

# Human gastric alcohol dehydrogenase activity: effect of age, sex, and alcoholism

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## Abstract

As various isoenzymes of gastric alcohol dehydrogenase exist and as the effect of sex and age on these enzymes is unknown, this study measured the activity of gastric alcohol dehydrogenase at high and low ethanol concentrations in endoscopic biopsy specimens from a total of 290 patients of various ages and from 10 patients with chronic alcoholism. Gastric alcohol dehydrogenase was also detected by immunohistological tests in biopsy specimens from 40 patients by the use of a polyclonal rabbit antibody against class I alcohol dehydrogenase. A significant correlation was found between the immunohistological reaction assessed by the intensity of the colour reaction in the biopsy specimen and the activity of alcohol dehydrogenase measured at 580 mM ethanol. While alcohol dehydrogenase activity measured at 16 mM ethanol was not significantly affected by age and sex, both factors influenced alcohol dehydrogenase activity measured at 580 mM ethanol. Young women below 50 years of age had significantly lower alcohol dehydrogenase activities in the gastric corpus and antrum when compared with age matched controls (SEM) (6.4 (0.7) v 8.8 (0.6) nmol/min/mg protein;  $p < 0.001$  and 6.0 (1.3) v 9.5 (1.3) nmol/min/mg protein;  $p < 0.001$ ). Over 50 years of age this sex difference was no longer detectable, as high  $K_m$  gastric alcohol dehydrogenase activity decreases with age only in men and not in women. In addition, extremely low alcohol dehydrogenase activities have been found in gastric biopsy specimens from young male alcoholics (2.2 (0.5) nmol/min/mg protein), which returned to normal after two to three weeks of abstinence. The activity of alcohol dehydrogenase in the human stomach measured at 580 mM ethanol is decreased in young women, in elderly men, and in the subject with alcoholism. This decrease in alcohol dehydrogenase activity may contribute to the reduced first pass metabolism of ethanol associated with raised ethanol blood concentrations seen in these people.

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Recent studies in men<sup>1-3</sup> and in rats<sup>4,5</sup> have shown that a significant fraction of ingested ethanol fails to reach the blood and that it is not retained in the gastrointestinal tract but is metabolised by first pass metabolism. The enzyme responsible for ethanol oxidation in the human stomach is alcohol dehydrogenase. Pestalozzi *et al*<sup>6</sup> reported the presence of a low  $K_m$  alcohol dehydrogenase in the human stomach by using immunohistology. Most recently, however, alcohol

dehydrogenase isoenzymes with much lower affinity to ethanol have been detected in surgical specimens from the stomach<sup>7,8</sup> and in gastric biopsy specimens.<sup>9,9</sup> As it was reported that the activity of gastric alcohol dehydrogenase correlates with the first pass metabolism of ethanol,<sup>10</sup> and as the first pass metabolism was decreased in women,<sup>10</sup> in the alcoholic,<sup>2</sup> and after cimetidine treatment<sup>11</sup> leading to raised blood ethanol concentrations,<sup>2,10-12</sup> we investigated the effect of sex, age, and chronic alcohol consumption on low and high  $K_m$  alcohol dehydrogenase activity in biopsy specimens from the corpus and antrum of the stomach.

## Methods

### PATIENTS

A total of 290 patients had gastroscopies and biopsy specimens (wet weight: 3-12 mg) were taken from apparently normal areas of the gastric corpus, or antrum, or both. Of the 290 patients, 118 were men and 172 were women with an age between 18 and 83 years. All patients required gastroscopy because of medical indications including dyspeptic symptoms and tumour exclusion. None of the patients were receiving H<sub>2</sub> receptor antagonist treatment. Patients with *Helicobacter pylori* infection and with moderate or severe gastritis determined by light microscopy were excluded from the study. The gastroscopy was carried out with an Olympus GIF Q endoscope and the biopsy specimen was taken by a forceps type FB3K. The study was approved by the ethics committee of the Department of Medicine, University of Heidelberg, Germany. The patients did not have any history of chronic alcohol abuse. In addition, biopsy specimens were taken from nine male alcoholics (age: 30-75 years) and from one female alcoholic (age: 45 years) at the time of hospital admission. All of these patients reported an intake of more than 100 g ethanol per day and all of them had raised serum  $\gamma$ -glutamyltransferase activities. In addition, all patients had histologically moderate to severe gastritis. Three of these patients had another biopsy during their stay in hospital after 14 and 19 days of abstinence from ethanol. At this time, gastric morphology returned to normal.

