Examination of a Lognormal Distribution Equation for Describing Distributions of Diameters of Bovine Adipocytes

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EXAMINATION OF A LOGNORMAL DISTRIBUTION EQUATION FOR DESCRIBING DISTRIBUTIONS OF DIAMETERS OF BOVINE ADIPOCYTES[1]

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Summary

Samples of subcutaneous, intermuscular and mesenteric adipose tissues from beef steers were fixed with osmium tetroxide, and freed adipocytes were counted with an automatic particle counter to determine whether a lognormal distribution function would describe adipocyte size distributions more accurately than a normal distribution function. Modes and medians of size distributions generally were larger than means for adipocyte size distributions modeled with a lognormal distribution function. Normalized third and fourth moments of predicted lognormal distributions often were close to 0 and 3, respectively, which are expected values for a normally distributed population. Considerable variation was observed in the skewness of adipocyte size distributions. Both normal and lognormal models for adipocyte size distribution yielded similar means. The lognormal model yielded a greater standard deviation than the normal model for adipocyte size distributions. Smaller chi-square values were found for size distributions modeled with a lognormal than with a normal distribution function. Results suggest that a lognormal distribution function more accurately models the size distributions of bovine adipocytes.

(**Key Words:** Adipocytes, Osmium Tetroxide, Lognormal Distribution Equation, Adipose Tissue, Cattle.)

Introduction

Determination of adipocyte size and number is necessary to understand growth and development of adipose tissue of meat animals. One method of measuring adipose tissue cellularity involves fixation with osmium tetroxide of fat cells in adipose tissue slices, followed by counting and sizing of fixed adipocytes with an automatic particle counter (Hirsch and Gallian, 1968). The method has several advantages, including the ease and accuracy with which great numbers of cells can be counted and the accuracy with which the distribution of adipocyte sizes can be determined. Sjöstrom *et al.* (1971) suggested that adipocytes of different sizes are normally distributed, but distributions of adipocytes presented by some workers are skewed (Hood and Allen, 1975; Hood and Thornton, 1979). Because skewed size distributions of adipocytes are observed, the size distribution of adipocytes may not be more accurately described as a normal or a Gaussian distribution function. A possibility is that populations of adipocytes are lognormally distributed.

Therefore, the objective of this study was to examine the use of a lognormal distribution function to describe the size distributions of bovine adipocytes. A computational form of the lognormal distribution described by Siano (1969) and Siano and Metzler (1969) was used to model size distributions of adipocytes determined with an automatic particle counter.

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Materials and Methods

Adipose Tissue Sampling and Fixation. Adipose tissue samples were collected from beef steers exsanguinated after stunning and were kept in a .9% NaCl solution at 37 C until prepared for fixation. Animals ranged from 11 to 15 months of age, and all were fed *ad libitum* a diet consisting of 72% ground shelled corn (IFN 4-02-931), 22% alfalfa-brome haylage (IFN 3-08-147) or oatlage (IFN 3-03-298) and 6% of a pelleted 32% protein supplement. Subcutaneous adipose tissue was taken from either the brisket region or the fat covering above the *longissimus* of the 10th through 12th rib section. Intermuscular adipose tissue was separated from between the *iliocostalis thoracis* and the *longissimus* of the 10th through 12th rib section. Mesenteric adipose tissue was collected after evisceration from tissue between the caudal end of the cecum and the small intestine. Between 30 and 45 min elapsed between animal death and fixation of subcutaneous and intermuscular adipose tissues. Fixation of mesenteric adipose tissues was done at about 90 min after animal death. Adipose tissue samples from several anatomical sites were taken so that the general applicability of the proposed method could be tested.

A weighed section of adipose tissue (approximately 200 mg) was fixed with 5 ml of 2% osmium tetroxide in 50 mM collidine hydrochloride buffer solution at pH 7.6 as described by Hirsch and Gallian (1968). Connective tissue debris was solubilized with 8 M urea as described by Etherton *et al.* (1977), and adipocytes were freed by washing with a .9% NaCl solution containing .01% Triton X-100, pH 10, through a 250-μm nylon mesh filter[7] and collected onto a 20-μm nylon mesh filter[7] Adipocytes were washed from the 20-μm nylon mesh filter into a weighed 400-ml beaker with .9% NaCl. Additional NaCl solution was added to increase volume to 350 milliliters. Beaker plus contents then were reweighed. All NaCl solutions or the NaCl solution containing Triton X-100 that were used for washing and counting adipocytes were filtered before use through a .22-μm filter.[8]

Adipocyte Counting. Distribution of adipocyte sizes was determined by counting samples with a model ZB Coulter Counter attached to a Channalyzer[9] in the expanded or logarithmic mode. A 400-μm aperture was used, and a 90.5-μm standard of polystyrene beads[10] was used to determine the volume for each of the instrument's 100 channels. Standard particles and the fixed adipocytes were assumed to be spherical. The edit feature of the particle counter was not used.

