British Journal of Nutrition (2008), **99**, 1226–1229
doi: 10.1017/S0007114507864828

Short Communication

The association between serum copper and anaemia in the adult Second National Health and Nutrition Examination Survey (NHANES II) population

Mary Ann Knovich[1], Dora Il'yasova[2], Anastasia Ivanova[3] and István Molnár[1,4]*

[1]*Section on Hematology and Oncology, Department of Internal Medicine, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC 27157, USA*
[2]*Cancer Control & Prevention Program, Department of Community & Family Medicine, Duke University, Durham, NC, USA*
[3]*Department of Biostatistics, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*
[4]*Comprehensive Cancer Center of Wake Forest University, Winston-Salem, NC, USA*

(Received 14 March 2007 – Revised 4 September 2007 – Accepted 15 October 2007 – First published online 21 November 2007)

Though common in older adults, anaemia is unexplained in about one-third of cases. As a rare cause of anaemia and neutropenia, Cu deficiency could account for some cases of unexplained anaemia. We examined the relationship between serum Cu and unexplained anaemia among 11 240 participants in the Second National Health and Nutrition Examination Survey (NHANES II): 638 (5·7 % of all adults) were anaemic; 421 (3·7 %) were not explained by deficiencies of vitamin B_{12}, folate or Fe, chronic illness or renal disease. Spline regression showed a U-shaped relationship between serum Cu levels and unexplained anaemia, indicating that both high and low serum Cu levels are associated with unexplained anaemia in adults. Chronic inflammation and mild Fe deficiency could account for the association between unexplained anaemia and elevated Cu levels. On the other hand, the finding of hypocupraemia in a subset of adults with unexplained anaemia suggests that Cu deficiency may be a common reversible cause of anaemia in adults.

Unexplained anaemia: Copper: NHANES II

Anaemia is common in older adults. Although most cases are due to nutritional deficiencies, chronic illness or renal disease, anaemia remains unexplained in about one-third of cases[(1)]. As Cu deficiency has been reported to be a rare cause of anaemia and neutropenia[(2)], we hypothesised that hypocupraemia could account for some proportion of unexplained anaemia cases in adults. As part of the Second National Health and Nutrition Examination Survey (NHANES II), serum Cu levels, a widely available indicator of Cu status, were obtained in over 10 000 healthy adults. We used the publicly available data from the NHANES II to examine the relationship between Cu levels and Hb in clinically healthy individuals older than 15 years of age. These data present a rare opportunity to examine the relationship between serum Cu and unexplained anaemia on a large scale, since Cu levels are not routinely obtained and hypocupraemia is unexpected in nutritionally replete adults.

Study design

The NHANES II was conducted in 1976–1980 on a nationwide probability sample of approximately 28 000 individuals aged 6 months to 74 years from the civilian, non-institutionalised population of the USA[(3)]. For the present analysis we used publicly available data on 20 322 individuals who were both interviewed and underwent medical examination[(4)]. We excluded 5134 individuals less than 15 years of age, an additional 768 individuals with missing values for Hb, and 3180 individuals with missing values for serum Cu. A total of 11 240 NHANES II participants were included in the present analysis. The upper age range was 74 years. Menopausal status of female participants was not reported.

We used the same inclusion criteria for the anaemia subgroup set forth by the NHANES II investigators to define subjects in whom analyses of vitamin B_{12}, erythrocyte and serum folate and ferritin were performed[(5)]. Briefly, in males aged 15 years or older, anaemia was defined as an Hb level $<$ 135 g/l (13·5 g/dl); in females aged 15 years or older, as an Hb level $<$ 115 g/l (11·5 g/dl). The following definitions were used for classification of explained anaemia: (1) Fe deficiency – if two or three of the following criteria were met – (a) transferrin saturation rate $<$ 15 %, (b) serum ferritin concentration $<$ 12 ng/ml, and (c) erythrocyte protoporphyrin concentration $>$ 1·24 μM[(6)]; (2) vitamin B_{12} deficiency – serum B_{12}

Abbreviation: NHANES II, Second National Health and Nutrition Examination Survey.

* **Corresponding author:** Dr István Molnár, fax + 1 336 716 5687, email imolnar@wfubmc.edu

concentration < 147 pM (200 pg/l); (3) folate deficiency – erythrocyte folate concentration < 232 nM (102·6 ng/ml); (4) anaemia of chronic illness or inflammation – serum Fe < 600 μg/l in subjects who were not Fe deficient. We excluded patients with chronic renal failure, defined as estimated creatinine clearance ≤ 30 ml/min as calculated by Cockcroft–Gault[7]. Detailed methods for determination of laboratory values are described elsewhere[4,5].

