Plasma molybdenum reflects dietary molybdenum intake

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Abstract

The relationship between plasma molybdenum (Mo) and dietary intake has not been investigated in humans. We developed an isotope dilution method to determine molybdenum in 0.5 mL blood plasma by ICP-MS and conducted a study to determine the effect of dietary intake on plasma molybdenum. Twelve young men consumed a very low Mo diet (22 μg/day) for 24 days while confined to the WHNRC metabolic research unit and plasma molybdenum was monitored. 97 Mo was infused in four of the subjects (Group 1) to follow its clearance from the blood. The other eight remained in unit for 120 days (an additional 96 days). Four consumed the 22 μg/day molybdenum diet for 102 days followed by 467 μg/day for 18 days (Group 2), and four consumed five levels of dietary molybdenum for 24 days each (Group 3). 100 Mo was added to the diet one or more times at each dietary level. Total plasma molybdenum and 100 Mo were monitored throughout the study. Plasma molybdenum in the 12 subjects decreased from 8.2 ± 0.5 to 6.1 ± 0.5 nmol/L after 13 days of low molybdenum intake and was 5.1 ± 0.5 nmol/L after 24 days. In Group 2, average plasma molybdenum was 7.8 ± 0.9 nmol/L at the beginning of the study, 5.4 ± 0.4 nmol/L during the 102 days low molybdenum period, and 16.5 ± 0.6 nmol/L during the high molybdenum period. Plasma molybdenum in Group 3 was 4.2 ± 2.1 nmol/L at 22 μg/day; 5.8 ± 2.5 nmol/L at 72 μg/day; 6.6 ± 2.3 nmol/L at 121 μg/day; 19.7 nmol/L ± 2.1 at 467 μg/day; and 43.9 ± 2.1 nmol/L at 1490 μg/day. The results demonstrate that, in contrast to most other essential minerals, plasma molybdenum reflects low and high dietary molybdenum intakes within 14 days and may a useful indicator of low and high dietary intakes. Published by Elsevier Inc. All rights reserved.

Keywords: Molybdenum; Plasma; Dietary intake; Stable isotope

1. Introduction

A wide range of plasma molybdenum concentrations have been reported, with average values ranging from 58 to 2680 nmol/L (5.6 to 257 μg/L) prior to 1973 [1]. Molybdenum measurements are subject to contamination from syringes, glassware, environment and reagents, contributing to the higher values reported. In addition, in early analyses, instrumentation was not adequate for reliable measurements. The molybdenum concentration in blood is low and below the detection limits of many instruments. When contamination is controlled and reliable methods for measuring molybdenum in blood, neutron activation analysis [2] and ICP-MS [3,4], are used, concentrations range from about 3 to 11 nmol/L (0.3 to 1.1 μg/L).

High plasma molybdenum was observed in hemodialysis patients compared to controls, 60 vs. 8 nmol/L (5.79 vs. 0.81 μg/L) [5], but no systematic studies have been reported that examine the effect of dietary intake on plasma molybdenum in humans. One study examined the effect of molybdenum supplements on whole blood molybdenum [6] using the only feasible, economical method available at the time, graphite furnace AAS. Blood concentrations were a factor of 10 higher than when measured with methods now considered reliable, but molybdenum concentration increased following the administration of a 2.9 mg dose of molybdenum in the form of ammonium molybdate.

Our investigation of the effect of dietary molybdenum on plasma molybdenum was conducted as part of studies designed to determine molybdenum absorption and balance over a broad range of dietary molybdenum intakes [7] and during depletion and repletion [8]. We were not able to determine plasma molybdenum in samples from those studies at the time because the concentration was very low and we lacked the necessary instrumentation. We developed a reliable ICP-MS method to determine molybdenum and its stable isotopes in small samples of blood plasma [9], which allowed us to carry out the analysis. The results are reported in this paper.
2. Materials and methods

2.1. Subjects

Twelve men within the age range 22 to 33 y, with heights of 165 to 183 cm and weights of 75 to 93 kg, were included in the study. They were recruited as previously described [8] and lived in the Western Human Nutrition Research Center’s Metabolic Research Unit (MRU) throughout the study. They were randomly assigned to treatments before the start of the study. The experimental protocol was reviewed and approved by the Letterman Army Medical Center Institutional Review Board and the USDA Human Studies Review Committee.

