Studies on the Hepatic Accumulation of Triglyceride
Induced by Chronic Ethanol Administration in Rats Fed
with Various Diets*

Moo Youn Cho**, Beon Sook Choi and Yoon Soo Kim

Department of Biochemistry, Yonsei University College of Medicine, Seoul, Korea

Investigation in our laboratory has been undertaken to study the effect of ethanol on the triglyceride (TG) content in the liver, the free fatty acid (FFA) content in the serum and the glycogen in the liver of rats which were fed on various diets.

Four hours after administration of a single dose of glucose (5g/kg BW) and ethanol (6g/kg BW) by gavage tube to rats fed a normal diet for 20 days then fasted for 18 hours, TG content in the liver increased by 80%, 10% compared to the control. When a single dose of equal amounts of both glucose and ethanol were administered to another group, TG content in the liver was 42% higher than the control. There was no great change in serum FFA content in the glucose treated group as compared with the control, however, there was an increment of serum FFA content in the ethanol treated group and in the group treated with both ethanol and glucose by 81% and 71% of the control, respectively. The results indicate that ethanol administration had an inhibitory effect on the TG accumulation in the liver of rats fed by glucose. There is a correlation between TG accumulation in the liver and FFA content in the serum, and it appears that the ethanol administration did not induce the TG accumulation in the liver but the increment of serum FFA content in rats is probably due to the increased fatty acid mobilization from adipose tissue. However, countercurrent results were observed in the glucose treated group as compared with the ethanol treated group suggesting that glucose administration does induce TG accumulation in the liver but does not increase the serum FFA content in rats. The increment of serum FFA content in rats. The increment of serum FFA content by ethanol treatment was not ameliorated by glucose administration.

In the liver perfusion experiment with rats fed both ethanol and various other diets, the results of incorporation of ethanol-1-14C into the total lipid in the high carbohydrate, high fat, low carbohydrate and control diet group were 1925±257 (cpm/g liver), 1237±76, 1269±105, 2041±74, respectively. The results indicate that amount of dietary carbohydrate and high fat had an effect on the total lipid accumulation derived from ethanol-1-14C molecule in the liver. Liver glycogen content in the control on rats, high fat, low carbohydrate and high carbohydrate diets were 91.5±7.9 (mg%), 93.0

---

* Received May 29, 1980
** This study supported by the Faculty Research Fund (1979) of Yonsei University College of Medicine.
±1.8, 99.1±4.4, and 153.7±26.0, respectively. There were no great differences between each dietary group and the rest control group except in the case of the high carbohydrate group which was over 1.5 times greater than that of the control. The incorporation of labelled ethanol into liver glycogen in the control rats and those on high fat diet, low carbohydrate diet and high carbohydrate diet were 525, 401, 351 and 806 cpm/g liver, respectively. The increased incorporation of ethanol-1-14C into liver glycogen in the high carbohydrate diet group is thought to be due to the increased gluconeogenesis from acetyl CoA derived from 14C from ethanol because rats were fasted for 18 hours before perfusion. It might be the result of increased gluconeogenesis of acetyl CoA derived from ethanol-1-14C by spare action of high carbohydrate on acetyl CoA.

During the liver perfusion, 14CO2 production from ethanol-1-14C was higher in the high fat diet and low carbohydrate diet groups than in the control group, however, no great difference was observed between the high carbohydrate and control groups. The higher production of 14CO2 from the single ethanol-1-14C dose in rats on the high fat diet and low carbohydrate diet groups than in the control group is probably due to the increased metabolism of ethanol through Kreb's cycle rather than the incorporation of it into the liver fat.

