ESTIMATION OF THE CONSTANCY OF DENSITY OF THE FAT-FREE BODY *

By EDWARD J. WERDEIN † AND LAURENCE H. KYLE

(From the Department of Medicine, Georgetown University School of Medicine, the Georgetown University Hospital, Washington, D. C., and St. Mary's Hospital, Rochester, N. Y.)

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Implicit in the estimation of body fat by densitometric measurement is the assumption that the fat-free body mass of normal persons possesses a constant density. This is true whether one utilizes the concept of Behnke, Feen and Welham (1), who visualized a lean body mass of specific gravity 1.1, containing a small amount of essential lipids, or the standard man of Keys and Brôzek (2), who is neither starved nor obese. Estimation of body fat by determination of total body water is, as pointed out by Messinger and Steele (3), subject to the same basic assumption. The formula of Siri (4), developed to avoid the assumption of a constant water content of the fat-free body and to permit correction for abnormal hydration, is nonetheless predicated on the basis of a known and constant density of the nonfat solids of the body.

Unusual deviations in the density of the fat-free body are to be expected in conditions of abnormal hydration of the body and with significant loss or gain of protein and minerals. Consideration must also be given to the possibility that there exists considerable deviation of density of the fat-free body in normal subjects, if for no other reason than the known biological variability in other spheres. Such deviation would not necessarily involve hydration but could be the result of variation of density of the mineral mass of the body or deviation from the estimated mineral: protein ratio.

Although no direct approach is available, indirect methods can provide some evidence as to the variability of fat-free body density. Significant deviation of percentage of water in the fat-free body of normally hydrated subjects, estimated by parallel measurements of body density and total body water, would indicate abnormal density of the fat-free body. Such study was conducted in a group of subjects of varying fat content and in patients selected because of known abnormality of their mineral mass.

MATERIALS AND METHODS

Subjects of the study were normal hospital personnel and selected patients with a wide range of body build who were considered to be "normal" as far as body constituents and compartments were concerned. Because of the facilities for underwater weighing, the subjects were predominately male and with two exceptions, noted in Table I, ranged in age from 18 to 39. To highlight deviation from the normal on the basis of abnormal skeletal density, measurements were also made in osteoporosis and in a man with osteosclerosis (marble bone disease).

Body density (D) was measured by underwater weighing, with correction for residual lung volume by the helium dilution technique, as described from this laboratory (5). Total body water (TBW) was measured by volume distribution of antipyrine or D_2O as outlined previously (5). Radio-antipyrine was also used in a few subjects as described by Talso and associates (6). Body fat (F) was estimated by substitution into the Pace-Rathbun equation (2), corrected for temperature and density of fat:

$$\% \text{ fat} = \frac{5.120}{D} - 4.684. \qquad (A)$$

Weight of the fat-free body (FFB) was determined by subtracting the amount of fat from the total body weight. Hence, the water content of the fat-free body was calculated:

$$\% \text{ water} = \frac{TBW}{FFB}$$

and used as an index of the degree of deviation of the density of the fat-free body from the norm, not with the idea that there existed abnormality of hydration, but with the assumption that abnormally high or low percentage of water would reflect variability of the fat-free body density.

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† Former Research Fellow of the National Institutes of Health. Present address: St. Mary's Hospital, Rochester, N. Y.

The measured values for D and TBW were substituted into the fat prediction equation of Siri (4) to minimize any possible error in fat estimation introduced by a true abnormality of hydration:

$$\% \text{F} = \left(\frac{2.118}{\text{D}} - 0.780 \text{ TBW} - 1.354 \right) \times 100. \quad \text{(B)}$$

The density of the fat-free body (D_{ffb}) was obtained by dividing fat-free body weight by its volume.

RESULTS

Table I summarizes the results of the outlined determinations in 30 normal subjects. Body density ranged from 0.962 in an extremely obese woman to 1.079 in a thin, well-conditioned man. Percentage of water in the fat-free body, as determined by independent measurements of body density and total body water (A) was 67.5 ± 5.5 but the range extended from 53.1 to 74.8 per cent. Calculations based on substitution of D and TBW in Siri's fat prediction equation demonstrated, as would be expected by the basic assumptions of the equation, a higher mean value and less spread of percentage of water in the fat-free body.

