Metallothionein Expression Is Increased in Monocytes and Erythrocytes of Young Men during Zinc Supplementation1,2,3

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ABSTRACT The metallothionein gene is transcriptionally regulated by zinc. Consequently, metallothionein has potential for serving as an index of dietary zinc status in humans. To examine this possibility, an enzyme-linked immunoassay (ELISA) based on a sandwich approach that utilizes monoclonal and chicken egg yolk antibodies was used to compare the response of erythrocyte metallothionein protein levels with the response of monocyte metallothionein mRNA levels as measured by competitive reverse transcriptase–polymerase chain reaction (CRT-PCR) during zinc supplementation. Young male subjects participated in an 18-d supplementation study in which zinc was provided at 50 mg/d. Control subjects received a placebo. The zinc supplement resulted in significantly greater erythrocyte metallothionein levels by d 8 of supplementation compared with controls. Monocyte metallothionein mRNA levels were significantly greater than those of controls by d 2 of supplementation. Both remained elevated through d 18. They returned to base line by 8 and 4 d after supplementation, respectively. The plasma zinc concentration was significantly greater than in controls by d 6 and had returned to control levels by d 22 of supplementation. The results presented here show that both monocyte metallothionein mRNA and erythrocyte metallothionein protein concentrations change in human subjects in response to elevated dietary zinc intake and that monocyte metallothionein mRNA responds more rapidly to elevation of dietary zinc status than erythrocyte metallothionein protein. Consequently, both erythrocyte metallothionein and monocyte metallothionein mRNA may prove to be measures useful for assessment of either zinc depletion or the bioavailability of zinc supplements. J Nutr. 128: 707–713, 1998.

KEY WORDS: • humans • metallothionein • zinc • monocytes • erythrocytes

The assessment of nutritional status usually is based on either the level of the nutrient in a blood component or a measurable variable associated with a function that responds to dietary intake and/or body stores of that nutrient. Zinc status assessment has presented a particular challenge. Measurement of zinc levels in plasma or blood cells, although technically straightforward with the use of atomic absorption spectrophotometric methods, does not appear to be a reliable sensitive predictor of zinc status. The plasma zinc concentration is homeostatically regulated at 10–15 μmol/L. There appears to be an adaptation to low zinc intake that decreases zinc excretion to achieve a conservation of this micronutrient and maintenance of a normal plasma zinc concentration. This is achieved through a variety of zinc transporters and intracellular binding proteins that regulate absorption and cellular zinc influx and efflux. In contrast, a variety of mediators, including endotoxin and interleukin 1, act to redistribute zinc to specific tissues by a process that involves metallothionein (MT) synthesis and most likely the activities of various cellular zinc transporters (reviewed in Cousins 1996, King 1990, Mills 1989).

Functions of zinc can be categorized as catalytic (metalloenzymes), structural (e.g., zinc finger domains of proteins) and regulatory (e.g., metal response elements of gene promoters) (Cousins 1996). Regulation of the metallothionein gene reflects this latter function. Metallothionein expression has been shown in numerous animal and cell experiments to be regulated by zinc and some other metals (Cousins 1994, Dunn et al. 1987, Hamer 1986). Furthermore, dietary zinc content is a factor that determines the level of metallothionein gene expression (Blalock et al. 1988, Cousins and Lee-Ambrose 1992, Sato et al. 1984).

We developed an ELISA for human metallothionein for the
purposes of examining metallothionein expression in humans (Grider et al. 1989). Erythrocyte metallothionein levels were shown to respond to zinc depletion and zinc supplementation (Grider et al. 1990) in much the same manner as demonstrated in animals (Sato et al. 1984). For example, experiments with rats have shown that erythrocyte metallothionein is a reflection of synthesis that is programmed in the bone marrow during erythropoiesis (Huber and Cousins 1993a and 1993b, Robertson et al. 1989). Metallothionein levels are highest in erythrocytes and decrease as the red cells mature and enter the peripheral circulation (Huber and Cousins 1993b). Unfortunately, factors that alter erythropoiesis also influence erythrocyte metallothionein (Huber and Cousins 1993b, Robertson et al. 1989). The decrease in erythrocyte metallothionein is particularly effective in identifying moderate to severe zinc depletion in humans (Grider et al. 1990, Thomas et al. 1992), but this effect has not been extensively investigated.

