Changes in fatty acid composition in nutritional fatty degeneration of the liver

2.* Effect of realimentation after starvation†

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Fasted adult rats have been shown capable of synthesizing large amounts of fat during realimentation. Longenecker (1939*a*) found that when fasted rats were given high-carbohydrate or high-protein diets they deposited a third of their total weight gain as fat. On initial refeeding after starvation, an increase in liver fat was observed in growing rats by Soberón & Sánchez (1961) and in growing chickens by Summers & Fisher (1960). By an examination of the fatty acid composition of liver fat we have attempted to determine the origin of the fatty liver condition which arises on refeeding after starvation. As a result of these investigations it was also found that appropriate adjustments in the diet offered for realimentation would prevent the development of this type of fatty liver.

EXPERIMENTAL

Procedure. Female chickens, and in one instance male chickens, 2 weeks old, from a Columbian × New Hampshire cross, were used in these experiments. During an initial 2-week feeding period a standard maize–soya-bean growing diet (Feigenbaum & Fisher, 1963) was given. The birds were next starved for a 5-day period and then refed. Table 1 shows the composition of the basal diet used throughout these studies. The source and quantity of fat as well as the variation in level of energy (adjusted with dietary cellulose) is shown in the tables of results. All dietary changes were made at the expense of glucose.

For the analyses, birds were selected in groups of five to represent the mean weight of the original group and were killed with chloroform. The livers were prepared and the fat was analysed, as previously described (Feigenbaum & Fisher, 1963).

Expt 1. In this experiment the source of dietary fat and the energy level were studied in relation to the fatty liver degeneration appearing upon refeeding starved chickens for 3 days. The basal diet was supplemented with 5% glycerol or coconut, olive or maize oil, with or without 20% cellulose. In this experiment birds were killed and their livers analysed after the starvation period or after 3 or 10 days of refeeding.

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Expt 2. This experiment included groups of male chickens from the same hatch and of similar body-weight as the females for comparison of possible sex differences. Since the replacement of glycerol in the diet in Expt 1 by an equal weight of fat reduced the accumulation of liver fat in most instances, Expt 2 was designed to study the effect of higher levels of fat. Maize oil was used, since it resulted in a much smaller accumulation of liver fat than did olive oil. Coconut oil, although as potent in this respect as maize oil, must be considered in a separate category (to be discussed later) because of its content of short-chain saturated fatty acids. In Expt 2, we also explored the possibility that the different behaviour of maize oil and olive oil might be due to the greater degree of unsaturation of the former. To do so, we included cod-liver oil, one of the more highly unsaturated fats, in this study in addition to the maize oil. In this experiment birds were killed at the same stages as in Expt 1. However, since all signs of fatty liver degeneration had disappeared after 10 days of refeeding, values for the 10-day analyses are not given in the tables for Expts 2–4.

Table 1. *Composition of basal diet*

Ingredient	Amount (%)	Ingredient	Amount (%)
Defatted soya-bean meal (50% protein)	40·00	Antioxidant §	0·01
Mineral mixture*	4·94	Cellulose ‖	Varied
Methionine hydroxy analogue†	0·30	Glycerol or fat	Varied
Choline chloride	0·20	Glucose monohydrate	To 100
Vitamins‡	0·25		

* For composition see Fisher, Griminger, Leveille & Shapiro (1960).
† MHA, Monsanto Chemical Co., St Louis, Mo, USA.
‡ For composition see Fisher & Johnson (1956).
§ Santoquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline), Monsanto Chemical Co., St Louis, Mo.
‖ Solka Floc, Brown and Co., Berlin, New Hampshire, USA.

Expt 3. This experiment was designed to study further the production and prevention of the fatty liver condition observed on refeeding starved chickens in relation to the role of specific dietary fatty acids. Olive oil and triolein, with their high oleic acid content, were used, since liver oleic acid (monoenoic acid) seemed to reflect the extent of the fat accumulation. Safflower-oil fatty acids were also used in this experiment as a rich source of dienoic acid (72·9%) to test whether the beneficial effects of maize oil might be related to its dienoic acid content. The three supplements were supplied in the diet at two levels (5 and 15%). In addition, these levels of triolein were also given in combination with 2% safflower-oil fatty acids. Birds were killed and their livers analysed as described in Expt 1.

Expt 4. This study was designed to explore possible interrelationships between dietary linoleic acid and triolein. Such an interrelationship was suggested by the results of Expt 3. Several levels of the two fatty acids were given individually or in combination. Again birds were killed as described for Expt 1.