Analysis of Particle Counter Data. Particle counter data were analyzed by fitting the number of cells of each diameter to a normal distribution function and a lognormal distribution function. The form of the normal distribution function used is shown by equation 1:

$$f(t) = y_0\,(1/2\Pi)^{1/2}\,\exp\,(-t^2/2), \qquad (1)$$

where f(t) is the number of cells in a diameter class, y_0 is the parameter adjusting the height of the distribution and t is defined by equation 2:

$$t = (x - \mu)/\sigma. \qquad (2)$$

In equation 2, the parameter μ is the mean diameter of the population of cells, σ is the standard deviation for the population and x is the diameter of adipocytes. A nonlinear regression program was used to fit particle counter data to equation 1 (Barr *et al.*, 1979).

A computer program described by Siano (1969) and Siano and Metzler (1969)[11] for the analysis of electronic absorption spectra was used to fit particle counter data to a lognormal distribution function (equation 3):

$$g(z) = y_0\,\exp\,\{-\ln(2)/\ln(\rho)\cdot \{\ln\{[(z - z_0)(\rho^2 - 1)/w\rho] + 1\}\}^2\}, \qquad (3)$$

where g(z) is the number of cells of a particular diameter, y_0 is the maximal number of cells, ρ is a skewness estimate (equation 4), z is the diameter of the adipocyte, z_0 is the diameter at the maximal number of cells and w is the width of the curve at half the maximal number of cells (equation 5).

[7] Small Parts Inc., Miami, FL.
[8] Millipore Corp., Bedford, MA.
[9] Coulter Electronics, Inc., Hialeah, FL.
[10] Lot No. 5005, Coulter Electronics.
[11] A fortran program was obtained through the courtesy of Dr. D. E. Metzler, Dept. of Biochem. and Biophys., Ames, IA. The program models data to equation 3, and, for the modeling of adipocyte distributions, computations were performed in double precision.

Equations 4 and 5 are:

$$\rho = (z_2 - z_0)/(z_0 - z_1), \quad (4)$$

$$w = z_2 - z_1. \quad (5)$$

The parameters z_1 and z_2 are the diameters at the half maximal number of cells of the left and right sides of the curve, respectively (figure 1). Equation 3 is a modification of a form of the lognormal frequency function described by Yaun (1933). Calculations of the mean, mode, median, standard deviation and higher moments are discussed by Yaun (1933). For the fitting of either normal or lognormal models, the residual sum of squares was minimized.

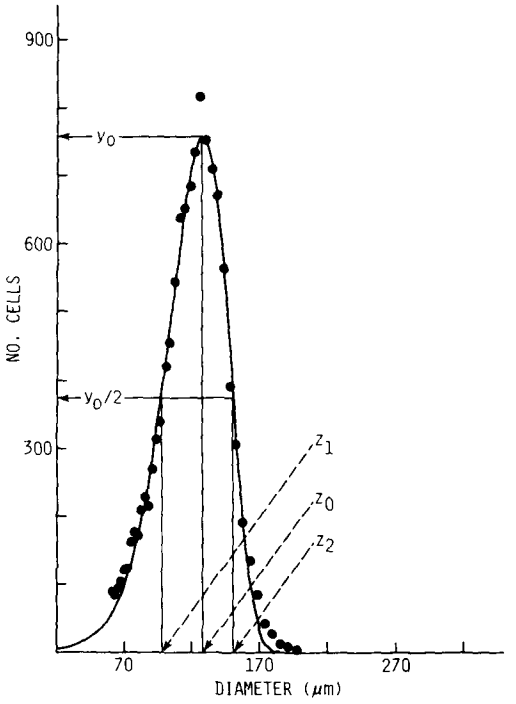

Figure 1. Adipocyte diameter distribution in intermuscular adipose tissue of 13-month-old steer (animal 347 in table 1). Points represent observed counts from particle counter. Solid line represents predicted curve from lognormal model. Osmium-fixed adipocytes (n = 1,000) were sized with a Nikon model M inverted microscope to determine smallest adipocyte; counts below that size were not included during fitting and are not shown. Computational parameters were: y_0, peak height; $y_0/2$, half height; z_1 and z_2, diameters at half height, and z_0, diameter at peak height (see text).

