March 25, 2015 Dear Editor, Please find enclosed the edited manuscript in Word format (file name:

16308-review.doc).

Title: Wilson Disease with Hepatic Presentation in an 8-month-old Boy

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The manuscript has been improved according to the suggestions of reviewers: 1
Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewer 1: Abuduxikuer et al have reported a case about a 8 month old Chinese boy that has been diagnosed with Wilson disease. The case report is interesting and factual. I would like them to expand their discussion by explaining how over consumption of copper can induced Wilson disease (if known). Answer: There is no direct evidence that over consumption of copper through high dietary and environmental exposure induce Wilson disease. However, this patient came from highly industrialized coastal region of China with potentially high exposure to copper, and there is evidence that high copper intake in healthy individuals lead to significantly higher amount of copper retention in the body. We expanded our discussion by adding the following content: "Over consumption of copper could be considered as one of the causes of early WD onset. An epidemiologic study of serum copper levels in 8 provinces of China revealed that serum copper levels in people from eastern China were significantly higher than that of middle and western China[4]. Authors also conducted a survey proving that more frequent sea food consumption led to significantly higher copper levels in the body. Dietary and environmental factors may have played a role since coastal regions in eastern China are more industrialized, and people living there consumes more sea food than the people from other parts of the country. There is also evidence that long-term high copper intake in healthy men led to significantly higher copper retention in the body, and homeostatic regulation was not sufficient to maintain a normal copper absorption[5]. Our patient came from the coastal Shandong Province in eastern China, and potentially higher exposure to dietary and environmental copper, coupled with potentially severe disruption of copper homeostasis caused by ATP7B gene mutation plus SNPs may have led to enough copper accumulation, and caused liver damage at this young age. However, further studies need to be done in order to elucidate the complex interplay among genotype, phenotype, and environmental factors (such as diet and pollution)."

1 Pan X, Ding C, Pan Y, Zhang A, Wu B, Huang H, Zhu C, Liu D, Zhu B, Xu G, Shao H, Peng S, Jiang X, Zhao C, Han C, Ji H, Yu S, Zhang X, Zhang L, Zheng Y, Yan H. Distribution of copper and zinc in blood among general population from 8 provinces in China. Zhonghua Yu Fang Yi Xue Za Zhi. 2014 Feb;48(2):109-13. PMID: 24746004. DOI: 10.3760/cma.j.issn.0253-9624.2014.02.007 [Article in Chinese]

2 Turnlund JR, Keyes WR, Kim SK, Domek JM. Long-term high copper intake: effects on copper absorption, retention, and homeostasis in men. Am J Clin Nutr. 2005 Apr;81(4):822-8. PMID: 15817858.

(2) Abuduxikuer and colleagues did a good work to report the case of 8-month-old Boy with Wilson Disease. In the report they stated that the weight of the body is below the normal during the period of 8-35 months. Please record these data. Answer: We had the weight and height data on 23 months of age, and 35 months of age, we clearly recorded these data in comparison with WHO growth standards, and added these data in the manuscript with the following content: "At the age of 23 months, the patient was slightly undernourished with a weight of 11.5kg (below 50th percentile by The WHO Child Growth Standards: http://www.who.int/childgrowth/standards/en/) and a height of 83cm (below 15th percentile). At the last follow-up when the patient was 35 months of age, liver function test was normal. Linear growth had significantly improved with weight for age reaching above the 50th percentile (14.5kg), and height for age reaching above the 15th percentile (93cm)."

3 References and typesetting were corrected

We tried adding PMID and DOI citation to all reference lists, and changed the reference 1 to more recent one that has both PMID and DOI. Reference 5 does not have DOI citation, we provided the first page of the paper on page 3 of this document.

Referrences:

1 Patil M, Sheth KA, Krishnamurthy AC, Devarbhavi H. A review and current perspective on Wilson disease. J Clin Exp Hepatol. 2013 Dec;3(4):321-36. DOI: 10.1016/j.jceh.2013.06.002. PMID: 25755520.

2 Shimizu N, Nakazono H, Watanabe A, Yamaguci Y, Hemmi H, Aoki T. Molecular diagnosis of Wilson's disease. Lancet. 1997 Jun 21;349(9068):1811-2. DOI: 10.1016/S0140-6736(05)61691-1. PMID: 9269219.

3 Kim JW, Kim JH, Seo JK, Ko JS, Chang JY, Yang HR, Kang KH. Genetically confirmed Wilson disease in a 9-month old boy with elevations of aminotransferases. World J Hepatol. 2013 Mar 27;5(3):156-9. DOI: 10.4254/wjh.v5.i3.156. PMID: 23556051.