2.2. Experimental design

The experimental design for the study is depicted in Fig. 1. The first part of the study was conducted for 24 days in all twelve volunteers. Characteristics of the 12 subjects and the three subgroups are shown in Table 1. All consumed a very low molybdenum diet containing 22 μg/day Mo. The diet has been described in detail [8]. It was a 3-day rotating menu comprised of low molybdenum foods, supplemented with a vitamin tablet and a liquid drink to provide the recommended intake of vitamins and minerals lacking in the low molybdenum foods. It was necessary to recrystallize all salts used in the diet to reduce the molybdenum content of the diet. The drink also contained additional energy to meet the energy requirement of each individual. Blood was collected on days 1, 14, and 25 to determine plasma Mo.

2.2.1. Group 1

On day 1 or d 7 of the study 33 μg of 97Mo was administered intravenously to four of the subjects to follow its metabolic fate. Blood was collected for plasma molybdenum and 97Mo concentrations at 0, 5, 15, 30, 60 min and 24 and 48 hr following the infusion.

2.2.2. Group 2

Another four subjects continued the study for a total of 120 days. Following the first 24-day period, these subjects continued on the low molybdenum diet for another 96 days (a total of 102 days) followed by 18 days on a molybdenum intake of 467 mg/day. The diet remained the same, but additional molybdenum as ammonium molybdate was added to the liquid drink consumed with each meal. 24 μg of 100Mo was included in the drink on d 13, 49, and 91 of the low molybdenum period and 428 μg of 100Mo of was substituted for the usual unenriched molybdenum in the diet on day 109, during the repletion period. Blood was collected at the beginning of the study, at the end of each treatment period and on the day following 100Mo administration for determination of total molybdenum and 100Mo concentrations.

2.2.3. Group 3

Another 4 subjects also continued the study for 120 days. This part of the study had 5 dietary treatment periods. The first 24-day period with dietary molybdenum intake of 22 μg/day, was followed by four additional 24-day periods with dietary intakes of 72, 121, 467, and 1490 μg/day. The increased molybdenum intakes were achieved by adding an ammonium molybdate solution molybdenum to the liquid drink fed at each meal. At the midpoint of each treatment period (study days 13, 37, 61, 85, and 109), 100Mo was substituted for the unenriched molybdenum added to the liquid drink on other days of the study. at the midpoint of each treatment period (study days 13, 37, 61, 85 and 109). During the lowest intake level, no natural molybdenum was added to the drink, so none could be removed and the molybdenum intake for that day was increased by the amount of isotope fed. The isotope was substituted for an equivalent amount of natural unenriched molybdenum, divided equally among the meals of the day. Amounts substituted were 24, 48, 95, 428, and 1378 μg of 100Mo. Blood was collected for the determination of 100Mo and total molybdenum concentrations at the beginning of the study.

![Fig. 1. Experimental design. n=4 per group. Arrow for Group 1 indicates days of infusion of 33 μg of 97Mo (two subjects on d 1 and two on d 7). Arrows for Groups 2 and 3 indicate days 100Mo was fed. It was added to the diet when diet contained only 22 μg/d and replaced the molybdenum in the diet at all other times.](image-url)
3. Methods

The molybdenum isotopes used and the preparation procedures for administration have been described [7,8]. Enriched molybdenum powders were dissolved in a 3:1 solution of HCl and HNO₃ and diluted in deionized water. After ⁹⁷Mo was prepared for intravenous administration by filtering, adjusting pH and testing for sterility and endotoxins, it was infused into an arm vein over a period of 1 min through a venous catheter. ¹⁰⁰Mo was prepared for oral administration and added to the liquid drink 1 hr before each meal on the day of administration.

