06</td>
<td>0.56</td>
</tr>
<tr>
<td>Left ventricular posterior wall (systolic thickness) (mm) (LVPW)</td>
<td>30 4.65</td>
<td>1.12</td>
<td>8 2.56</td>
<td>0.90</td>
</tr>
<tr>
<td>LVIDd/LVPW ratio</td>
<td>31 2.82</td>
<td>0.74</td>
<td>8 7.80*</td>
<td>3.34</td>
</tr>
<tr>
<td>IVS/LVPW ratio</td>
<td>30 0.99</td>
<td>0.15</td>
<td>8 0.93</td>
<td>0.14</td>
</tr>
<tr>
<td>Left ventricular shortening fraction</td>
<td>32 0.65</td>
<td>0.14</td>
<td>6 0.14</td>
<td>0.15</td>
</tr>
</tbody>
</table>

n= number of measurements
* = P<0.05 compared with value in control turkeys
† = P<0.01 compared with value in control turkeys
Left ventricular shortening fraction = \( \frac{LVIDd - LVIDs}{LVIDd} \)

spheres were felt to be suitable for measurement of regional blood flow. Blood flow values in non-cardiac tissues for control and RHD turkeys are compared in table 3.

Although renal, hepatic, and duodenal flows tended to be lower and splenic flow tended to be higher in RHD turkeys, the values were not significantly different from control values (all \( P > 0.05 \)). Flows to the right and left kidneys were similar, suggesting adequate mixing of spheres with blood after left ventricular injection. The values for non-cardiac regional flows were similar to values in the mature turkeys.

The atrial, right ventricular and left ventricular myocardial blood flows and the left ventricular subendocardial/subepicardial blood flow ratios are compared in table 4.

Although the atrial, right ventricular and left ventricular myocardial blood flow in RHD turkeys tended to be lower than control values, they were not significantly different (all \( P > 0.05 \)). However, the left ventricular subendocardial/subepicardial blood flow ratio of 0.89 in RHD turkeys was significantly less than the value of 1.19 for control birds (P<0.05), indicating relative subendocardial underperfusion in this group. The ventricular weight (right
and left ventricular free walls and interventricular septum) to body weight ratio (g·kg⁻¹) in the RHD turkeys of 7.28 ± 0.38, was found to be significantly greater than the value in control birds of 5.00 ± 0.41, (P < 0.01). The body weights of control and RHD turkeys were similar.

**ECHOCARDIOGRAPHIC MEASUREMENTS.**

Young turkeys with RHD have a characteristic electrocardiographic finding characterised by a frontal plane axis shift to the right. This characteristic electrocardiographic abnormality was used to screen turkeys for RHD. The electrocardiographic

**FIG 3 Comparison of the left ventricular echograms from a control (Bird No 15) and RHD (Bird No 24) turkey of similar age and body weight. The time and depth calibrations are shown. Marked ventricular dilatation was present in the RHD bird. Although the ventricular weight in the RHD bird was increased, the left ventricular posterior wall thickness in the birds are similar. (Note that not all complexes are adequate for measurement. A linear artefact is recorded on the left ventricular side of the septum and the dimensions of the septum are not well defined in this particular portion of the echogram from the RHD bird.)**

QRS axis in all RHD turkeys (except one in which it was indeterminate) had a rightward shift in the frontal plane to between +45 to +90 from a normal axis of approximately -90 in the control turkeys. In fig 3, the echograms obtained in a 37 day control (top) and a 37 day RHD (bottom) turkey of similar body weight (543 and 561 g, respectively) are compared. The bird with RHD has an axis of +56 compared with the -90 axis of the control bird. The transducer is angled below the level of the mitral valve leaflets in both cases. The diastolic left ventricular internal dimension in the RHD turkey is approximately twice that of the control. Although the diastolic thickness of the left ventricular posterior wall is essentially equal in these two birds, the ventricular weight to body weight ratio in the RHD bird of 8.66 g·kg⁻¹ is substantially greater than the value of 4.84 g·kg⁻¹ in the control bird.

Table 5 contains values for the echocardiographic measurements in 42 unanaesthetised, 17 to 37 day control and RHD turkeys. In this table, one standard deviation (SD) rather than one standard error is shown. Body weights of the control turkeys (mean = 395 g; range 223 to 731 g) and RHD turkeys (mean = 441 g; range 304 to 608 g) were similar, (P > 0.05).