TABLE I

Relationship between total body water and fat-free body *

Subject	Weight	D	TBW	H_2O/FFB (A)†	H_2O/FFB (B)‡	Sex	Age
	kg			%	%		*yrs*
1	78.8	1.059	35.5	53.1	63.8	M	28
2	75.9	1.064	37.0	55.9	65.6	M	26
3	81.0	1.049	36.6	56.3	65.7	M	24
4	76.1	1.060	39.2	60.3	67.9	M	27
5	85.5	1.037	40.2	62.9	69.2	M	28
6	85.0	1.038	40.5	63.5	69.6	M	30
7	93.1	1.019	39.5	64.3	69.9	M	45
8	79.5	1.044	40.1	64.7	70.2	M	32
9	66.1	1.064	37.5	65.1	70.5	M	28
10	72.9	1.079	45.1	65.8	70.8	M	27
11	71.5	1.037	35.9	67.2	70.7	M	31
12	63.4	1.046	33.8	67.6	71.6	M	35
13	74.3	1.056	42.1	67.8	71.7	M	29
14	70.8	1.017	31.5	68.5	71.9	F	28
15	179.5	0.995	66.2	68.5	73.4	M	28
16	63.2	1.062	37.6	69.0	72.2	M	30
17	83.7	1.057	48.6	69.1	72.3	M	26
18	74.9	1.046	41.0	69.4	72.4	M	33
19	121.4	0.962	30.5	69.5	72.3	F	18
20	73.0	1.043	39.5	69.8	72.6	M	29
21	81.9	1.050	46.2	69.8	72.6	M	27
22	171.5	0.976	52.4	69.8	72.8	M	24
23	65.5	1.041	36.2	72.1	73.7	F	23
24	66.0	1.009	29.3	72.7	73.8	F	39
25	59.2	1.051	35.0	72.8	73.8	M	27
26	77.1	1.048	45.1	73.2	74.2	M	33
27	56.8	0.979	19.0	73.6	73.9	F	14
28	50.4	1.046	29.3	73.6	74.4	F	21
29	129.4	1.028	68.0	74.7	74.6	M	20
30	72.3	1.070	48.6	74.8	74.8	M	31
Mean				67.5	71.4		
SD				±5.5	±2.7		

* D = density, TBW = total body weight; FFB = fat-free body.
† Modified Pace-Rathbun equation.
‡ Siri formula; see Methods for these equations.

TABLE II

Reproducibility of body partition studies

Case	Weight	D	TBW	H_2O/FFB (A)	H_2O/FFB (B)
	kg			%	%
31	62.3	1.051	36.7	72.5	73.8
	63.1	1.046	36.0	72.3	73.5
	70.3	1.042	36.2	66.9	71.3
	66.8	1.043	34.7	67.0	71.4
	69.3	1.035	36.0	70.5	72.7
3	81.0	1.049	36.6*	56.3	65.7
	81.3	1.045	36.0*	56.6	65.9
10	72.9	1.079	45.1	65.8	70.8
	74.1	1.080	45.6	65.2	70.4

* Antipyrine space	I^{131} A-P space	D_2O space
36.6	36.6	37.8
36.0	37.2	37.5

Table II summarizes repeated studies conducted in three subjects. Case 31 (not shown in Table I) was measured five times over a period of 15 months, at various stages of nutrition. Although there was change in body density, attributable largely to fluctuation in body fat, there was minimal change in percentage of water of the fat-free body. The variations noted are consistent with the expected error of the methods and stand in sharp contrast to the much greater variation among the entire group of normal subjects.

Case 3 (Table II) summarizes two measurements, one year apart, of density and TBW in a healthy subject demonstrating an extremely high density of the fat-free body. Because the latter was so far above "normal," careful recheck was made of body density (underwater weighing), and all three methods—antipyrine, I^{131}-antipyrine, and D_2O—were utilized to determine total body water.

Case 10 (Table II) had the highest body density of the normal subjects. Replicate determinations of D and TBW showed again a lesser degree of variation in percentage of water and density of

TABLE III

Effect of altered mineral mass upon total body water and fat-free body

	Weight	D	TBW	H_2O/FFB (A)	H_2O/FFB (B)	D_{ffb}*
	kg			%	%	
Osteosclerosis	48.6	1.114	21.1	†	55.0	1.189
Osteoporosis	49.1	1.008	29.4	99.0	83.1	1.057
Average "normals"				67.5	71.4	

* D_{ffb} = density, of fat-free body.
† Body fat had a negative value.

the fat-free body than was noted among the group of normal subjects.

