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Research Article

TOTAL EXCHANGEABLE POTASSIUM AND CHLORIDE AND TOTAL BODY WATER IN HEALTHY MEN OF VARYING FAT CONTENT

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During the past few years various attempts have been made to develop an improved standard of reference for comparison of those parameters of body composition which refer largely to lean body mass. Weir measured carcass chloride in dogs and found poor correlation between carcass chloride and body weight (1). He found, however, better correlation between carcass chloride and fat-free carcass weight (2). Corsa and co-workers (3) pointed out the theoretical desirability of relating total exchangeable potassium (K_e) to lean body mass or to a parameter closely related to lean body mass. Cheek and West (4) found that K_e, carcass potassium, Cl_e, and carcass chloride in rats were closely related to both fat-free carcass weight and to total body water (TBW). Muldowney, Crooks, and Bluhm (5, 6) measured K_e, Cl_e, antipyrine space, plasma volume, and red cell mass in adult human beings and compared all these except antipyrine space with lean body mass, derived from antipyrine space, and with creatinine excretion. They found that the correlation of each parameter with the derived lean body mass was significantly better than its correlation with body weight. McMurrey and co-workers (7) measured total exchangeable chloride, potassium, and sodium $(Cl_e, K_e, and Na_e)$, red cell mass, plasma volume, and TBW in a group of ten healthy men and ten healthy women. Their data show much better correlation of these parameters with TBW than with body weight.

Recently, O'Meara, Birkenfeld, Gotch, and Edelman (8) have found that the mixing of administered K^{42} is prolonged in subjects who have edema. It was concluded by these authors that equilibration times should be extended to 40 hours when measuring K_e in sick patients. Because of the short half-life of K^{42}, this makes the measurement much more difficult to perform. Recently, however, apparatus has become available that makes it convenient to measure K_e after a mixing time of 40 hours, using a dose of only 50 microcuries of K^{42} (9, 10).

In order to study the inter-relationships of parameters of body composition, these should be determined simultaneously in each subject. Data permitting such comparisons for healthy adults are scarce. Also, values for K_e in healthy adults with equilibration times of 40 hours are few. Finally, the effect of obesity on these inter-relationships is not known. In order to gather additional values permitting the study of these relationships, the present study was undertaken. Thirty-five healthy men were studied. They were chosen so that a wide range of fat content was encompassed. Simultaneous estimations of TBW, K_e, and Cl_e were made using tritiated water, K^{42}, and Br^{82} as the tracers, respectively. The results confirm the close relationships previously reported between the magnitude of K_e and Cl_e, estimated by Br^{82}, and the magnitude of TBW. The ratio $K_e:TBW$ was significantly higher in muscular men (weight-lifters) than in randomly selected or obese men. In addition, the values for the ratio $Cl_e:TBW$ tended to be higher in obese than in average men, and higher in average than in lean men.

METHODS

Subjects. Thirty-seven healthy men were studied. The first 21 were selected at random. Subsequent selections were made so that only obese or unusually lean men were selected. Food and water were allowed at will during the first 16 hours of the experiment. A diet low in potassium was prescribed for the remaining 24 hours.

Dosage. Each person received an intravenous injection of 50 microcuries of K^{42}, given 40 hours prior to equilibrium sampling, and an intravenous injection of 5 microcuries of Br^{82}, given 24 hours prior to equilibrium sampling. An intravenous injection of 1 millicurie of H^3 as tritiated water was given each subject on the morning

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equilibrium of K^{42} and Br^{82} distribution occurred. All injections were sterile and isotonic and were administered with calibrated syringe-pipettes, modified after Chaney (11) and delivering $20.0 \pm .01$ ml. The radiation dose from these three doses of isotope is less than 0.16 rad.

Sampling. Subjects saved their urine during the entire experiment for estimation of tracer excretion. On the morning of equilibrium sampling, neither food nor water was permitted. Two successive spot urine samples were collected, usually at 9:30 and 11:30 a.m., for determination of potassium equilibrium specific activity. Equilibrium venous whole blood samples were withdrawn. The serum was separated and used to determine equilibrium Br^{82} : Cl specific activity. At this time the tritiated water dose was given. Two and 3 hours later, venous whole blood was collected, and the serum was separated and stored in a freezer for tritiated water analyses.

Counting. All samples containing Br^{82} were counted as 15-ml aliquots in a NaI : Tl well counter. Each serum was counted in triplicate. For each, 10,000 counts were collected at counts: background ratios of about 4:1. Urine excretion samples were counted in duplicate. Duplicate dilutions of the injected material were counted in triplicate.

The samples containing K^{42} were counted as 80-ml volumes in a beta well counter (9, 10). To those urine samples containing both K^{42} and Br^{82}, a measured excess of $AgNO_3$ was added and the samples were filtered through asbestos. This removed all detectable Br^{82}. The filtrate was then used for K^{42} counting. Excretion samples were counted in duplicate. Equilibrium samples were counted in quadruplicate, with at least 10,000 counts for each sample. Counts: background ratios varied between 4:1 and 12:1.