Several laboratories have demonstrated that the evidence pointing to chief source of lipogenesis of liver is dietary lipid and carbohydrate, however, metabolic changes of liver lipid with diets have been appreciated associate with diet composition(Gearter and Carroll, 1967; Tepperman and Tepperman, 1964). It has been reported that lipogenesis in liver and adipose tissue was reduced by a high fat diet(Whitney and Robert, 1955; Hill et al., 1960) but high fat diet caused the reduction of carbohydrate concentration in the liver(Hill et al., 1958; Hausberger and Milstein, 1955). However, dietary fat has a specific inhibitory effect on hepatic lipogenesis regardless of carbohydrate intake according to Hill et al. (1960). Dietary protein also effects hepatic lipogenesis and inhibits the biosynthesis of fatty acid in the liver(Masoro et al., 1950; Cohen and Teitelbaum, 1966) and in adipose tissue(Leveille et al., 1962), furthermore a high fat plus a low carbohydrate diet reduces the biosynthesis of fatty acid(Kornacker and Lowenstein, 1965). Lipogenesis is altered in liver in response to the diet comosition, but lipid accumulation in the liver is primarily the result of the net biosynthesis of lipid.

Alcoholism also leads to fat accumulation in the liver, hyperlipidemia and ultimately cirrhosis. The exact action of alcohol together with the influence of various dietary composition is complex and still uncertain. According to Jones and Greene(1966), ethanol administration caused fat accumulation in the liver of rats fed on a high fat diet. Lieber (1973) reported that low fat diets with alcohol caused the decrease in lipid origining in the liver but the capacity of the fatty liver was increased by ethanol and increased protein ingestion reduced the induction of fatty liver by ethanol.

The present study has been undertaken to study the response due to ethanol on the triglyceride(TG) content in the liver, and
the free fatty acid (FFA) content in the serum and the glycogen content in the liver of rats which were fed various diets, and also liver perfusion experiments which rats fed both ethanol and various diets has been undertaken to study how the ethanol-1-\(^{14}\)C incorporated into total lipid and other intermediate metabolite in perfused liver system.

**MATERIALS AND METHODS**

**Effect of glucose and ethanol on hepatic triglyceride and serum free fatty acid**

Experiment 1. Albino male rats about 150 g each were purchased from the local supplier and maintained on 18% casein diets prepared by the procedure described by De Carli and Liber (1967). Rats were fed with a normal diet for 20 days and fasted for 18 hours before the experiment. All diets contained adequate amounts of nutrients including vitamins and minerals. Four hours after a single dose of glucose (6 g/kg, BW) or ethanol (6 g/kg, BW) or a single dose of equal amounts of glucose and ethanol were administered by stomach tube, the rats were sacrificed by decapitation and the blood and liver were obtained. The control group was given 2 ml of saline.

The triglyceride contents in the liver was measured by the micromethod of Handel and Zilversmit (1957) and the serum free fatty acid was measured by the Duncombe (1963) method.

Experiment 2. Albino male rats weighing 110 to 120 g were fed with various diets (Table 1) prepared by the modified DeCarli and Liber method (1967) for 60 days. During this period, ethanol (6 g/kg, BW) was administered by stomach tube every day. Rats were fasted for 18 hours and whole liver was removed. The isolated liver was perfused by Miller's (1951) method. The experiment was done for 3 hours and 0.2 M of bicarbonate buffer (pH 7.4) was used and Ringer solution (Biochemical hand book) was used as perfusate. After 10 min's perfusion, ethanol-1-\(^{14}\)C (500 nCi) was administered to perfuse as a single dose and respiratory \(^{14}\)CO\(_2\) was trapped in ethanolamine (methylcellulose, 2; monothanolamine, 1) solution. \(^{14}\)C incorporated from ethanol-1-\(^{14}\)C into total lipid, \(^{14}\)CO\(_2\) and liver glycogen were measured. Total liver glycogen was measured by the anthrone method of Hassid and Abraham (1957). Radioactivity was counted by the Packard Tricab Liquid Scintillation Spectrometer. The scintillation cocktail was composed of 1.2 g of 2,5-diphenyloxzasol (PPO) in 120 ml of methylcellulose and 240 ml of toluene.

