Adipose Hypocellularity in Cyanotic Congenital Heart Disease

DAVID BAUM, M.D., AND MICHAEL P. STERN, M.D.

SUMMARY Height, weight, total body fat, adipocyte size (cellular lipid content), total adipocyte number, and lean body mass were studied in two groups of similar aged children with congenital heart disease. One group was comprised of 19 children cyanotic from infancy. The second group was made up of 16 asymptomatic, acyanotic patients and served as the basis for age and sex adjusted comparisons.

Cyanotic patients were lighter (P < 0.001) and had less total body fat (P < 0.001). Although there was no difference in adipocyte size, total adipocyte number was less in the cyanotic children (P < 0.001), suggesting that the reduced body fat found with cyanosis is due to adipocyte hypocellularity. The additional observation that cyanotic patients were shorter (P < 0.003) and had less lean body mass (P < 0.001) than noncyanotic children implies that early hypoxemia produces widespread abnormalities which impair growth.

CHILDREN SURVIVING INFANCY with serious cyanotic congenital heart disease tend to be short and underweight.1-4 When underweight, these young patients appear to have subnormal amounts of adipose tissue.1-4 Theoretically, this reduced body fat results from a decrease in either the total number of adipocytes or average adipocyte size. In this report, we present data suggesting that reduced body fat in children with cyanotic congenital heart disease as compared to acyanotic, asymptomatic congenital heart disease, is primarily due to diminished adipocyte number, and not to reduced adipocyte size.

Methods and Materials

Thirty-five children, ages 2 through 14 years, with congenital heart disease were the subjects of this investigation. The patients were divided into two groups. One group consisted of 19 children with cyanotic congenital heart disease who were hypoxic from infancy. These children all had either arterial oxygen saturations of 85% or below, or pO2 of 50 torr or less, or both. The second group was composed of 16 children with acyanotic, asymptomatic congenital heart disease. These acyanotic patients had arterial oxygen saturations greater than 93% or pO2 above 70 torr, or both. The group of acyanotic children served as the basis for comparison to reduce the importance of congenital heart disease per se as a variable. No child in either group had other known congenital defects.

Adipose tissue samples weighing approximately 100 mg were obtained from the groin through incisions performed for cardiac catheterization or for cardiovascular surgery. For comparison, additional fat samples were taken from the arm and/or chest wall in several patients. Fat cells from these other sites were very similar to those from the groin.

Fat cell size and number were determined by the method of Hirsch and Gallian8 as modified by Stern and Conrad.8 After washing, a portion of the tissue sample was placed in a flask containing 5 ml 2% solution of osmium tetroxide and .5 M collidine buffer and incubated at 37°C for 72 hours. Intact osmium-fixed cells were separated from the tissue matrix during this period and were then washed with water through a 250 μ Nitex screen. These cells were then collected quantitatively on a 25 μ Nitex screen, after which they were transferred to a beaker containing a known amount of saline. They were then counted in a Cellscope Automatic Particle Counter and the total number of cells and, in selected cases, their distribution according to size were determined. In those cases where the distribution was plotted, there was only a single large peak well away from the upper and lower limits of the measurement method. Lipid content per wet tissue weight was determined in the remaining portion of the adipose tissue specimen. After homogenizing the tissue and extracting with hot Folch reagent, lipid content was determined gravimetrically. Cell size (average lipid content/cell) was calculated from the equation:

\[ \text{lipid content/cell} = \frac{\% \text{ lipid} \times \text{weight of sample}}{\text{total number of cells in sample}} \]

Total adipose cell number for each patient was estimated by dividing the total body fat by the average cell lipid content.

Total body fat was estimated by two independently derived equations. The first was developed by Friis-Hansen7 and relates patient height and weight measurements to total body water from which lean body mass and ultimately total body fat can be computed. This method was originally validated by measuring total body water with the deuterium oxide dilution method, and later by determining lean body mass from total body potassium-40 measurements.6 The second equation employs skinfold measurements and was described by Brook6 who validated it with measurements of total body water using deuterium oxide. All skinfold measurements in this study were performed with Harpenden calipers6 and were made by one of the investigators. Comparison of total body fat estimated by the two methods in 71 children, including many subjects in this report, showed a strong correlation (r = 0.873). In this report, total body fat based on the Friis-Hansen method is used.

The male/female ratio was 8/1 in the cyanotic group and 9/7 in the acyanotic group. When data from all 35 patients were pooled, significant positive age trends were noted for height (P < 0.001), weight (P < 0.001), body fat (P < 0.001), adipocyte size (P < 0.03), adipocyte number...
(P < 0.001), and lean body mass (P < 0.001). Therefore, in the statistical comparisons to be presented, three-way analysis of covariance (SPSS computer program) was used to simultaneously adjust for both the main effects and any interactions between age and sex, and the other variables of interest. A difference was considered statistically significant when the P value was less than 0.05.

