Determination of Fat Content of a Controlled Weight Reduction Diet

by

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Introduction

In many laboratories conducting experiments involving the preparation of diets, significant differences have been observed between the caloric values as calculated from tables of food composition and those obtained by the application of conversion factors to the values for carbohydrates, protein and fat determined by proximate analysis.

In the research project concerning the utilization of calories and protein by overweight college women on a weight-reduction diet, and conducted by the Foods and Nutrition Department of Michigan State College, discrepancies were observed between the food calories as computed from the tables and as determined from the bomb calorimeter.

As the diets were characterized by a high fat content, a likely variation in the actual fat content of the diet from the values computed from the food tables, was considered an important factor in causing the discrepancy. The purpose of this study therefore was to determine the fat content of the composite diets used in the above-mentioned research project, by quantitative analysis. A comparison of the values thus obtained with the fat content as calculated from the food composition tables, was also a matter of consideration.

It might be added that some of the fecal samples of the subjects were also analyzed for their fat content.

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Review of Literature

Various methods have been developed and used for the extraction of fat from foods, ranging from direct extraction from the dried product by solvents, saponification of the fat followed by extraction of the liberated fatty acids by light petroleum, to acid treatment followed by extraction with mixed ethers (Bransby et al. 1948).

The first method fails to extract the fat completely from many cooked starchy foods, such as bread and biscuit. In the second method the calculation of the fat content rests on the assumption that triglycerides are the only compounds of fatty acids present in the material originally taken. Besides, since light petroleum takes up sterols as well as fatty acids steps should have to be taken for their removal.

The prescence of lower fatty acids in butter, margarine and hydrogenated coconut oil introduces errors since these acids are appreciably volatile under the conditions required to drive off the extracting solvent and to dry the recovered fatty acids. Further, as butyric is miscible with water, and other acids are appreciably soluble in it, they are not completely extracted by solvents.

The third method consists in treating the sample with hydrochloric acid followed by alcohol and extracting the fat with ethyl ether and light petroleum. The extract obtained by this method includes glycerides, phosphatids, sterols and free fatty acids.

A reliable method of fat-analysis is by continuous extraction with ether in a soxhlet apparatus, although it takes a long time. Extraction determines crude fat, including neutral fats, phospholipids and other substances in small amounts. Extraction method is convenient, and considered more suitable if the sample is properly ground and dried. (FAO, 1947)

This method was adopted for the present investigation.

Nickelsen et al. (1947) analyzed batches of three different low-fat diets used in rotation over a period of six months as part of a large experiment on semi-starvation. Results of analysis of different batches of the same diet showed that the apparent fat content was subject to the greatest day-to-day variation. Using the soxhlet method of extraction with ether, they found that an addition of a detergent (.5% Duponol) to the food collected for fat analysis resulted in a very considerable increase in the weight of material extracted by ether. This was explained as being due to the homogeneity produced in the sample by the surface active agent. The difference amounted to as much as 30 per cent in a dry food mixture and to as much as 300 per cent in a mixture analyzed in the moist state.

Shannon (1949) reported that the length of drying time after extraction influenced the final percentage of fat obtained.

Bransby et al. (1948) in their study of the comparison of the estimation of the food intake for 3 days of a number of children obtained by weighing and calculating, and by chemical analysis found that calculation from tables over-estimated the fat content. In another study on the methods of individual dietary survey Bransby et al. (1948) found that for individual diet differences between the values found by calculations and by chemical analysis were in many instances so large as to throw doubt on the usefulness of the individual results obtained by calculation.

Hummel and others (1942) found that the results obtained from the analysis of foodstuffs varied widely from the standard figures. The few foods other than butter and milk which contained fat in appreciable amounts, were concentrated types of foodstuffs which are difficult to sample accurately. In addition they were not uniform as purchased, though

every possible precaution was observed to control variations which might arise at the source of supply. For instance, shoulder beef was recommended as the most uniformly lean meat to be obtained, and all purchases were made from one kitchen, but samples were found to vary as much as 1-15 per cent in fat content. The energy values of beef confirmed the difference in composition. Hummel found that individual samples of a given food vary from values reported in standard tables, but when analysis of composite diets are compared with dietary figures calculated from the literature, there is fairly good agreement in fat content.

