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Amanda Wood

Acquired copper deficiency post Roux-en-Y gastric bypass surgery:
A retrospective review

Amanda Wood

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Lingtak-Neander Chan, Chair

Tiana Colovos

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Abstract

Acquired copper deficiency post Roux-en-Y gastric bypass surgery:
A retrospective review

Amanda Wood

Chair of the Supervisory Committee:
Associate Professor, Lingtak-Neander Chan, PharmD, BCNSP
Interdisciplinary Faculty of Nutritional Sciences

Copper is an essential trace element which operates as a vital cofactor in enzymatic reactions crucial to normal function of the hematologic, vascular, skeletal, and the nervous systems. It is also a key component of the antioxidant system. The site of copper absorption in humans is primarily the stomach and the proximal small intestine. Copper deficiency is associated with a spectrum of aberrations including neurologic manifestations such as sensory ataxia secondary to dorsal column dysfunction, gait difficulties, proprioceptive deficits, and paresthesias; hematologic abnormalities such as hypochromic anemia with neutropenia and leukopenia; as well as myeloneuropathy. The neurologic symptoms may closely resemble the myeloneuropathy indicative of a vitamin B₁₂ deficiency and may be irrevocable if not treated. However, there is currently no consensus on an appropriate copper repletion regimen making awareness and early diagnosis critical. Though hypocupremia is rare in the general public it has been described in the setting of gastric-bypass surgery.

The Roux-en-Y gastric bypass (RYGB) procedure is a successful surgical treatment for morbid obesity. The number of RYGB surgeries performed in the United States is dramatically increasing in tandem with the country's rising obesity rates. Most RYGB procedures bypass the

duodenum as well as 100-200 cm of the proximal jejunum, where copper absorption takes place. Little information on how this procedure affects copper status in the long term is currently available. Most studies are focusing on the micronutrient status of more common deficiencies such as vitamin B₁₂, iron, thiamine, folate, vitamin D, and folate; both pre-operative status or the first five years post-operatively. With symptoms mimicking several of these deficiencies, a copper deficiency may in fact be unrecognized and under-reported in the bariatric population. With a burgeoning number of patients undergoing malabsorptive surgical procedures for obesity treatment, this strongly suggests the incidence of copper depletion and hematologic and neurologic derangements will increase in the future.

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Introduction

For over a decade, obesity and its comorbidities have been the leading cause of preventable death for millions of Americans nationwide (1). Obesity may lead to type 2 diabetes, high blood pressure, hyperlipidemia, arthritis, osteoporosis, joint problems, cancer, limited mobility, and social deficits including low self-esteem, depression, and discrimination (2). Recent data from the National Health and Nutrition Examination Survey indicate more than one-third, 35.7%, of men and women and 17% of children and adolescents in the United States were obese in 2009-2010 (1). Moreover, the self-reported class III obesity rates have increased by 50% for people with a body mass index (BMI) $>40 \text{ kg/m}^2$ and by 75% for those with a BMI $>50 \text{ kg/m}^2$ between 2000 and 2005 (3). Despite urgent warnings from scientists, government officials, media groups, and researchers, this costly epidemic continues to propagate. In 2008, the combined direct and indirect health care costs of obesity were estimated to be \$147 billion; \$1,429 higher per person than those of normal weight (4).

In recent years, efforts to stifle this skyrocketing rate of obesity have shifted upstream. Weight loss and weight maintenance via lifestyle modification has been recommended by both the National Institute of Health (5) and the World Health Organization (6) for the treatment of obesity. Unfortunately, the core issues causing this epidemic are deeply complex and multifactorial with implications at nearly every level of society ranging from individual families, to communities, the built environment, schools, worksites, the food industry, healthcare organizations, and even government agencies. In its simplest form, obesity is a result of decreased energy expenditure – most likely due to modern conveniences and technologies - in conjunction with increased energy intake - related to the availability of energy-dense, nutrient

poor foods. This resultant spike in obesity has sparked a profound demand for and interest in effective weight-loss solutions.

With diet, exercise, and overall lifestyle change failing to keep pace with rapidly expanding waistlines, the popularity of bariatric surgery has taken a commanding presence in recent years. In 1993, only 14,000 bariatric surgeries were performed nationwide (2). Since then, the American Society for Metabolic and Bariatric Surgery estimates the number of bariatric procedures has nearly quintupled to the current record high of 220,000 surgeries performed annually (7). Of these procedures, approximately 65% are gastric bypass, 20% laparoscopic adjustable gastric banding, 10% sleeve gastrectomy, and <5% duodenal switch procedures (8).

Bariatric surgery is a unique tool which aids the adoption of healthy and permanent lifestyle changes. These changes may help slow and may even reverse obesity and some related comorbidities. Studies show bariatric surgery leads to sustainable long-term weight loss and may even reverse diabetes and sleep apnea (2). Bariatric surgery is indicated for individuals with a BMI \geq 40 or BMI \geq 35 and with serious obesity-related comorbidities including hypertension, type 2 diabetes, congestive heart failure, or obstructive sleep apnea (9). Potential candidates would also include individuals who did not have adequate clinical responses to other weight loss interventions - for example, consumption of a low-calorie diet and increased physical activity. Inadequate results often include insufficient weight loss and/or maintenance of weight loss for morbid obesity (10). Candidates for bariatric surgery must also possess the appropriate motivation and psychological stability to understand the risks and benefits of the procedure. Most importantly, patients must pledge a commitment to lifelong postoperative lifestyle changes and medical surveillance (2). In 2011, approximately 21 million individuals in the United States met these criteria (8).

Roux-en-Y gastric bypass surgery

The Roux-en-Y gastric bypass (RYGB) is the most commonly performed weight-loss surgery in the United States making up approximately 65% of all bariatric procedures performed. It is considered the gold-standard bariatric procedure (11). First, the surgeon alters the gastrointestinal tract by reducing the native stomach to a less than one ounce or 20-30 mL gastric pouch (12). This small pouch limits food volume and leads to decreased, restrictive intake. Next, the procedure re-routes ingested food by bypassing the duodenum and approximately 100-200 cm of the proximal jejunum (13). This is achieved by dividing the jejunum and reattaching it to the gastric pouch to form the roux limb; typically 50-150 cm long (14). The distal aspect of the biliopancreatic limb is then anastomosed further down the small intestine. The location of the jejunojejunal anastomosis will determine the length of the roux limb and the common channel. Digested food moving from the gastric pouch and into the roux limb will now have less small intestine available for mixing bile and pancreatic secretions. This will inhibit some absorption of fatty acids, amino acids, and small peptides (12). The longer the roux limb and shorter the common channel, the greater the malabsorption. Therefore, the RYGB is both a restrictive and malabsorptive procedure (10,12-13,15).

Like all surgeries, the RYGB is not without risk. Complications of the RYGB include a less than 1% mortality risk within the first 30 days post-operatively (11). Higher mortality rates have been reported in patients over 65 years of age compared with younger patients (16). Pulmonary embolism is the most common cause of mortality accounting for over 50% of deaths in the perioperative period. Technical complications such as anastomotic leaks make up the second most common cause of mortality perioperatively (17). Early complications seen within the first few weeks post-operatively include bleeding in 0.5-4% of patients (18-19), anastomotic

leaks in up to 6% of patients (20,21), wound infection in up to 20% of patients (2), deep vein thrombosis, as well as cardiovascular complications such as cardiac failure and myocardial infarctions; and pulmonary complications such as respiratory failure (22). Other early risks include persistent nausea and vomiting, rapid weight loss, food aversions, and malabsorption (2).

Later complications, several weeks to months post-operatively, include stomal stenosis in 6-20% of patients (23), marginal ulcers in 0.6-12.5% (24-25), cholelithiasis in 38% within six months of surgery (26), the dumping syndrome in up to 50% (2,27), and incisional hernias in 5% (2,20-21). These complications impair oral intake and alter absorption of food. It is important for patients to adhere to specific dietary recommendations. Weight loss is most rapid in the first six months after surgery, but typically continues at a slower pace for up to two years. During this first six months, if intake is significantly impaired and weight loss too rapid, this can deplete internal nutritive stores. Thus, the potential for nutritional derangements is substantial following the RYGB procedure (14).

The most common micronutrient deficiencies after RYGB include vitamin B₁₂, iron, thiamin, folate, and vitamin D (10,12,28). The etiology of post-surgery nutritional deficiencies stems predominantly from altered dietary intake in conjunction with changes in gut hormone signaling and malabsorption of ingested nutrients. After the surgery, patients typically struggle with meeting nutritional requirements related to nausea and vomiting, the restrictive nature of the gastric pouch, and the development of dysgeusia, dumping syndrome, and food aversions post-operatively (2). Dumping syndrome is triggered when high levels of simple carbohydrates enter the small intestines too rapidly causing swift fluid shifts from the plasma into the bowel. This causes abdominal pain, diarrhea, nausea, and sometimes tachycardia (29). Approximately 50% of post-RYGB patients experience dumping syndrome post-operatively (27). In conjunction, the

new anatomical rearrangement alters gastric mixing and preparation and decreases the intestinal absorptive surface area. Thus, micronutrient supplementation is imperative to prevent clinical deficiencies. Dalcanale et al, however, found that of 75 patients followed for 5 years after RYGB, only 12% regularly took their multivitamin supplement and 16% never took a supplement (30).

Interestingly, recent studies have also found that the prevalence of at least one micronutrient deficiency preoperatively is approximately 57% in obese individuals (12). Consequently, the risk of developing a micronutrient deficiency post-operatively may be much greater than previously expected. As a result, tremendous focus is currently being placed on this pre-operative and very short-term, < 5 years, post-operative period regarding micronutrient status (13). Very little information is known about vitamin and mineral stores in the long term post-operatively.

With burgeoning numbers undergoing the RYGB procedure every year, an extensive, rapidly growing segment of the population may in fact be underdiagnosed with less known micronutrient deficiencies such as copper.

Copper

Optimal growth, development, and health of the human body are reliant on several key minerals present in minute amounts within the body. Copper is an important essential trace mineral which operates as a cofactor in enzymatic reactions vital to normal human metabolism (13). The copper content of the adult human body is approximately 50 to 150 mg. It is found in either two redox states, the cuprous state (Cu^{1+}) or cupric state (Cu^{2+}) (31). However, the cupric state is most prevalent in biological systems (32). Concentrations of copper are highest in the liver, brain,

heart, and kidney. Skeletal muscle contains a relatively low level of copper, but due to its large mass, this makes up approximately 40% of all the copper in the body (9). In blood, tissues, and cellular fluids, copper primarily exists in the cupric state. It is bound to organic components such as the amino acids of food proteins.

Absorption

The specific site of copper absorption in humans is unclear (13,32). The stomach and ileum appears to possess some absorptive capacity (13,32-36). The acidic environment of the stomach facilitates the transport of copper across the gastric mucosa; however, animal studies indicate copper is primarily absorbed throughout the proximal small intestines, particularly the duodenum (13). The release of food-bound copper from dietary sources is facilitated by gastric hydrochloric acid and pepsin. Proteolytic enzymes located in the small intestine further release copper by hydrolyzing food proteins (31).