TABLE I

Body composition in healthy men *

Subject	Age	Body weight	TBW		Cl_e			K_e			$Cl_e:K_e$	K^{42} s.a. difference
		kg	L	% wt	mEq	mEq/L TBW	mEq/kg	mEq	mEq/L TBW	mEq/kg		%
ARB	56	93.6	48.5	51.8	2,561	52.8	27.3	4,242	87.5	45.3	0.604	2.45
GC	21	62.7	39.1	62.4				3,277	84.3	52.3		1.90
BV	32	73.6	38.8	52.7				3,378	87.1	45.9		9.20
NW	20	64.3	37.6	58.5	1,804	48.0	28.0	3,534	94.2	55.0	0.510	
MS	43	52.4	30.6	58.4	1,532	50.1	29.2	2,434	79.5	46.4	0.629	
MO	45	82.2	44.8	54.4	2,375	53.0	28.9	3,976	88.9	48.4	0.597	0.90
SF	36	69.0	38.4	55.6	1,947	50.7	28.2	3,377	87.9	48.9	0.576	4.19
ALS	45	75.4	40.0	53.0	2,117	52.9	28.0	3,316	82.9	44.0	0.638	3.40
JD	22	81.8	50.0	61.1				4,404	88.1	53.8		0.53
EBK	26	74.3	43.4	58.4				4,010	92.4	54.0		2.11
MK	24	79.5	41.7	52.4	2,248	54.0	28.3	3,700	88.8	46.5	0.607	1.49
JS	21	74.1	44.3	59.8	2,276	51.4	30.7	3,781	85.4	51.0	0.602	1.08
RO'D	23	62.7			2,001		31.9	3,682		58.7	0.543	2.12
AY	34	85.4	49.0	57.4	2,665	54.4	31.2	4,008	81.8	48.9	0.665	0.00
MSh	30	87.0	46.8	53.8	2,248	48.0	25.8	3,988	85.2	45.8	0.563	1.78
DF	22	73.6	43.0	58.4	2,240	52.1	30.4	3,862	89.8	52.5	0.580	1.60
KF	28	89.5	52.4	58.5	2,686	51.2	30.0	4,572	87.2	51.1	0.587	1.93
TC	34	81.2	45.0	55.4	2,326	51.8	28.6	3,830	85.1	47.2	0.607	0.41
NS	23	77.6	48.8	62.9	2,373	48.6	30.6	4,307	88.2	55.5	0.551	1.52
DJ	25	77.7	43.2	55.7	2,235	51.7	28.8	3,662	84.8	47.1	0.610	3.38
OS	29	67.6	38.8	57.4	1,955	50.4	28.9	3,313	85.4	49.0	0.590	5.16
HC	37	58.2	38.0	65.3	1,863	49.0	32.0	3,019	79.5	51.9	0.617	1.42
TA	39	98.6	47.9	48.6	2,480	51.8	25.1	3,940	82.2	40.0	0.629	0.37
JF	25	70.9	45.5	64.2	2,236	49.1	31.5	4,265	93.7	60.2	0.524	0.30
LG	35	74.8	46.5	52.2	2,304	49.5	30.8	4,287	92.0	57.2	0.539	0.54
KJ	24	94.6	57.5	60.8	2,897	50.4	30.6	5,196	90.4	54.9	0.558	0.62
TC	26	79.3	49.7	62.7	2,299	46.3	30.0	4,611	92.9	58.1	0.499	1.69
EH	22	83.4	51.6	61.9	2,463	47.8	29.5	4,956	96.0	59.4	0.497	1.99
JH	30	78.6	53.0	67.4	2,801	52.9	35.6	4,625	87.3	58.8	0.606	
JB	41	130.7	61.1	46.7	4,021	65.8	30.7	5,282	86.4	40.4	0.761	0.27
JD	28	104.2	50.4	48.3	3,311	65.7	31.8	4,329	85.9	41.5	0.765	0.12
RR	26	124.6	52.5	42.1	3,617	68.9	29.0	4,378	83.4	35.1	0.826	0.76
FF	31	111.9	49.5	44.2	2,638	53.3	23.5	4,211	85.1	37.6	0.626	2.80
CR	27	83.6	45.8	54.8	2,458	53.7	29.4	4,164	90.9	49.8	0.590	0.30
JBe	28	122.3	54.9	44.9	3,058	55.7	25.0	4,709	85.8	38.5	0.649	0.36
LC	39	170.4			3,562		20.9	6,006			0.593	0.5
KT	35	115.0	53.7	46.7	2,886	53.8	25.1	4,774	88.9	41.5	0.604	1.33
Mean	30.6	86.1	46.3	56.0	2,384	51.2	28.8	4,092	87.3	48.7	0.586	1.8
				r =0.83		r =0.95	r =0.90		r =0.96	r =0.79	r =0.90	

* TBW =total body water, Cl_e =total exchangeable chloride, K_e =total exchangeable potassium, and s.a. =specific activity.