A new approach to zinc status assessment from a functional perspective is to measure metallothionein mRNA in cells. Among the various leukocytic cells, monocytes have the highest level of metallothionein expression (Harley et al. 1989, Pauwels et al. 1994) and the highest zinc concentration (Goode et al. 1989). In the experiments reported here, monocYTE metallothionein mRNA was measured by a newly developed competitive reverse transcriptase–polymerase chain reaction (C-RT-PCR) method (Sullivan and Cousins 1997). This C-RT-PCR method has shown that zinc supplementation increases monocyte metallothionein mRNA levels. The results of the longitudinal zinc supplementation study reported here demonstrate that monocyte metallothionein mRNA levels increase within 2 d of zinc supplementation at 50 mg/d and remain elevated until supplementation is withdrawn, whereupon the mRNA rapidly returns to basal levels. A newly developed sandwich ELISA for human metallothionein is described in detail. Results with this ELISA show that erythrocyte metallothionein protein levels do not respond as rapidly to the same changes in zinc intake. The magnitude of the changes in these levels during zinc supplementation are comparable to those observed with monocyte metallothionein mRNA, however. These methods may serve as useful measures of changes in zinc metabolism and zinc status assessment, and as a measure of zinc bioavailability from various food sources or dietary supplements.

SUBJECTS AND METHODS

Subjects and experimental design. Twenty-five male subjects between the ages of 19 and 35 y (mean = 24 y) with a mean weight of 72 kg were selected from volunteers. The study was approved by the Institutional Review Board of the University of Florida. Written informed consent was obtained from each subject before any tests or other participation took place. After an initial screening interview, subjects who consumed a typical nonvegetarian diet and had normal blood chemistry profiles (SMAC 25; SmithKline Beecham, Collegeville, PA) were used. All subjects agreed to refrain from drinking alcohol, to avoid eating specific zinc-rich foods (such as oysters and other shellfish), to consume no mineral or vitamin supplements, to limit their intake of caffeine and to disclose any illness that developed during their participation.

The 36-d study was divided into three phases. The first phase was a 7-d acclimation phase (from d –7 to d 0). For the zinc supplementation phase (d 1–18), subjects were randomly divided into either a treatment group that received 50 mg of zinc (as zinc gluconate) per day or a control group that received 50 mg sodium carbonate per day. Zinc supplementation was before the morning meal and was under supervision to ensure compliance. During the post-supplementation phase, subjects received no supplement or placebo for 12 d (d 19–30). All blood samples were drawn after an overnight fast between 0700 and 0745 h. Venous blood samples (20 mL) were drawn into tubes (Becton Dickinson Vacutainer No. 6457; Fisher Scientific, Pittsburgh, PA) containing EDTA on d –7, 0, 2, 4, 6, 8, 10, 15, 18, 22, 26 and 30. For measurement of plasma zinc and copper concentrations on some of those days, blood was drawn into trace element–free tubes (Becton Dickinson Vacutainer #369735; Fisher) containing heparin. Plasma was diluted 1:5 with glass distilled deionized (dd) H2O. Plasma zinc and copper concentrations were measured by air acetylene flame atomic absorption (FAA).

Preparation of erythrocyte lysate and monocytes. Whole blood was mixed (10:1) by inversion with Dextran 500 (60 g/L) in 154 mmoL NaCl and allowed to stand for 40 min before isolation of the erythrocytes. The erythrocyte lysate was prepared as described previously (Thomas et al. 1992). The lysate was stored at –70°C.