The profound effects of pathological degrees of increased or decreased bone density on *in vivo* dissection by densitometric or volume distribution techniques are illustrated in Table III. Measurements of the type outlined above in a subject with osteosclerosis and one with osteoporosis are compared with the mean value of the normal subjects. The very low percentage of water in osteosclerosis and the high value in osteoporosis reflect the significant role of bone density in the estimation of total body fat. When calculations are extended to determine density of the fat-free body, a marked deviation from the norm is noted. These striking abnormalities are, however, merely the extremes of a broad spectrum seen in the normal subjects.

DISCUSSION

Keys and Brôzek (2) have pointed out that the density of the fat-free body is unknown. Short of dissection of a large number of human bodies after *in vivo* analysis, it is unknowable. Furthermore, because adipose tissue contains both water and protein in addition to its larger component of pure fat, density of the lean body could deviate from any selected norm with overfeeding or starvation.

Data of the type reported here, bearing on body constituents, have been furnished by Messinger and Steele (3) who measured 9 normal subjects, and by Osserman, Pitts, Welham and Behnke (7) who studied 81 normal servicemen. Both of these groups reported mean values for percentage of water in the FFB consistent with the predictions of McCance and Widdowson (8) and the animal studies of Rathbun and Pace (9). There was, however, considerable range even in these relatively homogeneous groups and the even greater range in our subjects could be attributed to several factors. These include systematic error, true abnormality of body water, and wide variation of the density of the dry fat-free solids. The minimal methodological error previously reported from this laboratory (10), the error of both D and TBW reported by others, and the similarity of replicate determinations in the subjects shown in Table II attest to the fact that the wide range of values must involve factors other than methodological error. Data derived from both *in vivo* measurement and direct tissue analysis indicate that major deviation of percentage of water in the FFB is to be expected only with profound abnormality of hydration. Consequently, it would appear that the range of variability noted in this study must be attributed to a not inconsiderable range of density of the dry fat-free solids. This probability is supported by the even greater variability demonstrated in the patients with known skeletal abnormality. Considering the biological variation of human subjects in other spheres, it would be surprising to expect a constant weight per volume ratio of the fat-free body, particularly in view of the fact that an individual's supporting tissue must reflect that which it supports and the stresses to which it must become accustomed. Although muscle mass might vary to some degree, and thus offer partial explanation for deviation of density of the fat-free body, the much greater density of mineral mass would render this body constituent a more significant determinant of lean tissue density.

It is apparent that any disorder that affects skeletal density would prohibit accurate fat estimation by either densitometric or volume distribution techniques, both of which depend upon a constant density of the fat-free body or a constant mineral-protein ratio. With realization that material loss of minerals may occur before there exists radiological indication of such decrease in bone density, it seems logical that fat estimation from densitometric or volume distribution methods could be erroneous in older patients. Decrease in bone density, as would occur in mild osteoporosis, could affect the standard of reference to such a degree that an overestimation of body fat could be expected in patients over 40.

It must be pointed out, however, that much less error would accrue from serial determinations conducted to estimate change in total body fat (11). Because of the slow mineral turnover and minimal effect of protein change on body density, satisfactory estimation of loss or gain in fat is feasible even though the original estimation of total fat is in error. As pointed out by Siri, and by Keys and Brôzek, concern regarding a constant density of the fat-free body should serve to stimulate development of methods for measurement of body fat independent of those outlined above. While *change* in fat is more accurately estimated by densitometric techniques, the independent method of coupling TBW with balance study appears suitable (11). Densitometric and volume distribution methods have stimulated interest in the lipid component of the body, but precise assay of body fat remains dependent upon truly independent methods of quantitative analysis.

SUMMARY

The *in vivo* estimation of total body fat has provided useful information, particularly in young healthy males. When current techniques were applied to a heterogeneous group of subjects with apparently normal hydration, but who had varying degrees of physical fitness and obesity and who varied in age, the inherent weakness of our present techniques and some of their basic assumptions become apparent. Such discrepancies are highlighted in subjects with decreased or increased density of the skeleton. While useful for the estimation of *change* in total body fat, densitometric and volume distribution methods possess the inherent weakness of dependency upon the assumption that the fat-free body in normally hydrated individuals demonstrates a constant density. This study indicates that fat-free body density varies considerably among normal individuals and to an extreme degree in patients with bone disease.

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