Thomas et al. (1950) in a study of the nutritional status of children evaluated the accuracy of calculated intakes of food components with respect to analytical values. For fat and some other nutriments, 28 of 33 differences showed smaller contents by analysis than were indicated by calculation. The fat intakes estimated from food composition tables were in no close agreement with the actual amounts of fat ingested, emphasizing the great variations in the concentration of this constituent in foods. Thus a comparison of data obtained by analysis and by calculation from standard tables of food composition showed that in diets eaten by two groups of children, in fall and in spring, the results for fat were significantly different in both the seasons. Consistent differences indicated that values selected from the tables did not approximate the true values in either season.

The analyzed values for the fat content of diets containing large amounts of complex recipes, could be different from values obtained by calculation from tables. In calculating the diets of older women using the tables of Donelson and Leichsenring (1945), Ohlson (1950) found that the calculated values for nitrogen, calcium and phosphorus did not correspond to the analyzed values for these diets which contained a high proportion of processed meats or complex cooking mixtures.

Experimental Procedure

Experimental Background:

The diets analyzed for their fat content, were those used in the metabolism study on weight reduction carried on six overweight college women, by the Department of Foods and Nutrition of Michigan State College.

The general pattern of the weight reduction diet was: high protein, high fat and low carbohydrate, with a total caloric intake of 1,600 - 1,700 calories per day. (The National Research Council recommends an average of 2,000 calories daily for healthy, moderately active young women)

The subjects were maintained on a self-selected diet for a two-week observation period, preliminary to the period of weight loss. During the second week a balance study was made to establish calcium retention of the subjects. After two weeks on the weight reduction diet, another balance period was maintained for a week. Six balance periods were completed during the entire study which lasted from January, 1951 to June, 1951.

Experimental diet:

Seven menus were planned for the weight-reduction diet, and these were repeated each week. The meals were prepared and served in the Home Economics Department. All foods eaten were weighed on a Hansen dietetics scale.

Sampling of food:

During the second week of the self-selection diet, all servings were weighed and a weighed aliquots equal to one-fifth of the food eaten were frozen, after each meal. A similar procedure was followed for the weight reduction diet. Liquids and solids were saved separately for convenience in handling. The seven-day collection period was divided into A. 4-day period and B. 3-day period for convenience. The samples were thawed before

blending at the completion of each week. The food was blended in a Waring food blender for five minutes (until mixture became homogenous), transferred to a 2-liter volumetric flask and made to volume with distilled water. After mixing the slurry, two samples were measured into 250 milliliter volumetric flasks, then transferred to weighed evaporating dishes and dried in an oven at 40 degrees centigrade for 3 to 4 days until dry. The samples were stirred at intervals to permit more even drying, and after drying to constant weight, the sample was scraped from the dish, transferred to a bottle and stored in a desiccator until analysed.

During the balance periods daily fecal collections were made in waxed containers. At the end of each balance period the fecal collectionswere transferred to a blender and blended for five minute then made to volume in a two-liter volumetric flask. Three samples were measured into 100 ml. volumetric flasks, transferred to weighed evaporating dishes and dried partially over a steam bath and then under infra-red lamps. After drying to constant weight the sample was scraped, ground and stored in bottles in a desiccator until required for analysis.

The average weekly loss of weight was one kilogram per person.

Fat - extraction method:

Approximately two grams of the dried sample was weighed out directly in an ether-extracted extraction thimble (weighed previously) in an analytical balance. The samples were then extracted in a soxhlet apparatus for twelve hours.

The soxhlet apparatus consists of a wide glass tube with a side siphon, connected at the bottom to a receiver flask and fixed to a condenser at the top. The apparatus is fitted up on a water bath and the condenser cooled with a running stream of water from

the tap.