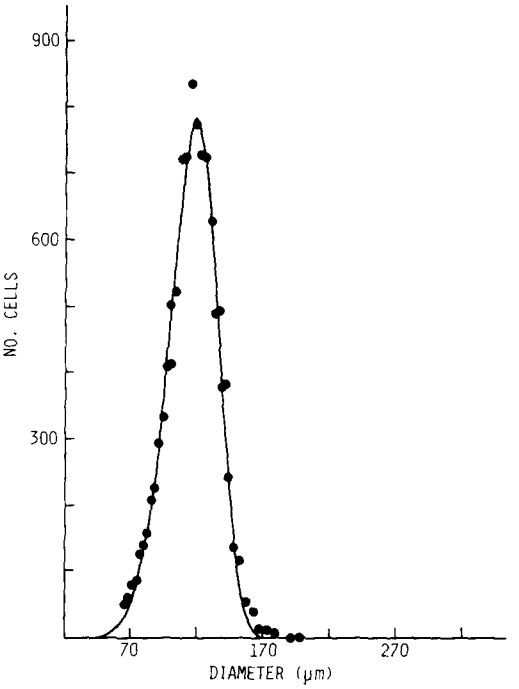

Figure 2. Adipocyte diameter distribution in intermuscular adipose tissue of a 13-month-old steer (animal 242 in table 1). Points indicate observed counts from particle counter. Solid line represents predicted curve from lognormal model. Osmium-fixed adipocytes (n = 1,000) were sized with a Nikon model M inverted microscope to determine smallest adipocyte; counts below that size were not included during fitting and are not shown.

Results

Examples of adipocyte size distributions for various depots are illustrated in figures 1 to 3. For each distribution, the predicted curve for the lognormal model (equation 3) is shown with a solid line. Figures 1 and 2 represent samples of intermuscular adipose tissue from two 13-month-old steers. Figure 3 presents data from a sample of subcutaneous adipose tissue from a 15-month-old steer in which the adipocytes were of a single mode. For each of these three adipocyte distributions, cells less than 70 μm in diameter were not observed microscopically (see figure legends). Some investigators have observed cells in the range from 20 μm to 70 μm in bovine adipose tissue samples (Hood and Allen, 1975; Etherton *et al.*, 1977; Robelin, 1981). We cannot explain these observational differences. In each of the three

figures, there is a small "shoulder" on the larger diameter side of the peak.

Population parameters estimated by fitting a lognormal model to adipocyte size distributions are presented in table 1. With four exceptions, modes and medians of adipocyte size distributions were larger than the means. The mode of a distribution is the location of the maximum of the distribution function, and the value above or below which half of the distribution lies is referred to as the median (Mood *et al.*, 1974). For a normal distribution, the mean, mode and median are equal. The mean adipocyte diameter for all samples of subcutaneous adipose tissue was 102 μm, which is similar to the value obtained by Hood and Allen (1975) for subcutaneous adipocytes of Holstein steers 14 months of age (106 μm).

The normalized third and fourth moments are measures of skewness and peakedness (kurtosis); the values obtained for these parameters by fitting a lognormal model to size distributions of adipocytes are shown in table 1. With three exceptions, the third and fourth moments were close to 0 and 3, respectively. Values of 0 and 3 are expected values for the normalized third and fourth moments of a normal distribution (Snedecor and Cochran, 1967).

The parameter ρ (equation 4) also is an indicator of skewness and would equal 1 for a normal distribution (Siano, 1969). Observed values of ρ were less than 1 for seven of the adipocyte samples examined, indicating that negative skewing was present. Observed values of ρ were greater than 1 for three of the adipocyte distributions, indicating that positive skewing was present.

