THE EFFECT OF DIFFERENT DIETARY FATS ON LIVER
FAT DEPOSITION*

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When weanling rats are fed a ration containing 9 per cent casein with
sucrose as the carbohydrate and with an adequate supply of the known
lipotropic factors, fatty infiltration of the liver occurs (1, 2). The addi-
tion of 0.36 per cent of dl-threonine to the diet causes a considerable de-
crease in liver fat deposition but does not reduce the liver fat content to
as low a level as is found in rats fed an 18 per cent casein diet. Glycine
fed at a level of 1.5 per cent of the diet also causes a reduction in the de-
position of liver fat when it is fed either with or without supplemental
threonine.

In a study of the effect of increased levels of dietary fat (3) it was found
that the corn oil normally used in the diet could be increased from 5 to 20
per cent without causing any greater accumulation of liver fat. In fact,
the liver fat content of rats fed the higher level of corn oil was slightly
lower. The liver fat content of rats fed a low protein diet containing 20
per cent of butter fat was higher than that of rats fed a similar diet con-
taining 20 per cent of corn oil. When threonine was added to the high
fat diets, the difference between the levels of liver fat of the corn oil group
and that of the comparable butter fat group was greater although the total
amount of liver fat was reduced in each case.

Channon et al. (4, 5), in 1936, studied the effect of different dietary fats
on liver fat deposition in rats fed low protein diets deficient in choline.
Adult rats were used in their work and diets containing 5 per cent of casein
and 40 per cent of fat were fed. They found the highest liver fat values
for rats fed butter fat and the lowest for those fed olive oil; the values for
those fed coconut oil were intermediate. From a comparison of the
amounts of the different fatty acids in these fats they concluded that the
increased infiltration of fat was proportional to the percentage of saturated
C\textsubscript{14} to C\textsubscript{18} acids in the dietary fat.

Their work was complicated by both a very low protein intake and a

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choline deficiency. They did not determine to what extent the accumulation of liver fat could be decreased by supplementation with choline or additional protein. The fatty acids were not fed free of the other components of the fats to implicate them directly in the increased liver fat deposition. The present work was undertaken to clarify these observations.

Methods

Male weanling rats of the Sprague-Dawley strain weighing 40 to 50 gm. were used in all of the experiments. They were divided according to weight into similar groups of six and housed in individual cages with raised screen bottoms. The animals were fed ad libitum and were weighed weekly during the 2 week experimental period.

The composition of the basal diet was as follows: sucrose 66.6, casein 9.0, DL-methionine 0.3, Salts IV (6) 4, choline chloride 0.15, and the fat specified in Table I 20 per cent. Vitamins were included to provide, in mg. per 100 gm. of ration, thiamine hydrochloride 0.5, riboflavin 0.5, niacin 1.0, calcium pantothenate 2.0, pyridoxine 0.25, biotin 0.01, folic acid 0.02, vitamin B₁₂ 0.02, and inositol 10.0. 2 drops of halibut liver oil fortified with vitamins E and K (1) were administered orally each week. Alterations in the amount of sucrose compensated for the alterations in the basal diet.

At the end of the 2 week experimental period the animals were sacrificed for the determination of liver fat. The animals were stunned by a blow on the head and decapitated. The livers were removed and stored at -4° until the fat determinations could be carried out. Liver fat was determined by extraction of the dried and ground liver with ether (7).

All of the fats used were commercial products. Both the butter and the margarine were melted and the fat layers were decanted and strained through cheese-cloth.

The unsaponifiable fraction of butter was prepared by heating 450 gm. of butter with 3 liters of 1 N alcoholic potassium hydroxide under reflux for 2 hours. An equal volume of water was added and the resulting solution was extracted twice with diethyl ether. The ether layer was washed three times with water and heated to dryness at 80°. The yield was 2.8 gm. or 0.62 per cent of the butter fat. This was fed as 0.14 per cent of the diet.

The water layer and washings from the previous procedure were acidified with concentrated hydrochloric acid and the fatty acid layer was separated. The water and alcohol were removed by heating at 80°. The fatty acids of corn oil were prepared by the same procedure.

For the esterification of fatty acids, 180 gm. of the fatty acids were
heated with 38 gm. of glycerol, and the progress of the reaction was followed by observing the amount of water which was distilled. Nitrogen gas was bubbled through the mixture during the heating period to prevent oxidation. A trace of zinc (8) was added as a catalyst. The product was taken up in ether and washed twice with water. Ether and water were removed under a vacuum. The resulting product was fed at a level of 20 per cent in the diet.

