THE INFLUENCE OF C^{14}-LABELED GEOMETRICAL ISOMERS OF OLEIC ACID ON LIPID METABOLISM IN THREONINE IMBALANCED RATS

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ABSTRACT

THE INFLUENCE OF C^{14}-LABELED GEOMETRICAL ISOMERS OF OLEIC ACID ON LIPID METABOLISM IN THREONINE IMBALANCED RATS

By

Marylee Taylor

The accumulation of fat in the liver of rats fed lipotropic deficient diets can be influenced by the nature of the diet fat. Prior to 1956 an inverse relationship between liver fat accumulation and the unsaturation or iodine value of the diet fat was postulated.[1,2,3] However, Morris[4] in 1965 reported that the hydrogenation of corn oil to an iodine value of 74 significantly reduced liver fat content when fed to threonine imbalanced animals. As hydrogenation results in increases in both the saturation and percent trans isomers of the corn oil, the following study was undertaken to ascertain the effect of trans isomers on liver lipids in threonine imbalanced animals when saturation of the diet fat is not a variable.

Weanling male Sprague-Dawley rats were fed a 9% casein, 30% fat, 0.50% choline diet that was imbalanced with respect to threonine. The diet fat was either a 1:1 mixture of corn oil and olive oil or a 1:1 mixture of corn oil and olive oil that had been 90% elaidinized. At the end of four weeks the animals were given by gavage approximately 1 ml of corn oil containing 20 μC per 165 grams of body weight of either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid. The animals were sacrificed by cardiac puncture at 4 and 8 hours after

intubation. The liver, epididymal fat pads, gastrocnemius muscle, intestines, feces, urine, serum and expired carbon dioxide were analyzed for radioactivity. In addition, the fatty acid composition of the fat pads, liver lipids, and liver triglyceride, phospholipid and cholesterol ester fractions was determined, as was the distribution of label within these liver lipid classes and within the serum VHD, LD, HD and VHD lipoprotein fractions.[5]

From this study the following observations were made:

1. Elaidinization had no effect on food consumption or weight gain; however, it significantly reduced the coefficient of digestibility.

2. Elaidinization caused a significant reduction in the accumulation of fat in the liver.

3. The intubation of 1 ml of non-radioactive corn oil had no apparent effect on liver lipid content. However, the 4 and 8 hour intervals spent without food resulted in a significant increase in liver moisture content in both the olive oil and elaidinized olive oil fed animals and a significant increase in fat pad moisture in the elaidinized olive oil fed animals.

4. Elaidinization of olive oil resulted in a significant increase in the percent of octadecenoic acid (C18:1) in the left epididymal fat pad at the expense of palmitic acid (C16:0). The starvation period and/or the intubation of corn oil resulted in an increase in fat pad octadecenoate in both the olive oil and elaidinized olive oil fed animals.

5. Elaidinization had little effect on the fatty acid composition of the liver triglyceride and cholesterol ester fractions. In the phospholipid fraction, however, it caused a significant increase in the percent of octadecenoate at the expense of octadecanoate.

6. Elaidinization resulted in a significant increase in the quantity of liver triglyceride and a significant decrease in the quantity of liver cholesterol at the end of 4 hours but not at 8. Starvation and/or the ingestion of corn oil had no effect on the composition of lipid classes in animals fed elaidinized olive oil; however, either or both caused an increase in the quantity of triglyceride present at the expense of the phospholipid content between 4 and 8 hours in the olive oil fed animals.

7. C^{14}-elaidic acid was absorbed slightly more rapidly than C^{14}-oleic acid during the first 4 hours. Just the opposite was true during the second 4 hours.

8. More C^{14}-oleic acid than C^{14}-elaidic acid was oxidized to carbon dioxide and a significantly greater quantity was excreted in the urine.

9. Four hours after intubation, there was more C^{14}-elaidic acid in the serum, liver, epididymal fat pads and muscle than C^{14}-oleic acid. After 8 hours, there were no differences in the distribution of C^{14}-elaidic acid and C^{14}-oleic acid in these tissues.

10. Between 4 and 8 hours after intubation, elaidinized olive oil fed animals were transporting C^{14}-elaidic

acid out of the liver at a rate commensurate with the influx of this fatty acid. The olive oil fed animals were transporting more C^{14}-oleic acid into the liver than out, during this same time interval.

11. C^{14}-elaidic acid was preferentially incorporated into the liver phospholipid fraction. C^{14}-oleic acid was incorporated predominantly into the triglyceride and cholesterol ester fractions.

12. Elaidinization resulted in a significantly greater amount of serum LDL at 4 hours but not at 8. There was also a significantly greater incorporation of C^{14}-elaidate into the LDL and HDL fractions.

13. Elaidinized olive oil fed animals hydrolyzed more of the circulating lipoprotein lipid per unit time.

14. The metabolism of either C^{14}-oleic acid or C^{14}-elaidic acid was not significantly influenced by dietary fat.

A limited accumulation of liver lipid was observed in threonine imbalanced rats fed elaidinized olive oil as a result of an initial preferential uptake of the trans isomer in adipose tissue, an increase in liver lipoprotein synthesis and/or release, and a faster rate of hydrolysis of circulating lipoprotein lipid. The enhancement of lipoprotein synthesis appeared to be mediated by a preferential incorporation of the trans isomer into the liver phospholipids. The mode of enhancement was postulated to be via 1) the physical characteristics of the liver lipid

micelles containing the trans isomer, 2) an acceleration of triglyceride micellerization or 3) a direct influence on lipoprotein synthesis.

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[1] Benton, D. A., A. E. Harper and C. L. Elvehjem, 1956. The effects of different dietary fat on liver fat deposition. J. Biol. Chem. 218: 693.

[2] Best, C. H. and M. E. Huntsman, 1935. Choline and fat Metabolism. J. Biol. Chem. 83: 255.

[3] Channon, H. J., S. W. F. Hanson and P. A. Loizides, 1942. The effect of variations of diet fat on dietary fatty livers in rats. Biochem. J. 36: 214.

[4] Morris, L., D. A. Arata and D. C. Cederquist, 1965. Fatty livers in weanling rats fed low protein, threonine deficient diet. 1. Effect of various diet fats. J. Nutr. 85: 362.

[5] VLDL = lipoproteins of density less than 1.019
LDL = lipoproteins of density from 1.019 to 1.063
HDL = lipoproteins of density from 1.063 to 1.21
VHDL = lipoproteins of density greater than 1.21

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INTRODUCTION

The amount of fat present in the liver at any one time is the composite of the rates of hepatic lipid uptake, synthesis, oxidation and metabolism, and excretion or transport out of the liver. These four phases of liver lipid metabolism are normally in a state of dynamic equilibrium. An increase or decrease in one phase is usually followed by a compensatory change in another. For example, an increase in liver lipid uptake after the ingestion of a fatty meal will be followed by a concomitant decrease in lipid synthesis and an increase in transport of lipid out of the liver. Likewise an increase in synthesis after the ingestion of excess carbohydrate will cause a simultaneous increase in transport of the newly synthesized lipid out of the liver. Both of these examples illustrate the interdependence of the four phases of liver lipid metabolism for the maintenance of constant lipid levels.

Fat enters the liver primarily as either chylomicrons formed in the intestines during the digestion of fat or as free fatty acids bound to serum albumin by the adipose tissue (66). Free fatty acid release by the adipose tissue is initiated by fasting, epinephrine and ACTH (46, 106, 124). The amount released into the circulation in a fasting state is dependent upon the nutritional state of the animal, as is the extent of liver uptake: the more extensive the fast, the greater the amount released and the greater the uptake by the liver.

Chylomicrons are found only in the postprandial circulation. Triglyceride represents from 81% to 86% of the chylomicrons (94). Since the liver does not need lipoprotein lipase for chylomicron uptake (25), the extent of hepatic uptake is dependent upon the amount of fat ingested, upon the pinocytotic or other uptake control mechanisms and upon the amount of circulating chylomicron triglycerides taken up by extrahepatic tissues. Of 33 mg of chylomicrons injected into rats, the liver absorbed 36% to 40% of the dose within ten minutes (91). When rats were injected with 64 to 123 mg of chylomicrons, the liver uptake was 23% to 31% of the dose (91). Stein et al. (113) injected 200 mg of chylomicrons and the resulting liver uptake was only 10%. The percent of the circulating chylomicrons taken up by the liver appears to be dose dependent.

Hepatic fatty acid biosynthesis is dependent upon a supply of carbohydrate and/or amino acid precursors, adenosine triphosphate, and reduced pyridine trinucleotides (123). The stimulation of the enzyme acetyl-CoA carboxylase by citrate denotes the strong dependence of the system on a calorie excess. An accumulation of fatty acid Co-A products will inhibit the enzyme, thus serving to control the amount of carbohydrate and amino acids converted to lipid. Dietary triglyceride will also inhibit the synthetic system. Hill et al. (65) reported that hepatic lipogenic activity was more sensitive to fat in the diet than to carbohydrate. Increasing the amount of dietary fat over a range

of 0% to 10% greatly inhibited the conversion of C^{14}-labeled carbohydrate to fat. Since both newly synthesized fatty acids and triglyceride both inhibit the biosynthetic enzyme, the overall extent of synthesis is dependent upon the rate at which fat is transported out of the liver.

Triglyceride along with sterols and phospholipids are transported out of the liver in the form of plasma lipoproteins (28, 63, 113). Borgstrom and Olivecrona (26) showed that without a functioning liver, the normal recirculation of injected free fatty acids as glycerides did not occur.

The plasma lipoproteins are commonly classified according to density, although electrophoretic mobility has also been employed. The very low density lipoproteins (VLDL) of density less than 1.019 gm per ml (62) or of flotation rates S_f 20-400 (39) are involved in the transport of endogenous glycerides (35). This lipoprotein class contains approximately 50% triglyceride, 18% phospholipid, 12% cholesterol ester, 7% unesterified cholesterol and 8% protein. The low density class (LDL) of density from 1.019 to 1.063 gm per ml or S_f 0-20 is rich in cholesterol ester. The composition is 11% triglyceride, 22% phospholipid, 37% cholesterol ester, 8% unesterified cholesterol and 21% protein. The high density lipoproteins (HDL) of density from 1.063 to 1.21 gm per ml are rich in protein: 8% triglyceride, 22% phospholipid, 14% cholesterol ester, 3% free cholesterol and 50% protein (94).

In spite of volumes of literature on the serum lipoproteins, the biosynthetic mechanisms are just beginning to be elucidated. Triglyceride ingestion results in a marked increase in the low density ($d < 1.063$) lipoproteins (74, 119); however, the mode of stimulation is not yet completely understood. Until as recently as 1964 it had been thought that the entire lipoprotein molecule is synthesized de novo and released immediately into the plasma pool. In 1959 Marsh and Whereat (84) observed a net synthesis of 125 μg of LDL per liver per hour in rat liver slices, and a subsequent study (85) published as late as 1963 showed the incorporation of labeled amino acids into lipoproteins in liver homogenates. This latter work corroborated the studies of Radding et al. (96) showing the incorporation of labeled amino acids into low and high density lipoproteins in rat liver slices. The high density lipoproteins isolated from the rat liver slices were the same as those isolated from the serum.

However, the more recent work in this area of lipoprotein synthesis suggests that the protein moiety may be synthesized separately. Eder et al. (44) postulated apolipoproteins that recycle and serve as carriers in lipid transport. This postulate was supported in subsequent liver perfusion studies showing the release of cholesterol, triglyceride and phospholipid predominantly in the form of low density lipoproteins into the perfusate when the latter contained plasma. When erythrocytes suspended in Ringer's

solution or albumin was perfused, the release of lipoproteins was greatly diminished. Another argument against the total de novo synthesis of lipoproteins might be the variation in plasma half-life between the lipid-protein constituents of the individual lipoprotein fractions (101, 105). Also, the in vitro synthesis of apo-lipoproteins by rat liver microsomes has been recently demonstrated by using immunological techniques (29). The isolated apo-lipoproteins were capable of binding the lipid extracted from rat liver.

Whether synthesis of lipoproteins entails separate or unit processes, the precise mechanism of coupling of the lipid with the protein fraction is still not known. Trams and Brown (118) proposed that the lipid component of the lipoproteins is determined by the information contained in the protein portion of the molecule, the final lipid composition being a result of variation in hydrophobic and lipophilic areas of the lipoprotein precursors. They also suggested that the specificity of the protein moiety towards a certain lipid composition might be increased as various lipids were added to the structure. This latter proposal had previously been suggested by Holman (68). He stated that the coupling of the protein and lipid probably is dependent not only on the protein, but also on the fatty acid composition of the glycerides, phospholipids and sterol esters that comprise the lipid moiety. In spite of the mechanism by which coupling occurs, unless the process takes place, little of the liver lipid is released into the serum.

And even if incomplete lipoproteins could be secreted, the extrahepatic tissues could not assimilate the lipid. Triglyceride cannot be directly taken up by these tissues, but must first be hydrolyzed. The hydrolyzing enzyme, lipoprotein lipase, released by the extrahepatic tissues requires the presence of intact lipoproteins for effective substrate binding (77).

Lipid uptake, synthesis, oxidation and metabolism and excretion must all operate effectively in the liver as separate entities and in harmony as interdependent processes to maintain constant liver lipid levels. The rate of activity of any one phase is determined by and reflective of the activities of the other phases. Malnutrition, disease, toxins, drugs and endocrine malfunctioning can result in an upset in one or more of these phases of lipid metabolism and displace the lipid equilibrium to a higher level, resulting in the accumulation of large amounts of fat in the liver. This review will be limited to fatty livers caused and corrected by dietary means, the corrective factors being termed lipotropic or dietary substances that decrease the rate of deposition or synthesis and/or accelerate the removal of abnormal amounts of lipid in the liver.

NUTRITIONAL FATTY LIVERS

The study of dietary fatty livers began with the works of Banting and Best on depancreatized dogs. The resulting fatty livers were attributed to the digestive enzymes lost when the pancreas was removed until Hershey and Soskin (64)

found that crude lecithin could prevent the onset of liver fat accumulation in depancreatized dogs. In 1932, Best, Hershey and Huntsman (16) found that choline was the active component of lecithin, and a dose-response curve was subsequently published relating daily choline intake to the reduction of liver lipids in the rat (17). Two years later Best, Channon and Ridout (14) identified the fat accumulating in livers of choline deficient rats as triglyceride. They also noted that the amount of choline-containing phospholipids in the liver was not reduced during choline deficiency. This finding has consistently been substantiated by many different research groups (27, 88, 99).

The liver triglyceride accumulation seen in choline deficiency might be due to depressed oxidation of fatty acids by liver tissues. Working with rat liver slices, liver homogenates and particulate fractions, Artom (8) reported that livers from choline deficient animals could not oxidize C^{14}-labeled fatty acids to carbon dioxide as well as could choline supplemented controls. On the other hand, Stetten and Salcedo (114) viewed the metabolic fault to be one of decreased transport of lipid out of the liver. Rats were fed D_2O to label the fat stores and were then placed on choline deficient diets. Dilution of the label in the liver but not in the fat depots indicated that choline deficient fatty livers arose from fat synthesized in the liver that is not transported to the depots.

Studies on the etiology of the choline deficient fatty

liver since 1944 have consistently supported the conclusions of Stetten and Salcedo. Wilgram et al. (127) found that after four weeks on a choline deficient diet, rats showed a marked decrease in the amount of serum S_f 70-400 lipoproteins. Choline supplemented animals had 34.5 mg per 100 ml serum of this lipoprotein fraction, whereas the choline deficient animals had only 6.1 mg per 100 ml. The deficiency also resulted in a slight lowering in the S_f 1-20 class which is high in phospholipid and cholesterol esters. In isolated liver perfusion studies, Haines and Mookerjea (55) showed that choline deficient livers failed to release triglycerides to the perfusate, whereas these livers had no difficulty in taking up the triglycerides. In the livers of the choline supplemented animals, on the other hand, the release of triglyceride was greater than the uptake. In in vivo studies, these same authors reported that after two days on a choline deficient diet there was a great rise in hepatic esterified fatty acids and a simultaneous decrease in plasma triglyceride, phospholipid and cholesterol esters. Olson et al. (92) and Forbes et al. (50) have also corroborated the theory that choline deficiency leads to fatty livers because the plasma lipoproteins are not produced and/or released from the liver in sufficient quantities; and since the turnover of these serum proteins is so fast (9), serum lipid levels fall rapidly with a simultaneous increase in liver lipid levels.

Since, in choline deficiency, less phospholipid is

theoretically leaving the liver as part of the lipoproteins and since the total amount of choline containing phospholipid (lecithin) in the liver does not decrease, there must be an impaired synthesis and a subsequent slower hepatic turnover rate of lecithin. Using N^{15} labeled choline, Boxer and Stetten (27) showed that choline deprivation resulted in a marked retardation in the rate of incorporation of choline into phosphatides. When choline was supplied in the diet at a level of 50 mg per rat per day, approximately 3.9 mg of the new choline entered the phosphatides per day. When no supplementary choline was given, the rate dropped to 1.3 mg. They concluded that the development of fatty liver is related to the rate at which choline phosphatides are turned over. This has since been substantiated by both Yoshida and Harper (131) and Lombardi, Ugazio and Raick (81, 82) who found a decreased rate of incorporation of labeled palmitate into liver phospholipids in choline deficient rats, yet the total amount of phospholipid present was not different from that in choline supplemented rats.

