Variations of Fat Tissue Fraction in Abnormal Human Bone Marrow Depend Both on Size and Number of Adipocytes: A Stereologic Study

By Ciril Rozman, Joan-Carles Reverter, Evarist Feliu, Lluis Berga, Maria Rozman, and Carme Climent

Studies dealing with the number or size of individual adipose cells in abnormal human bone marrow are lacking. To ascertain whether variations in fat tissue fraction depend on the size of individual adipocytes or their number or both, a stereologic study of 30 human bone marrow specimens (10 with aplasia, 10 with hyperplasia, and 10 with dysplasia) was performed. A total of 23,435 adipocyte profiles were measured and two stereologic parameters were obtained in each specimen: mean diameter and number of cells per mm² of bone marrow. The fat tissue fraction correlated positively with the size (r = .79; P < .001) and the number/volume (r = .77; P < .001) of adipocytes. The significance of both adipocyte size and adipocyte number/volume was confirmed by stepwise multiple regression, in which the size alone explained 62.5% of fat tissue fraction and both size and number/volume explained 95.8% of fat tissue fraction. These results are discussed from a pathophysiologic point of view.

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Although adipose tissue is a major structural component of the bone marrow, it has been relegated during many years to a purely passive role as a space filler in response to hematopoietic variations. It seems clear that fat cells are actively involved in the hematopoietic process, serving as a direct metabolic support tissue rather than as a purely mechanical filler. There is increasing interest in studying the changes of the marrow adipose cells in different pathologic conditions, as far as their morphological, functional, and biochemical features are concerned.

When the bone marrow adipose tissue fraction is studied in humans, their variations are expressed in percentage with respect to the marrow surface or volume. Investigations on number or size of individual adipose cells in human bone marrow are scant. In a previous stereologic study, we showed that age-related variations of fat tissue fraction in normal human bone marrow depend both on size and number of adipocytes. The purpose of the present study is to analyze, by stereologic methods, whether the variations of marrow fat tissue fraction in abnormal human bone marrow are due to changes either in size or number, or both, of the fat cells.

MATERIALS AND METHODS

Thirty bone marrow specimens were studied: 10 with aplasia, 10 with hyperplasia, and 10 with dysplasia. As a source of aplastic bone marrow, biopsy specimens obtained from 10 patients with aplastic anemia were used. The hyperplastic group was composed of 10 biopsy specimens obtained from patients with chronic myeloproliferative disorders: 5 with essential thrombocytopenia, 3 with chronic granulocytic leukemia, and 2 with polycythemia vera. The dysplastic group was represented by 10 biopsy specimens corresponding to patients with myelodysplastic syndromes: 4 with refractory anemia with excess of blasts (RAEB), 4 with RAEB in transformation, 1 with sideroblastic refractory anemia, and 1 with 5q– syndrome.

Biopsy specimens were taken from the anterior iliac crest with Jamshidi needle. Plastic (methyl-methacrylate) embedding of undecalcified cores was used. Sections 3 μm in thickness were stained with Gill’s hematoxylin. From each specimen, 8 to 10 random microphotographs were obtained and enlarged to final magnification of ×150 for the stereologic analysis. The magnification was controlled by means of a microscopic calibration device, Carl Zeiss 5 ×100/100 mm.

The stereologic analysis was performed with a digitizer (9111 A Graphic Tablet, Hewlett Packard) connected to a computer (9845B, Hewlett Packard). Specific software was developed for this research. The minimum sample size for each group of micrographs was determined by using the progressive mean technique with a confidence limit of ±5%. A total of 23,435 adipocyte profiles were measured with a mean of 781.2 per specimen. Because even small adipocytes are easily identified in plastic embedded sections, no special stain was used for this purpose. On the other hand, as detailed later, a compensation for “optically lost caps” was routinely performed.

In each specimen, the following morphometric parameters were determined: (1) percent of marrow fat tissue fraction; (2) mean adipocyte diameter (D); (3) number of adipocytes/volume unit (Nv), expressed per cubic millimeter. Percentage of marrow fat tissue fraction was calculated by fractional area (Aa) measurement. The area of each marrow space was measured by direct digitizing. The fat tissue area within each marrow space was obtained from the sum of areas corresponding to adipose cells. The latter were estimated from n a/2 b/2 where a and b are diameters of each adipocyte profile.

For the calculation of D, we used the Giger and Riedwyl method suitable for objects of spherical shape and normal distribution. Mean profile axial ratio of adipocytes ranged from 1.24 to 1.35, limits within which the objects can reasonably be treated as spherical. The adequacy of the Giger and Riedwyl method was further confirmed by the fact that adipocyte diameter did not significantly depart from gaussian distribution.