### DETERMINATION OF ALCOHOL DEHYDROGENASE IN GASTRIC BIOPSY SPECIMENS

The biopsy specimens were frozen immediately and kept at  $-80^{\circ}\text{C}$  until used. The tissue was homogenised in 100 mM glycine buffer, pH 9.6 using a specially designed homogeniser for

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Eppendorf vials and finally centrifuged at 27 000 *g* for 15 minutes. The supernatant was used to determine alcohol dehydrogenase activity at 22°C in 100 mM glycine buffer, pH 9.6 with a final ethanol concentration of either 16 mM or 580 mM, and a nicotine adenine dinucleotide concentration of 2.6 mM. The human stomach contains three isoenzymes of alcohol dehydrogenase: The  $\gamma$ -alcohol dehydrogenase forms (class I) with a  $K_m$  of 1–2 mM at pH 10.0, the  $\sigma$ -alcohol dehydrogenase (class IV) with a  $K_m$  of 11 mM at pH 10.0, and  $\chi$ -alcohol dehydrogenase (class III), which cannot be saturated with ethanol. At 16 mM ethanol  $\gamma$ -alcohol dehydrogenase isoenzymes are saturated,  $\sigma$ -alcohol dehydrogenase is partially saturated, and  $\chi$ -alcohol dehydrogenase is not active. At 580 mM ethanol  $\gamma$ -alcohol dehydrogenase is partially inhibited by excess of substrate,  $\sigma$ -alcohol dehydrogenase is saturated, and  $\chi$ -alcohol dehydrogenase has also a small contribution to the activity measured. The complexity of the system makes it impossible to differentiate clearly between the activities corresponding to each enzyme. Therefore, in this paper alcohol dehydrogenase activities measured at 16 mM and 580 mM ethanol are referred to activities of alcohol dehydrogenase with low or high ethanol concentrations.

The soluble protein content in the supernatant fraction was measured according to the method of Lowry *et al.*<sup>13</sup> using bovine serum albumin as standard.

#### IMMUNOHISTOLOGY OF GASTRIC ALCOHOL DEHYDROGENASE

**Chemicals** – protein A and sepharose CL-4B were obtained from Pharmacia Inc (Uppsala, Sweden), and horseradish peroxidase from Sigma Chemical Co (St Louis, Mo). Protein A-peroxidase was prepared as described.<sup>14</sup>

**Immunochemicals** – pyrazole sensitive human liver alcohol dehydrogenase was purified and characterised as described and used to elicit antibodies in rabbits.<sup>15</sup> Anti-alcohol dehydrogenase antibodies were purified by affinity chromatography: human liver alcohol dehydrogenase was immobilised on CNBr-activated sepharose CL-4B according to the method described previously.<sup>16</sup> A mixture of 10 ml antiserum and 10 ml of alcohol dehydrogenase-sepharose was incubated at 4°C overnight. The suspension was poured into a 0.9 cm diameter chromatography column. The gel was washed with 0.05 M phosphate buffered saline, pH 7.4, followed by 2 M sodium chloride in phosphate buffer.<sup>17</sup> Antibodies bound in the column were eluted with 3 M thiocyanate, pH 6, immediately dialysed against phosphate buffered saline (3 × 2 × 1), and concentrated in an Amicon ultrafiltration gel (Amicon Coop, Danvers, Mass) with PM-10 membrane to a protein concentration of 3–4 mg/ml. All elutions were carried out at a flow rate of 80 ml/h. The breakthrough fraction of the affinity chromatography column – that is, antiserum depleted of specific anti-alcohol dehydrogenase antibodies, was concentrated to the original volume and used as negative control (immuno-adsorbed antiserum).

**Tissue section** – gastric biopsy specimens were immediately fixed in 4% phosphate buffered formaldehyde, pH 7.4, embedded in paraplast, sectioned at a thickness of 5 micron and mounted on microscopic slides. Three to five sections of each specimen were tested for the alcohol dehydrogenase content with the following immunohistochemical staining method: alcohol dehydrogenase was localised with anti-alcohol dehydrogenase antibodies. Bound antibodies were detected with a protein-A-peroxidase conjugate. The intensity of the staining was graded independently by a pathologist.

#### STATISTICAL ANALYSIS

Results were expressed as mean (SEM). The statistical significance of the differences in alcohol dehydrogenase activity were assessed by the Student's unpaired *t* test. *p* Values under 0.05 were considered to show statistical significance. The correlation between alcohol dehydrogenase activity and the intensity of the immunoreaction was analysed by the Spearman non-parametric method.