It appears that the decreased lecithin synthesis and turnover seen in choline deficiency result in decreased synthesis of low density lipoproteins and subsequent accumulation of liver fat. However, this theory has been resistant to proof since the total amount of lecithin in the liver does not fall in choline deficiency. Why the lecithin present in the choline deficient liver is not used for lipoprotein synthesis is a question not yet answered. Possibly

this level of lecithin represents structural phosphatide and therefore a minimal concentration commensurate with hepatic function. In order for the liver to sustain a significant production of transport lipoproteins, Boxer and Stetten's (27) newly synthesized lecithin must be immediately incorporated into transport lipoproteins. If this hypothesis is correct, then the liver lecithin which is serving as one of the precursors for the synthesis of transport lipoproteins would be too transitory to affect the total concentration of lecithin in liver tissues and thus not measurable.

Liver triglyceride accumulation as a result of decreased lipoprotein synthesis or release can also be induced by many chemicals such as orotic acid, ethionine, carbon tetrachloride, azaserine and phosphorus. Prior to the liver triglyceride accumulation seen in animals fed one of these chemicals, there is a drop in hepatic ATP levels and a subsequent depression in lipoprotein synthesis (72, 111, 128, 129). However, Simon et al. (109) failed to observe a comparable drop in hepatic ATP levels in choline deficient rats. Therefore, the mechanism whereby choline deficiency affects lipoprotein metabolism is presumably different from that of orotic acid, ethionine, carbon tetrachloride, azaserine and phosphorus.

In addition to choline, the quantity and quality of dietary protein can also exert a lipotropic effect. One of the first reports of this phenomenon was by Best and

Huntsman (18) who showed that feeding only sucrose to rats with fatty livers resulted in an increase in hepatic lipid content, whereas the addition of 20% of casein did not. Choline contamination of the casein was suspected until Channon and Wilkinson (33) using alcohol extracted casein that was essentially choline free substantiated the work of Best and Huntsman (18).

Dried egg white was reported to be equal to casein in lipotropic activity (15), powdered dried beef muscle was less active and gelatin was inactive. Since proteins differ markedly in their lipotropic effect, attention was focused on the amino acid patterns in various proteins (120, 121). In 1937 Tucker and Eckstein (120) added 0.5% methionine to a 5% casein, 40% lard diet and observed a 41% decrease in liver lipid content. On the basis of this experiment, they suggested the previously observed lipotropic effect of casein may have been due to its methionine content. Du Vigneaud (42) subsequently reported that rats could grow normally with no choline but adequate methionine. To explain the lipotropic effects of protein and methionine, he suggested that methionine might furnish methyl groups for the synthesis of choline, a compound already identified as lipotropic. His theory was substantiated by feeding rats methionine with the methyl group labeled with deuterium and analyzing for the label in choline (43). This explained why choline was essential in the diet only when the protein and thus methionine intake was low or when the intake of fat was high.

This interrelationship of methionine, choline and protein was soon complicated when it was learned that protein could itself exert a lipotropic effect not attributable to its methionine content. Best and Ridout (20) found that a 30% casein diet was more effective in lowering liver fats than were equivalent amounts of the free amino acids methionine and cysteine. Beveridge et al. (21) found that at dietary levels of casein below 22%, free methionine exerted the greater lipotropic effect, and above 22%, the equivalent amount of casein was more efficient. The apparently greater lipotropic effect of free methionine might be due to the larger amounts of methionine required for the synthesis of tissue protein in the casein fed group, creating a decrease in the amount available for lipotropic purposes. At the higher level of casein, the diet supplied not only adequate growth essentials, but also an excess of methionine which could exert a maximal lipotropic effect. They concluded that the lipotropic effect of protein is determined not only by its methionine and cysteine content, but also by the quantity and nature of the other essential amino acids. The authors did not exclude, however, the presence in casein of other essential amino acids which might also have lipotropic action.

The situation depicted thus far is such that protein exerts a lipotropic effect by supplying methionine, which donates methyl groups used in the synthesis of choline. Additional protein exerts a lipotropic effect presumably by supplying additional amino acids for the synthesis of

enzymes involved in fat metabolism and/or the synthesis of the protein moiety of the lipoprotein molecule. However, this picture of lipotropic interrelationships became exceedingly confused by the observation that in some circumstances, methionine exerts an antilipotropic effect (56, 57, 59, 60). Harper et al. (58) reported that feeding weanling rats a 9% casein diet supplemented with 0.1% DL-tryptophan and 0.15% choline chloride produced liver fats of 4.2% wet weight of the liver; whereas the addition of 0.3% DL-methionine increased the liver lipid content to 9.7%. When the methionine content was lowered to 0.2%, the liver lipid content was lowered to 8.3%; a further reduction of methionine to 0.1% resulted in 7.8% liver fat. However, the addition of 0.36% DL-threonine along with methionine prevented lipid deposition. A subsequent investigation showed, in the situation just described, that methionine supplementation created an amino acid imbalance with respect to threonine, the second most limiting amino acid in casein.

Singal, Hagan, Sydenstricker and Littlejohn (110) studied the fatty livers produced by threonine deficiency. A threonine devoid diet did not cause liver fat deposition. Maximum liver fat deposition (14.8% wet weight of liver) did not occur until the diet contained 0.7% DL-threonine. When threonine was increased to 1.1%, the liver lipid content was within the normal range. Massive doses of choline (5 times normal) reduced liver lipids in these animals, although not as effectively as threonine. The authors concluded that

there were two separate, yet related phenomenon in liver fat production: 1) a primary methyl group or choline deficiency in which threonine was without effect and 2) a primary threonine deficiency in which choline was effective only when supplemented at very high levels.

Histological evidence supports Singal's theory on the two types of nutritional fatty livers. There is a centrolobular[1] distrubution of fat in methyl group deficient rats, whereas the feeding of a threonine imbalanced diet causes a periportal or peripheral distribution of fat in the liver lobule (61, 107, 108). Also, in the fatty liver induced by a deficiency of methyl groups, the individual cell contains droplets of fat in its cytoplasm which later fuse into large globules that displace the nucleus to one side of the cell. In livers from threonine deficient animals, however, the fat accumulates inside intracytoplasmic compartments which do not fuse together and cause nuclear displacement.

Whereas a choline deficient fatty liver appears to be due to a decreased hepatic phospholipid turnover, decreased phospholipid and lipoprotein synthesis and possibly a concomitant impairment in oxidation, the mechanism inciting liver fat accumulation in a threonine imbalanced rat remains to be elucidated. From studies reported to date, increased synthesis of neutral fat, decreased oxidation and decreased

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[1]fatty infiltration around the central vein

transport all seem to be involved. Whether one single underlying mechanism will be uncovered remains to be seen.

Arata et al. (5, 6) concluded that oxidation was impaired in the threonine imbalanced rat by studying several of the oxidative and electron transport enzymes. Weanling rats were fed a 9% casein, 5% corn oil diet that was made imbalanced in threonine by the addition of 0.3% DL-methionine and 0.1% DL-tryptophan. Choline was adequate. Threonine supplemented animals served as controls. The pyridine nucleotides, NAD and NADP, and endogenous respiration were studied, and both parameters were found to be significantly reduced in the imbalanced animals. In subsequent studies, Arata et al. (7) and Carroll et al. (30) studied the effects of the same diets on liver levels of both oxidized and reduced pyridine nucleotides and labile phosphorus from ATP and ADP, on the activity of the NAD cytochrome C-reductase system and the fatty acid oxidase system. These parameters were studied in relation to liver fat accumulation with time. Liver fat deposition was at a maximum at two weeks, and at six weeks the liver lipids were approaching normal values. As liver fat deposition increased, the fatty acid oxidase activity decreased, reaching a minimum of 43% of the supplemented animals just prior to maximum fat deposition. Thereafter the fatty acid oxidase activity recovered and liver lipid concentration receded. The labile phosphorus and oxidized pyridine nucleotides were consistently lower than the control group as was cytochrome C-reductase

activity. On the other hand, the reduced pyridine nucleotides were greater than 2 1/2 times that of the supplemented controls. The authors concluded that some portion of the electron transport system was a primary target of the threonine imbalance. The significance of the reduced labile phosphorus in relation to the studies of toxin induced fatty livers in which decreased ATP levels are also seen (72, 111, 128) is not known; however, it is possible that the underlying mechanism behind a threonine imbalanced fatty liver and a toxin (such as ethionine or orotic acid) induced fatty liver may be the same, especially since an ethionine or orotic acid fatty liver is initially periportal in distribution (45). The temporal changes of the fatty acid oxidase system point it to be a non-specific response, and the authors suggest that the liver fat deposition might be a compensatory mechanism to allow the recovery of the enzyme system, as indeed it does. They gave an alternate theory to explain the impairment of the fatty acid oxidase system centered on the apparent defect in the ability of tissues from deficient animals to reoxidize reduced pyridine nucleotides. Since NAD acts as a cofactor for the fatty acid oxidase system, any interference in the generation of NAD would be reflected in a reduction in activity of the oxidase system.

Yoshida and Harper (131), using the same diet as Arata and co-workers, injected acetate-1-C^{14} into both the imbalanced and supplemented rats at the time of maximal fat

deposition. The incorporation of acetate into neutral fat was significantly higher in livers from deficient animals than in those from supplemented animals, suggesting that the liver fat deposition was the result of increased synthesis of fat. Perhaps the raised NADPH levels (7) incite synthesis to a greater extent than the build up of fatty acids and triglycerides, acting as feedback inhibitors, stop it. There was no significant difference in the ability of the imbalanced and supplemented animals to incorporate acetate into liver phospholipids, although there was a trend toward greater incorporation by the imbalanced animals (8.7×10^{-3} versus 4.0×10^{-3} total CPM phospholipid/body weight). There was a similar trend for the incorporation of labeled acetate into carcass fat. This finding along with the fact that total carcass fat was significantly greater in the imbalanced animals indicates that the adipocytes are stimulated into synthetic activity by the imbalance and/or there is no impairment in the transport ability of the liver for fat. Transport may be functioning at a normal level, but it obviously is not keeping up with enhanced synthesis and depressed oxidation.

Viviani, Sechi and Lenaz (122) studied lipid metabolism in threonine imbalanced rats that had been fed a low-protein rice diet. Corroborating Yoshida and Harper (131) they found the excess lipids deposited in the liver were predominantly neutral fat with the absolute amount of phospholipid the same for the imbalanced and supplemented groups.

However, contrary to Yoshida and Harper, they found a marked depression in the ability of the imbalanced animals to incorporate labeled acetate into phospholipid. This, coupled with an observed decrease in serum phospholipids, suggests a defect in transport out of the liver.

DIETARY FAT AND FATTY LIVERS

While Best and his associates were studying the lipotropic effect of choline, they also studied the effect of different types of dietary fats on fatty livers. In 1934, Best (13) reported that beef fat was more effective than butter fat in producing fatty livers in rats. Hershey and Soskin (64) had reported prior to this work that beef fat promoted greater accumulation of liver fat than cod liver oil when fed to depancreatized dogs. They attributed the effect of the beef fat to the higher degree of saturation when compared with the fatty acids present in cod liver oil. Channon and Wilkinson in 1936 (33) compared the effects of supplements at 40% of the diet of butter fat, beef fat, palm oil, coconut oil, olive oil and cod liver oil on liver lipids in rats fed choline deficient diets containing 5% casein. They concluded that the severity of the liver fat accumulation was dependent not only on choline but also on the iodine value of the fat fed and the amount of C14 to C18 fatty acids consumed. Butter fat (IV 33) caused six times the degree of fat infiltration as cod liver oil (IV 145).

To further test the relationship between the iodine value of fats and liver fat deposition, Channon et al. (32) fed choline deficient rats varying levels of hydrogenated linseed oil. The fat was incorporated into a 5% casein diet at a level of 40%. Natural linseed oil (IV 175) gave liver fat values of 7.2 gm fat/100 gm fresh liver weight. Linseed oil hydrogenated to an iodine value of 12.3 and containing 4.7% iso-oleic acid resulted in 13.2 gm fat/100 gm fresh liver, and that hydrogenated to an iodine value of 55 and 11.9% iso-oleic acid resulted in 13.0 gms fat. The authors stated that these data supported earlier conclusions that liver fat deposition was inversely related to the iodine value of the dietary fat. However, the possible adverse effects of the iso-oleic acid formed during hydrogenation was not explored.

To determine if the physical state of the diet fat, solid or liquid, affected liver fat accumulation, rats were fed olive oil into which varying amounts of elaidin were incorporated (32). With olive oil at 40% of the diet, the total fat solids being 12.0%, liver fats were 0.8 gm/100 gm rat. With 30% olive oil and 10% elaidin, liver fats were 0.68 and fat solids were 27.8% of the fat. When elaidin represented 30% of the diet and olive oil 10%, the total fat solids were 59.3% and liver fats were 0.6 gm/100 gm rat. More than doubling the fat solids without changing the iodine value did not increase liver fat levels. This experiment showed not only that liver lipid accumulation is not

proportional to the solid content of the diet fat but also that elaidin may have a beneficial effect, since it did tend to lower liver fats.

A study of the effect of chain length on liver lipids of choline deficient rats was reported by Stetten and Salcedo (114). The ethyl esters of even numbered fatty acids from butyric to stearic were incorporated into a hypolipotropic diet at a level of 35%. The severity of liver fat accumulation increased markedly as the chain length decreased from 18 to 16 to 14 carbon atoms, but severely fatty livers were not encountered when less than 12 carbon atoms were fed. However, these data may be of questionable significance because food consumption and weight gain markedly dropped when the animals were fed the ethyl esters.

A protective effect of hydrogenated fat (Crisco) against the development of cirrhosis in choline deficient rats was noted by Gyorgy and Goldblatt (54). Rats fed choline deficient diets containing lard (20-40%) had a higher incidence of cirrhosis than animals whose diet contained comparable quantities of Crisco.

Benton et al. (11) studied the effects of dietary fat on the liver lipids in rats fed 9% casein diets adequate in choline with and without threonine supplementation. Butter fat and lard increased the percent liver fat, regardless of threonine supplementation. Use of margarine as diet fat yielded lipid values comparable to corn oil in the threonine deficient animals, but considerably lower in the threonine

supplemented group, 12.9% dry weight of liver versus 19.4% in the corn oil fed animals. Butter fat was then separated into liquid and solid fractions by lead salt separation and fed to animals deficient in both choline and threonine. The liquid fraction at a level of 20% of the diet yielded liver fat levels of 44.6% dry weight of the tissue, whereas the solid portion gave 66.8% fat. The authors concluded that long chain saturated fatty acids resulted in increased liver fat deposition in animals fed diets limited in either choline or threonine.

In a subsequent study, Benton et al. (12) determined the effect of different dietary fats on the choline requirement of the animal. Butter fat and corn oil incorporated into 8% casein, 12% gelatin diets at a level of 30% were compared. Normal liver fats were obtained with corn oil fed animals when choline chloride was supplemented to the extent of 0.12% of the diet. However, the dietary choline had to be increased to 0.15% to achieve normal liver fats in animals fed butter fat. Similar results were obtained when the casein content of the diet was raised to 9% with or without methionine and tryptophan supplementation to improve growth. The authors concluded that the substitution of butter fat for corn oil in diets containing 30% fat and 8% or 9% casein increased the amount of choline required for the attainment of normal liver fat levels.

Best, Lucas, Patterson and Ridout (19) studied the effects of different dietary fats and different levels of

choline on the accumulation of hepatic lipids. At a level of 20% in a choline free diet, olive oil, butter fat, coconut oil or lard gave higher liver lipids than did sunflower oil, corn oil Margene (oleomargarine) or Primex (hydrogenated cottonseed oil). The addition of 0.02% choline chloride to the diet did not significantly alter these results. Between 0.06% and 0.12% choline chloride, the trends reversed, with butter fat and lard giving lower liver fats. When the level of choline chloride was 0.36%, there were no differences in liver fats regardless of the type of fat in the diet. However, these differences were small and not statistically significant.

The effects of olive oil, cottonseed oil, hydrogenated vegetable oil (Maxim, IV 74) and hydrogenated corn oil (Proctor and Gamble, IV 74) were compared with corn oil with respect to the accumulation of liver lipid in rats fed threonine imbalanced diets (89, 90). The diet was the same as that originally used by Harper et al. (58) except that the fat content was increased from 5% to 30%. To avoid a caloric enrichment of the diet by the higher quantity of fat, Morris' diets were made isocaloric to Harper's by the addition of cellulose. Olive oil slightly increased liver fats as compared to corn oil (25.6% vs. 22.5% dry weight of the tissue); whereas the hydrogenated vegetable oils considerably lowered liver fats (Maxim yielded 17.6% fat and hydrogenated corn oil yielded 13.7% fat). This work contradicts the earlier hypothesis that liver fat accumulation

is inversely related to the unsaturation or iodine value of the dietary fat. In this study, liver fat levels tended to be lower as the fat is made more saturated by the hydrogenation process.

Woolcock (130) used the same diet as Morris to determine whether the ability of the hydrogenated fat to lower liver lipids was the result of chemical treatment of the fat. She compared untreated natural lard, commercially treated lard in which the fat had been molecularly rearranged without changing the fatty acid composition, hydrogenated corn oil and corn oil. The commercially treated lard had no effect on liver fats as compared to either the corn oil or natural lard controls. Hydrogenated corn oil again reduced liver fats. Since chemical treatment of lard had no effect on liver fat levels, and since the major difference between the fatty acid composition of chemically treated lard and hydrogenated corn oil is the geometrical configuration of the double bonds, the author concluded that the effect of hydrogenated corn oil was not due to chemical treatment, but rather to the presence of the trans isomers introduced during the hydrogenation process.

