Eur J Nutr (2005) 44 : 157–162
DOI 10.1007/s00394-004-0507-x

ORIGINAL CONTRIBUTION

Alexandr Parlesak
Kathi Ellendt
Kai O. Lindros
Christiane Bode

Acute but not chronic ethanol exposure impairs retinol oxidation in the small and large intestine of the rat

Received: 19 August 2003
Accepted: 31 March 2004
Published online: 11 May 2004

A. Parlesak, Ph.D. (✉) · K. Ellendt, M.Sc. · C. Bode, Ph.D.
Hohenheim University (140)
Dept. Physiology of Nutrition
Garbenstraße 28
70593 Stuttgart, Germany
Tel.: +49-711/459-4184
Fax: +49-711/459-3947
E-Mail: parlesak@uni-hohenheim.de

K. O. Lindros, Ph.D.
National Public Health Institute
Alcohol Research Center
Helsinki, Finland

Summary *Background and aim* Ethanol has been shown to inhibit retinol oxidation at the level of alcohol dehydrogenase in liver and colon but not previously in the small intestine. In the present study we investigated how chronic alcohol feeding and acute ethanol exposure affects retinol dehydrogenase activity in the colon and small intestine of the rat. *Methods* Rats were fed ethanol in a liquid diet for six weeks. Control rats received a similar diet but with ethanol isocalorically replaced by carbohydrates. Retinol dehydrogenase was analyzed from cell cytosol samples from the small and the large intestine with respect to maximum activity (V_{max}), Michaelis-Menten constant (K_m), and inhibition by ethanol (2–43 mM) in vitro. *Results* Both the V_{max} and the catalytic efficiency (V_{max}/K_m) were found to be significantly higher in the colon than in the small intestine (2.9–3.6 and 54–70 times higher, respectively). While chronic alcohol feeding did not affect these parameters, acute ethanol exposure reduced V_{max} and V_{max}/K_m dose-dependently ($p < 0.001$) in both intestinal segments. *Conclusions* The present data demonstrate that ethanol markedly inhibits *in vitro* cytosolic retinol oxidation in the small intestinal mucosa, which is considerably lower than that found in the colon. Considering the vital importance of retinol on intestinal integrity, our finding suggests that this might contribute to the ethanol-induced increase in intestinal permeability.

Key words ethanol – retinol oxidation – alcohol dehydrogenase – chronic alcohol feeding – intestine

Introduction

Vitamin A (retinol) is required for the integrity and proper function of epithelial cells of the intestine. Hypovitaminosis A leads to impaired cell differentiation of enterocytes and to reduced protein synthesis by the intestinal mucosa [1–3]. For biological function, intracellular oxidation of retinol to retinoic acid is required [4]. Retinoic acid acts by binding either to retinoic acid receptors (RAR α, β, γ) or in its 9-cis form to retinoid X receptors (RXR α, β, γ) [5].

Retinol is a substrate for cytosolic alcohol dehydrogenases (ADH, EC 1.1.1.1) both in rats [6] and humans [7]. The crucial role of ADH in the oxidation of retinol has been demonstrated in several studies [8]. Thus inhibition of ADH markedly reduces retinoic acid synthesis in rodents [9] and mice lacking genes encoding ADH1 ($Adh1^{-/-}$) (nomenclature as suggested by Duester [10]) or ADH4 ($Adh4^{-/-}$) have significantly reduced plasma levels of retinoic acid [11]. Although previous studies indicated that ADH3 was not involved in the catalysis of retinol oxidation [6, 7, 12], recent experiments with $Adh3^{-/-}$ mice also demonstrate an important role of

European Journal of Nutrition (2005) Vol. 44, Number 3

ADH3 in retinoic acid synthesis from retinol in vivo [13, 14].

The inhibitory effect of ethanol at concentrations occurring after social drinking in man on intracellular retinol oxidation has been shown for liver enzymes of man and rats [7, 12, 15, 16] and for retinol dehydrogenases in the colon mucosa of the rat [16]. From these experiments, an ethanol-induced limited availability of retinoic acid as a result of a reduced retinol oxidation was suggested. However, to our knowledge, no information is available on the effect of chronic alcohol feeding on changes in the capability of mucosal cells of the small and large intestine on cytosolic retinol oxidation.