Ringer Solution

<table>
<thead>
<tr>
<th>NaCl</th>
<th>KCl</th>
<th>KH(_2)PO(_4)</th>
<th>MgSO(_4) 7H(_2)O</th>
<th>NaCHO(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9%</td>
<td>1.15%</td>
<td>2.11%</td>
<td>3.8%</td>
<td>1.3%</td>
</tr>
<tr>
<td>(0.154M)</td>
<td>(0.154M)</td>
<td>(0.154M)</td>
<td>(0.154M)</td>
<td>(0.154M)</td>
</tr>
<tr>
<td>83ml</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
0.16M Na-Pyruvate 4
0.1M Na-Fumarate 7
0.16M Na-Glutamate 4
2.3M Glucose 5
0.1M Na-Phosphate buffer (pH 7.4) 18

RESULTS

1) The effect of chronic ethanol administration on growth in the rat fed with various diets

Albino rats weighing 110 g to 120 g were used in this experiment. On arrival of the rats, they were put into the control and experimental groups randomly. Designated food and tap water were supplied ad libitum throughout this study and single doses of equal amounts of ethanol (6 g/kg, BW) were administered by stomach tube to the rats of each group. The body weight of the experimental and control groups are presented in figure 1. After feeding no the designated diet for 60 days, the body weights were measured for the normal diet, high fat diet group, low carbohydrate diet group and averaged 150, 145 and 110 g, respectively, while, in the high carbohydrate diet group, 10 g of body weight was lost.

![Graph showing weight of rats fed on designated diets plus ethanol.]

Fig. 1. Weight of rats fed on designated diets plus ethanol.

Table 2. Concentration of hepatic triglyceride and serum free fatty acid in rats (in vivo)

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of animal</th>
<th>Triglyceride (mg/g of liver)</th>
<th>Fatty acid (μ mole/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>11.02±1.32</td>
<td>199.5±16.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>4</td>
<td>19.50±1.07</td>
<td>190.3±34.1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4</td>
<td>12.55±1.41</td>
<td>363.4±33.1</td>
</tr>
<tr>
<td>Glucose plus ethanol</td>
<td>4</td>
<td>15.76±1.53</td>
<td>342.8±33.8</td>
</tr>
</tbody>
</table>

* All rats were fed with a normal diet as used by DeCarli and Liber (1967). Exp. procedures are described in the text. All values are mean±standard error.

Table 3. Incorporation of ethanol-1-14C into total lipid in the perfused liver of rats fed on various diets.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of animal</th>
<th>14C in the lipid of liver</th>
<th>% of infused 14C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cpm/g of liver</td>
<td>cpm/whole liver</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>2041±74</td>
<td>25108±981</td>
</tr>
<tr>
<td>High fat</td>
<td>4</td>
<td>1237±76</td>
<td>15144±1450</td>
</tr>
<tr>
<td>Low carbohydrate</td>
<td>4</td>
<td>1269±105</td>
<td>13843±1455</td>
</tr>
<tr>
<td>High carbohydrate</td>
<td>3</td>
<td>1925±257</td>
<td>19382±1928</td>
</tr>
</tbody>
</table>

* All diets are isocaloric with adequate vitamins and minerals. Values are mean±standard error.
Table 1. Incorporation of ethanol-1-\(^{14}\)C into total glycogen in the perfused liver of rats fed on various diets.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of animal</th>
<th>glycogen-(^{14})C (cpm/g of liver)</th>
<th>Liver glycogen (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4</td>
<td>525±44</td>
<td>91.5±7.9</td>
</tr>
<tr>
<td>High fat</td>
<td>4</td>
<td>401±29</td>
<td>93.0±1.8</td>
</tr>
<tr>
<td>Low carbohydrate</td>
<td>4</td>
<td>347±45</td>
<td>99.1±4.4</td>
</tr>
<tr>
<td>High carbohydrate</td>
<td>3</td>
<td>737±81</td>
<td>153.7±26.0</td>
</tr>
</tbody>
</table>

Fig. 2. \(^{14}\)CO\(_2\) production from ethanol-1-\(^{14}\)C by rat liver in the perfusion system.