Monoclonal antibodies. A monoclonal antibody specific for metallothionein was developed. Lyophilized, purified human metallothionein-1 or metallothionein-2 suspended in complete Freund’s adjuvant. Two additional injections were given in incomplete Freund’s adjuvant at 2-wk intervals. Eggs were collected and separated yolk was mixed with polyethylene glycol (5.5 mmoL/L) and polyethylene glycol (3.5 mmoL/L) to form a 1:1 ratio. The yolk was homogenized in 1 mmol/L Tris HCl and 3 mmol/L NaCl, pH 7.4 (Buffer A). The homogenate was centrifuged at 40,000 × g for 10 min at 4°C. The supernatant was heated in a 100°C water bath for 5 min and centrifuged for 30 min at 40,000 × g. The supernatant was then applied to a Hi-Load 16/60 Superdex 75 column (Pharmacia Biotech, Piscataway, NJ) equilibrated with Buffer A. The homogenate of the longitudinal zinc supplementation study reported here was eluted with dd H2O, pooled and concentrated by ultrafiltration and stored at –80°C. Earlier experiments showed that protease inhibitors did not have to be added to retain metallothionein stability. The plasma anduffy coat were removed, layered over NycoFrep 1.068 (Gibco BRL, Gaithersburg, MD), and centrifuged at 600 × g for 15 min. The monocytes were collected at the interface, and the cells were washed twice with 154 mmoL/L NaCl containing 130 g/L EDTA and 10 g/L bovine serum albumin (BSA). Monocyte purity was determined by flow cytometry and found to be ~80% (Sullivan and Cousins 1997).

Human metallothionein. Human liver was obtained through the Pathology and Laboratory Medicine Department of the College of Medicine, University of Florida, and from the NIH-supported Liver Tissue Procurement and Distribution System at the University of Minnesota. Liver metallothionein was purified by high performance liquid chromatography gel filtration and ion exchange basically as described previously (Grider et al. 1989). Briefly, liver tissue was homogenized in 1 mmoL/L Tris HCl and 3 mmoL/L NaCl, pH 8 (Buffer A). The homogenate was centrifuged at 40,000 × g for 10 min at 4°C. The supernatant was heated in a 100°C water bath for 5 min and recentrifuged for 30 min at 40,000 × g. The supernatant was then applied to a Hi-Load 16/60 Superdex 25 column (Pharmacia Biotech, Piscataway, NJ) equilibrated with Buffer A at 4°C. The metallothionein-containing peaks were detected by FAA, pooled and applied to an Econo-Pak Q ion exchange cartridge (BioRad, Hercules, CA). Metallothionein isomers were separated by using a step gradient of 20 mmoL/L Tris HCl followed by 50 mmoL/L Tris HCl. Fractions containing both isomers were identified by FAA. Those were pooled separately, concentrated by ultrafiltration and stored at –80°C.

Chicken antibodies. All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee. Young laying hens were inoculated with 100 μg of either human metallothionein-1 or metallothionein-2 suspended in complete Freund’s adjuvant. Two additional injections were given in incomplete Freund’s adjuvant at 2-wk intervals. Eggs were collected and yolk immunoglobulin G (IgY) was isolated using a method adapted from Hensgens et al. (1994) by mixing egg yolk with calcium chloride. Each separated yolk was mixed with polyethylene glycol (5.5 mmoL/L) solution. The supernatant (3600 × g for 1 h) was placed on a Sepha-rose CL4B (Pharmacia) hydrophobic interaction column. The IgY was eluted with dd H2O, pooled and concentrated by ultrafiltration. Protein fractions were identified by measuring absorbance at 280 nm. The chicken IgY fraction was biotinylated with the use of a Protein Biotinylation System (Gibco BRL). The molar ratio of biotin to IgY averaged 30:1.