Chronic alcohol consumption leads to an enhanced intestinal permeability to macromolecules such as endotoxin [17], which was hypothesized to contribute significantly to the development of alcohol-induced liver disease [18, 19]. Due to the crucial role of an intact retinol metabolism in proper mucosal function, a causative role of the ethanol-induced impairment of retinol oxidation seems plausible. Therefore, this study was conducted to investigate the effect of both acute ethanol exposure in vitro and chronic alcohol feeding on the enzyme activity of the cytosol of mucosal cells from the small and the large intestine of male Wistar rats.

Animals, materials and methods

Animals

The design of the chronic ethanol feeding study and the diet composition has been described in detail before [20]. The study was approved by the Committee of Animal Experimentation of the National Public Health Institute, Helsinki. In brief, twelve adult male Wistar rats (Mollegaard, Ejby, Denmark; 220 to 240 g) were individually kept in cages with stainless steel wire bottoms. Animals (n = 6) received either an alcohol-containing diet (34.5 % of calories) with a high content of fat (44 %) and a low percentage of carbohydrates (5.5 %) or a control diet in which ethanol was replaced isocalorically by maltodextrose (40 % in total) [21]. Both of the diets contained 16 % of calories as protein. Control and ethanol-receiving animals were pair-fed for six weeks. For organ removal, animals were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg). The small and the large intestine were washed extensively with ice-cold KCl solution (1.15 % m/v) and stored at –70 °C until further proceeding.

Tissue preparation and incubation experiments

The isolated mucosal cells were homogenized in 12 ml ice-cold KCl solution and centrifuged (105,000 × g, 60 minutes, 4 °C). The supernatant (termed consequently as 'cytosol') was used for the measurement of retinol dehydrogenase activity. Protein concentration in the cytosol was determined by the method of Smith [22] using bovine albumin as standard.

Retinol dehydrogenase activity was measured as described earlier [16]. Retinol was suspended in an aqueous albumin solution (65 mg/mL) (both: Sigma, Taufkirchen, Germany) and added to the buffered cytosol solution with 2.5 mM NAD^+ [16] to give a final concentration of 11, 22, 44, 88, 175, and 349 µM. Samples (total volume of 500 µL) were incubated either without alcohol or with 2.7 mM (0.0125 % m/v), 11 mM (0.05 %), and 43 mM (0.20 %) ethanol for 30 minutes at 37 °C. The reaction was stopped by addition of 50 µL of an acidic stock solution [16] and 600 µL of ethanol (all organic solvents: Merck, Darmstadt, Germany) with tetramethylbutylphenol (TMBP) as internal standard.

Analysis of retinoids

Retinoids and TMBP were extracted with 700 µL of toluene containing 1 mg/mL 2,6-di-tertbutyl-p-cresol (Sigma, Steinheim, Germany) to prevent autoxidation and analyzed by high performance liquid chromatography (HPLC) (Gynkotek, Germering, Germany) [16].

Data evaluation and statistics

In experiments with identical ethanol concentrations, V_{max} and K_m values were calculated from the single specific enzyme activities at each retinol concentration by applying non-linear regression (Levenberg-Marquardt algorithm) presuming the reaction follows the rules of Michaelis and Menten. Constants for uncompetitive (K_{iu}) and competitive (K_{ic}) inhibition were calculated as described previously [16, 23].

If not indicated otherwise, all data are given as means ± standard error of the mean (SEM). After logarithmic transformation (to achieve homogeneity of variances), values were compared by analysis of variances (ANOVA; within-subject design for determination of differences induced by ethanol in the incubation medium) and Tukey's HSD post hoc test. Comparisons of data originating from the control and ethanol group were done with the Mann-Whitney U test.

Results

During incubation for the determination of retinol dehydrogenase activity, average applied protein concentrations were 13.3 ± 2.9 mg/mL in samples with cytosol from the small intestine and 1.47 ± 0.52 mg/mL in those

with cytosol from the colon. Specific retinol dehydrogenase activity at substrate saturation (V_{max}) was significantly lower in the small intestine compared to that of mucosal cytosol from the colon (Table 1). In both feeding groups, the Michaelis-Menten constant (K_m) was about 20-fold higher in the small intestine compared to that measured in the colon (Table 1). Consequently, the difference in catalytic efficiency (V_{max}/K_m) was even more pronounced (about 50 times/70 times higher in the colon than in the small intestine of rats from the control group or the alcohol feeding group, respectively).

