

## The Role of Fat in the Diet of Rats

### 5. Influence of Supplementation with Raw Skim Milk, Linoleic Acid or both on Food and Fluid Consumption and Urine Production

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This paper concerns the correlation between fluid intake, urine output, calorie intake and growth rate in rats on diets with different fat contents with and without supplementation with linoleic acid and raw skim milk.

#### EXPERIMENTAL

The rats and diets used in this study were those previously described (Aaes-Jørgensen & Dam, 1954*c*). The food and fluid consumption as well as the urine production were measured throughout the experimental period. The technique used in collecting the urine for 2-day periods has already been described (Aaes-Jørgensen & Dam, 1954*b*).

#### RESULTS AND DISCUSSION

The results are presented in Table 1.

*Fluid balance.* The table shows that the difference between fluid intake and urine output, both expressed in ml./sq.m surface/animal/day, was markedly increased on diets with 7 and 28% hydrogenated peanut oil and water (series A and E), compared with the corresponding lard and peanut-oil diets.

In series B, on a 7% fat level supplemented with linoleic acid and with water as drinking fluid, the difference between fluid intake and urine production was fairly small in all three groups. The fluid intake per sq.m surface was very low in group 48 (7% hydrogenated peanut oil, Table 1).

In series F with water as drinking fluid the 28% dietary fat level was supplemented with linoleic acid, and the difference between fluid intake and urine output was also small.

With raw skim milk at the 7% dietary fat level (series C), the difference between fluid intake and output was extraordinarily low on the peanut-oil diet (group 41) probably owing to the high diuresis in this group.

Supplying raw skim milk as drinking fluid with the 28% dietary fat level (series G) increased the difference between fluid intake and output to a high degree on hydrogenated peanut oil (group 53), owing to a big increase in the fluid intake per sq.m surface area in this group.

On diets with a 7% fat level, supplemented with linoleic acid and raw skim milk (series D) the difference between fluid intake and output was small; the highest value occurred in the hydrogenated peanut-oil group (group 50).

Table 1. Mean values for surface area, and mean daily values for gain in weight, food, fluid and calorie intake and urine production for groups of six rats for the last 6 weeks of the experiment