After the water had been allowed to run for some time, ether was poured into the receiver flask and the latter was fixed tightly to the soxhlet. The steam was turned on the water bath. The ether evaporated, was condensed in the condensor and allowed to drop into the soxhlet tube with the extraction thimble and the sample in it. The ether dissolved the fat in the sample, and when enough of it collected, it was automatically siphoned back into the receiver. Thus the extraction was continuous. After the 12-hour extraction, the steam was turned off, the apparatus disconnected and the thimbles carefully removed with a pair of tongs, and left in beakers to dry in the air for an hour. They were dried in the disiccater afterwards for 18 hours before being weighed. The difference in weights gave the weight of fat extracted from the sample. The percentage of fat in the given sample was calculated from this and from the recorded weight of the dried material in the 250 ml. aliquot of the blended composite, the weight of fat in it was obtained. Finally the grams of fat in the weight reduction diet for the given period per day was calculated as shown in Table 1.

Fat extractions were made of duplicate samples of both A and B parts of the following weighed controlled periods:
11, 12, 14, 15, 17, 20 and 22.

The conditions for drying were standardized in order to eliminate any variation in moisture content.

Fat extractions of the fecal samples of one of the subjects 'L' for the following balance periods were made:
2, 6, 13, and 21.

The amount of fat in the feces per day was calculated as shown in Table 2.

Results and Discussion

The average weights of fat in the diets as obtained from analysis by ether-extraction are given in Table 3. The fat contents of the diet as calculated from the food tables (U. S. Department of Agriculture, 1950) are given in Table 4.

The daily average weight of fat in the weight-reduction diet as obtained by extraction was 92.8 grams, while that calculated from food composition tables was 100.2 grams as shown in Table 5. This result is in accordance with that obtained by Thomas et al. (1950) who also found that the fat content of the diets was smaller when obtained by analysis than the values obtained from food composition tables.

The fat content of the diets analyzed for the seven different periods varied from 82.2 to 101.5 grams per day (Table 5). The actual fat content of meat, and rather intricately prepared recipes containing high fat, has been found to vary and fluctuate considerably from the values given in good composition tables. Although conditions of preparation, the source and cuts of the meat (as all other foodstuffs used in the diets) were standardized as far as possible, it is likely that some factors of variables could not be eliminated. Furthermore, there is no proof that the fat extraction values are absolutely correct. Repeated extractions of duplicate samples need to be done in order to get appreciably accurate results. The moisture content is an important factor in causing variation in results. Although the conditions for moisture content were standardized, in the present experiment, it cannot be claimed that they were the same as those conducted in other laboratories.

In the weight reduction diets, an average of 1,650 calories per day was supplied, of which about 360 calories were provided from approximately 90 grams of protein, 460 calories from about 115 grams of carbohydrate and the remaining 840 calories by about 90 grams of fat.

The average daily weight of fat in the diets obtained by analysis was actually 92.8 grams which provided 835 calories which is nearly 49 per cent of the total calories (average for the weight control periods analyzed) in the day's diet. The latter determined by the bomb calorimeter, was 1,708 calories.

The fecal samples of Subject L from the four balance periods analyzed gave the results tabulated in Table 6. The fat extracted from the feces ranged from 2.65 to 3.65 grams per day for periods II, VI, XIII. The validity of the results obtained for period XXI is questionable since the ~~continual~~ fecal sample was too small, for convenient preparation of the dry sample. It was found that 31.5 to 46.5 per cent of the total calorie output in the feces was provided by the fat content of the feces.

Table I

Calculation of grams of fat per day in the weight reduction diet for period 20. (A = first four days of the week. B = next 3 days).