The specific mechanisms for absorption across the brush border are not completely understood. It appears copper enters the mucosal surface by both active carrier-mediated transporters and by a nonsaturable, passive diffusion process (9). When concentrations of dietary copper are low, it is transported primarily through the active carrier-mediated pathway; whereas the passive diffusion process accommodates higher dietary concentrations. Copper is mostly absorbed into the enterocyte by carriers such as Ctr1 and divalent mineral transporter 1 (DMT1); Ctr1 being the main copper transporter. Ctr1 is present in several other body tissues with highest expression in the liver, heart, and pancreas and with intermediate expression in the intestines (37-40). In regards to DMT1, competition between copper ions and other divalent cations exists (9). Numerous studies have described competition inhibition of DMT1 transporter between iron and

copper for enterocyte brush border membrane absorption (32).

On the surface of the brush border, most copper is reduced to the cuprous state by cytochrome reductase activity before being absorbed (41). This is facilitated by ascorbate in the lumen of the gastrointestinal tract (31). The cuprous ion is then thought to be transported across the brush border membrane into the enterocyte by DMT1 in a $\text{Cu}^{1+}/\text{H}^{+}$ symport mechanism or by Ctr1 (41-42). Net absorption of dietary copper ranges from 25% to over 50% (32,41,43-44). The efficacy of copper absorption changes in order to maintain a stable total body copper status. Total absorption is regulated by the amount of copper already retained in the body as well as dietary copper availability. Therefore, the percentage of absorption decreases with increased intake and, vice versa (9). For example, when copper intake is $>5\text{mg/day}$ absorption efficacy may average 20%; whereas, when copper intake is $<1\text{ mg/day}$, copper absorption increases to over 50% (41-44).

Once inside the enterocyte, copper is stored, utilized within the intestinal cell, or chaperoned through the cytosol. If chaperoned, the copper will be destined for subsequent transport across the basolateral membrane and into the portal system for transport to other tissues. Once in the body, copper is always bound to proteins. Unbound copper ions, may participate in the formation of reactive oxygen species which damage cells via Fenton and Haber Weiss reactions (31,42). Metallothionein serves as an intracellular binding ligand for copper. Thionein is saturated with cysteine residues which bind metal with high affinity. Once copper is bound to thionein, the protein is called metallothionein. The copper stored as metallothionein in the enterocytes is usually lost with the sloughing of intestinal cells occurring approximately

every two to three days. To be utilized before cell turnover, the complex must be degraded and copper freed (31).

Copper is chaperoned by various proteins such as glutathione, Ctr2, Atox1, copper chaperone for superoxide dismutase (CCS), cyclooxygenase 17 (cox17), and cyclooxygenase 11 (cox11) which direct the copper for use within the cell. Amino acids and glutathione are purported Cu^{1+} carriers. Ctr2 chaperones copper into vesicles for temporary storage within the intestinal cell cytoplasm. CCS is found within the mitochondria and cytosol which delivers Cu^{1+} for the conversion of a superoxide dismutase into superoxide dismutase. Cox 17 is found in the cytosol and cox11 is located in the mitochondria. Both transport Cu^{1+} for cytochrome c oxidase synthesis (37-40,45). Other chaperones may include murr1 in the liver; APP found in a few cell membranes; and sco1 and sco2 are present in mitochondria (45). Atox1 is responsible for chaperoning Cu^{1+} to P-type ATP-ases called ATP7A for the active transport of copper. It is not clear whether ATP7A pumps copper into a vesicle for exocytosis or merely pumps copper directly out of the cell (46).

The transport mechanism described above is not unique to copper. Many organic and nonorganic compounds share these absorptive pathways and therefore may interfere with the bioavailability of copper. Fiber and phytate may inhibit copper absorption (9). Phytate is found mainly in plant foods such as cereals and legumes and is known to inhibit other minerals such as iron, zinc, and calcium. Several minerals such as zinc, iron, and molybdenum are also known to impede copper absorption (31).

Zinc has been shown to attenuate copper absorption and decrease copper stores. This can occur with daily supplementation of zinc in amounts $>40\text{mg/day}$ and as low as 18.5mg/day (47).

Zinc has higher binder affinity to thionein than copper in the enterocytes (9). Specifically, zinc stimulates thionein synthesis which has a stronger affinity to copper. Thus, more copper is captured into metallothionein and is excreted through intestinal cell sloughing before it can enter the body. Suboptimal copper status or acquired copper deficiency can occur if over supplementation continues (47). As mentioned previously, iron competes with copper for DMT1 absorption at enterocytes' brush border membrane (32). Iron ingested in high amounts has been shown to reduce copper absorption in rats and humans (48-49). Molybdenum in form of tetrathiomolybdate has also been shown to produce an insoluble complex when bound to copper. This binding inhibits copper's absorption in the gastrointestinal tract of rats and ruminants. Whether this occurs in humans is still unclear (31).

Other micronutrients have also been shown to impair copper absorption such as calcium, phosphorous, and vitamin C. One study described significantly increased urinary copper losses when a high-calcium, high-phosphorous diet is consumed compared to a moderate-calcium, moderate-phosphorous diet. Additionally, 2,382 mg of calcium gluconate and 2,442 mg of glycerol phosphate were shown to significantly increase fecal copper excretion compared to moderate amounts of both supplements (50). Vitamin C might also reduce copper to its cuprous state making it less absorbable. Moreover, a high intake of ascorbic acid in the order of 1500 mg/day may decrease copper retention within the body (9,51). Finally, as stated previously, copper is more efficiently absorbed in an acidic environment. In a more alkaline environment, copper binds to hydroxides which form insoluble complexes; therefore, chronic use of antacids may impair copper's absorption as well (31).

Transport

Once copper is transported across the basolateral membrane and out of the enterocyte via ATP7A, it is bound to albumin and ferried to the liver in the portal system. It may also be transported bound to transcuprein and to amino acids such as histidine and cysteine (9,46). Carrier proteins, such as Ctr1, on the surface of hepatocytes bring in the copper. From here, the copper is distributed intracellularly into one of three pathways via Cox17, Atx1, and CCS1 for incorporation into cytochrome C oxidase, ATP7B, and copper/zinc superoxide dismutase respectively (36). Copper is also transferred to apoceruloplasmin to form ceruloplasmin. Ceruloplasmin requires six copper ions in both the cuprous and cupric state to function. Three copper ions are involved in electron transfer and three ions function at the catalytic site. Without sufficient copper status, ceruloplasmin's activity would be diminished or absent. Any excess copper not incorporated into enzymes or stored is immediately bound to metallothionein for cellular protection (31).

Ceruloplasmin is then released back into the portal system to deliver copper to other tissues. Approximately 90% of all circulating copper is bound to ceruloplasmin; the other 10% being loosely bound to albumin, transcuprein, and amino acids (31,9). Circulating copper levels tend to be higher in women than in men, but are greatest in the neonate (9).

Ceruloplasmin binds to specific receptors on extrahepatic cells. The copper ions not bound to ceruloplasmin's oxidase site are reduced from Cu^{2+} to Cu^{1+} and released. As mentioned previously, ascorbic acid aids in copper's reduction and thus enhances copper's transfer. Copper is then free to enter the extrahepatic cells either through channels or Ctr1, 2, or 2 protein transporters (52). Copper is then free to be utilized or stored within the extrahepatic cells (31).

Storage

Copper is not stored in significant quantities within the body; typically in amounts < 150 mg (53). The liver and the kidney are the major extractors of copper from the blood, but the brain, heart, bone, muscle, skin, intestine, spleen, hair, and nails all contain copper. The liver is the main organ in which whole-body copper homeostasis is achieved and is the main storage site of copper (31). Here, it is bound to predominantly metallothionein which can store up to 12 copper atoms at a time. This protects the cells from damaging superoxide and hydroxyl radicals. Copper may also be bound to amino acids, proteins, and chaperones. Copper homeostasis is mainly controlled through ceruloplasmin synthesis; copper's incorporation into metallothionein, and through copper's excretion into the bile (36).

Excretion

Approximately 98% of copper is excreted in the bile and only 2% via the urine (36,53). Dietary copper intake directly stimulates biliary copper excretion. For example, low dietary copper attenuates fecal copper excretion (44). At a copper intake of 1.4 mg/day, endogenous fecal copper is about 2.4 mg/day (46). When cellular copper is high in the liver, ATP7B transports copper to the trans-Golgi network for insertion into apoceruloplasmin or into compartments for excretion in the bile. The chaperone protein, murr1, is thought to exocytose the copper-containing vesicles into hepatic caniculi where they then exit the body via the bile (36). Once in the gastrointestinal tract, copper may be reabsorbed or secreted in feces depending on the body's need for copper (9).

The kidneys, on the other hand, excrete only a small, constant amount of copper in the urine; less than 20µg per day. Unlike biliary copper excretion, the volume of copper excreted

daily in the urine does not change significantly with dietary copper intake (44). Other modes of copper loss include small amounts from sweat ($<50\mu\text{g}$) as well as trace amounts in desquamation of skin cells, menstruation; semen; and loss of hair and nails (31).

Biological Functions

Copper's essentiality is attributed to its role as a catalytic cofactor in numerous reactions vital to human metabolism and also as an allosteric regulator of enzymes. In many instances, copper functions as an intermediate in electron transfer (31). It participates in a variety of metalloenzymes involved in processes such as iron metabolism (hephaestin, ceruloplasmin); the functioning of the central nervous system (54); free radical scavenging (superoxide dismutase, ceruloplasmin); energy generation (cytochrome c oxidase); providing great tensile strength of connective tissues (lysyl oxidase); and pigmentation (tyrosinase) (13,31-32,36).

The function of copper as part of hephaestin and ceruloplasmin is critical to iron metabolism. Iron must first be oxidized before it can bind to transferrin for transport in the blood. Hephaestin is located in the intestinal cells and ceruloplasmin can be found throughout the body. The main transporter protein of copper, ceruloplasmin, is known here as ferroxidase I. Both hephaestin and ferroxidase I catalyze the oxidation of ferrous iron to its ferric form which can then bind to transferrin in the plasma. Copper deficiency may result in iron accumulation in the liver and intestines and reduced iron transport to tissues. As a result, there is an insufficient availability of iron to support formation of normal red blood cells resulting in microcytic hypochromic anemia (31-32). In this scenario, the hematologic symptoms of an iron deficiency may mask a true copper deficiency.

Moreover, copper also functions essentially as an allosteric structural component for amine oxidases. This is critical to the function of the central nervous system. Amine oxidases are found in the blood and in body tissues and catalyze the oxidation of amines such as tyramine, histamine, serotonin, norepinephrine, and dopamine to form aldehydes and ammonium ions. This is very important in regards to neurotransmission. Here, copper helps reduce molecular oxygen to produce hydrogen peroxide. Copper also play an essential, not well understood, role in nerve myelination. (9,31).