| Series no. | Group | Diet characteristics                                      | Final weight at the end of the experiment (18 weeks) (g) | Surface area (sq.cm) |      | Fluid intake |      | Food intake (g) | Total calorie intake (d) |           | Urine production |      | Difference: fluid intake - output (ml./sq.m) | Calories used for evaporation of water* (c) (Cal./sq.m) | Calories used for growth (a-c) (Cal./sq.m) | Weight gain (g) | Approximate calorie cost of weight gain (Cal./sq.m/g) |
|------------|-------|---|--|----------------------|------|--------------|------|-----------------|--------------------------|-----------|------------------|------|--|---|--|-----------------|---|
|            |       |   |  | ml.                  | sq.m | ml.          | sq.m |                 | Cal.                     | Cal./sq.m | ml.              | sq.m |  |   |  |                 |   |
| A          | 31    | 7% lard, water  | 190.3 ± 4.7  | 382                  | 33.4 | 875          | 10.8 | 44.2            | 1157                     | 13.1      | 343              | 532  | 324  | 833   | 0.57                                       | 1462            |   |
|            | 39    | 7% peanut oil, water                                      | 208.0 ± 11.0   | 406                  | 33.6 | 827          | 11.2 | 46.1            | 1135                     | 11.2      | 276              | 551  | 335  | 800   | 0.55                                       | 1455            |   |
|            | 47    | 7% hydrogenated peanut oil, water                         | 156.5 ± 5.3  | 336                  | 31.3 | 931          | 11.6 | 47.5            | 1414                     | 4.6       | 137              | 794  | 483  | 931   | 0.28                                       | 3325            |   |
| B          | 32    | 7% lard, linoleic acid, water                             | 185.2 ± 7.8  | 375                  | 31.3 | 835          | 10.4 | 42.6            | 1136                     | 9.3       | 248              | 587  | 357  | 779   | 0.43                                       | 1812            |   |
|            | 40    | 7% peanut oil, linoleic acid, water                       | 212.5 ± 9.7  | 412                  | 35.0 | 850          | 12.7 | 52.0            | 1262                     | 13.3      | 323              | 527  | 321  | 941   | 0.64                                       | 1470            |   |
|            | 48    | 7% hydrogenated peanut oil, linoleic acid, water          | 181.7 ± 4.5  | 371                  | 24.9 | 671          | 11.4 | 47.0            | 1267                     | 6.5       | 175              | 496  | 302  | 985   | 0.48                                       | 2011            |   |
| C          | 33    | 7% lard, raw skim milk                                    | 214.0 ± 5.2  | 414                  | 38.1 | 920          | 8.1  | 46.3            | 1118                     | 10.5      | 471              | 449  | 273  | 845   | 0.76                                       | 1112            |   |
|            | 41    | 7% peanut oil, raw skim milk                              | 228.8 ± 8.5  | 433                  | 40.8 | 944          | 8.7  | 49.5            | 1143                     | 31.3      | 723              | 221  | 134  | 1000  | 0.76                                       | 1328            |   |
|            | 49    | 7% hydrogenated peanut oil, raw skim milk                 | 203.2 ± 13.0   | 399                  | 43.1 | 1008         | 9.2  | 52.7            | 1321                     | 20.5      | 514              | 494  | 301  | 1020  | 0.77                                       | 1325            |   |
| D          | 34    | 7% lard, linoleic acid, raw skim milk                     | 227.0 ± 11.4   | 430                  | 52.2 | 1214         | 6.6  | 44.9            | 1044                     | 38.8      | 902              | 312  | 190  | 854   | 0.83                                       | 1029            |   |
|            | 42    | 7% peanut oil, linoleic acid, raw skim milk               | 222.3 ± 9.8  | 424                  | 40.6 | 960          | 9.5  | 52.7            | 1243                     | 26.8      | 632              | 328  | 200  | 1043  | 0.85                                       | 1228            |   |
|            | 50    | 7% hydrogenated peanut oil, linoleic acid, raw skim milk  | 206.7 ± 12.1   | 404                  | 41.7 | 1003         | 8.7  | 50.0            | 1238                     | 23.9      | 592              | 411  | 250  | 988   | 0.66                                       | 1497            |   |
| E          | 35    | 28% lard, water   | 237.0 ± 14.5   | 443                  | 30.0 | 677          | 9.2  | 47.3            | 1060                     | 8.1       | 183              | 494  | 301  | 768   | 0.88                                       | 873             |   |
|            | 43    | 28% peanut oil, water                                     | 260.2 ± 14.6   | 471                  | 30.8 | 653          | 9.7  | 50.1            | 1065                     | 9.7       | 206              | 447  | 272  | 703   | 0.92                                       | 862             |   |
|            | 51    | 28% hydrogenated peanut oil, water                        | 134.2 ± 7.5  | 302                  | 30.5 | 1001         | 9.4  | 48.3            | 1599                     | 3.7       | 133              | 876  | 534  | 1065  | 0  | —               |   |
| F          | 36    | 28% lard, linoleic acid, water                            | 244.2 ± 17.1   | 452                  | 26.2 | 580          | 10.2 | 52.6            | 1164                     | 6.9       | 153              | 427  | 260  | 904   | 0.85                                       | 1064            |   |
|            | 44    | 28% peanut oil, linoleic acid, water                      | 215.3 ± 10.2   | 415                  | 28.5 | 686          | 8.7  | 44.7            | 1077                     | 8.4       | 202              | 484  | 295  | 782   | 0.60                                       | 1303            |   |
|            | 52    | 28% hydrogenated peanut oil, linoleic acid, water         | 196.5 ± 6.6  | 391                  | 26.3 | 673          | 11.9 | 61.5            | 1573                     | 6.1       | 186              | 517  | 315  | 1258  | 0.38                                       | 3311            |   |
| G          | 37    | 28% lard, raw skim milk                                   | 246.2 ± 10.5   | 454                  | 28.7 | 632          | 8.3  | 52.7            | 1161                     | 14.1      | 311              | 321  | 195  | 966   | 0.82                                       | 1178            |   |
|            | 45    | 28% peanut oil, raw skim milk                             | 254.0 ± 12.8   | 464                  | 26.1 | 504          | 7.8  | 40.0            | 1056                     | 11.0      | 256              | 308  | 187  | 869   | 0.90                                       | 965             |   |
|            | 53    | 28% hydrogenated peanut oil, raw skim milk                | 180.2 ± 8.7  | 369                  | 41.9 | 1137         | 9.0  | 60.5            | 1649                     | 10.0      | 271              | 866  | 527  | 1113  | 0.41                                       | 2715            |   |
| H          | 38    | 28% lard, linoleic acid, raw skim milk                    | 239.3 ± 10.2   | 445                  | 35.8 | 805          | 7.2  | 49.2            | 1107                     | 25.8      | 580              | 225  | 137  | 970   | 0.87                                       | 1115            |   |
|            | 46    | 28% peanut oil, linoleic acid, raw skim milk              | 256.0 ± 14.4   | 466                  | 28.6 | 613          | 7.8  | 50.0            | 1073                     | 14.3      | 307              | 306  | 186  | 887   | 0.87                                       | 1020            |   |
|            | 54    | 28% hydrogenated peanut oil, linoleic acid, raw skim milk | 212.5 ± 4.8  | 412                  | 39.2 | 953          | 10.5 | 67.3            | 1633                     | 21.4      | 519              | 434  | 264  | 1369  | 0.84                                       | 1630            |   |

\* 608.5 Cal./kg for evaporation of water at 29°.

