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Density of Fat and Bone Mineral of the Mammalian Body

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The density at 37 C. of adipose, muscle, bone marrow and brain fat was determined by pyknometry. Adipose fat showed a density of 0.9008 for rabbit and 0.9009 for dog, with coefficients of thermal expansion of 1.16×10^{-3} and 1.00×10^{-3}, respectively. The density of muscle fat was 0.9279 for rabbit and 0.9327 for dog, with coefficients of thermal expansion of 0.85×10^{-3} and 0.88×10^{-3}, respectively. The density of bone marrow fat of the dog was 0.9034, and the density of brain fat was 1.0258 for the same mammal. The coefficient of thermal expansion for these fats were 0.69×10^{-3} and 0.16×10^{-3}, respectively. Differences in densities were explained in part by differences in cholesterol and phospholipid composition. The value of 0.900 for the density of total body fat, commonly used in body composition calculations, is a good approximation, despite differences in density of fat extracted from different tissues of the mammalian body, because adipose fat accounts for such a large portion of total body fat. The density of bone mineral, prepared from beef and dog whole bones by extraction with ethylene diamine, was also determined by pyknometry. The density of beef bone mineral was 3.000 at 36 C., and the density of dog bone mineral was 2.965 at 36.7 C. The value of 3.000, commonly used for the density of bone mineral in body composition calculations, is therefore accurate.

ALTHOUGH BODY COMPOSITION in living animals has been determined by a variety of different indirect approaches, densitometry has been widely used.[1-4] The density of the individual body components, however, taken into consideration in the calculations of body composition is not well known. Spivak in 1915[5] recognized the need for more information in this respect in order to permit with a simple equation, involving body density, the quantitative determination of the main components of the "human alloy."

The density and thermal expansion coefficient of the mammalian muscle have been recently determined[6] and the theoretic value for the density of "fat-free cells" has been calculated from the composition and density relationship. Fidanza et al.[7] have investigated the density of fat extracted from subcutaneous and internal mammalian adipose tissue. Values for the density of bone mineral have been derived from the density of dental tissue[8,9] and from bone preparations.[9]

The present study investigated the density of fat extracted from muscle, brain and bone marrow of different rabbits and dogs and also the density of bone mineral prepared from beef and dog bones.

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Material and Methods

The animals were obtained from the University Animal Hospital. They were killed either by a blow on the head (rabbits) or exsanguination (dogs). The subcutaneous adipose tissue from the abdominal region was rapidly dissected. The muscles from the thighs and legs were carefully dissected and all visible fat, extraneous connective tissue and tendons were removed. Composite rabbit muscle samples were necessary in order to obtain the amount of fat required for all the determinations. Each sample of fat extracted from rabbit tissues represented three animals. Brain tissue and bone marrow were obained by opening the bone structures with a saw.

The tissue samples were ground in a meat grinder when necessary and slurred in a Waring blendor with acetone. The slurries were dried in a vacuum oven at 50 C. and about 15 mm. Hg. pressure. Dried cakes were pulverized in a porcelain mortar and dried again in the vacuum oven.

The fat was obtained by continuous extraction with anhydrous ethyl ether in a large Soxhlet apparatus. The ether extracts were dried in vacuum after removal of the solvent.

The density of fat was determined in small pyknometers with a capacity of about 3.3 ml. using the method described by Fidanza et al.[7] Adjustments to the mark were done in a thermostatically controlled incubator with glass door, through an opening on the top, with kerosene previously warmed to the same temperature.

The coefficient of thermal expansion was obtained by determining the density at about 37 C. as explained above, and then readjusting to the mark with more kerosene at about 22 C. after thermal equilibrium was reached. Exact temperatures at each adjustment were recorded. Values for the coefficient of thermal expansion (K) were computed from the equation: $V_1 - V_2 = K(T_1 - T_2)$, in which V_1 and V_2 are the specific volumes at temperatures T_1 and T_2.

The total cholesterol determination in the fat samples was carried out using the method of Phil[10] and the Liberman-Burchard reagent described by Abell et al.[11] The phosphorus concentration was determined by the modification suggested by Ammon and Hinsberg[12] to the method of Fiske and SubbaRow.[13] Phospholipid concentration was obtained by multiplying the phosphorus value by the usual factor of 25. All determinations were done in duplicate.

