Naval Medical Research Center Building 503 503 Robert Grant Avenue Silver Spring, Maryland 20910-7500



NMRC 00-02 June 2000

BODY COMPOSITION ANALYSIS IN U.S. NAVY DIVERS

D. D. Gummin

Bureau of Medicine and Surgery Department of the Navy Washington, DC 20372-5120

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20060630357

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996."

This technical report has been reviewed by the NMRC scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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REPORT DOCUME	NTATION PAGE		Form Approved OMB No. 0704-0188					
	Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and							
	reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of the collection of information, including suggestions for reducing this burden to Washington Headquarters Services. Directorate for							
information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302 and to the								
Office of Management and Budget. I								
1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE		PORT TYPE AND DATES COVERED					
	June 2000	Techni	cal $6/92 - 5/93$					
4. TITLE AND SUBTITLE			5. FUNDING NUMBERS					
Body composition analysis in U	J.S. Navy divers.							
6. AUTHOR(S)			PE - 63713N					
			PR – M0099 TA – 01A/01B					
Gummin, D.D.*			WU = 1003/1428					
7. PERFORMING ORGANIZATION NAM	E(S) AND ADDRESS(ES)		8. PERFORMING ORGANIZATION					
			REPORT NUMBER					
NAVAL MEDICAL RESEARCH	CENTER		NMRC 00-02					
(CODE 00) 503 ROBERT GRANT AVENUE			NWIKC 00-02					
SILVER SPRING, MARYLAND 2	0910-7500							
9. SPONSORING/MONITORING AGENC		(ES)	10. SPONSORING/MONITORING AGENCY REPORT					
			NUMBER					
BUREAU OF MEDICINE AND S	URGERY		D) 1277011 (D) 1044515					
(MED-02)			DN377011/DN244515					
2300 E STREET, NW WASHINGTON, DC 20372-5300								
11. SUPPLEMENTARY NOTES								
*Current Address: Toxikon Consortiu	ım, 1900 W. Polk St., Sui	te 500, C						
12a. DISTRIBUTION/AVAILABILITY STATEMENT 12b. DISTRIBUTION CODE								
Approved for public release; distribution is unl	imited							
13. ABSTRACT (Maximum 200 wo								
)							
Physical readiness and body makeup	are considered fundament	tal attribu	tes of U.S. Navy divers. Methods to objectively					
determine body makeup are fraught with shortcomings and can be technically challenging, particularly in field operations.								
Two potential field methods of determining body composition, densitometry and deuterium oxide dilution, were assessed								
			unteered for testing by both techniques. Body					
			d data. Significant variability was found in					
			pefficient of variation 4.6%). Normalizing this					
			hydration "constant" for lean body mass of 0.833 \pm					
			but without substantive experimental support.					
			all changes in body composition. Published					
anthropometric data are unreliable if not specifically analyzed and interpreted in the context of the population studied.								
14. SUBJECT TERMS		15. NUMBER OF PAGES						
body composition, field method	ls, densitometry, deut	terium	28					
oxide dilutions, total body wate	r		16. PRICE CODE					
17. SECURITY 18. SECURI		JRITY	20. LIMITATION OF ABSTRACT					
CLASSIFICATION OF REPORT CLASSIFICA	TION OF THIS CLASSIF	ICATION OF	7					
Unclassified PAGE Unclassified	ABSTRA		Unclassified					
NSN 7540-01-280-5500		<u> </u>	Standard Form 298 (Rev 2-89)					
			Prescribed by ANSI S10 239-98					
			298-102					

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ACKNOWLEDGEMENTS

The author expresses gratitude to the divers of Naval Medical Research Institute Diving Medicine Department who participated in the study. Special thanks to HMC James Mancuso and the other technicians in the Hyperbaric Environmental Adaptation Program for their extensive technical support. This research was partially supported by the Naval Medical Research and Development Command work units 63713N M0099-01A.1003 and 63713N M0099-01B.1428. The opinions and assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

The research protocol employing human subjects in this study has been reviewed and approved by the Naval Medical Research Institute's Committee for the Protection of Human Subjects.

