Body Cell Mass and Lean Body Mass in the Growing Beagle[1] (36982)

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Several methods have been used in an attempt to measure the amount of primary energy-exchanging mass of tissue in the body. An early and still used measure of this cell mass was based on the concept of lean body mass (LBM). This mass has been estimated by several methods using at least one of the following assumptions: (a) that LBM has a constant percentage of water, and (b) that the specific gravity of the LBM is constant. Conceptually, while LBM differs from fat-free wet weight (FFWW) of the animal, the difference is not large, probably amounting to no more than 2–3% of the body weight (1). Therefore, for the purpose of this paper, LBM is considered synonymous with FFWW.

Inasmuch as LBM includes extracellular supporting structures as well as tissues primarily involved in energy exchange, the concept of body cell mass (BCM) has been suggested as a more realistic measure of the mass of cells involved with physical and chemical work. One method of determining the BCM is to calculate the volume of intracellular water (ICW) and then assume that the average water content of cells is 70% (1). Body cell mass has been calculated also by measuring the total amount of radioisotope ^{42}K which exchanges with the total potassium pool (1). This method is based on a number of assumptions: (a) at equilibration, complete mixing of the injected ^{42}K has occurred; (b) the total amount of K in the body is a linear function of BCM; (c) the average K–N ratio of the cells is 3 mEq/g; and (d) nitrogen makes up 4% of the cell mass.

The calculation of both LBM and BCM requires the use of "constants," which were calculated from data from adult animals. Therefore, up to the present, calculation of these two parameters was limited to animals that had reached "chemical maturity," and these "constants" should not be used in the young growing animal without experimental validation. However, the necessary data for such calculations became available recently for the beagle, and in this paper, analyses of both LBM and BCM from birth through the whole growth period are presented.

Materials and Methods. A detailed presentation of the management of the beagle colony has been published (2). The volume of ICW was calculated from the difference between the volumes of total body water (TBW) and extracellular water (ECW) measured with tritiated water and sodium thiocyanate, respectively (3). A detailed description of the procedures used to obtain the chemical data on the beagle has been reported (4). The body was dissected into different parts which were washed with deionized water, homogenized in a known volume of water, and stored in vials in a deep freeze. Aliquots of each portion were ashed to a constant weight at 550° in a muffle furnace and the ash was analyzed for K by flame photometer. The fat in the tissues was extracted with methylene chloride. The amount of protein in the tissues was analyzed by the method of Lowry *et al.* (5), and 16% of the total weight of protein was assumed to be nitrogen. The total amount of K, fat and protein in the beagle was the sum of each constituent of all the tissues.

The BCM of the beagle was calculated using the following equations (1). The first one, BCM = ICW/0.70, assumed that the average water content of cells was 70%. The second equation, BCM = [K/(K/N)] ×

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TABLE I. Body Cell Mass (BCM) and Lean Body Mass (LBM) of the Beagle During Growth

