Abnormal gut fermentation: Laboratory studies reveal deficiency of B vitamins, zinc, and magnesium

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Gut fermentation in the colon is a normal phenomenon whereby soluble non-starch polysaccharides are metabolized to short-chain fatty acids. Abnormal fermentation may be associated with clinical symptoms and is generally assumed to take place in the small bowel. It may be established by ethanol production after a sugar challenge in the fasting subject, which produces maximum production of ethanol 1 hour after sugar challenge. This timing is compatible with the dose acting in the small bowel, but not the large. It was noted that patients with abnormal gut fermentation established by gut fermentation ethanol production tests tended to have low levels of vitamins and minerals, and it was therefore decided to make a prospective study of patients with the condition to determine if this was so.

Patients were tested for ethanol production together with standard functional analysis techniques for vitamins B_1, B_2, and B_6 and zinc and magnesium concentrations by sweat analysis using air/acetylene flame atomic absorption. Fifty normal subjects (group A) were analyzed against 30 positive patients by alcohol testing (group B). Statistical analysis, using the Wilcoxon Sum of Ranks test, revealed a remarkable and consistently high difference for vitamins and minerals between the two groups. In group B, 19 of 30 patients had four of five or five of five nutrients abnormal, and no subject with a positive alcohol test had less than two abnormal nutrients.

It is concluded that the syndrome that causes abnormal gut fermentation appears to have adverse effects on levels of B vitamins, zinc, and magnesium. As yet it is not clear whether this is a result of malabsorption, over-utilization, or excessive excretion. The level of ethanol production in this condition is low, but the presence of the nutritional deficits implies that the syndrome may cause quite significant adverse effects on health. More research in this area is required to replicate and extend these studies. (J. Nutr. Biochem. 4:635–638, 1993.)

Keywords: gut fermentation; B vitamins; zinc; magnesium; deficiency

Introduction

Normal gut fermentation has been known for many years and more recently has been extensively studied. It is performed by commensal bacteria in the large bowel, where soluble non-starch polysaccharides are fermented to higher alcohols and short-chain fatty acids, which are then absorbed and contribute to human energy intake.[1] Abnormal gut fermentation, presumably taking place in the small bowel, has been studied for a much longer period. It was described in 1912 as "germ carbohydrate fermentation,"[2] "intestinal carbohydrate dyspepsia" by Hurst in 1931,[3] and enjoyed a popularity in the 1930s and 1940s. Recent attempts in the United States have been made on a hypothetical basis to link a similar symptom complex to yeast overgrowth.[4,5] Recently, a laboratory test has been devised that enables trace alcohol measurements to be estimated,[6] and a gut fermentation working party has now been set up to initiate further studies to establish laboratory and clinical factors that are relevant.[7]

The symptoms associated with gut fermentation require further definition, but often there is fatigue, nonspecific malaise, and depression. Irritable bowel is common, as might be expected, but bladder function disorders and catarrhal problems are not uncommon.[7]

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Received September 17, 1992; accepted June 8, 1993.

In the 1940s, authors writing about intestinal carbohydrate dyspepsia stated that vitamin deficiencies appeared to be present.[8,9] At that time, reliable laboratory measurements of B vitamin status were not available, and tests for tissue levels of trace minerals did not exist. This study, therefore, addresses the question of whether such deficiencies do occur in patients with a positive gut fermentation alcohol test.

Methods and materials

Patients

Successive referrals to one of the authors (K.K.E.) when the diagnosis of abnormal gut fermentation was suspected on clinical grounds were entered in this trial. At this time, all patients were consuming generally average United Kingdom diets. The intake of the nutrients we studied for average adults on such diets from all sources and from food sources alone is shown in *Table 1*.[10] No patient ws either an alcoholic or a regular daily consumer of alcohol, with the majority tending to abstain or consume single drinks only on rare occasions, as most were aware that alcohol upset them. The only excess related to cravings for sweet foods, usually chocolates and biscuits, and for bread. These foods were often consumed in a "binge," followed by a brief period of abstention. The patients were submitted for laboratory testing for abnormal gut fermentation; functional analysis for vitamins B1, B2, B6; and sweat mineral profiles. No "binges" were recorded around the time of testing. The results were recorded at the next consultation.