Expt 5. This experiment was designed to study the daily changes in liver fat accumulation and subsequent regression. Changes in carcass fat were also studied in

the hope that they would shed further light on the origin of the fatty liver degeneration. The birds, which had been starved in the usual manner, were given a diet containing 5 % glycerol without added cellulose. The glycerol diet was used since it had previously given rise to the largest accumulation of liver fat. Carcass and liver fat were determined daily for a 10-day period, since by the end of this time liver fat content had essentially returned to normal as shown earlier in Expt 1 (Table 2). Each carcass with the liver removed was frozen and finely ground. Five ground carcasses from each group were then thoroughly mixed together and samples freeze-dried. The dried samples were again finely ground and appropriate amounts analysed in the same way as for the liver.

RESULTS

Expt 1. Table 2 gives the results. Columns 2 and 3 show the mean values for normal chickens and for birds starved for 5 days (Feigenbaum & Fisher, 1963). In comparison with those of the normal and the starved groups, all other dietary treatments gave rise to fatty livers. With the high-energy diets (those without added cellulose) the livers were larger and contained more fat than did comparable livers from birds given the low-energy diets. Comparison of iodine values showed that birds on the high-energy diets produced a more saturated liver fat than those on the low-energy diets. On comparing the relative amounts of fatty acids (as a percentage of fat), we found, however, that the high-energy diets produced liver fat that was less saturated than that produced by comparable low-energy diets. These seemingly contradictory results can easily be explained by shifts within the unsaturated fatty acid fractions: thus, the monoenoic acid levels were higher, and all the polyunsaturated acid levels lower, in the liver fat of the chickens that had received the high-energy diets than in the liver fat of those that had received the corresponding low-energy diets.

In terms of deposition of liver fat, the dietary fat supplements fell into two groups, with glycerol and olive oil resulting in much greater fat accumulation than either coconut or maize oil. A similar effect of dietary fat on the monoenoic acid fraction was noted, particularly when it was expressed in absolute terms (mg/g liver).

Comparison of the normal and starved chickens on the one hand and the refed birds on the other showed that the relative amount of saturated fatty acids (as a percentage of fat) was lower in the refed birds than in the normal or starved birds, whereas in absolute terms (mg/g liver), the amount of the saturated fatty acid fraction was higher, especially with the high-energy diets. Of all the fatty acids, the monoenoic acid fraction showed the greatest increase during refeeding of previously starved birds and appears to be a reasonably good index of the degree of accumulation of liver fat. The concentrations of the polyunsaturated fatty acid fractions, which increased during starvation (Feigenbaum & Fisher, 1963), decreased during refeeding, and for the most part approached those of the normal group once again.

Table 2 also lists some of the liver measurements made after 10 days of refeeding. It will be noted that with all diets the fatty liver condition has greatly subsided and that some of the groups, particularly those on the low-energy diets, had a nearly normal liver fat content (cf. controls, column 2).

Table 2. *Expt* 1. *Effect of various dietary fats* (5 % *of the diet*) *on liver fat and its fatty acid composition during refeeding of chickens after starvation*