Dog No. and Sex	Body Weight (kg)	ECW[a] (g)	FFDW[b] Skeleton (g)	ECT[c] (g)	K_f[d] (mEq)	N_f[e] (g)	BCM/N[f] (g/g)	$BCM = \frac{K}{K/N} \times 25$ (% B. Wt)	$BCM = \frac{ICW^g}{0.70}$ (% B. Wt)	BCM = LBM − ECT (% B. Wt)	LBM (% B. Wt)
BL1 ♂	0.31	179	27	206	6.9	5	19.8	18	47	26	92
BL2 ♀	0.36	134	29	163	10.4	5.8	37.9	23	45	50	96
AQ3 ♀	0.28	187	15	202	6.8	4.5	15.0	19	36	20	92
BD3 ♀	0.25	186	16	202	6.6	4.0	12.2	21	30	17	97
BC1 ♂	0.40	205	47	252	10.9	6.4	26.6	22	52	32	95
BH5 ♂	0.33	179	28	207	9.1	5.6	22.0	22	53	32	95
BJ5 ♀	0.52	277	40	317	13.2	7.7	25.7	20	46	30	92
BG6 ♀	0.36	183	35	218	10.1	6.1	21.1	22	37	31	91
BK1 ♂	0.57	283	47	330	16.4	8.8	27.5	23	54	36	93
BK3 ♀	0.47	251	38	298	16.2	7.2	27.7	28	52	34	95
AY1 ♂	1.03	479	72	551	41.1	20.0	18.5	32	41	31	85
AX1 ♂	0.77	329	83	412	28.2	14.2	25.2	29	52	38	92
AW1 ♂	0.99	482	70	552	33.5	19.7	19.2	27	47	32	88
BG2 ♀	1.11	500	133	633	34.8	22.1	19.2	25	68	32	89
BI4 ♀	1.64	635	143	778	42.3	25.9	30.8	21	78	40	88
BF3 ♂	2.07	883	223	1116	60.1	38.4	28.5	23	44	29	83
AY4 ♀	1.57	820	133	953	53.3	23.7	13.7	27	43	18	79
AX2 ♂	1.54	1064	105	1169	50.7	26.6	5.3	26	32	8	83
AW2 ♂	1.63	942	205	1147	47.1	26.7	13.1	23	65	16	87
AK1 ♂	2.95	1282	270	1552	71.4	57.8	18.0	19	32	27	80

AK6	♂	2.69	1397	336	1733	70.5	41.1	12.8	21	21	15	80
AZ3	♀	1.07	547	136	683	29.0	18.2	20.4	22	59	28	91
BE7	♀	2.55	744	444	1188	62.4	46.4	36.5	20	40	40	87
AS4	♀	2.22	1070	313	1383	63.1	39.8	21.3	23	49	29	91
AE4	♀	4.1	1786	500	2286	164.6	74.9	24.9	32	55	35	91
AD2	♂	4.7	1565	656	2221	135.4	90.6	24.5	23	42	38	85
AC1	♂	4.6	1792	572	2364	127.4	82.4	22.4	22	45	32	84
TT4	♀	5.8	2273	683	2956	225.3	131.5	16.5	31	36	31	83
WW8	♂	6.0	2632	855	3487	276.5	139.8	14.8	37	41	29	87
UU5	♀	5.8	2344	787	3131	323.0	135.4	12.6	45	55	29	84
OO1	♂	8.9	2532	946	3478	572.5	262.7	18.9	51	38	50	89
NN5	♂	12.2	3125	1625	4750	660.2	269.4	27.2	43	37	50	89
KK7	♂	7.2	2740	1002	3742	371.9	182.7	16.4	41	60	35	87
KK3	♀	6.2	2516	660	3176	315.4	168.2	12.2	41	51	30	81
PP6	♀	9.2	3120	780	3900	474.2	241.0	13.5	41	39	31	74
JJ2	♀	7.4	2331	782	3113	436.1	224.2	13.9	47	52	37	79
T1	♂	7.4	2279	911	3190	320.5	166.4	19.4	35	61	34	77
S4	♂	8.0	2717	733	3450	378.1	191.5	22.0	38	42	46	89
R5	♀	6.9	2222	572	2794	362.0	176.2	18.8	42	76	44	85
I7	♂	8.4	2874	977	3846	452.1	275.2	14.4	43	50	41	87
G6	♀	8.3	1969	1003	2972	463.5	242.9	21.4	45	61	54	89
G1	♂	8.7	2083	904	2987	454.2	216.5	25.3	42	65	57	91

[a]Extracellular water (thiocyanate space) [b]Fat-free dry weight [c]Extracellular tissues (ECW + FFDW skeleton) [d]Total potassium [e]Total nitrogen [f]BCM = LBM – ECT [g]Intracellular water

25, assumed that 1/25 of the BCM was nitrogen and that the K/N ratio was 3.0 mEq/g (1). As data were available for the amount of total body protein, the BCM could have been calculated directly from protein if a "constant" for the amount of protein in the cells was assumed. However, as no indirect method has been developed for the measurement of total body protein, and as exchangeable K presumably measures total body K (1), the above equation was used.

