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The Effect of Oral Fructose on Ethanol - Induced Changes in Plasma and Hepatic Lipids

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Summary. **The effect of fructose on ethanol — induced hyperlipidemia and triacylglycerol accumulation in the liver was studied in adult albino rabbits using a colorimetric procedure. The results showed that the treatments produced a minimal increase in plasma cholesterol, but gradually increased plasma triacylglycerol (TAG) from a basal value of 0.600 ± 0.017 to 0.800 ± 0.020 mmol/L and 0.810 ± 0.025 mmol/L, respectively, at the end of the 15-week exposure period. While ethanol treatment significantly increased the basal hepatic TAG level from 0.342 ± 0.037 mmol/L to 1.780 ± 0.060 mmol/L (P<0.05), ethanol+ fructose treatment insignificantly increased the same to 0.440 ± 0.020 mmol/L (P>0.05). It appears that the metabolism of oral fructose in the presence of ethanol operates a mechanism that either prevents or delays the accumulation of TAG in the liver. Thus, a long- term chronic study is required to establish the more likely possibility.**

Key words—cholesterol, ethanol, fructose, lipids, triacylglycerol.

INTRODUCTION

The cytosol of hepatocytes contains alcohol dehydrogenase (ADH; EC 1.1.1.1.) that converts ethanol to acetaldehyde. Further oxidation of acetaldehyde to acetate is predominantly catalysed by the mitochondrial, low K_m acetaldehyde dehydrogenase (ALDH; EC 1.2.1.3) enzyme. The catalytic activities of ADH and ALDH respectively increase cytosolic and mitochondrial nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide (NADH/NAD^+) ratios.

The increased NADH/NAD^+ ratio raises the concentration of ∝-glycerophosphate which favors hepatic triacylglycerol accumulation by trapping fatty acids[1)]. In addition, excess NADH may promote fatty acid synthesis from different sources which may also accumulate as fat in the liver.

The metabolism of fructose in the presence of ethanol has been shown to be shunted to NADH-requiring pathways due to unavailability of NAD^+[2)], and this provides a rapid means of directly re-oxidizing the ADH and ALDH generated NADH to NAD^+ for further alcohol oxidation in the liver cells. Thus, oral fructose intake could maintain the NADH/NAD^+ redox usually altered by alcohol and so, theoretically, prevent the development of a fatty liver.

The reduction in the [lactate] / [pyrurate] ratio in blood would therefore be indirect evidence of a decrease in the NADH/NAD^+ ratio. However, Mascord et al.[3)] noted an increase (rather than decrease) in blood lactate/pyruvate ratios after fructose and concluded that the direct re-oxidation of NADH to NAD^+ did not provide an explanation for the "fructose effect". Yamamoto et al.[4)] showed that oral fructose administration enhances hepatic adenosine-5'-triphosphate (ATP) (energy) utilization --particularly in the presence of alcohol -- by temporarily trapping its high energy phosphate as fructose-6-phosphate and other phosphorylated glycolytic intermediates. A fall in ATP content of the cell would presumably activate mitochondrial electron

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Abbreviations—ADH, - alcohol dehydrogenase; ALDH, - aldehyde dehydrogenase; ATP, - adenosine - 5'- triphosphate; NaCl, - sodium chloride; NAD^+, - nicotinamide adenine dinucleotide (oxidized); NADH, - nicotinamide adenine dinucleotide (reduced); TAG, - triacylglycerol.

transport, which would in turn expedite NADH oxidation to NAD^+. In view of these conflicting reports, this present study attempts to investigate the effect of oral fructose on the changes in blood and hepatic lipids induced by a short-term chronic administration of ethanol.

MATERIALS AND METHODS

Animals and feeding

Sixteen adult male albino rabbits with an initial mean weight of 1.50±0.10 kg were purchased from Yoha Farms, Warri, Nigeria. The rabbits were housed singly in clean metal hutches and acclimatized on growers' mash, a product of Bendel Feed and Flour Mills (BFFM) Ltd, Ewu, Nigeria, for 10 days before commencing the experiment. The rabbits were then divided into four groups with four animals each, and labeled as group C (control), group E (ethanol-treated rabbits), group EF (ethanol + fructose treated rabbits) and group F (fructose-treated rabbits). Group C animals took normal saline, while the E-group animals orally received 1.5 g (40%) ethanol/kg body weight as a single daily dose. Group EF rabbits also received the same ethanol dose, but were additionally given 0.25 g fructose/kg body weight after about 10 min of the ethanol dose. The F-group animals were orally given 0.25 g fructose/kg body weight as a single daily dose. The animals were exposed to these treatments along with their usual feeding pattern – about 80 g wet weight of feed/kg body weight/day – for a continuous period of 15 weeks. The dosing regimen was based on previous experience with ethanol and / or fructose[5]. They were allowed to drink clean water *ad libitum*, and the feeds were mixed with water in a ratio of 10:1 (w/v) so as to achieve a texture acceptable to the animals. Stale feed remnants were regularly discarded, and the feeding experiment was conducted at room temperature (about 29°C) in a 12-hr day light cycle.