When ethanol was added to the incubation medium, V_{max} decreased dose-dependently and K_m increased with increasing ethanol concentrations (Table 1, Fig. 1). Consequently, V_{max}/K_m decreased markedly in both the small intestine (at 43 mM ethanol: –68 % in controls, –75 % in alcohol-fed animals) and the colon (at 43 mM ethanol: –92 % in both controls and ethanol-fed rats) (Fig. 2). In both feeding groups, non-competitive and competitive inhibition constants increased with increasing ethanol concentration in both intestinal segments (Table 1). Both inhibitory constants were lower in the colon.

Chronic alcohol feeding had no significant effects on either the K_m or V_{max} of retinol oxidation catalyzed by the cytosol of mucosal cells from the small intestine and the colon at all applied ethanol concentrations (Table 1). Although moderately lower, V_{max}/K_m of retinol oxidation turned out to be insignificantly ($p > 0.4$) changed in cells of the small intestine of animals from the ethanol-fed group compared to that of the control animals (Fig. 2). No difference ($p > 0.9$) was measured when V_{max}/K_m of colon cells from both groups were compared (Fig. 2).

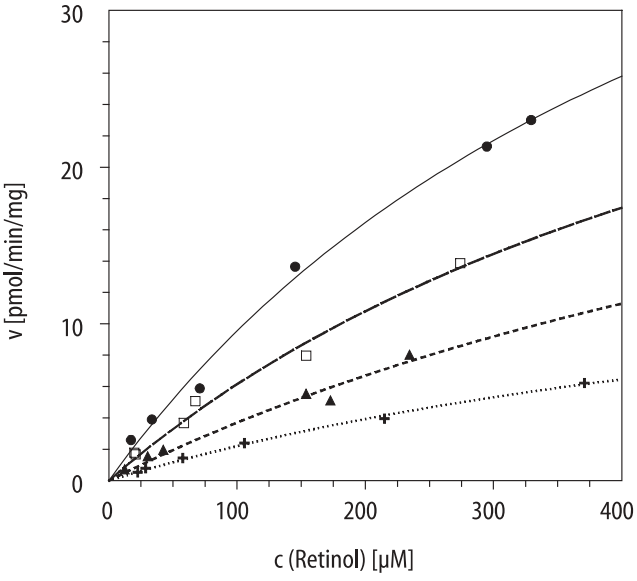

Fig. 1 Dose-dependent inhibition of retinol oxidation in small intestinal cytosol from mucosa of a rat from the control group. The graph shows the specific retinol dehydrogenase activity without alcohol (● solid line), with 2.7 mM (□ long-dashed line), with 11 mM (▲ short-dashed line), and with 43 mM (+ dotted line) ethanol

Discussion

In contrast to previous studies [16], in the present experiments we were able to measure both V_{max} and K_m of retinol oxidation in the cytosol of cells from the small intestine. The present data demonstrate a considerably higher activity of retinol dehydrogenase in the cytosol of the colon mucosa as compared to that of the small intestine. Within the cytosol, retinol is nearly completely bound to cellular retinol binding protein (CRBP I) [28]. As CRBP-bound retinol is not a substrate for ADH [29], out of the total cytosolic concentration of about 5 µM,

Table 1 Enzyme activities at substrate saturation (V_{max}) and Michelis-Menten constants (K_m) of retinol oxidation in the cytosol of the mucosa from the rat's small and large intestine depending on ethanol concentrations [c(EtOH)] added to the incubation medium. Values (means ± SEM) are given for both animals from the control group ('Con') and from the ethanol-fed group ('EtOH') . No effect of chronic alcohol feeding on V_{max} or K_m was observed, but the presence of ethanol in the incubation medium decreased V_{max} and increased K_m in the cytosol of both intestinal segments significantly (within-group comparisons vs. no addition of ethanol: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) . K_{iu} and K_{ic} noncompetitive and competitive inhibition constants

