

Decreased Serum Zinc in Fructose Malabsorbers, Maximilian Ledochowski,^{1*} Bernhard Widner,² Christian Murr,² and Dietmar Fuchs² (¹ Department of Clinical Nutrition and ² Institute of Medical Chemistry and Biochemistry, University of Innsbruck, A-6020 Innsbruck, Austria; * address correspondence to this author at: Universitätsklinik Innsbruck, Abteilung für Ernährungsmedizin, Anichstrasse 35, A-6020 Innsbruck, Austria; fax 43-512-504-2017, e-mail maximilian.ledochowski@uibk.ac.at)

Fructose malabsorption syndrome is a disease that was first described less than 15 years ago (1,2). In some patients, fructose malabsorption probably reflects a defect in the duodenal fructose transporter GLUT-5, a facilitative hexose transporter with limited capacity (3). In patients with fructose malabsorption, large quantities of fructose reach the colon where they are broken down by bacteria into short fatty acids, CO₂, and H₂ (4). Bloating, abdominal discomfort, and sometimes osmotic diarrhea are induced by the degradation products produced by the colonic bacteria. It is believed that ~36% of the European population has fructose malabsorption in a more or less severe form, and approximately one-half of those affected are symptomatic (5).

We recently found that fructose malabsorption is associated with early signs of mental depression (6), folic acid deficiency (7), and lower plasma tryptophan concentrations (8). We have examined whether serum zinc and iron are changed in fructose malabsorbers compared with lactose maldigestors.

One hundred forty-seven otherwise healthy adult outpatients (104 women and 43 men), ages 16–76 years (mean ± SD, 44.7 ± 13.2 years), who visited a physician's office for a medical health check-up and reported gastrointestinal complaints in a health questionnaire were studied. None of the patients showed signs of inflammatory bowel disease or any other chronic or infectious diseases, and none was taking medication except for contraceptives in some women. All 147 patients underwent H₂ breath testing after an oral load of fructose, and 128 patients underwent oral load with lactose 1 week apart. Blood samples were taken after an overnight fast for determination of zinc, iron, transferrin, and ferritin. To rule out non-H₂-producers, a single H₂ breath test or a lactulose H₂ breath test was performed in nonfasting individuals some days before fructose H₂ breath testing. Non-H₂-producers were excluded from the study.

Breath hydrogen (H₂) was measured with an analyzer (9,10) from Bedford Ltd. (Lahner, Salzburg). All tests were performed between 0800 and 0830, and body weight and height were measured. After a 12-h overnight fast, a baseline H₂-breath test was performed. An oral dose of 50 g of fructose or 50 g of lactose was given in 250 mL of tap water, and H₂ exhalation was monitored in 30-min intervals for at least 2 h. Maximum H₂ exhalation after the sugar load was monitored, and the differences from baseline concentrations (ΔH₂) were calculated.

Blood samples were drawn with a 7.5-mL trace-element-free syringe with a zinc-free needle from fasting

subjects for serum zinc, iron, transferrin, and ferritin measurements. Zinc was measured colorimetrically (Wako Chemicals) according to the manufacturer's instructions (11). Iron and albumin were measured by a BM/Hitachi 917 system (Boehringer Mannheim), and transferrin and ferritin were measured by immunoturbidimetric assays (Boehringer Mannheim). Iron-binding capacity was calculated using the formula: Fe (μg/dL)/transferrin (mg/dL) × 100. Cutoff values were 10.7 μmol/L for zinc deficiency, 600 μg/L for iron deficiency, and 15 μg/L for ferritin deficiency. The reference interval for calculated iron-binding capacity was 20–50%.

The cutoff point for the diagnosis of fructose malabsorption or lactose maldigestion was an increase of breath H₂ concentrations ≥20 ppm over baseline (1). The serum concentrations of zinc, iron, and ferritin in the two corresponding groups were compared by the Student *t*-test, and the correlation of zinc deficiency with fructose malabsorption was calculated by the Fisher exact test, using a standard PC statistical program (STATISTICA for Windows, Ver. 6.0) (12).

