Biochemical Pharmacology, Vol. 38, No. 3, pp. 503–508, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

ENHANCED STIMULATORY EFFECT OF HIGH DENSITY LIPOPROTEINS (HDL) AND OTHER AGONISTS ON VASCULAR PROSTACYCLIN PRODUCTION IN RATS FED ALCOHOL-CONTAINING DIETS*

MAURICIO GUIVERNAU, ENRIQUE BARAONA, JOHNNY SOONG and CHARLES S. LIEBER†
Alcohol Research and Treatment Center, Bronx Veterans Administration Medical Center and Mount Sinai School of Medicine, New York, NY, U.S.A.

(*Received* 4 *April* 1988; *accepted* 5 *July* 1988)

Abstract—Chronic administration of liquid diets containing 36% of energy as ethanol to rats increased the serum level of high density lipoproteins (HDL) by 40% and potentiated 2 to 3-fold the stimulatory effect of these lipoporteins on the production of prostacyclin by aortic rings, as compared to pair-fed controls given isocaloric carbohydrate instead of ethanol. Cross-incubations between aortic rings and HDL from either alcohol-fed or pair-fed control rats revealed two factors operating in opposite directions. On the one hand, the predominant mechanism for the potentiation was increased reactivity of the vessel to these lipoproteins. This increased reactivity was also apparent with other agonists of prostacyclin formation. On the other hand, for equal amounts of either cholesterol or protein, the HDL from alcohol-fed rats were less stimulatory than those from controls. This was associated with a smaller content of arachidonate in the HDL of alcohol-fed rats. These ethanol effects on prostacyclin, a potent vasodilator and platelet antiaggregator, may contribute to the decreased incidence of ischemic heart disease observed in moderate alcohol drinkers.

The possibility of an association between alcohol consumption and decreased incidence of atherosclerosis has been debated since the beginning of the century [1]. Only recently, most of the epidemiologic studies [2, 3] have agreed that moderate alcohol consumption is associated with decreased risk of ischemic heart disease. The mechanism for such an effect remains unknown, but it has been postulated to be linked to the fact that chronic alcohol consumption increases the serum levels of high density lipoproteins (HDL) [4]. The assumption that the increase in serum HDL-cholesterol reflects increased tissue cholesterol mobilization for biliary excretion was not supported by our findings (in alcohol-fed primates) that most of the increase is due to retention of HDL-cholesterol esters in the plasma [5]. The possibility, however, that tissue cholesterol could be mobilized as HDL-free cholesterol remains unsettled [5]. Since it has been shown that these lipoproteins stimulate vascular production of prostacyclin [6–8], a potent vasodilator and platelet antiaggregator [9], we studied the influence of chronic alcohol consumption on this stimulatory effect of HDL.

MATERIALS AND METHODS

Materials. The 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) radioimmunoassay kit was purchased from the Amersham Corp. (Arlington Heights, IL). Sodium arachidonate, cholesterol ester hydrolase, cholesterol oxidase, horseradish peroxidase, sodium cholate, 4-aminoantipyrine and the calcium ionophore A23187 were obtained from the Sigma Chemical Co. (St. Louis, MO). Other materials were obtained as follows: serum cholesterol standards from Beckman Instruments (Palo Alto, CA), Carbowax 6000 from Schwarz-Mann Research Laboratories (Orangeburg, NY) and prostaglandin endoperoxide H_2 from the Cayman Chemical Co. (Ann Harbor, MI). The composition of the liquid diets has been reported previously [10]. They provide 1% fiber and 18% of energy as protein, 35% as lipids, 11% as carbohydrate and 36% either as ethanol or as additional carbohydrate, and were purchased from Dyets, Inc. (Bethlehem, PA).

Animal procedures. Weanling male rat littermates of a Sprague–Dawley strain (CRL-CD(SD)BR) were purchased from the Charles River Breeding Laboratories (Wilmington, MA) and fed rat Chow 5012 diet (Ralston Purina Co., St. Louis, MO) *ad lib.* until they reached a weight of 140–160 g. Then the animals were housed in individual cages and pair-fed the liquid diets described above for 4 weeks. Alcohol-fed animals developed blood ethanol levels of 10–30 mM. To equalize the rate of diet consumption prior to the measurements, one-third of their mean daily ration was administered at 9:00 a.m. and two-thirds at 5:00 p.m. the day before the animals were killed. In addition, 6 ml of the corresponding diets per 100 g body weight was administered by gastric tube 90 min before sacrifice. This provided 1/5 to 1/4 of the usual daily dietary intake. In the alcohol-fed rats, this represented an acute administration of 3 g of ethanol/kg body weight. Rats fed Chow diet *ad lib.* were used as additional controls and also to test the linearity of prostacyclin production with

* Supported by the Veterans Administration and DHHS Grant AA 03508.