Furthermore, the functions of copper are critical to immune function. Ceruloplasmin also modulates the inflammatory process as an acute phase reactant as well as scavenges oxygen radicals. During the acute phase of an inflammatory reaction, ceruloplasmin and copper concentrations in the blood rise (32). Superoxide radicals and other damaging compounds are generated via phagocytosis by white blood cells of foreign organisms. Ceruloplasmin plays a role in eliminating these threats to prevent further damage to cells (31,54). Therefore, copper is essential for an efficient immune response (32).

Superoxide dismutase (SOD) is also copper-dependent and assumes a very important protective role in the body. It converts the superoxide anion to hydrogen peroxide. During the reaction, copper is reduced along with the oxygen radical to initially generate molecular oxygen and then, via reoxidation, hydrogen peroxide is formed. Superoxide radicals can cause peroxidative damage to the phospholipid bonds of cell membranes. They may even form more destructive hydroxyl radicals which damage unsaturated double bonds in cell membranes, fatty acids, and other molecules in cells. SOD is found in the cytosol of cells and extracellularly bound to heparin sulfate in arterial walls. To function, SOD must bind both copper and zinc to its

histidine and aspartate residues. Copper, in the cuprous state, is found at the active site of the enzyme where superoxide substrates bind. Copper deficiency has shown increased peroxidation of cell membranes (31). Knockout mice for SOD show damage to neuromuscular junctions in the hind limbs (32).

Additionally, the cytochrome c oxidase complex employs three copper atoms. Two copper atoms on one subunit transfer electrons and the other copper atom functions on the second subunit in reducing molecular oxygen. In the mitochondrial electron transport chain, cytochrome c oxidase is responsible for passing an electron that reducing molecular oxygen to form water molecules. In this terminal oxidative step, enough free energy is generated to permit ATP production. A chronic copper deficiency can ultimately impair the activity of cytochrome c oxidase and impact mitochondrial energy production (31).

Copper continues to function essentially in the development of great tensile strength of connective tissues. Here, copper operates as a cofactor for lysyl oxidase. Lysyl oxidase is secreted by connective tissue cells and is responsible for creating lysine-derived cross-linking of collagen and elastin (9). It catalyzes the oxidative deamination of lysyl and hydroxylysyl residues of collagen and elastin polypeptides. It also oxidizes the terminal carbon atom of an aldehyde to form cross-links; imparting stabilization to the extracellular matrix. Copper deficiency decreases lysyl oxidase's activity, weakening connective tissues (55).

Copper furthermore plays an essential role in tyrosine metabolism. Tyrosine is first metabolized to form dopamine which is then converted into norepinephrine via dopamine monooxygenase. This enzyme requires up to eight copper atoms per molecule for its function. A

copper-dependent hydroxylase is also required in tyrosine catabolism to convert p-hydroxyphenylpyruvate to homogentisate (31).

Hormone metabolism is additionally reliant on copper-dependent enzymes. Peptidylglycine α -amidating monooxygenase is predominantly found in the brain and is copper-dependent. When activated, this enzyme cleaves a carboxy terminal glycine off peptide hormones, leaving a terminal amide and producing the oxidized residue, glyoxylate. In this reaction, Cu^{1+} is oxidized to Cu^{2+} . Peptide hormones involved in this reaction include cholecystokinin, gastric, calcitonin, and bombesin (31).

Copper plays essential roles in other bodily processes that are not well understood. These include the development of new blood vessels, more functions in the immune system, and endorphin action. Copper also influences gene expression through binding to specific transcription factors which then bind to promoter sequences on DNA. Once bound to DNA, transcription may be enhanced or suppressed (31).

Finally, copper has been shown to interact with other nutrients in the body, most notably zinc and iron. The strong, antagonistic relationship between zinc and copper has been described previously. Zinc intakes ranging from as low as 18.5 mg to 300 mg daily has been shown to induce copper deficiency (47,56-58). One study demonstrated that cessation of zinc supplementation and oral copper repletion for 2 months failed to correct the deficiency. A 5-day regimen of an intravenous administration of a cupric chloride solution for a total of 10 mg was needed to resolve the hematological abnormalities and raise serum copper and ceruloplasmin levels to normal ranges. This showed that excess zinc is slow to be eliminated and until then, intestinal copper absorption is blocked (47).

The synergetic relationship of copper and iron metabolism has also been described previously. With a copper deficiency, microcytic anemia develops from reduced ceruloplasmin and hephaestin activity; mimicking an iron deficiency. Moreover, a high iron intake appears to interfere with copper mobilization from tissue stores, copper's use in the body, and copper absorption in infants and children. As a result, repletion of a suspected iron deficiency may in fact worsen the biochemical, hematological, and neurological abnormalities of a true copper deficiency. (48-49,59-60).

Assessment

In vivo copper stores are best assessed using multiple indicators. Typically, copper is assessed by measuring total serum copper in conjunction with ceruloplasmin levels. The reference range for serum copper is 70-140 µg/dL and 80-155 µg/dL for men and women respectively. The reference range for ceruloplasmin is 20 – 35 mg/dL. The ratio of ceruloplasmin enzyme activity to protein concentration is thought to be better than any measurement alone. The changes in plasma or serum copper concentration when inadequate amounts of copper are consumed can be quite disparate between individuals (31). If total serum copper drops below 30% of normal levels, that is indicative of a copper deficiency (42).

Dietary Reference Intakes

In 1989, 1.5 to 3 mg/day was estimated to be a safe and adequate range for copper intake (61). Today, according to the Institute of Medicine's Food and Nutrition Board, the average daily dietary intake level of copper for both adult men and women was determined to be 900 µg/day or 0.9 mg/day. Recommendations during pregnancy and lactation are 1,000 µg/day and 1,300 µg/day respectively (62).

Food Sources

Copper is a mineral found in both plant and animal foods (9); however the precise content may vary widely depending on the food origin and how it was grown, handled, and prepared (31). Most diets easily provide between 0.6 and 2 mg/day (9). Foods richest with copper include shellfish, particularly oysters; organ meats such as liver and kidney; muscle meats; poultry; nuts and seeds; cocoa; cereal grains; legumes; potatoes; cherries; and dried fruits (9,31). Generally most fruits and vegetables contain little copper. Cow's milk is also a poor copper source; containing only 0.015 to 0.18 mg/L. In contrast, human breast milk contains 0.15 to 1.05 mg/L and is well absorbed. Infants supplemented with cow's milk may be at risk for copper deficiency due to its low copper content (9). Copper can also be absorbed from drinking water; especially when copper water pipes are corroded (36).

Toxicity

The tolerable upper limit for copper is 10 mg/day for adults (62). Although quite rare in the United States, copper toxicity has been described from acute poisonings through water contamination and accidental consumption (31). High levels of copper can lead to neurological disorders; perhaps via derangements of the amine oxidase function leading to neurotransmission problems (42). Copper intake of 64 mg or 250 mg of copper sulfate resulted in symptoms of epigastric pain, nausea, vomiting, and diarrhea (63). Other symptoms of copper toxicity may include liver and kidney damage resulting in hematuria, oliguria or anuria, and jaundice. Consumption of 1,000 times the normal dietary intake of copper can even be fatal (31).

Deficiency

Acquired copper deficiency is rare in developed countries given its low daily requirement and abundance in the food supply (13,31). But, it has been described in a handful of cases including malnutrition, excessive zinc supplementation, myelodysplastic syndrome, prolonged parenteral and enteral nutrition, and ingestion of copper-chelating agents (13,33).

The first reported case of copper deficiency induced anemia and neutropenia secondary to gastric surgery was published in 1995. A 42 year old woman presented with severe anemia and neutropenia despite having normal serum concentrations of iron, vitamin B₁₂, and folate. She had a remote surgical history of a vagotomy, pyloroplasty, and antrectomy for peptic ulcer disease. After extensive testing, it was later confirmed her symptoms were a result of copper malabsorption. Her serum copper concentration was 12 µg/dL (Normal: 80-155 µg/dL) and serum ceruloplasmin < 5 mg/dL (Normal: 22-66 mg/dL). She was treated intravenously with elemental copper 3 mg twice weekly for 10 weeks with complete normalization of all hematological abnormalities (64).

Since then, copper deficiency after gastrointestinal surgery has been rarely reported in the literature. The second truly notable case was published several years later in 2001 which reported the first case of copper deficiency myelopathy in humans. In this case, a 46 year old woman presented with profound spastic tetraparesis, painful paresthesias, impaired sensory sense for all qualities, and microcytic anemia. Her MRI showed a hyperintense T2 signal in the cervical cord which is characteristic of a vitamin B₁₂ deficiency. Also seen was a lesion in the dorsal and medial part of the cervical cord. She had decreased serum iron, but normal transferrin, vitamin B₁₂, and folate levels. Her past medical history was significant for a partial gastrectomy for

gastric ulcers over 20 years ago. Despite normal levels, the patient was supplemented intravenously with vitamin B₁₂ and folate for 18 months. Her prognosis continued to worsen. After 18 months, physicians finally discovered her serum copper and ceruloplasmin levels were deficient at 30.8 µg/dL (Normal: 80-155 µg/dL) and 11mg/dL (Normal: 22-66 mg/dL) respectively. She too was treated with intravenous elemental copper 2 mg daily for 5 days. Though she successfully achieved normalization of all hematologic abnormalities and resolved the paresthesia, the tetraparesis did not improve. As a result, this case showed myeloneuropathies related to copper deficiency may be profound and potentially irreversible despite adequate repletion (13,65).

Thesis statement

Since 2001, there have been very few case reports in the literature summarizing the specific manifestations of acquired copper deficiency in humans after gastrointestinal surgery. In addition, there are also limited data on the clinical presentation of micronutrient deficiencies arising years to decades post weight loss surgery. This strongly suggests copper deficiency may actually be unrecognized and thus underreported in this patient population. With such a large segment of the population undergoing the RYGB every year, it is essential to identify the clinical presentations of hypocupremia after RYGB.

Aims

This thesis project sought to:

1. Define the diagnostic criteria of acquired copper deficiency after RYGB.
2. Compare RYGB dataset to other case reports with hypocupremia and a history of gastrointestinal surgery to investigate similarities.
3. Propose a review of the clinical essentials of acquired copper deficiency in post-RYGB patients with emphasis on clinical presentation, laboratory findings, diagnostic tests, repletion modalities, and patient outcomes.

Methods

The study was approved by the University of Washington Institutional Review Board. Waivers of Consent and HIPAA authorization were obtained.

Phase 1 – UWMC Case search

Reviews of electronic medical records were performed to obtain pertinent clinical, laboratory, and historical data. Data included patients after RYGB and diagnosed with copper deficiency between January 1, 2010 and January 1, 2013. Copper deficiency was defined as serum copper < 80 µg/dL for women and serum copper < 70 µg/dL for men. All laboratory findings were measured using standard clinical laboratory methods at UWMC Laboratory Medicine. Four cases (n=4) were identified and patient data was collected from electronic medical records of the University of Washington Medical Center. Data on demographics, indications for copper deficiency, length of roux limb, post-operative complications, laboratory investigations, results of diagnostic tests, micronutrient supplement regimens, copper repletion therapies, and patient outcomes were extracted and recorded on designated forms. Data was then entered in a database. Outcome was recorded as latest reported follow-up.

