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ANALYSIS OF ADIPOSE TISSUE AS A KEY TO THE UNDERSTANDING OF MECHANISM OF BODY WEIGHT LOSS IN THE HUMAN

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ABSTRACT

Changes in the composition of the whole body and of subcutaneous adipose tissue have been studied during weight reduction in 2 human subjects. The whole body lost tissues ranging in density from 0.92-0.96 gms/ml at body temperature, consisting of 55-85% fat. The adipose tissue fat contents fall during weight loss from 80-85% to 65-80% of the whole tissue, or by about 50% when related to the dry, de-fatted residue. The densities of fat depots increased, therefore, as a result of the excess loss of tissue fat. Adipose tissue densities calculated from chemical composition agreed well with the values obtained by direct determination. From these observations it is concluded that while adipose tissues probably contributed the greatest portion of the fat and body weight loss during weight reduction, other soft tissues also contribute significantly to the body weight decrease by losing non-fat components. Adipose tissue components are not lost uniformly during body weight loss in the human, as had been thought previously.

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SUMMARY

The Problem

Previous investigators have noted that tissues lost or gained in the human during dietarily-induced weight change have densities close to those of adipose tissues. From these comparisons they have concluded that fat depots participate as an anatomical unit in their response to body weight changes. However, these conclusions are unsupported by density or chemical analyses on adipose tissues, before and after weight change, nor have such comparisons been performed in the same individuals. Such data have been obtained in the present study, allowing more precise observations and conclusions to be obtained.

Findings

During body weight loss in 2 subjects, tissues lost had densities of 0.92-0.96 gms/ml, corresponding to 55-85% fat contents. Adipose tissues contained 80-85% fat before weight reduction, and their densities were 0.93 gms/ml, measured by direct determination or by calculation from the chemical analyses. Adipose tissue densities rose following whole body weight loss, due to a greater relative loss of fat than of non-fat components. Related to the dried, de-fatted residue, fat content decreased by 50% in the depots. However, the whole body lost relatively less fat than the original proportions in the fat depots. These observations indicate that while adipose tissues probably contribute the major loss of fat and total body weight, other soft tissues contribute significant losses of non-fat components.

Recommendations

Measurements of the density and composition of fat depots have provided key data in the interpretation of mechanisms of weight loss in humans. The earlier view that adipose tissue components participate together in body weight loss is contradicted by our observations.

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INTRODUCTION

The increasing awareness of the consequences of excessive caloric intake leading to overweight, obesity and accelerated degenerative changes has led to increased interest in the variations in gross body composition under different nutritional states and in diseased conditions. Quantitative estimations of gross body composition of man, initiated by Behnke and co-workers (1,2) and extended by a number of other investigators (3,4) have shown that the bulk of the alteration in body composition is accounted for by changes in the amount of body fat. However, the studies of Keys and co-workers (3) indicated that material other than fat also changed. In fact, Keys' studies on body weight gain led them to conclude that the tissue gained had a density of 0.948 and a composition which approximated 14 per cent extracellular fluid, 62 per cent fat and 24 per cent cellular matter (3). The material lost during weight reduction of 19 kg in 7 months (one individual) was reported by Behnke (1) to have a specific gravity of 0.94. And from the data obtained on 10 men who lost weight on a daily intake of 900 calories (5) Keys and Brozek (3) calculated the total weight loss to be made up of about 65 per cent fat, 23 per cent cellular matter and 12 per cent extracellular fluid. Thus it is not unreasonable to suggest that the tissue gained during dietary weight gain may resemble in composition the tissue lost during dietary weight loss.

Von Dobeln (4) has compared the densities of the tissue lost or gained, as reported in the above studies, to the directly determined density of adipose tissue reported by Schubler and Kapff (6) to be 0.971 and to the density of adipose tissue of 0.936 as calculated from the data of Forbes et al (7) on the chemical composition of human adipose tissue. From these comparisons von Dobeln concluded that the gained tissue does not differ significantly

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from adipose tissue in an anatomical sense, and that the part of the body changing through simple overeating is adipose tissue.

In the present study an attempt has been made to obtain additional information on the nature of the alterations produced during dietarily-induced weight reduction. The composition of the tissue lost, based on calculations from data on body weight, body water and body volume, has been compared to the composition of adipose tissue determined by chemical methods on biopsy samples taken before and after weight reduction.