† Address for correspondence and reprint requests: Charles S. Lieber, M.D., Alcohol Research and Treatment Center, VA Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468.

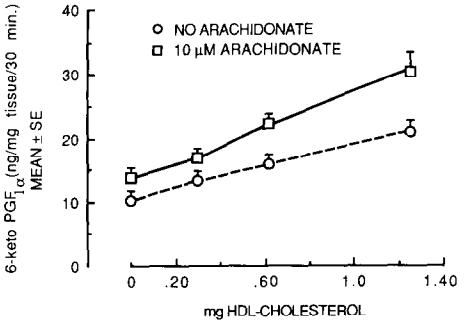

Fig. 1. Effects of HDL concentration and sodium arachidonate supplementation on prostacyclin production by rat aortic rings. Aortae from five Chow-fed rats were incubated with various amounts of HDL (expressed as HDL-cholesterol) with and without supplementation of the medium with 10 μM sodium arachidonate, a concentration previously shown to produce maximal stimulation of prostacyclin production by rat aortic rings [19]. All results are expressed per initial wet weight.

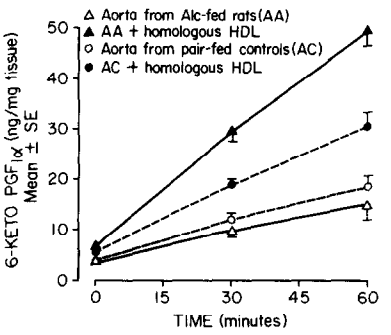

Fig. 2. Effects of homologous HDL and incubation time on prostacyclin production by aortic rings from ten rats pair-fed 36% of energy either as ethanol or additional carbohydrate. Prostacyclin production was assessed by radioimmunoassay of its stable breakdown product, 6-keto $PGF_{1\alpha}$, in aliquots of the medium obtained at various times of incubation. The amount of HDL corresponds to that isolated from 0.6 ml of plasma. Basal production was slightly ($P < 0.05$) lower in alcohol-fed rats, whereas the increase produced by HDL (over basal values) was 3-fold greater ($P < 0.001$) in the alcohol-fed animals.

increasing amounts of tissue and HDL-cholesterol concentrations, with time of incubation, and to assess possible effects of ether anesthesia as compared with decapitation.

Analysis of the blood samples. Blood was drawn under ether anesthesia from the abdominal aorta and collected in tubes containing 1 mg of EDTA, tetrasodium salt, per ml of blood. The plasma was immediately separated by centrifugation at 680 *g* for 20 min at 4°. Duplicate samples of 1.5 ml of plasma were adjusted to a density of 1.300 g/ml by addition of NaCl and KBr, transferred into 5-ml Quick-Seal (R) centrifuge tubes (Beckman), and overlaid with a 1.006 density solution (0.15 M NaCl containing 0.01% EDTA). The tubes were sealed and centrifuged at 360,000 *g* for 45 min at 10° in a Beckman L8-80 ultracentrifuge with a vertical rotor (model VTi 65), as described by Chung *et al.* [11]. After centrifugation, the fractions were recovered by piercing the bottom of the tube with a needle in a Beckman Fraction Recovery System and pushing the contents from below with a KBr solution of density 1.346 g/ml. The optimal elution conditions were established by vertical gradient centrifugation of the lipoprotein fractions isolated by sequential centrifugation and stained with activated Fat Red 7B, as previously reported [5]. Moreover, in every animal, the cholesterol profile of the elution fractions, which permits a clear identification of the low density lipoproteins (LDL) and HDL, was determined with cholesterol oxidase [12]. As previously reported [5], all the serum cholesterol was recovered in a major HDL fraction, a minor LDL fraction, and only traces in very low density lipoproteins (VLDL). The zone of overlapping between the LDL and the HDL peaks was determined by triangulation and excluded from the HDL fraction. The addition of 30 mM ethanol to aliquots of pooled plasma from chow-fed rats prior to the ultracentrifugation did not change the recovery of HDL-cholesterol (626.1 ± 10.3 mg/ml plasma vs 645.9 ± 39.4, without ethanol; df 7; NS). HDL fractions were pooled and dialyzed overnight against Krebs–Ringer bicarbonate buffer, pH 7.4. In the experiments done with Chow-fed rats, which involved the use of multiple HDL concentrations, we pooled plasma from similar rats for the isolation of large amounts of HDL. In the experiments done on pair-fed animals, HDL were prepared from individual members of each pair and used the following day on the aorta of homologous and heterologous members of the next pair of littermates. Their protein content was determined by the method of Lowry *et al.* [13]. HDL lipids were extracted by the method of Bligh and Dyer [14] and dried under nitrogen. In five controls and five alcohol-fed rats, cholesterol esters were separated by thin-layer chromatography [15]. Methanolysis of cholesteryl esters with boron fluoride–methanol was carried out at 100° for 45 min according to Morrison and Smith [16], and arachidonate was measured by gas liquid chromatography using pentadodecanoic acid as internal standard.