Phase 2 – PubMed database search and case collection

The PubMed database was searched from inception through March 2013 by combining the search terms “bariatric surgery” or “gastric bypass” or “roux-en-y gastric bypass” or “gastrointestinal surgery” or “gastric surgery” and “copper deficiency” or “hypocupremia.” English language article titles and abstracts were screened and the appropriate articles were reviewed regarding their demographics, etiology, biochemical parameters, diagnostic results,

treatment, and outcome. Additional articles cited by original references were also reviewed for additional cases.

The UWMC and literature after RYGB groups were hypothesized to be clinically and statistically similar. This would allow grouping into a cohort to create a database for further defining hypocupremia after RYGB.

Statistics

Descriptive statistics were used to characterize most common clinical features of acquired copper deficiency in patients after RYGB surgery. The unpaired Student's *t*-test was used to establish statistical significance between continuous variables. Fisher's Exact Test was utilized to establish statistical significance between categorical variables. Statistical significance was established at p -value < 0.05 . Analyses were performed using Microsoft Excel 2010 software (Microsoft, Redmond, WA) and Stata 11.0.

Results

Included Studies

Literature search identified a total of 233 bariatric cases as potentially relevant. On first pass, 183 cases were excluded due to individual case data not available. This left 11 RYGB cases and 39 non-RYGB cases as potentially relevant. On second pass, an additional 14 cases were excluded related to insufficient individual case data (n=5) and duplicate cases found (n=9). Final cases included in systematic review included 6 RYGB and 30 non-RYGB (Figure 1).

Patient Characteristics

All four patients from UWMC who developed hypocupremia after receiving RYGB were female. The mean age at presentation was 53.5 ± 4.9 years with a mean 52% body weight lost post-operatively. The median time interval between initial surgery and diagnosis of hypocupremia was 1.8 years (range 1.2 to 3.4 years). Their mean Roux limb length was $132.5 \text{ cm} \pm 20.6 \text{ cm}$ and a mean serum copper concentration was $66.3 \pm 11.0 \text{ ug/dL}$. There were no significant differences between the UWMC and literature RYGB hypocupremia cases in regards to their patient characteristics; except for serum copper levels. The 6 literature post-RYGB patients were significantly more deficient in serum copper compared to the UWMC group with a mean $31 \pm 22.5 \text{ ug/dL}$ (Table 1).

Clinical presentation of hypocupremia after RYGB

Clinical features of hypocupremia were denoted as common in the UWMC group if $n \geq 2$ cases exhibited that feature. The common clinical features of hypocupremia in the UWMC group are summarized in Figure 2. In comparison with the literature group, 75% of UWMC cases presented with hair changes whereas none in the literature group had hair changes (p -value 0.01).

All other clinical features of hypocupremia were similar between UWMC and those reported in the literature. Since both groups were clinically and statistically similar, they were then grouped as a cohort to create a database for further defining hypocupremia after RYGB.

The top clinical features of the RYGB recipients who developed hypocupremia (n=10) are described in Figure 3. Clinical features were common in the cohort if $n \geq 3$ cases exhibited that feature. Other less frequently reported characteristics (n=2) included general symptoms of fatigue, pallor, clumsiness, and dizziness; dysphagia; and neurological abnormalities of absent vibratory sensation in lower limbs bilaterally up to knees, vision changes (blurred, worsening night vision, double vision), dysesthesias, numbness in upper and lower extremities, light/fine touch decreased in stocking distribution, absent vibratory sensation in lower extremities up to knees, abnormal lower limb reflexes, and past-pointing in finger to nose test. All other clinical characteristics of hypocupremia described (n=1) are listed in Appendix I.

Clinical presentation of hypocupremia after RYGB (n=10) versus after non-RYGB (n=30)

Myelopathy (90%) and proprioceptive deficits (70%) were seen significantly more frequently in the after non-RYGB cases compared to the after RYGB cases. Conversely, more frequently described in the post -RYGB group was gait ataxia (p -value 0.047), unbalanced/unsteady (p -value < 0.01), and weight loss (p -value 0.04). Other presentations including anemia, paresthesia, weakness, visibly malnourished/cachexia, wheel chair bound, and peripheral neuropathy were not significantly different between the groups (Table 2).

Clinical presentation of hypocupremia after RYGB (n=10) versus after bariatric surgery (n=8)

Patient characteristics of the after bariatric surgery hypocupremia cases are described in Table 3. Hypocupremia after bariatric surgery was diagnosed significantly later after surgery compared to

RYGB cases at a median of 17.4 years (range 7 years to 25 years) (Figure 4). Additionally, these cases presented in a significantly more deficient state with a serum copper mean of 10.9 ± 7.1 ug/dL. Despite these differences, the presentation of symptoms in the after bariatric surgery group was overall statistically similar to the after RYGB group with the exception of myelopathy (Table 4). All after bariatric surgery cases presented with myelopathy compared to only 40% after RYGB (p -value 0.01).

Investigations on laboratory findings

The presentation and type of anemia seen concordant with hypocupremia after RYGB and after bariatric surgery were similar. Sixty percent of RYGB cases and 63% of bariatric surgery cases presented with some form of anemia. The most common type was normocytic anemia (67% and 80% of each group respectively) followed by macrocytic anemia (33% and 20%) and even fewer cases of leukopenia (17% and 40%), neutropenia (33% and 20%), and thrombocytopenia (0% and 20%). Microcytic anemia was not seen in either group (Table 5).

In conjunction with deficient serum copper and ceruloplasmin levels, both the RYGB and bariatric surgery groups presented with concomitant deficiencies in other micronutrients (Table 6). The mean vitamin A level was deficient in both groups at 132.8 ± 78.5 ug/L after RYGB and 151 ug/L after bariatric surgery. The bariatric group was also deficient in vitamin D with a mean serum level of 7 ± 1.4 ng/mL whereas the RYGB group was not. However, this difference was not significant (p -value 0.112).

Diagnostic Investigations after RYGB (n=10)

Furthermore, magnetic resonance imaging (MRI) of the brain and spine showed a characteristic T2-weighted hyperintensity in specific regions in three out of four (75%) cases after RYGB.

Specific regions included hyperintensity in the central spinal canal from T4-T7 (n=1); the posterior columns of the spinal cord from T1 to the skull base (n=1); and in the white matter of the brain (n=1) (Table 7). An x-ray of the upper gastrointestinal region depicted in 100% (n=3) of cases an intestinal obstruction. Two cases were obstructed via stenosis of the gastrojejunal anastomosis and one due to a lesion just distal to the jejunostomy. Next, a computer tomography of the chest, abdomen, and pelvis of three cases after RYGB revealed inflammation of the gastrojejunal anastomosis (n=1) and a hernia (n=1). Finally, one out of two (50%) bone marrow biopsies demonstrated vacuolated precursors and ringed sideroblasts (Table 7).

Clinical Outcomes of hypocupremia after RYGB (n=10)

The five cases who received an initial intervention of oral copper, had serum copper levels normalize within 2 months of treatment. Anemia, neutropenia, leukopenia, ceruloplasmin, zinc, and vitamin E normalized as quickly as one week but taking upwards of three months.

Additionally, this group saw improvement in their symptomology as soon as one week but taking at most three months to resolve. The three cases that received intravenous copper initially and then transitioned to an oral regimen saw a much faster resolution. Their serum copper levels normalized within one week. Improved hematologic indices and symptomology were both visualized within one week and up to two months. Finally the two cases who received intravenous elemental copper initially and then a hybrid intravenous and oral copper regimen achieved hematologic normalization including serum copper levels within 1 month and 10 months and improved symptomology in one week and one month. Regardless of treatment modality, copper deficiency can be reversed in as soon as one week but taking upwards of three months despite the degree of symptomatic and hematologic derangement.

However, in spite of the normalization of serum copper and other hematologic indices, some cases did exhibit persistent symptoms. Most notable were continued sense of unsteady/unbalanced (n=1), paraesthesias (n=1), vibratory sensation and proprioception remained absent in lower extremities (n=1), muscle weakness (n=1), and abnormal reflexes (n=1).

Repletion Modalities

Of the 10 after RYGB hypocupremic cases, five received oral elemental copper 2 mg daily up to 4 mg three times a day for three to four weeks as their initial intervention. The other five received intravenous elemental copper 1 mg – 4 mg daily for one to six days. Of these five, three received a secondary treatment of oral copper 6 mg daily for an additional three weeks and the other two received a hybrid intravenous and oral copper regimen – intravenous elemental copper 2.4 mg to 3.8 mg weekly in addition to oral elemental copper 4 mg twice daily for a few weeks up to four months. Appendix II lists all initial, secondary, and tertiary treatment interventions conducted prior to diagnosis of hypocupremia.

Discussion

This systematic review identified ten cases of hypocupremia after RYGB. The most common clinical features presented included an overwhelming 90% with gait ataxia, 70% with significant weight loss, 60% with anemia and paraesthesia, 50% generalized weakness, 40% myelopathy, and 30% presented with cachexia, proprioception deficits, peripheral neuropathy, nausea, vomiting, or were wheel chair bound due to the profound nature of their neurological derangements. Neurologic symptoms were primarily localized bilaterally in the lower extremities in a stocking distribution. These hallmark neuropathies are a direct consequence of posterolateral column deficits demonstrated by an increased T2-weighted hyperintensity in the dorsal cord as seen on MRI. Choi and colleagues argue the damage is a result of wallerian degeneration, gliosis, and demyelination of the spinal tract directly caused by copper deficiency (66).

The clinical picture of acquire copper deficiency at large is most likely a down-stream result of complications stemming from the RYGB itself as well as its malabsorptive properties. Based on the common diagnostic findings in the RYGB group, complications included inflammation and/or ulceration of the gastrojejunostomy with stenosis. These complications likely caused barriers to adequate intake which resulted in significant weight loss; either short-term post-operatively or re-initiating more than two years post-operatively when weight status should have been stable. Furthermore, animal studies indicate the stomach and proximal small intestines – particularly the duodenum – may be the main absorptive sites of copper (13, 67). Unfortunately, there was limited data available on the length of the roux limb post-operatively. Generally, the longer the roux limb the greater the malabsorption due to impaired biliary mixing and decreased intestinal absorptive surface area (28). Whether the rate of weight loss versus the

decreased absorptive capacity of copper increased the potential for a copper deficiency after RYGB is unclear based on this data.

The clinical presentation of acquired copper deficiency in RYGB recipients appears to be different from other GI procedures. Clinical features significantly different between the groups included gait ataxia, unbalanced/unsteady, weight loss, myelopathy, proprioceptive deficits, nausea, and vomiting. However, group assignment into the after gastrointestinal surgery cohort possessed major hidden confounders. Though the non-RYGB group represented all other gastrointestinal procedures apart from the RYGB, the cases initially assigned to this group were not statistically comparable. Within this non-RYGB group, other bariatric procedures – with the goal of weight loss - were grouped with gastrointestinal surgeries treating peptic ulcer disease, gastric adenocarcinoma, duodenal ulcer, and Zollinger-Ellison Syndrome – where the goal was not weight loss. As mentioned previously, recent studies have found that the prevalence of at least one micronutrient deficiency is approximately 57% in obese individuals (13). Therefore, these non-RYGB case studies were not comparable in their pre-surgery micronutrient status. This greatly skews the risk of developing a micronutrient deficiency post-operatively as well as the overall resultant clinical presentations within the group. Furthermore, the percentage of weight loss experienced post-operatively would also statistically skew study variables within the group. This is due to the nature and purpose of the different surgeries; some seeking weight loss and some not.