METABOLISM OF TRANS FATTY ACIDS

Ever since the popularization of hydrogenation, extensive reports on the metabolism of trans isomers have accumulated, much of which show the trans bonds to be metabolized quite differently than the cis counterpart (37, 38, 73, 97).

Neither the trans-octadecenoic, nor trans, trans-, nor trans, cis-, nor cis, trans-octadecadienoic fatty acids possess any essential fatty acid activity (22, 23, 40, 67, 69, 76). In fact, some workers have suggested an anti-essential fatty acid activity for these isomers, but the literature on this point is conflicting. Holman (67) suggested that the trans isomers of linoleic acid compete for the enzyme systems involved in the conversion of linoleic acid to arachidonic acid and thus increase the requirement for linoleic acid. Holman and Aaes-Jorgensen (69) showed that the dermatitis associated with essential fatty acid activity in rats was worsened when the animals were fed small amounts of linoelaidic and trans-linoleic acid. However Alfin-Slater and Deuel (1) fed partially hydrogenated triolein to essential fatty acid deficient rats and found that this supplemental fat did not interfere with growth, did not aggravate the deficiency symptoms and did not interfere with the curative effect of linoleate.

Blank and Privett (22) showed that dietary methyl cis, trans-linoleate was converted to a trans-eicosatetraenoic fatty acid with the double bonds in the 5,8,11,14-positions, the same positions as the C20:4 derivative of cis,cis-linoleate; however, the former had no essential fatty acid activity. Methyl cis,trans-linolenate was converted primarily to an eicosapentaenoic derivative, whereas its all cis counterpart is lengthened to an eicosahexaenoic (C20:6) fatty acid.

Privett, Nutter and Lightly (95) studied the influence of geometric isomers of linoleic acid on the structure of liver triglycerides and lecithin. The α-position of the liver triglycerides was filled predominantly with trans, trans-linoleate and oleate. The primary fatty acids found in the triglyceride β-position were cis,cis-linoleate and oleate. Palmitoleic and linolenic acids were randomly distributed. In liver lecithins, the predominant fatty acids in the β-position were linolenic, cis,cis-linoleic and cis,trans-linoleic acids. The α-position contained palmitic, stearic and trans,trans-linoleic acid and oleic acid when cis,cis-linoleic acid was in the β-position.

Coots (34) compared the metabolism of elaidic, oleic, palmitic and stearic acids in the rat by the use of radioisotopes. The rats were given the fatty acids transesterified into soybean oil by stomach tube. In this study the geometry of the double bond made no difference in the animals' ability to oxidize elaidate to carbon dioxide. After 51 hours, 66.0% of the administered oleic acid had been excreted as carbon dioxide and 66.3% of the elaidic acid had followed this route. However, in a later article (4) it was reported that oleic acid was more readily oxidized to carbon dioxide than its trans counterpart. During the peak of absorption, elaidic acid showed a slightly greater tendency to enter the lymph phospholipid fraction than did oleic. Stearic had a strong tendency to be incorporated into the lymph phospholipids. Thus elaidic acid and stearic acid

have a strong metabolic resemblance here, explainable most likely on the basis of carbon number and conformation as both have a straight chain conformation.

Dhopeshwarkar and Mead (41) fed rats radioactive methyl elaidate. Although transformation to other fatty acids was poor, more label appeared in stearate than in palmitate. Due to a preponderance of label in the carboxyl carbon of stearate, they suggested that elaidate might undergo a direct transformation to this fatty acid rather than β-oxidation to acetyl-CoA and subsequent total biosynthesis of stearic acid.

W.E.M. Lands (78) stated that phospholipid acyltransferase can be quite selective in terms of chain length and the degree of unsaturation. In 1965 he reported a study designed to see if acyl transferase could discriminate between geometrical isomers of octadecenoate and whether the more linear trans isomer behaved more like a saturated fatty acid. 1- and 2-acylglycerophosphocholine were prepared and incubated with stearate, elaidate or oleate acyl CoA and the acyl transferase enzymes. The reactions were followed spectrophotometrically. Incorporation into 1-acyl GPC was the following: C18:0, 3.0 μ moles/min/mg protein; C18:1 (trans), 15.5; and C18:1 (cis), 13.5. Incorporation into the 2-acyl GPC was C18:0, 28.5; C18:1 (trans), 42.0; and C18:1 (cis), 1.0. The acyl transferase catalyzing esterification at the 2-position did not discriminate between the cis and trans isomers of octadecenoate; however,

the enzyme adding at the 1-position discriminated markedly between the geometric isomers and treated the trans-octadecenoate almost as if it were the more linear saturated octadecanoate. Raulin et al. (98) showed also that the trans isomer was principally incorporated into the primary positions of depot triglycerides of rats that were fed a diet of elaidinized peanut oil for 2 to 7 months.

The metabolism of dietary fatty acids appears to be influenced by the geometry of the double bond. This effect may explain the reduction in liver fats in rats fed threonine deficient diets containing hydrogenated corn oil (89), the failure of margarine (11) or Margene (19) and Primex to increase liver fats in rats fed choline deficient diets or the reduction in liver fats in rats fed choline deficient diets containing varying amounts of elaidin (33), as all of these diet fats contained substantial amounts of trans fatty acids. To explore the possibility that the geometry of the double bond could influence liver fat accumulation, it was decided to trace the metabolism of two isotopically labeled isomeric fatty acids in rats fed low protein, threonine imbalanced diets.

PART I

THE EFFECT OF ELAIDINIZATION OF DIETARY OLIVE OIL ON LIVER FAT ACCUMULATION IN THREONINE IMBALANCED RATS

INTRODUCTION

That dietary fat can affect the accumulation of lipid in nutritional fatty livers was first shown by Hershey and Soskin (64) in depancreatized dogs, in which beef fat promoted greater accumulation of liver fat than cod liver oil. Best (18) subsequently stated that beef fat was more effective than butter fat in producing fatty livers in choline deficient rats. Channon and Wilkinson (32) reported that butter fat caused six times the degree of fat infiltration as cod liver oil in rats fed 5% casein, 40% fat, choline deficient diets. Benton (11) found that butter fat also increased liver lipids in threonine imbalanced, choline supplemented rats. Throughout this time span (1931-1956) it was hypothesized that liver fat accumulation in rats fed low protein, high fat diets was directly related to the percent of long chain fatty acids of the dietary fat and inversely related to the iodine value of the dietary fat.

Contradicting this hypothesis is the study of Morris et al. (89, 90), in which the hydrogenation of corn oil to an iodine value of 74 significantly reduced the liver lipid content of rats fed 30% fat, threonine imbalanced, choline supplemented diets. The authors attributed the effect to the presence of the trans fatty acids formed during hydrogenation. However, this evidence is far from conclusive because of the concomitant increase in the amount of saturation of the fatty acid constituents with changes in the

geometry of the double bond as a consequence of the hydrogenation process.

As the iodine value was reduced to 74 during the hydrogenation of the corn oil in Morris' work, there was a five-fold increase in C18:0 (stearic acid) and a two and one half-fold increase in C18:1 (cis or trans oleic acid). Both of these increments were at the expense of C18:2 (linoleic acid). Concomitant with the increase in saturation was a 45% increase in the number of trans bonds present. Hence, the tendency of the hydrogenated corn oil to reduce the liver fat content, as seen in Morris' work (89), cannot be directly pinpointed to either the increase in the saturation of the fat or to the presence of the trans bonds.

In a subsequent study, Woolcock (130) examined the effect of the fatty acid composition of dietary fat on the accumulation of liver lipids in threonine imbalanced rats. She employed the same basic diet as did Morris. Coconut oil and olive oil when fed to rats at a level of 30% of the diet resulted in a significantly greater accumulation of liver lipid than did corn oil; whereas hydrogenated corn oil and cocoa butter caused a significant reduction. When safflower oil or lard was used in the diet, there was no difference in liver fats from the corn oil fed animals. As a result of these data, no correlations can be drawn between the degree of saturation of the dietary fat and the accumulation of fat in the livers of threonine imbalanced rats. Hydrogenated corn (IV 74) and cocoa butter (IV 8.5) reduced liver lipids

when fed in place of corn oil, whereas lard (IV 55) caused an increase in fat accumulation. Woolcock suggested that stearic acid and elaidic acid, which are large constituents of cocoa butter and hydrogenated corn oil respectively, may have a sparing effect on the lipotropic action of threonine. The mode whereby this effect might have occurred could not be ascertained from her study.

In order to discriminate between the increase in saturation or the increase in trans isomers as the variables in the hydrogenated corn oil responsible for the reduction of liver lipids, the following experiment was designed in which saturation of the fat was eliminated as a variable by a comparison of the elaidinized olive oil and natural olive oil. Olive oil after elaidinization has essentially the same fatty acid composition as the starting material except that approximately 90% to 95% of the oleic acid has been converted to elaidic acid. In order to make this compound more comparable to those fed by Morris and Woolcock, the olive oil and elaidinized olive oil used in this study were diluted 50% with corn oil. Under these conditions, if the animals fed the elaidinized olive oil threonine imbalanced diets showed a reduction in liver lipids from the olive oil fed controls, then this reduction should be attributable only to the presence of the elaidic acid. And such a reduction would support Morris' original hypothesis that trans isomers prevented liver fat accumulation in threonine imbalanced rats.

METHODS

Weanling male Sprague-Dawley rats were divided into two groups such that the average weights of each group did not differ by more than 0.5 grams. They were housed in individual cages with raised screen bottoms in a temperature controlled room that was maintained with 12 hours of light and 12 hours of dark. They were fed the following basal diet: casein, 9.0: sucrose, 24.65; salts[1], 4.0; fat, 30.0[2]; choline chloride, 0.50; vitamin mix, 0.25; DL-methionine, 0.30; DL-tryptophan, 0.10; alphacel 31.2 grams/100 grams diet. Incorporated into sucrose the vitamin mix provided in mg/100 grams diet: vitamin A concentrate (500,000 IU/gm), 0.4; calciferol (40,000,000 IU/gm), 0.0383; thiamine hydrochloride, 0.5; riboflavin, 0.5; niacin, 1.0; pyridoxine, 0.25; calcium pantothenate, 2.0; inositol, 10.0; folic acid, 0.02; vitamin B_{12} (0.1% trituration), 2.0; biotin, 0.01; para-aminobenzoic acid, 1.0; menadione, 0.38. The diets were made fresh every other week and stored at 4°C., to insure stability of the dietary fat. Food and water were allowed ad libitum.

The dietary fat for the control animals was corn oil (Mazola) and olive oil (Fisher) in a ratio of 1:1. The

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[1]Wessen, L.G. Science 75 (1932) 339. Obtained from Nutrition Biochemicals Corporation, Cleveland, Ohio.

[2]Contains 75 mg of α-tocopherol per kilogram diet.

experimental group was fed corn oil and elaidinized olive oil (1:1). Olive oil was elaidinized in a 3 liter resin reactor under a constant nitrogen atmosphere according to the method of Litchfield, Harlow, Isbell and Reiser (80). The reaction was followed by periodically spotting methyl esters of the reacted fat on 0.3 mm thin layer plates impregnated with silver nitrate (20%). The solvent system used was hexane: diethyl ether (9:1 v/v). Separations were visualized by sulfuric acid charring, and the reaction was terminated when greater than 50% of the reacted fat moved with the methyl elaidate standard.

The nitric acid and sodium nitrate were quickly washed from the fat in a 2 liter separatory funnel with water and petroleum ether (B.P. 30-60°C) as solvents. The fat was then dried over anhydrous sodium sulfate and the nitrogenous by-products were removed by passing the fat dissolved in petroleum ether through a 4 foot column of silicic acid (Mallincrodt). To hasten the purification, the columns were attached to an aspirator. This quadrupled the flow rate and also served to keep the purified fat well below room temperature.

The percent trans bonds of the purified fat was raised by four successive recrystallizations: once from petroleum ether at -8°C; twice from methanol at 4°C; and once from acetone at 4°C. All solvents were redistilled prior to use. Two thousand grams of olive oil were reacted at one time and all batches were pooled prior to analyses and incorporation

into the diets. The final yield was approximately 25%.

The percent trans bonds were determined on methyl esters (87) of the reacted and purified fat according to the method of Firestone and LaBouliere (47). The absorbance was taken from 10.02 to 10.59 μ on a Perkin-Elmer model 337 infrared spectrophotometer in a variable path length KBr cell. A 0.1 mm KBr cell was used for a reference. Pure methyl elaidate (Applied Science) was used as a standard and the percent trans bonds were read off of a standard curve.

The fatty acid composition of the dietary fats was determined on an F&M model 400 gas liquid chromatograph. The solid support, 80-100 mesh Diatoport S (F&M), and the liquid phase, diethyleneglycol succinate (5%), were packed into a 6 foot by 1/8 inch (id) glass U-tube. Helium was used as the carrier gas at 40 psi. The oven temperature was programmed from 168°C to 210°C at a rate of 5° per minute. The injection port temperature was maintained at 230°C; detection temperature at 220°C. The retention time for arachidonic acid (C20:4) was approximately 6 inches of chart paper or 12 minutes. The percent of each fatty acid was determined by triangulation. All analyses were done in duplicate.

Since the 6 foot column would not separate the cis and trans isomers, samples of the fat were sent to Northern Regional Laboratories at Peoria, Illinois, where they were put through a 100 foot capillary column which separated the fat into its isomeric components.

The isomerized olive oil was analyzed for nitrogen

content on a Coleman micro nitrogen analyzer (53). Sixty to 80 mg samples were weighed out on a Cahn electrobalance, and the final combustion cycle at 810°C was lengthened by three minutes to insure complete pyrrolysis of the sample.

Three days prior to the termination of the experiment fecal samples were collected for digestibility determinations. The entire three day collection for each animal was ground with a mortar and pestle. A 1.5 to 2.0 gram aliquot was dried to constant weight (± 0.5 mg) in a tared alundum cup in a vacuum oven at 60°C. The fat content was determined by continuous chloroform-methanol extraction on a Goldfish apparatus. Endogenous fecal fat was determined by feeding 10 rats an essentially fat free diet; it provided approximately 60 mgs of linoleic acid per rat per day. All fat digestibilities were corrected for the presence of endogenous fecal fat.

At the end of four weeks, all animals were sacrificed by decapitation. The livers were promptly excised, blotted and weighed. They were subsequently homogenized in distilled water in a Potter-Elvehjem homogenizer and dried to constant weight at 85°C. The dried samples were ground in a Wiley mill, and a 1.0 gram aliquot was taken for fat determination via continuous diethyl ether extraction on a Goldfish apparatus. The diethyl ether was previously redistilled under nitrogen and over cooper wire. The nitrogen content of the dried, fat extracted liver tissue was determined on a 5 mg sample on a Coleman micro nitrogen analyzer. Samples were

analyzed in duplicate.

Error was estimated by calculating the standard error of the mean. The significance of differences was determined with the student's t-test.

RESULTS AND DISCUSSION

Elaidinization resulted in a complete loss of linoleic acid (c,c-C18:2) activity (Table 1). However, the dilution of the elaidinized olive oil by 50% with corn oil, which contains 59% linoleic acid, should make this loss insignificant. Similarly, the addition of the corn oil to both the olive oil and elaidinized olive oil diluted the presence of the oleic acid that was not isomerized. Assuming the purification procedures were complete, as evidenced by the nitrogen content, and considering the similarity of the fatty acid compositions after dilution with corn oil, it can be fairly safely concluded that the primary difference between the two dietary fats is the geometry of the double bond of the octadecenoic acid.

The data in Table 1 do not, however, give any indication of the presence of positional isomers, but their existence in the elaidinized olive oil must be acknowledged. Allen and Keiss (2) have stated that the number of positional isomers increases as the percent of trans bonds increases during hydrogenation. Since such isomers form in any reaction in which a pi bond is temporarily broken, the assumption must be made that a significant number of positional isomers are produced during elaidinization. During hydrogenation of oleic acid (2) the quantity of fatty acids containing double bonds at positions 8 and 10 equalled those with double bonds at positions 7 and 11 and these amounts were always

considerably less than the amount of 9 isomer present. More likely than not, this same process of movement of the double bond position up and down the carbon chain prevails in a similar manner during elaidinization. While the extent of positional isomerism after elaidinization was not ascertained it can be suggested that there were fewer positional isomers in elaidinized olive oil than in Morris' hydrogenated corn oil because the reaction conditions are milder in the former process. Moreover, the presence of positional isomers should be of less significance when compared to natural olive oil due to the dilution of both fats with corn oil.

Isomerization of olive oil produced no difference in weight gain or food consumption as compared to the olive oil fed animals (Table 2); however, it did markedly affect the digestibility (corn oil:olive oil, 90.0% and corn oil:elaidinized olive oil, 84.2% digestible). The reduction in digestibility as a result of elaidinization is in general agreement with Crockett and Deuel (36) who found the coefficient of digestibility inversely related to the melting point of the fat. Olive oil is liquid at room temperature, whereas its elaidinized counterpart is solid, consistent with the fact that the straight configuration of the trans bond melts at a considerably higher temperature than the folded shape of the cis bond. Mattson (86) however, felt that the coefficient of digestibility was more specifically related to the amount of trisaturated triglyceride present. As palmitic and stearic acid combined constitute less than

15% of the total fatty acids in elaidinized olive oil, it is doubtful that there is much trisaturated triglyceride present, and so the decrease in digestibility must be due to the change in the geometry of the double bond and the resulting increase in melting point.