After oral fructose administration, 102 of 147 (69%) patients (33 men and 69 women; 45.0 ± 13.1 years) presented with a ΔH₂ ≥20 ppm. Breath tests after lactose loading were positive (ΔH₂ ≥20 ppm) in 25 of 128 (20%) subjects (6 men and 19 women; 44.6 ± 13.0 years).

Serum zinc was significantly lower in fructose malabsorbers (13.1 ± 2.2 μmol/L) than in normal fructose absorbers (14.1 ± 2.1 μmol/L; *P* = 0.007; Fig. 1). Serum zinc concentrations had a slight tendency to be lower in lactose maldigestors (13.0 ± 1.9 μmol/L) compared with normal lactose digestors (13.5 ± 2.2 μmol/L), but this difference was not significant. There was no influence of sex or age.

The mean serum albumin concentration in fructose malabsorbers was 46.8 mg/L (± 3.2 mg/L) compared

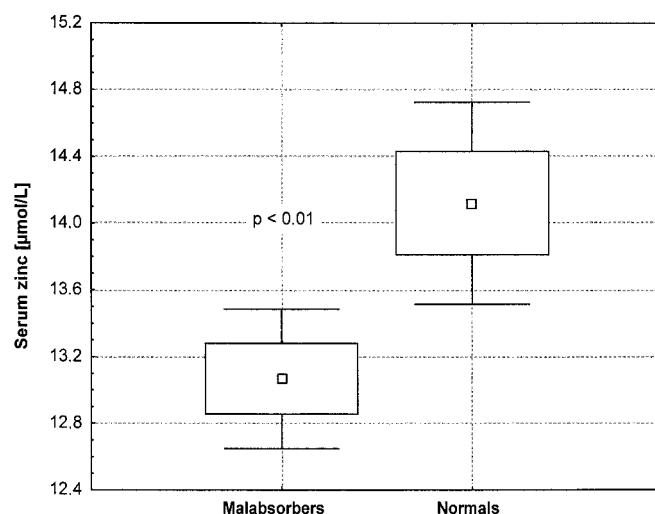


Fig. 1. Serum zinc concentrations in fructose malabsorbers compared with normal absorbers.

□ indicate means; larger rectangles indicate ± 1.00 SE; bars indicate ± 1.96 SE.

with 47.1 mg/L (\pm 3.1 mg/L; *P*, not significant) in normal absorbers.

In fructose malabsorbers, plasma iron was 787 μ g/L (\pm 307 μ g/L), transferrin was 3260 mg/L (\pm 524 mg/L), and ferritin was 65.7 μ g/L (\pm 71.8 μ g/L), which did not differ from normal fructose absorbers [plasma iron, 800 μ g/L (\pm 300 μ g/L; *P*, not significant); transferrin, 3438 mg/L (\pm 643 mg/L; *P*, not significant); ferritin, 82.3 μ g/L (\pm 78.9 μ g/L; *P*, not significant)]. Iron-binding capacity was 24.5% (\pm 9.9%) in fructose malabsorbers and 24.8% (\pm 10.1%; *P*, not significant) in normal fructose absorbers.

Ten of 102 subjects (9.8%) with fructose malabsorption had zinc deficiency (serum zinc concentration \leq 10.7 μ mol/L), whereas in the group with normal fructose absorption, 45 of 45 subjects had normal serum zinc concentrations (Fisher exact test, *P* = 0.02).

Patients with chronic diarrhea are known to exhibit signs of zinc deficiency (13). Fructose malabsorption is one of the most common causes for chronic diarrhea (4). The decreased zinc that we found in subjects with fructose malabsorption was not an effect of differences in sex or age. Ten subjects presented with zinc deficiency, and all suffered from fructose malabsorption. None of these 10 subjects took oral contraceptives or hormones.