BACKGROUND

Body composition is an essential feature in evaluating an individual's fitness to dive, as evidenced by the prolific history of projects dedicated to this issue (1-22). Fitness-to-dive qualifications include adherence to the physical readiness and body fat standards stated in the Manual of the Medical Department (23). The readiness standards set down in the naval instruction (24) were recently updated (25) though the body composition criteria for personnel readiness remain essentially unchanged.

More essential to undersea medicine is an appreciation of the role of individual body tissues in systemic physiologic processes. These include metabolic activity; pharmacologic distribution and processing; nutritional and hydrational regulation; distribution, absorption and retention of inert gas burdens; and the degree of involvement of each tissue in each of these processes. Body composition has long been purported to affect an individual's ability to decompress safely from dives or submarine escape scenarios (8-10,14,19). Human decompression experience has not borne this out, nor is there strong epidemiologic support for this concept (7,22).

To assess currently available indices of body composition in U.S. Navy divers, two analytical techniques were evaluated: deuterium oxide (D₂O) dilution and hydrostatic weighing (or densitometry). Healthy divers at the Naval Medical Research Institute (NMRI) were solicited to undergo body composition analysis by these two widely accepted techniques.

1

METHODS

Ten healthy male U.S. Navy diver volunteers aged 22-44 ate a standardized diet for 24 h prior to each day of the study. All subjects had a current diving medical examination on file in their medical record, were free of cardiovascular, pulmonary or renal disease, and were qualified to participate by the Head of the Health Monitoring Division at NMRI. The diet was a typical mixed American diet that provided approximately 3300 Cal, 3.5 gm of sodium, and 4.4 gm of potassium. Caloric intake consisted of 15% protein, 30% fat, and 55% carbohydrates. Although fluid intake was *ad libitum* over the 24 h preceding the day of the study, additional calories, caffeine, and alcohol intake were not permitted. All subjects were hydrostatically weighed to calculate body density in the manner described by Brozek et al. (3).

Densitometry

While immersed, each subject's pulmonary residual volume was determined by taking the mean of two measurements of oxygen-dilution as described by Wilmore et al.²¹:

$$RV = VO_2 \cdot \frac{(b-a)}{(c-d)}$$
 [Eq. 1]

where, RV = pulmonary residual volume in ml; $VO_2 =$ volume of O_2 in the bag at the beginning of the procedure; $a = \% N_2$ impurity in the original O_2 breathed; $b = \% N_2$ in the mixed gas in the bag at equilibrium (= 100% - % O_2 - % CO_2); $c = \% N_2$ in the alveolar air at the beginning of the test; and $d = \% N_2$ in the alveolar air during the last maximal breath (= b + 0.2% N₂). Simplified, this becomes

$$RV = \frac{VO_2 \cdot b}{79.8 - b}$$
 [Eq. 2]

To then determine body density, accounting for lung residual volume, subjects were hydrostatically weighed. Taking the standard equation for body density from Brozek (3) adjusting for residual volume, and correcting for water density at the temperature of the test (35.5 °C):

$$D_B = \frac{BM}{\frac{BM - Wetwt}{(D_c)} - RV} \quad [Eq. 3]$$

where $D_B = Body$ density in gm/cm³; BM = body mass of the subject in air; Wet wt = weight of the subject weighed when immersed; RV = lung residual volume in ml; and D_w = density of water in the tank at the temperature of the test.

Body density was then used to calculate percent body fat as:

$$\% BF = \frac{4.570}{D_{B}} - 4.142 \cdot 100$$
 [Eq. 4]

with ^{*} = Constants from Brozek. Lean body mass was then calculated as:

$$LBM = BM - \frac{BM \cdot \% BF}{(100)} \quad [Eq. 5]$$

Total Body Water

Total body water (TBW) was determined by D₂O dilution on 5 individual days, each following a day of dietary control. Determination of TBW content was performed for both plasma and urine samples. Because one subject was unable to participate in the last trial of the study and several others gained weight before the last trial, the fifth trial of the data set was excluded from analyses of TBW determinations.