The liquid and solid fractions of the fatty acids of butter were prepared by the lead salt separation as described by Hilditch (9). The fatty acid fractions were esterified by the procedure given above.

Results

The results of each experiment are presented separately in order that each group may be compared with the control in the same experiment. Although the liver fat values are usually consistent within experiments, considerable variation is encountered among similar groups in different experiments.

In Experiment 1, the liver fat values for the groups fed corn oil and margarine were considerably lower than those for groups fed butter fat or lard. Although threonine reduced the level of liver fat in each case, this difference occurred whether threonine was added or not. The liver fat value for Group 2 which received corn oil and threonine was higher than those of similar groups in the other experiments. The liver fat values for the groups that received 10 per cent of corn oil and 10 per cent of butter fat were intermediate between those for the groups receiving corn oil and the groups receiving butter fat. The levels of liver fat of Groups 11 and 12 which received the 18 per cent casein diet were very low, and there was no difference between the liver fat levels of the groups receiving the different fats.

A supplement of 1.5 per cent of glycine caused a reduction in the level of liver fat in the animals that received threonine and in those that did not (Experiment 2). However, the difference between the liver fat content of those receiving corn oil and those receiving butter fat was reduced only when both threonine and glycine were included in the diet.

In Experiment 3, when the unsaponifiable fraction of butter was fed with corn oil, the level of liver fat was not increased. In an experiment not presented in Table I, 75 mg. per cent of cholesterol or 2 per cent of sodium butyrate was found to have no effect on liver fat deposition when fed with 20 per cent of corn oil. Also, the food consumption of the groups receiving corn oil and butter fat was found to be identical, although there was a significant difference in the level of liver fat.

The fatty acids of butter and of corn oil were esterified with glycerol and
<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Group No.</th>
<th>Fat, 20%</th>
<th>L- Threonine</th>
<th>Changes in basal diet</th>
<th>Rate of gain*</th>
<th>Liver fat*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Corn oil</td>
<td>0.36 per cent</td>
<td></td>
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<tr>
<td></td>
<td>2</td>
<td>&quot; &quot;</td>
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<td></td>
<td>15.1 ± 1.2</td>
<td>22.4 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Butter fat</td>
<td></td>
<td></td>
<td>21.0 ± 1.4</td>
<td>19.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>&quot; &quot;</td>
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<td></td>
<td>12.7 ± 0.9</td>
<td>32.6 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Margarine</td>
<td></td>
<td></td>
<td>18.4 ± 0.8</td>
<td>24.2 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>&quot; &quot;</td>
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<td>13.5 ± 0.8</td>
<td>22.7 ± 1.4</td>
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<tr>
<td></td>
<td>7</td>
<td>Lard</td>
<td>0.36 per cent</td>
<td></td>
<td>19.7 ± 1.6</td>
<td>12.9 ± 0.7</td>
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<td>8</td>
<td>&quot; &quot;</td>
<td></td>
<td></td>
<td>13.8 ± 0.6</td>
<td>23.2 ± 1.3</td>
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<td>Butter fat, 10%</td>
<td>0.36 per cent</td>
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<td>20.0 ± 0.7</td>
<td>20.3 ± 1.4</td>
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<td>Corn oil, 10%</td>
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<td></td>
<td>12.8 ± 0.8</td>
<td>23.0 ± 1.8</td>
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<td>11</td>
<td>&quot; &quot;</td>
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<td>18% casein†</td>
<td>19.0 ± 1.2</td>
<td>19.8 ± 1.4</td>
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<td>12</td>
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<td>0.36 per cent</td>
<td></td>
<td>12.6 ± 0.3</td>
<td>3.5 ± 0.2</td>
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<tr>
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<td>13</td>
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<td>34.8 ± 1.2</td>
<td>14.8 ± 1.0</td>
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<td>14</td>
<td>Corn oil</td>
<td>0.36 per cent</td>
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<td>21.4 ± 0.6</td>
<td>17.5 ± 0.7</td>
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<tr>
<td></td>
<td>15</td>
<td>Butter fat</td>
<td>0.36 per cent</td>
<td></td>
<td>20.0 ± 0.8</td>
<td>22.6 ± 1.7</td>
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<tr>
<td></td>
<td>16</td>
<td>Corn oil</td>
<td>0.36 per cent</td>
<td>1.5% glycine</td>
<td>21.8 ± 1.3</td>
<td>16.3 ± 0.9</td>
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<td>24.9 ± 0.9</td>
<td>17.2 ± 2.1</td>
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<tr>
<td></td>
<td>18</td>
<td>Butter fat</td>
<td>0.36 per cent</td>
<td>1.5% &quot;</td>
<td>16.3 ± 1.3</td>
<td>19.1 ± 0.5</td>
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<tr>
<td></td>
<td>19</td>
<td>Corn oil</td>
<td>0.36 per cent</td>
<td></td>
<td>16.9 ± 1.0</td>
<td>26.6 ± 2.2</td>
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<tr>
<td></td>
<td>20</td>
<td>&quot; &quot;</td>
<td></td>
<td></td>
<td>21.1 ± 0.4</td>
<td>15.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>Butter fat</td>
<td>0.36 per cent</td>
<td>1.5% &quot;</td>
<td>20.3 ± 0.5</td>
<td>13.2 ± 1.0</td>
</tr>
</tbody>
</table>