Collection of blood sample

After the 5^{th} , 10^{th} and 15^{th} week of treatment, whole blood was drawn from an ear vein of each rabbit into lithium-heparinized tubes, using sterile disposable, 21-guage hypodermic needles. The whole blood sample was centrifuged at 1,200 × g for about 5 min at room temperature to separate the plasma, which was then removed, stored refrigerated, and analyzed within 48 hr.

Preparation of liver extracts

At the end of the 15-week exposure, the rabbits were sacrificed by cervical dislocation and their livers were quickly excised and rinsed in cold normal saline (0.9% NaCl solution). They were then prepared for biochemical study using an aqueous extraction technique.

Estimation of analytes

Triacylglycerol levels in the plasma and liver extracts were estimated using the enzymatic-endpoint colorimetric method[6]. Plasma total cholesterol was colorimetrically quantified in both forms of samples as described by Allain et al.[7]. Plasma and liver HDL- cholesterol was determined by the enzymatic-colorimetric method[8]. LDL- cholesterol was mathematically estimated using fasting data[9].

Statistical analysis

The repeat measure analysis of variance, ANOVA, was used to compare similar mean values, and the level of significance was established at the 5% probability level.

RESULTS

The results obtained are shown in Tables 1 and 2. They respectively show the changes in plasma and hepatic lipids obtained from rabbits in the different treatment groups.

The data indicate that the plasma triacylglycerol (TAG) level for the EF group gradually increased beyond the similar trends induced in either the E or F group (Table 1). The level of TAG in the hepatic tissue of the E group was significantly ($P< 0.05$) increased when compared with the values obtained from the other groups using ANOVA (Table 2). Although the level of plasma TAG for the EF group increased at the end of the 15-week exposure period, this did not appear to accumulate in the liver of the animals as judged by the mean level of TAG in the hepatic tissue (Table 2).

Changes in plasma lipoprotein-cholesterol content showed that ethanol progressively induced an increase in plasma HDL-cholesterol. However, the proportion of the increases was greater in the presence of fructose, which produced a significantly different HDL-cholesterol value at the end of the 15-week treatment (Table 1). Undulating and statistically insignificant ($P>0.05$) changes in mean plasma LDL-cholesterol were observed for all the groups, but the plasma LDL- cholesterol obtained during the 15th week for the EF group was significantly the lowest ($P<0.05$).

Table 1. Changes in mean plasma lipids induced by the different treatments in experimental rabbits

Treatment period (wk)	Treatment	Changes in plasma lipid values (mmol/L) 0	5	10	15
Plasma Triacylglycerol (TAG)	Normal saline	0.550 ± 0.010	0.540 ± 0.015	0.450 ± 0.030	0.540 ± 0.015
	Ethanol	0.600 ± 0.017	0.650 ± 0.020	0.715 ± 0.015	0.800 ± 0.020
	Ethanol + Fructose	0.601 ± 0.021	0.715 ± 0.015	0.750 ± 0.010	0.810 ± 0.025
	Fructose	0.581 ± 0.021	0.603 ± 0.017	0.616 ± 0.013	0.623 ± 0.021
Plasma total cholesterol	Normal saline	2.350 ± 0.030	2.100 ± 0.066	2.300 ± 0.021	2.200 ± 0.047
	Ethanol	2.400 ± 0.040	2.550 ± 0.018	2.700 ± 0.051	2.710 ± 0.020
	Ethanol + Fructose	2.300 ± 0.020	2.600 ± 0.038	2.320 ± 0.041	2.150 ± 0.052
	Fructose	2.416 ± 0.023	2.323 ± 0.026	2.426 ± 0.037	2.510 ± 0.043
HDL-cholesterol	Normal saline	0.860 ± 0.030	0.760 ± 0.040	0.820 ± 0.050	0.800 ± 0.030
	Ethanol	0.900 ± 0.027	0.980 ± 0.080	1.130 ± 0.040	1.180 ± 0.032
	Ethanol + Fructose	0.880 ± 0.036	0.940 ± 0.072	1.200 ± 0.066	1.260 ± 0.046*
	Fructose	0.843 ± 0.028	0.836 ± 0.071	0.852 ± 0.044	0.847 ± 0.042
LDL-cholesterol	Normal saline	1.240 ± 0.015	1.095 ± 0.080	1.275 ± 0.073	1.155 ± 0.062
	Ethanol	1.227 ± 0.012	1.275 ± 0.062	1.218 ± 0.080	1.166 ± 0.072
	Ethanol + Fructose	1.147 ± 0.025	1.335 ± 0.058	0.779 ± 0.036	0.522 ± 0.035*
	Fructose	0.933 ± 0.021	1.213 ± 0.060	1.294 ± 0.052	1.380 ± 0.056