Zinc deficiency seems to play a role in cellular turnover of the gastrointestinal mucosa (14) and also has been linked to poor appetite and mental disturbances (15); the latter is interesting because earlier we found signs of depression in subjects with fructose malabsorption (6).

Plasma iron and ferritin showed a tendency toward lower concentrations in fructose malabsorbers, but the differences were not significant. Zinc and iron are likely to be absorbed by the same duodenal metal transporter, DMT-1 (NRAMP-2), which is also responsible for the absorption of other divalent cations, including Cu^{2+} , Mn^{2+} , Co^{2+} , Cd^{2+} , and Pb^{2+} . Because iron is also absorbed via a $\beta(3)$ integrin and mobilferrin pathway that is not shared by other nutritional metals (16), a defective DMT-1 transporter system could be masked by compensatory enhancement of the integrin and mobilferrin pathway for iron metabolism, leaving only the absorption of zinc decreased. Thus, our data cannot exclude that zinc deficiency in fructose malabsorbers is attributable to a limited transport capacity of DMT-1.

Because serum zinc is bound to proteins, especially albumin, we determined serum albumin concentrations in a subgroup of 102 individuals. Mean serum albumin concentrations were not decreased in fructose malabsorbers. Because malnutrition is very unlikely in the population studied, the differences in zinc concentrations between fructose malabsorbers and normal absorbers may thus be attributable to the malabsorption of alimentary zinc.

Overproduction of cytokines may be associated with decreased blood zinc concentrations (17) because of increased renal loss of zinc (18). We previously have shown that fructose malabsorption is a promoting factor for small intestinal bacterial overgrowth syndrome and is associated with higher serum neopterin concentrations

(19), an indicator of immune activation. The different bacterial composition of the gut could thus be a promoting factor for zinc deficiency in fructose malabsorbers by chronic immune stimulation. It has been shown that dietary carbohydrates, such as sucrose and fructose, reduce the bioavailability of copper by 30% in humans, but not in rats (20). Because zinc is handled very much like copper and high intestinal fructose concentrations are seen in fructose malabsorption, the same phenomenon could play a role in low zinc status in fructose malabsorption.

In summary, fructose malabsorption is associated with lower serum zinc concentrations; in this study, 10 of 147 subjects (6.8%) had zinc deficiency, and all 10 suffered from fructose malabsorption. Although this is probably not attributable to a shortened transit time of a defective DMT-1-transporter, the exact mechanism still remains to be elucidated. Fructose malabsorption may thus reflect only part of a more complex malabsorption syndrome. Because fructose malabsorption can be seen in approximately one-third of the Western European population, fructose malabsorption could be a major etiology of low zinc status.

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ELISA Methodology for Detection of Modified Osteoprotegerin in Clinical Studies, *De Chen, Nihal A. Sarikaya, Han Gunn, Steven W. Martin, and John D. Young* (Department of Pharmacokinetics and Drug Metabolism, Amgen, Inc., One Amgen Center Dr., Thousand Oaks, CA 91320; * author for correspondence: fax 805-499-4953, e-mail dchen@amgen.com)

Osteoprotegerin (OPG), also known as osteoclast inhibitory factor, is a soluble receptor of the tumor necrosis factor receptor superfamily. The protein is secreted as a covalent, disulfide-linked homodimer, which is the predominant extracellular form (1), and is expressed in multiple tissues (1–3). OPG-mediated pathways might have a role in osteoporosis (3–6) because estrogen increases OPG gene expression (4, 5). OPG maintains the structure of healthy bone and inhibits osteoclast activation and differentiation (3, 7). In the vascular system, OPG inhibits pathological calcification in the media intima (3). OPG has been proposed for therapy of osteopenic disorders, such as postmenopausal osteoporosis, Paget disease, rheumatoid arthritis, hypercalcemia, and lytic bone metastases (8).