Serum samples containing H^3 were kept frozen between collection and assay. They were then thawed, vacuum distilled from the frozen state, and counted in a liquid scintillation spectrometer, by a method similar to that of Leibman, Gotch, and Edelman (12).

Chemical determinations. Potassium determinations were done by internal standard flame photometry, with a Baird Associates flame photometer. Dilutions were prepared in duplicate, each dilution was read on each of 2 days, and all values were averaged. Serum chloride determinations were done in duplicate on each of 2 days by a potentiometric titration based on the method of Sanderson (13).

Calculations. In calculating K_e, all beta well counter data were first corrected for background and for radioactive decay. A specific gravity correction was necessary (10, 14). The reduced data were used to calculate K_e by the standard formula (15).

In calculating Cl_e, the NaI : Tl scintillation well counting rates were corrected for background. Since the Br^{82} samples also contained K^{42}, it was necessary to correct for this. For the Br^{82} excretion urine sample, the correction was based upon the ratio, K^{42} counts per minute per 15-ml sample (NaI : Tl well counter) : K^{42} counts per minute per 80-ml sample (beta well counter).

This method for separating K^{42} and Br^{82} was sufficiently precise for use in estimating the urinary excretion of K^{42} and Br^{82}. For the equilibrium Br^{82} serum samples, the correction for the K^{42} contribution to the NaI : Tl rate was also based upon this ratio. It must be emphasized

that this correction was always small, never more than 17 cpm for any of these subjects, compared with the total serum counting rate of 400 to 600 cpm. Consequently, errors in the estimation of these K^{42} contributions to the NaI : Tl counting rates could have no significant effect upon the values for Cl_e.

TBW was calculated by the standard formula (15). The values represent water at 20° C.

RESULTS

The values for K_e, Cl_e, and TBW are given in Table I. The age and weight of each subject is listed. Values are given for K_e, Cl_e, TBW, and for the ratios Cl_e : weight, Cl_e : TBW, K_e : weight, K_e : TBW, Cl_e : K_e, and TBW : weight. The mean of each parameter and each ratio is given. Correlation coefficients are listed. The difference between the K^{42} : K^{39-41} specific activity of the first equilibrium urine sample in each subject and that of the second sample, voided about 2 hours later, is given as per cent of the mean specific activity for those two samples.

DISCUSSION

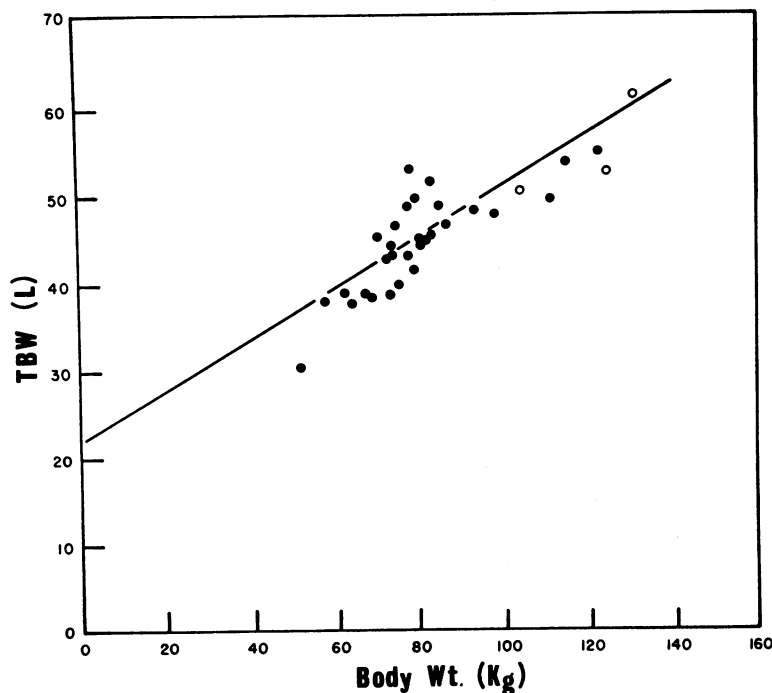The regressions of TBW, K_e, and Cl_e on body weight in this group of men all intersect the ordi-