Bimodal distributions of adipocytes have been observed (Allen *et al.*, 1974; Hood and Allen, 1975). Figure 4 represents a bimodal population of adipocytes observed in a sample of subcutaneous adipose tissue from an 11-month-old steer. The sample were modeled as the sum of two lognormal distributions, and the summed distributions are indicated by curve A. Curves B and C represent the two individual curves that were summed to produce curve A. Microscopic examination of the osmium-fixed adipocytes for the sample used in figure 4 are shown as a histogram in figure 5. While the size ranges in figures 4 and 5 are similar, a somewhat greater proportion of adipocytes larger than 170 μm was observed by microscopic examination of the sample. The reason for this observation is unknown.

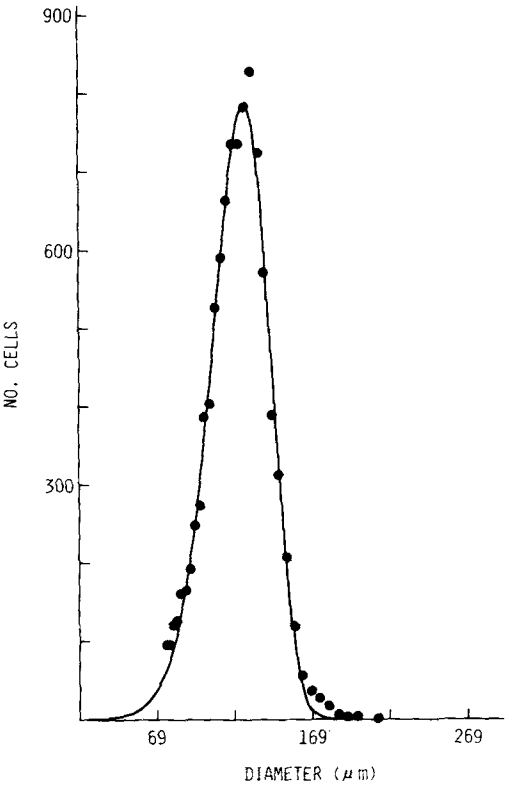

Figure 3. Adipocyte diameter distribution in subcutaneous adipose tissue (backfat) of a 15-month-old steer (animal 487 in table 1). Points indicate observed counts from particle counter. Solid line represents predicted curve from lognormal model. Osmium-fixed adipocytes (n = 1,000) were sized with a Nikon model M inverted microscope to determine smallest adipocyte; counts below that size were not included during fitting and are not indicated.

Population parameters estimated for individual peaks from a bimodal population of adipocytes modeled with a lognormal distribution function are shown in table 2. Means for the two peaks were 34.7 and 96.9 μm and were smaller in both instances than the respective modes or medians. The standard deviation and normalized third moment for the peak with a mean of 34.7 μm (B) were smaller than the same parameters for the peak with a mean of 96.9 μm (C). The normalized fourth moment was smaller for peak C. Negative skewing was indicated for both peaks.

To compare normal and lognormal distribution functions for modeling size distributions of adipocytes, we fitted particle counting data for samples indicated in table 1 to equation 1;

TABLE 1. PARAMETERS ESTIMATED BY FITTING LOGNORMAL DISTRIBUTION TO AUTOMATIC PARTICLE COUNTER DATA FOR VARIOUS ADIPOSE TISSUE LOCATIONS IN BEEF STEERS

Item	Animal									
	543	535	527	487	347	242	214	127	080	060
Age, months	13	13	13	15	13	13	11	13	13	11
Carcass weight, kg	261	248	246	298	234	278	222	221	264	219
Location	SQ[a]	ME[b]	SQ[a]	SQ[c]	IN[d]	IN[d]	ME[b]	ME[b]	SQ[a]	IN[d]
Mean, μm	90.1	112.6	113.4	120.7	118.7	114.6	106.2	127.5	81.9	107.0
Mode, μm	93.9	125.5	106.4	125.6	126.7	117.5	106.4	127.0	101.5	104.8
Median, μm	91.4	123.6	111.1	122.4	121.5	115.6	106.3	127.3	88.9	106.2
Third moment	.03	.10	.19	.26	.51	.10	.00	.01	1.59	.05
Fourth moment	3.05	3.18	3.33	3.47	3.92	3.18	3.00	3.01	5.95	3.09
ρ[e]	.93	.88	1.18	.82	.76	.88	.99	1.03	.64	1.09

[a]Subcutaneous adipose tissue (brisket region).

[b]Mesenteric adipose tissue.

[c]Subcutaneous adipose tissue (backfat).