Measurement	Normal chickens*	Starved chickens†	Mean values for duplicate analyses of one group of five birds: Without cellulose G	C	O	M	With 20 % added cellulose G	C	O	M
After 3 days' refeeding										
Body-weight (g)	166 ± 5	113 ± 6	200	180	186	191	176	187	194	195
Liver weight as percentage of body-weight	4·6 ± 0·2	2·6 ± 0·1	7·0	5·4	5·3	4·7	4·1	4·3	4·3	3·9
Liver fat:										
as percentage of wet liver weight	5·2 ± 0·2	4·8 ± 0·3	22·2	15·4	19·9	14·5	11·0	7·7	11·6	8·5
as mg/liver	401 ± 39	134 ± 7	3130	1505	1971	1304	805	605	972	648
iodine value	71 ± 1	86 ± 4	62	64	66	72	71	75	73	78
Fatty acids:										
as percentage of fat										
saturated	52·4 ± 2·4	60·5 ± 2·1	36·4	38·5	34·4	36·2	35·3	43·3	36·1	43·6
monoenes	31·1 ± 2·9	18·0 ± 2·8	60·8	56·3	61·3	53·9	57·6	43·3	55·1	39·6
dienes	8·2 ± 0·7	6·4 ± 0·4	1·3	2·9	2·4	6·4	3·3	6·7	4·2	9·8
trienes	1·3 ± 0·1	0	0·5	0·7	0·5	0·9	1·0	1·3	1·2	1·1
tetraenes	3·4 ± 0·3	5·9 ± 0·5	0·4	0·8	0·6	1·5	1·4	2·5	2·1	3·7
pentaenes	1·0 ± 0·1	1·7 ± 0·1	0·1	0·3	0·2	0·3	0·4	0·8	0·4	0·8
hexaenes	2·0 ± 0·2	7·5 ± 0·6	0·4	0·6	0·5	0·8	1·0	2·0	1·0	1·4
as mg/g liver										
saturated	27·2 ± 1·3	28·7 ± 2·1	80·8	59·1	68·5	52·5	38·9	33·2	41·8	37·2
monoenes	17·1 ± 2·1	9·0 ± 1·7	135·0	86·5	122·1	78·1	63·5	33·2	63·8	33·8
dienes	4·1 ± 0·3	3·0 ± 0·2	2·9	4·5	4·8	9·3	3·6	5·1	4·9	8·4
trienes	0·7 ± 0·05	0	1·1	1·1	1·0	1·3	1·1	1·0	1·4	0·9
tetraenes	1·8 ± 0·1	2·8 ± 0·2	0·9	1·2	1·2	2·2	1·5	1·9	2·4	3·2
pentaenes	0·5 ± 0·05	0·8 ± 0·05	0·2	0·5	0·4	0·4	0·4	0·6	0·5	0·7
hexaenes	1·0 ± 0·1	3·5 ± 0·2	0·9	0·9	1·0	1·2	1·1	1·5	1·2	1·2
After 10 days' refeeding										
Body-weight (g)			298	282	289	283	245	275	296	284
Liver weight (as percentage of body-weight)			5·0	4·1	3·7	3·7	3·2	3·7	3·6	3·8
Liver fat (as percentage of wet liver weight)			9·3	6·2	6·3	6·1	5·7	5·1	5·0	5·4

G, glycerol (no added fat); C, coconut oil; O, olive oil; M, maize oil.

* Mean values with their standard errors for sixteen groups of 2-week-old female chickens before starvation.

† Mean values with their standard errors for ten groups of chickens starved for 5 days (weight before starvation 168 ± 12 g).

Expt 2. The results in Table 3 again show a greater liver fat accumulation on the high- than on the low-energy diets. A comparison of the intake of food and metabolizable energy by birds given high- and low-energy diets showed that the latter groups consumed 14% more food but 11% less energy than did the former groups. A similar pattern was observed in all experiments in which low- and high-energy diets were compared.

The birds given either maize oil or cod-liver oil in the presence of 20% cellulose had an essentially normal liver fat content. Whereas the increase in the level of dietary fat was very effective in preventing the accumulation of liver fat, the greater degree of unsaturation of cod-liver oil compared with maize oil was of no additional consequence. It is noteworthy that the dietary maize oil had relatively little effect in altering the fatty acid composition of liver fat, whereas the changes in the liver fatty acids that occurred on the cod-liver oil diet were, although more substantial, still small in comparison with the large quantities of polyunsaturated fatty acids contributed by the oil. The comparison between male and female chickens showed that the male birds had less liver fat (which was also more saturated) under all dietary conditions except on the high-calorie, glycerol régime. This finding is in agreement with earlier observations of a more saturated liver fat in male birds after a 5-day starvation period (Feigenbaum & Fisher, 1963).

Expt 3. The results given in Table 4 again clearly indicate that, with the exception of olive oil, the higher level of dietary fat (15%) was more effective in preventing a great accumulation of liver fat. The diet with 15% olive oil produced almost as high a liver fat content as did the diet with 5% olive oil. Again, the concentration of the monoenoic acid fraction was well correlated with the degree of accumulation of liver fat. Despite the fact that triolein supplies monoenoic acid exclusively (in contrast to olive oil which also contains other fatty acids), it is surprising that the content of the monoenoic acid fraction of the liver fat of birds given the 15% triolein diet was appreciably lower than in the corresponding group that had been given 15% olive oil. This finding was even more striking on an absolute basis (mg/g liver), since it was the only large difference, with virtually no changes in any of the other fatty acids studied.