The aortic root dimension, and the interventricular septum/left ventricular posterior wall (IVS/LVPW) ratio in control and RHD turkeys were similar. However, the left atrial internal dimension, and therefore, the left atrial/aortic ratio, the diastolic left ventricular internal dimension (LVIDd) and the LVIDd/LVPW ratio were all significantly increased (see table 5). The thickness of the interventricular septum and left ventricular posterior wall were both slightly reduced in RHD birds as compared to control turkeys. Of special interest, left ventricular function, as reflected by the left ventricular shortening fraction, was markedly reduced in the RHD turkeys when compared to the control turkeys (that is 0.14 vs 0.65, P < 0.01). Motion of the interventricular septum was noted to be paradoxical in two of eight RHD birds while paradoxical motion was not present in any of the control turkeys. In addition, reduced systolic thickening of both the interventricular septum and left ventricular posterior wall was found in RHD turkeys.

The effect of anaesthesia on echocardiographic parameters was evaluated in 13 control and one RHD turkey. The values in these birds are not included in table 5. The combination of ketamine hydrochloride-acepromazine anaesthesia used in our study had no significant effect on the left ventricular shortening fraction and only a minimal depressant effect on heart rate. The heart rate in control birds was reduced slightly from 332 ± 9 to 313 ± 8 beats·min⁻¹ between 5 and 20 min following
injection (n=13, P<0.05). The left ventricular shortening fraction was, however, unchanged from the pre-anaesthesia value of 0.59 ± 0.02 in 10 of the 13 birds in which it could be accurately measured on both echocardiograms. In the one bird with cardiac dilatation, heart rate dropped slightly from 318 to 306 beats·min⁻¹ following anaesthesia, however, the left ventricular shortening fraction was unchanged from the reduced pre-anaesthesia value of 0.12. Anaesthesia also had no effect on left atrial size, aortic root dimension, or the left ventricular posterior wall and interventricular septal dimensions.

Table 6 compares post-mortem measurements to echocardiographic measurements obtained immediately before sacrifice in 12 of the 13 control turkeys. A technically satisfactory echocardiogram could not be obtained in only one control turkey. It can be seen that the post-mortem measurements of the left ventricular posterior wall and interventricular septum consistently overestimated the diastolic measurements obtained from the echocardiogram. However, the systolic measurements, especially for the left ventricular posterior wall were quite similar. In addition, the cavity diameter measured at post-mortem was similar to the systolic left ventricular internal dimension measured echocardiographically.

In the one turkey with ventricular dilatation, very little change in the left ventricular internal dimension occurred between diastole (17 mm) and systole (15 mm). The cavity diameter measured at post-mortem of 16 mm fell between these two values.

The post-mortem measurement of left ventricular posterior wall thickness of 2 mm also fell between the echocardiographically measured diastolic (1.5 mm) and systolic (2.5 mm) measurements.

**MYOCARDIAL WATER CONTENT**

Table 7 compares the regional myocardial tissue water content in 11 RHD and 12 control 28 to 37 day turkeys. Since the right and left atrial values were similar, they have been combined in Table 7. There was no significant difference between RHD and control turkeys in any region.

Table 8 compares the various regional heart weights to body weight ratios in this same group of turkeys. In addition, the left ventricular (that is, left ventricular free wall plus interventricular septum) to right ventricular weight ratio is shown. Myocardial mass in RHD turkeys was increased in all cardiac regions. Whether the increased mass was secondary to hyperplasia or to hypertrophy of the individual muscle fibres, or to a combination of hypertrophy and hyperplasia was not addressed in the present study.

**Discussion**

Round Heart Disease, a naturally occurring cardiomyopathy was first described in turkeys by Magwood and Bray in 1962.14 The cardiac dilatation in RHD primarily involves the left ventricle and is first evident grossly at 3 to 4 weeks of age. As previously stated, inbreeding of affected birds markedly increased the incidence of congestive cardiomyopathy. Mortality in this inbred flock approaches 30% by 10 days of age compared to less than 5% in commercial flocks. At this time the heart appears flabby but not dilated. Further deaths occur at later ages but do not exceed an additional 10%. These later deaths are secondary to congestive heart failure. The pathological spectrum present in RHD turkeys closely parallels the disease process as reported in man and therefore permits sequential investigations of parameters of function that are not ordinarily possible in the human subject.

Numerous hypotheses have been proposed for the development of congestive cardiomyopathy with endocardial fibroelastosis. These include chronic impairment of cardiac lymph drainage, myocardial, endocardial, and elastic hyperpla- sia, interstitial myocarditis, autoimmune disease, genetic disorders, maternal toxins, primary muscle dysfunction15 and intramyocardial arterial occlusive vascular disease.16 It is probable that the gross and microscopic structural changes merely reflect the end stages of subendocardial ischaemia and/or hypoxia secondary to multiple inciting events.