† Liver fat expressed as per cent of dry weight.
| 4 | 22 | Corn oil | 0.36 | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;24.1 ± 1.4 16.6 ± 1.5 5.0 ± 0.5 |
| 23 | Butter fat | 0.36 | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;24.4 ± 1.2 24.3 ± 1.0 7.3 ± 0.3 |
| 24 | Fatty acids of butter† | 0.36 | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;19.7 ± 0.8 22.8 ± 3.2 7.1 ± 1.2 |
| 25 | “ “ “ corn oil‡ | 0.36 | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;17.0 ± 0.5 15.5 ± 0.6 4.3 ± 0.2 |
| 26 | Corn oil | 0.36 7% casein† | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;14.4 ± 1.1 17.2 ± 1.1 4.9 ± 0.5 |
| 27 | Butter fat | 0.36 7% “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;10.1 ± 0.6 29.5 ± 3.2 9.4 ± 1.3 |
| 28 | Corn oil | 0.36 7% “ ; no choline | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;12.8 ± 0.7 38.2 ± 2.9 13.4 ± 1.5 |
| 29 | Butter fat | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;18.4 ± 1.2 55.1 ± 1.8 23.0 ± 1.2 |
| 30 | Corn oil | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;9.6 ± 0.4 34.5 ± 1.8 11.7 ± 0.8 |
| 31 | Butter fat | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;12.6 ± 1.1 58.2 ± 2.2 25.0 ± 1.7 |
| 32 | Fatty acids of butter ‡ | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;11.9 ± 0.8 59.0 ± 2.5 25.7 ± 2.0 |
| 33 | Liquid “ “ “ ‡ | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;14.1 ± 0.6 44.6 ± 2.8 16.3 ± 1.5 |
| 34§ | Solid “ “ “ | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;9.7 ± 0.3 66.8 ± 0.9 31.4 ± 0.5 |
| 5 | 35 | Corn oil | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;13.1 ± 1.3 34.2 ± 1.7 11.5 ± 0.7 |
| 36 | Butter fat | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;14.2 ± 0.8 59.0 ± 1.5 26.1 ± 1.1 |
| 37 | Oleic acid‡ | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;10.2 ± 0.6 28.6 ± 2.2 9.2 ± 0.8 |
| 38 | “ + stearic acids§ (1:1) | 0.36 7% “ “ “ | &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;11.8 ± 0.5 52.7 ± 1.8 21.1 ± 0.9 |

* The values represent the mean ± the standard error of the mean for six animals.
† This is the total amount of casein in the diet.
‡ The fatty acids were esterified with glycerol and the product was fed as 20 per cent of the diet.
§ One animal died and another lost weight during the 2nd week. The results presented are for only four animals.
fed to rats as 20 per cent of the diet in Experiment 4. The levels of liver fat of rats fed these preparations were the same as those of the groups receiving the fats from which they were prepared.

When the level of casein was decreased (Groups 26 and 27) from 9 to 7 per cent, the difference between the liver fat content of the groups receiving corn oil and of those receiving butter fat was increased. An even greater difference was obtained when the lower level of casein was fed and choline was omitted from the diets (Groups 28 and 29). There was also a marked increase in the level of liver fat in both groups when choline was omitted.

In the remaining experiments, the 7 per cent casein diet without choline was used to insure larger and more consistent differences. The level of liver fat of the groups fed the glycerides of the fatty acids from butter in Experiment 5 was as high as that of the group which received butter fat. The solid or long chain saturated fatty acids of butter, fed as the glycerides, caused a higher level of liver fat than did butter fat. The level of liver fat of the rats receiving the liquid fatty acid glycerides was below that of the group receiving butter fat, but was still above the level of those receiving corn oil.