Expt 4. The results in Table 5 show a marked effect of the highest level of linoleic acid (5%) in lowering liver fat content, whereas no such effect was observed with any of the levels of triolein. An interaction was noted for the groups given diets containing combinations of linoleic acid and triolein. The largest accumulation of liver fat was noted in birds given the diet with the combination of 3·5% linoleic acid and 1·5% triolein and the lowest amount of liver fat was found with the diet supplying equal amounts of each of the two supplements. As in all previous experiments, the monoenoic acid content expressed on an absolute basis (mg/g liver) again was a good index of the extent of liver fat accumulation. In the group given the combination that gave rise to the largest amount of liver fat, it appeared that, in addition to an increase in the absolute amount of monoenoic acid (mg/g liver), the saturated and dienoic acid fractions were also markedly increased.

In order to understand the effect of the combination of dietary fatty acids, the liver fatty acids of this group must be compared with those of the groups receiving the

Table 3. *Expt 2. Effect of level (5 or 15%) and type of dietary fat on liver fat and its fatty acid composition after 3 days of refeeding of chickens after starvation*

(Mean values for duplicate analyses for one group of five birds)

	Females										Males					
	Without added cellulose					With 20% added cellulose					Without added cellulose			With 20% added cellulose		
	5%			15%		5%			15%		5%		15%	5%		15%
Measurement	G	M	C	M	C	G	M	C	M	C	G	C	C	G	C	C
Liver weight as percentage of body-weight	6·5	4·3	5·3	3·6	4·9	4·9	3·8	4·3	3·3	4·1	6·1	4·5	4·5	4·6	4·1	4·1
Liver fat:																
as percentage of wet liver weight	15·7	10·4	14·4	8·5	9·9	11·7	7·9	7·2	5·2	6·2	16·2	9·9	7·8	7·5	6·3	4·3
as mg/liver	1848	847	1321	578	769	992	552	506	330	428	1707	780	590	566	428	297
iodine value	66	60	80	57	99	68	82	87	81	107	70	73	89	69	84	89
Fatty acids:																
as percentage of fat																
saturated	31·6	47·3	26·6	60·2	35·3	34·2	42·2	42·0	58·1	43·9	25·9	39·1	49·7	32·7	49·0	57·8
monoenes	65·8	44·8	68·5	25·8	50·3	61·1	39·4	45·3	16·9	36·2	72·5	54·2	33·8	62·6	35·2	22·5
dienes	1·3	4·6	1·2	9·0	2·4	2·1	10·8	2·6	13·5	3·4	0·8	1·5	3·1	2·2	5·3	4·3
trienes	0·2	0·7	0·0	0·7	0·0	0·4	1·2	0·0	0·7	0·0	0·0	0·0	1·4	0·2	0·0	1·0
tetraenes	0·5	1·5	0·6	2·7	1·4	1·0	3·9	1·4	6·8	1·8	0·5	0·8	1·4	1·2	1·8	1·8
pentaenes	0·2	0·3	0·8	0·5	4·0	0·3	0·8	3·0	1·6	5·0	0·1	1·6	3·9	0·3	2·8	4·5
hexaenes	0·4	0·9	2·2	1·0	6·6	0·8	1·6	5·7	2·4	9·7	0·2	2·8	6·7	0·7	6·0	8·1
as mg/g liver																
saturated	49·5	49·4	38·2	51·2	35·1	40·0	33·2	30·1	30·4	27·0	42·0	38·6	38·8	24·6	31·0	24·7
monoenes	103·1	46·8	98·4	21·9	50·0	71·5	31·0	32·5	8·8	22·3	117·7	53·5	26·4	47·1	22·2	9·6
dienes	2·0	4·8	1·7	7·7	2·4	2·5	8·5	1·9	7·1	2·1	1·3	1·5	2·4	1·7	3·3	1·8
trienes	0·3	0·7	0·0	0·6	0·0	0·5	1·0	0·0	0·3	0·0	0·0	0·0	1·1	0·2	0·0	0·4
tetraenes	0·8	1·5	0·9	2·3	1·4	1·2	3·1	1·0	3·5	1·1	0·8	0·8	1·1	0·9	1·1	0·8
pentaenes	0·3	0·3	1·1	0·4	4·0	0·4	0·6	2·2	0·8	3·1	0·2	1·6	3·0	0·2	1·8	1·9
hexaenes	0·6	1·0	3·2	0·9	6·6	0·9	1·2	4·1	1·3	6·0	0·3	2·8	5·2	0·5	3·8	3·5

G, glycerol (no added fat); M, maize oil; C, cod-liver oil.

individual fatty acid components. On a relative basis (as a percentage of fat) the liver fatty acid pattern (specifically the saturated, mono- and di-enoic fractions) for the group receiving the combination was the same as for that receiving 3·5% linoleic acid. Since the addition of 1·5% triolein to 3·5% linoleic acid resulted in increased fat accumulation, we would expect and in fact observed markedly higher absolute levels (mg/g liver) of the three fatty acid fractions previously specified. A further comparison between the group given the combination and the group given 1·5% triolein showed equal absolute (mg/g liver) amounts of monoenoic acid. On the basis of these comparisons we can conclude that the interaction has resulted in higher absolute levels of saturated and dienoic acid, and that the change in the latter was related to a homoeostatic mechanism involving the maintenance of the physical characteristics of liver fat.

