Use of dietary saturated fatty acids and vitamin E in the treatment of alcoholic liver disease

Amin A Naji, MD, FRCP
Dept of Pathology, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA, USA

Several lines of evidence indicate that dietary fat has the potential to affect the severity of alcoholic-induced liver injury. Aside from altering the threshold for alcoholic liver injury, saturated fat can be used to treat established alcoholic liver disease. Diets enriched in saturated fatty acids (palm oil and medium chain triglycerides) inhibit cytokine production, P450 2E1 and lipid peroxidation and ameliorate established alcoholic liver disease. Vitamin E may also play a role in modulating lipid peroxidation and liver injury. Additionally, both estrogens and pro-inflammatory cytokines are also downregulated after treatment with saturated fatty acids. Thus saturated fatty acids are a potential therapeutic intervention in inflammatory liver injury.

Introduction

Evidence shows that dietary lipid is an important determinant of alcoholic-induced liver injury. For example, epidemiologic observations suggest that saturated fat is relatively protective against alcoholic liver disease1. Also, dietary lipid can modulate the severity of alcoholic liver injury in rats3. None of the pro-inflammatory factors known to be involved in alcoholic liver injury develop in rats fed ethanol and saturated lipid whereas fatty acid liver, necrosis and fibrosis develop in rats fed ethanol and lipid enriched in polyunsaturated fatty acids. Several investigations have proposed that polyunsaturated fatty acids promote alcoholic liver injury by inducing cytokine synthesis P450 2E1 (CYP 2E1) and lipid peroxidation4.

With these facts in mind, different strategies could be employed to decrease lipid peroxidation and treat alcoholic liver disease. One approach would be to use dietary saturated fatty acids because both CYP 2E1, fatty acid composition of the liver and lipid peroxidation are sensitive to dietary manipulation4. Therefore, studies were carried out in which rats with alcoholic liver injury were treated with diets enriched in saturated fatty acids (palm oil and medium chain triglycerides) or a diet enriched in polyunsaturated w-3 fatty acids (fish oil). In addition to being a rich source of saturated fatty acid, palm oil contains tocopherols and tocopherol which inhibit lipid peroxidation5. Palm oil also modulates esophagitis metabolism in a manner in which the ratio of vasodilator to vasoconstrictor prostanoids is increased6.

Materials and methods

Experimental design

Males Wistar rats weighing between 225 and 250 grams were fed a liquid diet by continuous infusion through permanently implanted gastrotubeces as previously described7. The rats were administered their total nutritional intake by intragastric infusion. The percentage of calories derived from fat was 35% of total calories. Vitamins and minerals were as described previously8. The amount of ethanol given was modified to maintain high levels of blood ethanol (150-300 mg/dL) throughout the day. This amount was initially 5 g/kg/day and was increased up to 16 g/kg/day as tolerance developed. Each ethanol-fed rat had at least two measurements of blood alcohol level.

Table 1. Pathologic changes in different experimental groups

<table>
<thead>
<tr>
<th>Duration of fatty acid treatment (weeks)</th>
<th>Fatty acid</th>
<th>Necrosis Inflammation of liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Fish-oil</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>6</td>
<td>Fish-oil</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>8</td>
<td>Fish-oil</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>Fish-oil</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>12</td>
<td>Fish-oil</td>
<td>0.10 ± 0.01</td>
</tr>
</tbody>
</table>

Discussion

The problem of treating alcoholic liver injury has remained intractable. Although diets high in protein and calories have been used to reverse the protein-calorie malnutrition that often accompanies alcoholic liver disease, little effort has been directed toward developing a dietary strategy that might treat the underlying disease. Our results show that when the dietary fat was switched from fish oil that is rich in polyunsaturated fatty acids to diets rich in saturated fatty acids and tocopherol, the alcohol-induced liver injury was reversed to normal. When rats were continued on fish oil, the liver pathology persisted.
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With these facts in mind, different strategies could be employed to decrease lipid peroxidation and treat alcoholic liver disease. One approach would be to use dietary saturated fatty acids because both CYP 2E1, fatty acid composition of the liver and lipid peroxidation are sensitive to dietary manipulation. Therefore, studies were carried out in which rats with alcoholic liver injury were treated with diets enriched in saturated fatty acids (palmitic and medium chain triglycerides) or a diet enriched in polyunsaturated w3 fatty acids (fish oil). In addition to being a rich source of saturated fatty acid, palm oil contains tocopherols and tocotrienols, both of which inhibit lipid peroxidation. Palm oil also modulates esoxaloside metabolism in a manner in which the ratio of vasodilator to vasoconstrictor prostanoids is increased.