In vitro *studies.* The aortae were immediately dissected from the iliac bifurcation up to the arch, washed in saline, trimmed free of fat and connective tissue, and manually cut to produce approximately 1 mm thick rings. Aortic rings, weighing 6–12 mg, were incubated at 37°, under 95% O_2 and 5% CO_2, in sealed 25-ml flasks containing 2.5 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, [17], with or without HDL, for 30 min. At 30 min, aliquots of the incubation medium were immediately frozen in liquid nitrogen and stored at −20° for the radioimmunoassay of 6-keto-$PGF_{1\alpha}$ [18]. Dilutions of 1:15, 1:30 and 1:60 were prepared to produce concentrations of 6-keto-$PGF_{1\alpha}$ that fell in the linear part of the radioimmunoassay. Results were expressed per mg of initial wet weights. Wet weight/dry weight ratios of aortic rings from alcohol-fed rats were similar to those of controls (2.74 ± 0.05 vs 2.85 ± 0.08, in controls; df 15; NS).

In five Chow-fed rats, the effects of several

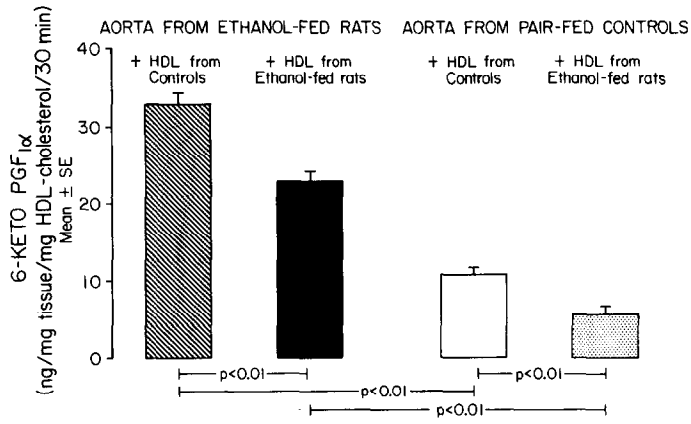

Fig. 3. Contribution of HDL and aortic response to the stimulatory effect of chronic alcohol consumption on vascular prostacyclin production. The results indicate the increases (over basal production) of 6-keto $PGF_{1\alpha}$, expressed as ng per mg of tissue and per mg HDL-cholesterol during 30 min. Two-way ANOVA revealed significant effects ($P < 0.01$; df 1,20) of both the source of the aorta and the source of HDL.

concentrations of HDL-cholesterol on aortic prostacyclin production were measured with and without *in vitro* supplementation of the medium with 10 μM sodium arachidonate, a concentration previously shown to produce maximal stimulation of prostacyclin production [19]. All other studies were done without arachidonate supplementation.

In a first series of ten pair-fed littermates, the vessels were incubated with homologous HDL in amounts equivalent to those existing in 0.6 ml of plasma. After demonstration of linearity with the concentration of HDL and the existence of a greater content of HDL in the plasma of alcohol-fed rats, all subsequent comparisons were done with equal amounts of HDL-cholesterol.