In order to further investigate these confounders the individuals who had undergone any bariatric procedure other than the RYGB (e.g. a pre-surgery obese individual who had weight loss surgery) were extracted from the non-RYGB group. This after bariatric group was then

compared to the after RYGB group to investigate similarities in their presentation of hypocupremia. Overall, the data demonstrated acquired copper deficiency presents similarly irrespective of the bariatric procedure performed and slightly differently compared to other gastrointestinal, non-weight loss surgeries.

The resultant clinical features of acquired copper deficiency after RYGB identified in this study make physiological sense. Without adequate copper intake or supplementation, the ability of copper to perform its crucial functions in human metabolism becomes compromised. The clinical symptoms described directly reflect errors in copper's essential operations as a co-factor. These include aberrations in iron metabolism, free radical scavenging, energy generation, functioning of the central nervous system, and the tensile strength of connective tissues. Copper, however, is not the only essential micronutrient functioning vitally in these metabolic pathways. Moreover, several micronutrient deficiencies share these same characteristics. To ensure the clinical checklist described above is hypocupremia specific, it must be validated by comparing it to established clinical checklists describing common micronutrient deficiencies after RYGB.

As mentioned previously, the top most-common micronutrient deficiencies after RYGB include deficiencies of vitamin B₁₂, iron, thiamin, folate, and vitamin D (10,12,28); all of which play very important roles in the pathways vital to human metabolism. To determine if the clinical picture demonstrated in this study is specific to hypocupremia, findings were compared to established criteria describing vitamin B₁₂, iron, thiamin, and folate deficiencies respectively. Vitamin D was not included in this comparison because it does not play essential roles in the central nervous system.

In regards to vitamin B₁₂, some studies report >60% of patients may develop this deficiency within 5 years after RYGB (10,14). As a result, vitamin B₁₂ is rigorously supplemented and monitored post-operatively (2). Like copper, Vitamin B₁₂ plays a critical role in the growth and replication of cells as well as nervous system functioning (12). Vitamin B₁₂ deficiency commonly develops in the presence of barriers to adequate intake including nausea, vomiting, food intolerances, aversions, and taste changes which may cause significant weight loss (14). Deficiency typically presents as sub-acute combined degeneration of the dorsal and lateral spinal columns. This is mainly caused by a defect in myelin formation and produces a bilaterally neuropathy; usually affecting the legs more than the upper extremities. The neuropathy manifests first as paraesthesias, loss of vibratory sense, and proprioception degradation. It then progresses to severe weakness, gait disturbances, and peripheral neuropathy (68). Even more comparable, an MRI of the brain and spine typically demonstrates a T2-weighted hyperintensity (66).

However, unlike hypocupremia after RYGB, vitamin B₁₂ deficiency may also cause glossitis, cognitive difficulties, and depression which were not seen in this clinical study. More objectively, vitamin B₁₂ deficiency causes characteristically elevated homocysteine and methylmalonic acid levels which were normal in hypocupremia cases after RYGB (67). In summary, thorough objective evaluations must be made to correctly diagnose hypocupremia versus vitamin B₁₂ deficiency after RYGB given their highly congruous clinical picture. The neurologic deficits are clinically indistinguishable from each other and the hematologic abnormalities are not specific. Most importantly, hypocupremic patients do not respond to vitamin B₁₂ treatments. Symptomology typically worsens until further investigations reveals low serum

copper status. Consequent to the delay, neurologic aberrations may be too profound to ameliorate.

The second most common deficiency after RYGB is iron deficiency with an estimated incidence of 20-50% (10,14,69). Overall, the established clinical picture of iron deficiency compares very little to that of copper. Iron deficiency presents with a very characteristic iron deficiency anemia which typically manifests in the presence of barriers to adequate intake. Unlike copper deficiency, iron deficiency also may present with symptoms of chest pain, irritability, brittle nails, pica syndrome, shortness of breath, and more objectively low ferritin levels (69). The ferritin levels of the hypocupremic cases depicted in this study were within normal ranges (n=8). Despite these inconsistencies, three of the 10 (30%) cases after RYGB received iron repletion as their initial intervention. Studies in ruminants (70-71), guinea pigs (72), and children (32) have actually demonstrated iron, ingested in high amounts, adversely affects copper status. Specifically, it interferes with copper mobilization from tissue stores and copper's use in the body. This can ultimately worsen the hematological abnormalities of a true copper deficiency (32) which is exactly what occurred in the three hypocupremic cases in this study.

Furthermore, thiamin deficiency is also prevalent after RYGB with a prevalence estimated at 18.3% within the first year post-operatively. Some studies report up to 49% of post-RYGB patients become deficient at some point in time (10,12,28). Thiamin plays a major role in carbohydrate metabolism. Despite this essential function, tissue storage of thiamin is limited. With low intake, in vivo thiamin may be used in 18-20 days (14). Similar to copper deficiency, thiamin deficiency may develop in the presence of persistent vomiting, low dietary intake, and

rapid weight loss. The clinical features of thiamin deficiency, also known as beriberi, may be broken down into several subtypes. Those similar to hypocupremia include symptoms of gait disturbances, paresthesias, muscle weakness, and visual changes. Unlike hypocupremia, however, thiamin deficiency is also very notable for edema, mental confusion, aggression, convulsions, cardiac abnormalities, megacolon, glossitis, and lactic acidosis (73). Overall, the clinical pictures are quite different between the two deficiencies. Furthermore, all hypocupremia cases tested for serum thiamin (n=2) and transketolase activity (n=1) were normal.

Lastly, the prevalence of folate deficiency after RYGB ranges from 3.6% - 13% of patients (14,35,73). Folate functions vitally as a cofactor in the synthesis of methionine, thymidine nucleotides, purine nucleotides, and the coenzyme tetrahydrofolate. Deficiency typically results in a mixed anemia with increased red cell distribution width, weakness, anorexia, and weight loss. Folate deficiency has been associated with neural tube defects and cardiovascular disease (10,12). Deficiency after RYGB stems from decreased food intake, surgical bypass of the primary absorption site, and a concomitant vitamin B₁₂ deficiency – which is required as a coenzyme to convert folate to its active form (35). As mentioned earlier, the common neurological manifestations of hypocupremia resemble the sub-acute combined degeneration seen with vitamin B₁₂ deficiency. As a result, a folate deficiency could be a potential cause of similar hematologic and neurologic manifestations.

Conclusions

Acquired copper deficiency after RYGB is preventable with adequate supplementation and regularly monitoring intake. Post-RYGB patients should be required to take a complete multivitamin and mineral supplement with 100% dietary reference intake for copper. Both

chewable and gummy vitamins should be discouraged because they are not complete and do not contain minerals. Acquired copper deficiency must be included in the differential diagnosis particularly when neurologic symptoms are present. Both vitamin B₁₂ and folate deficiencies may yield similar clinical pictures. For non-critically ill patients, an initial treatment regimen of oral elemental copper 2 mg daily up to 4 mg three times a day for three weeks has been proven adequate in reversing hypocupremia. For critically-ill patients, intravenous elemental copper is warranted; 1 mg to 4 mg for one to six days and then transitioned to the oral elemental copper regimen mentioned previously. A follow up plan with regular monitoring of symptoms (particularly neurologic symptoms) is absolutely critical. Monitoring should also include serum copper and ceruloplasmin levels to ensure repletion is successful; typically achieved in one week but taking up to three months.

Implications of research

From a public health standpoint, this is an important issue. The number of RYGB surgeries performed in the United States is dramatically increasing in tandem with the country's rising obesity rates. Though the surgery is intended for weight loss, it may profoundly impact a patient's nutritional status depending on the level of nutrient intake and supplementation regimen. With symptoms mimicking a vitamin B₁₂ deficiency – a very common deficiency after RYGB – a copper deficiency may actually be under-reported and undiagnosed in the bariatric population. Additionally, little information on copper depletion after RYGB is currently available. This strongly suggests that the incidence of copper depletion and hematologic and neurologic derangements will increase in the future. These findings hopefully raise awareness, help avoid diagnostic delays, and improve treatment outcomes for hypocupremia after gastric bypass surgery. Unfortunately, these current data do not allow us to identify which patients will develop hypocupremia after bariatric surgery; whether related to the rate or percentage of weight loss as well as degree of malabsorption from the surgery. Therefore, rigorous, prospective trials on the incidence and prevalence of copper deficiency and its associated morbidity in the RYGB patient population are warranted.

References

1. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of Obesity in the United States, 2009-2010. NCHS Data Brief. U.S. Department of Health and Human Services. Centers for Disease Control and Prevention. 2012; 82: 1-8.
2. Virji A, Murr M. Caring for patients after bariatric surgery. *American Family Physician*. 2006; 73: 1403-1408.
3. Btaiche IF, Yeh AY, Wu IJ, Khalidi N. Neurological dysfunction and pancytopenia secondary to acquired copper deficiency following duodenal switch: case report and review of the literature. *Nutr Clin Pract*. 2011; 26: 583-592.
4. Finkelstein EA, Trogon JG, Cohen JW, and Dietz W. Annual Medical Spending Attributable To Obesity: Payer-And Service-Specific Estimates *Health Affairs*, 28, no.5 (2009):w822-w831 (published online July 27, 2009; 10.1377/hlthaff.28.5.w822)
5. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults—the evidence report. National Institutes of Health. *Obes. Res.* 6 (Suppl. 2), 51S–209S (1998).
6. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ. Tech. Rep. Ser.* 894, 1–253 (2000).
7. Mitka M. Surgery for Obesity: demand soars amid scientific, ethical questions. *JAMA*. 2008; 14: 289.
8. Martindale RG. Bariatric Surgery: The good, the bad, and the ugly. Managing nutritional and metabolic complications. Coram Specialty Infusion Services. 2011.
9. Mahan KL, Escott-Stump S. Krause’s Food & Nutrition Therapy. 12th Ed. Elsevier. 2008.
10. Bal BS, Finelli FC, Shope TR, Koch TR. Nutritional deficiencies after bariatric surgery. *Nat. Rev. Endocrinol.* 2012; 8: 544–556.
11. DeMaria EJ, Pate V, Warthen M, Winegar DA. Baseline data from American Society for Metabolic and Bariatric Surgery-designated Bariatric Surgery Centers of Excellence using the Bariatric Outcomes Longitudinal Database. *Surg Obes Relat Dis.* 2010; 6: 347–355.
12. Bal BS, Finelli FC, Koch TR. Origins of and recognition of micronutrient deficiencies after gastric bypass surgery. *Curr Diab Rep.* 2011; 11:136–141.
13. Griffith DP, Liff D, Ziegler TR, Esper GJ, Winton EL. Acquired copper deficiency: A potentially serious and preventable complication following gastric bypass surgery. *Obesity.* 2009; 17(4): 827-831.
14. O’Donnell K. Severe micronutrient deficiencies in RYGB patients: Rare by potentially devastating. *Practical Gastroenterology.* 2011; 11: 13-27.
15. Gletsu-Miller N, Broderius M, Frediani JK, Zhao VM, Griffith DP, Davis SS, Sweeney JF, Lin E, Prohaska JR, Ziegler TR. Incidence and prevalence of copper deficiency following roux-en-y gastric bypass surgery. *International Journal of Obesity.* 2012; 36: 328-335.
16. Livingston EH, Langert J. The impact of age and Medicare status on bariatric surgical outcomes. *Arch Surg* 2006; 141:1115.
17. Melinek J, Livingston E, Cortina G, Fishbein MC. Autopsy findings following gastric bypass surgery for morbid obesity. *Arch Pathol Lab Med* 2002; 126:1091.