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Table 1: Pathologic changes in the different experimental groups (n=6).

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration fatty acid</th>
<th>Necrosis Inflammation of liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Fish oil-ethanol (FE)</td>
<td>4.06±0.0 1.44±0.4</td>
</tr>
<tr>
<td>Group 2</td>
<td>Fish oil-cholesterol (FE)</td>
<td>3.81±0.4 1.24±0.3</td>
</tr>
<tr>
<td>Group 3</td>
<td>Fish oil-cholesterol (FE)</td>
<td>2.2±0.4 0.7±0.3</td>
</tr>
<tr>
<td>Group 4</td>
<td>Fish oil-cholesterol (FE)</td>
<td>6.3±0.5 1.4±0.5</td>
</tr>
<tr>
<td>Group 5</td>
<td>Fish oil-cholesterol (FE)</td>
<td>1.6±0.5* 0.5±0.1*</td>
</tr>
</tbody>
</table>

* a.p<0.05 vs. fish oil-ethanol in the same group
b.p<0.05 vs. fish oil-cholesterol in the same group
c.p<0.01 vs. fish oil-ethanol in the same group

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Note: Portions of these studies have been previously reported in Gastroenterology 1995; 109; 547-554 and J Pharmacol Exp. Thet. 1996; 277: 1694-1700.

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follows: steatosis (the percentage of liver cells containing fat) was scored using a 1-4 scale of the cells containing 0, 1-25%, 25-50%, 50%, and >75%. Necrosis was evaluated as the number of necrotic foci per square millimeter, and inflammation was scored as the number of inflammatory cells per square millimeter. At least three different sections were examined per sample of liver. The pathologist evaluating the sections was unaware of the treatment groups when assessing the histology.

Measurement of blood alcohol
Blood was collected from the tail vein, and ethanol concentration was measured using an alcohol dehydrogenase kit from Sigma Chemical Co (St. Louis, MO).

Determination of thiorbituric acid reactive substances
Levels of liver thiorbituric acid reactive substances (TBARS) were measured according to the method of Okhota et al. Briefly, 0.2 mL sodium dodecyl sulfate (8.1%), 1.5 mL 20% acetic acid (5.5 mL), 0.8% thiorbituric acid were added to 200 mL of liver homogenate. After addition of distilled water, the tubes were vortexed and placed in boiling water for 1 hour. The reaction was stopped by immersion of tubes in a cold water bath. After addition of 15:1 (vol/vol) butanol-pyridine and centrifugation, the upper phase was removed and absorbance at 532 nm was determined. Butyldihydropyridine (BHT) (90 mmol) was added to prevent the formation of TBARS in vitro.

Measurement of conjugated dienes
Conjugated dienes in the total lipid extracted from liver homogenates were determined by their optical density of between 220 nm and 300 nm as described by Reichenek and Glende.

Aniline hydroxylase activity
Aniline hydroxylase activities were performed according to the method of Imal et al. with the following modification. Liver microsomes were homogenized for 60 minutes at 27°C in 0.1 M Tris, pH 7.4 containing 8 mM L-arginine and 1 mM EDTA. Reactions were terminated with 50 mL of 40% trichloroacetic acid. Samples were then placed on ice for 10 minutes before being centrifuged to remove inorganic material. The supernatant (0.3 mL) was mixed with 10% NaCl (0.24 mL) and 2% phenol (0.36 mL). Abs values were determined after incubation for 45 minutes in the dark. Specific activities were calculated from a standard curve prepared with the reaction product 4-aminohippuric acid (Addich, Milwaukee, WI).

Results
No differences were found in the amount of weight gained during the week period of ethanol feeding or during the 2 week period after switching to the experimental diets. No significant difference was found in blood alcohol levels.