Elaidinization had no effect on liver moisture (Table 3), but it did cause a significant reduction in liver fat (22.9% versus 18.0% on a dry weight basis) and an increase in liver nitrogen content on a wet weight basis. The increase in nitrogen content of liver tissues (2.54% compared to 2.40%) is small and most likely can be accounted for by the differences in liver fat content.

The reduction in liver fat content as a result of elaidinization, as percent of liver tissues or as grams of fat per 100 grams body weight (Table 3), was not as marked as that produced by the hydrogenation of corn oil (89, 90). This might be the result of several simultaneously operating factors. First, threonine imbalanced fatty livers are not consistent with respect to fat content from experiment to experiment (57).

A second factor might be the exclusion of the saturated fatty acid variable that was prevalent in Morris' work. Woolcock (130) showed that cocoa butter containing almost 33% stearic acid could reduce liver lipids when fed to threonine imbalanced rats. Since hydrogenation of corn oil resulted in a five-fold increase in this fatty acid, the liver fat reducing effect of the hydrogenated corn oil

observed by Morris might have been attributable to the combination of stearic and elaidic acids present, a combination not found in the corn oil:elaidinized olive oil mixture. Regardless of whether the increased liver lipid values of Table 3 as compared to Morris' work are due to an overall increase in both groups and/or to the absence of stearic acid, the fact remains that elaidinized olive oil does reduce or limit the liver fat accumulation in threonine imbalanced rats.

SUMMARY

The elaidinization of olive oil (86% of the double bonds isomerized) resulted in the following differences when fed to weanling rats at a level of 15% in a 9% casein, 15% corn oil threonine imbalanced diet as compared to untreated olive oil fed animals:

1. a significant reduction ($\alpha<.01$) in digestibility,
2. a significant reduction in liver fat content ($\alpha<.01$),
3. a significant increase in liver nitrogen content.

Table 1. Analysis of Dietary Fats

A. Percent fatty acid composition on F&M 400 GLC

Diet Fat	C16:0 (palmitic acid)	C18:0 (stearic acid)	C18:1 (oleic acid)	C18:2 (linoleic acid)
Corn Oil (Mazola)	12.2	1.8	27.7	58.6
Olive Oil (Fisher)	10.0	2.7	79.6	7.8
Elaidinized Olive Oil	12.5	1.9	79.4[1]	6.2

[1] Contains both oleic and elaidic acid

B. Nitrogen and trans bonds content of dietary fats

Diet Fat	% N_2	% trans bonds
Corn Oil (Mazola)	----	0.0
Olive Oil (Fisher)	0.36	0.0
Elaidinized Olive Oil	0.37	86.0

C. Percent fatty acid composition on Capillary GLC[2]

Diet Fat	C16:0	C18:0 & c-C18:1	t-C18:1	c,c-C18:2	c,t-C18:2	t,t-C18:2 & t,c-C18:2
Elaidinized Olive Oil	12.5	20.6	58.4	---	1.6	7.0

[2] Schofield, Northern Regional Research Laboratories, Peoria, Illinois

Table 2. Food consumption, weight gain and diet fat digestibility of male rats fed a threonine imbalanced diet containing two sources of diet fat for four weeks.

Diet Fat	Food Consumption	Weight Gain	Digestibility of Diet Fat
	gm/week	gm/week	%
Corn Oil: Olive Oil (1:1)	73.1 ± 2.2[1]	21.7 ± 0.8	90.0 ± 0.6
Corn Oil: Elaidinized Olive Oil (1:1)	73.6 ± 1.8	21.0 ± 0.7	84.2 ± 0.7[2]

[1] Mean ± SEM of 16 animals

[2] Significantly different from Corn Oil:Olive Oil fed animals at $\alpha<.01$

Table 3. Liver composition of male rats fed a threonine imbalanced diet containing two sources of diet fat for four weeks post weaning.

Diet Fat	Moisture	Fat		Nitrogen
		% dry weight	Grams/100 grams body weight	% wet weight
Corn Oil: Olive Oil (1:1)	69.2 ± 0.3[1]	22.9 ± 1.0	0.392 ± .024	2.40 ± .04
Corn Oil: Elaidinized Olive Oil (1:1)	69.6 ± 0.3	18.0 ± 1.0[2]	0.297 ± .019[2]	2.54 ± .02[2]

[1] Mean ± SEM of 16 animals

[2] Significantly different from Corn Oil:Olive Oil fed animals at $\alpha<.01$

PART II

A COMPARISON OF THE METABOLISM OF GEOMETRICAL ISOMERS OF C^{14}-Δ^9-OCTADECENOIC ACID IN THREONINE IMBALANCED RATS

INTRODUCTION

Elaidic acid (trans-Δ^9-octadecenoic acid) can limit the extent of liver fat accumulation in rats fed 9% casein, threonine imbalanced diets, as shown in Part I. The mechanism whereby the geometrical configuration of a fatty acid double bond could influence liver fat accumulation is not known. Presumably, the trans bond must in some way influence or alter one or more of the pathways that affect lipid metabolism. Such altered metabolic pathways could include enhanced oxidation to carbon dioxide, enhanced transport of fat out of the liver and/or by-passing the liver as chylomicrons for direct uptake by adipose tissue and muscle, resulting in a decreased deposition of liver fat.

In order to elucidate the mode by which the trans isomer can limit liver lipid accumulation in threonine imbalanced rats, it was decided to trace its metabolic pathways with radioisotopes and compare the results with that of the cis counterpart. Tracing the temporal changes in the radioactivity of the epididymal fat pads, intestines, serum lipoproteins, muscle, liver lipids, excreted carbon dioxide and urine after the ingestion of C^{14}-labeled cis- or trans-Δ^9-octadecenoic acid should provide some insights into the possible mechanism(s) of the elaidic acid effect. A difference in radioactivity between the cis- and trans-C^{14} dosed animals should suggest any alteration in one or more metabolic pathways which is dictated by the trans configuration.

However, elaidic acid and elaidinized olive oil are not naturally occurring; and it is, therefore, reasonable to suspect that the deposition of C^{14}-elaidic acid in an animal that had previously consumed a diet containing elaidinized olive oil for four weeks might be altogether different from that of another animal pre-fed an elaidic acid free diet. In other words, the results of the isotope experiment just described would give no indication whether the parameters involved represent inherent or adapted pathways. Since some of the affected enzyme systems can eventually adapt to the threonine imbalance (30), distinguishing between adapted and inherent pathways could be important in determining the effect of the elaidic acid. In order to do this, some of the animals fed olive oil for a period of four weeks were dosed with C^{14}-elaidic acid and some of the animals fed elaidinized olive oil for an equal period were dosed with C^{14}-oleic acid. If the pre-fed diet fat has no effect on the distribution of radioactivity, then that distribution must represent an inherent metabolic pathway.

In order for the C^{14}-fatty acids to accurately represent the metabolic pathways of oleic and elaidic acids whether adapted or inherent, the experimental conditions must simulate normal; there must be no isotope effect or carrier effect and the isotope must be administered by gavage. The molecular weight of octadecenoic acid is high enough and the energy of C^{14} is low enough to exclude any isotope effect. However, as the carrier of these long chain

fatty acids must be another fat, it is vital to know if the carrier itself has any effect on the lipid metabolism being studied. Hence, the effect of the carrier was examined prior to studying the metabolic distribution of the isotope.

SECTION A

THE EFFECT OF 1 ML CORN OIL GIVEN BY GAVAGE ON LIVER AND EPIDIDYMAL FAT PAD LIPIDS IN THREONINE IMBALANCED RATS

Methods

Sixty-four weanling male Sprague-Dawley rats were divided into two groups of 32 each and fed the 9% casein, 30% fat diets as described in Part I. In one group corn oil:elaidinized olive oil (1:1) provided the diet fat while corn oil:olive oil (1:1) was incorporated into the diet fed the second group of 32 animals. The same experimental conditions used in Part I prevailed.

On the morning of the 28th day, food cups were removed at 7:00 A.M. A 2 hour interval was then allotted to allow for partial clearing of food already present in the GI tract. At 9:00 A.M. each animal was given 1 ml of corn oil (Mazola) by gavage. The gavaging apparatus consisted of a 2 1/2 ml syringe joined to 6 inches of PE-60 polyethylene tubing by a luer-lock to tubing coupler (Clay-Adams). The rats were allowed to swallow the tubing while their jaws were held open to prevent their biting the tubing. The animals then underwent a 4 or 8 hour period with access to water but not to food. Sixteen animals from each group were used for each time period. At the end of each time period, the animals were lightly anaesthetized with ether and sacrificed by cardiac puncture. The livers and right epididymal fat pads were quickly removed, blotted

and weighed.

The livers were analyzed as described in Part I.

The epididymal fat pads were cut into small pieces and then dried to constant weight in tared aluminum pans at 85°C. The dried fat pads were subsequently subjected to 6 hours of continuous ether extraction on a Goldfish apparatus for the determination of total fat content. The dried, fat free residue was then ground with a mortar and pestle and analyzed for nitrogen content on a Coleman micro nitrogen analyzer.

All analyses were done in duplicate.

Results and Discussion

Four and eight hours after the gavaging of 1 ml of corn oil, there was no detectable change in the total liver lipid content of rats pre-fed diets containing either corn oil:olive oil (1:1) or corn oil:elaidinized olive oil (1:1), as indicated in Table 4. This lack of effect persisted whether the liver lipids were expressed on a percent dry weight or grams of liver fat per 100 grams body weight basis. In fact, the total liver lipids for both groups remained remarkably constant in spite of such variables as the ingestion of 1 ml of corn oil and the 4 to 8 hour period spent without food (see Table 3, page 45). The time required for the accumulation of lipids in the livers of threonine imbalanced rats is evidently greater than 8

hours, even after the consumption of large quantities of fat.

Either the intubated corn oil and/or the 4 to 8 hour time span spent without food had an effect on the percent liver moisture (Table 4), since the moisture content increased from 0 to 4 hours after intubation (69.2% to 70.5% and 69.6% to 70.3% in olive oil and elaidinized olive oil pre-fed animals, see Table 3, page 45), and from 4 to 8 hours after intubation (70.5% to 72.9% and 70.3% to 72.2% in the olive oil and elaidinized olive oil pre-fed animals). The anticipated results were a decrease in liver moisture content as the intubated corn oil entered the liver and diluted the water present. However, the unexpected finding of an elevated percentage of moisture in liver tissues probably reflects an increased mobilization of glycogen and fat from the liver when food is unavailable. In support of this suggestion is the apparent decrease with time in liver fat when expressed on a wet weight basis.

The liver nitrogen content did not change appreciably with either time or intubation (Table 4).

Neither variable, giving corn oil by gavage or time without food, appeared to have had an effect on the moisture, percent fat dry weight, or nitrogen content of the right epididymal fat pad in the olive oil fed animals (Table 5). In the elaidinized olive oil fed animals, however, there was a large increase in fat pad moisture as the fat content, expressed as grams of fat per 100 grams body weight,

markedly decreased (0.583 grams fat to 0.377 grams fat per 100 grams body weight). As 8 hours is ample time for the ingested corn oil to be deposited in the epididymal fat pads, this drop in fat content again conflicts with the expected results and must be related to the interrelationship between body fat and the 8 hour starvation period. Apparently, the animals fed elaidinized olive oil are more capable of utilizing lipid from fat pads during an 8 hour period without food than are the olive oil fed animals.

Of all the parameters measured, none appeared affected by the ingestion of 1 ml of corn oil. It was concluded that 1 ml of corn oil would produce no carrier effect if it were used to carry C^{14}-fatty acids in an experiment tracing the metabolism of cis and trans fatty acids. On this basis, the second study was designed.

Table 4. Liver composition of male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number	Diet Fat	Hours after gavaging	Moisture	Fat		Nitrogen
			%	% dry weight	Grams/100 grams body weight	% wet weight
1	Corn Oil:Olive Oil (1:1)	4	70.5 ± 0.5[1]	21.3 ± 0.9	0.326 ± .018	2.40 ± .04
3	Corn Oil: Elaidinized Olive Oil (1:1)	4	70.3 ± 0.4	18.9 ± 0.8[3]	0.288 ± .014[3]	2.54 ± .02[2]
5	Corn Oil:Olive Oil (1:1)	8	72.9 ± 0.5[2]	22.8 ± 0.9	0.324 ± .016	2.45 ± .05
6	Corn Oil: Elaidinized Olive Oil (1:1)	8	72.2 ± 0.3[4]	18.8 ± 1.0[5]	0.256 ± .018[5]	2.51 ± .08

[1] Mean ± SEM of 16 animals

[2] Significantly different from Group 1 at $\alpha<.01$

[3] Significantly different from Group 1 at $\alpha<.05$

[4] Significantly different from Group 3 at $\alpha<.01$

[5] Significantly different from Group 5 at $\alpha<.01$

Table 5. Right epididymal fat pad composition of male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number	Diet Fat	Hours after gavaging	Moisture	Fat		Nitrogen
			%	% dry weight	Grams/100 grams body weight	% wet weight
1	Corn Oil:Olive Oil (1:1)	4	25.6 ± 0.8[1]	95.1 ± 0.3	0.433 ± .016	0.47 ± .003
3	Corn Oil: Elaidinized Olive Oil (1:1)	4	21.0 ± 0.8[2]	96.2 ± 0.6	0.583 ± .031[2]	0.41 ± .070
5	Corn Oil:Olive Oil (1:1)	8	26.4 ± 1.5	95.7 ± 0.1	0.407 ± .023	0.41 ± .006
6	Corn Oil: Elaidinized Olive Oil (1:1)	8	25.9 ± 1.3[3]	96.3 ± 0.4	0.377 ± .025[3]	0.37 ± .040

[1] Mean ± SEM of 16 animals

[2] Significantly different from Group 1 at $\alpha<.01$

[3] Significantly different from Group 3 at $\alpha<.01$

SECTION B

A COMPARISON OF THE METABOLISM OF GEOMETRICAL ISOMERS OF Δ^9-OCTADECENOIC ACID IN THREONINE IMBALANCED RATS

Methods

Thirty-six weanling Sprague-Dawley male rats were fed the basal diet described in Part I. The diet fat fed to one-half of the animals was corn oil:olive oil (1:1 w/w) and that fed to the other half was corn oil:elaidinized olive oil (1:1, w/w). The same conditions used in Part I prevailed. At the end of the fourth week, food cups were removed at 7:00 A.M. and the animals were weighed and put into plastic metabolism cages for a 2 hour adjustment period. At 9:00 A.M. each animal was given by gavage 20μC per 160 grams body weight of either oleic acid-1-C^{14} or elaidic acid-1-C^{14}.

The oleic acid-1-C^{14} was purchased from Volk Radiochemicals (Burbank, California). The specific activity was 165.0μC per mg. Both the chemical and radiopurity were greater than 99% as determined by gas chromatography, reverse phase paper chromatography, and thin layer chromatography. The elaidic acid-1-C^{14} was purchased from Applied Science Laboratories (State College, Pennsylvania). The specific activity was 181.0 μC per mg; and the radio-chemical purity, determined by thin layer chromatography, was greater than 99.5%. The isotopes were dissolved in corn oil, 1 ml

of corn oil containing 20 μC of radioactive fatty acid. Corn oil was used as the carrier since it had been shown in Section A, Part II that the intubated oil had no apparent effect on liver fat concentrations.

After intubation the animals were returned to metabolism cages which were promptly sealed with an O-ring door.[1] The cages had been previously checked for leaks with freon and none were detectable. While in the metabolism cages, the animals had access to water, but not to food. Oxygen was supplied from a tank of compressed air. The gases in the cages were continuously aspirated out and through 1) sixteen inches of tygon tubing containing drierite to absorb the exhaled water vapor, 2) two successive Swartz U-tubes filled with 1.0 M Hyamine-hydroxide (Amersham Searle, Chicago, Illinois) to trap the expired CO_2 and 3) a 100 ml gas washing bottle filled with CO_2-free 1.0 N sodium hydroxide to trap any residual carbon dioxide. The purpose of this last step was to determine the trapping efficiency of the Hyamine hydroxide, which was found to be approximately 90%. The floor of the metabolism cages was large wire mesh. Beneath this was wire screening of smaller mesh size for the collection of feces, and to the very bottom of the cage was attached a scintillation vial for the direct collection of urine.

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[1]made to specifications by Plastics Mfg., Inc., Lansing, Michigan

The animals were dosed with radioactive fatty acids according to the following chart.

DIET FAT	ISOTOPE	NO. OF ANIMALS	LENGTH OF CO_2 COLLECTION
Corn Oil: Olive Oil (1:1)	Oleic acid-1-C^{14}	6	4 hours
	Oleic acid-1-C^{14}	6	8 hours
	Elaidic acid-1-C^{14}	6	4 hours
Corn Oil: Elaidinized Olive Oil (1:1)	Elaidic acid-1-C^{14}	6	4 hours
	Elaidic acid-1-C^{14}	6	8 hours
	Oleic acid-1-C^{14}	6	4 hours

Twelve of the animals fed olive oil were dosed with oleic acid-1-C^{14}. In six of these animals, carbon dioxide was collected for 8 hours and in the other six animals it was collected for 4 hours. The remaining six animals of the olive oil fed group were dosed with elaidic acid-1-C^{14} and carbon dioxide collection was 4 hours long. The elaidinized olive oil fed group was treated similarly. Six animals were dosed with oleic acid-1-C^{14} with a 4 hour carbon dioxide collection period; twelve animals were dosed with elaidic acid-1-C^{14} with 4 and 8 hour collection periods.