FIG. 1. LINEAR REGRESSION OF TBW AGAINST BODY WEIGHT FOR WHOLE GROUP. For values represented by o in Figures 1–4 and 7 see Discussion. cussion.
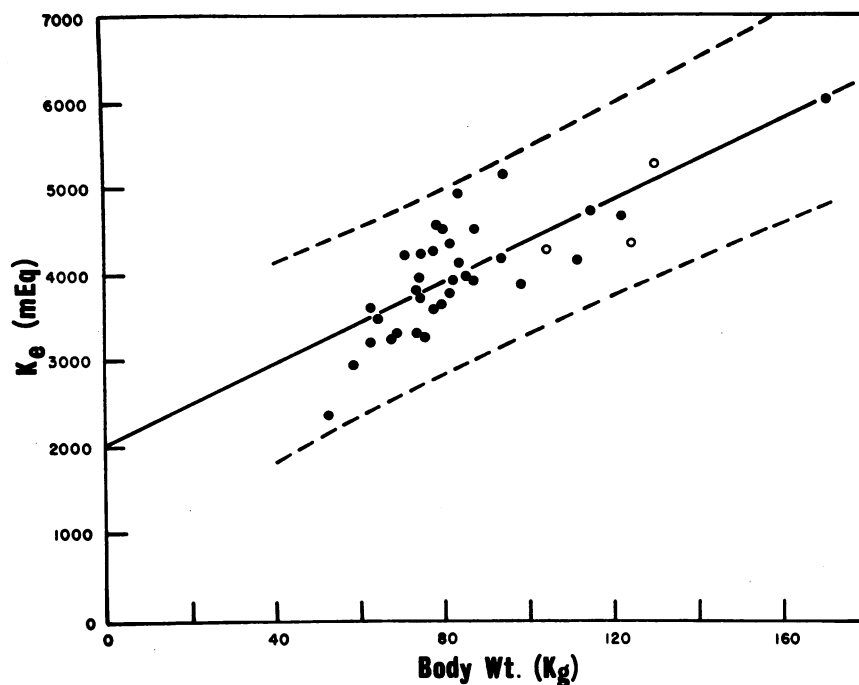
FIG. 2. LINEAR REGRESSION OF K_e AGAINST BODY WEIGHT FOR WHOLE GROUP. 95 per cent confidence limits for individual values shown.

nate substantially above zero. The regression equations are: a) TBW = 0.288 weight + 22.08 L, b) K_e = 23.16 weight + 2098 mEq, and c) Cl_e = 16.28 weight + 1008 mEq (Figures 1–3). The regression coefficients would be larger, and the zero-weight intercepts smaller, if the group did not contain a large proportion of obese individuals. Thus, when these regressions are computed excluding those eight men who were deliberately selected because they were obese, the resulting rgressions are: a) TBW = 0.500 weight + 6.43 L, b) K_e = 48.77 weight + 198 mEq, and c) Cl_e = 26.98 weight + 210 mEq. It is seen that all three regression lines pass substantially closer to the origin when the eight markedly obese men are excluded. The origin lies within the 95 per cent confidence limits for all three lines.

In contrast, the regression of K_e on TBW for the entire group, including the fat men, passes close to the origin (Figure 4). The same is true for Cl_e against TBW (Figure 5). The regressions are: a) K_e = 92.97 TBW − 297 mEq (r = 0.96), and b) Cl_e = 54.53 TBW − 149 mEq (r = 0.95). The origin lies within the 95 per cent confidence limits for both regression lines.

In all of the graphic presentation, the data from subjects JB, JD, and RR, representing the determinations from 1 week, are plotted as open circles. These points lie clearly within the group when the comparisons TBW : weight, K_e : TBW, and K_e : weight are considered. However, for these comparisons in which Cl_e appears, i.e., for Cl_e : weight, Cl_e : TBW, and Cl_e : TBW against TBW : weight, they are clearly different from the group. These points are not seen in Figure 5 because all 3 fall completely outside the scale which was chosen to depict Cl_e against TBW. Because of this extreme divergence, these values were not used in the statistical analysis. Although no clue could be found, it is probable that a systematic laboratory error occurred in the measurement of Cl_e for these 3 subjects.