18. Papasavas PK, Hayetian FD, Caushaj PF, et al. Outcome analysis of laparoscopic Roux-en-Y gastric bypass for morbid obesity. The first 116 cases. *Surg Endosc* 2002; 16:1653.
19. Schaurer PR, Ikramuddin S, Gourash W, et al. Outcomes after laparoscopic Roux-en-Y gastric bypass for morbid obesity. *Ann Surg* 2000; 232:515.
20. Nguyen NT, Goldman C, Rosenquist CJ, et al. Laparoscopic versus open gastric bypass: a randomized study of outcomes, quality of life, and costs. *Ann Surg* 2001; 234:279.
21. Higa KD, Boone KB, Ho T. Complications of the laparoscopic Roux-en-Y gastric bypass: 1,040 patients--what have we learned? *Obes Surg* 2000; 10:509.
22. Gagner M, Milone L, Yung E, et al. Causes of early mortality after laparoscopic adjustable gastric banding. *J Am Coll Surg* 2008; 206:664.
23. Schneider BE, Villegas L, Blackburn GL, et al. Laparoscopic gastric bypass surgery: outcomes. *J Laparoendosc Adv Surg Tech A* 2003; 13:247.
24. Sanyal AJ, Sugerman HJ, Kellum JM, et al. Stomal complications of gastric bypass: incidence and outcome of therapy. *Am J Gastroenterol* 1992; 87:1165.
25. Sapala JA, Wood MH, Sapala MA, Flake TM Jr. Marginal ulcer after gastric bypass: a prospective 3-year study of 173 patients. *Obes Surg* 1998; 8:505.
26. Shiffman ML, Sugerman HJ, Kellum JM, et al. Gallstone formation after rapid weight loss: a prospective study in patients undergoing gastric bypass surgery for treatment of morbid obesity. *Am J Gastroenterol* 1991; 86:1000.
27. Ukleja A. Dumping syndrome: pathophysiology and treatment. *Nutr Clin Pract* 2005; 20:517.
28. Gehrler S, Kern B, Peters T, et al. Fewer nutrient deficiencies after laparoscopic sleeve gastrectomy (LSG) than after laparoscopic Roux-Y-gastric bypass (LTYGB)—a prospective study. *Obes Surg*. 2010, 20(4): 447–53.
29. Matthews DH, Lawrence W, Poppell JW, et al. Change in effective circulating volume during experimental dumping syndrome. *Surgery* 1960; 48:185.
30. Dalcanale L, Oliveira C, Faintuch J, et al. Long-Term Nutritional Outcome after Gastric Bypass. *Obes Surg*, 2010;20:181-187.
31. Gropper SS, Smith JL, Groff JL. *Advanced nutrition and human metabolism*. 5th Ed. Wadsworth Cengage Learning. 2009.
32. Arredondo A, Nunex MT. Iron and copper metabolism. *Molecular Aspects of Medicine*. 2005; 26: 313-327.
33. Jaiser SR, Duddy M. Copper deficiency masquerading as subacute combined degeneration of the cord and myelodysplastic syndrome. *Adv Clin Neurosci Rehabil*. 2007; 7: 20-21.
34. Baker DH. Cupric oxide should not be used as a copper supplement for either animals or humans. *Journal of Nutrition*. 1999; 129: 2278-2279.
35. Shankar P, Boylan M, Sriram K. Micronutrient deficiencies after bariatric surgery. *Nutrition*. 2010; 26:1031-1037.
36. Wijmenga C, Klomp LWJ. Molecular regulation of copper excretion in the liver. *Proceedings of the Nutrition Society*. 2004; 63: 31-39.
37. Huffman DL, O'Halloran TVO. Function, structure, and mechanism of intracellular copper trafficking proteins. *Ann Rev Biochem*. 2001; 70:677-701.

38. Harrison Md, Jones CE, Dameron CT. Copper chaperones: Function, structure and copper-binding properties. *JBIC*. 199; 4:145-53.
39. Cullotta VC, Lin SJ, Schmidt P, Lomp LW, Casareno RL, Gitlin J. Intracellular pathways of copper trafficking in yeast and humans. *Adv Exp Med Biol*. 1999; 448: 247-54.
40. Rosenzweig AC. Copper delivery by metallochaperone proteins. *Acc Chem Res*. 2001; 34: 119-28.
41. Knopfel M, Solioz M. Characterization of a cytochrome b(558) ferric/cupric reductase from rabbit duodenal brush border membranes. *Biochem Biophys Res Com*. 2002; 291: 220-225.
42. Michael Rosenfeld, Ph.D. University of Washington, Department of Nutritional Sciences, NUTR 522; Nutrition and Metabolism III, Lecture 6 – April 18,2011.
43. Linder M, Hazegh-Azam M. Copper biochemistry and molecular biology. *Am J clin Nutr*. 1996; 63: 797S-811S.
44. Turnlund J, Keyes W, Kim S, Domek J. Long-term high copper intake: Effects of copper absorption, retention, and homeostasis in men. *Am J Clin Nutr*. 2005; 81: 822-828.
45. Prohaska J, Gybina A. Intracellular copper transport in mammals. *J Nutr*. 2004; 134:1003-06.
46. Linder MC, Wooten L, Cervesa P, Cotton S, Shulze R, Lomeli N. Copper transport. *Am J Clin Nutr*. 1998; 67:965S-71S.
47. Hoffman H, Phyliskey R, Felming C. Zinc-induced copper deficiency. *Gastroenterology* 1988; 94:508-512.
48. Yu S, West C, Beynen A. Increasing intakes of iron reduce status, absorption and biliary excretion of copper in rats. *Brit J Nutr*. 1994; 71: 887-95.
49. Haschke F; Ziegler E, Edwards B, Fomon S. Effect of iron fortification of infant formula on trace minerals absorption. *J Pediatr Gastroenterol Nutr*. 1986; 5: 768-73.
50. Snedeker S, Smith S, Greger J. Effect of dietary calcium and phosphorous levels on the utilization of iron, copper, and zinc by adult males. *J Nutr*. 1982; 112:136-43.
51. Finley E, Cerklewski F. Influence of ascorbic acid supplementation on copper status in young adult men. *Am J Clin Nutr*. 1983; 37: 553-56.
52. Percival S, Harris E. Copper transport from ceruloplasmin: Characterization of the cellular uptake mechanisms. *Am J Physiol*. 1990; 258:C140-146.
53. Pineles SL, Wilson CA, Balcer LJ, Slater R, Galetta SL. Combined optic neuropathy and myelopathy secondary to copper deficiency. *Survey of ophthalmology*. 2010; 55; 386-392.
54. Balsa JA, Botella-Carretero JI, Gomez-Martin JM, Peromingo R, Arrieta F, Santiuste C, Zamarron I, Vazquez C. Copper and zinc serum levels after derivative bariatric surgery: Differences between roux-en-y gastric bypass and biliopancreatic diversion. *Obes Surg*. 2011; 21: 744-750.
55. Werman M, Bhatherna S, Turnlund J. Dietary copper intake influences skin lysyl oxidase in young men. *J Nutr Biochem* 1997; 8: 201-4.
56. Festa M, Anderson H, Dowdy R, Ellersieck M. Effect of zinc intake on copper excretion and retention in me. *Am J clin Nutr*. 1985; 41: 285-92.
57. Fosmire G. Zinc toxicity. *Am J clin Nutr*. 1990; 51: 225-27.
58. Sandstead H. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. *Am J Clin Nutr*. 1995; 61: 621S-24S.

59. Barclay S, Aggett P, Lloyd D, Duffty P. Reduced erythrocyte superoxide dismutase activity in low birth weight infants given iron supplements. *Pediatr Res.* 1991; 29: 297-301.
60. Morais MD, Fisberg M, Suzuki HV, Amancio OMS, Machado NL. Effects of oral iron therapy on serum copper and serum ceruloplasmin in children. *J Trop Pediatr.* 1994; 40: 51-52.
61. National Research Council. *Recommended Dietary Allowances*, 10th ed. Washington, DC: National Academy Press, 1989; 224-230.
62. Institute of Medicine, Food and Nutrition Board: *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*, Washington, DC, 2001, National Academies Press.
63. Chuttani H, Gupta P, Gulati S, Gupta D. Acute copper sulfate poisoning. *Am J Med.* 1965; 39: 849-54.
64. Hayton BA, Broome HE, Lilenbaum RC. Copper deficiency-induced anemia and neutropenia secondary to intestinal malabsorption. *Am J Hematol* 1995;48:45–47.
65. Schleper B, Stuerenburg HJ. Copper deficiency-associated myelopathy in a 46-year-old woman. *J Neurol* 2001;248(8):705–6.
66. Choi ER, Strum W. Hypocupremia-related myeloneuropathy following gastrojejunol bypass surgery. *Ann Nutr Metab.* 2010; 57: 190-192.
67. Shankar P, Boylan M, Sriram K. Micronutrient deficiencies after bariatric surgery. *Nutrition.* 2010; 26: 1031-1037.
68. Khambatta S, Nguyen DL, Wittich CM. 38-year-old with increasing fatigue and dyspnea. *May Clin Proc.* 2010; 85(4): 392-395.
69. John S, Hoegerl C. Nutritional deficiencies after gastric bypass surgery. *JAOA.* 2009; 11: 601-604.
70. Standish JF, Ammerman CB, Simpson CF, Neal FC, Palmer AZ. Influence of graded levels of dietary iron, as ferrous sulfate, on performance and tissue mineral composition of steers. *J Anim Sci.* 1969; 3: 496-503.
71. Humphries WR, Philloppo M, Young BW, Bremner I. The influence of dietary iron and molybdenum on copper metabolism in calves. *Br J Nutr.* 1983; 49(1): 77-86.
72. Smith CH, Bidlack WR. Interrelationship of dietary ascorbic acid and iron on the tissue distribution of ascorbic acid, iron, and copper in female guinea pigs. *J Nutr.* 1980; 110(7): 1398-1408.
73. Rudnicki SA. Prevention and treatment of peripheral neuropathy after bariatric surgery. *Curr Treat Options Neurol.* 2010; 12(1): 29-36.