At the end of the metabolic period, the animals were removed from the metabolism cages, lightly anaesthetized with ether and sacrificed by cardiac puncture. The liver, right

and left epididymal fat pads, left gastrocnemius muscle were excised, blotted and weighed. The intestines were removed and bisected at the cecum. The feces were pooled with the large intestines, and the luminal contents were washed from the small intestines with 0.9% saline. The intestines, luminal contents, fat pads and muscle were frozen to await further analyses. The blood drawn from cardiac puncture, approximately 4 to 6 mls, was allowed to clot over a 6 hour period. After retraction, the clot was loosened and spun down. Serum was removed with a capillary dropper and frozen until further analyses.

After excision, the liver was dropped into a 250 ml Virtis homogenizing flask containing 40 ml of cold chloroform:methanol (2:1, v/v). It was homogenized for one minute and the liver fat was then extracted by the addition of 150 ml of chloroform:methanol according to the method of Folch, Lees and Sloane-Stanley (49). The extract was filtered through a 150 ml sintered glass filter into a 250 ml graduated cylinder, washed with 0.02% $CaCl_2$, flushed with nitrogen and allowed to separate into two phases at 4°C. The interface was then washed three times with upper layer solvents and the extract was brought to volume with methanol. An aliquot was removed for gravimetric determination of total lipid. If the remaining extract could not at that time be analyzed, the chloroform:methanol was removed on a rotary evaporator and the liver fat was stored in petroleum ether under nitrogen at -8°C.

A 20 mg aliquot of the liver lipid extract was assayed for total radioactivity in a Nuclear-Chicago liquid scintillation counter (Unilux I). The scintillation fluor consisted of 0.1% PPO (2,5-phenyloxazole) and 0.04% POPOP (1,4-di [2,5-phenyloxazolyl] benzene) in toluene. Counts per minute were converted to disintegrations per minute by the Channel's Ratio method. The samples were counted to at least 10,000 counts to minimize the counting error to less than 1%. Each sample was counted three times.

The liver lipids were then assayed for the distribution into lipid classes. Total cholesterol and cholesterol esters were determined by the method of Huang et al. (70, 71) using tomatine to precipitate the unesterified cholesterol. Phospholipids were determined by the method of Taussky and Shorr (117). A conversion factor of 25 was used to convert lipid phosphorus to phospholipid. Triglycerides were determined by the method of Sardesai and Manning (103). The absorbance of the phosphorus was read on a Bausch and Lomb spectronic 20. That of the cholesterol and triglycerides was read on a Beckman model DB spectrophotometer. All analyses were done in duplicate.

A 60-80 mg aliquot of the liver lipid extracted 4 hours after gavage was applied to a 20 X 20 cm glass plate coated with 1.5 mm of Silica Gel G according to Stahl (Brinkman). The plate had previously been activated at 120°C. for 4 hours. The spotted plate was developed in a glass tank saturated with hexane: diethyl ether:acetic acid (73:25:2, v/v/v).

The separated lipids were visualized with 2,7-dichlorofluoroscene, and the lipid zones were scraped off the plate into 50 ml sintered glass filters. Cholesterol esters, triglycerides and free cholesterol were eluted with 25, 50 and 25 ml of chloroform respectively. Phospholipids were eluted with 50 ml of methanol. Aliquots of each were removed for scintillation counting, spectrophotometric quantitation and methylation for GLC analysis. The same scintillation fluor and conditions as used for the assay of total liver radioactivity were employed. The agreement between the determination of the liver lipid classes on the total liver lipid extract and that separated by TLC ranged from 88% to 102%. The values obtained by thin layer chromotography, corrected to 100%, were accepted as being the more accurate as each class was quantified without the interference of other classes. All but the free cholesterol were methylated according to the method of Karmen and White (75) for analysis of fatty acid composition in an F&M 400 gas liquid chromatograph (conditions as described in Part I).

The left epididymal fat pads were cut into small pieces and ground with a mortar and pestle and a small volume of chloroform:methanol (2:1, v/v). The suspension was transferred to a 100 ml Virtis homogenizing flask and the fat extracted according to the method of Folch et al. (49). An aliquot was taken for gravimetric quantification, an aliquot for scintillation counting and an aliquot for methylation for fatty acid analysis. Previously described procedures were employed.

The gastrocnemius muscle was minced with a scalpel. A 20 mg aliquot was digested in a scintillation vial overnight in 1 ml of NCS-solubilizer (Amersham-Searle, Chicago) at 65°C. and counted using previously described procedures.

The large intestines plus feces, small intestines and lumen wash were all subjected to a Folch extraction using a 250 ml Virtis homogenizing flask. The large intestines, feces and small intestines were each cut into small pieces and ground with a mortar and pestle prior to individual extraction. The lumen wash was extracted unaltered. Aliquots of each were taken for scintillation counting using previously described procedures.

The urine was digested for three days at 50°C. in 1 ml of Hyamine hydroxide. It was subsequently counted in a scintillation fluor consisting of 1% PPO, 0.05% POPOP and 5% naphthalene in 5 parts dioxane and 1 part ethylene glycol monoethyl ether.

An aliquot of the carbon dioxide trapped in Hyamine hydroxide was counted in 0.1% PPO and 0.04% POPOP in toluene. A 5 ml aliquot of the carbon dioxide trapped in sodium hydroxide was taken and treated with 5 ml of 1.0 M barium chloride. The resulting precipitate was centrifuged, slurried in ethanol and transferred to a scintillation vial using repeated ethanol washings. The slurry was then suspended in a thixotropic gel, Thixin (Baker Castor Oil Company), containing 0.3% PPO in toluene according to the procedure of White and Helf (126) and counted.

A 0.1 ml aliquot of the serum was digested overnight with 1 ml of NCS-solubilizer. It was counted in 0.1% PPO and 0.04% POPOP in toluene. Of the remaining serum, 1.5 ml from each animal was pooled with that of another animal in the same group. Serum lipoproteins were isolated from the pooled serum according to the method of Havel, Eder and Bragdon (62) in a No. 50 rotar of a Spinco model L preparative ultracentrifuge. The very low density (VLD) lipoprotein fraction ($d<1.019$) was flotated by spinning the serum for 18 hours at 126,992 X g. at 4°C. in a salt solution of 1.0307 gm per ml. The density of the serum was taken to be 1.0073 gm per ml (82) and that of the salt solution was determined by pycnometry. The top 2 ml were removed. From the remaining 8 ml, the low density (LD) lipoproteins ($1.019<d<1.063$) were isolated by spinning for an additional 18 hours at the same speed in a salt solution of 1.239 gm per ml. The top 5 ml were removed. From the remaining 5 ml, the high density (HD) lipoproteins ($1.063< d<1.21$) were isolated by spinning at the same speed for 24 hours in a salt solution of 1.357 gm per ml. The top 4 ml were removed. The remaining 6 ml were considered to be the very high density (VHD) lipoproteins in combination with albumin fraction.

An aliquot of each lipoprotein fraction was digested in 1 ml of NCS-solubilizer for subsequent scintillation counting in 0.1% PPO and 0.04% POPOP in toluene. The lipoprotein fractions were quantified by determining the amount

of protein in each fraction according to the method of Lowry et al. (83). Crystalline bovine albumin (Armour and Company, Chicago) was used as the standard. The LD, VLD and HD fractions were read at 750 mμ on a Beckman model DB spectrophotometer. The residual very high density fraction (d>1.21) was read at 500 mμ on the same spectrophotometer. All analyses were done in duplicate.

Results and Discussion

1. Absorption of isotope

Within each diet group, a smaller percentage of the fatty acid dose was present in the lumen wash after 4 hours when elaidic acid was given compared with when oleic acid was administered (Table 6). For example, in olive oil fed animals (Groups 1 and 2) 24.0% of the 1-C^{14}-oleic acid was still in the lumen, but 21.3% of the 1-C^{14}-elaidic acid was present. A similar trend was observed in animals fed elaidinized olive oil (compare Groups 3 and 4). On the other hand, the higher concentration of C^{14}-elaidic acid in the intestinal wall in Group 2 vs. Group 1 (11.3% vs. 6.4%) and in Group 4 vs. Group 3 (8.1% vs. 6.0%) reflect a delay in the absorption of elaidic acid into the body cavity, regardless of diet fat. In spite of the slightly faster passage of C^{14}-elaidic acid from the lumen into the intestinal wall and the somewhat slower exit of C^{14}-elaidic acid from the intestinal wall into the body proper, the overall trend was towards a comparable net absorption of both fatty acids at

the end of 4 hours in groups fed the same diet fat (69.6% vs. 67.4% and 73.2% vs. 75.2% in Groups 1 vs. 2 and Groups 3 vs. 4).

Although the intubation of isomeric forms of octadecenoic acid did not influence net absorption, prolonged feeding of these fatty acids did affect net absorption. The elaidinization of olive oil resulted in an apparently greater net absorption 4 hours after intubation of both oleic and elaidic acids (Groups 3 and 4 vs. 1 and 2).

Between 4 and 8 hours there was a more rapid exit of oleic acid from the lumen to the intestinal wall in olive oil fed animals (24.0% to 3.5%) than of elaidic acid in elaidinized olive oil fed animals (16.7% to 12.0%). Likewise, this same effect was seen in the net absorption of the C^{14} fatty acids (69.6% to 93.8% for oleic acid in olive oil fed animals vs. 75.2% to 82.5% for elaidic acid in elaidinized olive oil fed animals, Table 6).

These trends towards slightly differing absorption rates seen 4 and 8 hours after intubation cannot be indicative of varying hydrolysis rates as the radioactive isomers were given in the free fatty acid state. They may, however, reflect differing rates of esterification within the intestinal wall. Coots (34) reported a tendency for elaidic acid to be preferentially incorporated into the phospholipid fraction of the intestinal lymph. As chylomicrons are less than 10% phospholipid (94), rapid saturation of available esterification sites might explain the slower passage of

elaidic acid from the intestinal wall into the body seen at 4 and 8 hours, and the subsequent back-up of this fatty acid within the intestinal lumen, seen at 8 hours.

The tendency for a slower passage of elaidic acid from the small intestinal wall into the body, though present, is small enough to doubt that it plays a significant role in the ability of elaidinized olive oil to limit liver accumulation.

2. Excretion of isotope

The amount of radioactivity in the combined large intestines and feces at the end of 4 hours is the same for both C^{14}-oleic acid and C^{14}-elaidic acid dosed animals, regardless of the diet fat (Table 7). Eight hours after intubation more C^{14}-oleic acid than C^{14}-elaidic acid, but not a statistically significant amount, was found in the large intestines and feces (4.3% of the C^{14}-oleic acid found in the lower GI tract of the olive oil fed animals compared to 2.4% of the C^{14}-elaidic acid in the elaidinized olive oil fed animals). This is not surprising as the corn oil carrier contained 28% oleic acid, which would greatly increase the amount of this fatty acid to be absorbed. The quantity of unabsorbed C^{14}-oleic acid (4.3%) is also in good agreement with the overall digestibility of a corn oil:olive oil mixture (1:1) fed to animals at a dietary level of 30% (see Table 2, page 44), if the additional oleic acid from the corn oil carrier that is undoubtedly present in the

large intestines and feces is considered.

The figure of 2.4% of the C^{14}-elaidic acid present in the large intestines and feces at the end of 8 hours (Table 7) is not in agreement with the overall digestibility of this fatty acid (see Table 2, page 44). However, in comparing the amount of C^{14}-elaidic acid given by gavage (20μC at a specific activity of 181 μC per mg per 160 grams body weight) with the amount of C^{12}-elaidic acid normally ingested, this figure becomes plausible, since digestibility is apparently dependent upon the size of the dose (34, 86). When Mattson (86) fed rats 2-stearoyl diolein as the sole source of dietary fat, the coefficient of digestibility was only 83%. However, when Coots (34) fed trace amounts of C^{14}-stearic acid that had been randomly rearranged in soybean oil, the digestibility was greater than 97%.

Quite evident in Table 7 is the ability of all animals, regardless of diet fat, to oxidize more C^{14}-oleic acid to carbon dioxide. Although these values are not statistically significant, there is a strong trend which persists throughout the entire 8 hour metabolic period. Since absorption rates have a strong bearing on oxidation rates, a more slowly absorbed fatty acid would be oxidized more slowly because of a longer transport time to tissues. And the absorption of a fatty acid as a constituent of chylomicron phospholipid instead of triglyceride would also greatly retard its oxidation rate as phospholipids turn over much more slowly than triglycerides. Therefore the enhanced phospholipid incorporation

in the lymph and the slower absorption rate between 4 and 8 hours might explain the depressed oxidation rate of the elaidic acid. However, the possibility persists that the different geometries of these two compounds may be directly responsible for differences in oxidation rates.

Anderson (3) reported a study of the oxidation of geometric isomers of Δ^9-octadecenoic acid by isolated rat liver mitochondria. The oxidation of uniformly labeled C^{14}-oleic acid exceeded that of uniformly C^{14}-labeled elaidic acid at all substrate concentrations (4.10^{-5} to 40.10^{-5} M) and at all incubation times (5 to 160 minutes). The oxidation of 1-C^{14}- and 10-C^{14}-oleic acid occurred at essentially the same rate, but the 10-C^{14}-elaidic acid was oxidized more slowly than the 1-C^{14}-elaidic acid. Hence, the total oxidation of the trans isomer was slower than the cis isomer because of the slower oxidation of the alkyl chain on the methyl side of the trans bond. Struijk and Beerthuis (116) compared the rates of isomerization of Δ^3cis and Δ^3trans-dodecenoyl CoA by a beef liver isomerase, as it had earlier been shown that Δ^9 cis oxidation involved conversion of the Δ^3cis intermediate to a Δ^2trans acid (115). Struijk and Beerthuis showed that the Δ^3trans intermediate was isomerized more slowly than the Δ^3cis isomer. These differences in isomerization rates may account for the differences in mitochondrial oxidation rates of oleic and elaidic acid observed by Anderson.

In a subsequent paper, Anderson and Coots (4) reported the results of a study of the oxidation rates of uniformly labeled oleic and elaidic acid in intact rats. The cis isomer was oxidized to a greater extent than the trans isomer (70% vs. 65%) 51 hours after ingestion of the isotopes, and this effect was independent of the level of the isotope over a wide range.

As a consequence of these studies, it becomes necessary to postulate three possible factors in explaining the differing rates of oxidation of elaidic and oleic acid to carbon dioxide in threonine imbalanced rats: 1) an increased Δ^3 trans to Δ^2 trans isomerase time when elaidic acid is the substrate; 2) an increased incorporation of elaidic acid into the more slowly turning over lymph phospholipids; and 3) the reduced absorption rate of elaidic acid after 4 hours.

Concomitant with increased oxidation of oleic acid compared with elaidic acid is an increased excretion of this fatty acid and/or its metabolites in the urine (Table 7). This result is not only significant at $\alpha<.01$, but also it is consistent throughout the entire 8 hour metabolic period, regardless of the diet fat. The same mechanisms responsible for the greater oxidation of oleic acid may also be operative in the increased excretion via the urine.

From the carbon dioxide and urine excretion data, it can be concluded that elaidic acid does not reduce liver lipids by increased excretion via either of these routes.

It might be surmised, on the other hand, that the drop in digestibility as a result of elaidinization as seen in Part I (corn oil: olive oil fed animals, 90.0%; corn oil: elaidinized olive oil fed animals, 84.2%, Table 2) might explain the elaidic acid effect: less fat entering the body, therefore less fat being deposited in the liver. However, much evidence contradicts this theory. Threonine imbalanced fatty livers have been produced with 5%, 20% and 30% fat in the diet (11, 58, 89). The loss of 5.7% of the diet fat is more likely related to the slightly lowered food efficiency ratio of the elaidinized olive oil fed group (see Part I) and not the decreased liver fat.

3. Deposition of C^{14}-fatty acids within tissues

Four hours after gavaging, there is a significantly greater deposition of C^{14}-elaidic acid than C^{14}-oleic acid ($\alpha<.01$ in olive oil fed animals and $\alpha<.05$ in elaidinized olive oil fed animals, Tables 8 and 9) in the liver and serum of the animals dosed this labeled fatty acid, regardless of diet fat. Moreover, there is a general tendency towards a greater accumulation of elaidic acid in both of the epididymal fat pads and the gastrocnemius muscle, (Tables 8 and 9). Though the differences in the fat pads and muscle C^{14}-elaidic acid vs. C^{14}-oleic acid concentrations are not statistically significant, they become substantially larger if the entire muscle and adipose tissue

is considered.[1] The greater deposition of C^{14}-elaidic acid may reflect its initially more rapid absorption and slower excretion via carbon dioxide and urine, as previously discussed, for after 8 hours all differences in deposition of these two fatty acids disappear; and/or it may reflect a more rapid tissue uptake of the trans isomer. In the case of the epididymal fat pads, the tendency toward greater deposition of C^{14}-elaidic acid may indicate a direct uptake of chylomicrons without prior passage through the liver. In support of this theory are the data in Table 10, which reflect the tendency toward greater deposition of C^{14}-elaidic acid than of C^{14}-oleic acid in the fat pads on a DPM per mg fat basis. This is suggestive of a preferential uptake of elaidic acid over other fatty acids. The fatty acid composition of the left epididymal fat pad[2] (Table 11) at the end of 4 hours further shows this enhanced uptake. There is significantly more octadecenoic[3] acid in the fat pads of the elaidinized olive oil fed animals; and considering the radioactivity data, a considerable amount of this C18:1 fatty acid must be the trans isomer.