Finally, on the assumption that the body weight of an animal was made up of BCM, fat, and extracellular tissues (ECT), the BCM was calculated by the equation BCM = LBM — ECT (1). The ECT included a fluid component, the extracellular water (ECW), and a solid component, the skeleton. For the calculation, it was assumed that the sum of the extracellular water and the fat-free dry weight of the skeleton constituted the ECT.

Results. The data on BCM are given in Table I. The mean BCM was 33% of body weight from birth to 1 yr of age when calculated from LBM — ECT. However, the manner in which the data were distributed suggested that there might be a significant change in BCM after a body weight of 5.7 kg (after the age of 4 mo). Recalculation of the data gave a mean BCM of 30% of body weight from birth through the fourth month and 41% from 4 mo to 1 yr. The difference was significant at $p < 0.01$ level.

The mean BCM was 31% of body weight for the whole growth period when calculated from total potassium by the equation BCM = [K/(K/N)] × 25 when the K/N ratio was assumed to be 3 mEq/g and 25 was the "nitrogen coefficient" on the assumption that 1/25 of BCM was nitrogen. Again, the distribution of the data suggested that a change occurred at a body weight of 5.7 kg. The mean BCM was 24% of body weight from birth to 3 mo old and 41% from 4 mo to 1 yr old. The difference was significant at $p <$ 0.01 level.

The equation BCM = ICW/0.70 gave a mean BCM of 48% of body weight for the 1-yr period; 47% for 0 day to 4 mo and 51% after 4 mo. These differences were not statistically significant.

The mean LBM (FFWW) of the beagles decreased rapidly from a value of 97% of body weight at birth to 85% at 1.2 kg at an age of 1–1.5 mo. Above a body weight of 1.2 kg, the LBM remained at 85% of body weight.

Discussion. Most of the measurements of BCM have been made on the adult human using exchangeable potassium, and a mean of 33% for females and 40% for males has been reported (1). Because the human data were obtained presumably after chemical maturity was attained, any comparison of the data on the dog with that of the human should be confined to the period after the beagles had reached chemical maturity at the age of 4 mo (4). In addition, it must be remembered that any differences between the results could represent a species difference. Finally, no attempt was made to differentiate between the BCM of the sexes in beagles as was done for the human because of the insufficient number of dogs used.

After chemical maturity the mean BCM was 41% of body weight when calculated from either LBM — ECT or (K_t/3.0 × 25), a value comparable to the 40% reported for the male human (1). However, before chemical maturity, the equation LBM — ECT gave a BCM of 30% of body weight, which was significantly different ($p < 0.01$) from the 24% obtained from (K/3.0) × 25. Thus, both methods gave similar results after chemical maturity, but not before.

The K/N ratio of 3.0 mEq/g and "nitrogen coefficient" of 25 used for the calculation of BCM in the human need not necessarily be applicable to the dog. In spite of this, after chemical maturity in the dog, these "constants" gave a result identical to that calculated from LBM — ECT. The latter calculation required only the assumption that ECW and fat-free wet weight of the skeleton constituted ECT, while calculation of BCM from total potassium required the use of two constants whose values were estimates only. If it was assumed that LBM — ECT gave a reasonable estimate of BCM, then the identical result obtained with total potassium suggested that the constants used in the latter

TABLE II. Comparison of Mean BCM Expressed as Percentage of Body Weight Calculated by Three Methods.

Mean BCM	0–3 mo N = 27	4 mo–1 yr N = 15
LBM − ECT	30.0 ± 1.7[a]	41.0 ± 2.4
$\frac{K_t}{3.0\ mEq/g} \times 25$	24.0 ± 0.7	41.0 ± 1.2
$\frac{ICW}{0.70}$	47.0 ± 2.3	51.0 ± 3.0

[a] Mean ± SE.

calculation must be valid after chemical maturity, but not before.

