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Ethanol has an acute effect on bile acid biosynthesis in man

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A single dose of ethanol, 0.4 g/kg body weight, was found to give a 5–15 fold increase of the plasma concentrations of 7α-hydroxy-cholesterol and 7α-hydroxy-4-cholesten-3-one in humans. The rise was maximal 4 h after ethanol ingestion, was dose-dependent and was not seen in a cholecystectomized subject. The effect was selective for these and some other 7α-hydroxylated C_{27}-intermediates in bile acid biosynthesis. The changes are compatible with an acute stimulation of cholesterol 7α-hydroxylase possibly due to an ethanol-induced inhibition of gallbladder contraction resulting in an interruption of the enterohepatic circulation of bile acids. The effect is of interest in relation to the influence of ethanol consumption on cardiovascular and gallstone diseases.

Alcohol; Bile acid; Cholesterol; Human; Blood

1. INTRODUCTION

Alcohol has many effects on lipid metabolism in man [1]. An influence on cholesterol and bile acid metabolism may be suspected from the negative correlation between the intake of small daily doses of alcohol and cardiovascular and gallstone disease [1–5]. The mechanisms behind such effects are poorly understood. The elimination of cholesterol and bile acids was unchanged during long-term intake of ethanol in normolipidemic humans [6,7], whereas bile acid formation was inhibited by chronic ethanol feeding in rats [8]. Since ethanol metabolism alters the hepatic redox level [9] and bile acid formation involves NADH-dependent oxidations catalyzed by microsomal 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase/isomerase [10] we studied the effect of a small single dose of ethanol on the levels in plasma of 3β-hydroxy-Δ^5-C_{27}- and 3-oxo-Δ^4-C_{27}-steroids which are potential intermediates in the bile acid biosynthesis. We then observed that ethanol caused a dramatic increase of the levels of the bile acid precursors 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one reaching a maximum about 4 h after the alcohol intake. In this paper, we describe some of the characteristics of this new effect of alcohol in man. Probable mechanisms and possible implications for cholesterol metabolism are discussed.

2. MATERIALS AND METHODS

2.1. *Subjects and experimental conditions*

Four healthy volunteers, two women (GG and AS, 37 and 45 years old, respectively) and two men (MA and JS, 40 and 60 years old, respectively) participated in the study. In addition, one man, 40 years old, who had been cholecystectomized 2 years prior to the study participated. The fasting subjects ingested ethanol (0–0.4 g/kg body weight) in 0.1–0.2 l orange juice as a single dose at about 8.30–9.00 am and a light lunch was permitted about 4.5 h later. In some experiments repeated doses were given. Blood samples were collected in 10 ml heparinized tubes immediately before and at appropriate intervals after the alcohol intake. Following centrifugation, plasma was separated and stored at −20°C until analyzed.

2.2. *Analytical procedures*

2.2.1. *Ethanol.* A small aliquot of plasma was taken for determination of ethanol concentration by gas chromatography on a column of 0.2% Carbowax 1500 on Carbopack C (Supelco Inc., Bellefonte, PA, USA) using *n*-propanol as internal standard [11].

2.2.2. *Cholesterol metabolites.* The analytical procedures have been described previously [12–14]. Briefly, bile acids and their acidic precursors were extracted from plasma (diluted twice with 0.5 M triethylamine-sulphate, pH 7) on a small column of octadecylsilane-bonded silica at 64°C [12]. This sorbent retains bile acids and polar sterols but only little cholesterol, which is the major contaminant. When neutral intermediates were analyzed, plasma was added to ethanol to dissolve steroids and precipitate proteins prior to the liquid-solid extraction step [14]. Group separation and purification of neutral and acid intermediates were achieved on a lipophilic anion exchanger [12]. Excess cholesterol in the neutral fraction was removed by straight-phase chromatography on a Sep-Pak silica cartridge [14]. Following methylation with diazomethane and/or trimethylsilylation, steroid derivatives were analyzed by gas-liquid chromatography and gas chromatography-mass spectrometry using a 25 m fused-silica capillary column coated with cross-linked methyl silicone [12]. High-performance liquid chromatography with UV-detection was employed for quantitation of 7α-hydroxy-4-cholesten-3-one in plasma [13]. Levels of 25-hydroxyvitamin D_3 were determined simultaneously with this method. 7-Dehydrocholesterol was analyzed by a similar method.