[d]Intermuscular adipose tissue.

[e]Parameter ρ is an estimate of skewness at half height; ρ would equal 1.0 if skewness was absent.

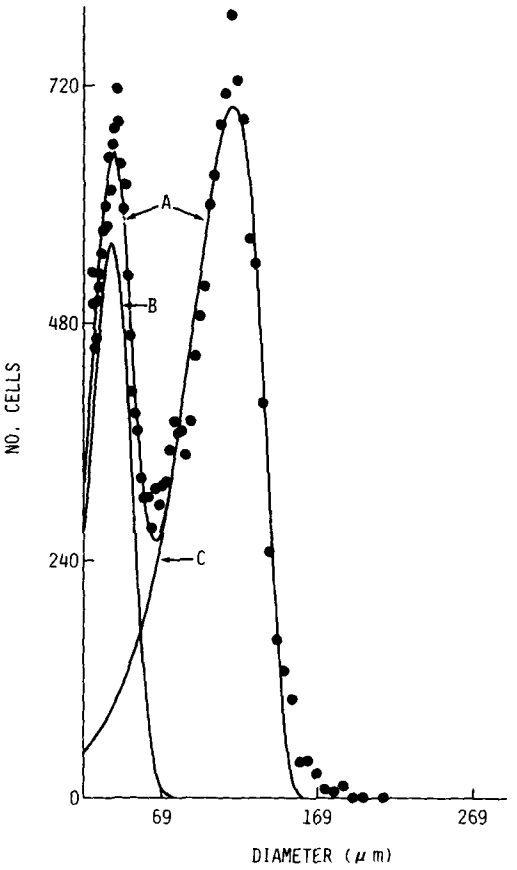

Figure 4. Adipocyte diameter distribution in subcutaneous adipose tissue (brisket region) of an 11-month-old steer (table 2). Points are observed counts from particle counter. Curve A represents predicted curve for sum of two lognormal distributions. Curve B represents predicted lognormal curve for adipocytes of smaller mode. Curve C represents predicted lognormal distribution for adipocytes of larger mode. Note that curves A and C cannot be separated into diameter regions greater than 80 micrometers.

the results are shown in table 3. In general, there was little difference between the mean adipocyte diameter estimated with a normal model of the adipocyte populations and that estimated with a lognormal model. When data were modeled with a normal distribution function, the standard deviation for the adipocyte populations was smaller.

To determine whether the normal or the lognormal model of adipocyte size distributions was better, we calculated chi squares for each; values are presented in table 3. Chi-square values generally were greater for adipocyte size distributions modeled with a normal distribution, but large chi-square values were observed for both models.

The large chi-square values can be attributed to specific regions of the populations of adipocytes, as shown in table 4, which lists the contribution to chi square for three regions of the adipocyte size distributions. For each adipocyte distribution examined in both models, contributions to calculated chi-square values were greater in the two tails of the distribution than in the central region. On the left side (smaller diameters) of the adipocyte distributions, no general trend for either model to make smaller contributions to chi square was observed; on the right side (large diameter) of the adipocyte distributions, the lognormal model generally made larger contributions to chi square. In the center region of the size distribution, the lognormal model was observed to provide a better fit to the adipocyte size distribution, as evidenced by the usually smaller contribution to chi square.

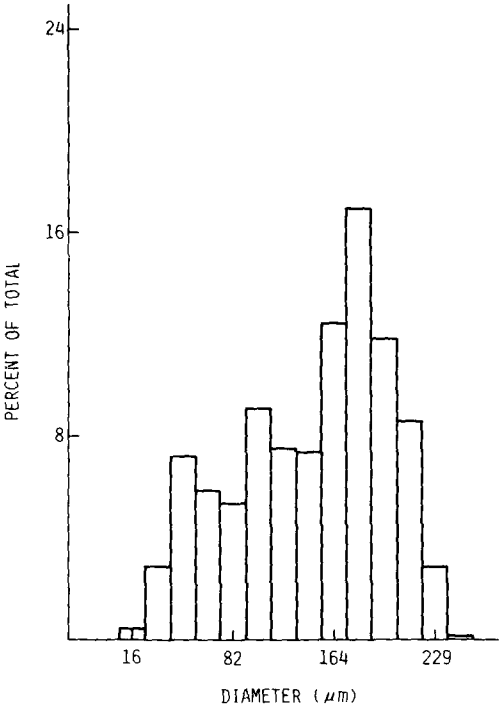

Figure 5. Adipocyte diameter distribution in subcutaneous adipose tissue of 11-month-old steer. Data were obtained by sizing osmium-fixed cells (n = 500) with a Nikon model M inverted microscope. Sample was same as that used with the particle counter in figure 4.