Values are expressed as mean ± SD (n=4 rabbits per group), * Significantly different ($P<0.05$) from the value obtained from normal saline treated (control) group, Normal saline treatment, Ethanol treatment, 1.5 g(40%) ethanol/kg body weight; Ethanol + fructose treatment, 1.5 g(40%) ethanol + 0.25 g fructose/kg body weight Fructose treatment, 0.25 g fructose/kg body weight.

Table 2. Mean hepatic lipid levels at the end of the 15-week exposure period

Analyte	Treatment	Lipid levels in hepatic tissue (mmol/L)
Triacylglycerol	Normal saline	0.360 ± 0.040
	Ethanol	1.780 ± 0.060*
	Ethanol + Fructose	0.440 ± 0.020
	Fructose	0.382 ± 0.020
Total cholesterol	Normal saline	0.620 ± 0.040
	Ethanol	0.900 ± 0.050
	Ethanol + Fructose	0.703 ± 0.015
	Fructose	0.682 ± 0.019

Value are expressed as mean ± SD (n=4 rabbits per group); *Significantly different ($P<0.05$) from the value obtained from normal saline - treated (control) rabbits; Basal values- Triacylglycerol, 0.356 ± 0.033; Total cholesterol, 0.611 ± 0.042.

DISCUSSION

The effect of ethanol on blood lipid levels has been studied primarily in order to elucidate the mechanism of alcoholic hyperlipidemia. In this study, ethanol administration insignificantly ($P>0.05$) increased the amount of cholesterol in both plasma HDL- and LDL-fractions when compared with the control value at the end of the 15-week exposure. These in turn, increased

the plasma total cholesterol, though not to a statistically significant level (P>0.05). However, ethanol+fructose treatment significantly (P<0.05) increased plasma HDL-cholesterol but reduced plasma LDL-cholesterol (P<0.05) at the end of the exposure period. These lipoprotein-cholesterol changes culminated in an insignificant lowering of the plasma total cholesterol at the 5% level.

Ethanol intake has also been reported to cause an increase in both plasma[10] and hepatic[11] TAG levels. The observation in this present study vis- à -vis the increase in plasma and hepatic TAG - induced by ethanol – appears to support the previous findings, indicating that a rabbit study could be a good model for predicting possible human effects. Although the [NADH] / [NAD^+] ratio and ADH/ALDH activity values were not determined in this study, previous reports have implicated the ethanol-induced changes in NADH/NAD^+ redox to be responsible for the increase in both plasma and hepatic TAG levels[12]. However, for the EF group, the plasma level of TAG was observed to be similar in proportion to that produced by ethanol alone, but with a different trend in the liver (Table 2). The observations with ethanol + fructose seem to suggest that the metabolism of oral fructose may not directly recycle NADH to NAD^+ in blood during alcohol oxidation, as earlier reported[2], since it could not ameliorate the ethanol - induced increase in plasma TAG.

The observed trends in the cholesterol contents of plasma HDL- and LDL- fractions for the E and EF groups (Table 1) have been reported to be beneficial in reducing the risk of cardiovascular diseases[13], and, in addition, the significant (P< 0.05) change in the EF group at the end of the 15 - week exposure period is accompanied by the reduced possibility of TAG deposition in the liver (Table 2). The mechanism underlying the changes in the plasma cholesterol levels induced by ethanol + fructose treatment and the detailed reason(s) why ethanol or fructose treatment alone did not cause such changes are yet be fully understood. However, approximately 30% of alcoholics develop liver diseases[14] and cardiovascular complications[15] which can lead to death. A fatty liver has been claimed to be the most common form of alcoholic liver disease although it has been demonstrated to be reversible with abstinence[16].

If rabbit-to-man extrapolation is permissible, the results of this sub longitudinal study show that the metabolism of oral fructose in the presence of alcohol might involve mechanism(s) that could either prevent or delay the accumulation of TAG (fat) in the liver. However, a longitudinal study is required to establish the probable occurrence.

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