		Small intestine				Large intestine			
c(EtOH) [mM]	Group	V_{max} [pmol/min/mg]	K_m [µM]	K_{iu} [mM]	K_{ic} [mM]	V_{max} [pmol/min/mg]	K_m [µM]	K_{iu} [mM]	K_{ic} [mM]
0	Con	40.6±13.6	475±90			116±29.5	26.3±3.0		
	EtOH	30.4±9.6	486±71			109±28.7	22.4±1.1		
2.7	Con	35.0±11.0	622±99**	17.0	5.2	57.0±15.1***	29.5±1.7	2.6	2.1
	EtOH	26.0±6.6	671±77	16.1	4.4	55.4±15.0***	32.3±1.5*	2.8	1.5
11	Con	29.4±10.4***	638±110**	28.7	12.8	41.0±11.0***	45.8±2.8**	5.9	2.8
	EtOH	24.3±9.1*	756±63*	43.0	11.5	37.6±10.6***	46.1±2.2***	5.7	2.2
43	Con	21.8±8.4***	755±138***	50.2	22.1	24.5±6.5***	74.6±7.8***	11.6	3.5
	EtOH	21.3±6.9*	1326±181***	101	15.0	22.3±6.0***	64.4±7.5***	11.2	3.3

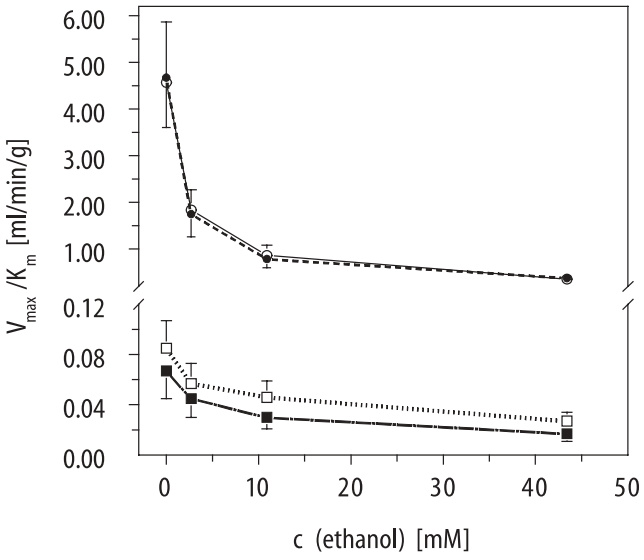

Fig. 2 Effect of ethanol addition on the catalytic efficiency V_{max}/K_m [ml/min/g] of retinol oxidation by the cytosol obtained from colon mucosa from animals fed the control diet (○ solid line) and those fed the ethanol diet (● dashed line) and from the small intestine of rats fed the control diet (□ dotted line) and those fed the ethanol diet (■ dashes and dots). Addition of ethanol resulted in highly significant depression of catalytic efficiency at all ethanol concentrations (ANOVA for both intestinal segments: $p < 0.001$)

only 2.5 nM are in the unbound state [30, 31] and therefore available for ADH-mediated oxidation. To approach the binding of retinol to CRBPI within the cell, in contrast to previous studies [16] we applied retinol bound to protein as substrate in the present assay. This resulted in a decreased V_{max} of cytosolic retinol oxidation in the colon, but, evidently, the reduction of V_{max} by acute exposure to ethanol occurs independently of the applied formulation of retinol (Table 1). With respect to the low concentration of free retinol in the cell, the catalytic efficiency (V_{max}/K_m), which represents the slope of the enzyme activity vs. substrate concentration at very low substrate concentrations [23], is the most favorable parameter to discuss changes in enzymatic retinol oxidation. The present data demonstrate that V_{max}/K_m of retinol oxidation in cells from the colon is about 50 to 70 times higher compared to that of the mucosa from the small intestine.

The huge difference in cytosolic retinol oxidation between the mucosa of the small and large intestine is paralleled by findings on ADH subclass localization in the intestine using immunohistochemical and Western blot techniques [24, 25]. In these studies, ADH3 was detected in both the small and large intestine of rodents with a significantly higher level of ADH1-protein expression in the colon than in the small intestine, while ADH2 and ADH4 were not detectable with these methods [24, 25]. Regarding the immunohistochemical and enzyme kinetics data, the expression of ADH1 in the colon, which is practically absent in the small intestine [24], seems to be responsible for the markedly higher cytosolic retinol oxidation in the colon while ADH3 seems to mediate retinol oxidation in the small intestine [24]. Both rodent and human ADH3 were previously considered to be inactive in ethanol oxidation [6, 12]. Nevertheless, as compared to wild-type mice, animals in which the gene for ADH3 was deleted, retinoic acid synthesis was reduced by the factor 3.8 [13] and although low in activity, ADH3 was clearly shown to contribute significantly to systemic retinoic acid synthesis in vivo [14]. A possible explanation for the absence of retinol dehydrogenases in the small intestine may be the fact that – besides the liver – the rodent's small intestine is the only organ where retinal can be formed from carotenoids cleaved by 15,15'-dioxygenase [32] and the availability of retinoic acid may therefore not depend on the synthesis of retinal from retinol.