Tables and Figures

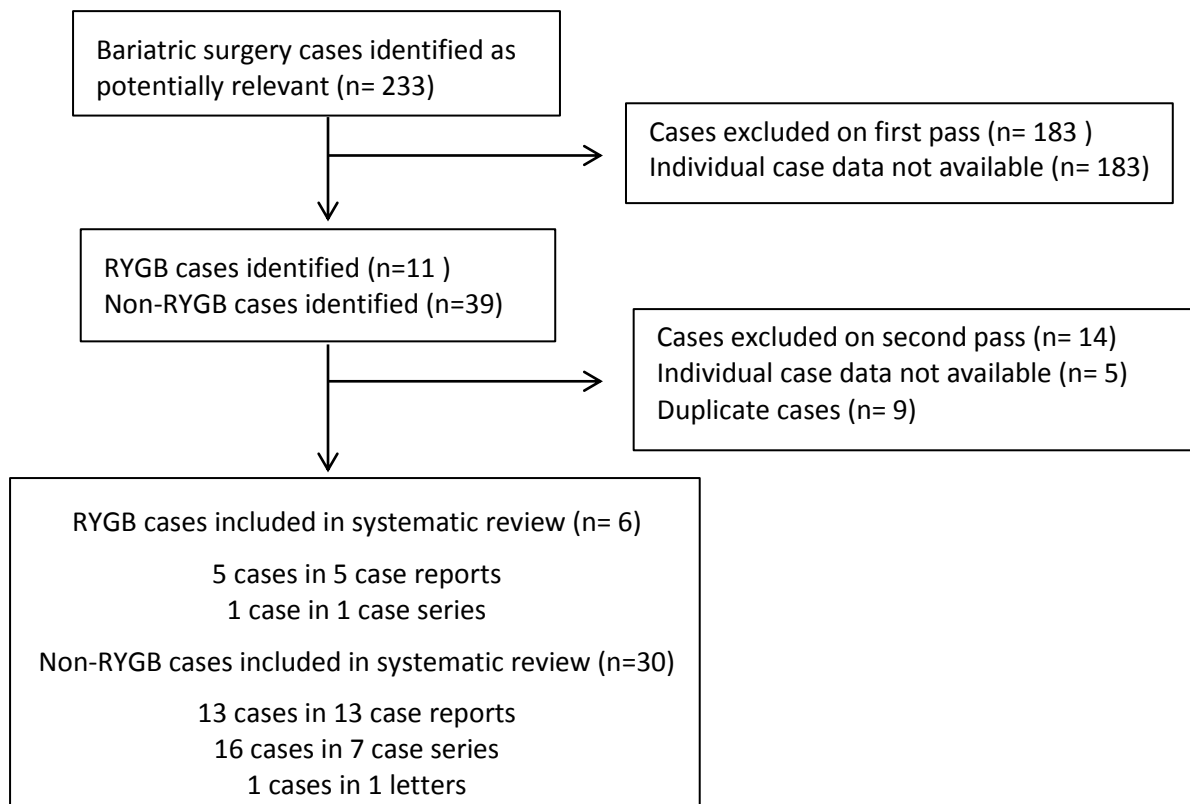


Figure 1. Case selection.

Table 1

UWMC & Literature RYGB Patient Characteristics	UWMC RYGB Patients (n=4)	Literature RYGB Patients (n=6)	p-Values
Sex			
Male	0	1	0.40
Female	4	5	0.40
Age at Presentation			
Mean ± Standard Deviation	53.5 ± 4.9	48.5 ± 11.8	0.45
% Body weight lost post-op			
Mean ± Standard Deviation	52% ± 0.1	59% ± 0.2	0.57
Years between initial surgery and hypocupremia diagnosis			
Mean ± Standard Deviation	2.0 ± 1.0	9.6 ± 8.3	0.11
Median (Range)	1.8 (1.2-3.4)	9.5 (0.4-21)	
Duration of neurological symptoms before hypocupremia diagnosis			
Mean ± Standard Deviation	N/A	2.4 ± 3.8	N/A
Roux limb length, cm			
Mean ± Standard Deviation	132.5 ± 20.6	N/A	N/A
Copper, serum (80-155 ug/dL)	66.3 ± 11.0	31 ± 22.5	0.03
Ceruloplasmin, serum (22.0-66.0 mg/dL)	N/A	3.4 ± 3.9	N/A
<i>Student's t-test, unpaired 2 tailed, homoscedastic.</i>			

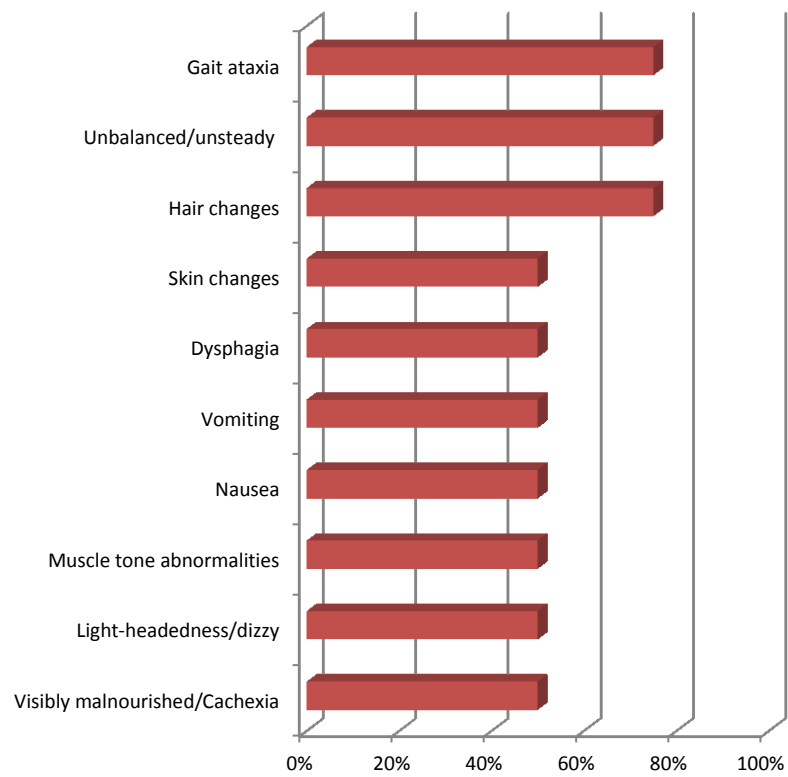


Figure 2 Clinical Presentations of Hypocupremia in UWMC RYGB Patients (n=4).

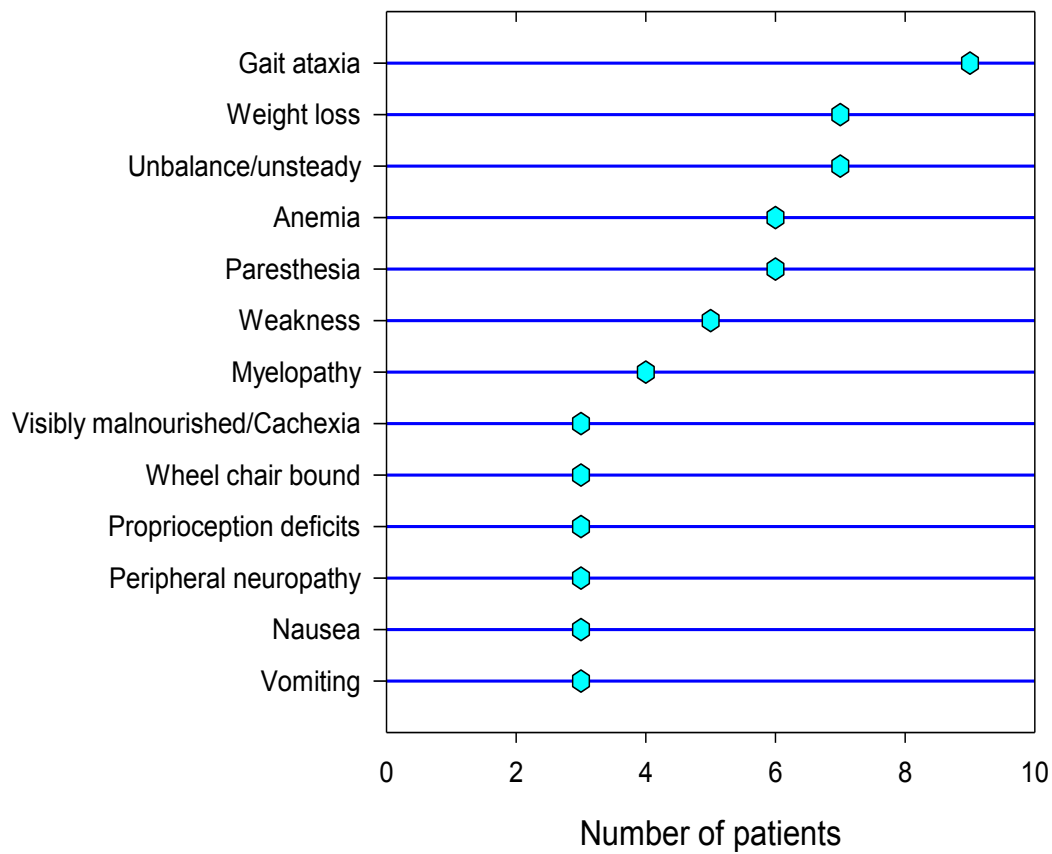


Figure 3 Clinical features reported in hypocupremia cases after RYGB (n=10). Gait described as wide or broadly based. Range of weight loss was 65 lbs. in 3 weeks to 212 lbs. in 1.5 years. Paresthesia localized primarily in lower limbs, 50% in upper extremities. Weakness primarily in lower extremities. Proprioception deficits in lower extremities up to knees.