Effects of experimental diets on liver pathology
Feeding the fish oil-ethanol diet for 6 weeks resulted in fatty infiltration, inflammation and necrosis (Table 1, Figure 1). There was minimal improvement when the ethanol was stopped and the rats switched to the fish oil-dextrose diet for 2 weeks. Addition of vitamin E to the fish oil-dextrose diet resulted in an improvement in the liver, necrosis and inflammation. The degree of fatty liver, necrosis and inflammation were all markedly improved when the rats were switched to palm oil-dextrose or MCT-dextrose diet. Treatment with diets containing saturated fatty acids led to complete normalization of liver histology (Figure 2).

Diabetic mediation of lipid peroxidation
We had hypothesized that feeding saturated fatty acids would result in decreased levels of lipid peroxidation. The levels of TBARS and conjugated dienes were significant lower in the diabetic groups treated with saturated fatty acids (Table 2). Part of the explanation for the decrease in lipid peroxidation could be related to changes in CYP 2E1 activity. The activity of aniline hydroxylase, which reflects the activity of CYP 2E1, is shown in Table 2. The activity of aniline hydroxylase in the MCT-dextrose and palm oil-dextrose treated groups was significantly lower than in other treatment groups.
Adipose tissue expansion and the development of obesity: influence of dietary fat type

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Recent studies indicate that the prevalence of obesity in adults has increased by 30% or more in the past decade, with increases in both genders and in all ethnic and racial populations and age groups. Obesity is associated with many chronic diseases and alterations in physiological function including cardiovascular disease, hypertension, diabetes mellitus, gallbladder disease and certain types of cancer. Much attention regarding dietary influences on obesity development or prevention has focused on high-fat diets. Many studies have confirmed that high fat feeding leads to an increase in adipose tissue mass through an increase in fat cell size and to the subsequent development of obesity. However, there is little definitive information on the effect of type of dietary fat, especially palm oil, on adipose tissue cellularity and the development of obesity. These studies were designed to determine whether dietary fat of different sources vary in their ability to produce obesity and to begin to elucidate the mechanism by which such divergence occurs. Male Osborne-Mendel rats were fed either a low fat (15% dietary fat) or one of three high fat diets (65% calories) for 12 weeks. The predominant fat sources in the high fat diets were either soybean oil, tallow, or palm olein (a fraction of palm oil). Final body weight was not influenced by fat level or type, however percent carcass lipid and fat pad weights were higher in soybean oil and tallow fed rats than in low fat and palm-olein fed rats. Fat pad specific increases in cell size and cell number were observed for tallow and soybean oil fed compared to low fat and palm-olein fed rats. Serum triglycerides were higher in the tallow and palm-olein fed rats compared to low fat fed rats, no significant effects of dietary fat type on serum cholesterol were observed. These results indicate that palm-olein, unlike tallow and soybean oil, were comparable to a low fat diet concerning fat pad weight, body composition and adipose tissue cellularity when fed for twelve weeks at 65% of energy intake. The lower fat storage in the palm-olein-fed rats may be associated with a stall trend in serum triglyceride uptake and/or a reduced fat cell proliferative capacity. The influence of dietary fat type on the proliferative capacity of the pre-adipocytes and on the production of a local or systemic adipogenic factor is being determined in subsequent studies.

Key words: Palm oil, fat cell size, fat cell number, rats

Introduction

Obesity is associated with many chronic diseases and alterations in physiological function including cardiovascular disease, hypertension, diabetes mellitus, gallbladder disease and certain types of cancer. Obesity is a major public health problem in the United States and Europe and is becoming increasingly important in many other areas of the world. The prevalence of obesity in adults in the U.S. has increased by 30% or more in the past decade, with increases in both genders and in all ethnic and racial populations and age groups. It is now estimated that one third of the adult population in the US are obese. Obesity is very common in western society, with a prevalence of considerably greater than 10% based on anthropometric and perimetric body mass indices. Recent studies indicate a strong correlation between the increasing prevalence of obesity and decreased chronic disease rates in developing countries including China, Pacific Island populations and Brazil.