EXPERIMENTAL

Subjects - Two normal, healthy men ages 53 (Subject B) and 44 (Subject E) established constant weights somewhat above their "ideal normal" weights by the ingestion of free-choice diets. Subject B then ingested various diets as indicated in Table I such that weight loss occurred. Subject E ingested a liquid diet at a level which maintained body weight then arbitrarily cut the diet in half without altering its composition.

Diets - During the ingestion of free-choice diets no attempt was made to control the type of food ingested. The amounts of food ingested, however, were recorded and the calories estimated. The liquid diets were prepared from the constituents listed in Table I by admixture with water using a Waring Blendor. Subject B ingested water ad lib but the water intake of Subject E, except for a few days during the experimental period, varied between 3200-3300 ml.

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Whole Body Measurements - Before, during and after weight reduction determinations of body weight, body water and body volume were made. Body water was determined by the tritium dilution technique and body volume by use of a helium chamber (8). We are indebted to Dr. William Siri, University of California, Berkeley, for measurements of body water and body volume. These data, recorded in Table II, were used for calculation of body density and composition of tissue lost (Table III).

Adipose Tissue Biopsy - Prior to and after weight reduction biopsy samples of subcutaneous abdominal adipose tissue were obtained under local procaine anesthesia. Care was taken to keep the injected solution away from the biopsy sample. Care was also taken to obtain 5-10 grams of adipose tissue free from connective tissue. A small portion of the tissue was placed in formalin for histologic study and the remainder was cut into 2-3 pieces for determinations of density and subsequent chemical analysis.

Measurement of Adipose Tissue Density - The adipose tissue sample was suspended by a thin copper wire attached to the beam of an analytical balance. The tissue was then weighed, first in air and again while completely immersed in distilled water maintained at 24-25°C. Readings were made in less than 0.5 minute after immersion to avoid excess wetting of the tissue. Weighings were made on 2-3 portions of the biopsy samples. After correction for the weight of the wire, the specific gravity of the sample was calculated as:

$$\text{Sp. Gr.} = \frac{Wa}{Wa - Ww}$$

where Wa and Ww represent weight in air and distilled water respectively. Density of adipose tissue (Da) was expressed as:

$$Da = \text{Sp. Gr.} \times D_W/T$$

where D_W/T represents the density of water at the temperature of the measurement (24-25°C).

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Chemical Analysis of Adipose Tissue - After the weighings for density calculations, excess water was removed from the tissue samples by blotting with saline-moistened gauze and the tissue was immersed in 20 volumes of a chloroform-methanol mixture (2:1). The solvents were boiled under reflux for 3 hours, after which the tissue was transferred to extraction thimbles (Whatman, seamless, single thickness) and extracted in a Soxhlet apparatus with chloroform-methanol solvent used above for about 50 hours to remove the fats completely. The chloroform-methanol extract was transferred to a 100 ml volumetric flask and made to volume with additional solvent mixture. The residue, still in the thimble, was dried first in air, then in vacuo over P_2O_5, and finally removed to a piece of tared aluminum foil and dried to constant weight. Deoxyribonucleic acid (DNA) was determined on the residue by the method of Dische (9) after extraction of the DNA according to the method of Schneider (10). We are indebted to Dr. K. L. Jackson for performing the DNA analyses.

The fat content of duplicate aliquots of the chloroform-methanol extract was determined gravimetrically after purification of the extract by the method of Folch, et al (11), removal of excess solvent and drying over P_2O_5. In the purification procedure the "fluff" layer was left with the fat fraction, which was evaporated just to dryness over a hot plate, under a stream of nitrogen, precautions being taken to avoid excess heating. The fat was re-dissolved by heating with 3 successive small portions of $CHCl_3$ - MeoH, each portion being filtered through fat-free filter paper (Whatman No. 42, Soxhlet - extracted) into tared 50 ml Erlenmeyer flasks, and the combined extracts were evaporated almost to dryness on a steam bath. After further drying under a stream of nitrogen, the samples were freed of traces of solvent and water by storing in vacuo over P_2O_5 and paraffin for 1-2 days, until reaching constant weight.

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Water was determined as the difference between the adipose tissue weight in air and the combined weight of total tissue fat plus residue.

RESULTS

The data on the measurements of body weight, body water and body volume are presented in Table II, along with a calculation of the body fat, based on the assumption that the fat-free body mass is equal to body water/0.72 (2,12,13). It is shown that the periods of dietary restriction resulted in decreases of all measurements and in body fat.