The Wilcoxon–Mann–Whitney rank test was used to examine differences in the distributions of serum Cu between non-anaemic participants and those with different types of anaemia. To quantify the associations between serum Cu and unexplained anaemia, we fitted a logistic regression model. Unexplained anaemia was entered into the model as the dependent variable and log-transformed serum Cu level as the independent variable. Other independent variables included age (years), sex, and race (white, black, others). To explore the dose–response shape of the association, we allowed a non-linear effect of log-transformed serum Cu levels and forced the linear effect of the rest of the variables. A non-linear effect of log-transformed serum Cu was fitted using cubic splines with nodes placed at tertiles. Possible correlation within primary sampling units was accounted for by using generalised estimating equations with clustering by primary sampling unit. The model was fitted using procedure GENMOD in SAS (SAS Institute, Cary, NC, USA). The estimates of the OR and point-wise 95 % CI were computed from the model, with the reference level being the median value of log-transformed serum Cu level.

We calculated the proportion of subjects with low leucocyte levels (<4000/μl) in three groups: participants with low serum Cu levels (<700 μg/l), normal serum Cu levels (700–1400 μg/l) and elevated serum Cu levels (>1400 μg/l).

Results and discussion

In this adult subset of the NHANES II population, 638 (5·7 %) met the study's definition of anaemia. More men (*n* 422; 7·8 %) than women (*n* 216; 3·7 %) were anaemic. Fe deficiency accounted for most cases of explained anaemia in women (seventy-five of 105; 71 %), whereas in men, 27 % of those with explained anaemia were Fe deficient (thirty-one of 112) (Table 1). Folate deficiency accounted for anaemia in twelve or 0·1 % of adults. There were no cases of vitamin B_{12} deficiency. Anaemia of chronic inflammation was present in 0·4 % of females (*n* 25) and 1·3 % of males (*n* 68) and accounted for ninety-three of the 217 cases (43 %) of explained anaemia. Exclusion of the above causes for anaemia left a substantial proportion of cases unexplained: 3·7 % of all participants (*n* 421), including 1·9 % of women (*n* 111) and 5·8 % of men (*n* 310).

Serum Cu levels were significantly higher in all participants with anaemia (median 1260 μg/l), with explained anaemia (1330 μg/l) and with unexplained anaemia (1220 μg/l) compared with non-anaemic participants (1190 μg/l) (Table 1). Fig. 1 and Table 2 show the U-shaped dose–response in the association between serum Cu (log-scale) and unexplained anaemia obtained using spline regression. Compared with participants with the median serum Cu level (1190 μg/l), those at the lowest 10th percentile of Cu distribution (920 μg/l) had increased odds of having unexplained anaemia of 1·19 (95 % CI 1·05, 1·33). The odds of unexplained anaemia were even higher in patients at the 90th percentile (1610 μg/l): 1·84 (95 % CI 1·58, 2·16). The number of unexplained anaemia cases in subjects with low serum Cu (<700 μg/l) is three out of sixty-two (prevalence of 48·4 per 1000) and in subjects with elevated serum Cu (>1400 μg/l) is 124 out of 2386 (prevalence of 51·9 per 1000).

Underdetection of chronic inflammation and mild Fe deficiency could explain the association between elevated levels of serum Cu and unexplained anaemia. In this analysis, a strict definition for anaemia of chronic inflammation could lead to misclassification of these cases as unexplained anaemia. Because chronic inflammation is associated with increased serum levels of Cu[8–10], such misclassification could contribute to the association between unexplained anaemia and high serum Cu. For example, in the analysis of the NHANES II data, Dallman *et al.* showed that the prevalence of anemia in elderly men was among the highest of all groups (4·8 %), and that inflammation was the primary aetiology[11]. Also, serum Cu levels are higher in the elderly in general, whether healthy or

Table 1. Anaemia in the adult (>15 years of age) Second National Health and Nutrition Examination Survey (NHANES II) study population

	Adults		Female		Male	
	n	%*	*n*	%*	*n*	%*
Subjects (*n*)	11 240	100	5854	52·1	5386	47·9
Non-anaemic (*n*)	10 602	94·3				
Anaemia (*n*)	638	5·7	216	3·7	422	7·8
Explained anaemia†	217	2·0	105	1·8	112	2·2
Fe deficiency	106	0·9	75	1·3	31	0·6
Folate deficiency	12	0·1	3	0·1	9	0·1
Renal failure	11	0·1	3	0·1	8	0·1
ACI	93	0·8	25	0·4	68	1·3
Unexplained anaemia (*n*)	421	3·7	111	1·9	310	5·8

ACI, anaemia of chronic inflammation.

* Percentage is calculated using the total number of subjects in the column.

† The total number of explained anaemia cases is smaller than the sum resulting from adding all the categories. Each category of the explained anaemia has cases that belong to more than one category: (a) in addition to Fe-deficiency anaemia, two subjects are classified as having renal failure and one subject is folate deficient; (b) one subject has folate deficiency and renal failure; (c) one subject is classified as having renal failure and ACI. The total number of cross-classified subjects is five.