**REGIONAL MYOCARDIAL BLOOD FLOW**

In patients with congestive cardiomyopathies, several studies have measured total left ventricular myocardial blood flow. Both normal17–18 and reduced19–23 flows per gram of tissue have been

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**Table 6. Comparison of post-mortem and echocardiographic measurements in 12 control turkeys.**

<table>
<thead>
<tr>
<th></th>
<th>Echocardiogram (mm)</th>
<th>Post-mortem (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVPW (diastolic)</td>
<td>2.75 ± 0.13</td>
<td>4.63 ± 0.12</td>
</tr>
<tr>
<td>(systolic)</td>
<td>4.63 ± 0.12</td>
<td>4.79 ± 0.20</td>
</tr>
<tr>
<td>IVS</td>
<td>2.72 ± 0.16</td>
<td>4.02 ± 0.22</td>
</tr>
<tr>
<td>(diastolic)</td>
<td>4.02 ± 0.22</td>
<td>4.96 ± 0.20</td>
</tr>
<tr>
<td>(systolic)</td>
<td>4.95 ± 0.89</td>
<td>4.04 ± 0.50</td>
</tr>
</tbody>
</table>

Values given are mean ± SE in 12 turkeys.

LVPW = left ventricular posterior wall
IVS = interventricular septum
LVID = left ventricular internal dimension

† = P<0.001 for comparison made between echocardiographic and post-mortem measurement.
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**TABLE 7** Tissue water content (TWC) expressed as fraction of wet weight in different region of the turkey heart

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Round heart disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
</tr>
<tr>
<td>Atrial TWC</td>
<td>13*</td>
<td>0.828</td>
</tr>
<tr>
<td>RV TWC</td>
<td>12</td>
<td>0.806</td>
</tr>
<tr>
<td>LV Endo TWC</td>
<td>12</td>
<td>0.801</td>
</tr>
<tr>
<td>LV Epi TWC</td>
<td>12</td>
<td>0.799</td>
</tr>
</tbody>
</table>

*Includes one additional control turkey in which the right and left atrial tissue water content were not measured separately.

n=number of measurements
TWC = tissue water content \(\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}}\)
RV = right ventricular free wall
LV Endo = subendocardial half of the left ventricle (free wall and interventricular septum)
LV Epi = subepicardial half of the left ventricular myocardium

**TABLE 8** Myocardial weight to body weight ratio in the different regions of the turkey heart

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Round heart disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>mean</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>13</td>
<td>381</td>
</tr>
<tr>
<td>Atrial/BW (g kg(^{-1}))</td>
<td>13</td>
<td>0.38</td>
</tr>
<tr>
<td>LV/BW (g kg(^{-1}))</td>
<td>12</td>
<td>3.30</td>
</tr>
<tr>
<td>RV/BW (g kg(^{-1}))</td>
<td>12</td>
<td>0.80</td>
</tr>
<tr>
<td>LV/RV (g g(^{-1}))</td>
<td>12</td>
<td>4.32</td>
</tr>
</tbody>
</table>

n=number of measurements
Atrial/BW = combined atrial weight to body weight ratio
LV/BW = left ventricular (free wall and interventricular septum) to body weight ratio
RV/BW = right ventricular free wall to body weight ratio
LV/RV = left ventricular to right ventricular weight ratio
\(^\dagger\) = P<0.01 compared with value in control turkeys

reported. However, when inert gas (that is nitrous oxide, Xenon-133, or helium) washout techniques are used in patients to measure mean left ventricular blood flow, it is not possible to determine the presence or absence of subendocardial ischaemia as these techniques are insensitive to areas of low flow which might exist in parallel with larger areas of normal flow. Henry et al\(^{20}\) suggested, however, the patients with left ventricular dysfunction may have non-uniform distribution of coronary blood flow. In his study, the coronary venous washout curves in patients with left ventricular dysfunction deviated from a single exponential, suggesting the presence of non-uniform distribution of coronary blood flow. Our observations support this hypothesis. In RHD turkeys, left ventricular myocardial blood flow was found to be slightly, but not significantly, reduced. However, the left ventricular subendocardial/subepicardial blood flow ratio was significantly reduced, indicating the presence of relative subendocardial underperfusion.

Neither intramyocardial arterial occlusive vascular disease nor impairment of cardiac lymphatic drainage appears to be operative in this congestive cardiomyopathy model. The factors which appear to play a part in the development of decreased perfusion to the subendocardial region of the left ventricle are the presence of reduced aortic diastolic pressure, a rapid heart rate and left ventricular dilatation,\(^{14\text{-}28}\) all of which are observed in this animal model.