2) Effect of single dose of glucose and ethanol on triglyceride and serum free fatty acid

Four hours after administration of a single statadose of glucose (5 g/kg BW.) and ethanol (6 g/kg. BW.) by stomach tube to rats fed with a normal diet for 20 days, then fasted for 18 hours, triglyceride content in the liver increased by 80% and only 10% in the control group. When a single dose of equal amounts of both glucose and ethanol were administered to another group, TG content in the liver was increased by 42%, our that of the control. There was no great change in serum FFA content in the glucose treated group as compared with the control, however, there was an increment of serum FFA content in the ethanol treated group and in the group treated with both ethanol and glucose by 81% and 71% of the control, respectively (Table 2). The results indicate that ethanol administration had an inhibitory effect on the TG accumulation in the liver of rats fed by glucose.

3) Incorporation of ethanol-1-\(^{14}\)C into total lipid, total glycogen and CO\(_2\), in the isolated perfused rat liver

In the liver perfusion experiment with rats fed with both ethanol and using various designated diets, the results of incorporation of ethanol-1-\(^{14}\)C into the total lipid were similar in the high carbohydrate diet group and the control group (2041±74 cpn/g liver vs 1925±257 cpn/g liver, respectively) and also similar in high fat diet group (1237±76 cpn/g liver) and in low carbohydrate diet group (1269±105 cpn/g liver), but were 38% of the control (Table 3). The results indicate that the amount of dietary carbohydrate and high fat had an effect on the total lipid biosynthesis from acetyl CoA derived from ethanol-1-\(^{14}\)C molecule in the liver. Liver glycogen content in the rats of the control, high fat, low carbohydrate and high carbohydrate diet group were 91.5±7.9, 93.0±1.8, 99.1±4.4, and 153.7±26.0
mg\%, respectively.

There were no great differences between each diet group except in the high carbohydrate group which was over 1.5 times greater than that of control. The incorporation of labelled ethanol into liver glycogen in rats of the control group, high fat diet, low carbohydrate diet and high carbohydrate diet were 525, 401, 351 and 806 cpm/g liver, respectively (Table 4). The increased incorporation of ethanol-1-\(^{14}\)C into liver glycogen in the high carbohydrate diet group is thought to be due to the increased gluconeogenesis from acetyl CoA derived from the \(^{14}\)C of the ethanol, because rats were fasted for 18 hours before perfusion. It might be the result of increased gluconeogenesis of acetyl CoA derived from ethanol-1-\(^{14}\)C by sparing action of high carbohydrate on acetyl CoA.

During the liver perfusion, \(^{14}\)CO\(_2\) production from ethanol-1-\(^{14}\)C was higher in the high fat diet and low carbohydrate diet groups than in the control group, however, no great difference was observed between the high carbohydrate and control groups (Fig. 2). The higher production of \(^{14}\)CO\(_2\) from ethanol-1-\(^{14}\)CO\(_2\) from ethanol-1-\(^{14}\)C in rats perfused by a stat dose in the high fat diet and low carbohydrate diet groups than in the control group is probably due to the increased metabolism of ethanol through Kreb's cycle rather than the incorporation of it into the liver fat.