Gut fermentation alcohol test

This tests the ability of a subject to produce trace levels of ethyl alcohol (EtOH) from a small dose of sugar given orally. Normal patients are not able to do this. The patients were instructed to fast for 3 hours prior to testing and to abstain from alcohol for 24 hours. In original studies concerning the production of alcohol, we found that the peak EtOH concentration was reached at 1 hour, returning to undetectable levels within 3 hours. It was therefore decided that the fasting period of 3 hours was sufficient to ensure that any endogenous alcohol was eliminated. A fasting 2-mL blood sample was taken into fluoride/oxalate anticoagulant and analyzed for glucose and alcohol concentrations. Glucose was measured enzymatically using the glucose oxidase/peroxidase/chromogen system [GOD - Perid Kit (Cat. No. 1244028, Boerhinger, Lewes, W. Sussex, U.K.)]. Alcohol was also measured enzymatically using the blood alcohol dehydrogenase method (Sigma Cat. No. 332-A, Sigma Chemical Co., St. Louis, MO USA). The method was modified to increase sensitivity by adjusting the sample and reagent volumes to bring the detection limit to 0.5 mg/dl.[6] The patient was then administered 1 g of glucose in a hard gelatin capsule along with 100 mL of 4% glucose solution in water to ensure passage to the small bowel. A further blood sample for glucose and alcohol was taken after 60 minutes. In the initial stages of development of the gut fermentation alcohol test, it was considered appropriate to measure glucose levels pre- and post-test, should there be any consequence.[6] However, in over 500 consecutive measurements only two pre-diabetic levels were observed, and it was not considered that the practice of glucose measurement was relevant to the alcohol test (Hunnisett, A., unpublished observations, 1990). An increase of blood alcohol over the test period was considered to be positive. More recently we have been using a gas-chromatography technique to improve the sensitivity further and to increase the diagnostic scope of the test.[11]

Measurements of B vitamin status

Vitamin B_1 (thiamin), B^2 (riboflavin), and B_6 (pyridoxine) were measured in blood by standard functional analysis techniques, i.e., methods employing coenzyme stimulation of vitamin-dependent enzymes in the red blood cells. The assays rely on the native vitamin present in the subjects' blood samples to act as cofactor to an enzyme reaction in vitro. In concurrent essays, an enzyme activity that requires the vitamin of interest as a cofactor is measured in a standard reaction mixture using the subjects' native vitamin concentrations (A) and in a system with excess exogenous vitamin (B). The increase in activity, if any, shown in B is expressed as a percentage of A. Clearly,

Table 1 Average adult (age 16–64 years) daily intakes

		Male				Female			
		All sources*		Food†		All sources*		Food†	
Zinc	mg.	11.4	(5.7) (19.0)		NA NA	8.4	(3.6) (13.6)		NA
Magnesium	mg.	323	(156) (548)		NA	237	(105) (441)		NA
B_1	mg.	2.01	(0.79) (3.29)	1.7	(0.79) (2.81)	1.61	(0.56) (3.09)	1.24	(0.55) (2.06)
B_2	mg.	2.29	(0.92) (4.32)	2.08	(0.92) (3.65)	1.84	(0.59) (4.04)	1.57	(0.59) (2.94)
B_3 niacin equivalents	mg.	40.9	(21.6) (67.4)	39.9	(21.6) (62.2)	30.3	(13.9) (51.2)	28.5	(13.7) (46.4)
B_6	mg.	2.68	(1.2) (5.35)	2.48	(1.2) (4.47)	2.84	(0.71) (10.46)	1.57	(0.71) (2.62)

*All sources, including food supplements
†Food sources only, excluding supplements
Mean average value (lower 2.5 percentile: upper 2.5 percentile).

if the endogenous cofactor is deficient, the activation of B over A will be higher than it would be if there were sufficient endogenous cofactor. Patients with deficiency tend to show elevated results. The normal range is 0–15%: 15–25% represents a borderline deficiency, and above this is a poor status for the vitamin assayed. The ranges are the same for B_1, B_2, and B_6. The enzymes measured were erythrocyte transketolase (ETK - B_1 dependent), erythrocyte glutathione reductase (EGR - B_2 dependent), and erythrocyte glutamate-oxalocetate transaminase (EGOT - B^6 dependent).[12]

Measurement of mineral status by sweat testing

Zinc and magnesium were measured in extracted sweat samples by a standard air/acetylene flame atomic absorption technique using a Phillips PU9000 instrument (Phillips Scientific, Cambridge, U.K.), per manufacturer's recommendations.[13,14] The technique for the collection of sweat has been reported elsewhere.[15] Patients with deficiency tend to show reduced mineral levels.[15]

Statistical analysis

Thirty patients with a positive gut fermentation alcohol test were analyzed against 50 normal patients. Some of the positive patients had additional symptoms of chronic fatigue syndrome as defined by standard United Kingdom research criteria.[16,17] Sets of data for vitamins and minerals were compared for patients not able to produce alcohol with those able to produce alcohol. The graphic distributions of data were mostly considered to be not normal, and the comparison variances were largely unequivalent as shown by the *F* test. Consequently, the Wilcoxon Sum of Ranks test, adjusting for ties, was used for the analysis.

Because the means and medians were very close, the *t* test (two-tail) was sufficiently robust to give the same result. However, standard deviations should be used with caution because several of the distributions, with the exception of alcohol values, were not normal. For this reason, the actual experimental ranges are given. The analysis shows a remarkable and consistently very high level of significant difference between alcohol-normal and alcohol-abnormal patients for the vitamins and minerals.

