The Noncollagen Protein in Adipose Tissue as an Index of Cell Number[1] (36552)

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The determination of adipose tissue cellularity is an important adjunct in the study of experimental and human obesity (1, 2). In 1968, Hirsch and Gallian (3) described a method of adipose tissue fixation and cell counting that utilized a Coulter electronic counter. This method obviated some of the problems of previously described techniques. In particular, the estimation of DNA in adipose tissue is inexact due to the large numbers of nonadipocyte nuclei in the supporting tissues (4).

The technique described by Hirsch and Gallian depends on the recovery of all adipose cells and that each cell contains sufficient lipid to become well fixed by osmium tetroxide. Cells containing a small amount of lipid (< 0.01 μg/cell) could be lost during preparation (3).

Since most of the protein in adipose tissue is intracellular and the extracellular protein is primarily in collagen, the determination of noncollagen protein should be equivalent to the adipocyte protein (5). If the intracellular protein content in adipocytes is constant, the measurement of the percentage of noncollagen protein should correlate well with the cell number per gram of adipose tissue. This hypothesis was tested in the following experiment.

Clinical material. Seventeen normal infants and children ranging in age from 6 months to 17 years were randomly selected and served as the control group. They were patients admitted to the Childrens Medical and Surgical Center of the Johns Hopkins Hospital undergoing routine inguinal herniorrhaphies. Informed consent was obtained for a biopsy of adipose tissue. In addition, nine patients undergoing muscle or adipose tissue biopsy for other reasons were included in the study. Five of these patients had myopathies, three were obese, and one patient had hypopituitarism. The control group consisted of 14 males and 3 females, while the abnormal group consisted of 6 males and 3 females. A sample of adipose tissue weighing approximately 800 mg was excised in each case.

Methods. The adipose tissue samples were immediately divided and one half was placed in a preweighed glass vessel. The other half was cut into 4 pieces and placed in a beaker containing isotonic saline at 37°. The pieces were individually transferred to tared circular discs of nylon mesh (pore size 250 μ) and washed with warm (37°) isotonic saline through a Nalgene filter unit.[3] The bottom of this unit had been cut away to allow easy filtration. Each of the filter discs were removed and rapidly blotted on the undersurface with absorbent paper and the wet weight of the adipose tissue obtained. Three of the pieces of adipose tissue on their respective nylon discs were then immediately immersed in a plastic counting vial containing 30 ml of 2% osmium tetroxide in 0.05 *M* collidine–HCl buffer at pH 7.4 and 37°. Fixation at 37°

[1] This work was supported in part by Grants HD 00126-08 and HD 02761 from the National Institute of Child Health and Development and a Centennial Fellowship of the Medical Research Council of Canada.

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[3] Available from the Nagle Company Inc., Rochester, NY.

TABLE I. Source and Composition of Normal Adipose Tissue.

No.	Sex	Age (years)	Biopsy site	% H_2O	% Fat	% FFDS[a]	% Protein	% Collagen	% NCP[b]	Cell no./g $\times 10^6$
C 1	F	1.72	Inguinal	40.5	47.3	12.3	10.48	3.52	6.96	3.80
C 2	F	2.35	Inguinal	30.3	62.0	7.7	6.80	2.78	4.02	2.12
C 3	F	4.86	Inguinal	43.3	40.5	16.1	15.35	9.37	5.97	3.59
C 6	M	8.10	Inguinal	17.0	73.8	9.2	7.87	2.44	5.43	5.18
C 9	M	0.75	Inguinal	38.7	41.1	20.2	12.44	8.87	3.57	3.53
C 11	M	0.58	Inguinal	15.0	68.0	16.9	13.68	6.13	7.55	3.80
C 12	M	2.33	Inguinal	30.0	48.2	21.7	11.05	2.06	8.98	4.58
C 13	M	2.45	Inguinal	29.1	49.3	21.6	10.54	5.29	7.99	3.51
C 14	M	0.50	Inguinal	28.6	42.5	28.9	12.99	6.21	6.78	4.48
C 15	M	4.18	Inguinal	16.0	69.1	14.9	4.02	1.71	2.33	3.27
C 16	M	13.6	Inguinal	35.0	53.8	11.2	9.17	3.95	5.23	3.12
C 17	M	0.60	Inguinal	26.7	57.4	15.9	12.29	7.05	5.24	3.54
C 20	M	3.50	Inguinal	22.9	43.2	34.0	10.06	6.45	3.60	2.20
C 25	M	12.7	Inguinal	30.5	59.6	9.9	7.27	2.02	4.25	3.02
N 61	M	15.2	Buttock	13.9	81.3	4.8	4.12	2.28	1.84	2.50
N 62	M	15.8	Buttock	25.8	66.1	8.2	7.63	5.00	2.63	1.85
N 63	M	17.5	Buttock	16.4	78.6	5.0	4.46	1.49	2.96	1.99

[a] FFDS, fat-free dry solid.
[b] NCP, noncollagen protein.

was allowed for 48–72 hr. The remaining piece of tissue and nylon disc were placed in a stoppered test tube for subsequent lipid extraction.