Several authors have suggested an association between a viral illness and the subsequent development of a congestive cardiomyopathy,\(^{27\text{-}28}\) or endocardial fibroelastosis.\(^{29\text{-}30}\) Although light microscopic examination of the hearts of birds from our inbred flock shows occasional focal areas of lymphocytic infiltrates within the myocardium, there is not good correlation between the intensity of these findings and the severity of the disease process. These observations are similar to other reported studies of
experimental myocarditis where it has been shown that very discreet interstitial cellular infiltrates and eosinophilic staining of muscle cells may be the only microscopic finding, despite a significantly increased mortality and morbidity in infected animals when compared to controls.\(^{51-53}\) It is thus apparent that altered myocardial function with resultant cardiac dilatation and failure cannot always be related to the degree of cellular infiltrates or histological evidence of injury.

In RHD, deposition of fibroelastic tissue begins in the subendocardial region of the left ventricle after 3 weeks of age in association with left ventricular dilatation. In this study, two of four RHD birds demonstrated increased subendocardial fibrous tissue at 28 days of age. It is suggested that relative subendocardial underperfusion of the left ventricular wall precedes the development of endocardial fibroelastosis in this cardiomyopathy model.

It has been suggested that tissue oedema, known to produce perfusion abnormalities in skeletal muscle,\(^{34}\) may contribute to left ventricular dysfunction in patients with cardiomyopathies.\(^{35}\) The subendocardial region of the left ventricle is felt to be the earliest site of reduced myocardial contractility in left ventricular failure.\(^{36}\) Since myocardial oedema could have contributed to the perfusion abnormality and resultant left ventricular dysfunction, as well as the increase in ventricular mass in RHD turkeys, this was evaluated in our study (tables 7 and 8). The regional myocardial tissue water content in control and RHD turkeys was similar suggesting that myocardial oedema does not contribute to either the perfusion abnormality or the increased ventricular weight in this cardiomyopathy model. Of interest, our value for left ventricular water content in turkeys is similar to reported values for canine left ventricular myocardium.\(^{10,36}\)

**ECHOCARDIOGRAPHIC OBSERVATIONS**

Both invasive and noninvasive techniques have been used in the evaluation of left ventricular performance of patients with congestive cardiomyopathies. In these individuals with diffuse myocardial disease the ejection phase contractile indices (that is, ejection fraction, mean V\(_{\text{cf}}\)) have been shown to be superior to isovolumic indices (i.e. V\(_{\text{max}}\), peak dP/dt) for assessment of the basal myocardial contractile state.\(^{37}\) Ejection phase indices can be accurately measured noninvasively by standard echocardiographic techniques\(^{38}\) and echocardiography has been shown to be a useful clinical tool for both the diagnosis and serial evaluation of patients with either hypertrophic or congestive cardiomyopathies.\(^{39}\) Characteristic echocardiographic and/or angiographic findings in patients with congestive cardiomyopathies include: a markedly reduced left ventricular shortening fraction, V\(_{\text{cf}}\), and ejection fraction, elevated left ventricular end diastolic and end systolic volumes, poor interventricular septal and posterior wall motion\(^{22,40}\) and abnormal left ventricular systolic time intervals characterised by an increase in the left ventricular pre-ejection period and a shortening of the left ventricular ejection time.\(^{41}\) Recent echocardiographic studies\(^{42}\) suggest that the left ventricular shortening fraction may be a particularly useful index of left ventricular function, since unlike V\(_{\text{cf}}\), it is independent of heart rate. The echocardiographic features which characterise RHD in turkeys (left atrial and left ventricular dilatation, a normal interventricular septum/posterior wall ratio, an increased diastolic left ventricular internal dimension/left ventricular posterior wall ratio, and a markedly reduced left ventricular shortening fraction) are similar to echocardiographic observations in congestive cardiomyopathies in humans.

As in most patients with congestive cardiomyopathies, septal motion was flat (that is essentially no systolic thickening) in six of eight RHD birds. Paradoxical septal motion was seen in the remaining two birds. Bahler et al\(^{43}\) reported two patients with paradoxical septal motion, unassociated with a right ventricular volume overload, and suggested that this may represent an early age of a congestive cardiomyopathy. His observations are consistent with the echocardiographic findings in the turkeys with early RHD. Longitudinal assessment of the turkey with RHD by echocardiography, may, therefore, be a useful tool in the study of the pathophysiology of congestive cardiomyopathy.

The etiology of RHD in turkeys is unknown. The presence of a viral-like particle in the cisternae of the sarcoplasmic reticulum suggests that a functional defect of this organelle may be present. Preliminary studies to evaluate the function of this membrane suggest that the initiating event may be related to a defect in calcium transport resulting in primary muscle dysfunction (Staley et al, unpublished observations). Thus myocardial dysfunction followed by chronic cardiac failure (that is, systemic hypotension, left ventricular dilatation) would lead to subendocardial underperfusion and eventual development of endocardial fibroelastosis.

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