2.2.3. *Hormones.* The concentrations of the following hormones were determined by the immunometrical methods in routine use at the clinical chemistry laboratory: glucagon, insulin and C-peptide, cor-

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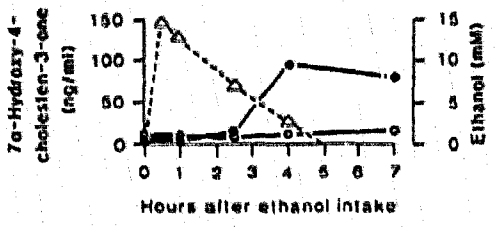

Fig. 1. Concentrations of 7α-hydroxy-4-cholesten-3-one in plasma from a healthy mean (JS, ●—●) given ethanol (0.4 g/kg) in the morning and a woman (AS, ○—○) not taking ethanol. The concentrations of ethanol in blood from JS are also shown (△···△).

tisol, growth hormone (GH), thyrotropin (TSH), prolactin, follitropin (FSH) and lutropin (LH).

2.2.4. *Other plasma components.* Small aliquots of plasma were taken for analyses of glucose, triglycerides, cholesterol, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and γ-glutamyltransferase by a dry slide technique using a Kodak Ektachem 700 Analyzer (Eastman Kodak Co., Rochester, NY, USA).

3. RESULTS AND DISCUSSION

3.1. *Effects of ethanol on bile acid precursors in plasma*

Previously described acute effects of ethanol on steroid metabolism parallel and depend on the alcohol-induced change of the redox state, i.e. the NAD^+/NADH-ratio in the liver [15]. In order to study metabolic relationships between potential bile acid precursors, the levels of compounds constituting redox couples, were measured in plasma before and during the metabolism of ethanol 0.4 g/kg body weight. Instead of an expected decrease of 3-oxo-Δ^4 intermediates, alcohol caused a dramatic increase (5–15 times) of the level of 7α-hydroxy-4-cholesten-3-one reaching a maximum about 4 after the alcohol intake (Fig. 1). The levels remained unchanged in controls given orange juice only. Since the maximal effect occurred several hours after the peak of the blood alcohol curve at a time

Table I

Concentrations of 7α-hydroxy-4-cholesten-3-one in plasma of healthy subjects before and 4 h after a single dose of ethanol

Subject[a]	Ethanol dose[b]	Steroid concentration (ng/ml)	
		Before ethanol	After ethanol
GG	0.1	31	115
GG	0.4	28	140
MA	0.1	21	50
MA	0.4	10	107
AS	0.2	3	25
AS	0.4	3	47
JS	0.2	8	24
JS	0.4	7	96

[a] Details on subjects are given in section 2
[b] g/kg body weight

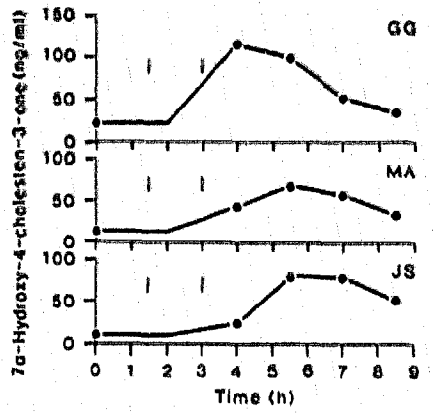

Fig. 2. Concentrations of 7α-hydroxy-4-cholesten-3-one in plasma from 3 healthy subjects (GG, MA and JS) given 3 small doses of ethanol (0.1 g/kg) at 0, 1.5 and 3 h.

when ethanol had almost disappeared, a direct relationship to the redox state of the liver could be excluded. The effect was highly reproducible and was dose-dependent (Table I). As little as 0.1 g ethanol/kg increased the levels of 7α-hydroxy-4-cholesten-3-one.

The response to 3 repetitive small doses of alcohol (0.1 g/kg) varied between subjects from an apparently

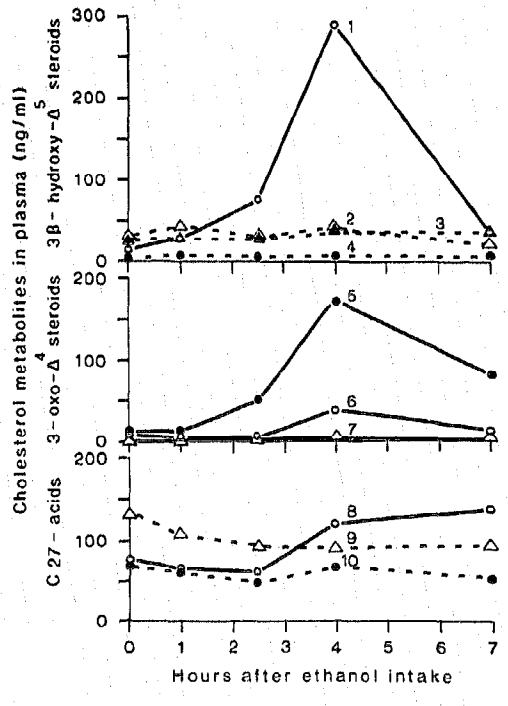

Fig. 3. Concentrations of cholesterol metabolites in plasma from a woman (GG) given ethanol (0.4 g/kg) in the morning. 1 = C^5-3β,7α-ol, 2 = C^5-3β-ol-7-one, 3 = C^5-3β,26-ol, 4 = C^5-3β,7β-ol, 5 = C^4-7α-ol-3-one, 6 = C^4-7α,26-ol-3-one, 7 = C^4-7α,12α-ol-3-one, 8 = CA^4-7α-ol-3-one, 9 = CA^5-3β-ol, 10 = CA^5-3β,7α-ol. For abbreviations, see Table II. Metabolites dependent on cholesterol 7α-hydroxylase for their formation are indicated by solid lines.