**Results**

**Clinical Data**

The patients’ age, sex, cardiovascular defect, and arterial oxygen saturation and/or pO2 are listed in Table 1. There was no significant age difference between the cyanotic and acyanotic groups. Tetralogy of Fallot and transposition of the great arteries were the most common cardiovascular anomalies in the cyanotic patients while small ventricular septal defect and mild aortic stenosis were the most common in the acyanotic, asymptomatic group. Of the 19 cyanotic children, only five (R.H., C.W., G.M., S.D.S. and A.M.C.) developed congestive heart failure.

**Table 1. Clinical Data**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Cardiac defect</th>
<th>Art. O2 sat. (%)</th>
<th>Art. pO2 (torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Cyanotic Congenital Heart Disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.S.</td>
<td>2.95</td>
<td>M</td>
<td>T/F</td>
<td>80</td>
<td>—</td>
</tr>
<tr>
<td>R.H.</td>
<td>4.35</td>
<td>M</td>
<td>TGA</td>
<td>—</td>
<td>50</td>
</tr>
<tr>
<td>M.C.</td>
<td>4.86</td>
<td>F</td>
<td>A-V canal + PS</td>
<td>80</td>
<td>46</td>
</tr>
<tr>
<td>C.W.</td>
<td>8.29</td>
<td>F</td>
<td>TGA</td>
<td>80</td>
<td>—</td>
</tr>
<tr>
<td>E.B.</td>
<td>5.00</td>
<td>F</td>
<td>T/F</td>
<td>75</td>
<td>—</td>
</tr>
<tr>
<td>M.E.</td>
<td>5.55</td>
<td>M</td>
<td>T/F</td>
<td>76</td>
<td>42</td>
</tr>
<tr>
<td>D.S.</td>
<td>6.55</td>
<td>F</td>
<td>T/F</td>
<td>—</td>
<td>44</td>
</tr>
<tr>
<td>G.M.</td>
<td>3.78</td>
<td>F</td>
<td>Tricuspid atresia + TGA</td>
<td>83</td>
<td>—</td>
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<tr>
<td>S.D.S.</td>
<td>14.00</td>
<td>F</td>
<td>Common ventricle + TGA</td>
<td>85</td>
<td>50</td>
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<tr>
<td>A.M.C.</td>
<td>8.39</td>
<td>F</td>
<td>TGA + VSD</td>
<td>81</td>
<td>47</td>
</tr>
<tr>
<td>T.C.</td>
<td>3.84</td>
<td>F</td>
<td>DORV + PS</td>
<td>64</td>
<td>33</td>
</tr>
<tr>
<td>P.H.</td>
<td>12.25</td>
<td>F</td>
<td>T/F</td>
<td>85</td>
<td>50</td>
</tr>
<tr>
<td>P.R.</td>
<td>13.19</td>
<td>M</td>
<td>T/F</td>
<td>83</td>
<td>50</td>
</tr>
<tr>
<td>K.S.</td>
<td>10.82</td>
<td>M</td>
<td>T/F</td>
<td>76</td>
<td>48</td>
</tr>
<tr>
<td>S.G.</td>
<td>4.25</td>
<td>F</td>
<td>T/F</td>
<td>75</td>
<td>—</td>
</tr>
<tr>
<td>T.E.</td>
<td>5.73</td>
<td>M</td>
<td>T/F</td>
<td>84</td>
<td>50</td>
</tr>
<tr>
<td>D.R.</td>
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<td>M</td>
<td>T/F</td>
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<td>48</td>
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<tr>
<td>D.M.</td>
<td>10.63</td>
<td>F</td>
<td>Hypoplastic RV + VSD + ASD</td>
<td>85</td>
<td>—</td>
</tr>
<tr>
<td>R.M.</td>
<td>3.60</td>
<td>M</td>
<td>DORV + PS</td>
<td>77</td>
<td>44</td>
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<tr>
<td>B. Acyanotic Congenital Heart Disease</td>
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<tr>
<td>L.S.</td>
<td>2.32</td>
<td>M</td>
<td>VSD</td>
<td>95</td>
<td>—</td>
</tr>
<tr>
<td>M.J.</td>
<td>4.05</td>
<td>M</td>
<td>PS</td>
<td>96</td>
<td>89</td>
</tr>
<tr>
<td>K.C.</td>
<td>4.66</td>
<td>F</td>
<td>ASD</td>
<td>96</td>
<td>—</td>
</tr>
<tr>
<td>V.C.</td>
<td>6.26</td>
<td>M</td>
<td>Coarctation</td>
<td>100</td>
<td>102</td>
</tr>
<tr>
<td>M.C.</td>
<td>6.66</td>
<td>F</td>
<td>A-V canal</td>
<td>98</td>
<td>—</td>
</tr>
<tr>
<td>E.V.</td>
<td>13.75</td>
<td>F</td>
<td>VSD</td>
<td>94</td>
<td>74</td>
</tr>
<tr>
<td>D.A.</td>
<td>9.53</td>
<td>F</td>
<td>PDA</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>E.C.</td>
<td>10.92</td>
<td>F</td>
<td>PS</td>
<td>96</td>
<td>—</td>
</tr>
<tr>
<td>S.F.</td>
<td>4.85</td>
<td>F</td>
<td>PDA</td>
<td>95</td>
<td>93</td>
</tr>
<tr>
<td>R.K.</td>
<td>6.66</td>
<td>M</td>
<td>VSD</td>
<td>96</td>
<td>94</td>
</tr>
<tr>
<td>T.C.</td>
<td>6.17</td>
<td>M</td>
<td>VSD</td>
<td>96</td>
<td>—</td>
</tr>
<tr>
<td>T.S.</td>
<td>4.66</td>
<td>M</td>
<td>VSD + RV band</td>
<td>94</td>
<td>—</td>
</tr>
<tr>
<td>D.C.S.</td>
<td>7.06</td>
<td>M</td>
<td>AS</td>
<td>95</td>
<td>—</td>
</tr>
<tr>
<td>R.L.</td>
<td>7.16</td>
<td>M</td>
<td>AS</td>
<td>97</td>
<td>95</td>
</tr>
<tr>
<td>K.B.</td>
<td>12.92</td>
<td>F</td>
<td>ASD</td>
<td>97</td>
<td>93</td>
</tr>
<tr>
<td>D.M.</td>
<td>12.47</td>
<td>M</td>
<td>ASD, mitral prolapse</td>
<td>95</td>
<td>74</td>
</tr>
</tbody>
</table>