Item	Sample AY	AY	PX	Sample BY
Weight of beaker & thimble & sample	51.5040 gm.	51.4245 gm.	51.3516 gm.	51.8314 gm.
Weight of beaker & thimble	49.4100	49.3950	49.3339	49.8026
Weight of sample	1.7950	2.0295	2.0177	2.0288
Weight of beaker & thimble & sample after extraction	50.9022	50.7270	50.6025	51.2805
Weight of fat extracted	0.6018	0.7075	0.7491	0.7509
Percentage of fat in sample	34.7 %	34.7 %	37.1 %	37.0 %
Weight of dried material in 250 ml. of blended composite	28.2	28.5	21.1	21.3
Weight of fat in 250 ml. of composite	9.78	9.88	7.83	7.88
Weight of fat in 2000 ml. of composite	78.24	79.04	62.64	63.04
Fat per day in 1/5 serving	19.56	19.76	20.88	21.01
Fat per day, in grams	97.80	98.80	104.4	105.05

Average fat per day 101.6 gms.

Table II

Calculation of grams of fat per day in the feces obtained from subject I for the balance period 13.

Item	Sample X	Sample Y
Weight of beaker + thimble + sample	52.0912	52.0616
Weight of beaker + thimble	50.1660	50.0450
Weight of sample	1.9252	2.0166
Weight of beaker + thimble + sample after extraction	51.6723	51.6151
Weight of fat extracted	0.4189	0.4465
Percentage of fat in the sample	21.7 %	22.1 %
Weight of dried material in 100 ml. aliquot	4.3	4.3
Weight of fat in 100 ml.	0.93	0.95
Weight of fat in 2000 ml.	18.6	19.0
Grams of fat in feces per day	2.6	2.7

Table III

Fat content of the diets as obtained by analysis.

Period	Sample AX	AY	Ave.	Sample EX	EY	Ave	Average gms. fat/ day for p
11	92	106	99	78	76	77	88
12	87	89	88	111	97	104	96
14	95	95	95	100	99	99.5	97.5
15	95	95	95	100	97	98.5	96.7
17	81	81	81	94	94	94	87.5
20	98	99	98.5	104	105	104.5	101.5
22	86	89	87.5	78	76	77	82.2

Total average wt. of fat per day from periods 11 - 22 = 92.8

Table IV

Fat content (calculated from tables) of foods included in menus during seven day period of weight reduction diet.

Food	Quantity. wt. in gms.	Gms. fat
MONDAY		
Grapefruit and orange juice	100	0.1
Eggs - 2	124	11.5
Bread	20	0.6
Butter	10	8.0
Milk	450	17.5
Pork Chop	125	32.5
Broccoli	100	0.2
Hamburg	125	37.5
Creamed potatoes	100	2.0
Total fat content of the day's diet		109.9
TUESDAY		
Orange juice	100	0.2
Eggs	124	11.5
Bread	20	0.6
Butter	10	8.0
Milk	450	17.5
Lamb Patties	125	35.0
Pears, drained	100	0.1
Lettuce	15	-
Roast veal	125	15.0
Peas	75	0.3
Butter	10	8.0
Apple sauce	100	0.2
Total fat content		96.4

Table IV - continued

Food	Quantity. wt. in gm.	Gm. fat
WEDNESDAY		
Orange juice	100	0.2
Eggs - 2	124	11.5
Butter	20	16.0
Bread	20	0.6
Milk	450	17.5
Meat Loaf	125	40.0
Cottage cheese	20	0.1
Fresh tomatoes	30	-
Lettuce	10	-
Roast beef	125	16.2
Boiled potato	80	-
Sweet cherries	80	0.4
Total fat content of the day's diet		102.5
THURSDAY		
Grapefruit juice	100	0.1
Eggs - 2	124	11.5
Butter	15	12.0
Bread	20	0.6
Milk	450	17.5
Swiss steak, with	125	16.2
fat for broiling	5	5.0
Cauliflower	100	0.2
Roast pork	125	32.5
Beets	100	0.1
Apricots	100	0.1
Total fat content of the day's diet		95.8