In vitro, the presence of ethanol was shown to inhibit ADH-associated retinol oxidation in the cytosol from human liver and cattle retina [15] as well as that of isolated alcohol dehydrogenases from both the rat (ADH1) [34] and man (ADH1, ADH2, ADH4) [7, 12, 29]. Furthermore, retinol oxidase activity in the cytosol from colon cells of the rat was measured to be significantly inferior at ethanol concentrations occurring in man after social drinking [16], a result that is in accordance with the results of the present study. The inhibition is evidently based on a direct interaction between the protein and ethanol and is of mixed type (non-competitive and competitive) with constants for competitive inhibition between 1.5 mM ethanol in the colon and 22 mM in the small intestine (Table 1).

In the present study, we were able to show for the first time that although quite low, the activity in the cytosol from cells of the small intestine is inhibited significantly by low ethanol concentrations (Figs. 1 and 2). Presuming fast ingestion, a concentration of 2.7 mM ethanol in the aqueous phase of the body in a 'standard' human (70 kg body mass) is already achieved after drinking about half a glass of wine (~90 ml, 11 % ethanol v/v) [35]. This concentration is sufficient to block about 33 % of cytosolic retinol oxidation in the small intestine and about 60 % of that in the cytosol of colon mucosa (Fig. 2).

In mice, systemic hypovitaminosis A leads to an impaired functionality of the intestinal mucosa, a thinner mucus layer, histological abnormalities and a reduced cellular division rate of cells in the small intestine [36–39]. Hypovitaminosis A is associated with a higher intestinal permeability in mice and man and can be reduced by supplementation with retinol [40, 41]. The observed reduction of retinol oxidation and the consequently reduced availability of the active vitamin A metabolite retinoic acid might therefore contribute to a higher permeability of macromolecules through the intestinal wall, as measured in rats after chronic alcohol

feeding [42] and in patients with chronic alcohol abuse [17]. In fact, in the present study a higher concentration of endotoxin was measured in the peripheral blood of the alcohol-fed animals as compared to the control rats [20], which may be of relevance because of the important role of the permeation of endotoxins from the intestine in the development of alcohol-induced organ damage [43, 44].

The results of our experiments suggest that retinol oxidation in the cytosol of intestinal cells is impaired as long as ethanol is present in the cell. Chronic alcohol feeding did not change the basal enzymatic activity of the cytosol. Therefore, an unchanged expression of retinol-oxidizing enzymes, namely ADH, can be presumed. Contradictory results were reported on the effect of chronic alcohol feeding on changes of ADH activity in the rat liver. Hepatic ADH activity was shown to be induced after chronic alcohol feeding in rats [45]. In contrast, comparable experiments with female rats resulted in a reduced hepatic ADH activity with ethanol as substrate [46]. In the present study, we were not able to measure any effect of chronic ethanol feeding on total ADH activity with retinol as substrate either in the small or the large intestine (Fig. 2). Therefore, the role of ethanol-induced inhibition of retinol oxidation followed by impaired function of the intestinal mucosa can be viewed as a consequence of the prolonged presence of ethanol, a situation that may become detrimental in the long run in alcoholics.

Acknowledgements This study was generously supported by a grant (70-1881-Bo1) from Deutsche Krebshilfe (to A. P. and C. B.).