Table 4. *Expt* 3. *Effect of additional dietary fats and a fat combination on liver fat and its fatty acid composition after* 3 *days of refeeding of chickens after starvation*

(Mean values for duplicate analyses for one group of five birds)

	Olive oil		Safflower-oil fatty acids		Triolein		2% Safflower-oil fatty acids + triolein	
Measurement	5%	15%	5%	15%	5%	15%	5%	15%
Liver weight as percentage of body-weight	5·2	5·1	5·9	4·1	5·4	4·6	5·1	4·2
Liver fat:								
as percentage of wet liver weight	13·8	13·1	10·0	8·2	15·1	8·7	11·7	9·2
as mg/liver	1312	1219	1064	588	1448	695	1073	706
iodine value	70	73	65	71	67	64	67	66
Fatty acids:								
as percentage of fat								
saturated	30·4	29·4	40·3	45·4	32·0	43·4	38·4	43·3
monoenes	65·6	64·8	52·2	39·9	64·4	48·8	53·9	46·8
dienes	2·1	3·3	5·0	9·9	2·0	4·3	4·9	6·3
trienes	0·4	0·6	0·7	1·0	0·4	0·8	0·6	1·0
tetraenes	0·6	0·9	0·9	2·1	0·6	1·3	1·0	1·4
pentaenes	0·2	0·2	0·2	0·4	0·2	0·3	0·2	0·3
hexaenes	0·7	0·8	0·7	1·3	0·5	1·1	0·9	1·0
as mg/g liver								
saturated	42·0	38·6	40·4	37·1	48·7	37·6	45·0	39·9
monoenes	90·6	85·1	52·4	32·6	97·4	42·3	63·2	43·1
dienes	2·9	4·3	5·0	8·1	3·0	3·8	5·7	5·8
trienes	0·6	0·8	0·7	0·8	0·6	0·7	0·7	0·9
tetraenes	0·9	1·2	0·9	1·7	0·9	1·1	1·2	1·3
pentaenes	0·3	0·3	0·2	0·3	0·2	0·3	0·3	0·3
hexaenes	0·9	1·1	0·7	1·1	0·7	0·9	1·0	0·9

Expt 5. The results of this study are shown in Table 6. The increase in liver fat after 3 days of refeeding was almost half that found after 3 days in Expt 1, perhaps as a result of differences in body-weights at the beginning of the refeeding periods. For the two experiments under consideration this difference was in excess of 10%.

Table 5. *Expt 4. Effects of linoleic acid and triolein, alone and in combination, on liver fat and its fatty acid composition after 3 days of refeeding of chickens after starvation*

(Mean values for duplicate analyses for one group of five birds)

	L				T						
Measurement	1·5 %	2·5 %	3·5 %	5·0 %	1·5 %	2·5 %	3·5 %	5·0 %	3·5 % L + 1·5 % T	2·5 % L + 2·5 % T	1·5 % L + 3·5 % T
Liver weight as percentage of body-weight	5·2	4·6	4·8	4·6	5·2	5·6	5·2	5·3	5·4	4·5	5·0
Liver fat:											
as percentage of wet liver weight	9·9	9·1	9·0	6·6	11·3	11·7	12·4	13·0	13·1	9·0	11·0
as mg/liver	997	775	774	561	1127	1129	1253	1358	1293	760	1066
iodine value	66	64	63	60	65	62	65	64	62	67	72
Fatty acids:											
as percentage of fat											
saturated	39·5	43·0	42·4	54·6	36·5	39·7	35·7	37·8	42·3	41·4	32·4
monoenes	53·6	49·5	50·0	33·3	59·3	56·1	60·3	57·6	50·6	49·9	60·2
dienes	4·1	4·4	4·7	7·4	2·1	2·0	2·0	2·5	4·7	5·1	4·4
trienes	0·7	0·8	0·7	0·9	0·4	0·4	0·4	0·5	0·6	0·7	0·8
tetraenes	1·1	1·2	1·1	1·9	0·9	0·8	0·7	0·8	0·8	1·5	1·2
pentaenes	0·3	0·3	0·3	0·4	0·2	0·2	0·2	0·2	0·2	0·3	0·3
hexaenes	0·8	0·9	0·8	1·4	0·8	0·8	0·7	0·7	0·7	1·1	0·8
as mg/g liver											
saturated	39·3	39·0	38·2	35·9	41·2	46·4	44·3	49·0	55·4	37·1	35·6
monoenes	53·3	48·9	45·0	21·9	66·9	65·5	74·9	74·6	66·3	44·8	66·2
dienes	4·0	3·9	4·3	4·9	2·4	2·3	2·5	3·3	6·2	4·6	4·8
trienes	0·7	0·7	0·6	0·6	0·5	0·5	0·5	0·6	0·8	0·7	0·8
tetraenes	1·1	1·1	1·0	1·2	1·0	0·9	0·9	1·0	1·0	1·3	1·3
pentaenes	0·3	0·3	0·2	0·3	0·2	0·2	0·2	0·2	0·3	0·3	0·3
hexaenes	0·8	0·8	0·7	0·9	0·9	0·9	0·9	0·9	1·0	1·0	0·9