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[1] If the epididymal fat is taken to be 10.5% of total adipose (personal communication, R. Schemmel, Michigan State University), then the fat pad differences become significant.

[2] Only the left epididymal fat pad was analyzed for fatty acid composition.

[3] Octadecenoic acid is used throughout this paper to denote both cis and trans C18:1.

The temporal distribution of radioactivity (Figure 2) shows a significantly greater efficiency in the elaidinized olive oil fed animals to clear the serum of C^{14}-elaidic acid, as indicated by the slope of the line connecting the 4 and 8 hour radioactivity concentrations. In the olive oil fed animals, on the other hand, the serum radioactivity from C^{14}-oleic acid increases very slightly over the 4 hour period, suggesting no net clearance. Extra hepatic tissues (i.e. adipose tissue and muscle) must account for the majority of the C^{14}-elaidic acid cleared from the serum of the elaidinized olive oil fed animals, as the liver of these animals decreases in radioactivity over the 4 hour metabolic period.

The decrease in liver radioactivity of the elaidinized olive oil fed animals indicates the ability of these animals to transport fat out of the liver between 4 and 8 hours at a rate faster than liver uptake of fat. Also, the decrease in the percent of octadecenoic acid in the livers of the elaidinized olive oil fed animals between 4 and 8 hours (Table 8) again suggests the transport of elaidic acid out of the liver. The increase in radioactivity between 4 and 8 hours in the fat pads and muscle of the elaidinized olive oil fed animals most likely reflects clearance of serum lipoproteins synthesized in the livers of these animals (Tables 8 and 9). On the other hand, the livers of the olive oil fed animals are increasing in C^{14}-oleic acid content between 4 and 8 hours after gavaging. Obviously these animals must be transporting fat out of the liver as lipoproteins at a

slower rate than liver lipid uptake.

The temporal distribution of C^{14}-elaidic acid in the elaidinized olive oil fed animals, along with the high initial uptake of elaidic acid by the epididymal fat pads and the decrease in liver octadecenoic acid between 4 and 8 hours suggests that elaidic acid might reduce liver lipids by enhancing transport out of the liver and by by-passing the liver for direct uptake as chylomicrons by the adipose tissue.

4. Composition of liver lipids

Paralleling the temporal liver distribution of C^{14}-oleic acid and C^{14}-elaidic acid in the olive oil and elaidinized olive oil fed animals is the change in liver lipid composition with time (Table 12). As the livers of the olive oil fed animals take up a significant quantity of C^{14}-oleic acid between 4 and 8 hours, there is a concomitant increase in the percent of the liver lipids that is triglyceride. This is to be expected as the C^{14}-oleic acid would be taken up predominantly as chylomicron triglycerides. As the percent triglyceride increases in the livers of the olive oil fed animals, there is a significant reduction in the percent of phospholipid present. This reduction remains when the data in Table 12 is expressed on a mg fat per gm liver basis (Table 13). In the elaidinized olive oil fed animals, however, there is no net change in either the triglyceride or phospholipid levels on a percent or mg per gram liver basis.

Instead of representing a halt or slowdown in liver lipid metabolism, these values are probably indicative of transport of triglyceride out of the liver at a rate commensurate with or greater than influx, as evidenced by the radioactivity that is starting to decline over the 4 hour interim and the fall in the total liver cholesterol content during this same time interval.

The specific activity of the liver lipid classes 4 hours after intubation is given in Table 14 in DPM/mg lipid. C^{14}-oleic acid has been deposited primarily in the triglyceride and cholesterol ester fractions, regardless of the diet fat. On the other hand, C^{14}-elaidic acid has been predominantly incorporated into the phospholipid fraction, again irrespective of the diet fat. These differences are significant at $\alpha<.01$.

Although the diet fat consumed prior to gavaging did not influence the deposition of radioactive elaidic and oleic acids in the liver phospholipid fraction (Table 14), it did have a marked effect on the percent fatty acid composition of this liver lipid class (Table 15). There is a significantly greater amount of octadecenoic acid in the liver phospholipids of the elaidinized olive oil fed animals compared to that found in the phospholipids of the olive oil fed animals (28.47% vs. 11.86%, respectively). Considering that greater than 50% of the liver radioactivity from elaidic acid was found in the phospholipid fraction (on a DPM/mg lipid class basis, Table 14), it is reasonable to

assume that a considerable quantity of the phospholipid octadecenoic acid is in the trans configuration in animals pre-fed this fatty acid. In support of this assumption is the work of W.E.M. Lands (78) who showed extensive in vitro incorporation of elaidic acid but not oleic acid into the 1-position of the phospholipid molecule, and a small, but random incorporation of both fatty acids into the 2-position. In addition, he reported the incorporation of elaidic acid into the phospholipid molecule to be equal to that of stearic. Contrasting this result is the preferential incorporation of octadecenoic acid, much of which is presumably elaidic acid in place of stearic in the phospholipids of animals pre-fed elaidinized olive oil (Table 15). This may represent the very large ratio of elaidic acid to stearic acid in the diet fat of these animals and/or the consequence of altered lipid metabolism induced by the threonine imbalance.

Although elaidinization had a marked effect on the percent of octadecenoic and octadecanoic acid in the liver phospholipids, it had very little influence on the fatty acid composition of the liver triglyceride or cholesterol ester fractions (Table 16 and Table 17, respectively). In the cholesterol ester fraction, the only difference induced by elaidinization was an increase in palmitoleic acid in the elaidinized olive oil fed animals. This increase in C16:1 must be the result of increased synthetic activity, as palmitoleic acid was devoid from the diets of either group, and as the removal of a two-carbon fragment to form palmitoleic

acid from oleic or elaidic acid is not a major pathway (125).

A reduction in the quantity of arachidonic acid is the only result of elaidinization seen in the triglyceride fraction. Again, this is indicative of decreased synthetic activity, as arachidonic acid was not supplied in the diet. The significance of either of the differences in the cholesterol ester and triglyceride fractions is not known as both of the fatty acids in question are minor constituents.

5. Transport of C^{14}-Fatty Acids

From the changes in tissue radioactivity with time (Figure 2), the changes in liver lipid classes with time (Tables 12 and 13) and the changes in the total liver fatty acid composition with time (Table 18), it appears thus far that the effect of elaidic acid might be the facilitation of fat transport out of the liver. This theory is largely borne out by the lipoprotein data seen in Tables 19, 20, 21 and 22. Not only is there a significantly greater quantity of protein in the 4 hour serum LDL fraction ($1.019<d<1.063$) of the elaidinized olive oil fed animals (Table 21), but also this fraction contains significantly more radioactive elaidate in animals dosed this fatty acid (Table 19). However, the specific activity (DPM/mg protein) of this fraction is not significantly greater than that of the olive oil fed animals (Table 23), due to the increase in both protein and radioactivity. Concomitant with the increased LDL protein and radioactive elaidate is an increase in HDL

C^{14}-elaidate content in Groups 2 and 4 after 4 hours as compared to the HDL C^{14}-oleate content in Groups 1 and 3 in the same time period (see Table 20). This probably reflects the extensive incorporation of the C^{14}-elaidic acid into the liver phospholipids, as the HDL fraction contains greater than 26% phospholipid (94). Noteworthy are the trends towards an increased incorporation of C^{14}-elaidic acid compared to C^{14}-oleic acid into the VLDL fractions, although these differences are not statistically significant (Table 19). From Tables 19 and 21, it appears that elaidic acid is not only preferentially incorporated into the LDL fraction, but also can enhance the synthesis of this fraction when pre-fed to threonine imbalanced animals for four weeks. The foremost question is how is elaidic acid able to enhance lipoprotein synthesis as compared to oleic acid?

Very little is actually known of the relationship of the physical-chemical nature of the lipids present in hepatic tissues and the synthesis of the resulting lipoproteins. Whatever the coupling mechanism(s), it is imminently clear that both the lipid and protein moieties must be simultaneously present for synthesis to occur. Alteration in the protein synthesis precludes lipoprotein formation. This is a result of studies with compounds such as puromycin and orotic acid which block protein synthesis, and prevent the formation of lipoproteins, while having no influence on the rapidly accumulating liver lipids (74, 129). Additional evidence was obtained from studies of the hereditary

lipoproteinemias, such as abetalipoproteinemia (51). In this disorder, there is no synthesis of the B protein which comprises the protein moiety of the low density lipoproteins. The result is not only an impairment of the synthesis of low density lipoproteins, but also the appearance of abnormal chylomicrons and pre-beta lipoproteins. These two very low density fractions contain the B protein as well as other protein fractions, thus suggesting the importance of protein in determining lipoprotein structure. Holman (68) has discussed the importance of the fatty acid composition of the glycerides, phospholipids and cholesterol esters and on the proportions of these lipid classes that comprise the lipoprotein molecules in determining the ultimate structure of the lipoproteins. Evidence for this theory arises from the studies of nutritional fatty livers in which the dietary fat plays a major role.

The following diagram (Figure 1) depicts the assemblage and secretion of hepatic lipoproteins. From this model four specific areas (circled) can be postulated in which elaidic acid could affect lipoprotein synthesis: 1) esterification to cholesterol and subsequent enhanced incorporation into lipoproteins with possible influence on the other lipid classes being incorporated; 2) the same effect mediated through esterification into triglyceride; 3) facilitation of the micellerization of accumulated triglyceride globules that is necessary for their incorporation into lipoproteins or 4) esterification into phospholipid with subsequent

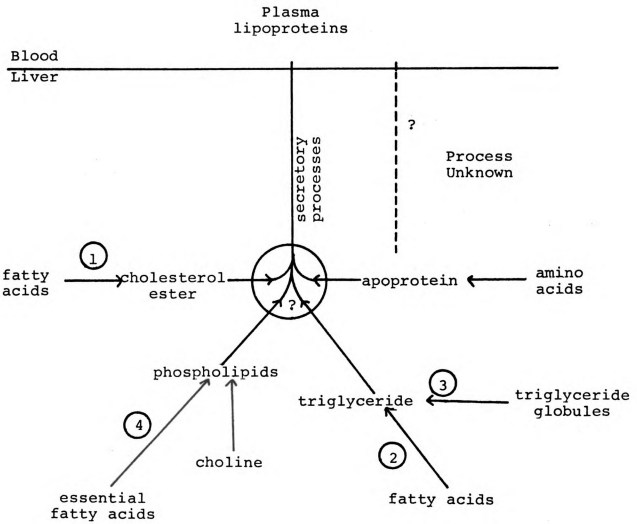


Figure 1. Lipoprotein synthesis and release. (Adapted from Harper, H. A., 1969. Review of Physiological Chemistry; Lange Medical Publishers, Los Altos, California, 303.)

enhanced incorporation into lipoproteins with possible influence on the other lipid classes being incorporated.

Although the liver C^{14}-elaidate was not concentrated within the cholesterol ester and triglyceride fractions, there is insufficient evidence to eliminate these classes as means by which elaidic acid can influence lipoprotein synthesis (Routes 1 and 2, Figure 1). If the size of the triglyceride class is taken into consideration, its role as a possible mediator of liproprotein synthesis increases. Four hours after gavaging, 62.2% of the liver lipids of the elaidinized olive oil fed animals is triglyceride; whereas the phospholipids comprise only 33.8% (Table 12). Hence, there is more radioactivity from C^{14}-elaidic acid in the triglycerides than in the phospholipids; Table 14 expresses the distribution of label on a DPM/mg lipid basis, which indicates concentration and not the percent of the total liver label.

How the presence of elaidic acid in liver triglycerides could enhance lipoprotein synthesis is a question open only to speculation since the synthetic mechanisms, let alone structure, of the lipoproteins are not yet known. Dietary triglyceride and subsequently liver triglyceride are strong stimulators of liver β-lipoprotein synthesis. Trams et al. (119) fed rats sesame oil or mineral oil and found that both oils markedly stimulated VLDL synthesis. The unresolved question is does all liver triglyceride enhance β-lipoprotein synthesis equally or are certain triglycerides more

stimulatory than others? If route 2 (Figure 1) is the pathway used by elaidic acid to enhance lipoprotein synthesis, then the answer to the above question would be that the lipoprotein stimulatory effect of liver triglycerides varies with the triglyceride; and the stimulatory effect of dietary elaidic acid would be due to the presence of the trans bonds in the liver triglycerides.

The liver triglyceride fraction consists of two pools, a rapidly turning over one in the microsome fraction and one which turns over more slowly in the soluble portion of the cytoplasm. Gross et al. (52) reported that the metabolically active pool in the microsome fraction turned over as a unit with greater than 60% of it being secreted into the plasma as Sf>20 triglycerides. The rest of the microsomal fraction was hydrolyzed. The turnover rate of the cytoplasmic portion is dependent upon its rate of entry into the microsomal fraction. Accumulated liver fat, as in threonine imbalance, consists of cytoplasmic globules of lipid. For the liver fat to be reduced, the globules must be micellerized, moved into the microsomes and then out of the liver as part of the β-lipoprotein structure. The extent of packing and the cohesiveness of triglyceride films is affected by the nature of the non-polar chains on the glycerol stem. Triglycerides containing trans unsaturated fatty acids will pack more closely together and form solid or semi-solid films, depending upon the other fatty acid constituents. On the other hand, the crimping introduced by

a cis configuration as in oleic acid hinders close packing (10). Hence the physical properties of the liver triglyceride may have an effect on globularization or micellerization. Perhaps the triglycerides containing the trans bonds are preferentially micellerized and deposited in the microsomal fraction, and in this manner stimulate lipoprotein synthesis via route 3.

Although the use of routes 1, 2 and 3 as a means by which elaidic acid can enhance lipoprotein synthesis cannot be discredited, the heavy concentration of C^{14}-elaidic acid as compared to C^{14}-oleic acid in the liver phospholipid fraction (Table 14) suggests route 4 might be the major pathway employed. Phospholipid is a key component of the serum lipoproteins, because its hydrophilic properties are necessary for the solubilization of the more hydrophobic lipids within the lipoprotein complex. Considering the effect of choline deficiency, the role of the phospholipids in the formation of the serum lipoproteins cannot be underestimated.

As the structure and synthesis of the serum lipoproteins and the relationship of phospholipid to this process are not yet understood, the mechanism by which preferential incorporation of elaidic acid into liver phospholipids could enhance lipoprotein synthesis cannot be deciphered. However, several theories can be postulated. One, as the triglyceride concentration in the livers of olive oil fed animals is increasing the phospholipid is proportionately

decreasing (Table 13), whereas the concentration of these two lipid classes appears to have plateaued in the elaidinized olive oil fed animals. This plateau of phospholipid concentration may be indicative of a faster lecithin turnover rate analogous to the theory proposed by Boxer and Stetten, see page 11. Secondly, as in the case of the triglycerides, the planar trans molecule may affect greater cohesion between the protein and non-polar lipid moieties of the lipoprotein molecules, resulting in a more closely packed and possibly more stable molecule. Finally, phospholipid is required for the micellerization of globular triglyceride; and the elaidic acid containing phospholipids may be more effective than those of the olive oil fed animals in this process.

Whether it is the strong incorporation of the trans bonds into the liver phospholipids or their presence in the liver triglycerides or whether it is an increased movement of the triglycerides from the cytoplasmic pool to the microsomal pool or whether it is a combination of these three, elaidinization did cause a significant increase in the synthesis of the LD serum lipoproteins, as evidenced by the increased protein content of this lipoprotein fraction. Important, however, in the reduction of the liver lipids is not only the synthesis of the low-density lipoproteins, but also the turnover rate of these lipoproteins once they have been released into the serum, or in other words, the extrahepatic tissues' ability to clear the circulating lipoproteins

of their lipid content.

Gross et al. (52) reported that the fractional turnover rate of the plasma $S_f>20$ triglycerides was two to three times that of liver triglycerides in normal dogs. Except in certain leucodystrophies, there is an upper limit to the amount of β-lipoprotein circulating at any one time. Hence, the faster the turnover rate, the greater is the animal's ability to remove liver triglyceride over a period of time. Figure 3 shows the concentration of the four different lipoprotein fractions in the serum with respect to time. In all fractions, except the VHD (d>1.21), the slope of the line indicating the decrease of radioactive fatty acid per mg of lipoprotein protein with time is greater in the elaidinized olive oil fed animals. This indicates that more of the serum lipoproteins are being cleared of their fat content per unit time in the animals fed the trans bonds. Hence, elaidinization of dietary fat not only incites lipoprotein synthesis, but also results in lipoproteins that are more rapidly hydrolyzed by the extrahepatic tissues in threonine imbalanced animals.