After fixation, the contents of the plastic containers were thoroughly washed through nylon mesh (250 μ) with distilled water and further washed on 25 μ nylon mesh and the cells were suspended in a known volume of saline. The cells were then counted with a Coulter counter.[4] The validation, advantages, and shortcomings of this method are discussed elsewhere (3). Knowing the count per unit volume of suspension, the cell number per gram of tissue can be calculated.

The portion of adipose tissue that was transferred to a tared weighing vessel was placed in an oven at 95° for 48 hr, cooled and reweighed. The tissue was then lipid extracted at room temperature using petroleum ether. Extraction was continued until all visible lipid was removed. The sample was dried and reweighed to determine the neutral lipid content. A 10–15 mg sample of the dried tissue was then digested for the determinations of L-hydroxyproline content according to the method of Prockop and Udenfriend (6). The nitrogen content in a sample of the dry tissue was determined using a Coleman nitrogen analyzer (7). The protein content was obtained by multiplying the nitrogen content by 6.25 and the collagen content by multiplying the L-hydroxyproline content by 7.46. The noncollagen protein was then calculated subtracting the collagen protein from the total protein.

Results. The correlation between the percentage of noncollagen protein and the cell number per gram of adipose tissue is shown in Fig. 1. The correlation coefficient is 0.70 and is highly significant ($p < .001$). The regression equation and the standard error of the estimate were calculated using the "least squares" technique on the normal data. The abnormal patients were plotted on the graph for comparison. It is of interest that the three obese patients had an extremely low cell number per gram and a corresponding low percentage noncollagen protein.

The chemical composition of the adipose tissue and the biopsy site in the normal con-

[4] Available from the Coulter Electronics Co., Hialeah, FL.

TABLE II. Source and Composition of Adipose Tissue.

Diagnosis	No.	Sex	Age (years)	Biopsy site	% H_2O	% Fat	% FFDS[a]	% Protein	% Collagen	% NCP[b]	Cell no./g $\times 10^6$
Hypopituitarism	C 19	M	10.0	Inguinal	8.8	88.4	2.74	2.15	0.44	1.71	1.07
Myopathy	C 21	M	12.4	Right arm	34.0	62.0	3.99	3.00	1.30	1.70	1.26
	C 22	M	8.40	Right leg	31.3	62.6	6.06	5.69	2.18	3.51	1.37
	C 23	M	6.90	Right leg	19.2	75.7	5.11	4.62	1.28	3.34	0.89
	C 24	M	9.60	Right arm	43.1	32.2	24.8	9.63	3.61	6.02	2.02
Obesity	O 61	F	14.2	Buttock	6.6	91.5	1.87	1.41	0.28	1.13	0.57
	O 62	F	11.0	Buttock	28.4	69.3	2.31	1.87	0.70	1.16	0.90
	O 63	M	17.7	Buttock	47.9	49.7	2.41	1.99	0.50	1.48	0.93
Muscle hypertrophy	S 18	M	6.00	Arm	27.8	66.2	5.98	4.56	2.62	1.93	5.11

[a] FFDS, fat-free dry solid.
[b] NCP, noncollagen protein.

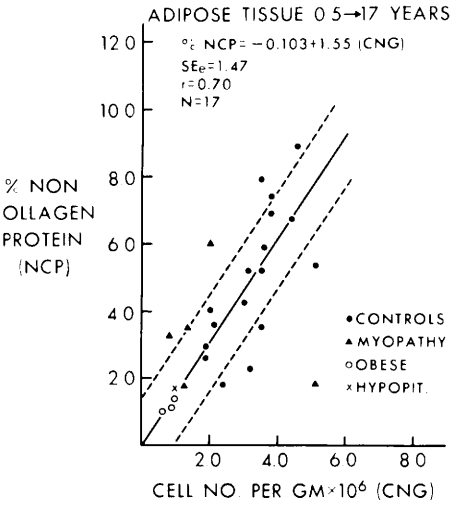

FIG. 1. The calculated regression line for the correlation between the percentage of NCP and the cell number per gram of adipose tissue (=) ±1 standard deviation. Only the normal values were used in the calculation.

trol patients and the abnormal patients is documented in Tables I and II, respectively. The percentage of fat correlated positively with both age and weight in the control patients ($r = 0.61$). However, the variability was large. In the abnormal patients the scatter was even greater.

The cellular components of normal adipose tissue were inversely correlated as illustrated in Figs. 2 and 3. The percentage of both water and protein decrease with increasing fat content. The abnormal patients showed a greater deviation from the mean than the normal patients.

The cell number per gram of adipose tissue did not show a significant correlation with age, weight, or height. Total fat and lean body mass were not measured in this study and total adipose cell number could not be computed.