Monoclonal antibodies. A monoclonal antibody specific for metallothionein was developed. Lyophilized, purified human metallothionein-1 was diluted in 100 mmoL/L 2-(N-morpholino)ethanesulfonic acid and 150 mmoL/L NaCl, pH 4.7. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was then added to form a metallothionein/EDC conjugate with keyhole limpet hemocyanin (KLH) as described by the manufacturer (Pierce, Rockford, IL). Balb C mice wereimmunized with the metallothionein/KLH conjugate in complete Freund’s adjuvant for the initial injection. Incomplete Freund’s adjuvant was used for injections over a 5-mo period. Mice with the highest titers were then killed for spleen B lymphocyte-myeloma cell fusions. A hybrid clone that cross-reacted with both metallothionein-1 and metallothionein-2 was selected and cultured in Dulbecco’s modified Eagle’s medium. The murine IgG was purified using Protein G Sepharose 4 fast flow (Pharmacia) chromatography before use.
**RNA PCR kit (Perkin Elmer Cetus, Foster City, CA) was used for**

\[\text{GTC CCA ACA TCA GGC } 3^*\]

**and MT3**

cDNA and the metallothionein cDNA from each monocyte RNA

\[\text{AAC TGC TCC TGC G } 3^*\]

**are absorbance at 405 nm produced at the concentrations indicated.**

**The interassay coefficient of variation was 9%**.

**for this C-RT-PCR method was 6%, whereas the intra-assay coefficient of variation was 18% and the intra-assay coefficient of variation was 6%.**

**Sandwich ELISA assay.** Nunc-immuno plates (Fisher) were coated with goat anti-mouse Fab antibody (Pierce). Nonspecific sites were then blocked with PBS, 30 g/L BSA and 3 mmol/L NaCl. The BSA was removed and monoclonal anti-human metallothionein-1/2 was added (1:600) in PBS for 2 h. The plate was washed with wash buffer (1 × PBS, pH 7.4; 4 mmol/L Tween 20; 3 mmol/L NaCl). Purified metallothionein or erythrocyte lysate (100 μL) was added. Plates were washed and biotinylated chicken anti-human metallothionein IgY was added (1:1000) for 2 h. Plates were washed and p-nitrophenyl phosphate (Pierce) was added. After an incubation period, absorbance was measured at 405 nm. The interassay coefficient of variation was 18% and the intra-assay coefficient of variation was 6%.

**Competitive reverse transcriptase–polymerase chain reaction (C-RT-PCR) for human metallothionein mRNA.** The C-RT-PCR has been described in detail (Sullivan and Cousins 1997). Briefly, purified monocytes from each subject were homogenized in 1 mL TRIzol reagent (Gibco BRL). RNA was either immediately extracted or stored overnight at −70°C and extracted the next day. Concentration and purity of the RNA samples were determined by measuring the absorbance in TE buffer (10 mmol/L Tris-HCl, pH 8.0; and 50 mmol/L EDTA) at 230, 260 and 280 nm.

The following PCR primers were used: 5' ATG GAT CCC AAC AAC TGC TCC TGC G 3' (MT5' primer); and 5' AGG GCT GTC CCA ACA TCA GGC 3' (MT3' primer). The GeneAmp RNA PCR kit (Perkin Elmer Cetus, Foster City, CA) was used for PCR amplification. Reverse transcription was performed with 200 ng RNA and 300 U reverse transcriptase (Ausbpel et al. 1995). A 180–bp competitor metallothionein cDNA was used. The MT5' and MT3' primers simultaneously amplify both the competitor cDNA and the metallothionein cDNA from each monocyte RNA sample. The known concentration of the competitor cDNA was used to establish the concentration of the metallothionein cDNA. The latter is a direct reflection of metallothionein mRNA levels (Sullivan and Cousins 1997). The interassay coefficient of variation for this C-RT-PCR method was 6%, whereas the intra-assay coefficient of variation was 9%.

**Protein quantitation.** Protein measurements for erythrocyte lysate, metallothionein and antibody concentrations were made by the method of Lowry et al. (1951) with the use of a BSA standard. Because sulphydryl groups interfere with the Lowry assay, a correction factor was used for all metallothionein measurements (Grider et al. 1989).