Results

The results of gut fermentation alcohol testing, vitamin, and mineral levels for both groups A and B are shown in *Table 2*.

When the values for normal subjects are compared with the symptomatic patients, it will be seen that for B_1 the mean falls outside the range of normal, although some subjects were within the range. B_2 gave a mean within the reference range, with fewer patients abnormal, and was the least likely of the nutrients studied to be adversely affected in our group. B_6, by contrast, was the most adversely affected, with a mean of 22.07 with fewer patients in the reference range. Magnesium levels, although frequently abnormal, were generally only slightly outside the reference range. Zinc is more difficult to report because of separate ranges for males and females, but both sexes showed means outside the reference range, and the majority of patients had evidence of deficiency.

Discussion

If these findings are corroborated by further studies, it would appear that abnormal gut fermentation associated with a measurable ethyl alcohol concentration in peripheral blood is associated with relatively severe alteration of vitamin and mineral concentrations. This alteration may represent absorptive failure, over-utilization, or excessive excretion, or a combination of any or all of these. Of the sample of 30 patients, 19 had four of five nutrients abnormal. No single subject with a positive alcohol test was without abnormal nutrient status or had only one abnormal nutrient. Five had only two abnormal nutrients.

Such findings may conceivably be associated with abnormalities in gut function, and a subsequent study will examine whether gut alcohol production is associated with evidence of intestinal malabsorption.

There is also clinical importance in establishing organic changes in patients who have abnormal gut fermentation. The symptoms expressed by this group are not easily defined,[7] and are such as to permit a non-organic diagnosis to be equally tenable for such patients unless the symptoms can be shown to correlate with measurably abnormal laboratory findings. Many members of the general public voluntarily purchase supplements that could be prescribed if there is a sound reason for doing so. Such preparations could have a powerful

Table 2

	Group A Normal values for adults not able to produce alcohol		Group B Abnormal values for adults able to produce alcohol	
	n	Mean (SD)	n	Mean (SD)
Age	50	35 (7.20)	30	39.8 (9.50)
Blood B1 (%)	50	9.14 (3.82)*	30	18.4 (5.80)*
Blood B2 (%)	50	8.66 (3.93)*	30	14.23 (4.08)*
Blood B6 (%)	50	8.32 (3.66)*	30	22.07 (7.53)*
Sweat Mg (mM/L)	50	0.23 (0.03)*	30	0.19 (0.03)*
Sweat Zn [M] (μM/L)	25	7.37 (1.37)*	6	4.96 (1.06)*
Sweat Zn [F] (μM/L)	25	8.52 (2.64)*	24	5.35 (0.80)*
Sweat Zn [M + F] (μM/L)	50	8.02 (2.18)*	30	5.28 (0.85)*
Blood EtOH (μM/L)	50	8.4 (4.9)*	30	640 (181)*

*Wilcoxon and *t* Test $P < 0.001$

placebo action in a group of patients with problems of psychological origin who are unwilling to accept a non-physical explanation for their illness. We have presented in this study evidence that an abnormal gut fermentation is accompanied not only by an abnormal alcohol production, but by multiple diminution of mineral concentrations and vitamin levels, as indicated by the higher activation coefficients in the alcohol-positive group when compared with the normal population. In some cases these deficiencies are at least low enough to be associated with mood changes (Zinc, B_1, B_6),[18–20] and muscular and circulatory symptoms (magnesium).[21,22] In patients with these findings the prescription of such supplements, where supported by adequate laboratory data, appears to have a logical basis.

It should be noted that in patients with abnormal gut fermentation the ethanol production is used solely as a diagnostic test. The levels produced do not exceed 5.0 mg/dL, and it is therefore improbable that these modest levels are the direct cause of the symptomatology. The vitamin and mineral deficiencies we have recorded are the consequence of the underlying disease process and not that of the alcohol levels. The fact that "binging" on sugary foods, which will increase glucose levels, is common in these patients may well be more relevant, as even moderate glucose loads have been shown to increase urinary excretion of magnesium.[23]

There are references in the alcohol ingestion literature to the adverse effects of ethanol intake on nutrient status, and specifically to zinc and magnesium. However, it is important to note that these refer to ethanol loads far in excess of the very modest levels recorded in the patients we studied.[24] However, one study does relate to low and moderate intake.[11] Further investigation of this condition is required. No clinical management programs have been adequately studied, further laboratory values need to be related to alcohol production, and microbiological studies have yet to be determined. Nevertheless, it may now be said that organic changes occur in patients hitherto considered on symptoms alone to have problems of purely psychosomatic origin.

Acknowledgments

The authors would like to express appreciation to members of the Gut Fermentation Working Party for advice and criticism in the preparation of this paper.

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