To distinguish whether the differences in vascular protacyclin production between alcohol-fed and control animals were due to the stimulatory capacity of HDL or to the response of the aorta, we incubated aortae from ten pair-fed animals with equal amounts of HDL obtained from either ethanol-fed or control rats.

To investigate the mechanism of the enhanced reactivity of the aorta from alcohol-fed animals, we compared the response of five of these vessels to other agonists of prostacyclin production with that of the corresponding pair-fed controls. We used either a phospholipase A_2 agonist, such as the calcium ionophore A23187 (10 μM at 37° for 30 min), or the immediate precursor of prostacyclin, namely prostaglandin endoperoxide H_2 (PGH_2), under conditions (5 μg/ml at 22° for 5 min) that take into account the instability of this compound [20].

Statistics. Results are expressed as means ± SE. Values obtained in the alcohol-fed animals were compared to those obtained in their pair-fed controls, and the mean of the individual differences was tested by Student's *t*-test (paired comparisons) [21]. When more than one treatment was applied to the same pair-fed animals, the significance of the differences was tested by two-way analysis of the variance [21].

RESULTS

The addition of HDL to the medium increased the production of prostacyclin by aortic rings in proportion to the amount of tissue (not shown), the amount of HDL (Fig. 1) and the duration of incubation (Fig. 2). The stimulatory effect of HDL persisted even in the presence of maximally stimulatory concentrations of sodium arachidonate (Fig. 1). There was no difference in prostacyclin production between the animals killed by decapitation and those killed under ether anesthesia.

The basal production of prostacyclin, in the absence of HDL, was somewhat less in aortic rings from alcohol-fed rats than in those from controls (Fig. 2). The addition of homologous HDL increased prostacyclin production both in alcohol-fed and control rats. However, the increase produced by HDL from equal volumes of serum in aortae from alcohol-fed rats was almost 3-fold greater than in controls. In the serum of alcohol-fed rats, we found a 40% increase in HDL when expressed either as HDL-cholesterol or as HDL-protein. The 40% increase in the amount of HDL per volume of serum clearly did not account for the approximately 300% increase in prostacyclin production found in alcohol-fed animals.

Per equal amount of cholesterol (or protein), the HDL from alcohol-fed rats stimulated prostacyclin production less than those from controls, in either aortae from ethanol-fed rats or aortae from controls (Fig. 3). The lesser stimulatory capacity of the HDL from alcohol-fed rats was associated with decreased amounts of arachidonate per HDL-cholesterol (Table 1). HDL-cholesteryl esters from alcohol-fed rats were impoverished in palmitate (C16:0) and arachidonate (C20:4) and enriched in oleate (C18:1) and linoleate (C18:2) (Table 2). However, when we compared the vessel response, the increase in prostacyclin produced by equal amounts of HDL-cholesterol was much greater in aortae from ethanol-fed rats than in aortas from pair-fed controls, regard-

Table 1. Effects of chronic ethanol administration on HDL*

	HDL-protein (mg/ml plasma)	HDL-cholesterol (mg/ml plasma)	HDL-arachidonate (mg/ml cholesterol)
Ethanol-fed rats	2.00 ± 0.15 (8)	1.41 ± 0.08 (8)	0.22 ± 0.04 (5)
Pair-fed controls	1.43 ± 0.08 (8)	1.01 ± 0.05 (8)	0.38 ± 0.08 (5)
P†	< 0.01	< 0.01	< 0.05

* Values are means ± SE (number of pairs of rats is given in parentheses) in rats pair-fed liquid diets containing 36% of energy either as ethanol or as carbohydrate for 4 weeks.
† Paired comparisons: Student's *t*-test.

Table 2. Effects of chronic ethanol administration on the fatty acid composition of HDL-cholesteryl esters*

	Fatty acid (%)				
	C16:0	C18:0	C18:1	C18:2	C20:4
Ethanol-fed rats	11.6 ± 3.5	3.5 ± 0.9	13.9 ± 0.7	20.4 ± 3.5	49.3 ± 0.7
Pair-fed controls	17.2 ± 0.7	5.1 ± 1.0	9.3 ± 1.6	7.3 ± 1.7	61.3 ± 3.2
P†	< 0.01	NS	< 0.05	< 0.01	< 0.01

* Values are means ± SE of the percentage of the fatty acids in ten rats pair-fed 36% of energy either as ethanol or as additional carbohydrate for 4 weeks.
† Paired comparisons; Student's *t*-test (df 4). NS = not significant.