**Statistical analysis.** Linear regression (Excel 4.0 software; Microsoft, Redmond, WA) was used to determine concentrations for monocyte metallothionein cDNA, erythrocyte metallothionein and plasma zinc. Tests for significance were performed by repeated measures ANOVA using mixed model methodology (Littell et al. 1996, SAS Institute 1985). Significance was established at \( P < 0.05 \).

**RESULTS**

**Erythrocyte metallothionein.** A variety of antibodies generated in our laboratory and some commercially available antibodies were tested in developing the sandwich ELISA. These included polyclonal sheep anti-metallothionein IgG (Grider et al. 1989) used previously, the chicken anti-human metallothionein IgY (described above), commercially available mouse monoclonal anti-metallothionein antibody and the monoclonal IgG described above. After considerable evaluation, a monoclonal anti-human metallothionein IgG, produced as we described above, that exhibited uniform cross-reactivity to both human isoforms was selected as the capture antibody, and the chicken anti-human metallothionein IgY derived from egg yolk was selected as the detection antibody.

Specificity of the ELISA was evaluated in many ways. A direct ELISA showing the reactivity of diluted chicken anti-metallothionein IgY against purified human metallothionein is shown in Figure 1. A second direct ELISA showing the reactivity of the chicken IgY against purified human, rabbit and horse metallothionein at a constant dilution of IgY is presented in Figure 2. There is also a quantitative additive response in the absorbance at 405 nm in the sandwich ELISA when purified human metallothionein was added to erythrocyte lysates (data not shown). A standard curve for human metallothionein is shown in Figure 3.
FIGURE 3 Standard curve of human metallothionein by sandwich ELISA. Mouse monoclonal anti-human metallothionein immunoglobulin G (IgG) was the capture antibody and chicken anti-human metallothionein yolk IgG (IgY) was the detection antibody. Data are means and are representative of several experiments. Values are absorbance at 405 nm produced by dilutions of human metallothionein.

In the longitudinal zinc supplementation study, the sandwich ELISA showed that there was a significantly greater erythrocyte metallothionein protein concentration (P < 0.05) by d 8 of supplementation when compared with control values (Fig. 4). Erythrocyte metallothionein levels of the zinc-supplemented men were also significantly greater than controls on d 10, 15 and 22. When zinc supplementation was discontinued, concentrations of the protein decreased and were not different at d 26 and 30 from those found in the control subjects. Erythrocyte metallothionein in the control group varied over time. Erythrocyte metallothionein concentrations in control subjects at d −7 and d 0 were significantly different (P < 0.01) from those of control subjects at d 10, 15 and 22.

Monocyte metallothionein mRNA. RT-PCR showed that there were greater monocyte metallothionein mRNA levels in zinc-supplemented subjects than in controls (Fig. 5). These mRNA levels (measured as metallothionein cDNA in picograms cDNA per nanogram of total monocyte RNA) were 2.6-fold greater (P < 0.01) in zinc-supplemented subjects than in the control group by d 2 of supplementation. Levels remained significantly elevated in the zinc-supplemented men through d 18. The highest mRNA levels were on d 8 and 18. These were approximately threefold greater than those obtained before supplementation. They were not significantly different from the other levels of the zinc-supplemented subjects during the supplementation period, however. Monocyte metallothionein mRNA levels returned to base-line values by d 22, 4 d after supplementation was discontinued. Monocyte metallothionein mRNA levels did not vary significantly in the control subjects during the comparison period.

Plasma zinc and copper. Plasma zinc concentration was significantly (P < 0.01) increased by 86% at d 6 of supplementation compared with control subjects (Table 1). By d 15 of supplementation, plasma zinc had decreased compared with that at d 6, but was still significantly elevated (P < 0.05) at 12% above the concentration found in control subjects. Plasma zinc concentrations in zinc-supplemented subjects were not different from those of control subjects by d 22 and remained at normal levels through d 30. Plasma zinc concentrations in control subjects were not significantly different throughout the experiment.