**DISCUSSION**

Administration of ethanol in moderate amounts simply contributes additional calories to the diet by virtue of its oxidation acetyl CoA, which metabolizes into CO\(_2\) plus water by Kreb's cycle, however, in large amount administration of ethanol creates a number of metabolic problems with ultimate tissue damage, the degree of tissue damage is variable depending on diet composition associated with adequate amounts of other nutrients individual tolerance and other factors such as mode of administration. One of the mechanism in the metabolic problem, fatty liver due to ethanol is not entirely cleared up to date, but several studies have demonstrated that elevated levels of FFA by excessive mobilization of triacyl glycerol from adipose tissue to the liver was caused in part by action of ethanol in triggering the release of catecholamine and ACTH (Brodie et al., 1961; Ellis, 1966) and by the increase of lipid absorption from the gastrointestinal tract (Baron and Lieber, 1970; Mendenhall, 1972; Estler and Ammon, 1967) and of glycerophosphate due to increased NADH by alcohol dehydrogenase (Nikkila, 1963). Consequently elevated FFA is deposited as TG in liver (Estler and Ammon, 1967; Horning et al., 1960; Cho and Kim, 1978). The decrement of FFA oxidation (Forsander et al., 1965; Lieber et al., 1961; Roboucas and Isselbacher, 1961) is due to the decreased pyruvate and decreased citric acid cycle (Rawat, 1968) caused possibly by an increased NADH/NAD ratio generally by the oxidation of ethanol by alcohol dehydrogenase, this causes a shift to the left in the equilibrium state of malate oxaloacetate, and a shift to the right in the equilibrium state of pyruvate lactate, which may reduce Kreb's cycle by this sparing action of ethanol oxidation on the utilization of liver triacylglycerol phosphate (Schlamp et al., 1974). Failure to synthesize sufficient lipoprotein for transporting triacylglycerol is another important factor to induce fatty liver, because the release of lipoprotein in diminished (Liber et al., 1966) and there is decreased
synthesis of plasma protein (NadKarni 1974).

In our experiment TG content in the liver increased by 80% and 14% of the control group. When a single dose of equal amounts of both glucose and ethanol were administered to another group, TG content in the liver was increased by 42% over the control. There was no great change in serum FFA content in the glucose treated group as compared with the control and this result coincides with that of Liber et al. (1966). However, there was an increment of serum FFA content in the ethanol treated group and in the group treated with both ethanol and glucose by 81% and 71% of the control, respectively. The result indicates that ethanol administration had an inhibitory effect on the TG accumulation in the liver of rats fed with glucose. Our experiment reveals that there is a correlation between TG accumulation in the liver and FFA content in the serum, and it appears that ethanol administration did not induce the TG accumulation in the liver but the increment of serum FFA content in rats is probably due to the increased fatty acid mobilization from adipose tissue. However, countercurrent results were observed in the glucose treated group as compared with the ethanol treated group suggesting that glucose administration does induce TG accumulation in the liver but does not increase the serum FFA content in rats. The increment of serum FFA content by ethanol treatment was not ameliorated by glucose administration.

In the liver perfusion experiment with rats fed with both ethanol and various designated diets for 2 months, the amounts of the incorporated ethanol-1-14C into the total lipid were similar in both the control and high carbohydrate diet group and also similar in the fat and low carbohydrate diet groups but the amounts were decreased compared to that of the control and high carbohydrate diet group. On the other hand, the amounts of 14CO₂ derived from ethanol-1-14C in the control and high carbohydrate diet groups were decreased but the amounts were increased in the high fat and low carbohydrate diet groups. The higher production of 14CO₂ from ethanol-1-14C in rats perfused by stat doses in the high fat diet and low carbohydrate diet groups than in the control group is probably due to the increased metabolism of ethanol through the Kreb's cycle rather than the incorporation into the liver fat. Therefore, we can conclude that when less ethanol-1-14C was incorporated into lipid, the 14CO₂ production in corresponding rat liver was increased. Since the rats were fasted for 18 hours before perfusion, incorporated liver glycogen contents of the rats were less than 1 g% except in the high carbohydrate group (1.54 g%), which is far less than the normal glycogen content in liver. There were no great differences between each dietary group except in the high carbohydrate group in which the glycogen was over 1.5 times greater than that of the control. The increased ethanol into liver glycogen, in the high carbohydrate diet group is probably due to the enhancement of Kreb's cycle by the increased amounts of acetyl CoA from carbohydrate, and the net increase of gluconeogenesis from ethanol metabolite. These results coincide with the results of CO₂ from carbohydrate which was decreased in the corresponding liver perfusion experiment in the high carbohydrate group.