On the morning of each day of the study, subjects first underwent a 2-hour prehydration period. During that period, each drank 2.5 ml/kg body weight of deionized water every 30 min. Thirty minutes following the last water consumption, subjects drank a 10-gram (gravimetrically weighed) dose of deuterated water (Deuterium Oxide, 99.9% enriched, Cambridge Isotopes, Woburn, MA). The cup was rinsed 3 times with a total of 100 cc of deionized water. A 2-hour equilibration period ensued during which subjects were seated upright and at rest. Following D₂O equilibration, and precisely 4 h after the hydration period began, plasma and urine specimens were obtained for ²H/H ratio analysis. Schoeller and colleagues (15) allowed a 6-hour equilibration period, but found that $H_2^{18}O$ equilibrated completely within 1-2 h for healthy subjects. This is consistent with previous findings for deuterium oxide (6).

Individual test days for the four TBW assays were over the course of a 48-day period, during which the subjects participated in a larger, more comprehensive hydration study. To ensure that no ordering or work-up effect would be present in that study, no fewer than 7 days intervened between test days. No subject underwent hyperbaric or exercise stress in the 24 h preceding body composition analysis. All subjects were counselled on the possible risks and benefits of participation in this study, after which each subject gave written, witnessed informed consent to participate.

Assays

Total body water was determined by correcting D_2O dilution space. Two hours after drinking a 10-gram dose of D_2O , serum and urine specimens were collected. These were submitted to a commercial laboratory (Metabolic Solutions, Inc., Acton, MA) for isotope ratio mass spectrometry. ²H/H ratio was determined in the reference standard, Acton tap water, and transposed to a ratio against Vienna Standard Mean Ocean Water (VSMOW). Total body water was calculated as follows (from Schoeller, et al., who cite a 1% coefficient of variation with this method (15)):

$$TBW = \frac{d}{mw} \cdot \frac{APE}{100} \cdot 18.02 \cdot \frac{1}{R_{std} \cdot \Delta\sigma^2 H} (kg) \quad [Eq. 6]$$

where, d = the dose of D₂O in grams, mw = the molecular weight of deuterium oxide, APE = percent enrichment deuterium, R_{std} = the ratio of ²H/H in VSMOW (= 0.00015576), and $\Delta\delta^{2}H$ = the difference between baseline and equilibrium deuterium enrichment in the samples. To correct for the 4% overestimation of TBW by the deuterium method, values calculated from the

 D_2O analysis were divided by 1.04 to get the reported values, as has been suggested from the literature (5,13,20).

Analyses

Systat, Version 5.0 for WindowsTM (Systat, Inc., Evanston, IL) was used for all statistical analyses. Data were analyzed by one-way and two-way repeated measures analysis of variance (ANOVA). Where significant changes were detected, a Newman-Keuls analysis was performed to identify those differences.

		Table 1. Mean H	Body Mass (kg)	
Subject	Trial A	Trial B	Trial C	Trial D	Trial E
	95.68	94.78	95.44	· 95.78	96.14
12	72.28	71.86	72.72	72.38	72.86
3	80.48	81.38	80.32	80.00	80.58
4	83.20	83.54	83.50	84.18	84.36
- 5	93.78	93.52	94.02	94.66	94.12
6	69.50	69.38	70.38	70.20	71.00
2	104.02	103.84	103.84	104.40	
8	71.88	71.92	71.77	71.64	71.32
9	80.08	81.20	81.36	80.90	82.24
¹ 10 └ └	81.88	81.80	82.28	83.12	83.66

p=0.007, F=4.333, df=4,32

RESULTS

Body mass determination

Subjects were weighed daily, after the hydration period, and immediately prior to D_2O administration. Those results are shown in Table 1. Mean body mass of 9 subjects in Trial E