L, linoleic acid; T, triolein.

Table 6. *Expt 5. Daily observations on the effect on liver and carcass fat and their fatty acid composition of refeeding starved chickens on a high-carbohydrate diet*

(Mean values for duplicate analyses for one group of five birds)

Measurement	Days of refeeding 0	1	2	3	4	5	6	7	8	9	10
Liver											
Weight as percentage of body-weight	3·3	5·7	7·6	6·3	8·1	6·9	7·3	7·0	6·3	6·3	6·5
Fat:											
as percentage of wet liver weight	4·0	7·2	10·6	12·0	12·4	10·7	9·4	9·1	8·1	7·9	6·9
as g/liver	0·13	0·50	1·12	1·20	1·82	1·46	1·54	1·51	1·28	1·36	1·32
iodine value	69	63	60	59	56	60	62	66	66	68	70
Fatty acids:											
as percentage of fat											
saturated	66·5	44·1	40·7	41·6	43·4	39·9	38·5	37·2	39·0	38·7	37·4
monoenes	15·6	49·9	55·7	55·0	53·8	56·5	57·8	57·4	54·9	54·4	54·6
dienes	6·8	2·5	1·9	1·7	1·5	1·8	1·8	2·6	2·9	3·3	3·8
trienes	0·0	0·4	0·4	0·3	0·4	0·5	0·5	0·9	1·1	1·1	1·3
tetraenes	4·5	1·4	0·7	0·6	0·5	0·6	0·6	1·0	1·1	1·3	1·5
pentaenes	1·5	0·4	0·2	0·2	0·2	0·2	0·2	0·3	0·4	0·5	0·5
hexaenes	5·1	1·5	0·5	0·6	0·3	0·4	0·4	0·6	0·7	0·8	0·9
as mg/g liver											
saturated	26·8	31·8	43·1	49·9	53·8	42·7	36·2	34·0	31·4	30·7	25·7
monoenes	6·3	35·9	58·9	66·0	66·6	60·5	54·3	52·4	44·2	43·1	37·5
dienes	2·7	1·8	2·0	2·0	1·9	1·9	1·7	2·4	2·3	2·6	2·6
trienes	0·0	0·3	0·4	0·4	0·5	0·5	0·5	0·8	0·9	0·9	0·9
tetraenes	1·8	1·0	0·7	0·7	0·6	0·6	0·6	0·9	0·9	1·0	1·0
pentaenes	0·6	0·3	0·2	0·2	0·2	0·2	0·2	0·3	0·3	0·4	0·3
hexaenes	2·1	1·1	0·5	0·7	0·4	0·4	0·4	0·5	0·6	0·6	0·6
Carcass											
Weight (g)*	99	120	138	159	181	199	226	237	252	274	295
Fat:											
as percentage of wet carcass weight	2·3	2·2	3·5	4·7	5·2	6·0	6·6	7·0	7·6	6·7	6·0
as g/carcass	2·28	2·66	4·86	7·43	9·36	11·96	14·96	16·50	19·15	18·33	17·67
iodine value	73	63	70	70	77	76	77	77	78	75	75
Fatty acids:											
as percentage of fat											
saturated	34·2	49·2	36·3	35·0	25·6	25·6	21·7	22·0	21·1	26·7	26·5
monoenes	56·1	39·5	54·3	56·6	67·2	67·2	72·9	72·5	73·3	66·7	66·7
dienes	6·7	7·0	6·7	6·1	5·3	5·6	4·1	4·1	4·2	5·0	5·0
trienes	0·9	1·2	0·8	0·7	0·6	0·6	0·5	0·6	0·5	0·7	0·8
tetraenes	0·9	1·3	0·8	0·7	0·7	0·6	0·5	0·5	0·4	0·5	0·5
pentaenes	0·4	0·5	0·3	0·4	0·2	0·1	0·1	0·1	0·1	0·2	0·2
hexaenes	0·9	1·2	0·8	0·5	0·4	0·3	0·2	0·2	0·4	0·3	0·3
as mg/g carcass											
saturated	7·9	10·9	12·8	16·3	13·2	15·4	14·4	15·3	16·0	17·9	15·9
monoenes	12·9	8·8	19·1	26·4	34·7	40·4	48·3	50·5	55·7	44·6	40·0
dienes	1·5	1·6	2·4	2·8	2·7	3·4	2·7	2·9	3·2	3·3	3·0
trienes	0·2	0·3	0·3	0·3	0·3	0·4	0·3	0·4	0·4	0·5	0·5
tetraenes	0·2	0·3	0·3	0·3	0·4	0·4	0·3	0·3	0·3	0·3	0·3
pentaenes	0·1	0·1	0·1	0·2	0·1	0·1	0·1	0·1	0·1	0·1	0·1
hexaenes	0·2	0·3	0·3	0·2	0·2	0·2	0·1	0·1	0·3	0·2	0·2