TABLE II

Concentrations of steroids in plasma of healthy human subjects (GG,MA,AS,JS)[a] before (0 h), and 4 h after intake of 0.4 g ethanol/kg body weight. In the control experiment, subjects MA*,AS* did not ingest ethanol.

Structures[b]	Steroid concentration in plasma (ng/ml)											
	GG		MA		AS		JS		MA*		AS*	
	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h	0 h	4 h
Unconjugated neutral steroids												
C^5-3β,7α-ol	13	292	15	72	15	66	13	112	14	15	11	7
C^5-3β,7β-ol	5	9	5	6	5	4	7	5	5	5	6	5
C^5-3β-ol-7-one	32	44	18	18	17	14	19	15	10	6	7	16
C^5-3β,26-ol	26	39	35	38	17	22	40	40	41	38	13	28
C^5-3β,7α,26-ol[c]	0.4	1	0.5	0.7	0.2	0.2	0.4	0.3	0.1	0.4	0.2	0.4
C^4-7α-ol-3-one	11	175	10	107	3	47	7	96	14	17	10	12
C^4-7α,12α-ol-3-one[c]	0.9	5	0.3	0.8	0.3	0.4	0.6	0.6	0.6	0.4	0.7	0.6
C^4-7α,26-ol-3-one	10	39	3	9	2	5	6	6	3	4	2	5
$C^{5,7}$-3β-ol	71	44	56	33	24	30	41	36	ND[d]	ND	ND	ND
D_3-25-ol	21	21	18	19	21	23	32	29	17	16	25	25
Unconjugated bile acids												
CA^5-3β-ol	131	90	141	126	70	53	85	74	130	168	99	96
CA^5-3β,7α-ol	67	67	52	53	40	40	30	35	52	58	45	44
CA^4-7α-ol-3-one	75	120	103	157	86	110	81	107	102	132	97	93
5β-B-3α,7α-ol	21	12	103	26	69	10	77	11	24	9	12	5
5β-B-3α,7α,12α-ol	19	6	24	19	77	5	40	7	9	5	8	5
5β-B-3α,12α-ol	183	59	82	50	30	15	116	63	36	12	31	14
Conjugated bile acids												
5β-B-3α,7α-ol	179	38	207	90	46	17	86	57	146	54	50	25
5β-B-3α,7α-12α-ol	81	43	88	134	78	31	37	27	67	48	51	13
5β-B-3α,12α-ol	144	32	45	24	24	13	28	22	47	12	16	10

[a] Details on subjects are given in section 2

[b] C = cholestane, D_3 = cholecalciferol, CA = cholestanoic acid, B = cholanoic acid; superscript indicates position of double bonds; Greek letters denote configuration of hydrogen or hydroxyl groups.

[c] Uncertain analysis due to low levels in plasma

[d] ND = not determined

non-additive to an additive effect, possibly related to the initial response (Fig. 2). Thus, there seemed to be individual differences in the sensitivity to ethanol.

In order to evaluate the selectivity of the ethanol effect, a number of cholesterol metabolites and bile acids were measured. The results, summarized in Table II, showed that only metabolites of 7α-hydroxylated cholesterol increased in parallel with 7α-hydroxy-4-cholesten-3-one (Fig. 3). The absence of changes of other non-polar sterols argues against nonspecific effects on liver cell membrane permeability or plasma lipid elimination. The results are compatible with an increased formation of intermediates in bile acid biosynthesis. Cholesterol 7α-hydroxylase is considered to catalyze the rate-limiting step in this synthesis and 7α-hydroxycholesterol is then oxidized to 7α-hydroxy-4-cholesten-3-one [10]. The latter is transformed to cholic acid via 7α, 12α-dihydroxy-4-cholesten-3-one [10] or to chenodeoxycholic acid via 7α,26-dihydroxy-4-cholesten-3-one and 7α-hydroxy-3-oxo-4-cholestenoic acid [14,16]. The levels of these bile acid intermediates are elevated in plasma of patients with enhanced bile acid production [14,17]. Furthermore, the levels of 7α-hydroxy-cholesterol and 7α-hydroxy-4-cholesten-3-one in plasma have been shown to reflect the activity of cholesterol 7α-hydroxylase in man [13,18,19]. Thus, the observed effect of ethanol is consistent with an acute stimulation of cholesterol 7α-hydroxylase and bile acid synthesis. The effect did not seem to be coupled to a simultaneous increase of the cholesterol biosynthesis, since the level of 7-dehydrocholesterol, which reflects the rate of cholesterol synthesis [20], did not increase (Table II).