4 Pan X, Ding C, Pan Y, Zhang A, Wu B, Huang H, Zhu C, Liu D, Zhu B, Xu G, Shao H, Peng S, Jiang X, Zhao C, Han C, Ji H, Yu S, Zhang X, Zhang L, Zheng Y, Yan H. Distribution of copper and zinc in blood among general population from 8 provinces in China. Zhonghua Yu Fang Yi Xue Za Zhi. 2014 Feb;48(2):109-13. DOI: 10.3760/cma.j.issn.0253-9624.2014.02.007. PMID: 24746004. [Article in Chinese]

5 Turnlund JR, Keyes WR, Kim SK, Domek JM. Long-term high copper intake: effects on copper absorption, retention, and homeostasis in men. Am J Clin Nutr. 2005 Apr;81(4):822-8. PMID: 15817858. (provided the first page of the paper on page 3 of this document)

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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Long-term high copper intake: effects on copper absorption, retention, and homeostasis in men[1–3]

Judith R Turnlund, William R Keyes, Soon Kyung Kim, and Joseph M Domek

ABSTRACT

Background: Numerous studies have examined the effect of low and adequate intakes of copper on absorption and retention, but little information is available on the regulation of absorption and retention of copper when intake is high.

Objective: A study was conducted in men to determine the effect of long-term high copper intake on copper absorption, retention, and homeostasis.

Design: Nine men were confined to a metabolic research unit (MRU) for 18 d and were fed a 3-d rotating menu containing an average of 1.6 mg Cu/d. They continued the study under free-living conditions for 129 d, supplementing their usual diets with 7 mg Cu/d. They then returned to the MRU for 18 d and consumed the same diet as during the first period, except that copper intake was 7.8 mg/d. The stable isotope ^{63}Cu was fed to 3 subjects and infused into the other 6 on day 7 of each MRU period, and complete urine and stool collections were made throughout the study. Total copper and ^{63}Cu were determined by inductively coupled plasma mass spectrometry. Copper absorption, excretion, and retention were calculated on the basis of dietary, urinary, and fecal copper and ^{63}Cu.

Results: Results were as follows when comparing the high copper intake with the usual intake: fractional copper absorption was significantly lower, but the amount absorbed was significantly higher; excretion of the infused ^{63}Cu was significantly faster; and total retention was significantly higher.

Conclusions: Homeostatic regulation of copper absorption and retention helped to minimize the amount of copper retained with high copper intake but was not sufficient to prevent retention of >0.6 mg Cu/d. *Am J Clin Nutr* 2005;81:822–8.

KEY WORDS Copper, absorption, retention, homeostasis, stable isotope, high copper intake

INTRODUCTION

An estimated average requirement for copper of 0.7 mg/d and a recommended dietary allowance (RDA) of 0.9 mg/d for adults were introduced in 2001 in the new dietary reference intakes (1). Before, sufficient data were not available to establish an RDA for copper, but an estimated safe and adequate daily dietary intake range was suggested. The new recommendations were based on several studies conducted with low and adequate copper intakes (2–4).

A tolerable upper amount was also established, but limited data were available to set this amount. Human data, based on chronic consumption of copper gluconate, were used (1) as a basis for the upper amount. In that study 10 mg Cu/d was fed for 12 wk and liver damage was not observed (5), whereas a case report indicated that long-term intake of 60 mg Cu/d resulted in acute liver failure (6). Because no liver damage was observed at 10 mg/d, it was suggested as the amount at which no adverse effects were observed, thus, an upper amount of 10 mg/d was established. Numerous studies reported gastrointestinal effects of copper intake < 10 mg/d, including abdominal pain, cramps, nausea, diarrhea, and vomiting, from consuming beverages or drinking water (1), and the incidence of these effects increases at copper concentrations >3 mg/L (7). However, those observations had limitations and the effects were considered transient. Little research has been done to examine the effect of high copper intake on copper status, metabolism, and functional effects under controlled conditions.

We have shown that, with a copper intake of 7.5 mg/d for 24 d, the efficiency of absorption was considerably less than with a lower intake, but the amount absorbed increased. Copper retention was high, averaging 0.9 mg/d, but the amount retained decreased over time (8). We hypothesized that over a longer period normal balance of close to 0 would be restored. A report on the effect of 6 mg Cu/d for 8 wk on adaptive responses found that 0.75 mg Cu/d was retained, more than when the diets contained less copper (9). The efficiency of absorption was not affected in that study, but the amount absorbed would have increased. Another study was reported in which a supplement of 6 mg Cu/d was given for 4 wk, but copper absorption and retention were not reported (10). This report describes the effect of long-term high copper intake on copper absorption, retention, and homeostasis.

SUBJECTS AND METHODS

Subjects

Eleven healthy men were selected to participate in the study. Recruitment, selection procedures, exclusion criteria, and procedures for informed consent have been described (11). The

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