It is of considerable interest that although monocyte metal-
Zinc-induced human metallothionein expression

**TABLE 1**

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1 Values are means ± SEM, n = 10–13 subjects for d 6, 15, 22 and 30; n = 20–26 subjects (groups pooled) for d 0 and 7. Significantly (**P < 0.01; *P < 0.05) different from unsupplemented (−) controls.
2 Zinc supplementation (+) was from d 0 to 18 of the study and was 50 mg Zn/d.

Metallothionein mRNA was elevated in Zn-supplemented men throughout the supplementation period, the plasma zinc concentration was not elevated consistently during that period. This suggests that the stimulus for the elevation in monocyte metallothionein expression is not the plasma zinc concentration. Perhaps the flux of zinc through a specific metabolic pool(s) provides the stimulus because the decrease in plasma zinc concentration observed in the zinc-supplemented subjects by d 15 suggests that these subjects are adapting to a high zinc intake.

There were no significant differences in plasma copper concentrations between control and zinc-supplemented subjects at any time during the study (Table 1), although there was a trend toward higher levels in both groups over time.

**DISCUSSION**

With the abundance of new information about the essentiality of zinc to human health, there is a need for specific, reliable indicators of zinc status and cellular responses to dietary zinc. However, several factors have made the identification of such markers difficult. Because zinc metabolism is under tight homeostatic control, the most commonly used variables for measuring nutrient status (reviewed in Cousins 1996, Mills 1989) such as zinc concentrations in body fluids and zinc metalloenzyme activities have not been reliable for the assessment of zinc status (King 1990). Consequently, marginal zinc deficiency, which may be prevalent in several populations throughout the U.S. and the world (Golden 1989, Rosado et al. 1997, Szczawal et al. 1996), is especially difficult to detect.

Metallothionein expression has shown promise as a potential index of zinc status in animals and humans. Blalock et al. (1988) showed that metallothionein mRNA expression in rat liver, intestine and kidney is regulated by zinc intake. Metallothionein protein levels in rat plasma and erythrocytes have been shown to correlate well with zinc intake (Bremner et al. 1987, Sato et al. 1984). By using a competitive ELISA, Grider et al. (1990) showed that erythrocyte metallothionein protein in humans also reflected alterations in dietary zinc. The competitive ELISA was an initial effort in the search for a method for assessing zinc status in humans based on this zinc-inducible protein. We showed that erythrocyte metallothionein was particularly effective as an index of severe zinc depletion (Thomas et al. 1992). Recently, Akintola et al. (1995) reported the development of a competitive ELISA using a rabbit anti-human metallothionein-1 antibody for the measurement of the protein in human plasma.

The sandwich ELISA described here uses a monoclonal antibody that does not exhibit appreciable nonspecific binding and shows high specificity for human metallothionein. In addition, a chicken anti-human metallothionein antibody was used as the detection antibody. Antibodies produced in avians can offer several important advantages over those produced in mammals. Because of the phylogenetic divergence of avians and mammals, there is greater potential of producing specific antibodies against human proteins in chickens compared with other mammalian species (Gassman et al. 1990). This is of particular relevance for a highly conserved protein such as metallothionein (Hamer 1986, Kagi and Kojima 1987).

Assays for metallothionein protein in human studies could utilize erythrocytes, leukocytes or plasma. Our data suggest that metallothionein protein levels in leukocytes are too low to be measured by ELISA using the number of cells that can be obtained routinely in survey studies. Specifically, we were unable to detect metallothionein protein in monocytes, where the protein is most abundant (Harley et al. 1989, Pauwels et al. 1994), by using the sandwich ELISA method in control subjects or subjects who received zinc supplementation at 50 mg/d for 10 d. Consequently, erythrocytes have been a focus of our studies on human metallothionein protein as a reflection of zinc intake. Robertson et al. (1989) showed that most of the metallothionein in the erythrocyte population of rats is accounted for by the reticulocytes. Huber and Cousins (1993b) showed that the source of erythrocyte metallothionein in rats is the early progenitor cells from the bone marrow and that induction of the protein in marrow will increase via cellular proliferation associated with acute blood loss and accelerated erythropoiesis. The increases in erythrocyte metallothionein in the control subjects of this study could be related to the number of blood samples (a total of 12) removed from each subject. This number of blood draws was for the purpose of defining the kinetics of monocyte metallothionein mRNA induction. The results presented here clearly show that zinc supplementation at 50 mg/d increases erythrocyte metallothionein levels. This supports the observations of Grider et al. (1990). Further studies are required to determine whether the sandwich ELISA is able to measure erythrocyte metallothionein during zinc depletion.