**Abbreviations:** DORV = double outlet right ventricle; PS = pulmonic stenosis; RV = right ventricle; T/F = tetralogy of Fallot; TGA = transposition of the great arteries; VSD = ventricular septal defect; ASD = atrial septal defect; PDA = patent ductus arteriosus; AS = aortic stenosis.

As seen in figures 1 and 2, cyanotic patients were shorter (P < 0.003) and weighed less (P < 0.001) than the non-cyanotic group. The difference in height did not change with age, whereas the difference in weight tended to widen as age increased (P = 0.038).

**Tissue Data**

The children’s total body fat, mean cellular lipid content (adipocyte size), total adipocyte number, and lean body mass are shown in figures 3–6. The cyanotic patients had less body fat compared with the acyanotic patients (P < 0.001), and this difference appeared to widen with increasing age (P < 0.006). There was no significant difference in the adipocyte size (cell lipid content) of cyanotic and acyanotic children. Although cell size appeared not to make an important contribution to the discrepancy in body fat between cyanotic and acyanotic children, this was not the case with fat cell number. Age and sex adjusted total fat cell number

**Figure 1.** Comparison of height in children with cyanotic and acyanotic, asymptomatic congenital heart disease.

**Figure 2.** Comparison of weight in children with cyanotic and acyanotic, asymptomatic congenital heart disease.
FIGURE 3. Comparison of total body fat in children with cyanotic and acyanotic, asymptomatic congenital heart disease.

FIGURE 4. Comparison of adipocyte size (cell lipid content) in children with cyanotic and acyanotic, asymptomatic congenital heart disease.

Discussion

Children in our study, hypoxic from infancy because of congenital heart disease, weighed less and were shorter than patients with asymptomatic, acyanotic congenital heart defects, confirming previous observations.1-4 The cyanotic group was found to have less calculated fat body than their acyanotic counterparts, contributing to their subnormal weights. Further comparison of the two groups of children revealed no significant difference in fat cell size. However, there were significantly fewer fat cells in the cyanotic patients. From these observations, we conclude that fat cell number is more important than fat cell size in reducing body fat in children with cyanotic congenital heart disease.