References

1. De Luca L, Wolf G (1969) Vitamin A and protein synthesis in mucous membranes. Am J Clin Nutr 22:1059–1062
2. Zile M, DeLuca HF (1970) Vitamin A and ribonucleic acid synthesis in rat intestine. Arch Biochem Biophys 140: 210–214
3. Zile M, Bunge C, DeLuca HF (1977) Effect of vitamin A deficiency on intestinal cell proliferation in the rat. J Nutr 107:552–560
4. Kim CI, Leo MA, Lieber CS (1992) Retinol forms retinoic acid via retinal. Arch Biochem Biophys 294:388–393
5. Chambon P (1996) A decade of molecular biology of retinoic acid receptors. FASEB J 10:940–954
6. Boleda MD, Saubi N, Farres J, Pares X (1993) Physiological substrates for rat alcohol dehydrogenase classes:aldehydes of lipid peroxidation, omega-hydroxyfatty acids, and retinoids. Arch Biochem Biophys 307:85–90
7. Yang ZN, Davis GJ, Hurley TD, Stone CL, Li TK, Bosron WF (1994) Catalytic efficiency of human alcohol dehydrogenases for retinol oxidation and retinal reduction. Alcohol Clin Exp Res 18: 587–591
8. Duester G (2000) Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid. Eur J Biochem 267: 4315–4324
9. Collins MD, Eckhoff C, Chahoud I, Bochert G, Nau H (1992) 4-Methylpyrazole partially ameliorated the teratogenicity of retinol and reduced the metabolic formation of all-trans-retinoic acid in the mouse. Arch Toxicol 66:652–659
10. Duester G, Farres J, Felder MR, Holmes RS, Hoog JO, Pares X, Plapp BV, Yin SJ, Jornvall H (1999) Recommended nomenclature for the vertebrate alcohol dehydrogenase gene family. Biochem Pharmacol 58:389–395
11. Deltour L, Foglio MH, Duester G (1999) Metabolic deficiencies in alcohol dehydrogenase Adh1, Adh3, and Adh4 null mutant mice: overlapping roles of Adh1 and Adh4 in ethanol clearance and metabolism of retinol to retinoic acid. J Biol Chem 274:16796–16801
12. Han CL, Liao CS, Wu CW, Hwong CL, Lee AR, Yin SJ (1998) Contribution to first-pass metabolism of ethanol and inhibition by ethanol for retinol oxidation in human alcohol dehydrogenase family – implications for etiology of fetal alcohol syndrome and alcohol-related diseases. Eur J Biochem 254:25–31
13. Molotkov A, Fan X, Duester G (2002) Excessive vitamin A toxicity in mice genetically deficient in either alcohol dehydrogenase Adh1 or Adh3. Eur J Biochem 269:2607–2612
14. Molotkov A, Fan X, Deltour L, Foglio MH, Martras S, Farres J, Pares X, Duester G (2002) Stimulation of retinoic acid production and growth by ubiquitously expressed alcohol dehydrogenase Adh3. Proc Natl Acad Sci USA 99:5337–5342
15. Mezey E, Holt PR (1971) The inhibitory effect of ethanol on retinol oxidation by human liver and cattle retina. Exp Mol Pathol 15:148–156
16. Parlesak A, Menzl I, Feuchter A, Bode JC, Bode C (2000) Inhibition of retinol oxidation by ethanol in the rat liver and colon. Gut 47:825–831
17. Parlesak A, Schäfer C, Schütz T, Bode JC, Bode C (2000) Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. J Hepatol 32:742–747
18. Bode C, Bode JC (1997) Alcohol's role in gastrointestinal tract disorders. Alcohol Health Res World 21:76–83
19. Thurman RG (1998) II Alcoholic liver injury involves activation of Kupffer cells by endotoxin. Am J Physiol 275: G605–G611
20. Jarvelainen HA, Fang C, Ingelman-Sundberg M, Lindros KO (1999) Effect of chronic coadministration of endotoxin and ethanol on rat liver pathology and proinflammatory and anti-inflammatory cytokines. Hepatology 29: 1503–1510
21. Lindros KO, Jarvelainen HA (1998) A new oral low-carbohydrate alcohol liquid diet producing liver lesions: a preliminary account. Alcohol Alcohol 33: 347–353
22. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85
23. Cornish-Bowden A (1995) Inhibition and activation of enzymes. In: Fundamentals of enzyme kinetics. Anonymous. Portland Press, London, pp 93–128
24. Haselbeck RJ, Duester G (1997) Regional restriction of alcohol/retinol dehydrogenases along the mouse gastrointestinal epithelium. Alcohol Clin Exp Res 21:1484–1490