The bone samples were cow's tibias obtained from a meat market and composite samples of tibia and femur from dogs obtained in the laboratory. The shafts were used in the preparation of the bone mineral samples. Bone mineral was prepared by extracting dried bone powder (40 mesh) with 1:1 ethyl alcohol-ether mixture in the Soxhlet extraction apparatus for 24 hours, and a second extraction with boiling ethylene diamine (85 per cent) under reflux during 48 hours with continuous bubbling of nitrogen through a capillary tube. The bone-solvent volume ratio used was 1 to 25. The bone mineral thus prepared was washed with CO_2-free water until it was free from ethylene diamine, and then dried under vacuum.

The density of the bone mineral was determined with the pyknometer technic as described for the density of fat, and the air trapped in the powder was eliminated by vacuum extraction prior to the final adjusment with kerosene. Temperature was recorded.

The ash content of bone mineral was determined by the official method for ash determination in foods described by the AOAC.[14]

Results

The density and the coefficient of thermal expansion of fat extracted from different tissues of rabbit and dog are presented in table 1. A value of 0.9008 was obtained at 37 C. for the density of fat from adipose tissue of the rabbit and a value of 0.9009 for the dog. The coefficients of expansion were 1.16×10^{-3} and 1.00×10^{-3}, respectively. Muscle fat from rabbit gave a density at 37 C. of 0.9279, while the density of fat extracted from dog's muscle was 0.9327 with coefficients of thermal expansion of 0.85×10^{-3} and 0.88×10^{-3}, re-

Table 1.—*Density of Fat (Ether Extract) Extracted from Different Tissues*

Sample no.	Tissue	Density at 37 C. Gm., cc^{-1}	Expansion coefficient x 10^{-4} Gm., cc^{-1}, C.$^{-1}$	Temperature range C.
		Rabbit		
1	Adipose	0.8987	1.20	21.7–35.2
2	Adipose	0.9015	1.10	22.0–36.0
3	Adipose	0.9022	1.17	21.8–35.7
	$\bar{x}$	0.9008	1.16	21.9–35.6
4	Muscle	0.9235	1.03	21.1–35.5
5	Muscle	0.9302	0.78	20.5–34.8
6	Muscle	0.9300	0.73	20.5–34.7
	$\bar{x}$	0.9279	0.85	20.7–35.0
		Dog		
1	Adipose	0.8994	1.28	23.0–36.2
2	Adipose	0.9022	0.72	24.8–36.3
3	Adipose	0.9011	—	——
	$\bar{x}$	0.9009	1.00	23.9–36.3
4	Muscle	0.9444	1.38	22.2–34.8
5	Muscle	0.9240	0.53	24.8–36.2
6	Muscle	0.9298	0.74	26.2–37.0
	$\bar{x}$	0.9327	0.88	24.4–36.0
7	Bone Marrow	0.9048	0.58	24.9–36.8
8	Bone Marrow	0.9020	0.80	25.5–36.8
	$\bar{x}$	0.9034	0.69	25.2–36.8
9	Brain	1.0225	0.12	24.6–37.6
10	Brain	1.0290	0.20	24.7–36.6
	$\bar{x}$	1.0258	0.16	24.7–37.1

spectively. The density of bone marrow fat from dog was 0.9034 with a coefficient of expansion of 0.69×10^{-3} while for brain fat was 1.0258 with 0.16×10^{-3} as the coefficient of thermal expansion.

In table 2 the composition of several fat samples is presented. The cholesterol and phospholipid content of brain fat is greater than in muscle or adipose fat. The cholesterol and phospholipid concentration is greater in rabbit muscle fat than in adipose fat. The cholesterol concentration of dog muscle fat is lower than that of rabbit muscle fat, but the phospholipids appear in greater concentration in dog muscle fat.

The density and ash content of bone mineral prepared from beef and dog bones are presented in table 3. Beef bone mineral showed a density between 2.9930 and 3.0066 with an ash content between 96.17 and 96.46 and dog bone mineral between 2.9624 and 2.9667 with an ash content between 95.17 and 95.47.