* With liver removed.

Liver size, as a percentage of body-weight, increased greatly until a peak was reached on the 4th day, after which time the rate of increase levelled off for the remainder of the experimental period. The increase in liver size was paralleled by a very sharp increase in fat content that reached a peak after 4 days and then declined gradually. The marked increase in the amount of fat in the liver, particularly during the first 3 days, was accompanied by a dramatic increase in the amount of the monoenoic acid fraction and by increases of small magnitude in that of the saturated acid fraction. These observations agreed with all our previous findings. The carcass fat composition strongly suggested that the origin of the fatty liver was *de novo* fat synthesis in the liver. Since the amount of dietary fat consumed was only 40–80 mg a day, this source cannot possibly account for the large increments of fat in both liver and carcass. An infiltration of carcass fat into the liver can be ruled out on the following grounds: (1) there were, between liver and carcass, marked differences in the rate of increase of fat, in liver fat a very sharp increase that occurred on the 1st day of refeeding, and in carcass fat only a very gradual increase; (2) there were considerable differences in the time after refeeding at which peaks in fat accumulation occurred, with a 4-day peak occurring with liver fat and an 8-day peak with carcass fat; finally (3) there were big differences in the fatty acid composition of liver and carcass fat expressed either on a relative (as a percentage of fat) or on an absolute (mg/g tissue) basis.

DISCUSSION

When the high food consumption of starved refed chickens is considered it is not surprising that a fatty liver condition arises in them. Our food records show that, on refeeding, the previously starved birds may consume twice the quantity of food that would be consumed by normal birds of similar body-weight. The food:weight gain ratios of the previously starved birds approached unity during early refeeding, indicating, therefore, retention of the excess energy consumed. This finding suggests *de novo* fat synthesis for the storage of the calories consumed in excess. Longenecker (1939*a*) also found *de novo* synthesis and showed that fasted rats deposited a third of their total weight gain as fat during refeeding on a high-carbohydrate diet. However, the consumption of excess calories *per se* will not fully explain the origin of the fatty liver, since we have shown that the development of this condition can be modified or prevented by the substitution of substantial quantities of certain dietary fats for carbohydrate. Thus, *de novo* fat synthesis or fatty acid conversion (saturation or desaturation) in the liver may be more important than high calorie intake.

Longenecker (1939*b*) has shown that dietary fat can by-pass the liver and be deposited directly in the fat depots of animals refed after starvation. He found that the depot fat laid down in fasted rats on a diet high in maize oil was almost identical in fatty acid composition with maize oil itself. When he gave an equicaloric diet containing sucrose in place of maize oil, the fatty acid composition of the depot fat was very different. The similarity of the depot fat to maize oil, and the different character of the depot fat resulting from substitution of sucrose, may be taken as clear evidence that dietary fat reduces *de novo* fat synthesis.