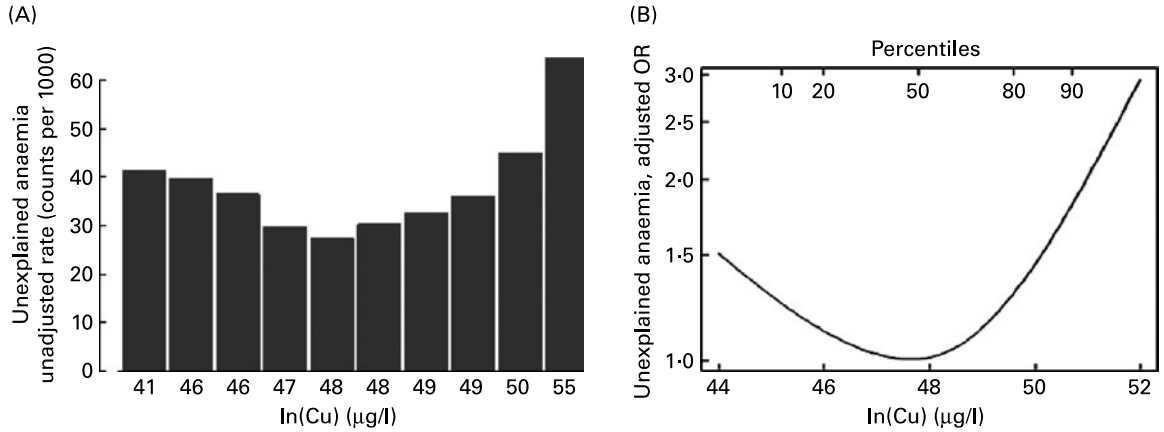

Fig. 1. Dose–response in the association between serum Cu and unexplained anaemia. (A) Rates of unexplained anaemia by decile; *x* axis shows median value for ln(serum Cu) in each decile. (B) Spline regression; OR are plotted against the values of serum Cu. Median serum Cu OR = 1 (reference).

acutely ill[12]. Cu levels are elevated in Fe-deficiency anaemia as well[13]. Thus, some cases of unexplained anaemia in this cohort might be secondary to early Fe deficiency but not captured by the stringent definition used here.

The pathophysiology of anaemia of inflammation (formerly known as anaemia of chronic illness) has been further characterised in recent years, and is now understood as resulting from a combination of Fe-restricted erythropoiesis, shortened erythrocyte lifespan, and erythropoietin resistance. In addition, inflammatory cytokines, especially IL-6, play a key role in the induction of hepcidin, a small peptide produced by the liver which directly promotes hypoferraemia. Of course, such markers were either unknown or unmeasured at the time of NHANES II data collection[14].

Severe Cu deficiency also causes leucopenia, which when combined with severe anaemia, can mimic myelodysplastic syndrome, a clonal bone marrow disease resulting in low blood counts[2]. We calculated the proportion of subjects with low leucocyte levels (<4000/μl) in three groups – participants with low, normal, and elevated serum Cu levels. The prevalence of low leucocyte counts (<4000/μl) among those with low levels of serum Cu (<700 μg/l) was approximately twice greater compared with the participants with normal serum Cu (700–1400 μg/l) and three-fold compared with those with high serum Cu (>1400 μg/l): the proportions of participants with low leucocytes were 0·048 (95 % CI 0·013, 0·120; *n* 62), 0·022 (95 % CI 0·019, 0·025; *n* 8076) and 0·017 (95 % CI 0·012, 0·022; *n* 2259) in the three groups, respectively.

The interesting finding of this analysis is the association between unexplained anaemia and lower (less than median) Cu levels. Classical causes of hypocupraemia (Wilson's disease, enteropathies, short-gut syndromes) would be uncommon or excluded in the NHANES II population. We propose that mild Cu deficiencies, possibly from chronic malabsorption, could contribute to the aetiology of the unexplained anaemia. Graham has noted that deficiency states may develop with Cu and/or caeruloplasmin loss into the gut in patients with enteropathies, if their diet does not replace the losses[15]. It is possible that we underestimated the prevalence of Cu deficiency in this population because serum Cu concentration is not an ideal test to assess total body Cu nutriture[16]. However, better indicators such as hepatic Cu concentration or erythrocyte superoxide dismutase activity[17] were not measured in NHANES II.

In conclusion, this analysis of the NHANES II data confirms that: (1) unexplained anaemia is common among US adults, and (2) that both low and high serum Cu levels are positively associated with unexplained anaemia. Further studies on the contribution of Cu deficiency to unexplained anaemia are needed.

Table 2. Odds ratios derived from spline regression of the 10th and 90th percentiles of the distribution of serum copper compared with the median value

	ln(serum Cu) (μg/l)	Cu (μg/l)	OR	95 % CI
Median (reference)	47·6	1170	1	
10th Percentile	45·1	910	1·26	1·00, 1·58
90th Percentile	50·7	1590	1·81	1·34, 2·43

Acknowledgements

M. A. K. and I. M. designed the research, analysed the data and wrote the paper. D. I. performed the research, analysed the data and wrote the paper. A. I. analysed the data. This research was supported in part by the Doug Coley Fund for Leukemia Research (I. M.), the Leukemia Research Fund of Wake Forest University School of Medicine (I. M.) and a career development award from Amgen Oncology Institute (M. A. K.). We appreciate the editorial assistance of Karen Klein, Office of Research, Wake Forest University Health Sciences. The study was presented in part at the 47th Annual Meeting of the American Society of Hematology, Atlanta, GA, USA, 10–12 December 2005.

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British Journal of Nutrition