Discussion

Although the average densities for adipose tissue fat reported in this paper are slightly lower than those reported by Fidanza et al.[7] for the same animals,

Table 2.—*Cholesterol and Phospholipid Composition of Fat (Ether Extract) Extracted from Different Tissues*

Sample no.	Tissue	Total cholesterol Gm.%	Phospholipids Gm.%	Ch./Pl.
		Rabbit		
1	Adipose	0.154	0.71	0.217
2	Adipose	0.138	1.02	0.136
3	Adipose	0.126	0.87	0.145
	$\bar{x}$	0.140	0.87	0.166
4	Muscle	4.138	11.37	0.364
5	Muscle	6.688	14.78	0.454
6	Muscle	6.799	15.46	0.440
	$\bar{x}$	5.875	13.87	0.419
		Dog		
1	Muscle	2.652	34.23	0.077
2	Brain	24.056	50.64	0.475

The iodine and saponification numbers[14] of rabbit adipose fat were 87.6 and 172.6; in rabbit muscle fat, 76.7 and 180.2; in dog adipose fat, 73.9 and 185.1; in dog muscle fat, 80.6 and 178.6; in dog bone marrow fat, 72.0 and 185.5.

Table 3.—*The Density and Ash Content of Bone Mineral*

Animal bone	Temperature C.	Density Gm., cc^{-1}	Ash* Gm.%
Cow tibia	36.0	2.9930	96.17
Cow tibia	36.0	3.0066	96.46
	$\bar{x}$	2.9995	96.32
Dog femur and tibia	36.7	2.9624	95.17
Dog femur and tibia	36.7	2.9667	95.47
	$\bar{x}$	2.9646	95.32

*The ash content corresponds to the same batch of sample of bone mineral, but does not represent the particular aliquot used in the determination of density.

the range of values is similar. Fat extracted from bone marrow gave also similar values. The density of fat from muscle is significantly higher than the density of fat from adipose tissue and bone marrow. Finally, the brain fat showed the highest density of all the fats studied. A quite marked difference in composition between the fats studied is shown in table 2. These differences could be responsible for the differences observed in fat densities. Thus, the density of muscle fat was calculated from the composition using the density of cholesterol as 1.067,[15] phospholipids, 1.035[16] and neutral fat as 0.900.[7] Neutral fat was obtained as the difference of the total mass and cholesterol and phospholipid concentration. The theoretic values calculated in this way for the density of muscle fat from rabbit ranged from 0.9195 to 0.9286, which approximates the average value of 0.9279 obtained in this study by the direct method. For dog muscle fat the theoretic value of 0.9463 is slightly higher than the ob-

served average value of 0.9327, but it is quite similar to the upper limit of 0.9446 reported here.

The cholesterol concentration reported by Del Vecchio et al.[17] for muscle of rabbit agrees very well with the findings in this study when compared on the basis of total ether extract.

So far it has been shown that the densities of the fats extracted from muscle and nervous tissues differ considerably from the values of the density of depot fat. It would be desirable to take into account these densities when dealing with the calculation of body components. Unfortunately, there is no way of determining precisely the relative amounts of muscle and nervous tissue fat in the individual body. On the other hand, in considering the total quantities of these fats in the body, it is evident from cadaver analyses[18] that they do not amount to more than a few hundred grams. Thus the total lipids of brain and main nervous tissue have been estimated as 200 to 300 grams for the adult man.[1] The lipids of muscle could amount to a similar quantity. If desired, it would be possible to make an approximation and correct for the density of these lipids, but this correction would not contribute greatly to a better estimation of the body components. It is concluded, therefore, that the value of 0.900 for the density of fat is a good approximation to be used in the calculation of body composition when body density is involved.

Various methods for preparing bone mineral have been described. Ashing of the bone has been excluded on the basis of the changes in composition of the mineral produced by ignition.[19,20] The classic method of Gabriel[21] and the modification of Crowell et al.[22] have the advantage that mineral components are not dissolved,[22,23] although calcium citrate may be changed to carbonate.[24] The method employed in this study using ethylene diamine gives the bone crystal without any apparent change in composition.[25]

The density of dental hard tissues have been reported by several authors. Manly et al.[8] found a density between 2.89 and 3.00 for dried enamel, 2.00 and 2.30 for air dried dentin and between 2.01 and 2.05 for cementum. Dallemagne and Melon[9] studied the densities of bone mineral from beef bone and dental tissues, prepared by the method of Gabriel.[21] Bone mineral from bone had a value of 2.99 and bone mineral prepared from different dental tissues had a value around 3.00. These authors concluded that the density of the bone mineral prepared from dental tissues is of the same order of magnitude as the density of bone mineral prepared from whole bone. The findings in the present study confirm the values reported by Dallemagne and Melon for the bone mineral prepared from whole bone.

It is evident, therefore, that the density of 3.00 generally used as the density of bone mineral in body composition calculations should be considered as the value of choice.

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