Individual adipocyte profile diameters were calculated from \(2(ab)^1/2\) and plotted as a histogram of 10 to 20 size classes. To compensate for “optically lost caps”, the histogram was extended to the left by linear extrapolation. The mean diameter (d) from the provisionally completed histogram was calculated, and a first estimate of the mean diameter was made: \(\bar{D} = 4d/r\). From the homogram devised by Giger and Riedwyl, a refined estimate \(\bar{D}\) was derived. This was used for a more accurate estimation of the smaller size classes obtained by linear extrapolation. If N is the total number of transsections measured, then the number \(N_k\) in the kth

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small size class may be calculated from

\[ N_v = d_a \frac{N}{(D^3)^2}, \]

where \( a = \) width of a histogram block, and \( d_a = \) mean diameter of transections in the \( k^{th} \) block. From this completed and refined histogram the analysis was repeated and fresh estimates of \( N, D, \) and \( B_k \) were obtained. This second \( B_k \) was considered as the final mean adipocyte diameter \((D)\).

For the determination of parameter \( N_v \) we used the method of Weibel and Gomez:19

\[ N_v = \frac{K}{\beta} \times \left( \frac{N_4}{V_4} \right)^{3/2}, \]

where \( K \) is a constant depending on the size distribution of the particles, and \( \beta \) is a shape constant. \( N_4 \) corresponds to number of profiles per section area, and \( V_4 \) to the volume fraction. Instead of using the Weibel and Gomez nomogram, the constant was computed according to Lindberg and Vorwerk.\(^{19}\)

To evaluate the relative contribution of \( N, D \) to the variations of fat tissue fraction, the ratio \( N_v/D \) was estimated in each case. As controls, data from our previous work\(^15\) were used.

Statistical methods. Significance of correlation coefficients and differences between means were evaluated by \( t \) statistics.\(^{13}\) Contribution of different variables to the percentage of fat tissue fraction was analyzed by means of stepwise multiple regression\(^{24}\) using the software 15010 on a 9845B computer (Hewlett Packard). Adequacy of fitting model was controlled by graphic representation of standardized residuals.

RESULTS

The results are presented in Table 1. The values of all three parameters (fat tissue fraction, mean adipocyte diameter, and number of adipocytes/mm\(^3\)) were significantly higher in aplasia as compared with hyperplasia and dysplasia, whereas the differences between the latter two groups were not significant. When the cases were classified in lower and higher fat tissue fraction group, both mean adipocyte diameter and number/volume were significantly lower in the former as compared with the latter group (Table 2). The fat tissue fraction correlated positively with the mean diameter \((r = .79; P < .001)\) and the number/volume \((r = .77; P < .001)\) of adipocytes. Thus, with increasing fat tissue fraction, there is an increase in both adipocyte size and number, whereas the reverse is also true. A stepwise multiple regression was performed using the fat tissue fraction as a dependent variable and the mean size of adipocytes \((D)\) and their number/volume \((N_v)\) as independent variables. The first parameter to enter the regression at a significant level was the mean size of adipocytes \((D)\) and their number/volume \((N_v)\) as independent variables. The first parameter to enter the regression at a significant level was the mean size of adipocytes \((D)\) and their number/volume \((N_v)\) as independent variables. The first parameter to enter the regression at a significant level was the mean size of adipocytes \((D)\) and their number/volume \((N_v)\) as independent variables. The first parameter to enter the regression at a significant level was the mean size of adipocytes \((D)\) and their number/volume \((N_v)\) as independent variables.

The study of relative contribution of adipocyte number and size to the variations of fat tissue fraction by means of \( N_v/D \) ratio showed (Table 3) that this was significantly increased in aplasia with respect to normal controls, whereas no significant difference was observed in cases of hyperplasia and dysplasia.