In our studies, all of the dietary fats used had some beneficial effect in reducing accumulation of liver fat. Olive oil was the least effective of the fats in alleviating the fatty liver condition. This small effect may have been due to the fatty acid composition of olive oil, which is characterized by a high content of oleic acid and also an appreciable amount of linoleic acid. The interaction between triolein and linoleic acid observed in Expt 4 supports this suggestion. In this experiment (Table 5) the two combinations supplying unequal amounts of linoleic acid and triolein resulted in greater accumulation of liver fat than did linoleic acid alone. The combination of equal parts of linoleic acid and triolein, which resembles the ratio of oleic and linoleic acid in maize oil, had the same beneficial effects in preventing a fatty liver as did maize oil in other experiments.

A comparison of the fatty acid composition of liver fat in the different experiments showed a high monoenoic acid fraction to be a common denominator of the fatty liver condition. This observation, taken in conjunction with the relatively small changes observed in the iodine values with different dietary fats, suggests that a homoeostatic mechanism involving the physical characteristics of liver fat may be at play. Owing to a substantial content of polyunsaturated fatty acids, 'normal' chicken fat has a relatively low melting point. With high-carbohydrate diets, low in polyunsaturated fatty acids (which the chicken is unable to synthesize), there appears to be greater synthesis of monoenoic acid in order to compensate for the absence of the polyunsaturated acids. Recently, Okey, Shannon, Tinoco, Ostwald & Miljanich (1961) reached a similar conclusion 'that there is a tendency [in rat liver] toward maintenance of physical properties of each lipid within a characteristic range'.

The effectiveness of coconut oil, particularly in comparison with olive oil, in reducing accumulation of liver fat deserves comment. A similar observation was made by Channon & Wilkinson (1936), who studied various dietary fats in relation to the dietary production of fatty livers in rats. With the exception of coconut oil, they observed an inverse relationship between fat deposition and degree of unsaturation, coconut oil behaving like the more unsaturated fats. These findings may be explained on the basis of the fatty acids in coconut oil, which, when not oxidized, would not require saturation or desaturation in the liver. We have previously shown (Feigenbaum & Fisher, 1959) the direct deposition of coconut oil in the fat depots of the hen but not in the egg-yolk lipids.

SUMMARY

1. Chickens, 2 weeks old, were starved for a period of 5 days and their liver fat was examined after 3 and 10 days of refeeding.

2. On a diet devoid of supplemental fat (but containing 5 % glycerol), a very high content of fat in the liver was noted after 3 days of refeeding. This fatty liver condition was of a temporary nature, with the liver fat returning to normal after 10 days of continued refeeding.

3. Our results show that the fat accumulation was due to the ingestion of excess calories as carbohydrate which was converted into fat by *de novo* synthesis in the liver.

It could be partly and even completely prevented by substituting fat for part of the dietary carbohydrate or by reducing the caloric density of the diet.

4. Of the fats studied, olive oil was less effective in this regard than safflower-oil fatty acids, or than maize, cod-liver, or coconut oil, and also less effective than pure triolein.

5. A comparison of certain mixtures containing linoleic acid and triolein showed a definite interaction, which might explain the small effect of olive oil.

6. The monoenoic acid fraction of liver fat generally served as a good indicator of fat accumulation.

7. The small changes observed in iodine values of liver fat with dietary fats of varying degree of unsaturation suggest a homoeostatic mechanism for the maintenance of certain physical characteristics of liver fat.

REFERENCES

Channon, H. J. & Wilkinson, H. (1936). *Biochem. J.* **30**, 1033.
Feigenbaum, A. S. & Fisher, H. (1959). *Arch. Biochem. Biophys.* **79**, 302.
Feigenbaum, A. S. & Fisher, H. (1963). *Brit. J. Nutr.* **17**, 31.
Fisher, H., Griminger, P., Leveille, G. A. & Shapiro, R. (1960). *J. Nutr.* **71**, 213.
Fisher, H. & Johnson, D. Jr. (1956). *J. Nutr.* **60**, 261.
Longenecker, H. E. (1939*a*). *J. biol. Chem.* **128**, 645.
Longenecker, H. E. (1939*b*). *J. biol. Chem.* **129**, 13.
Okey, R., Shannon, A., Tinoco, J., Ostwald, R. & Miljanich, P. (1961). *J. Nutr.* **75**, 51.
Soberón, G. & Sánchez Q., E. (1961). *J. biol. Chem.* **236**, 1602.
Summers, J. D. & Fisher, H. (1960). *J. Nutr.* **72**, 153.

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