REFERENCES

Baraona E, Lieber CS: Effect of chronic ethanol feeding on serum lipoprotein metabolism in the
Brodie BB, Butler WM, Jr, Horning MG, Maikekel
RM, Maling HM: Alcohol-induced triglyceride
deposition in liver through derangement of fat
Cho MY, Kim YS: Studies on the effect of
chronic ethanol administration on the activity of
malic enzyme and ATP-citrate lyase in rats
fed various diets. Korean Biochem J 11:29,
1978
Cohen AM, Teitelbaum A: Effect of different
levels of protein in sucrose and starch diets on
lipid synthesis in the rats. J Nutr 91:25,
1966
DeCarli LM, Liber CS: Fatty liver in the rat after
prolonged intake of ethanol with nutritionally
Ellir FW: Effect of ethanol on plasma cortisone
evels. J Pharmacol Exp Ther 153:121,
1966
Erller CJ, Ammon HPT: The influence of beta-
adrnergic blockade on the ethanol-induced der-
angement of lipid transport. Arch Int Phar-
macodyn Ther 166:333, 1967
Forsander OA, Raitha N, Salaspuro M, Manesna
P: Influence of ethanol on the liver metabolism
of fed and starved rats. Biochem J 94:259,
1965
Gaertner EB, Carroll C: Metabolic responses of white
rats to balanced and imbalanced protein fed with
different carbohydrates in 15% and 5% fat
Handel EV, Zilversmit DB: Micromethod for the
direct determination of serum triglyceride. J
Lab Clin Med 50:152, 1957
Hassid, Abraham: Method in enzymology. Academic
Press Inc. New York vol 3:34, 1957
Hausberger FX, Milstein SW: Dietary effects on
lipogenesis in adipose tissue. J Biol Chem 214
:485, 1955
Hill R, Webster J, Linazasoro JM, Chaikoff IL:
Time of occurrence of changes in the liver
capacity to cholesterol synthesis after fat feed-
ing. J Lipid Res 1:150, 1960
Hill R, Linazasoro JM, Chevallier F, Chaikoff IL:
Regulation of hepatic lipogenesis: The influ-
ence of dietary fats. J Biol Chem 233:305,
1958
Horning MG, Williams EA, Maling HM, Brodie
BB: Dipt fat as source of increased liver trig-
lycerides after ethanol. Biochem Biophys Res
Comm 3:635, 1960
Jones PP, Greene EA: Influence of dietary fat on
alcoholic fatty liver. Amer J Clin Nutr 18:
350, 1966
Kornacker MS, Lowenstein JM: Citarate and the
conversion of carbohydrate into fat. Biochem J
94:209, 1965
Leveille GA, Shockley TW, Sauberlich HE: Influence
of dietary protein level and amino acids on plasma cholesterol of the growing chick. J
Nutr 76:321, 1962
Lieber CS: Alcohol, Nutrition and the liver. Am J
Clin Nutr 26:1163, 1973
Lieber CS, Schimid R: The effect of ethanol on
fatty acid metabolism: Stimulation of hepatic
fatty acid synthesis in vitro. J Clin Invest 40
394, 1961
Lieber CS, Spritz N, DeCarli LM: Role of dietary,
adipose, and endogenously synthesized fatty
acids in the pathogenesis of the alcoholic fatty
Masoro EJ, Chaikoff IL, Chernickss, Feltz JM:
Previous nutritional state and glucose conver-
sion to fatty acids in liver slices. J Biol Chem
185:845, 1950
Mendenhall CL: Origin of hepatic triglyceride fatty
acids: Quantitative estimation of the relative
contributions of linoleic acid by diet and adipose tissue in normal and ethanol-fed rats. J
Lipid Res 13:177, 1972
Miller LL, Bly CG, Watson MC, Bale WP: The
dominant role of the liver in plasma protein
Nadkarni GS: Effect of acute ethanol administra-
tion on rat plasma protein synthesis. Biochem
Pharmacol 23:389, 1972
Nikkila EA, Ojaer K: Role of hepatic L-a-glycerop
Studies on the Hepatic Accumulation of Triglyceride Induced by Chronic Ethanol Administration in Rats Fed with Various Diets


Reboucas G, Isselbacker KJ: Studies on the pathogenesis of the ethanol-induced fatty liver: Synthesis and oxidation of fatty acids by the liver.

J Clin Invest 40:1355, 1961


- 17 -