DISCUSSION

An accurate histomorphometry of bone marrow relies on two factors: (1) plastic embedding of undecalcified specimens, and (2) use of stereologic techniques. It has been shown that paraffin embedding of bone marrow can lead to considerable shrinkage, amounting to approximately 15%,\(^{25}\) producing an under-estimation of the hematopoietic tissue

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fat Tissue Fraction (%)</th>
<th>Mean Adipocyte Diameter (µm)</th>
<th>No. of Adipocytes/mm(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SEM)</td>
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</tr>
<tr>
<td>A. Aplasia (N = 10)</td>
<td>71.10 (3.78)</td>
<td>62.77 (2.47)</td>
<td>5.219 (7.77 (622.99)</td>
</tr>
<tr>
<td>B. Hyperplasia (N = 10)</td>
<td>16.39 (1.60)</td>
<td>50.04 (1.23)</td>
<td>2.151 (0.90 (200.81)</td>
</tr>
<tr>
<td>C. Dysplasia (N = 10)</td>
<td>22.32 (5.05)</td>
<td>51.67 (1.98)</td>
<td>2.528 (0.09 (422.01)</td>
</tr>
<tr>
<td>All patients</td>
<td>36.61 (5.01)</td>
<td>54.83 (1.52)</td>
<td>3.299 (6.55 (359.01)</td>
</tr>
</tbody>
</table>

A v B P < .001
A v C P < .001
B v C P = NS

Abbreviation: NS, not significant.

Table 1. Results in Aplasia, Hyperplasia, and Dysplasia

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<td>Fat tissue fraction &lt;50% (N = 19)</td>
<td>50.3 (1.06)</td>
<td>2,284.14 (228.50)</td>
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<tr>
<td>Fat tissue fraction &gt;50% (N = 11)</td>
<td>62.65 (2.24)</td>
<td>5,124.08 (571.42)</td>
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\( P < .001 \)
A vD, P = .02; B vD, NS; C vD, NS (NS, not significant).

fraction26 and a disruption of the limits between bone trabeculae and marrow spaces. From the lack of such disruption in plastic-embedded undecalcified specimens, it can be assumed that the shrinkage is of no major importance in these conditions.

To obtain reliable histomorphometric measurements, the use of stereologic methods is mandatory. If these are not used, an important underestimation or overestimation of different parameters may result. For example, calculation of the mean diameter of adipocytes directly from transected profiles would lead to a striking underestimation of this parameter. A plane cut through the volume produces a section containing circular profiles whose diameters range from the maximum for equatorial sections through the largest adipocyte to near zero for tangential cuts. Thus, the corresponding correction by means of stereologic method is mandatory. In a previous study27 we demonstrated that real mean adipocyte diameter is more than 16% higher than mean diameter of transected profiles. On the other hand, it is well-known18 that direct counting of particle profile sections tends to produce an incorrect estimation of number/volume parameter. Stereologic methods accounting for size-frequency distribution and shape factor yield much more accurate results.

The inverse relationship between the prevalence of fat cells in red bone marrow and the activity of hematopoiesis is an established fact.28 On the other hand, reciprocal age-related variations of hematopoietic cellularity and fat tissue fraction are well-known.14,31,32 Whereas during the first decade of life there is a striking predominance of hematopoietic cellularity and relatively scanty fat tissue, the reverse is true in older age. In a previous stereologic study13 we showed that such age-related variations of fat tissue fraction in normal human bone marrow depend both on size and number of adipocytes.

Variations in fat tissue fraction may be due to changes in the size of individual adipocytes, or their number, or both. Very few data are available on the relative importance of these two factors in experimental animals, whereas before our studies pertinent data in humans were lacking. Bathija et al1 demonstrated, in rabbits, that the stimulation of erythropoiesis leads to a striking reduction in size of red marrow fat cells, whereas the volume of systemic fat cells remains unchanged. These findings suggest that increased hematopoiesis stimulates lipolysis of red marrow fat cells, and that both populations, hematopoietic cells and marrow fat cells, may be functionally related, probably the latter metabolically supporting the former. In a study of rat marrow, the stimulation of erythropoiesis over 7 days of hypoxia led to a great reduction in both size and number of marrow adipocytes, with normalization of these parameters after the cessation of experimental conditions.30 The reverse phenomenon, ie, an increase of marrow adipocyte size and/or number in states of depressed hematopoiesis, might be presumed; however, it has not yet been demonstrated.

In our previous study13 we showed that physiologic age-related variations of fat tissue fraction depend both on size and number of adipocytes. The present study is the first to demonstrate that this physiologic phenomenon is maintained during different physiopathologic situations such as bone marrow aplasia, hyperplasia, and dysplasia. An important degree of variation in size and number can be observed in these abnormal conditions. As expected, the capacity of adipocytes to expand and increase their size is not unlimited. In hyperplasia and dysplasia the relative contribution of adipocyte size and number to the variations of fat tissue fraction is not different from controls, but the ratio number/size increased significantly in aplastic patients, suggesting that after fat cells have expanded to their maximum, a disproportional increase in number is required to fill void marrow spaces. Additional stereologic studies on adipocytes may yield further insight on the relationship between hematopoiesis and its supporting tissue.

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