Ljunggren (16) measured TBW, K_e, a corrected thiosulfate distribution volume, and a distribution volume of Br^{82} in normal and obese men and women. From mathematical interpretations, he concluded that the sex differences evident when either chloride space, thiosulfate space, or K_e was compared with either weight or TBW, could be explained on the basis of variation in relative fat
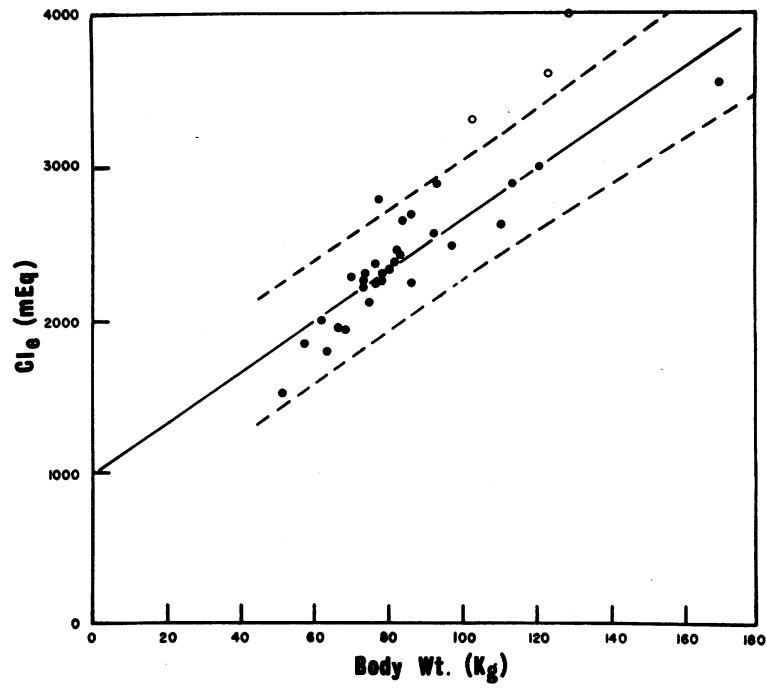
FIG. 3. LINEAR REGRESSION OF CL_e AGAINST BODY WEIGHT FOR WHOLE GROUP.
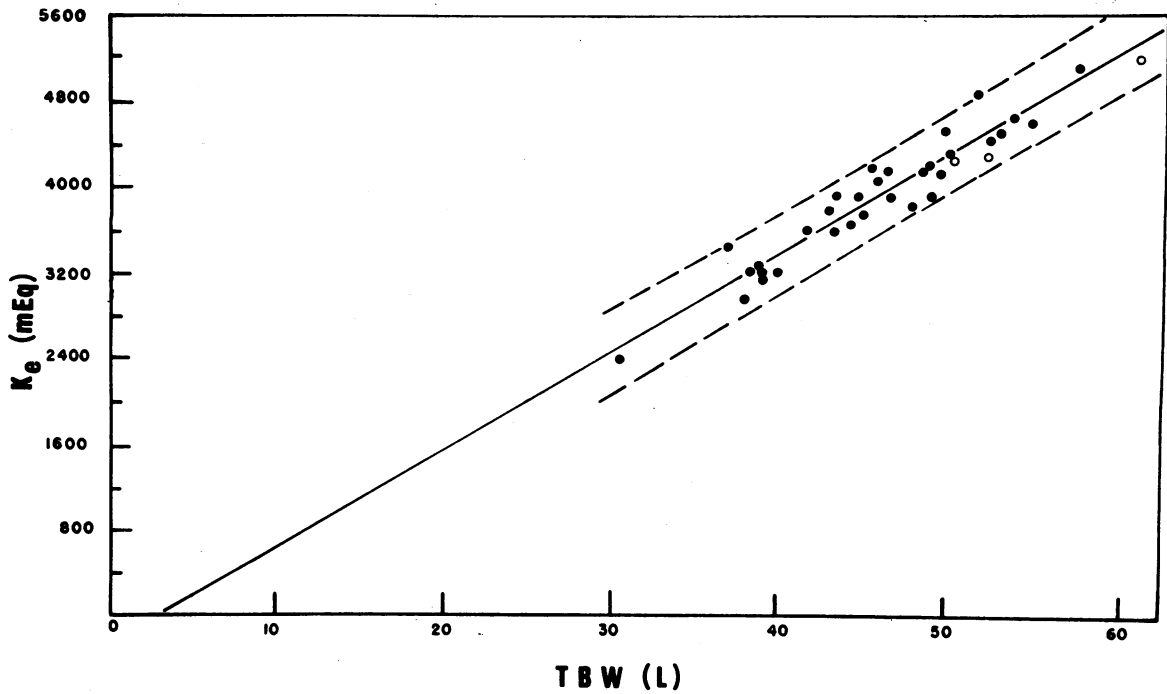95 per cent confidence limits for individual values shown.