Body Composition Changes Calculated From Whole Body Measurements - The data designated in Table III as "Total tissue lost since initial observation" at each interval were calculated by dividing the total body weight loss since the beginning of the experiment by the corresponding total body volume loss. The data on "tissue lost since previous observation" was calculated by dividing the body weight loss since the previous observation by the volume change during the same interval.

From the data on the total tissue lost it can be seen that the densities of the tissue lost varied between 0.92 and 0.96 gms/ml during weight reductions of 9-18%. It should be noted that even after a 16-week period of weight regain (84-86 Kg in Subject B) the density of the total tissue lost was still 0.92.

The tissue densities involved in weight changes during the different intervals listed point to discrete periods of weight change, characterized by loss (or gain) of tissue of light or denser composition. Thus in Subject B during the first 7-week period the tissue lost had a density of 0.93, whereas in the succeeding 9-week period, denser tissue, more resembling the non-fat

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portion of soft tissue, was lost. In the third period loss of materials of the same density as fat was noted, and during the single period of weight gain, the materials resembled protein in their density characteristics. In the final 6-week period of weight loss the tissue lost again had the density characteristics close to that of non-fat soft tissue.

From these density values, the compositions of tissues involved in the corresponding body weight changes were calculated. These weight changes were considered in terms of fat and non-fat components, and it was assumed that (1) only soft and adipose tissues, but not bone, are affected by weight alterations; (2) density of fat = 0.90 gms/ml (14); and (3) density of non-fat soft tissue = 1.06, calculated from a "representative" composition of water = 80%, protein = 19%, and mineral = 1%.

From these considerations, the body volume change ΔV, may be expressed as a function of body weight change, ΔW, and body fat change, ΔF, as:

$$\frac{\Delta F}{0.90} + \frac{\Delta W - \Delta F}{1.06} = \Delta V \qquad (1)$$

from which may be derived an expression for body fat change:

$$\Delta F = \frac{0.954\,(\Delta V) - 0.90\,(\Delta W)}{0.16} \qquad (2)$$

The values thus derived (Columns 7 and 8, Table III) indicate that fat comprised by far the greater portion of body weight loss in the 1st and 3rd intervals studied in Subject B, non-fat components dominated the weight loss in the 2nd and 5th periods, while the body weight gain during the 4th interval seemed to result from accumulation of non-fat components in the face of a continued loss of body fat. It may also be noted that the body weight loss in Subject E was quite evenly divided between fat and non-fat components.

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Since the calculation used for the data in Table III to study fat versus non-fat tissue changes may be too simplified a method of study, body fat contents were calculated by 4 different approaches and the fat changes for these same intervals have been compared in Table IV with those obtained by the shorter method. Two calculations of body fat were those of Keys and Brozek (3), and of Siri (15), using only body density. Two more calculations used the body water values (Table II); the equation of Siri also applying the body density (8), the other derived from the simple relationship of a 72% water content in the fat-free body (2,12,13).

From the data in Table IV it can be seen that the cumulative body fat losses, calculated by the simpler method (Table III) in the first 3 periods compared most favorably with the more elaborately derived formulas in which body density estimations were used (formulas 1 and 2). However, in the last two intervals the fat losses calculated by the simpler method agree most closely with the values obtained with the formulas in which body water values were used (formulas 3 and 4). It is significant that after the body weight gain during the 4th interval studied in Subject B (84-86.5 kilos), when the body fat would likewise be expected to increase, only the formulas (3 and 4) based on body water measurements gave indications of such an increase in fat content. This supports the greater reliability of the body fat determination involving body water.

Adipose Tissue Chemical Analyses - Table IV shows how the periods of diet affected the composition and density of subcutaneous abdominal adipose tissue in the 2 subjects. The chemical compositions reported are average values from analyses of 2 or 3 portions from each biopsy sample.

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Clearly shown is the decrease of fat content during body weight reduction in both subjects. These losses are expressed not only in terms of a percentage content decrease to 79% and 92% of the original fat contents, but also more markedly in the loss of fat per residue to about half of the original levels in both subjects. This latter measure related the fat to non-fat, non-water constituents, and thus provides a truer comparison to more constant portions of cellular material, especially protein.

The predominant loss of fat from the adipose tissues caused in both subjects an increase in the densities of the tissues. This is apparent not only in the values calculated from the chemical compositions, but also in the values obtained by direct measurement, which agree well with the former, which were obtained using figures of 0.91, 1.00, and 1.40 gms/ml, respectively, for the densities of fat, water, and residue, at room temperature.