25. Svensson S, Stromberg P, Hoog JO (1999) A novel subtype of class II alcohol dehydrogenase in rodents Unique Pro(47) and Ser(182) modulates hydride transfer in the mouse enzyme. J Biol Chem 274:29712–29719
26. Seitz HK, Oneta CM (1998) Gastrointestinal alcohol dehydrogenase. Nutr Rev 56:52–60
27. Estonius M, Svensson S, Hoog JO (1996) Alcohol dehydrogenase in human tissues: localisation of transcripts coding for five classes of the enzyme. FEBS Lett 397:338–342
28. Chytil F, Ong DE (1979) Cellular retinol- and retinoic acid-binding proteins in vitamin A action. Fed Proc 38: 2510–2514
29. Kedishvili NY, Gough WH, Davis WI, Parsons S, Li TK, Bosron WF (1998) Effect of cellular retinol-binding protein on retinol oxidation by human class IV retinol/alcohol dehydrogenase and inhibition by ethanol. Biochem Biophys Res Commun 249:191–196
30. Li E, Qian SJ, Winter NS, d'Avignon A, Levin MS, Gordon JI (1991) Fluorine nuclear magnetic resonance analysis of the ligand binding properties of two homologous rat cellular retinol-binding proteins expressed in Escherichia coli. J Biol Chem 266:3622–3629
31. Ross AC (1993) Cellular metabolism and activation of retinoids: roles of cellular retinoid-binding proteins. FASEB J 7:317–327
32. Paik J, During A, Harrison EH, Mendelsohn CL, Lai K, Blaner WS (2001) Expression and characterization of a murine enzyme able to cleave beta-carotene. The formation of retinoids. J Biol Chem 276:32160–32168
33. Koivusalo M, Baumann M, Uotila L (1989) Evidence for the identity of glutathione-dependent formaldehyde dehydrogenase and class III alcohol dehydrogenase. FEBS Lett 257:105–109
34. Julia P, Farres J, Pares X (1986) Ocular alcohol dehydrogenase in the rat: regional distribution and kinetics of the ADH-1 isoenzyme with retinol and retinal. Exp Eye Res 42:305–314
35. Kalant H (1996) Pharmacokinetics of ethanol: Absorption, distribution and elimination. In: Begleiter H, Kissin B (eds) The pharmacology of alcohol and alcohol dependence. Oxford University Press, New York, pp 15–58
36. Ahmed F, Jones DB, Jackson AA (1990) The interaction of vitamin A deficiency and rotavirus infection in the mouse. Br J Nutr 63:363–373
37. De Luca L, Wolf G (1972) Mechanism of action of vitamin A in differentiation of mucus-secreting epithelia. J Agric Food Chem 20:474–476
38. Meneghetti L, Ceriani T, Maccabelli G, Ferraris P, Gori S, Ventura U (2003) Potential difference and mucus content in the stomach of vitamin A deficient rats. Eur J Phys 415:30(abs)
39. Zile MH, Bunge EC, DeLuca HF (1981) DNA labeling of rat epithelial tissues in vitamin A deficiency. J Nutr 111: 777–788
40. Thurnham DI, Northrop-Clewes CA, McCullough FS, Das BS, Lunn PG (2000) Innate immunity, gut integrity, and vitamin A in Gambian and Indian infants. J Infect Dis 182(Suppl 1):S23–S28
41. Quadro L, Gamble MV, Vogel S, Lima AA, Piantedosi R, Moore SR, Colantuoni V, Gottesman ME, Guerrant RL, Blaner WS (2000) Retinol and retinol-binding protein: gut integrity and circulating immunoglobulins. J Infect Dis 182(Suppl 1):S97–S102
42. Bode C, Vollmer E, Hug J, Bode JC (1991) Increased permeability of the gut to polyethylene glycol and dextran in rats fed alcohol. Ann N Y Acad Sci 625:837–840
43. Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG (1995) Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology 108:218–224
44. Fukui H, Brauner B, Bode JC, Bode C (1991) Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. J Hepatol 12:162–169
45. He L, Ronis MJ, Badger TM (2002) Ethanol induction of class I alcohol dehydrogenase expression in the rat occurs through alterations in CCAAT/enhancer binding proteins beta and gamma. J Biol Chem 277:43572–43577
46. Keegan A, Batey R (1994) Ethanol consumption by rats is inversely related to hepatic alcohol dehydrogenase activity. J Gastroenterol Hepatol 9:205–206