Table 6. Radioactivity of small intestines from male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive[1] fatty acid	Hours after gavaging	Lumen wash % dose	Small intestines % dose	% dose absorbed[2]
1	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	4	24.0 ± 2.4[3]	6.4 ± 0.7	69.6
2	Corn Oil:Olive Oil (1:1)	1-C^{14}-elaidic acid	4	21.3 ± 5.2	11.3 ± 0.7	67.4
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-oleic acid	4	20.8 ± 1.5	6.0 ± 1.2	73.2
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	4	16.7 ± 1.2	8.1 ± 0.9	75.2
5	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	8	3.5 ± 1.6	3.7 ± 0.4	93.8
6	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	8	12.0 ± 3.6	5.5 ± 0.4	82.5

[1] 20 μC per animal
[2] % absorbed dose = 100 -(small intestines + lumen wash)
[3] Mean ± SEM of 6 animals

Table 7. Radioactivity of large intestines, urine and excreted carbon dioxide from male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive fatty acid[1]	Hours after gavaging	large intestines	urine	carbon dioxide
				% dose	% dose	% dose
1	Corn Oil:Olive Oil: (1:1)	1-C^{14}oleic acid	4	1.0 ± 0.5[2]	0.47 ± .07	5.1 ± 1.7
2	Corn Oil:Olive Oil (1:1)	1-C^{14}elaidic acid	4	1.1 ± 0.7	.054 ± .02[3]	2.2 ± 0.7
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}oleic acid	4	1.5 ± 0.5	.74 ± .10[4]	4.2 ± 1.3
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}elaidic acid	4	1.2 ± 0.6	.056 ± .008	2.0 ± 0.7
5	Corn Oil:Olive Oil (1:1)	1-C^{14}oleic acid	8	4.3 ± 0.6	.64 ± .10	9.2 ± 2.7
6	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}elaidic acid	8	2.4 ± 0.7	.021 ± .002[5]	6.4 ± 2.2

1 20 μC per animal
2 Mean ± SEM of 6 animals
3 Significantly different from Group 1 at $\alpha<.01$
4 Significantly different from Group 4 at $\alpha<.01$
5 Significantly different from Group 5 at $\alpha<.01$

Table 8. Radioactivity of gastrocnemius muscle and liver from male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive fatty acid[1]	Hours after gavaging	Gastrocnemius muscle	Liver
				DPM/mg wet weight	DPM/mg wet weight
1	Corn Oil: Olive Oil (1:1)	1-C^{14}-oleic acid	4	21 ± 2[2]	191 ± 30
2	Corn Oil:Olive Oil (1:1)	1-C^{14}-elaidic acid	4	23 ± 3	334 ± 38[3]
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-oleic acid	4	18 ± 2[4]	209 ± 28
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	4	24 ± .4	351 ± 7
5	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	8	33 ± 7	292 ± 17[3]
6	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	8	34 ± 4[4]	323 ± 38

[1] 20 μC per animal
[2] Mean ± SEM of 6 animals
[3] Significantly different from Group 1 at $\alpha<.01$
[4] Significantly different from Group 4 at $\alpha<.05$

Table 9. Radioactivity of right and left epididymal fat pads and serum from male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive fatty acid[1]	Hours after gavaging	Left EFP[2]	Right EFP[2]	Serum
				DPM/mg wet weight		DPM/ .1 ml
1	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	4	123 ± 21[3]	111 ± 20	5698 ± 184
2	Corn Oil:Olive Oil (1:1)	1-C^{14}-elaidic acid	4	174 ± 31	160 ± 27	9024 ± 825[4]
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-oleic acid	4	133 ± 23	122 ± 21	5570 ± 423[5]
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	4	207 ± 28	195 ± 23	8564 ± 374
5	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	8	273 ± 28	275 ± 30	6072 ± 1086
6	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	8	231 ± 16	219 ± 5	6684 ± 469[6]

[1] 20 μC per animal
[2] EFP = epididymal fat pad
[3] Mean ± SEM of 6 animals
[4] Significantly different from Group 1 at $\alpha<.01$
[5] Significantly different from Group 4 at $\alpha<.01$
[6] Significantly different from Group 4 at $\alpha<.05$

Table 10. Radioactivity of fat extracted from the liver and left epididymal fat pad from male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive fatty acid[1]	Hours after gavaging	Liver	Right EFP	Left EFP
				DPM/mg fat	DPM/mg fat	DPM/mg fat
1	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	4	2702 ± 300[2]	157 ± 31	171 ± 30
2	Corn Oil:Olive Oil (1:1)	1-C^{14}-elaidic acid	4	4312 ± 640[4]	225 ± 37	241 ± 44
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-oleic acid	4	2921 ± 307[6]	163 ± 30[6]	175 ± 33
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	4	4126 ± 268	255 ± 35	271 ± 36
5	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	8	3765 ± 254[3]	389 ± 38[3]	406 ± 40[3]
6	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	8	4108 ± 421	326 ± 13	328 ± 30

[1] 20 μC per animal
[2] Mean ± SEM of 6 animals
[3] Significantly different from Group 1 at $\alpha<.01$
[4] Significantly different from Group 1 at $\alpha<.05$
[5] Significantly different from Group 4 at $\alpha<.01$
[6] Significantly different from Group 4 at $\alpha<.05$

Table 11. Fatty acid composition of left epididymal fat pad from male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number[1]	1 & 2	3 & 4	5	6
Diet Fat	Corn Oil:Olive Oil (1:1)	Corn Oil:Elaidinized Olive Oil (1:1)	Corn Oil:Olive Oil (1:1)	Corn Oil:Elaidinized Olive Oil (1:1)
Hours after gavaging	4	4	8	8
Fatty Acid	%	%	%	%
C14:0	1.56 ± 0.10[2]	1.04 ± 0.07[3]	trace	trace
C16:0	22.96 ± 1.00	13.78 ± 0.20[3]	17.44 ± 0.5	15.12 ± 0.6[6]
C16:1	4.13 ± 0.25	2.82 ± 0.10[3]	2.16 ± 0.2	2.99 ± 0.2[6]
C18:0	2.04 ± 0.08	1.07 ± 0.04[3]	2.36 ± 0.18	1.74 ± 0.07[5]
C18:1	37.81 ± 1.00	46.62 ± 0.30[3]	49.71 ± 0.4	51.21 ± 0.60
C18:2	31.84 ± 0.9	34.70 ± 0.50[4]	28.32 ± 0.6	28.96 ± 0.50

[1] Groups 1 and 2 and Groups 3 and 4 (see Table 6) combined assuming the radioactive fatty acids (a dose of approximately 0.1 mg per animal) had no effect on lipid composition
[2] Mean ± SEM of 12 animals (Groups 1 & 2 and 3 & 4) or 6 animals (Groups 5 and 6)
[3] Significantly different from Group 1 & 2 at $\alpha<.01$
[4] Significantly different from Group 1 & 2 at $\alpha<.05$
[5] Significantly different from Group 5 at $\alpha<.01$
[6] Significantly different from Group 5 at $\alpha<.05$

Table 12. Liver lipid composition of male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number[1]	Diet Fat	Hours after gavaging	Triglyceride	Total Cholesterol	Esterified Cholesterol	Phospho-lipid
			% of total fat in the liver tissue[2]			
1 & 2	Corn Oil:Olive Oil (1:1)	4	55.8 ± 2.2[3]	4.7 ± .2	1.7 ± .1	39.6 ± 2.1
3 & 4	Corn Oil: Elaidinized Olive Oil (1:1)	4	62.2 ± 2.1[6]	4.1 ± .1[6]	1.8 ± .1	33.8 ± 2.0
5	Corn Oil:Olive Oil (1:1)	8	66.4 ± 1.3[5]	4.1 ± .2	--------[4]	29.5 ± 0.5[5]
6	Corn Oil: Elaidinized Olive Oil (1:1)	8	63.7 ± 1.5	3.5 ± .1[7,8]	--------[4]	32.8 ± 1.3

[1] Groups 1 and 2 and Groups 3 and 4 (see Table 6) combined assuming the radioactive fatty acids (a dose of approximately 0.1 mg per animal) had no effect on lipid composition.
[2] Values corrected for 100%.
[3] Mean ± SEM of 12 animals (Groups 1 & 2 and 3 & 4) or 6 animals (Groups 5 and 6)
[4] Samples not analyzed for Cholesterol Ester content
[5] Significantly different from Group 1 & 2 at $\alpha<.01$
[6] Significantly different from Group 1 & 2 at $\alpha<.05$
[7] Significantly different from Group 5 at $\alpha<.05$
[8] Significantly different from Groups 3 & 4 at $\alpha<.01$

Table 13. Liver lipid composition of male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number[1]	Diet Fat	Hours after gavaging	Triglyceride	Total Cholesterol	Esterified Cholesterol	Phospholipid
			mg fat/ gm wet weight of liver tissue			
1 & 2	Corn Oil:Olive Oil (1:1)	4	43.8 ± 4.1[2]	3.5 ± 0.1	1.3 ± 0.1	29.6 ± 0.9
3 & 4	Corn Oil: Elaidinized Olive Oil (1:1)	4	50.0 ± 4.2[5]	3.2 ± 0.2	1.4 ± 0.1	26.0 ± 1.1
5	Corn Oil:Olive Oil (1:1)	8	51.1 ± 2.7[4]	3.2 ± 0.1	---------[3]	22.8 ± 0.5[4]
6	Corn Oil: Elaidinized Olive Oil (1:1)	8	50.4 ± 4.1	2.6 ± 0.2	---------[3]	25.6 ± 1.3

[1] Groups 1 and 2 and Groups 3 and 4 (see Table 6) combined assuming the radioactive fatty acids (a dose of approximately 0.1 mg per animal) had no effect on lipid composition

[2] Mean ± SEM of 12 animals (Groups 1 & 2 and 3 & 4) or 6 animals (Groups 5 and 6)

[3] Samples not analyzed for Cholesterol Ester content

[4] Significantly different from Group 1 & 2 at $\alpha<.01$

[5] Significantly different from Group 1 & 2 at $\alpha<.05$

Table 14. Specific activity of liver lipid classes of male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive fatty acid[1]	Hours after gavaging	Triglyceride	Esterified Cholesterol	Free Cholesterol	Phospho-lipid
				DPM/ mg of lipid			
1	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	4	6878 ± 1123[2]	5240 ± 610	4901 ± 1715	3109 ± 264
2	Corn Oil:Olive Oil (1:1)	1-C^{14}-elaidic acid	4	2090 ± 370[3]	1769 ± 287[3]	3813 ± 1000	10028 ± 983[3]
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-oleic acid	4	4402 ± 729	4352 ± 736[6]	2909 ± 558	2791 ± 114[5]
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	4	2984 ± 280	1976 ± 193	3214 ± 581	9152 ± 329

[1] 20 μC per animal
[2] Mean ± SEM of 6 animals
[3] Significantly different from Group 1 at $\alpha<.01$
[4] Significantly different from Group 1 at $\alpha<.05$
[5] Significantly different from Group 4 at $\alpha<.01$
[6] Significantly different from Group 4 at $\alpha<.05$

Table 15. Phospholipid fatty acid composition of livers from male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number[1]	1 & 2	3 & 4
Diet Fat	Corn Oil:Olive Oil (1:1)	Corn Oil:Elaidinized Olive Oil (1:1)
Hours after gavaging	4	4
Fatty Acid	%	%
C16:0	21.25 ± 2.28[2]	16.84 ± 1.20
C16:1	1.27 ± 0.08	1.66 ± 0.18
C18:0	28.17 ± 1.93	17.53 ± 1.36[3]
C18:1	11.86 ± 1.09	28.47 ± 1.49[3]
C18:2	12.80 ± 1.00	14.65 ± 1.25
C20:4	25.59 ± 2.21	21.15 ± 2.96

[1] Groups 1 and 2 and Groups 3 and 4 (see Table 6) combined assuming the radioactive fatty acids (a dose of approximately 0.1 mg per animal) had no effect on lipid composition

[2] Mean ± SEM of 12 animals

[3] Significantly different from Group 1 & 2 at $\alpha<.01$

Table 16. Triglyceride fatty acid composition of livers from male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number[1]	1 & 2	3 & 4
Diet Fat	Corn Oil:Olive Oil (1:1)	Corn Oil:Elaidinized Olive Oil (1:1)
Hours after gavaging	4	4
Fatty Acid	%	%
C16:0	17.43 ± 0.32[2]	16.59 ± 0.27
C16:1	1.52 ± 0.08	2.45 ± 0.13
C18:0	1.97 ± 0.06	1.49 ± 0.10
C18:1	41.15 ± 0.84	43.77 ± 0.64
C18:2	32.83 ± 0.63	43.00 ± 0.46
C18:3 or C20:1[3]	0.92 ± 0.06	1.17 ± 0.04
C20:3 or C22:1[3]	trace	trace
C20:4	4.04 ± 0.27	0.99 ± 0.07[4]

[1] Groups 1 and 2 and Groups 3 and 4 (see Table 6) combined assuming the radioactive fatty acids (a dose of approximately 0.1 mg per animal) had no effect on lipid composition

[2] Mean ± SEM of 12 animals

[3] These two fatty acids have the same retention time

[4] Significantly different from Group 1 & 2 at $\alpha<.01$

Table 17. Cholesterol ester fatty acid composition of livers from male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number[1]	1 & 2	3 & 4
Diet Fat	Corn Oil:Olive Oil (1:1)	Corn Oil:Elaidinized Olive Oil (1:1)
Hours after gavaging	4	4
Fatty Acid	%	%
C16:0	10.46 ± .97[2]	12.14 ± 1.11
C16:1	2.63 ± .30	4.64 ± .34[4]
C18:0	8.18 ± 3.05	3.73 ± .86
C18:1	40.85 ± 4.05	43.41 ± 2.79
C18:2	19.78 ± 2.03	24.01 ± 1.92
X_1[3]	10.31 ± 1.23	13.54 ± 4.0
X_2[3]	5.59 ± .95	3.98 ± .48
C20:4	12.27 ± 1.73	8.62 ± 1.62

[1] Groups 1 and 2 and Groups 3 and 4 (see Table 6) combined assuming the radioactive fatty acids (a dose of approximately 0.1 mg per animal) had no effect on lipid composition

[2] Mean ± SEM of 12 animals

[3] Unidentifiable peaks on GLC

[4] Significantly different from Group 1 & 2 at $\alpha < .01$

Table 18. Total fatty acid composition of livers from male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number[1]	Diet Fat	Hrs. after gavaging	C16:0 %	C16:1 %	C18:0 %	C18:1 %	C18:2 %	C20:4 %
1 & 2	Corn Oil: Olive Oil (1:1)	4	18.67±.89[2]	trace	10.23±.62	32.23±.91	26.85±1.15	12.03±.60
3 & 4	Corn Oil: Elaidinized Olive Oil (1:1)	4	15.91±.25[3]	2.29±.1	4.39±.28[3]	39.06±.64[3]	30.05±.43[4]	8.25±.60[3]
5	Corn Oil: Olive Oil (1:1)	8	19.71±1.73	trace	13.00±1.24	31.70±1.75	24.33±.99	10.79±.70
6	Corn Oil: Elaidinized Olive Oil (1:1)	8	17.90±.46	1.17±.08	5.67±.25[5]	35.54±1.41	30.90±.72[5]	8.83±.60

[1] Groups 1 and 2 and Groups 3 and 4 (see Table 6) combined assuming the radioactive fatty acids (a dose of approximately 0.1 mg per animal) had no effect on lipid composition
[2] Mean ± SEM of 12 animals (Groups 1 & 2 and 3 & 4) or 6 animals (Groups 5 and 6)
[3] Significantly different from Group 1 & 2 at $\alpha<.01$
[4] Significantly different from Group 1 & 2 at $\alpha<.05$
[5] Significantly different from Group 5 at $\alpha<.01$

Table 19. Total radioactivity of low density serum lipoproteins isolated from male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive fatty acid[1]	Time after gavaging	VLDL[2]	LDL[2]
				Total DPM	
1	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	4	28,596 ± 4610[3]	26,600 ± 1847
2	Corn Oil:Olive Oil (1:1)	1-C^{14}-elaidic acid	4	33,633 ± 830	46,588 ± 2217[4]
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-oleic acid	4	39,603 ± 5082	20,517 ± 1246[5]
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	4	54,423 ± 7551	38,800 ± 2673
5	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	8	7770 ± 1034[4]	9746 ± 579[4]
6	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	8	12,585 ± 4370[5]	8567 ± 1167[5]

[1] 20 μC per animal
[2] VLDL = lipoproteins of density less than 1.019
[2] LDL = lipoproteins of density from 1.019 to 1.063
[3] Mean ± SEM of 3 samples pooled from 6 animals
[4] Significantly different from Group 1 at $\alpha<.01$
[5] Significantly different from Group 4 at $\alpha<.01$

Table 20. Total radioactivity of high density serum lipoproteins isolated from male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive fatty acid[2]	Time after gavaging	HDL[2]	VHDL[2]
				Total DPM	
1	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	4	35,400 ± 2720[3]	48,832 ± 3448
2	Corn Oil:Olive Oil (1:1)	1-C^{14}-elaidic acid	4	90,417 ± 9493[4]	52,220 ± 4261
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-oleic acid	4	26,667 ± 759[5]	42,186 ± 3139
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	4	55,617 ± 7017	44,217 ± 3975
5	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	8	28,263 ± 2920	32,913 ± 6013
6	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	8	38,240 ± 1326	41,568 ± 1543

[1] 20 μC per animal
[2] HDL = lipoproteins of density from 1.063 to 1.21
VHDL = lipoproteins of density greater than 1.21
[3] Mean ± SEM of 3 samples pooled from 6 animals
[4] Significantly different from Group 1 at $\alpha<.01$
[5] Significantly different from Group 4 at $\alpha<.01$

Table 21. Protein content of low density serum lipoproteins isolated from male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number[1]	Diet Fat	Hours after gavaging	VLDL[2]	LDL[2]
			mg protein/3 ml serum	
1 & 2	Corn Oil:Olive Oil (1:1)	4	0.519 ± .071[3]	2.54 ± 0.14
3 & 4	Corn Oil: Elaidinized Olive Oil (1:1)	4	0.564 ± .095	3.30 ± 0.16[4]
5	Corn Oil:Olive Oil (1:1)	8	0.393 ± .043	1.29 ± 0.12[4]
6	Corn Oil: Elaidinized Olive Oil (1:1)	8	0.431 ± .046	1.80 ± 0.30[5]

[1] Groups 1 and 2 and Groups 3 and 4 (see Table 6) combined assuming the radioactive fatty acids (a dose of approximately 0.1 mg per animal) had no effect on lipid composition

[2] VLDL = lipoproteins of density less than 1.019
LDL = lipoproteins of density from 1.019 to 1.063

[3] Mean ± SEM of 6 samples pooled from 12 animals (Groups 1 & 2 and 3 & 4) or 3 samples pooled from 6 animals (Groups 5 and 6)

[4] Significantly different from Group 1 & 2 at $\alpha<.01$

[5] Significantly different from Group 3 & 4 at $\alpha<.01$

Table 22. Protein content of high density serum lipoproteins isolated from male rats given 1 ml of corn oil by gavage and pre-fed a threonine imbalanced diet containing two sources of diet fat.