Table 3. Effects of chronic ethanol administration on the response of aortic prostacyclin production to calcium ionophore and prostaglandin endoperoxide H_2*

	Incubation time (min)	6-keto $PGF_{1\alpha}$ (ng/mg tissue)		
		Ethanol-fed rats	Pair-fed controls	P†
(A) A23187				
0 μM	30	10.1 ± 0.4	11.8 ± 0.3	< 0.05
10 μM	30	16.8 ± 0.5	14.3 ± 0.8	< 0.05
% Increase		66 ± 2	22 ± 6	< 0.02
(B) PGH_2				
0 μg/ml	5	4.3 ± 0.3	4.6 ± 0.2	NS
5 μg/ml	5	28.8 ± 1.6	19.5 ± 0.8	< 0.02
% Increase		567 ± 36	323 ± 21	< 0.02

* Values are means ± SE in two series of twelve rats pair-fed 36% of energy either as ethanol or as carbohydrate for 4 weeks.
† Paired comparisons; Student's *t*-test (df 5). NS = not significant.

less of the sources of HDL (Fig. 3). The aortic rings from rats fed Chow diet *ad lib.* behaved similarly to those from the pair-fed controls.

As expected, both the calcium ionophore and the prostaglandin endoperoxide H_2 increased prostacyclin formation (Table 3). However, the magnitude of the increase produced by both agonists was significantly greater in aortae from alcohol-fed rats than in those from pair-fed controls.

DISCUSSION

The results of the present study indicate that chronic ethanol administration markedly increases the reactivity of the vessels to agonists of prostacyclin production, including HDL. The serum concentration of these lipoproteins increases after chronic alcohol consumption both in humans [4] and in rats [22, 23]. However, the magnitude of the increase in vascular prostacyclin production induced by incubation of aortic rings from alcohol-fed rats was much greater than that accounted for by the increased serum levels of HDL and persisted even when the amounts of HDL-cholesterol were equalized. This suggested a change in the stimulatory capacity of the HDL, in the reactivity of the vessel, or in both. Alcohol feeding changed both factors in opposite directions.

Unexpectedly, the stimulatory capacity of the HDL of alcohol-fed rats was less than that of HDL

from control rats (Fig. 3). The mechanism for the decreased stimulatory capacity of the HDL from alcohol-fed animals is not fully elucidated. One feature associated with this difference was the relative deficiency in arachidonate of the HDL from alcohol-fed rats. Since the liquid diets used in this study provide linoleic but not arachidonic acid, ethanol could decrease the supply of this precursor by inhibiting hepatic desaturase activities [24, 25] responsible for the conversion of linoleic into arachidonic acid. However, it is not clear whether the stimulatory effect of HDL is due solely to its capacity to serve as a non-specific carrier of arachidonate. In fact, the stimulation persisted after supplemention of the medium with optimal concentrations of sodium arachidonate (Fig. 1). Both the lipid and the protein moieties of HDL were required for optimal stimulation of prostacyclin production by cultured endothelial cells [6]. It has been suggested that the arachidonate from cholesteryl esters may enter a cellular phospholipid pool more readily available for prostaglandin synthesis, since incorporation into prostaglandins was considerably greater when HDL were labeled with arachidonate esterified with cholesterol than with phospholipids [8].

Whatever the mechanism for the decreased stimulatory capacity of the HDL, this effect was fully offset by the much larger increase in the capacity of the vessels from alcohol-fed animals to respond to HDL, regardless of their origin (Fig. 3). This enhanced reactivity extended to the effects of other agonists of prostacyclin production (Table 3), suggesting that the changes in vascular reactivity induced by chronic alcohol consumption could be due, at least in part, to an increase in the activity of the prostacyclin synthetic pathway. The relatively modest increase in the response to the calcium ionophore, as compared to that of HDL, is consistent with the view proposed by others [8] that the arachidonyl cholesterol esters of HDL, by entering a special phospholipid pool, may be a highly efficient substrate for prostaglandin synthesis. Possible changes in endothelial permeability or in HDL receptors also need to be assessed. The small decrease in basal production of prostacyclin (in the absence of exogenous substrates) by aortic rings from ethanol-fed rats has been a constant feature in this, as well as in previous studies [19]. One possible explanation for this difference could be more rapid depletion of endogenous substrates due to the higher capacity for prostacyclin synthesis of the vessels from alcohol-fed rats, since such a difference disappeared when the medium was supplemented with exogenous arachidonate [19].