Stearic acid was chosen as a member of the solid fatty acid group to be tested for its effect on liver fat deposition. A mixture of stearic and oleic acids (1:1) was used to give a product with a lower melting point and this was compared with the glycerides prepared from oleic acid alone. The level of liver fat of the group that received stearic acid was nearly as high as that of the group fed butter fat, while the group which received oleic acid alone showed a level of liver fat below that of the corn oil group.

**DISCUSSION**

Previous work has shown that the level of corn oil fed to rats receiving the 9 per cent casein diet containing choline did not affect the severity of the fatty infiltration of the liver even when the level was varied from none to 20 per cent. Butter fat or lard, when fed as 20 per cent of the diet, caused a marked increase in the level of liver fat. When a low protein choline-deficient diet was fed, the difference between the liver fat content of the group receiving butter fat and that of the group receiving corn oil was greater than that observed when diets containing choline were used. Although differences (1, 10) have been observed between the fatty livers which result from a protein deficiency and those caused by a choline deficiency, in both cases the effect of different dietary fats on the level of liver fat is similar.

Butter fat does not affect the level of liver fat when the diet contains adequate levels of protein and choline. When either protein or choline is
inadequate, butter fat added to the diet increases the severity of the fatty infiltrations of the liver which result under these dietary conditions. In general, this effect of butter fat was more pronounced when the diet was made more deficient in lipotropic factors. The choline-deficient diet which was used in this work produced the highest level of liver fat. Also, the greatest difference between the liver fat content of the group receiving butter fat and of that receiving corn oil was found in the experiment in which this type of diet was used. This difference in liver fat content between the butter fat and corn oil groups was also increased when the level of protein in the diets containing choline was reduced from 9 to 7 per cent. This was accompanied by an increase in the level of liver fat in the group fed corn oil. This generalization did not hold for the groups receiving the 9 per cent casein sucrose diet containing choline and supplemented with threonine or glycine, for these compounds reduced the level of liver fat but did not reduce the difference between the liver fat levels in the groups fed butter fat and those fed corn oil.

Isolation and fractionation of the fatty acids of butter by the lead salt procedure clearly demonstrated that the fatty acids and in particular the long chain saturated fatty acids of butter cause an increase in liver fat deposition when fed at rather high levels in diets deficient in protein and choline. Corn oil contains a very small amount (about 13 per cent) of the long chain saturated acids and therefore should have little influence on the accumulation of liver fat as was found in this work. These results support the suggestion of Channon et al. (5) that the level of C₁₄ to C₁₈ saturated fatty acids affects the amount of liver fat which will accumulate.

Stearic acid was found to be active in increasing liver fat deposition but other saturated fatty acids have not been tested. The liquid fatty acids of butter caused a higher level of liver fat than did corn oil. This may be explained by the amounts of myristic and palmitic acids which remained in this fraction or may indicate an effect from the shorter chain saturated acids.

The marked increase in liver fat deposition caused by the long chain saturated fatty acids indicates that these acids are metabolized by the liver in a manner different from that of the unsaturated acids which do not affect the accumulation of fat in the liver. The present knowledge of the causes of fatty infiltration of the liver is not complete enough to permit any conclusion as to what the difference in the metabolism of the two groups of fatty acids may be.

This effect of fats containing large amounts of long chain saturated fatty acids on liver fat deposition would be of importance only when relatively large amounts of fat were fed to animals receiving diets deficient in either choline or protein. For example, the amount of fat accumulating in the
livers of two groups of rats receiving high fat diets containing the same suboptimal level of choline would be distinctly different if the dietary fats for the two groups differed appreciably in their content of saturated fatty acids. On the other hand when an adequate diet is consumed, as is usually the case under practical conditions when the intake of animal fats is high, the type of fat would be without effect on the deposition of liver fat.

SUMMARY

When different fats were fed at a level of 20 per cent in a low protein diet (9 per cent casein), the level of liver fat in the rats was high when butter or lard was fed and was low when corn oil or margarine was fed. This effect was increased when the level of protein in the diet was decreased and when choline was omitted from the diet.

The fatty acids of butter, which were isolated and fed as glycerides, caused liver fat to accumulate to the same extent as did butter fat. The solid fatty acid fraction of butter caused a much greater accumulation of liver fat than did the liquid fatty acid fraction, and stearic acid caused a much greater accumulation of liver fat than did oleic acid. The unsaponifiable material of butter was without effect.

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