The etiology of human obesity is quite complex, involving genetic, metabolic, behavioral and environmental factors. Although obesity is believed to have a strong genetic component[1], the increased incidence of obesity in specific population groups undergoing Westernization indicates the importance of dietary and lifestyle changes in the manifestation of this disease[2]. Among dietary factors, total energy intake and fat intake are significantly correlated with higher body mass index in these population groups[3]. However, increased intake of fat energy is associated with a greater extent in body mass than is increased intake of calories from non-fat sources. Therefore, much attention regarding dietary influences on obesity development or prevention has focused on high fat diets.

Many animal studies have confirmed that high fat feeding leads to an increase in adipose tissue mass through an increase in fat cell size and/or number and to the subsequent development of obesity[6]. Hyperphagia[7] and decreased energy expenditure (via changes in metabolic rate) induced by high fat feeding have been identified as contributing factors to the development of high fat diet induced obesity, while changes in adipose tissue cellularity influence the reversibility of this condition[8]. It is thought that once the peak capacity for storing lipids is reached in high fat fed animals, cell number is increased and that cell number is increased and that cell number is increased and that cell number is increased. These changes in fat cell number are permanent, as substitution of the high fat diet with a low fat diet leads to a reduction in fat cell mass, but not in cell number[2,8]. Recent evidence indicates that changes in adipose cellularity during the development of obesity in high fat fed rats is associated with the appearance of a locally induced factor(s) capable of stimulating adipose cell proliferation[9].

There is little definitive information on the effect of type of dietary fat on adipose tissue cellularity and the development of obesity. Alterations in dietary fat type have been shown to influence membrane composition, function and metabolic processes in many tissues[4,5]. Kirkland et al[10] observed that long-term feeding of high fat diets (20% containing beef drippings vs. maize oil had no influence on body weight gain or adipose tissue cellularity in pigeons. More recently, Shimomura et al[8] reported a decreased accumulation of body fat in rats fed safflower oil vs. beef tallow diet.

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Figure 2. Liver section from a rat treated with palm oil dextrose for 4 weeks after feeding of fish oil ethanol. There is no evidence of pathological changes.

Palm oil and MCT were effective in treating alcoholic liver injury primarily by normalizing content of polyunsaturated fatty acids and/or tocopherol content. Saturated fatty acids are not targets of free radical attack[1,2], therefore, lipolysis was minimized in rats fed the saturated fatty acids, especially palm oil with its extra vitamin E. In fact vitamin E alone was able to accomplish what palm oil did when the vitamin E was added to fish oil. Thus, the main protection would seem to be an antioxidant in the intragastic feeding model used for alcoholic liver disease, CYP 2E1 induction is associated with an increase in lipid peroxidation[8]. The reduction in lipid peroxidation in the saturated fat-treated groups was accompanied by a decrease in CYP 2E1 activity.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>TBAK</th>
<th>Conjugated dienes</th>
<th>Antilipase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE (w:z)</td>
<td>1.37±0.26</td>
<td>0.46±0.16</td>
<td>0.75±0.11</td>
</tr>
<tr>
<td>FE-FD</td>
<td>0.74±0.19</td>
<td>0.29±0.08</td>
<td>0.39±0.03</td>
</tr>
<tr>
<td>FE-FD</td>
<td>0.26±0.08</td>
<td>0.14±0.01</td>
<td>0.32±0.04</td>
</tr>
<tr>
<td>FE-FD-V E</td>
<td>0.30±0.13</td>
<td>0.54±0.07</td>
<td>0.25±0.02</td>
</tr>
<tr>
<td>FE-MCT</td>
<td>0.22±0.07</td>
<td>0.09±0.03</td>
<td>0.29±0.01</td>
</tr>
</tbody>
</table>

(a) p<0.02 vs. FE group; (b) p<0.01 vs. FE and FE-FD group; (c) p<0.01 vs. other groups except FE-MCT, FE-FD.

The therapeutic strategies employed in these studies are based on prior studies in which ethanol fed to rats with saturated fatty acids prevented both the induction of CYP 2E1, lipid peroxidation and liver injury. Polysaturated fatty acids, on the other hand, promote CYP 2E1 induction, lipid peroxidation and liver injury[10]. Regardless of the mechanisms involved, feeding saturated fatty acids or vitamin E results in a simple and effective means of reversing alcoholic liver injury. It is important to determine whether a lipid-based strategy is effective in clinical alcoholic liver disease.

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