An increase in the activity of enzymes participating in prostacyclin synthesis after chronic alcohol intake could occur as a result of cross-induction of microsomal cytochrome P-450-dependent mixed-function oxidases, such as the prostacyclin synthase [26] and the microsomal ethanol-oxidizing system (MEOS) [27]. Increases in MEOS activity are well documented not only in the liver, but also in extrahepatic tissues such as the small intestine [28] and the lungs [29]; this remains to be studied in vascular tissue. In support of this hypothesis, we have found recently that ethanol, which lacks the capacity to stimulate vascular prostacyclin formation in untreated animals, acquires such an effect after chronic alcohol administration; the latter action may be mediated by vascular oxidation of ethanol to acetaldehyde [19].

These findings, although observed *in vitro*, may have important implications for some of the *in vivo* vascular effects of alcohol consumption, which are probably multifactorial [30]. It is noteworthy that these effects of alcohol consumption on endothelial prostacyclin production, as well as on the diameter of coronary arteries [31], are opposite to those produced by cigarette smoking [32, 33]. In moderate drinkers, the effect of ethanol itself [19] could be added to the possible benefit of HDL-mediated stimulation of vascular prostacyclin production on the incidence of ischemic heart disease. In severe alcoholics, the benefit of drinking on ischemic heart disease disappears [2]. The lesser increase of serum HDL [34] and the decreased availability of arachidonate due to inhibition of the hepatic desaturase activities [35] in the heavy drinker may contribute to this dual effect of alcohol consumption.

REFERENCES

1. Cabot RC, The relation of alcohol on arteriosclerosis. *J Am Med Assoc* **43**: 774–775, 1904.
2. Yano K, Rhoads GG and Kagan A, Coffee, alcohol and risk of coronary heart disease among Japanese men living in Hawaii. *N Engl J Med* **297**: 405–409, 1977.
3. Klatsky AL, The cardiovascular effects of alcohol. *Alcohol Alcoholism* (Suppl 1): 117–124, 1987.
4. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A and Zukel WJ, Alcohol and blood lipids. The cooperative lipoprotein phenotyping study. *Lancet* **2**: 153–155, 1977.
5. Karsenty C, Baraona E, Savolainen MJ and Lieber CS, Effects of chronic ethanol intake on mobilization and excretion of cholesterol in baboons. *J Clin Invest* **75**: 976–986, 1985.
6. Fleisher LN, Tall AR, Witte LD, Miller RW and Cannon PJ, Stimulation of arterial endothelial cell prostacyclin synthesis by high density lipoproteins. *J Biol Chem* **257**: 6653–6655, 1982.
7. Pomerantz KB, Tall AR, Feinmark SJ and Cannon PJ, Stimulation of vascular smooth muscle cell prostacyclin and prostaglandin E_2 synthesis by plasma high and low density lipoproteins. *Circ Res* **54**: 554–565, 1984.
8. Pomerantz KB, Fleisher LN, Tall AR and Cannon PJ, Enrichment of endothelial cell arachidonate by lipid transfer from high-density lipoproteins: Relationship to prostaglandin I_2 synthesis. *J Lipid Res* **26**: 1269–1276, 1985.
9. Moncada S and Vane JR, Pharmacology and endogenous roles of prostacyclin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol Rev* **30**: 293–331, 1979.
10. Lieber CS and DeCarli LM, The feeding of alcohol in liquid diets: 1986 update. *Alcoholism Clin Exp Res* **10**: 550–553, 1986.
11. Chung BH, Wilkinson T, Geer JC and Segrest JP, Preparative and quantitative isolation of plasma lipoproteins: Rapid discontinuous density gradient ultracentrifugation in a vertical rotor. *J Lipid Res* **21**: 284–291, 1980.
12. Allain CC, Poon LS, Chan CSG, Richmond W and Fu PC, Enzymatic determination of total serum cholesterol. *Clin Chem* **20**: 470–475, 1974.
13. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.