Deoxyribonucleic acid analyses, expressed per residue weight, point to a maintenance of what may be regarded as "normal cellularity" in the 16-week diet interval of subject B, but a loss of this normal relationship in the next few months in this same subject and also in subject E. It is notable that the decrease in DNA per residue in the latter case is of the same relative magnitude as the water loss.

<u>Adipose Tissue Histologic Study</u> - Hematoxylin-eosin-stained sections of adipose tissues were studied before and after weight reduction in subject E. The numbers of nuclei per high-power field (x 440) were counted for 1000 fields on sections of each biopsy. These observations were kindly performed by Dr. Alfred Spivack of this Laboratory. Before dieting the data showed an average of 13.4 nuclei per field, while the sample after weight loss

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averaged 17.3. This increase of 4 nuclei per field was found significant at the 1% level (Student "t" test), and is of the same magnitude (29%) as was to be expected from the data of Table IV, where a decrease of 26% may be noted in the total amount of tissue material (fat, water, and residue) per DNA in subject E.

DISCUSSION

The results described here on the density of the material lost during weight reduction are in accord with those reported by other investigators, and support the conclusion that, although fat represents a large portion of the weight lost, other non-fat materials also participate in the loss. In addition, the data obtained by chemical analyses on biopsy samples taken before and after weight reduction leave no doubt that fat has been removed from adipose tissue. And, although it is apparent from direct analysis that non-fat materials also have been removed from adipose tissue, it seems unlikely that a great portion of the non-fat material lost from the body was derived from this source. The source of this non-fat material is not known.

If, as has been suggested[3,4], adipose tissues participate as a unit in human body weight reduction or gain, 2 principles should hold: (1) the density of tissues lost or added by the whole body should resemble that of adipose tissue, and (2) depot tissue density and composition should remain essentially unchanged after weight alteration. Previous data support the first of these conditions. The density of adipose tissue may be set at a value somewhere between 0.941 gms/ml, as may be calculated from the data of Forbes *et al*[7] and 0.96, as determined by Schubler and Kapff[6] and

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and corrected to body temperature by von Döbeln[4]. Between these figures lie most values obtained for the density of tissues lost or gained during body weight changes in the human, as reported by Behnke, Osserman and Welham[2], and by Keys and Brozek[3].

Likewise, the densities of adipose tissues in our 2 subjects were 0.93 gms/ml before weight reduction, both by direct determination and by calculation from chemical analyses (Table V). This value would correct to 0.92 gms/ml at body temperature. Similarly, the data in Table III indicate that body tissues lost during weight reduction have densities of 0.92-0.96 gms/ml at body temperature, figures quite close to those of the adipose tissue densities. From these values alone, we, too, might conclude that adipose tissue has participated uniformly in body weight loss.

However, careful analysis of the data indicates that this is not so. Firstly, tissues lost at most intervals in both subjects are denser than the original adipose tissues. This is true for the cumulative total tissues lost in both subjects (Table III) as well as the successive intervals in subject B, where values as high as 1.06 gms/ml were obtained for short periods of weight loss, just as has been recorded previously[2,16]. (Such data give values not as reliable as those for longer periods of weight loss, involving greater body weight and volume changes, due to the greater relative error in dealing with ratios of smaller numbers). This loss of whole body tissues consistently <u>denser</u> than the original depots points to a loss of a greater proportion of non-fat substances from the whole body than was originally present in the adipose tissue.

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That these non-fat components did not come, at least largely, from the depots themselves is indicated by the data of Table V, which show that the depots actually lost a greater proportion of fat than of non-fat materials. For the proportion of fat to have decreased as shown, fat must have been lost preferentially from the depots, a process further indicated by the increased fat tissue densities determined in Subject E. The small changes in water and DNA per residue also support this conclusion, especially in the face of a 50% drop in fat per residue in both subjects.

The apparent return of depot fat at the time of the last biopsy in subject B (Table V) is puzzling, especially in view of the continued weight loss by this subject (Table II). This would not seem to be a fault of sampling technique or analytical error, since good checks in chemical analyses and densities have always been obtained in duplicate or triplicate samples from the same biopsy specimen, while better correlations have even been seen in comparing analyses of certain depots in the rat. On the other hand, suspicion in this case lies in the fact that the subject had been on a pemmican-type diet for seven days previously, during which the diet, containing 45% each of protein and fat and practically no carbohydrate, led to the development of distinct ketonuria and ketonemia. The consequent weight loss consisted mostly of water (Table II), and it may well be that the disturbances in body fat metabolism, indicated by the excess blood and urine ketones, would certainly have complicated the picture of depot fat disposition.