Initially, we developed an antibody-based ELISA method with an anti-human OPG monoclonal antibody for capture and an anti-human OPG polyclonal antibody for detection. Yano et al. (9) raised the concern for us that we may not detect the active dimeric OPG with antibody capture because they reported that serum OPG increased with age and that the monomer was the predominant form of OPG in human serum. Although they used a different antibody-dependent ELISA method (monoclonal capture and detection), the results reported by Yano et al. (9) do not correspond with the work performed at Amgen (1, 3–5, 7, 8, 10–12). We reasoned that OPG ligand (OPGL) (2, 7, 8, 10–16), also known as osteoclast differentiation factor, is a potential alternative capture protein for an OPG assay. In an attempt to develop an assay that would measure all bioactive form(s) of OPG, we developed an ELISA assay that uses OPGL as the capture protein. To avoid problems posed by batch-to-batch variability of human serum pools for use as assay diluent, assay development was used to define a serum substitute.

Assay development was performed with AMGN-0007, a modified OPG. Calibrators and quality-control (QC) materials were prepared in human serum or serum substitute. Whereas calibrators were serially diluted, QC materials were prepared individually. Calibration curves were prepared using calibrators containing 0.020–500

$\mu\text{g/L}$ AMGN-0007. Each calibration curve contained at least nine points, including the zero calibrator.

OPGL, AMGN-0007, and murine monoclonal antibody were purified essentially as described previously (1, 7). OPGL was coated onto 96-well microtiter plates (Costar). Plates were blocked with 2 mL/L I-Block (Tropix) and 5 mL/L Tween 20 (Pierce) in phosphate-buffered saline (PBS). Assay buffer, calibrators, and QC materials were added to the wells. After all unbound substances were removed by washing, murine anti-human OPG monoclonal antibody was added to the wells. After another wash, goat anti-mouse IgG conjugated with horseradish peroxidase (IgG-HRP; Zymed) was added to the wells. After the final wash, KPL TMB Microwell Peroxidase Substrate (Kirkegaard & Perry Laboratories) was added to the wells. The colorimetric reaction was stopped with 0.812 mol/L phosphoric acid. The color intensity was measured at 450–650 nm with a ThermoMax Microtiter Plate Reader (Molecular Devices).

The full-length OPG homodimer (OPG-FLD) and the full-length OPG monomer (OPG-FLM) were purified from conditioned medium with Sepharose columns and concentrated into PBS. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed to confirm size and purity (95%) of the monomer and dimer. Calibrators and QC materials were prepared for each OPG analog: AMGN-0007, OPG-FLD, and OPG-FLM. The assay was performed as described above, except that we used HRP-conjugated murine anti-human OPG monoclonal antibody.

Prepared in PBS, human serum substitute buffers contained 30 mL/L human serum albumin (HSA; Bioreclamation, Inc.) and various concentrations (0–500 mL/L) of fetal bovine serum (FBS; Sigma Chemical Co.). Calibrators and QC materials were prepared and assayed as described above.

OPGL was immobilized on the surface of the microtiter plate. AMGN-0007 was then added to the plate at concentrations of 0.244–31.25 $\mu\text{g/L}$, the calibration curve range. The analyte was detected with murine anti-human OPG monoclonal antibody plus goat anti-mouse IgG-HRP. Defined as two times the zero calibrator signal, the detection limit was 0.244 $\mu\text{g/L}$. Other assay configurations, such as monoclonal capture with polyclonal detection and ligand capture with polyclonal detection, were studied and demonstrated low signal-to-noise ratios throughout the calibration curve. For the dynamic range of interest, OPGL capture followed by monoclonal detection produced the best signal-to-noise ratio throughout the calibration curve; most likely, the result was attributable to the specificity of both the ligand and the monoclonal antibody for AMGN-0007.

The abilities of OPG analogs to bind to solid-phase-bound OPGL were compared (Fig. 1). The signal at 50 $\mu\text{g/L}$ OPG analog for OPG-FLD (2.352 absorbance units) was very similar to that of AMGN-0007 (2.411 absorbance units), whereas OPG-FLM had signal strength of 1.693 absorbance units. Compared with AMGN-0007 and OPG-FLD, OPG-FLM demonstrated a curve shift to the right