FIG. 4. LINEAR REGRESSION OF K_e AGAINST TBW FOR WHOLE GROUP, INCLUDING FAT GROUP. 95 per cent confidence limits for individual values shown.
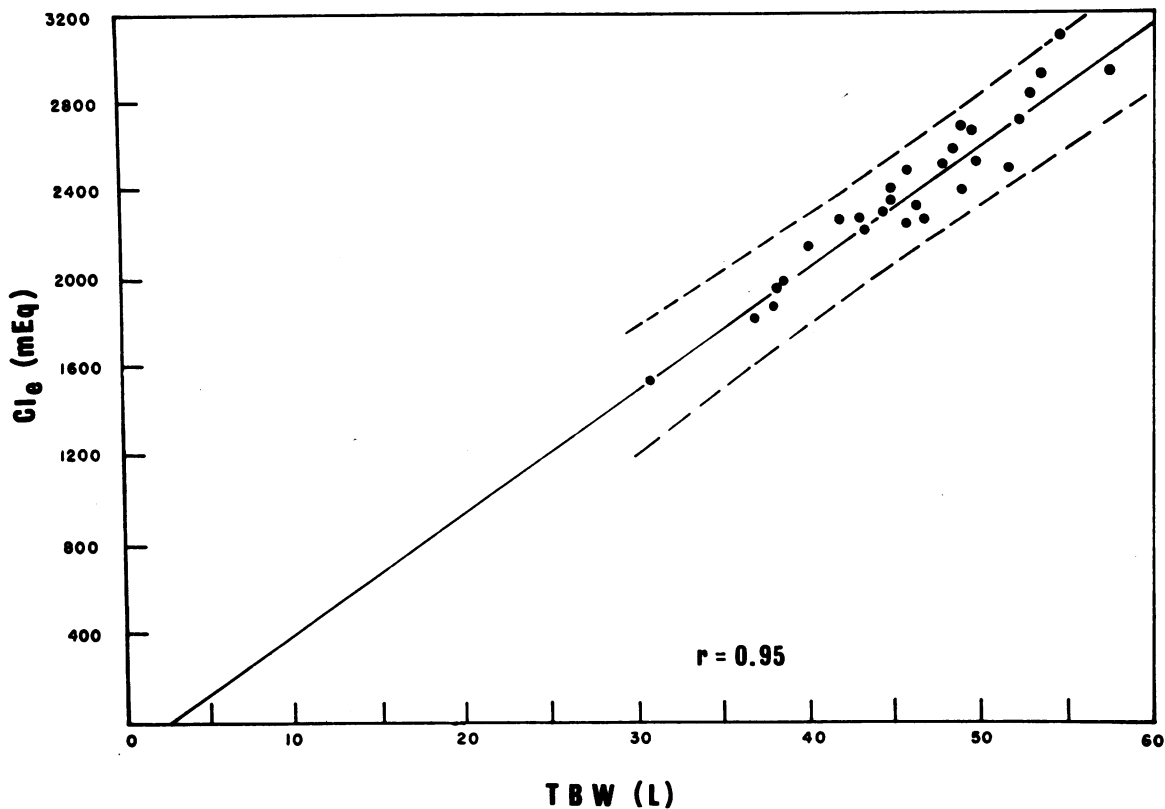
FIG. 5. LINEAR REGRESSION OF CL$_e$ AGAINST TBW FOR WHOLE GROUP, INCLUDING FAT GROUP. 95 per cent confidence limits for individual values shown.
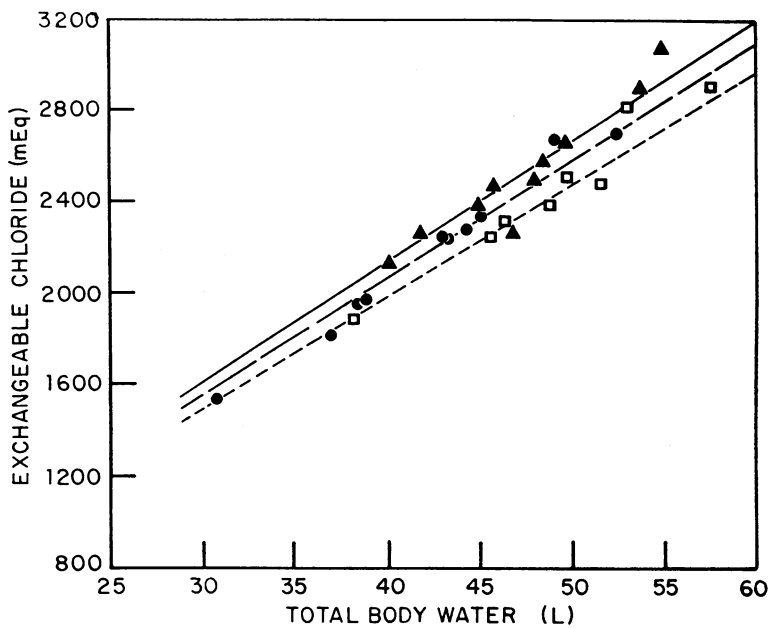

FIG. 6. LINEAR REGRESSION OF CL$_e$ AGAINST TBW FOR EACH OF 3 GROUPS. ▲ represent fat men; ●, average men; and □, thin men.

content, as could the differences between obese and normal women.

In order to test for the presence in our subjects of variations in Cl_e associated with obesity, the regression Cl_e against TBW was examined for 3 groups of subjects: a) those less than 55 per cent water by weight, who were considered fat; b) those from 55 to 60 per cent water by weight, who were considered average; and c) those more than 60 per cent water by weight, who were considered thin. Regression lines were computed for these groups, and are shown with the corresponding points in Figure 6. These regression lines are significantly different (p < .01).