differed from those of the 9 subjects in the previous trials, (p=0.007, F=4.333, df=4,32). A Newman-Keuls analysis identified Trial E as the sole outlier, which formed the basis for exclusion of this trial from repeated measures analyses. A pooled estimate of variance was calculated for body mass:

$$\sigma^2_{\text{pooled}} = \sum (n_i - 1) \cdot \sigma^2_I \text{ [Eq. 7]}$$

where n = the number of observations for each subject, i = the subject number, σ^2_i = the betweentrials variance, and σ^2_{pooled} = the pooled estimate of variance. The pooled variance for Trials A-D was 0.316 kg², giving a pooled standard deviation for these trials of 0.563 kg. The mean of the coefficients of variation (c.v.) was 0.005.

Densitometry

Table 2 gives the results determined by hydrostatic weighing for the 10 subjects.

			Table 2. D	ensitometry			
Subj	Underwater Body Mass (kg)	Height (cm)	Avg Pulmonary Residual	Underwater Weight (gm)	Body Density (gm/cm ³)	Body Fat (%)	Lean Body Mass [#]
	94.14	180.34	<i>Vol* (ml)</i> 1778	2485	1.04098	24.81	(kg) 70.79
2 [3	72.32	173.99 170.18	1423.5 1386.5	<u>3180</u> 2680	1.06124 1.04719	16:43 22:20	60.44 61.86
4 5	83.00	<u>185.42</u> 187.96	1380.5 1354 1291	<u>3865</u> 3180	1.06054 1.04379	<u>16.70</u> 23.63	69.13 71.18
6	<u>93.20</u> 68.70	170.18	1140	4115	1.07601	10.52	61.47
7 -8 [103.18 71.12	1 87.96 179.07	<u>1961</u> 1613.5	2650 2665	1.04040 1.05727	25.05 18.04	77.33 58.29
9 10 [79.52 81.56	171.45 180.34	<u>1192</u> 1054.5	<u>3140</u> 3645	1.05106 1.05450	20.60 19.14	<u>63.14</u> 65.95

*Average of two independent measures [#]Calculated from body density

Lean Body Mass

Because variance in total BM was so low across Trials A-D, it was assumed that body composition (i.e., body density) remained constant for each individual in each of these 4 trials. Lean body mass was then calculated from the initial body density and the measured body mass

on each day of Trials A-D, using Eqs. 4 and 5. These are tabulated in Table 3.

Table	• 3. Lean Boo	ly Mass (kg) Calculated	for Éach Tria	1 Day
Subject	Trial A	Trial B	Trial C	Trial D
	71.94	71.27	71.76	72.02
2	60.40	60.05	60.77	60.49
3	62.61	63.31	62.49	62.24
4	69.31	69.59	69.56	70.12
5	71.62	71.42	71.80	72.29
6	62.19	62.08	62.98	62.81
7	77.96	77.83	77.83	78.25
8	58.91	58.95	58.82	58.72
9	63.58	64.47	64.60	64.23
10	66.21	66.14	66.53	67.21

Deuterium oxide dilution

Using the equation from Schoeller (15) D_2O space was calculated for each subject. Total body water was then calculated by adjusting for the 4% overestimation of the D_2O dilution. Results for TBW determined from plasma D_2O are in Table 4, and for urine-determined TBW, in Table 5. Data were available for all study days from urine for 8 of the subjects, and for 9 subjects in Trials A-D.

Subject	Trial A	Trial B	Trial C	Trial D	Trial E
1	55.64	56.47	52.89	55.58	62.92
2	48.29	50.72	50.45	47.62	48.28
3	49.66	53.18	47.77	48.71	51.37
4	55.32	54.90	54.99	54.43	46.58
5	54.95	49.25	55.44	56.60	57.18
6	48.65	48.45	46.00	49.72	53.33
7	59.88	67.98	59.24	66.21	
8	45.53	54.68	48.10	52.79	44.84
9	53.66	51.33	52.65	46.50	53.49
. 10	54.38	56.06	62.10	60.72	50.22