However, calculation of these constants for the dog gave values different than those used for the human. The mean K/N ratio for the beagle was 1.7 mEq/g through the first 3 mo and 2.0 mEq/g for the remainder of the growth period, and they differed significantly ($p < 0.01$). The ratio of 2.0 mEq/g after chemical maturity was the same as that reported for the pig (7). The calculation of a nitrogen coefficient for the dog was based on the determination of BCM from LBM — ECT; consequently, the value obtained depended on the validity of that calculation. The nitrogen coefficient decreased from a value of 22 before chemical maturity to 18 after. Since the nitrogen coefficient was determined from (LBM — ECT)/N, the BCM calculated from (K/1.7) × 22 before chemical maturity and (K/2.0) × 18 after chemical maturity will be the same as that calculated from LBM — ECT.

In spite of the differences in the nitrogen coefficient and K/N ratio between the human and the beagle, calculation of the BCM of the beagle from the human constants gave a value identical to that calculated from LBM — ECT, but the agreement would appear to be fortuitous. In the human, the ratio N coeff $(K/N)^{-1}$ using a nitrogen coefficient of 25 and a K/N ratio of 3.0 mEq/g gave a constant of 8, while the data for the dog gave a constant of 9. Because the difference between the constants was small, the calculated BCM was therefore similar. However, before chemical maturity N coeff $(K/N)^{-1}$ gave a constant of 13 for the beagle; consequently, the use of the constant 8 underestimated the BCM.

Although both methods of calculating BCM before chemical maturity gave different values, they both showed a significant increase in BCM when the dogs were between 3 and 4 mo old. In the calculation of BCM from LBM — ECT, an increase in BCM from 30 to 41% occurred in the 3- and 4-mo-old dogs (Table II). There was no change in LBM (percentage of body weight) at this time, but there was a decrease in ECW (percentage of body weight) which resulted in a reduction of ECT. The increase in BCM from 24 to 41% when the equation (K/3.0) × 25 was used was due to a significant increase in total potassium (mEq/g) between the third and fourth months (4).

The BCM calculated by the ICW method remained constant throughout growth; not an unexpected finding, as it was reported previously that ICW was a constant percentage of the body weight (3). Also, the BCM when calculated from ICW was consistently higher (by a mean of 15% of body weight throughout growth) than that calculated from LBM — ECT. The difference in results may be explained in part by the use of the assumption in the ICW calculation that water constituted a mean of 70% of the cells (1). This assumption can at best be described as an educated guess, but if water constituted more than 70% of the cell, the calculated BCM from ICW would be decreased.

The calculation of LBM (as percentage of body weight) revealed a rapid decrease from birth to a body weight of 1.2 kg (1.5 mo of age), and then the LBM stabilized through the remainder of the growth period. The significant increase in BCM which occurred between a body weight of 4.7 and 5.7 kg when either K or LBM — ECT was used for its calculation was not detectable in the LBM. This discrepancy can be explained by the fact that LBM is made up of two components, BCM and ECT, and an increase in BCM could be obscured by a proportionate decrease in ECT. In the beagle, because both the LBM and ECW decreased significantly at the age of 1.5 mo (1.2 kg body wt), no change in the BCM would be detected.

Summary. The lean body mass (LBM)

was considered equivalent to fat-free wet weight (FFWW), and it decreased from 97% of body weight at birth to 85% when the pups were 1.5 mo old, after which it did not change significantly throughout the rest of the growth period. The body cell mass (BCM) was calculated by three methods: ICW/0.70, [K/(K/N)] × 25, and LBM — ECT. The BCM calculated from ICW/0.70 did not change significantly with growth, and a mean of 48% was calculated from 0 day to 1 yr. With the other two methods of calculating BCM, there was a significant increase between the third and fourth months. The equation [K/(K/N)] × 25 gave a mean BCM of 24% for 0 day to 3 mo and 41% from 4 mo to 1 yr, while the means calculated from LBM — ECT for the corresponding periods were 30 and 41%. Before chemical maturity the two methods gave significantly different results, but their agreement after chemical maturity was fortuitous. The increase in BCM between 3- and 4-mo-old dogs when calculated from total K was accounted for by a significant increase in K at chemical maturity, while the increase in BCM when calculated from LBM — ECT was the result of a reduction in ECW (percentage of body weight).

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