Discussion. The present study indicates a significant correlation ($r = 0.70$) between the cell number per gram and the percentage of noncollagen protein in normal adipose tissue. This relationship appears to be valid during active tissue growth from 0.5 to 17 years. We suspect that the correlation is of a higher order since there are variables in the techniques that need consideration. There

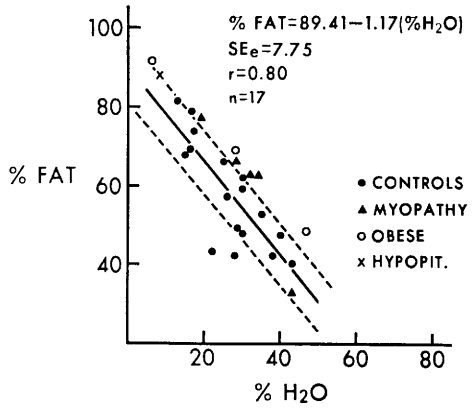

FIG. 2. Adipose tissue. The inverse correlation is shown between the percentage of fat and water. The correlation coefficient is very high and variability small. The majority of the abnormal samples are above 1 standard deviation.

were only three female patients in the control group and sex differences may be present if larger groups are analyzed. In addition, particular attention needs to be directed toward obtaining adipose tissue samples that are representative of the subcutaneous tissue. Connective tissue and facia content may be very high in the inguinal region. Although the fat content and the cell size of adipose tissue does not differ in various subcutaneous sites (1, 8), samples from deep sites have a smaller cell size (8). The abnormal patients do not have a specific pattern except that the

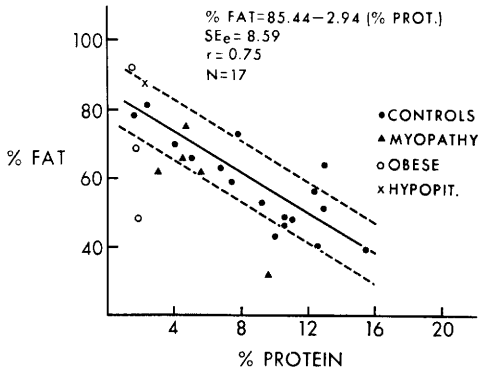

FIG. 3. Adipose tissue. A similar inverse correlation is shown between the percentage of fat and protein. The slope is not as steep compared with the percentage of water and the variability is greater. The abnormal samples do not have a specific pattern.

obese patients and the hypopituitary patient fit the normal relationship very well.

Examination of the adipose tissue composition indicates data consistent with that described by Baker (9). Obviously, it is important to handle the tissue in a consistent manner to insure minimal water loss during preparation. In normal patients there is a gradual increase with age or weight in the percentage of fat within adipose tissue and a concomitant decrease in the percentage of water and protein. Part of the variability in protein and collagen content may also relate to the biopsy site (9, 10).

Recent data of Salans and Dougherty (12) indicate a constancy of the protein per cell over a wide range of adipose cell size in the epididymal fat pad of rats. They reported an average value of 0.584 ng of protein/cell. The value we calculate for human tissue is 15.4 ng noncollagen protein/cell. The difference in these values may be related to protein contributed by fibroblasts, endothelial cells, red cells or plasma. However, the good correlation coefficient between the cell number per gram as calculated by electronic counting and the percentage of noncollagen protein suggests that most of the noncollagen protein is in fact associated with the adipocyte. Direct measurement of intact, isolated human adipose cell protein will be required to clarify this point. In addition, human adipose cells are larger than the adipose cells of the rat and this may account for some of the difference in protein content.

If the relationship between the percentage of noncollagen protein and the cell number per gram of adipose tissue can be strengthened by further data and meticulous attention to the site, size, and quality of the specimen then this method can be utilized in the determination and close approximation of cell number per gram of adipose tissue. This combined with estimates of body fat can give estimates of total adipose tissue cell number. Utilizing existing data, this relationship would predict a marked increase in adipose cell number in obese males (11). The increase in females is somewhat less. The cell number per gram is minimally reduced indicating a slight increase in cell size. These

predictions agree with the findings of Hirsch and Knittle (1).

Summary. Adipose tissue biopsies from infants and children ranging in age from 6 months to 17 years were examined. The composition of the adipose tissue was determined and the percentage of noncollagen protein computed. Duplicate adipose tissue samples were used for fat cell number determinations according to the method of Hirsch and Gallian (3). A highly significant ($p < .001$) positive correlation was found between the percentage of noncollagen protein (NCP) and the cell number per gram of tissue. This correlation may be improved by direct measurement of isolated fat cell protein. The regression equation can be an aid in prediction of total body fat cell number when body fat is known.

The authors gratefully acknowledge the surgical assistance of Dr. G. N. Peters and J. J. White, and the technical assistance of Miss Rachel Scott and Mrs. E. Higginbottom.

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Received Jan. 21, 1972. P.S.E.B.M., 1972, Vol. 140.