p = 0.548, F = 0.722, df = 3, 27

Subject	Trial A	Trial B	Trial C	Trial D	Trial E
1	60.77	53.74	54.07	47.15	57.71
2 [43.78	48.76	44.07	43.73	44.43
3	58.80	48.26	59.86	48.21	49.53
4	52.50	57.49	59.22	46.48	49.44
5	75.14	68,46	63.90	57.15	88.14
6 [50.11	47.05	44.88	45.60	44.20
7	58.43	71.13	43.57	59.88	
8	45.04	53.10	45.83	42.99	43.59
9	42.79	50.35	50.41	46.10	54.32
10 [50.94	61.80	53.10	Strand Mary States States	50.02

Analysis of TBW measurements showed no significant difference between the values determined on each of the trial days. This was true for the values calculated from the plasma samples (p = 0.548, F = 0.722, df = 3, 27) and from the urine samples (p = 0.086, F = 2.476, df = 3, 24). A two-way repeated measures ANOVA demonstrated no difference between the measured TBW values for plasma and urine (p = 0.892, F = 0.019, df = 1,16). Because urine data was not available for all 4 trials, one subject was excluded from the one-way ANOVA for the urine TBW test, as well as from the two-way ANOVA. The pooled variance for the plasma method was 12.683 kg², while the urine demonstrated a higher pooled variance, 56.852 kg². The mean coefficient of variance (c.v.) for the plasma method was 0.049.

To address possible changes in body composition over the course of the 48 days of the study, the absolute difference between TBW measurements was determined for each individual over the shortest and the longest intervals between assays. Those results are presented in Tables

Table 6. 1	Interval Changes in	n TBW (by Plasma	Method) between	Test Days
Subject	Shortest Interval (days)	∆ Total Body Water (kg)	Longest Interval (days)	∆ Total Body Water (kg)
1	9	3.58	37	0.06
2	8	0.27	34	0.67
3	8	5.41	35	0.94
4	8	0.09	39	0.89
5	12	5.70	48	1.66
6	10	3.72	36	1.08
7	10	8.73	44	6.33
8	8	6.58	40	7.26
9	8	1.32	35	7.16
10	8	6.03	35	6.34
$Mean \pm SE$		4.14 ± 0.91	· · ·	<i>3.24</i> ± 0.97

6 and 7 for both the plasma and the urine determination methods.

Subject	Shortest Interval (days)	∆ Total Body Water (kg)	Longest Interval (days)	∆ Total Body Water (kg)
1	9	0.33	37	13.62
2	8	4.70	34	0.06
3	8	11.60	35	10.59
4	8	1.73	39	6.02
5	12	6.68	48	17.99
6	10	0.72	36	4:51
7	10	27.56	44	1.45
8	8	8.17	40	2.05
9	8	0.06	35	4.21
10	8	8.70	22	2.16
Mean±SE		7.02±2.61		6.26±1.87

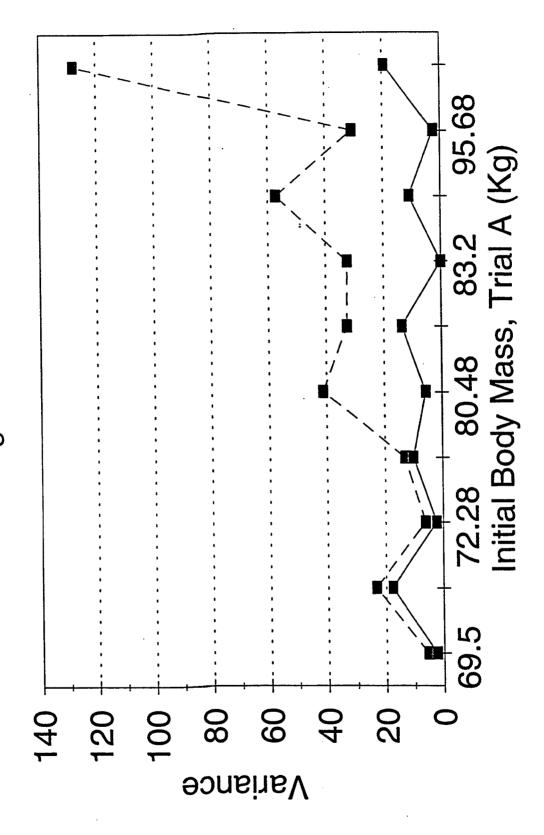
These data demonstrate at least as much variability in the data collected at relatively short temporal intervals as over the longer intervals. The smaller differences seen between measurements of TBW taken 22-48 days apart than between those taken 8-12 days apart is interesting, but not significant (p = 0.36, F = 1.115, df = 3, 27).