TABLE 2. PARAMETER ESTIMATES FOR BIMODAL ADIPOCYTE DISTRIBUTION IN SUBCUTANEOUS ADIPOSE TISSUE (BRISKET REGION) OF A BEEF STEER AT 11 MONTHS OF AGE[ab]

Item	Peak B	Peak C
Mean, μm	34.7	96.9
Mode, μm	37.5	115.2
Median, μm	35.6	103.6
SD[c]	25.3	48.2
Third moment	.18	2.25
Fourth moment	3.32	7.26
ρ	.84	.59

[a]The data were fitted to the sum of two lognormal distribution functions.

[b]Carcass weight of steer was 160 kilograms.

[c]SD = standard deviation.

Discussion

Lognormal distributions have been used to describe the size of particles from a crumbling cookie (Koch, 1966), ultraviolet spectra of molecules (Siano and Metzler, 1969), frequency distributions of colloidal particle sizes (Herdan, 1953) and the distribution of financial incomes (Aitchison and Brown, 1957). Koch (1969) has suggested that size distributions of bacterial cells approach lognormality. Many diverse phenomena seemingly can be described by a lognormal distribution.

In previous studies of the distribution of adipocyte sizes, Sjöstrom *et al.* (1971) concluded that adipocytes from rat adipose tissue were distributed normally, as determined by counts of osmium tetroxide-fixed adipocytes made with an automatic particle counter. Mersman *et al.* (1973) modeled adipocyte diameter distributions from swine adipose tissues with a Guassian distribution function and reported little skewness of the distribution of adipocyte sizes. Hood and Allen (1975) and Hood and Thornton (1979), however, have presented frequency distributions of adipocytes from bovine and ovine adipose tissues, respectively, that are skewed. Because differing degrees of skewness are observed for distributions of adipocyte sizes, some distribution function other than a normal distribution function may more adequately describe the distribution of adipocyte sizes.

In the present investigations, a lognormal distribution function was used to describe the distribution of bovine adipocytes determined with an automatic particle counter. The computing method allowed the estimation of many population parameters of the adipocyte distribution. Seven of the 10 samples examined displayed only slight departures from normality, as evidenced by the fact that the third and fourth moments were close to 0 and 3, respectively. The parameter ρ, which also measures skewness, was less than 1 for all seven distributions that were close to normality. Three size distributions of adipocytes showed considerably more departure from normality, as evidenced by third and fourth moments greater than 0 and 3, respectively; these same populations had skewness estimates (ρ) greater than 1, indicative of positive skewness. These results suggest that any distribution function chosen to describe size distributions of adipocytes should be capable to dealing with differing degrees of skewness. The method presented in this study is capable of dealing with the differing degrees of skewness observed.

Comparison of normal and lognormal models for describing size distributions of adipocytes suggest that a lognormal model is more appropriate for bovine adipocytes. Lower chi-square values generally were observed for size distributions modeled with a lognormal distribution function. Both models of adipocyte size distribution displayed weaker fits to the data in the left and right extremes of the populations. A greater standard deviation of the size distribution was estimated by a lognormal model, but both models estimated similar means for the size distribution.

Our results suggest that one should consider the lognormal distribution when modeling adipocyte size distributions. It is reasonable to ask, How might adipocyte sizes become lognormally distributed? A simple model that yields a lognormal distribution directly (Koch, 1966) would consider adipocyte size as a function of its previous size and of the sum of the magnitudes of the random factors, such as breed, sex, age, nutritional plane and hormonal responsiveness, that act on the growing adipocyte in a manner proportional to its size. Interestingly, Etherton *et al.* (1977) observed greater rates of glucose uptake per million cells in adipocytes 63 to 102 μm in diameter than in adipocytes 20 to 63 μm in diameter. Other

studies have suggested that rate of lipolysis (Jolly *et al.*, 1980), rate of palmitate esterification (Etherton and Allen, 1980), rate of glucose conversion to glyceride-fatty acids and glyceride-glycerol (Etherton *et al.*, 1981) and insulin sensitivity (Salans *et al.*, 1968) vary as functions of adipocyte size. These variations in adipocyte metabolism with size of adipocyte seem to indicate that the proposed model is plausible, but do not constitute proof.