At steady state, however, metallothionein protein levels

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**NOTE:** The image contains a page from a scientific journal article discussing the effects of zinc supplementation on metallothionein expression in human plasma. The text includes tables and figures that detail plasma zinc and copper concentrations, along with discussions on the implications of these findings. The article emphasizes the importance of metallothionein as a biomarker for zinc status and highlights the use of competitive and sandwich ELISA methods for its measurement. The text also references previous studies and highlights the potential advantages of using avian antibodies in the detection of human metallothionein.
should be small proportions of metallothionein mRNA levels. Because very small amounts of mRNA can be detected by RT-PCR (Alard et al. 1993, Tsai and Wiltbank 1996), we developed a PCR-based assay for metallothionein mRNA (Sullivan and Cousins 1997). Pauwels et al. (1994) successfully used semiquantitative RT-PCR to detect metallothionein mRNA in monocytes. An RT-PCR method was also developed for the detection of liver metallothionein in winter flounder (Jessen-Eller et al. 1994). We found that differences between zinc-supplemented men and controls were not evident when semi-quantitative RT-PCR was used. However, differences in monocyte metallothionein mRNA levels after supplementation with dietary zinc could be detected using C-RT-PCR (Sullivan and Cousins 1997). Monocyte production increases with illness in response to various colony-stimulating factors (Ganong 1995). Consequently, in such individuals, monocyte metallothionein expression may be altered. However, the C-RT-PCR approach described here is based on a finite amount of total cellular RNA. If only the number of monocytes changes, but a constant rate of metallothionein expression occurs, this assay will not be affected by conditions that increase monocyte production. Further experiments with this C-RT-PCR will be required before that question can be directly answered, however.

The kinetics of metallothionein induction in monocytes are very rapid (Fig. 5). This is likely a reflection of the rapid turnover of this class of leukocytes (Ganong 1995) and the rapid induction of metallothionein gene expression upon exposure to zinc (reviewed in Cousins 1994, Hamer 1986). With refinement, it may be possible to use this approach with very small numbers of cells as has been shown for other RT-PCR methods (Katz and Dong 1990, O'Brien et al. 1994). Alternatively, the demonstration that monocyte metallothionein mRNA is affected by zinc intake could lead to the development of PCR-based in situ methods for quantification or screening purposes in survey studies with the use of only a few cells (Li et al. 1996). Metallothionein expression in monocytes from human breast milk could also be used in studies to evaluate zinc status during lactation.

The results presented here were from a study that utilized zinc gluconate as the supplement. The rapid and marked response of metallothionein mRNA in monocytes suggests that this approach could also be used to compare zinc sources of differing bioavailability. Although the biological role of metallothionein has not been clearly defined, in vitro evidence suggests that it can act as an intracellular antioxidant as part of the host defense system (reviewed in Cousins 1996). This is supported by the induction characteristics of metallothionein that parallel the acute phase response. Consequently, induction of the protein in monocytes by zinc at a level found in over-the-counter zinc supplements suggests that dietary supplementation at this level of intake has measurable cellular effects.

In summary, we conclude from these studies that metallothionein expression, as measured by metallothionein mRNA and protein, is upregulated by zinc supplementation in humans. Further experiments are required to evaluate the use of the ELISA and C-RT-PCR approaches in dietary zinc status assessment and the variables that may influence those measurements, and for other research such as the evaluation of dietary zinc supplements.

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ZINC-INDUCED HUMAN METALLOTHIONEIN EXPRESSION