A more informative graphic presentation is made by comparing the ratio Cl_e : TBW, which refers Cl_e to TBW as an index of lean body mass, with the ratio TBW : weight, used here strictly as an index of obesity. In health, this latter ratio can be translated into fat : weight by the expression: Fat/weight $= 1 - (TBW/wt \times 0.72)$, which is derived from the Pace-Rathbun formula (17).

This comparison, Cl_e : TBW against TBW : weight, is seen in Figure 7. The regression line is shown with 95 per cent confidence limits for individual values. An increase in fat content, as shown by a decrease in TBW : weight, is associated with an apparent increase in the ratio Cl_e : TBW.

As Strauss, Lamdin, Smith, and Bleifer (18, 19) have pointed out, sodium excretion and retention are related in a definite way to dietary salt intake. Each day's salt intake is excreted more or less exponentially over several succeeding days. Since our values for Cl_e were obtained in men on a free-choice diet, it is possible that the regression shown in Figure 7 is at least partially related to differences in salt intake. Further elucidation of this point will await the measurement of Cl_e and TBW under circumstances that control this variable.

It may be pertinent to list other possible reasons for this apparent increase in the ratio Cl_e : TBW in obese men. Increase in the mean size of fat cells may be associated with an increase in the ratio of extracellular to total water in fat tissue. Also, obesity may be associated with a small change in the composition of that portion of the lean body mass that is not part of the fat depots. Further, it is possible that the intracellular chloride may vary sufficiently to cause a slight variation in exchangeable chloride, such as that seen in our data and the data from the literature. Lastly,
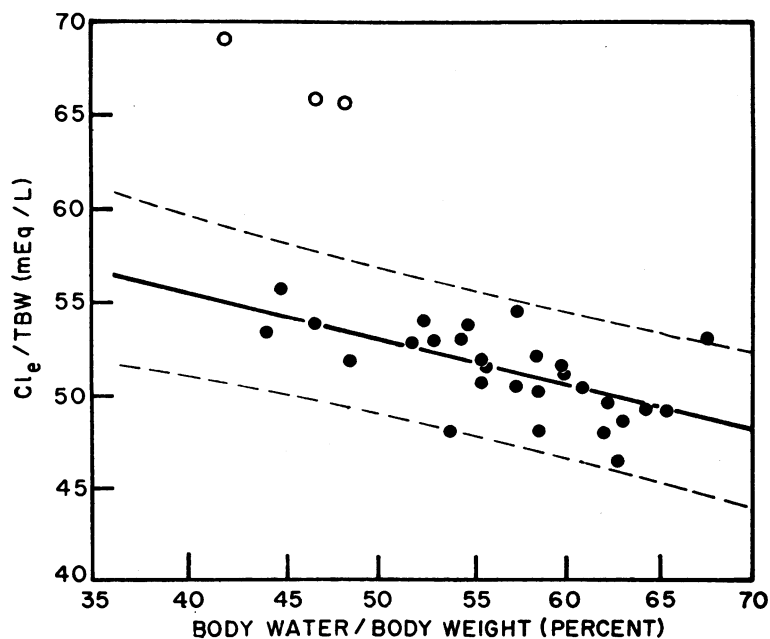

Fig. 7. Linear regression of Cl_e : TBW against TBW : weight. 95 per cent confidence limits for individual values are shown.
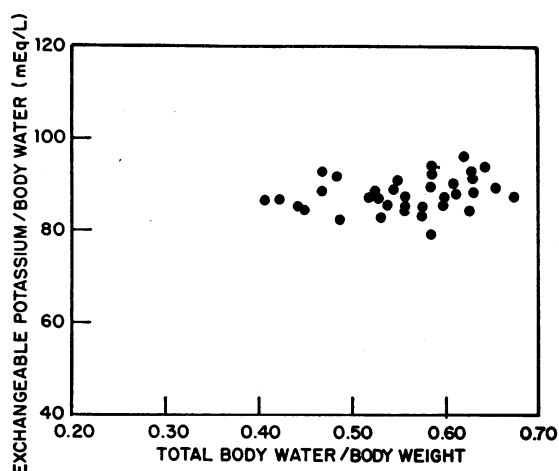
FIG. 8. RELATIONSHIP OF K_e: TBW TO TBW: WEIGHT.

TABLE II

Comparison of 3 groups of men

Group	K_e:TBW	SD
	mEq/L	
Randomly selected n = 21	86.4	±3.71
Obese n = 8	86.1	±2.61
Muscular n = 6	92.0	±2.96

the validity of the use of bromide as a chloride tracer has not been conclusively established, and the difference seen may be due to a difference in the distribution of bromide relative to chloride in the obese as against the lean subject.

Ljunggren (16) also concluded that the sex difference evident in the comparison K_e against weight could be eliminated by means of the multiple regression of K_e on TBW and weight. He

concluded that the sex differences evident in his comparisons could be explained on the basis of differences in fat content between men and women, and between men and thin women on the one hand, and obese women on the other.