#### Results

In biopsy specimens from the human stomach alcohol dehydrogenase can be detected immunohistochemically in parietal and mucus producing cells and to a lesser degree in chief cells. The intensity, however, of the immunoreaction illustrated as pigment development varies inter-individually (Fig 1). A significantly positive correlation was found between the intensity of the immunoreaction and the alcohol dehydrogenase activity measured with 580 mM ethanol by using the same biopsy specimen divided into two parts to perform both determinations (Fig 2).

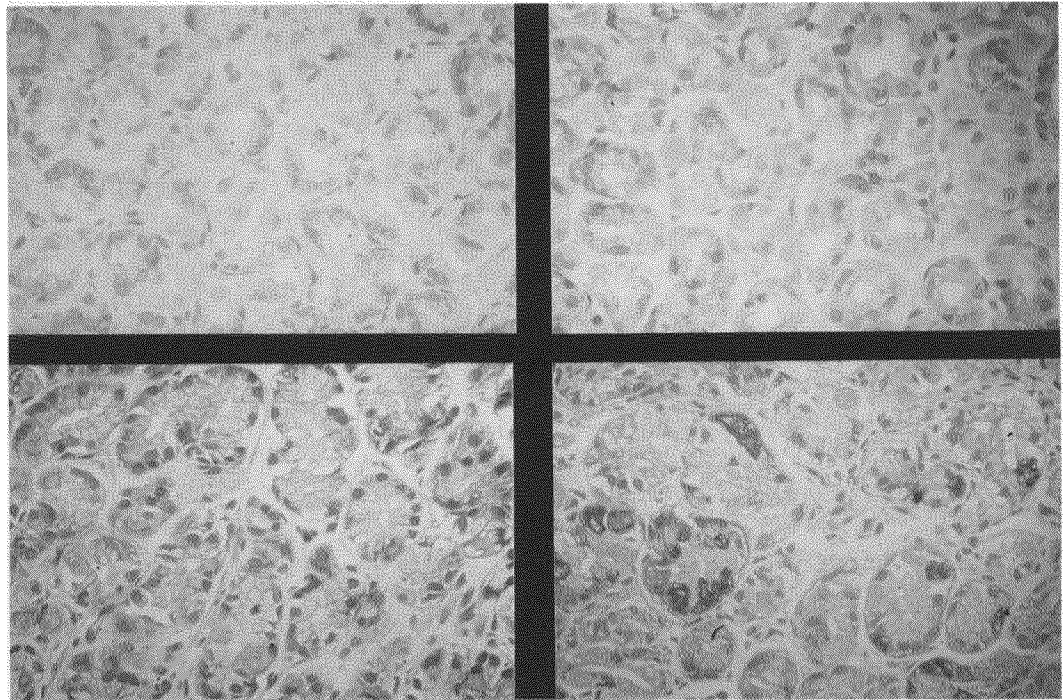
When alcohol dehydrogenase activity was measured in gastric biopsy specimens at an ethanol concentration of 580 mM no significant differences in the enzyme activity were found between the corpus and the antrum of the stomach (Table). Younger women, however, had a significantly lower alcohol dehydrogenase activity compared with age matched men, and elderly men showed a significantly lower alcohol dehydrogenase activity compared with younger men (Table). Figure 3 shows the effect of both age and sex on the activity of gastric alcohol

Effect of age and sex on gastric alcohol dehydrogenase activity

	Age 17–50 years (mmol/mg protein/min)	<i>p</i> Values	Age 50–85 years (nmol/mg protein/min)
Women Antrum*	6.0 (1.3)‡ (n=9)	NS	7.1 (0.6)‡ (n=14)
Men Antrum*	9.5 (1.3) (n=11)	<i>p</i> <0.001	3.7 (0.4) (n=9)
Women Corpus*	6.4 (0.7)‡ (n=14)	NS	6.2 (0.7) (n=19)
Men Corpus*	8.8 (0.6) (n=15)	<i>p</i> <0.001	4.7 (0.6) (n=14)
Women Corpus†	5.4 (0.8) (n=20)	NS	6.7 (0.8) (n=26)
Men Corpus†	NS	NS	NS
Men	6.7 (1.3) (n=12)	NS	5.2 (0.8) (n=18)

\*Alcohol dehydrogenase activity measured at 580 mM ethanol; †Alcohol dehydrogenase activity measured at 16 mM ethanol; ‡*p*<0.001 for the comparison with men in the same group; results expressed as mean (SEM).