Table 2

Clinical Presentations of RYGB versus non-RYGB Literature	All RYGB (n=10)	Non-RYGB Literature (n=30)	P-value
Gait ataxia	90%	80%	0.047
Weight loss	70%	33%	0.04
Unbalanced/unsteady	70%	13%	< 0.01
Anemia	60%	73%	0.43
Paresthesia	60%	57%	0.85
Weakness	50%	47%	0.86
Myelopathy	40%	90%	< 0.01
Visibly malnourished/Cachexia	30%	6.7%	0.05
Wheel chair bound	30%	20%	0.51
Proprioception deficits	30%	70%	0.03
Peripheral neuropathy	30%	43%	0.46
Nausea	30%	0%	< 0.01
Vomiting	30%	0%	< 0.01
<i>Fisher's Exact Test.</i>			

Table 3

RYGB & Bariatric Surgery Literature Patient Characteristics	RYGB (n=10)	Bariatric Surgery (n=8)	p-Values
Sex			
Male	1	1	0.87
Female	9	7	
Age at Presentation			
Mean ± Standard Deviation	50.5 ± 9.6	49.3 ± 9.9	0.79
% Body weight lost post-op			
Mean ± Standard Deviation	54.9% ± 16.1%	56%	
Years between initial surgery and hypocupremia diagnosis			
Mean ± Standard Deviation	6.6 ± 7.4	16.7 ± 8.1	0.02
Median (Range)	2.7 (0.4-21)	17.4 (7-25)	
Duration of neurological symptoms before hypocupremia diagnosis			
Mean ± Standard Deviation	2.4 ± 3.8	2.9 ± 2.5	0.75
Median (Range)	0.96 (10-0.02)	2.3 (6.0-0.5)	
Roux limb length, cm			
Mean ± Standard Deviation	132.5 ± 20.6	200	
Serum Copper (80-155 ug/dL)	48.6 ± 25.0	10.9 ± 7.1	< 0.01
Ceruloplasmin, serum (22.0-66.0 mg/dL)	3.4 ± 3.9	3.8 ± 0.9	0.86
<i>Student's t-test, unpaired 2 tailed, homoscedastic.</i>			

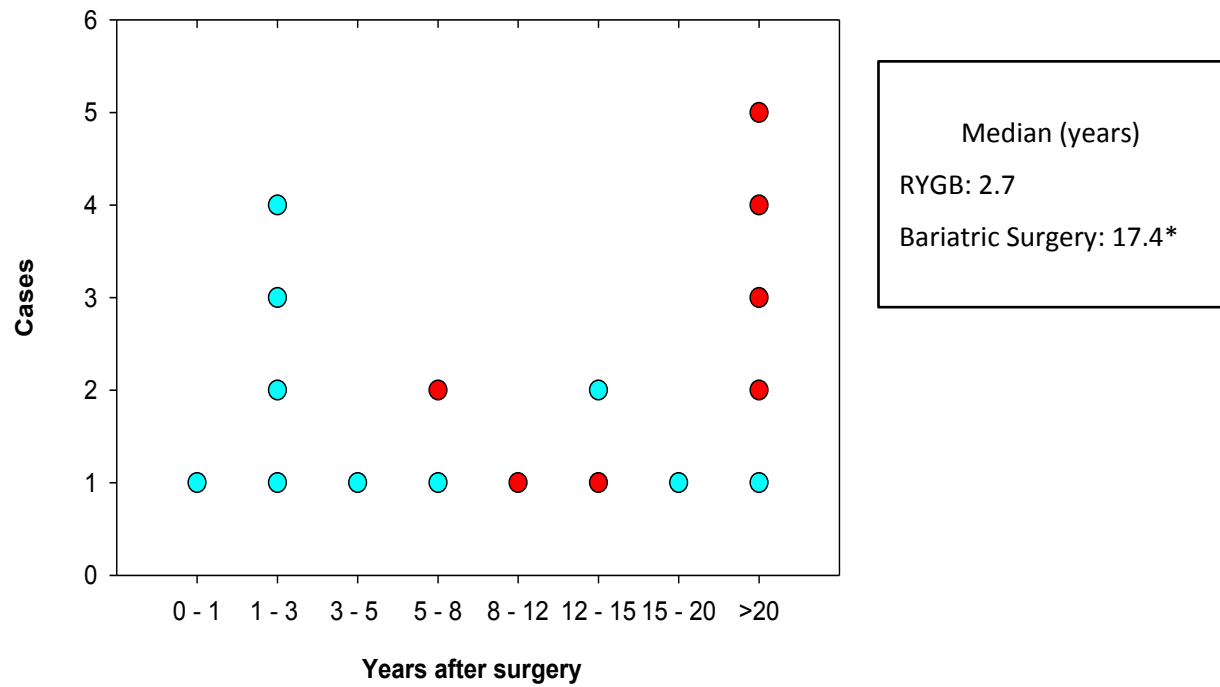


Figure 4 Diagnosis of acquired copper deficiency after bariatric surgery. Blue = RYGB patients. Red = all other bariatric surgery patients. * One bariatric surgery patient's data unavailable.

Table 4

Clinical Presentations of RYGB versus Bariatric Literature	All RYGB (n=10)	Bariatric Surgery Literature (n=8)	P-value
Gait Ataxia	90%	100%	0.36
Weight loss	70%	25%	0.06
Unbalanced/unsteady	70%	63%	0.74
Anemia	60%	63%	0.91
Paresthesia	60%	63%	0.91
Weakness	50%	63%	0.60
Visibly malnourished/Cachexia	30%	13%	0.38
Wheelchair bound	30%	38%	0.73
Proprioception deficits	30%	75%	0.06
Peripheral neuropathy	30%	38%	0.74
Nausea	30%	0%	0.09
Vomiting	30%	0%	0.09
Myelopathy	40%	100%	0.01
<i>Fisher's Exact Test.</i>			

Table 5

Anemia Breakdown	RYGB (n=6)	Bariatric Surgery (n=5)	p-Value
Microcytic	0%	0%	N/A
Normocytic	67%	80%	0.62
Macrocytic*	33%	20%	0.62
Leukopenia	17%	40%	0.39
Neutropenia	33%	20%	0.62
Thrombocytopenia	0%	20%	0.25

Fisher's Exact Test. *Vitamin B₁₂ and folate levels normal

Table 6

Vitamins & Minerals	All RYGB (n=10)	Bariatric Surgery Literature (n=8)	p- value
Vitamin A (retinol) (300-650 ug/L)	132.8 ± 78.5 [†]	151 [†]	N/A
Folate, serum (>5.8 ng/mL)	14.7	11	N/A
Vitamin B12 (180-914 pg/mL)	937.3 ± 387	430 ± 189	0.094
25-hydroxy Vitamin D Total (20.1-50.0 ng/mL)	24.5 ± 12.6	7 ± 1.4 [†]	0.112
α-Tocopherol (5-20 mg/L)	3.5 ± 0.3 [†]	4.8 ± 0.7 [†]	0.137
Zinc, serum (60-120 ug/dL)	116.8 ± 71.8	81.3 ± 27.5	0.285
Copper, serum (M: 70-140 ug/dL; F: 80-155 ug/dL)	48.6 ± 25.0 [†]	10.9 ± 7.1 [†]	< 0.01*
Copper, urine (15-60 ug/24 hr)	8.9 ± 5.4 [†]	19 ± 7.1	0.249
Ceruloplasmin (22.0-66.0 mg/dL)	3.4 ± 3.9 [†]	3.8 ± 0.9 [†]	0.858
<i>Student t-test, unpaired 2 tailed, homoscedastic</i>			
<i>† Vitamin or mineral deficiency</i>			

Table 7

Diagnostic Investigations in Hypocupremia RYGB cases (n=10)	Abnormal Test Result (%)	Typical Pathology
Esophagogastroduodenoscopy (EGD)	4/5 (80%)	Ulceration around gastrojejunal anastomosis with stenosis
MRI Brain & Spine	3/4 (75%)	T2-weighted hyperintensity in specific regions [†]
X-ray Upper GI	3/3 (100%)	Intestinal obstruction in specific regions*
CT Chest, Abdomen & Pelvis	2/3 (67%)	Inflammation of gastrojejunal anastomosis (n=1) hernia (n=1)
Bone Marrow Biopsy	1/2 (50%)	Vacuolated precursors and ringed sideroblasts

[†] Specific regions: central spinal canal from T4 –T7 (n=1); posterior columns of spinal cord from T1 to skull base (n=1); in white matter (n=1)
* Specific regions: stenosis of gastrojejunal anastomosis (n=2); a lesion just distal to jejunojunostomy (n=1)

Appendix I Documented Clinical Presentations

A. General Symptoms

1. Fatigue
2. Visibly malnourished/Cachexia
3. Weight loss
4. Pallor
5. Confusion
6. Clumsy
7. Unbalanced/unsteady
8. Wheel chair bound
9. Walker/Cane
10. Stiffness
11. Diet
12. Light-headness
13. Temperature (°C)
14. Voice
15. Emotional ability

B. Nervous System

1. Cranial nerve examination
2. Gait
3. Vision
4. Mental Status
5. Visual Acuity
6. Nystagmus
7. Square wave eye jerks
8. Kayser-Fleischer rings
9. Sensitivity to light
10. Hypoguesia
11. Hyposmia
12. “Legs fell asleep easily”
13. Shooting pain in lower extremities
14. Tremor
15. Paraparesis
16. Paresthesia
17. Dysesthesias
18. Numbness
19. Pin-prick Sensation
20. Light/Fine touch
21. Proprioception
22. Pain sensation
23. Temperature sensation

24. Sharp-dull discrimination
25. Vibratory sensation
26. Sensory Loss
27. Hyperesthesia
28. Reflexes
29. Plantar reflexes
30. Babinski's sign
31. Romberg sign
32. Heel to shin
33. Finger-to-nose test
34. Fine Finger movements
35. Pseudoathetosis
36. Myoclonus
37. Polyneuropathy
38. Myopathy
39. Neuropathies
40. Peripheral Neuropathy
41. Optic neuritis
42. Bypass arthropathy (initated)
43. Sensory Ataxia
44. Upper limb signs and symptoms
45. Sciatica
46. Extensor Spasms

C. Respiratory System

1. Dyspnea
2. Upper respiratory tract infections
3. Auscultation

D. Circulatory System

1. Pulse
2. Respiratory rate
3. Blood Pressure
4. O₂ saturation
5. Systolic ejection murmur over left sternal border
6. Congestive heart failure

E. Muscular System

1. Strength
2. Muscle tone
3. Bulk
4. Joint Pain
5. Myalgia

6. Hernia

F. Digestive System

1. Glossitis
2. Nausea
3. Stomach Cramping
4. Gastrointestinal bleeding
5. Abdominal pain
6. Constipation
7. Vomiting
8. Diarrhea
9. Dysphagia
10. Dumping Syndrome
11. Food Intolerances
12. Bowel resection
13. Red tongue
14. TPN
15. Tube feeding

G. Urinary System

1. Urinary Tract Infections
2. Urinary Incontinence
3. Urinary Urgency
4. Urinary Symptoms

H. Integumentary System

1. Cheilitis
2. Skin changes
3. Hair changes
4. Panniculectomy

I. Reproductive System

1. Yeast infections

J. Skeletal System

1. Broken Bone
2. Dentition

Appendix II Interventions pre- Hypocupremia Diagnosis

A. Initial

1. Oral iron replacement therapy
2. Packed red blood cells transfusions
3. Zinc, Oral
4. Vitamin A
5. Vitamin B complex
6. vitamin B12 supplements
7. Vitamin D
8. Calcium
9. Folate, Oral
10. Vitamin B6
11. Steroids, IV
12. Multivitamin Mineral Supplement, oral
13. Thiamin
14. TPN
15. G-tube placement
16. Proton Pump Inhibitors
17. Ig, IV
18. Pyridoxine therapy
19. Physiotherapy

B. Secondary

1. Vitamin B6
2. Vitamin B12
3. Multivitamin Mineral Supplement, oral
4. Plasmapheresis
5. Granulocyte colony stimulating factor
6. Erythropoietin

C. Tertiary

1. Multivitamin Mineral Supplement, oral
2. Rifamixin, oral
3. Surgical Revision