The data presented here reveal a distinct difference between the principal sites of fat and non-fat tissues lost during weight reduction. In the material lost from the body the proportion of non-fat substance was

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greater than that in adipose tissue while the proportion of fat was smaller. On the other hand, in the material lost from the depots the percentage of fat was greater than that originally present in the adipose tissue. We must conclude therefore that while adipose tissues probably contributed in greatest measure to the fat (and body weight) loss during weight reduction, other soft tissues also contribute significantly to the body weight decrease by losing non-fat components. Uniform participation of adipose tissue components in body weight loss, as previously concluded, is unsupported by our observations.

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SUMMARY

Gross body measurements in 2 subjects before and at various periods during weight reduction, have shown over-all losses of tissues ranging in density from 0.92 to 0.96 gms/ml at body temperature. Calculations indicate that fat comprises 55-85% of these total lost tissues. During successive intervals in one subject, densities of lost tissues were found to range from 0.93 to 1.06 gms/ml, corresponding to fat contents of from 2 to 90%.

Densities of subcutaneous adipose tissues rose from 0.93 gms/ml before weight reduction to 0.94-0.96 gms/ml after weight loss. Densities calculated from chemical composition agreed with those obtained by direct determination. Depot tissue fat content decreased from 80-85% of fresh tissue weight to 60-80%. Related to dried, de-fatted residue, fat content decreased by 50%, while water and DNA remained more constant.

Body weight loss caused fat to be lost from adipose tissues in greater proportion than was originally present in the depots. On the other hand, the whole body lost relatively less fat than was contained in the depots. These observations indicate that soft tissues other than fat depots lose significant portions of non-fat components, even though adipose tissues probably contribute the major portion of body fat and weight loss.

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TABLE I

COMPOSITION OF DIETS

Subject	Date Diet Started	Duration of Diet	Type of Diet	Calories per day[a]	Daily Dietary Intake			
					Milk Powder (gm)	Dextri Maltose (gm)	Corn Oil (gm)	Other
B	12-20-55	14 wks	Free choice	1400[b]	-	-	-	-
	3-30-56	10 wks	Free choice	2400 to 3000[b]	-	-	-	-
	6- 7-56	2 wks	Liquid-evap. milk	2320	-	120	45	Evap. milk, 60 gm Dry cereal, 170 gm
	6-21-56	8 days	Liquid	3180	274	200	120	Cereal, 102 gm
	6-29-56	1 wk	Liquid-butter	3000	274	200	-	Butter, 120 gm Cereal, 102 gm
	7- 6-56	13-1/2 wks	Free choice	3200[b]	-	-	-	-
	10- 8-56	8 days	Liquid-high CHO	3190	408	454	-	-
	10-16-56	3 wks	Free choice	3200[b]	-	-	-	-
	11- 5-56	4 days	Meat bar	1000	-	-	-	Meat bar, 170 gm
	11- 9-56	3 days	Meat bar dextrose	1190	-	50	-	Meat bar, 170 gm
	11-12-56	1 day	Free choice	3200[b]	-	-	-	-
	11-13-56	6 days	Liquid	2400	204	227	90	-
E	9-27-56	7 days	Liquid	2460	408	126	60	-
	10- 4-56	43 days	Liquid	1230	204	63	30	-
	11-16-56	10 days	Liquid-Margarine	1230	204	63	0	Margarine, 37 gm

(a) Calculation of calories based on caloric values for 1 gm of protein, carbohydrate, and fat of 4.0, 3.9, and 8.9, respectively.

(b) Estimated values.