Group Number[1]	Diet fat	Hours after gavaging	HDL[2]	VHDL[2]
			mg protein/3 ml serum	
1 & 2	Corn Oil:Olive Oil (1:1)	4	13.63 ± 0.95[3]	111.19 ± 1.9
3 & 4	Corn Oil: Elaidinized Olive Oil (1:1)	4	13.82 ± 0.55	101.36 ± 3.3
5	Corn Oil:Olive Oil (1:1)	8	13.19 ± 0.53	119.10 ± 3.3
6	Corn Oil: Elaidinized Olive Oil (1:1)	8	13.95 ± 0.45	121.90 ± 11.2

[1] Groups 1 and 2 and Groups 3 and 4 (see Table 6) combined assuming the radioactive fatty acids (a dose of approximately 0.1 mg per animal) had no effect on lipid composition

[2] HDL = lipoproteins of density from 1.063 to 1.21
VHDL = lipoproteins of density greater than 1.21

[3] Mean ± SEM of 6 samples pooled from 12 animals (Groups 1 & 2 and 3 & 4) or 3 samples pooled from 6 animals (Groups 5 and 6)

Table 23. Specific activity of low density serum lipoproteins isolated from male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive fatty acid[1]	Time after gavaging	VLDL[2]	LDL[2]
				DPM/mg protein	
1	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	4	55,110 ± 9392	9804 ± 879
2	Corn Oil:Olive Oil (1:1)	1-C^{14}-elaidic acid	4	65,653 ± 2243	17,322 ± 897
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-oleic acid	4	77,562 ± 11,832	6700 ± 128
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	4	81,927 ± 10,296	11,304 ± 314
5	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	8	23,586 ± 4231	7515 ± 841
6	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	8	25,586 ± 9083	3902 ± 262

[1] 20 μC per animal
[2] VLDL = lipoproteins of density less than 1.019
LDL = lipoproteins of density from 1.019 to 1.063
[3] Mean ± SEM of 3 samples pooled from 6 animals

Table 24. Specific activity of high density serum lipoproteins isolated from male rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced diet containing two different sources of diet fat.

Group Number	Diet Fat	Radioactive fatty acid[1]	Time after gavaging	HDL[2]	VHDL[2]
				DPM/ mg protein	
1	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	4	2789 ± 486[3]	435 ± 37
2	Corn Oil:Olive Oil (1:1)	1-C^{14}-elaidic acid	4	6045 ± 1353	492 ± 40
3	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-oleic acid	4	1815 ± 33	422 ± 40
4	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	4	4673 ± 495	472 ± 12
5	Corn Oil:Olive Oil (1:1)	1-C^{14}-oleic acid	8	2088 ± 255	272 ± 51
6	Corn Oil: Elaidinized Olive Oil (1:1)	1-C^{14}-elaidic acid	8	2733 ± 235	302 ± 24

[1] 20 μC per animal
[2] HDL = lipoproteins of density from 1.063 to 1.21
VHDL = lipoproteins of density greater than 1.21
[3] Mean ± SEM of 3 samples pooled from 6 animals

Figure 2. Temporal distribution of radioactivity in left epididymal fat pad, gastrocnemius muscle, serum and liver of rats given either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid four weeks after pre-feeding a threonine imbalanced diet containing two sources of diet fat. (O——O 1-C^{14}-oleic acid in rats pre-fed olive oil; Δ- - -Δ 1-C^{14}-elaidic acid in rats pre-fed elaidinized olive oil.)

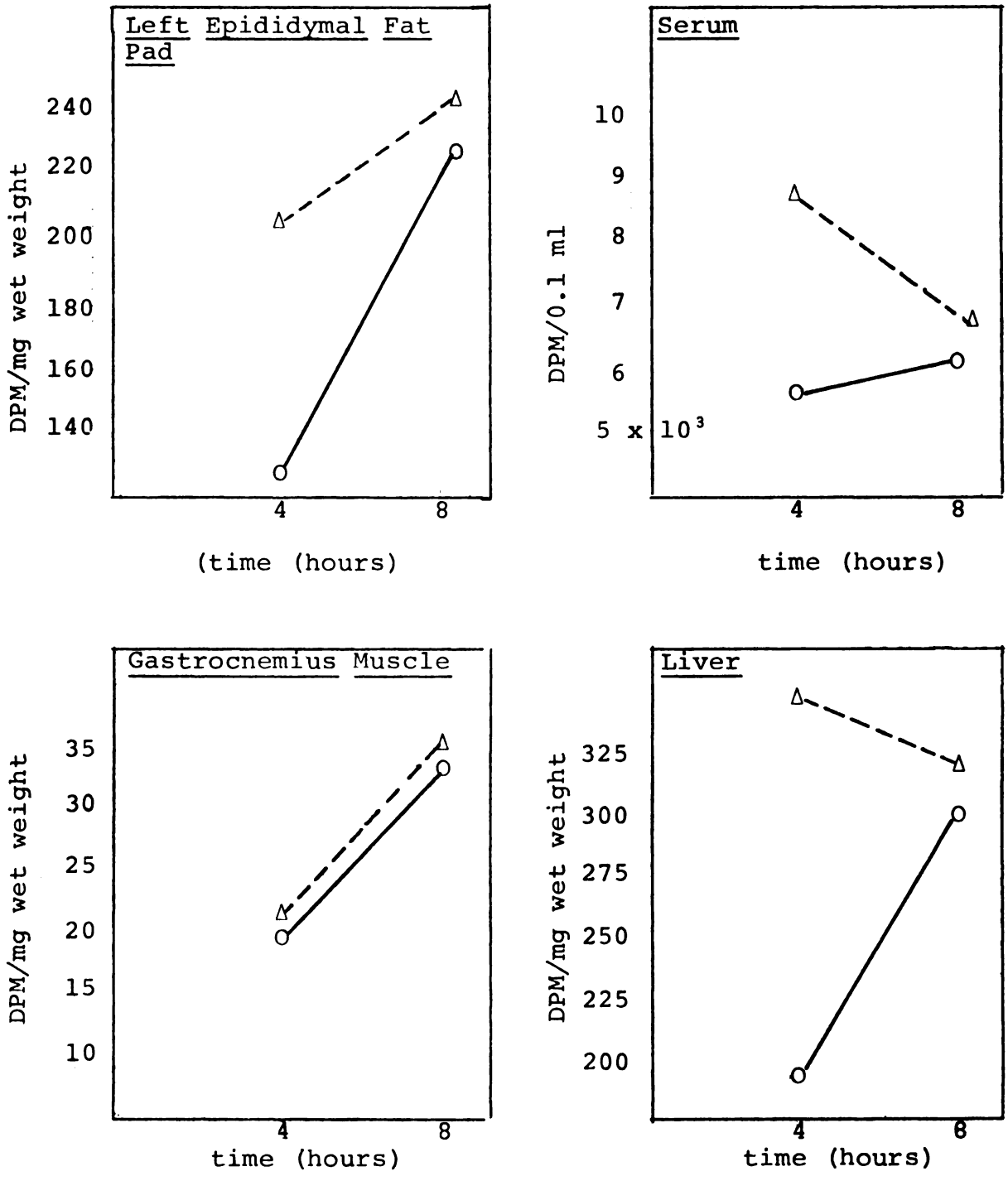
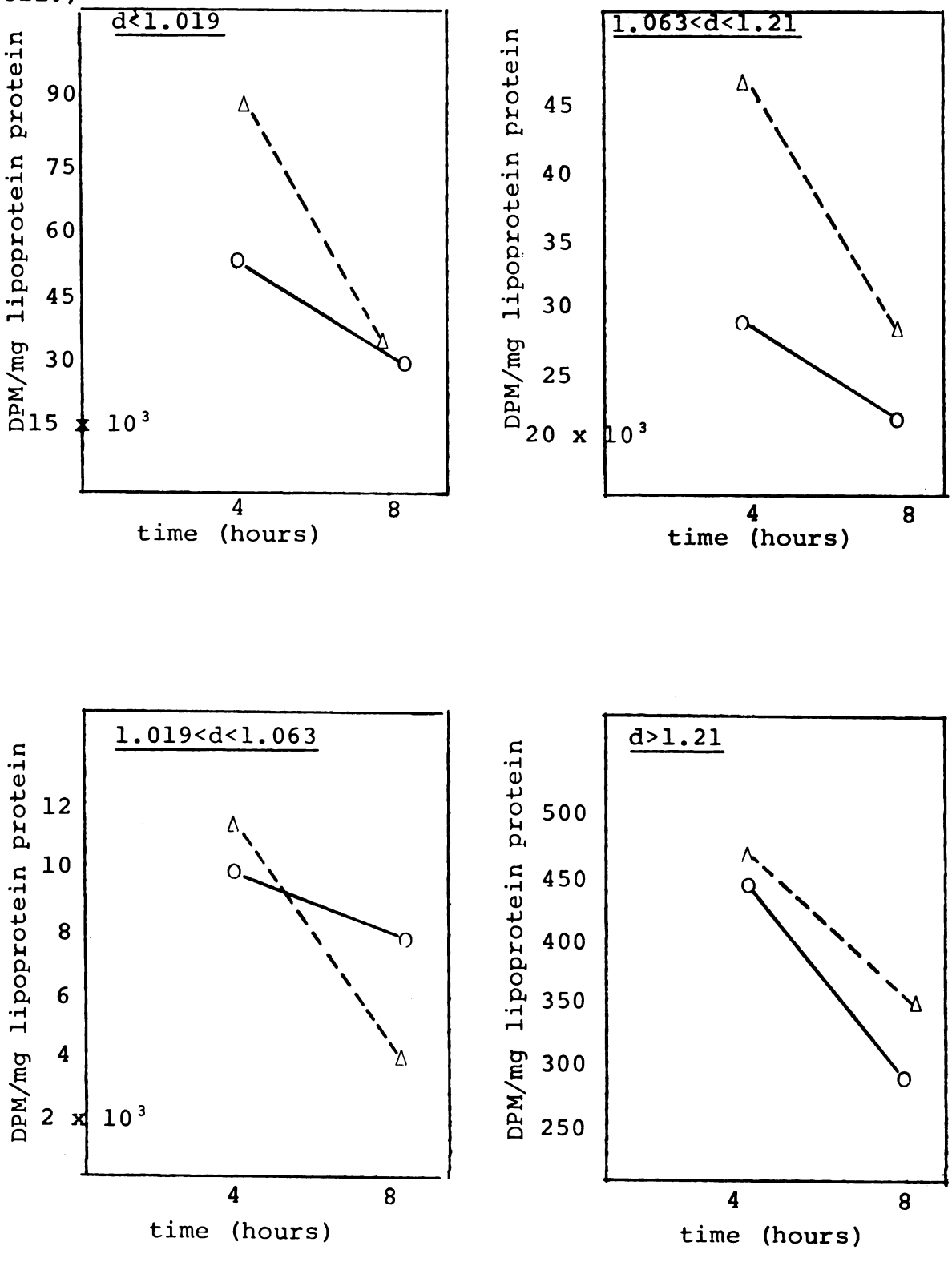


Figure 3. Temporal distribution of radioactivity in serum lipoproteins isolated from rats given either 1-C^{14}-oleic or 1-C^{14}-elaidic acid by gavage four weeks after pre-feeding a threonine imbalanced acid containing two sources of diet fat. (O——O 1-C^{14}-oleic acid in rats pre-fed olive oil, Δ– – –Δ 1-C^{14}-elaidic acid in rats pre-fed elaidinized olive oil.)



CONCLUSIONS AND SUMMARY

Weanling male Sprague-Dawley rats were fed a 9% casein, 30% fat, 0.50% choline diet that was imbalanced with respect to threonine. The diet fat was either a 1:1 mixture of corn oil and olive oil or a 1:1 mixture of corn oil and olive oil that had been 90% elaidinized. At the end of four weeks the animals were given by gavage approximately 1 ml of corn oil containing 20 μC per 165 grams of body weight of either 1-C^{14}-oleic acid or 1-C^{14}-elaidic acid. The animals were sacrificed by cardiac puncture at 4 and 8 hours after intubation. The liver, epididymal fat pads, gastrocnemius muscle, intestines, feces, urine, serum and expired carbon dioxide were analyzed for radioactivity. In addition, the fatty acid composition of the fat pads, liver lipids and liver triglyceride, phospholipid and cholesterol ester fractions was determined, as was the distribution of label within these liver lipid classes and within the serum VHD, LD, HD and VHD lipoprotein fractions.

From this study the following observations were made:

1. Elaidinization had no effect on food consumption or weight gain; however, it significantly reduced the coefficient of digestibility.

2. Elaidinization caused a significant reduction in the accumulation of fat in the liver.

3. The intubation of 1 ml of non-radioactive corn oil had no apparent effect on liver lipid content. However, the 4 and 8 hour intervals spent without food resulted in a

significant increase in liver moisture content in both the olive oil and elaidinized olive oil fed animals and a significant increase in fat pad moisture in the elaidinized olive oil fed animals.

4. Elaidinization of olive oil resulted in a significant increase in the percent of octadecenoic acid (C18:1) in the left epididymal fat pad at the expense of palmitic acid (C16:0). The starvation period and/or the intubation of corn oil resulted in an increase in fat pad octadecenoate in both the olive oil and elaidinized olive oil fed animals.

5. Elaidinization had little effect on the fatty acid composition of the liver triglyceride and cholesterol ester fractions. In the phospholipid fraction, however, it caused a significant increase in the percent of octadecenoate at the expense of octadecanoate.

6. Elaidinization resulted in a significant increase in the quantity of liver triglyceride and a significant decrease in the quantity of liver cholesterol at the end of 4 hours but not at 8. Starvation and/or the ingestion of corn oil had no effect on the composition of lipid classes in animals fed elaidinized olive oil; however, either or both caused an increase in the quantity of triglyceride present at the expense of the phospholipid content between 4 and 8 hours in the olive oil fed animals.

7. C^{14}-elaidic acid was absorbed slightly more rapidly than C^{14}-oleic acid during the first 4 hours. Just the opposite was true during the second 4 hours.

8. More C^{14}-oleic acid than C^{14}-elaidic acid was oxidized to carbon dioxide and a significantly greater quantity was excreted in the urine.

9. Four hours after intubation, there was more C^{14}-elaidic acid in the serum, liver, epididymal fat pads and muscle than C^{14}-oleic acid. After 8 hours, there were no differences in the distribution of C^{14}-elaidic acid and C^{14}-oleic acid in these tissues.

10. Between 4 and 8 hours after intubation, elaidinized olive oil fed animals were transporting C^{14}-elaidic acid out of the liver at a rate commensurate with the influx of this fatty acid. The olive oil fed animals were transporting more C^{14}-oleic acid into the liver than out, during this same time interval.

11. C^{14}-elaidic acid was preferentially incorporated into the liver phospholipid fraction. C^{14}-oleic acid was incorporated predominantly into the triglyceride and cholesterol ester fractions.

12. Elaidinization resulted in a significantly greater amount of serum LDL at 4 hours but not at 8. There was also a significantly greater incorporation of C^{14}-elaidate into the LDL and HDL fractions.

13. Elaidinized olive oil fed animals hydrolyzed more of the circulating lipoprotein lipid per unit time.

14. The metabolism of either C^{14}-oleic acid or C^{14}-elaidic acid was not significantly influenced by dietary fat.

A limited accumulation of liver lipid was observed in threonine imbalanced rats fed elaidinized olive oil as a result of an initial preferential uptake of the trans isomer in adipose tissue, an increase in liver lipoprotein synthesis and/or release, and a faster rate of hydrolysis of circulating lipoprotein lipid. The enhancement of lipoprotein synthesis appeared to be mediated by a preferential incorporation of the trans isomer into the liver phospholipids. The mode of enhancement was postulated to be via 1) the physical characteristics of the liver lipid micelles containing the trans isomer, 2) an acceleration of triglyceride micellerization or 3) a direct influence on lipoprotein synthesis.

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