In an attempt to further characterize sources of variance within the data, Figure 1 shows a plot of the subjects' initial body mass measurement (immediately prior to Trial A) vs. variance in the means from Trials A-D. For the urine determination method, where pooled variance was greater, the degree of variance appeared to be individual-dependent; in fact, the variance in the urine method increased with the size of the volunteer. A Pearson correlation analysis between subjects' characteristics and degree of variance using the urine method was carried out, and shown in Table 8.

Table 8. Subject Ch	aracteristics vs. Variance (Urine Method)
Characteristic	vs. Standard Deviation	vs. Variance
Age (Years)	0.317	0.372
% Body Eat	0.742	0.655
EBM _	0.813	0.8112
MeanTBW	0.768	0.666
Total BM	0.861	0.831

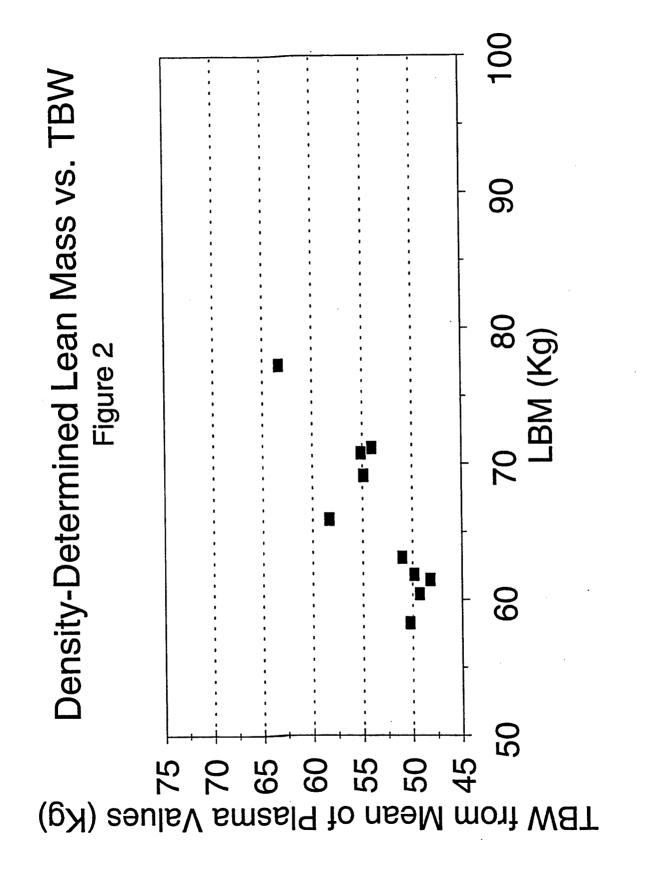
Because variance was smaller using the plasma method, only the plasma values for each subject were compared with densitometry data. Mean values for TBW correlated with densitometry-determined LBM (r = 0.866). Lean body mass calculated from the initial body density for each of Trials A-D also correlated with TBW on each of those trials (r = 0.938, 0.664, 0.684, 0.796, respectively). These correlation coefficients place confidence at the 90-96% levels.