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TABLE II

MEASUREMENT OF WHOLE BODY CHANGES

Subject	Date	Body Weight (kg)	Body Volume (l)	Body Density (gm/ml)	Body Water (l)	Body Fat (kg)[b]
B	12-20-55[a]	101.5	98.06	1.035	49.6	33.1
	2- 8-56	91.2	86.94	1.049	(49.0)	23.6
	4-10-56[a]	86.6	82.60	1.048	48.6	19.6
	6-29-56	84.4	80.15	1.053	47.5	18.9
	10-16-56	86.5	81.76	1.058	47.6	20.8
	11-12-56[a]	83.0	78.45	1.058	45.3	20.5
E	9-26-56[a]	89.1	86.67	1.028	49.8	20.4
	11- 5-56[a]	80.73	78.00	1.035		

(a) Dates when adipose tissue biopsies were also taken.
(b) Fat-free body weight was calculated as body water/0.72 and the value obtained was subtracted from the body weight to give the kg of body fat.

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TABLE III

DENSITY AND COMPOSITION OF TISSUE LOST OR GAINED

Subject	Body Weight (kg)	Total Tissue Lost Since Initial Observation					Tissue Lost Since Previous Observation				
		Composition[a]					Composition[a]				
		Density (gms/ml)	Fat kg	Fat percent	Non-Fat kg	Non-Fat percent	Density (gms/ml)	Fat kg	Fat percent	Non-Fat kg	Non-Fat percent
B	101.5										
	91.2	0.926	8.38	81	1.92	19	0.926	8.38	81	1.92	19
	86.6	0.964	8.38	56	6.52	44	1.060	0.00	-	4.60	100
	84.4	0.955	10.63	62	6.47	38	0.898	2.25	-	(0.05)[b]	-
	86.5	0.920	12.81	85	2.19	15	(1.304)[b]	2.18	-	(4.28)[b]	-
	83.0	0.943	12.88	70	5.62	30	1.057	0.07	2	3.43	98
E	89.1										
	80.7	0.965	4.56	54	3.81	46	0.965	4.56	54	3.81	46

(a) Composition of "Tissue Lost" calculated as described in text, assuming
1) Density of fat = 0.90
2) Density of non-fat soft tissues = 1.06
3) Changes involve only soft and adipose tissues, not bone.

(b) Indicates gain of tissue with this density or composition.

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TABLE IV

BODY FAT CONTENT CHANGES CAUSED BY DIETING

Subject	Body Weight (kg)	Cumulative Body Fat Loss (kg)				
		Formula 1- Keys (3)	Formula 2- Siri (15)	Formula 3- Siri (8)	Formula 4- Behnke (2)	Formula 5- This Paper
B	101.5	-	-	-	-	-
	91.2	7.46	8.75	9.13	9.5	8.38
	86.6	8.00	9.41	11.78	13.5	8.38
	84.4	10.03	11.76	13.24	14.2	10.63
	86.5	11.30	13.24	12.76	12.3	12.81
	83.0	11.86	13.86	13.22	12.6	12.88
E	89.1	-	-	-	-	-
	80.73	4.55	5.22	-	-	4.56

Formulas used to calculate body fat contents.

1. $F = \frac{4.201}{d} - 3.813$

2. $F = \frac{4.950}{d} - 4.500$

3. $F = \frac{2.057}{d} - 0.786\ W - 1.286$

4. L.B.M. = BW ÷ 0.72
   B.F. = TBWt - L.B.M.

5. Equation (2) in text, see also Table III.

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TABLE V

EFFECTS OF WEIGHT LOSS UPON HUMAN ADIPOSE TISSUE

	Subject B			Subject E	
	12-19-55	4-10-56	11-19-56	9-25-56	11-28-56
Body Weight (kg)	101.5	86.6	83.0	89.1	80.7
Adipose tissue analyses					
Fat (percent)	79.2	62.3	76.7	85.7	78.9
Water (percent)	18.0	32.4	19.0	12.5	17.7
Residue (percent)	2.8	5.3	4.3	1.8	3.4
Density (calc.)[a] (gms/ml)	0.935	0.957	0.940	0.926	0.937
Density (det'd.) (gms/ml)		(0.97)[b]	0.940	0.927	0.935
Fat/residue (gms/gm)	28.2	11.8	17.8	47.5	23.2
Water/residue (gms/gm)	6.4	6.1	4.4	6.9	5.2
DNA/residue (gms/gm)	.00414	.00402	.00194	.00523	.00397
Fat/DNA (mg/y)				9.11	6.22
Water/DNA (mg/y)				1.33	1.36
Res./DNA (mg/y)				0.188	0.259
Total content/DNA[c] (mg/y)				10.63	7.84

(a) Calculated from tissue constituents.
(b) This analysis was made by cruder technique than described in text (see Methods).
(c) For significance of this values, see text for discussion of histologic findings.