In order to test the relationship of K_e to TBW for the presence of an obesity-dependent correlation, our group (n = 35) was once again divided into the same 3 groups, fat, average, and thin, with respect to TBW: weight ratio. The regressions of K_e on TBW in these 3 groups were calculated and were not significantly different. The comparison K_e: TBW against TBW: weight is seen in Figure 8. No regression seems apparent.
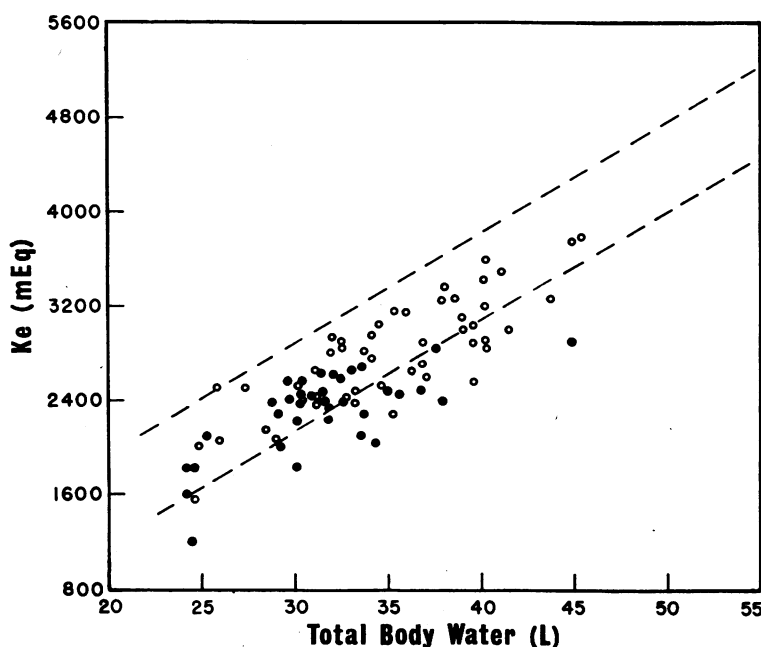

FIG. 9. DATA FOR K_e AGAINST TBW. For data from the literature, ● represent healthy men, and o, healthy women. 95 per cent confidence limits are shown for individual values (not shown here) of our data.

Our group of subjects included 6 muscular weight-lifters, each more than 60 per cent water by weight. These men will be referred to as the muscular group, and are compared with the obese group (n = 8) and the randomly selected group (n = 21) in Table II. It is seen that the randomly selected group and the obese group have essentially the same mean K_e: TBW ratio and essentially the same variation about the mean. The mean K_e: TBW ratio in the muscular group, however, is more than 2 standard deviations higher than the means of the other groups, indicating that this is a distinct difference. Thus, in our group of men, no obesity-dependent difference in K_e: TBW ratio was apparent, but a distinct difference in K_e: TBW ratio was associated with muscular hypertrophy.

The individual confidence limits for the data in our series of men are compared with the available data for simultaneously determined K_e and TBW in Figure 9. The 95 per cent confidence limits for our 35 subjects are shown. The available data (3, 6, 7, 20–22) are plotted. It is seen that the bulk of the data for men fall within our 95 per cent limits; of the values for women, however, only about half fall within our 95 per cent limits. It seems likely that there are sex differences in the K_e: TBW ratio that cannot be eliminated by systems of reference tending to control only the obesity variable. It seems more likely that a difference in K_e: TBW ratio between men and women is associated with the differing degree of muscular development usually seen.

SUMMARY

Total body water, total exchangeable potassium (K_e), and total exchangeable chloride (Cl_e) were estimated in a group of 35 healthy men of widely varying degrees of fatness, by use of tritiated water, K^{42}, and Br^{82} as tracers. The methods used permitted these measurements with isotope doses such that the radiation dose was only 0.16 rad. The values for K_e and Cl_e were compared with those for total body water. A high degree of correlation was found for both of these regressions. Correlation coefficients were 0.96 and 0.95, respectively. In addition, a regression was seen that tended to show a higher Cl_e: total body water (TBW) ratio associated with obesity. For 10

fat men each less than 55 per cent water by weight, Cl_e: TBW was 53.0 mEq per liter. For 10 average men between 55 per cent and 60 per cent water by weight, Cl_e: TBW was 51.5 mEq per liter. For 8 lean men containing more than 60 per cent water by weight, Cl_e: TBW was 49.3 mEq per liter. Although these differences are statistically significant $(0.01 > p > 0.005)$, it is not possible to conclude that the total exchangeable chloride was proportionately increased in these men, since the values for Cl_e were determined by observing the distribution of Br^{82}, which can only give approximate values for Cl_e. The relationship between K_e and TBW, on the other hand, was independent of the degree of obesity.

There was a significant difference in the ratio K_e: TBW between 6 very muscular subjects, all weight-lifters, and the remainder of the group. The data are discussed and are compared with data from the literature.

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