Subject Weight vs. TBW Variance Figure 1



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Linear regression using LBM (from body density) and individual daily weight as the independent variables, and TBW in Trials A-D as the dependent variables, reveals the best-fit regression line with a slope (\pm standard error) of 0.675 \pm 0.096 and y-intercept of 8.482 \pm 6.396. Because doubling the error for the y-intercept gives a confidence interval that subsumes the origin, it must be assumed that this parameter should be dropped. Extrapolating the y-intercept to (0,0) gives a regression line with a slope of 0.801 \pm 0.008. Determining a similar regression line for initial LBM measurements made at the time of densitometry against mean values for TBW determined for each of the 10 subjects, once again gives a non-significant y-intercept. The regression line determined through the origin for the latter values gives a slope equal to 0.809 \pm 0.012. The mean values are plotted in Figure 2.



SUMMARY

Fat mass (FM) can be defined as that portion of the body's mass that is comprised exclusively of lipid. The remainder of the body mass is treated as essentially free of lipid (though this is obviously not the case in nature). The other body compartment, the "lean" compartment, has alternatively been referred to as the fat-free body mass (FFBM), the fat-free body weight (FFBW), fat-free wet weight (FFWW), or the lean body mass (LBM). Behnke's original concept of the LBM was more specific in its stated relationship to actively metabolizing tissue (1). It is assumed here that the body can be separated into these two compartments exclusively, and that the definitions for the lean compartment can be used interchangeably. However, it is recognized that the LBM does contain lipid and that the FM maintains some degree of hydration and metabolic activity.

Determination of body composition by underwater weighing is rapid and is precise to within approximately 1%, assuming no error in weight measurement (5). This estimate of precision assumes a 70 kg reference man has 19.3% body fat. Other assumptions in determining body density by this technique are that all "fat-free" body tissues are of equivalent density and equally hydrated, and that adipose tissue is equally dense throughout and essentially anhydrous. There are 5 factors most likely to contribute to errors in precision: differences in pulmonary residual volume, differences in gastrointestinal gas volume, differences in degree of tissue hydration, and intra-individual variation in FM. Residual volume is usually measured directly, where possible. Gastrointestinal (GI) gas volume is assumed to be insignificant using the present approach (3), and a source of negligible error. Other approaches account for a fixed, small

amount of GI gas volume, thus adding a constant correction factor (5). Correction for buoyancy in air during weighing is typically neglected, but is likely to cause error on the order of 0.2% or less.

While only a single determination of body density was performed in the present study, the low variance in total BM across the 4 trials, coupled with strict adherence to dietary and hydrational control, probably justify the assumption of minimal changes in body composition throughout the trials. Inaccuracy in densitometry estimations of LBM likely reflect interindividual differences in composition of LBM (e.g., proportions of fat-free muscle mass, fat-free adipose mass, and fat-free bone mass); differences in the densities of these tissues; and differences in hydrational equilibrium state of each of these tissues (4). The accuracy of densitometry cannot be established without a suitable "gold standard" against which to normalize (Indirect and direct measures of body composition are impossible in the same living subject). Technical aspects of densitometry are fairly complex, requiring experienced personnel and accurately calibrated equipment. The test requires complete immersion of the subject. These factors make densitometry less than ideally suited for field studies. When safety, flexibility, and precision are considered, this may still be the most reliable index of body composition currently available. Newer techniques are promising (5,18).

Measurement of TBW by D₂O dilution is rapid, simple, and non-invasive (though small animal studies have shown mental status and metabolic changes with levels >10% (15)). The portability and the relative non-invasiveness of sampling make the method ideally suited for field studies. Sampling in plasma, urine, and saliva have been performed with equivalent ease and efficacy, though salivary sampling may be less precise (13). Determination of body composition with this method relies on the same assumptions stated above for body density, with the caveat that deuterium-hydrogen ion exchange in the body will cause the method to overestimate TBW by a quantifiable amount. Precision estimates for D₂O dilution techniques have been cited to be as modest as 1-2% (5,15). However, these values actually reflect the variation in quantification of deuterium in the specimens, rather than reproducibility of the technique in humans. Culebras and Moore (6) have eloquently determined the maximal overestimation of TBW from isotope exchange during isotope dilution to be 5.22% of the TBW, but did not report the error associated with their estimate. In the present study, the mean of the coefficients of variation was 4.6%. This value should be understood as applicable for plasma assayed at 2 h of equilibration in healthy males. Precision of the assay technique likely accounts for some part of this error.