Diminished total body fat with cyanotic congenital heart disease is likely to be nutritional rather than due to an associated congenital defect since the asymptomatic, acyanotic children with cardiac anomalies were normal sized6 and had quantities of body fat similar to those found in normal children.11 There are at least two explanations for nutritional deficiency in children with cyanotic congenital heart disease. First, young children with serious heart disease and severe hypoxemia or heart failure often feed poorly,12,13 a complaint commonly made by the parents of these patients. Secondly, they have large substrate requirements, probably because of increased cardiopulmonary work14,15 and abnormalities in thermoregulation.16

Existing knowledge of adipose tissue development suggests a mechanism for the adipocyte hypocellularity associated with early hypoxemia. Investigation of normal man has revealed that adipose tissue enlarges primarily by a rapid increase in the number of lipid-laden cells during infancy.16 Thereafter, the number of lipid containing cells continues to increase until the adult state is reached,4,17 but at a much slower rate.16 Fat cell number is essentially stable in adults with changes in total body fat resulting from changes in adipocyte lipid content.17 In rodents, the basic pattern of adipose tissue growth is similar except for the time period, which is compressed.18,19 Greenwood and Hirsch20 both support and help explain these observations with data obtained from the developing rat and employing a technique which involves the incorporation of tritiated thymidine by adipocyte DNA. These data demonstrate that the adult complement of adipocytes is determined early in life after which it is unalterable. Only a portion of the adipocytes contain lipid initially; the number containing lipid increases until maturity is reached, when the number stabilizes. However, the maximum potential number of lipid-laden cells is governed by the adipocyte complement which is fixed by the cessation of cell division long before maturity is attained.

*Stuart HC: Percentile charts for measurements of boys and girls. Constructed for use at the Children’s Medical Center, Boston, Massachusetts.
ADIPOSE HYPOCELLULARITY AND CYANOSIS/Baum, Stern

Early nutritional alterations known to permanently affect adipose cellularity suggest that oxygen deficiency in infancy has an important role in producing adipose hypocellularity in cyanotic congenital heart disease. Calorically restricting rats prior to weaning results in hypocellularity of epididymal fat pads which cannot be corrected by later ad libitum feeding. On the other hand, when nutrition prior to weaning has been adequate, caloric restriction after weaning leads to temporary reduction in lipid containing cells which is reversible if the nutritional deficiency is later corrected. Thus, permanent hypocellularity seems to result only if nutritional deprivation takes place prior to that critical time when the adult complement of cells has been determined. In addition, it has been shown that caloric restriction in mature animals and humans reduces adipose deposits by decreasing adipocyte lipid content rather than cell number. Human data indicate that permanent effects on adipose cellularity are most likely when nutritional influences operate during the critical early period of development. This critical period in man seems to approximate the first year of life. Since nutritional deficiency is associated with congenital heart disease and early hypoxemia, it is reasonable to hypothesize that chronic hypoxemia developing in infancy will result in potentially irreversible adipose tissue hypocellularity. For similar reasons, early congestive heart failure might also contribute to such adipose tissue changes. However, heart failure does not appear to be essential for these changes to develop since 14 of our 19 cyanotic patients were free of the condition.

Although it is possible that the smaller amount of body fat found in cyanotic patients is not the result of true adipocyte hypocellularity but is due to a pool of lipid-poor adipocytes and pre-adipocytes, we consider this unlikely for a number of reasons. First, our interpretation is consistent with existing animal and human data indicating that adipocyte cellular division occurs very early in postnatal development and is vulnerable to nutritional perturbation. Second, examination of the size distribution of counted adipocytes revealed only one peak per patient. Thus, although not ruled out, our data did not suggest a large pool of cells that was not counted. Finally, and perhaps most important, postoperative growth and weight gain following relatively late repair of transposition of the great arteries and tetralogy of Fallot, although impressive, result in children who are still undersized and underweight, implying a limiting factor such as permanent hypocellularity. If lipid-poor cells too small for detection exist in cyanotic patients, a normal complement of adipocytes should become detectable with postoperative weight gain and lipid filling of these pre-existing adipocytes regardless of the age at which repair is performed. Conversely, if permanent hypocellularity results from early nutritional deprivation, only children whose defects are repaired early should manifest a normal complement of adipocytes postoperatively, whereas those whose defects are repaired late should continue to show a subnormal amount of body fat and hypocellularity. Serial studies, including pre and postoperative determinations of adipocyte lipid content (cell size) and total adipocyte number, in children whose defects are repaired at various ages should clarify this issue.

It is possible that early hypoxia contributes to abnormal development of nonadipose organs as well. Diminished lean body mass in cyanotic patients supports this possibility. Recognizing that adipose organ size has an obvious role in determining weight but not height, the shortness of cyanotic patients argues further for the involvement of lean tissues. Pertinent to this respect are reports of cellular deficits in certain nonfat organs of rodents made hypoxic early in life and the inference of reduced skeletal muscle cell number in some patients with symptomatic congenital heart disease. These observations in man and animals indicate the need to examine other organs and to evaluate cellular function in children with cyanotic congenital heart disease. If functional abnormalities are associated with the morphologic changes it will become necessary to determine at what developmental stage these defects become irreversible. This type of information could prove invaluable in improving the criteria for reparative cardiovascular surgery and its timing.

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