3.2. *Mechanisms behind the ethanol effect*

The time-lag between the maximum of alcohol concentrations in blood and the observed increase of levels of bile acid precursors excludes a direct activation of cholesterol 7α-hydroxylase by alcohol, and indicates an induction process possibly involving protein synthesis. Such a process could be triggered by alcohol or its metabolism and be mediated via changes of stimulatory

or inhibitory factors. For this reason, the plasma levels of some hormones were measured. However, the concentrations of gastrointestinal (glucagon, insulin), pituitary (GH, TSH, prolactin, LH, FSH) and adrenal (cortisol) hormones did not seem to be affected by the small doses of ethanol. Levels of other components in plasma, including several liver enzymes, were normal and only small and inconsistent variations were seen with and without alcohol. These analyses also served as tests of normal liver function.

Another possibility was an effect of alcohol on the enterohepatic circulation of bile acids, resulting in a reduced feed-back inhibition of bile acid synthesis. The concentrations of unconjugated and conjugated C_{24} bile acids in plasma decreased during the first 4 hours of the experiments (Table II) but a similar decrease was also observed in the control subjects. Evidence that the gallbladder was involved was obtained from the observation that alcohol did not induce the elevation of bile acid precursor levels in plasma in a cholecystectomized subject (Fig. 4). An inhibitory effect of alcohol on gallbladder contraction is supported by a previous study showing that ethanol, 1 g/kg body weight, causes a marked diminution of bile acid output to the duodenum in man [21]. The present study indicates that this results in an acute interruption of the feed-back inhibition of cholesterol 7α-hydroxylase causing an activation/synthesis of the enzyme.

Acute effects of ethanol on cholesterol and bile acid turnover in man have not previously been reported. Chronic ethanol administration did not change the total excretion of cholesterol and bile acid metabolites in faeces in one study [6] but increased the bile acid excretion in 3 hyperlipidemic subjects in another [7]. While changes of intermediates in bile acid biosynthesis have not been reported, cholesta-4,6-dien-3-one was recently identified at elevated levels in the liver of alcoholics [22]. This compound can be formed in the liver from 7α-hydroxy-4-cholesten-3-one [23], now shown to increase in plasma upon acute intake of small doses of ethanol. Conflicting results on the effect of ethanol on the rate-limiting enzymes have been obtained in animal experiments. The activity of HMG-CoA reductase was found to increase [8] or decrease [24] in rats fed ethanol for 3 weeks or more, whereas the activity of cholesterol 7α-hydroxylase was reduced [24], consistent with a decreased bile acid synthesis [8]. No change of these enzyme activities were seen 2 h after an intraperitoneal injection of 3.2 g ethanol/kg body weight [24]. However, the rat has no gallbladder and since the hepatic handling of cholesterol may differ significantly between animals and man, it is difficult to extrapolate data from one species to another.

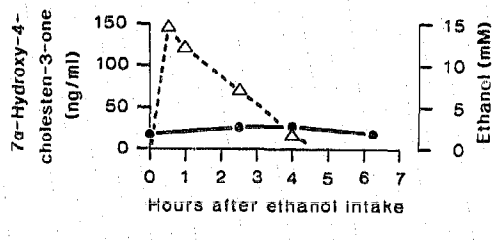

Fig. 4. Concentrations of 7α-hydroxy-4-cholesten-3-one in plasma from a cholecystectomized man (●—●) given ethanol (0.4 g/kg) in the morning. The concentrations of ethanol in blood are also shown (Δ---Δ).

Epidemiologic studies have shown that a moderate alcohol intake reduces risks of both cardiovascular and gallstone diseases (see [2,3,5]). The findings in this study may have some bearing on these observations. If small doses of ethanol inhibit the contraction of the gallbladder this can affect both the feed-back inhibition of bile acid synthesis and cholesterol absorption. When the bile acids are released and return to the liver, there may be a compensatory inhibition of cholesterol 7α-hydroxylase and bile acid synthesis. Changes of the short-term regulation of cholesterol and bile acid biosynthesis may alter the sources of cholesterol used for bile acid synthesis and possibly affect plasma lipoprotein distribution. The relationships between cholesterol and bile acids in bile could conceivably be affected if the relative rates of recirculation and synthesis of bile acids and cholesterol are altered by ethanol.

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