At the 28% dietary fat level, with linoleic acid and raw skim milk (series H) the difference in fluid intake and output was decreased on hydrogenated peanut oil (group 54), compared with that on 28% hydrogenated peanut oil and raw skim milk (group 53) and hydrogenated peanut oil and water (group 51), but still showed the highest value compared with the lard (group 38) and the peanut-oil (group 46) diets.

These findings are in agreement with the results previously obtained (Aaes-Jørgensen & Dam, 1954*b*).

*Calorie intake and growth rate.* With a fat level of 7% the calorie intake was highest with hydrogenated peanut oil when the drinking fluid was water or skim milk (series A and C). When linoleic acid was given, with water (series B) or skim milk (series D), the differences in calorie intake between peanut oil and hydrogenated peanut oil were almost nil.

With a fat level of 28% the calorie intake was very high for the hydrogenated peanut oil, irrespective of whether water or skim milk was given or the diets with water or skim milk were supplemented with linoleic acid (groups 51-54).

When lard or peanut oil was used, the calorie intake was nearly equal in all four series, with a tendency to slightly higher values in the lard groups (36-38).

The growth rates of these animals have been discussed previously (Aaes-Jørgensen & Dam, 1954*c*).

From Table 1 it is evident that a calorie intake of about 1100 Cal./sq.m may result in very varying growth rates. With fat levels of 7 as well as of 28%, the growth rate was higher with lard and peanut oil than with hydrogenated peanut oil. The higher growth rate was not due to higher calorie intakes on the lard and peanut-oil diets, as illustrated particularly strikingly at the 28% fat level.

The growth rate was about equal on lard and peanut oil. The linoleic-acid content of the lard was 6.7%\* and of the peanut oil 26.8\*. It means that the requirements of female rats for linoleic acid (about 20 mg/day, Greenberg, Calbert, Savage & Deuel, 1950) were covered by the 7% lard diets and greatly exceeded by the 28% lard and the peanut-oil diets (cf. Aaes-Jørgensen & Dam, 1954*c*, Table 2). The effect of supplementing these groups with 21.4 mg linoleic acid was probably negligible.

Comparison (Table 1) of differences in fluid intake and output with total calorie intake and growth rate showed that increased growth with increased dietary lard or peanut oil was not caused by the extremely small differences between fluid intake and urine output, leaving more calories for growth; neither was it caused by an increased calorie intake.

The poorest growth rate in all the eight series was invariably found in the animals fed on hydrogenated peanut oil.

The difference between fluid intake and output was very high with the 7 or 28% hydrogenated peanut-oil diet with water (series A and E, Table 1). Assuming that this amount of water was evaporated, there were still more calories available for growth in these two groups (47 and 51) than in the similar groups fed on lard or peanut oil (31, 35 and 38, 43) which were growing significantly better.

\* The determinations were made by F. Engel, using the alkali isomerization method as described in the *Report of the Spectroscopy Committee* (Stillman, 1949).

Supplementing the 7 and 28% hydrogenated peanut-oil diets with 21.4 mg linoleic acid/animal/day (groups 48 and 52) brought the difference between fluid intake and urine output to about the same level as found on the corresponding lard and peanut-oil diets (series B and F). Again in this instance the slower growth rate of the animals on hydrogenated fat could not be explained by a decrease in the calories available for growth due to an increased need of calories for evaporation of water.

These results apparently indicate a deleterious effect of hydrogenated peanut oil, assuming that the digestibility and absorption of fat were not decreased with hydrogenated peanut oil (m.p. 40–42°) in the diet as previously discussed (Aaes-Jørgensen & Dam, 1954*a*).

Changing the drinking fluid from water (groups 47 and 51) to raw skim milk (groups 49 and 53) in the groups fed 7 or 28% hydrogenated peanut oil increased the total calorie intake, compared with that of animals given lard or peanut oil, especially in group 53, where the calorie intake was of the same order as in group 51, drinking water, and in group 52, supplemented with linoleic acid. On the high fat level with hydrogenated peanut oil plus raw skim milk the amount of water evaporated was fairly high and the growth rate was low compared with that of the animals on the other diets in the same series (G); but it was significantly higher than for group 51 for which the drinking fluid was water, suggesting that the effect of giving raw skim milk was not related to the higher calorie intake but to the presence of a growth-promoting factor.