14. Bligh EG and Dyer WJ, A rapid method for total lipid extraction and purification. *Can J Biochem Physiol* **37**: 911–917, 1959.
15. Amenta JS, A rapid chemical method for quantification of lipids separated by thin-layer chromatography. *J Lipid Res* **5**: 270–272, 1964.
16. Morrison WR and Smith LM, Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. *J Lipid Res* **5**: 600–608, 1964.
17. DeLuca HF and Cohen PP, Suspending media for animal tissues. In: *Manometric Techniques* (Eds. Umbreit WW, Burris RH and Stauffer JF), pp. 131–133. Burgess Publishing, Minneapolis, MN, 1964.
18. Granstrom E and Kindahl H, Radioimmunoassay of prostaglandins and thromboxane. In: *Advances in Prostaglandin and Thromboxane Research* (Ed. Frolich JC), Vol. 5, pp. 119–210. Raven Press, New York, 1978.
19. Guivernau M, Baraona E and Lieber CS, Acute and chronic effects of ethanol and its metabolites on vascular production of prostacyclin in rats. *J Pharmacol Exp Ther* **240**: 59–64, 1987.
20. Moncada S, Gryalewsky R, Bunting S and Vane JR, An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* **263**: 663–665, 1976.
21. Snedecor GW and Cochran WC, *Statistical Methods*, 7th Ed. The Iowa State University Press, Ames, IA, 1980.
22. Baraona E and Lieber CS, Effects of chronic ethanol feeding on serum lipoprotein metabolism in the rat. *J Clin Invest* **49**: 769–778, 1970.
23. Hirayama C, Nosaka Y, Yamada S and Yamanishi Y, Effect of chronic ethanol administration on serum high density lipoprotein cholesterol in rat. *Res Commun Chem Pathol Pharmacol* **26**: 563–569, 1979.
24. Nervi AM, Peluffo RO, Brenner RR and Leikin AJ, Effect of ethanol administration on fatty acid desaturation. *Lipids* **15**: 263–268, 1980.
25. Wang DL and Reitz RC, Ethanol ingestion and polyunsaturated fatty acids: Effects on the acyl-CoA desaturases. *Alcoholism Clin Exp Res* **7**: 220–226, 1983.
26. Ullrich V, Castle L and Weber P, Spectral evidence for the cytochrome P450 nature of prostacyclin synthetase. *Biochem Pharmacol* **30**: 2033–2036, 1981.
27. Lieber CS and DeCarli LM, Hepatic microsomal ethanol-oxidizing system. *In vitro* characteristics and adaptive properties *in vivo*. *J Biol Chem* **245**: 2505–2512, 1970.
28. Seitz HK, Korsten MA and Lieber CS, Ethanol by intestinal microsomes: Increased activity after chronic ethanol administration. *Life Sci* **25**: 1443–1448, 1979.
29. Pikkarainen PH, Baraona E, Jauhonen P, Seitz HK and Lieber CS, Contribution of oropharynx microflora and of lung microsomes to acetaldehyde in expired air after alcohol ingestion. *J Lab Clin Med* **97**: 631–636, 1981.
30. Altura BM and Altura BT, Peripheral and cerebrovascular actions of ethanol, acetaldehyde, and acetate: Relationship to different cations. *Alcoholism Clin Exp Res* **11**: 99–111, 1987.
31. Barboriak JJ, Anderson AJ and Hoffmann RG, Smoking, alcohol and coronary artery occlusion. *Atherosclerosis* **43**: 277–282, 1982.
32. Fried LP, Moose RD and Pearson TA, Long-term effects of cigarette smoking and moderate alcohol consumption on coronary artery diameter. Mechanisms of coronary artery disease independent of atherosclerosis or thrombosis? *Am J Med* **80**: 37–44, 1986.
33. Reinders JH, Brinkman HJM, van Mourik JA and de Groot PJ, Cigarette smoke impairs endothelial cell prostacyclin production. *Arteriosclerosis* **6**: 15–23, 1986.
34. Duhamel G, Nalpas B, Goldstein S, Laplaud PM, Berthelot P and Chapman MJ, Plasma lipoproteins and apolipoprotein profile in alcoholic patients with and without liver disease: On the relative role of alcohol and liver injury. *Hepatology* **4**: 577–585, 1984.
35. Johnson SB, Gordon E, McClain C, Low G and Holman RT, Abnormal polyunsaturated fatty acid patterns of serum lipids in alcoholism and cirrhosis: Arachidonic acid deficiency in cirrhosis. *Proc Natl Acad Sci USA* **82**: 1815–1818, 1985.