A more sophisticated model for predicting the distribution of adipocyte sizes seems necessary. That need centers around the potential for increases in adipocyte number during the development of obesity (Johnson and Hirsch, 1972; Faust *et al.*, 1977; Robelin, 1981). The mechanisms controlling the adipocyte proliferation are unclear (Hausman *et al.*, 1980); however, models have been proposed for bacterial systems where increases in cell number are predicted with distributions simulating the lognormal distribution (Koch, 1969). That such models are appropriate for mammalian systems remains to be determined.

A difficulty with using a single distribution function to describe adipocyte sizes is the occasional appearance of bimodal distributions (figure 4 and Allen *et al.*, 1974; Hood and Allen, 1975). A potential solution to this problem is the reexpression of the distribution as a volume frequency distribution[12] (Allen *et al.*, 1974; Hood and Allen, 1975). Such reexpression produces a size distribution of a single mode and may allow for easier estimation of mean sizes for comparison of groups of animals or various treatments. Thus, a normal or lognormal model such as that examined in this paper could be used to estimate means, depending upon one's desire to deal with skewness of the distributions.

In summary, a lognormal distribution function seemed to describe the size distributions of adipocytes from beef steers more acurately than did other distribution functions tested. The lognormal distribution function described by Siano (1969) and Siano and Metzler (1969) provides a computationally convenient method for describing size distributions of adipocytes and obtaining estimates of population parameters such as the mean, mode, median, standard

TABLE 3. COMPARISON OF NORMAL AND LOGNORMAL MODELS FOR DESCRIBING POPULATIONS OF BOVINE ADIPOCYTES[a]

Item	Distribution	Animal								
		543	535	527	487	347	242	214	127	080	060
Mean, μm	Normal	92.4	123.7	108.5	122.8	122.6	115.7	105.9	126.8	92.7	105.7
	Lognormal	90.1	122.6	113.4	120.7	118.7	114.6	106.2	127.5	81.9	107.0
SD[b]	Normal	42.0	18.0	34.0	19.0	23.0	18.0	10.0	12.0	32.0	19.0
	Lognormal	83.0	35.0	73.0	35.0	42.0	34.0	20.0	24.0	59.0	40.0
χ^2 cd	Normal	102.0	783.0	152.0	430.0	838.0	347.0	1105.0	280.0	673.0	284.0
	Lognormal	73.0	1180.0	65.0	139.0	200.0	170.0	1422.0	261.0	229.0	231.0

[a]Normal model refers to equation 1, and lognormal model refers to equation 3 in text.
[b]SD = Standard deviation.
[c]Chi-square was calculated as Σ (residual^2/predicted).
[d]Predicted values less than 5 were omitted from the calculation of chi-square.

[12] See also Figure 2.6 in Allen *et al.* (1976).

TABLE 4. CONTRIBUTIONS TO CHI SQUARE FOR SPECIFIC REGIONS OF ADIPOCYTE SIZE DISTRIBUTION

Region	Model	Animal									
		543	535	527	487	347	242	214	127	080	060
Left	Normal	11[a]	536	10	360	730	296	365	65	132	2
	Lognormal	12	118	16	33	49	49	380	96	13	161
	Range of diameters, μm	<88	<88	<88	<88	<88	<88	<88	<97	<47	<88
Center	Normal	22	84*	31	68*	83*	36*	35*	13	123*	88*
	Lognormal	22	117*	22	47*	34*	26*	43*	17	54*	30*
	Range of diameters, μm	80 to 163	88 to 163	88 to 163	88 to 163	88 to 163	88 to 142	88 to 129	97 to 152	47 to 109	88 to 153
Right	Normal	69	162	111	2	24	14	705	202	417	193
	Lognormal	39	944	27	59	116	96	999	148	161	39
	Range of diameters, μm	>163	>163	>163	>163	>163	>142	>129	>152	>109	>153

[a]Values are Σ residual^2/predicted for specific regions.
*Chi-square is larger ($P<.05$).

deviation, normalized third and fourth moments and ρ, an estimator of skewness. An advantage of the method is that data collected with a particle counter can be used directly to obtain the population parameters of interest.

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