Precision may be jeopardized by allowing insufficient time for isotope equilibration, as was seen with the urine sampling in our larger subjects. As Schoeller and colleagues found (15) obese subjects appear to require 3-4 h to equilibrate in the urine. Based on the experience in the present study, this caution should be extended to include larger subjects (probably those greater than 75 kg total body mass). Subjects should void their bladders and be weighed immediately before administering the isotope. They should not eat or drink anything during the equilibration period, as new water taken in will not completely equilibrate in body tissues before obtaining samples. A more labor-intensive approach addresses the non-equilibrium state, as Coward et al. point out (5). The technique used for the present study quantifies the TBW at the *beginning* of the equilibration period. By the time sampling is performed, some of this water has already left

the body in the urine. For short equilibration times, the difference in TBW should be negligible. However, urinary loss of body water will be more significant with longer equilibration times.

The higher variability seen in this study for the urine assay probably relates to insufficient (variable) isotope equilibration, which was more marked in the subjects with higher body mass. This appears to indicate that distribution of deuterium is incomplete in the larger subjects, though differences in gastrointestinal absorption, renal excretion, or insensible losses cannot be excluded by this analysis. Schoeller and colleagues (15) found delayed isotope equilibration in the urine of obese subjects whom they studied. None of the subjects in the present study met criteria for obesity. In our subjects, body fat percentage correlated slightly less well with variance for the urine method than did total body mass. Both body fat content and body size are likely to affect distribution and therefore isotope equilibration. If the urine assay method is to be used, longer equilibration is recommended (3-4 h), particularly in larger subjects.

Because of the magnitude of variation in precision of the D₂O dilution technique, quantifying small changes in hydration or body composition with this technique would be difficult, as those changes are likely to fall within the confidence margins of the analysis. Effects of exercise, fluid administration, changes in insensible losses (e.g., as would be expected while in a hyperbaric chamber), or pharmacologic intervention during the equilibration period are not addressed here, but are likely to contribute additional error to the measurement.

Figure 2 shows a plot of the LBM determined from the hydrostatic weight vs. the mean values of TBW measured from D₂O equilibrated in plasma. Linear regression showed a best-fit slope equal to 0.801 ± 0.008 . This "constant" theoretically defines the proportion of LBM that is

water mass. Because the TBW values in this analysis were determined by lowering the actual values for D₂O dilution space by 4%, a similar linear regression analysis was done to determine the slope of the line relating LBM to D₂O space. As the confidence interval of the y-intercept subsumed the origin, the intercept term was dropped, giving the slope 0.833 ± 0.009 . Values for the constant reported in the literature range from 0.712 (16) to 0.808 (11), perhaps most commonly cited as 0.732 (5). This constant should always be interpreted on the basis of the population studied and techniques being compared. It should not be touted as a natural constant. At best, this "constant" can facilitate extrapolating values from one type of test to another.

In attempting to directly convert from TBW to LBM, one must be appreciative of the constants used to calculate both TBW and LBM. Deuterium oxide dilution space measured by the technique outlined here should reliably correlate to values giving similar confidence when referencing LBM calculated by the formulae and techniques used in the present study, assuming that the population of interest is similar. Using constants derived from animal studies with extrapolation to human data, vice-versa, or across species is unwarranted (16). Using different isotope dilution methods, e.g., DHO, H2¹⁸O, or tritium oxide (THO) will result in estimates for TBW that differ from those reported here, as different isotope exchange kinetics pertain. Using any of the many other constants available in the literature to determine body fat from density will give results dissimilar to those reported here.

It is hoped that this review of the precision and relative flexibility of each of these techniques will help guide future efforts in body composition analysis for diving and decompression research.

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