The last column in Table 1 gives the calorie cost of the weight gains in terms of calories available after subtraction of those used for evaporation of water. The approximate calculations of the calorie intake were made as described earlier (Aaes-Jørgensen & Dam, 1954*b*).

It is seen that the feeding of hydrogenated peanut oil at the 7% dietary level (group 47) was very uneconomical. However, supplementation with linoleic acid (group 48) decreased considerably the calories required per gram weight gain. Changing the drinking fluid from water to raw skim milk (groups 47, 49) was still more effective. These findings stress the fact that an improvement in calorie economy may also be obtained otherwise than by adding linoleic acid. The results with hydrogenated peanut oil at the 28% dietary level lead even more strikingly to the same conclusion. Comparison of groups 49 and 53 (given raw skim milk as drinking fluid) with groups 47 and 51 (given water) and groups 48 and 52 (given water and supplemented with linoleic acid) makes it difficult to explain the results merely on the basis of linoleic acid requirement. These findings are in accordance with the suggestion (Aaes-Jørgensen & Dam, 1954*c*) that a relation between fat and protein metabolism is perhaps involved.

From Table 1 it is seen that the calorie cost of the weight gains in terms of calories available after subtraction of those used for evaporation of water at the 7% dietary fat level in series A–D was much higher for the animals reared on the unsupplemented hydrogenated peanut-oil diet (group 47) than for those on any of the other diets. At the 28% dietary fat level the cost was increased on all the diets with hydrogenated peanut oil (groups 51–54), especially on the unsupplemented diet 51 (where it was

infinite because the animals in this group did not grow during the last 6 weeks in which the measurements were made). This suggests that the efficiency for growth of the calories consumed, even after subtraction of calories needed for evaporation, was low on the diets containing hydrogenated peanut oil; it was improved to some extent by supplementation with linoleic acid or by giving raw skim milk instead of water. The effects of these measures seemed to be additive under the conditions of the experiments. In this connexion it may be of interest to recall that Wesson & Burr (1931) found the B.M.R. increased in fat-deficient rats, whereas the basal R.Q. (0.91) and the rectal temperature were normal, and discussed a possible relation to the thyroid gland. Burr & Beber (1937) found that fat-deficient rats were not more active than normal rats and that their metabolic rate followed the course of that of stock rats but at a higher level.

Sinclair (1952) concluded that water passed with abnormal readiness through the skin in both directions in fat-deficient rats, and assumed that the excessive loss of water through the skin was responsible for both the increased metabolic rate (and consequent increased food consumption) and the increased consumption of water. However, in our calculations the calories needed for evaporation have already been subtracted, indicating that Sinclair's cannot be the whole explanation of the observed increased calorie intake.

The results of analysing the collected urine samples from the single animals were in accordance with the results reported previously (Aaes-Jørgensen & Dam, 1954*b*).

#### SUMMARY

1. Female rats were reared on diets with lard, peanut oil or hydrogenated peanut oil at two different levels. These diets were supplemented with linoleic acid, raw skim milk or both. Food and fluid intake and urine output were measured throughout the 18 weeks of the experiment.

2. The ratio of total calorie intake minus calories for water evaporation to average weight gain per animal per day was very high with hydrogenated peanut-oil diets. It decreased on supplementation with linoleic acid or with raw skim milk instead of water. An increased rate of evaporation was not a sufficient explanation of the increased calorie intake and poor growth rate.

3. The higher growth rate of animals on diets with lard or peanut oil instead of hydrogenated peanut oil was not the result of an increased calorie intake.

#### REFERENCES

- Aaes-Jørgensen, E. & Dam, H. (1954*a*). *Brit. J. Nutr.* **8**, 281.  
 Aaes-Jørgensen, E. & Dam, H. (1954*b*). *Brit. J. Nutr.* **8**, 290.  
 Aaes-Jørgensen, E. & Dam, H. (1954*c*). *Brit. J. Nutr.* **8**, 296.  
 Burr, G. O. & Beber, A. J. (1937). *J. Nutr.* **14**, 553.  
 Greenberg, S. M., Calbert, C. E., Savage, E. E. & Deuel, H. J. Jr. (1950). *J. Nutr.* **41**, 473.  
 Sinclair, H. M. (1952). *Symp. biochem. Soc.* no. 9, p. 80.  
 Stillman, R. C. (1949). *J. Amer. Oil Chem. Soc.* **26**, 399.  
 Wesson, L. G. & Burr, G. O. (1931). *J. biol. Chem.* **91**, 525.