Figure 1: Immunohistology of gastric alcohol dehydrogenase using a polyclonal rabbit antibody against human hepatic alcohol dehydrogenase and the PAP method. Different degrees of immunoreactivity of gastric alcohol dehydrogenase in human gastric biopsy specimens are seen: no alcohol dehydrogenase activity detectable (upper left), minimal immunoreaction (upper right), moderate immunoreaction (lower left corner), strong immunoreaction (lower right). Haematoxylin counterstain, original magnification  $\times 100$ .



dehydrogenase measured at 580 mM ethanol. While the activity of the enzyme stays fairly constant during the life time in women, a sharp decrease of the activity is seen in men between the age of 40 to 50 years. This is in contrast with the alcohol dehydrogenase activity measured at 16 mM ethanol (Table). Neither age nor sex had a significant effect on this enzyme activity, although a trend towards lower activities in younger women and in elderly men can also be detected. Finally, alcoholics had a strikingly low gastric alcohol dehydrogenase activity at 580 mM ethanol compared with non-alcoholics (Fig 4). The 45 year old woman with chronic alcoholism had a gastric alcohol dehydrogenase activity of 0.7 nmol/mg protein/min. Three of the nine male alcoholics could be biopsied again during hospital stay 14 to 19 days after abstinence

from alcohol. All three showed a complete return to normal of the enzyme activity.

### Discussion

The data presented show the presence of at least two types of alcohol dehydrogenase isoenzymes in endoscopic biopsy specimens from the human stomach with different activities. One isoenzyme has a low  $K_m$  for ethanol comparable with that of class I alcohol dehydrogenase isoenzymes as defined for the liver.<sup>18</sup> This enzyme has already been described in necropsy,<sup>19</sup> and in fresh surgical specimens.<sup>7,20</sup> By using fresh gastric mucosa, Hernandez-Munoz *et al*<sup>7</sup> found that this low  $K_m$  enzyme has an optimal pH of 10.6, is very sensitive to the inhibition by 4-methylpyrazole, and migrates cathodically on electrophoresis. This enzyme could also be detected here by immunohistology using a polyclonal rabbit antibody against human liver class I alcohol dehydrogenase. The other type of alcohol dehydrogenase has a much lower affinity to ethanol but with activities that become significant at ethanol concentrations commonly present in the human stomach after alcohol consumption.<sup>21</sup> It has been shown that one high  $K_m$  isoenzyme has a pH optimum of 10.5, is refractory to the inhibition by 4-methylpyrazole, and has kinetic properties corresponding to the class III (or  $\chi$ -alcohol dehydrogenase) isoenzyme.<sup>7,22</sup> In addition, a new alcohol dehydrogenase isoenzyme, named  $\sigma$ -alcohol dehydrogenase has been purified from the human stomach.<sup>9</sup> This enzyme is a class IV isoenzyme but differs from  $\pi$ -alcohol dehydrogenase, has a pH optimum of 9.9, and a  $K_m$  of 41 mM at pH 7.4.<sup>9</sup> This gastric isoenzyme has also been detected in the rat<sup>23</sup> and in the baboon<sup>24</sup> and therefore its occurrence may be widespread in mammals. Thus, three different types of alcohol dehydrogenase isoenzymes are capable of oxidising ethanol in the mucosa of the human stomach. To measure the overall metabolism of

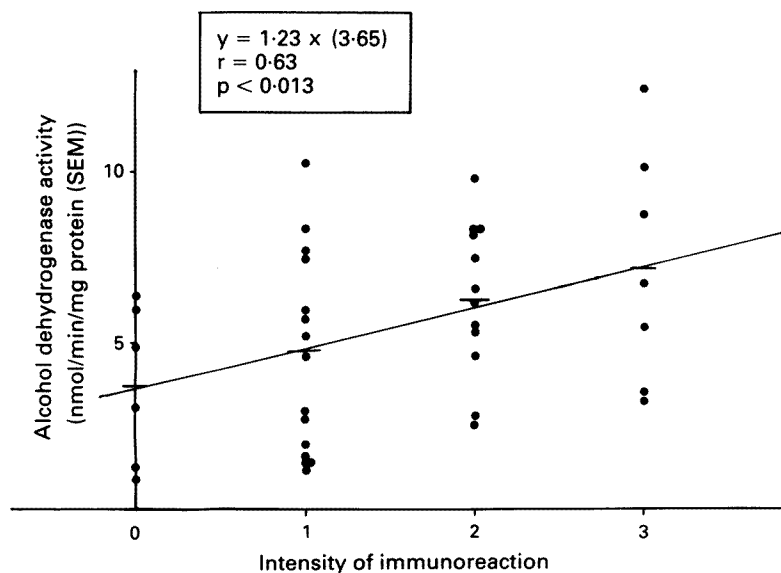


Figure 2: Correlation between immunoreactivity (expressed as no=0, minimal=1, moderate=2, and strong=3 immunoreactivity) of gastric alcohol dehydrogenase and the enzyme activity measured in the same gastric biopsy specimens at 580 mM ethanol.