Table IV - continued

FRIDAY		
Food	Quantity wt. in gm.	Gm. fat
Tomato juice	100	0.2
Eggs	124	11.5
Butter	30	24.0
Milk	450	17.5
Bread	20	0.6
Baked white fish (Haddock)	125	7.0
Sauce	15-	2.0
Baked sweet potatoes	100	0.9
Salmon (Pink)	125	7.7
Celery	25	-
Mayonnaise	25	19.5
Lettuce	10·	-
Green beans	100	0.2
Total fat content of the day's diet		91.1
Saturday		
Orange & grapefruit juice	100	0.1
Eggs	124	11.5
Butter	15	12.0
Bread	20	0.6
Milk	450	17.5
Hamburg	125	37.5
Tossed salad	100	0.2
French dressing	5	1.8
Pork roast	5	32.5
Peas	75	0.3
Lettuce	50	0.1
Canned peaches	100	0.1
Total fat content of the day's diet		114.2

Table IV continued

SUNDAY

Food	Quantity wt. in gm.	Gm. fat
Orange	One	0.3
Sweet roll	25	1.4
Butter	20	16.0
Milk	300	11.7
Bread	30	0.9
Roast chicken	150	18.9
Fresh tomato	100	0.3
Lettuce	50	0.1
French dressing	5	1.8
Cold meat loaf	125	40.0
Apple	150	0.6
Total fat content of the day's diet		91.1

Table V

Table showing the daily intake of fat on the weight reduction diet in a seven-day period, as calculated from food composition tables.

Day	Gm. fat/day
Mon.	109.9
Tues.	96.4
Wed.	102.5
Thurs.	95.8
Fri.	91.1
Sat.	114.2
Sun.	92.0
Daily average	100.2

Table V

Percentage of calories supplied by fat as determined by extraction, in the weight reduction diet.

1. Period	2. Fat content as obtained from extraction	3. Caloric value of (2)	4. Total caloric value of diet* determined gy bomb. cal./day	5. Percent cals. supplied by fat.
	gm/day	calories		./.
11	88	792	1637.34	48.3
12	96	864	1747.54	49.4
14	97.5	877.5	1774.87	49.4
15	96.7	870.3	1726.75	50.8
17	87.5	787.5	1692.8	46.9
20	101.5	913.5	1731.58	52.7
22	82.2	739.8	1647.76	44.9
Average	92.8	835	1708	48.9

Note * Obtained from Foods and Nutrition staff.

Table VI

Relationship between the percentage of caloric output calculated from fat excreted in feces, and total caloric output during balance periods for Subject L.

Period	Caloric intake per day*	Caloric output in feces per day*	Gms fat extracted from feces sample (1)	sample (2)	ave.	Calories. supp. by excreted fat	Percent cals. from fat in total output %
II	1788.5	70.79	3.9	3.4	3.65	32.85	46.5
VI	1692.02	92.37	2.96	3.57	3.27	29.43	31.5
XIII	1782.77	62.40	2.6	2.7	2.65	23.85	38.8
XXI ⊥	1298.84	11.65	0.44	0.43	.44	3.96	34.1

Note * Data obtained from bomb calonimeter records from the Staff of Foods and Nutrition Department.

⊥ The original sample collected was very small.

Summary and Conclusion

The fat contents of dried samples of controlled weight reduction diets for seven periods were determined by extraction with ether in a soxhlet apparatus, under standardized conditions. An average daily fat content of the diet was calculated from the seven-day menu using food composition tables. The average amount of fat in the diet as obtained by ether extraction was found to be 92.8 grams per day which was lower than the amount estimated by calculation from food tables -- this being 100.2 grams per day. As the diet contained a daily average of 1,650 calories, the fat provided nearly 49 per cent of these calories.

The discrepancy between the fat values of the diet obtained by the two methods could be due to various factors already cited in the review of literature. However, it must be noted that this difference is not too great.

The dried fecal samples of one of the subjects L on the fat reduction diet from four balance periods were also ether - extracted to determine the fat content of the feces. Approximately 3 grams of fat were excreted in the feces daily, and this formed 30 per cent of the total caloric output.

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