Blood was collected as described in experimental design. It was collected for Group 1 from the arm opposite the arm used for isotope administration at baseline, 5, 15, 30, and 60 min and 24 and 48 h following ⁹⁷Mo administration. Whole blood was collected into heparinized trace element free tubes and after 20 min was spun in a centrifuge. Plasma was separated from whole blood and stored frozen at −20°C for total molybdenum and isotope determinations. Precautions were taken to avoid trace element contamination at all steps of sample preparation and analysis [8,9].

It was necessary to carry out elaborate procedures during plasma digestion and analysis to minimize the contamination of plasma samples with molybdenum. These procedures, the precision and accuracy of the method of analysis, and quality control have been described [9]. Briefly, all materials used in sample processing were acid washed with 1:1 HNO₃ and rinsed with deionized water. Aliquots of 0.5 mL plasma were weighed into 10-mL PTFE beakers inside a clean room. All other sample handling was done inside a class 100 laminar flow hood within the clean room. The beakers were placed inside PFA digestion vessel liners for microwave digestion, with 8 mL of ultrapure concentrated acid. The acid in the base of the liner provided the volume needed for microwave energy absorption. After digestion the samples were diluted and placed in an enclosed autosampler for ICP-MS analysis. Corrections were made for processing blanks and analytical interferences.

⁹⁶Mo, a third isotopic tracer, was added to the samples as an isotopic diluent. Therefore, it was possible to determine total molybdenum and isotope concentrations with a single analysis. The concentrations of ¹⁰⁰Mo, ⁹⁷Mo, and total molybdenum, based on isotope dilution, were calculated as was described previously [10]. Data are missing for three plasma samples from group 2 and three from group 3. Two of the samples were not collected at the designated times and the others were lost or contaminated during sample preparation.

Urinary and fecal molybdenum content of samples from these studies were reported previously [7,8]. Correlations were calculated between dietary molybdenum and plasma, urinary, and fecal molybdenum.

3.1. Statistical analysis

Statistical analysis was performed with SAS version 6.12 (SAS/STAT Users Guide, Version 6. Cary, NC: SAS Institute, Inc., 1989). Descriptive statistics, including least squares means, standard errors, and correlations, were tabulated and compared. A one-way analysis of variance (ANOVA) model was used to determine the effects of the different molybdenum intakes over time on plasma molybdenum concentration. A significance level of P < 0.05 was used for all statistical tests. If a significant difference was found, the Tukey’s Studentized Range Test was used to determine which treatment means differed.

In addition, an ANOVA model with repeated measures over subjects was used to determine the effects of the different molybdenum intakes over time on plasma, fecal, and urinary molybdenum. Pearson correlations were examined between plasma, fecal, and urinary molybdenum.

4. Results

Mean plasma molybdenum in the 12 subjects (Table 2)

<table>
<thead>
<tr>
<th>Time</th>
<th>Intrinsic Mo</th>
<th>⁹⁷Mo</th>
<th>Total Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>6.9a</td>
<td>0.0a</td>
<td>6.9a</td>
</tr>
<tr>
<td>5 min</td>
<td>10.7b</td>
<td>16.3a</td>
<td>27.1b</td>
</tr>
<tr>
<td>15 min</td>
<td>10.5b</td>
<td>11.1b</td>
<td>22.1bc</td>
</tr>
<tr>
<td>30 min</td>
<td>13.1b</td>
<td>7.2a</td>
<td>20.2b</td>
</tr>
<tr>
<td>60 min</td>
<td>13.0b</td>
<td>4.0b</td>
<td>17.4b</td>
</tr>
<tr>
<td>24 hr</td>
<td>5.7a</td>
<td>0.3a</td>
<td>6.0a</td>
</tr>
<tr>
<td>48 hr</td>
<td>5.1a</td>
<td>0.2a</td>
<td>5.3a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.74</td>
<td>0.72</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Means within a column with different superscripts are significantly different from one another (p < 0.05).
declined significantly on the low molybdenum diet from 8.2 nmol/L at the beginning of the study to 6.1 nmol/L by d 14. It averaged 5.1 nmol/L after 24 days, which was not significantly lower than on d 14.

4.1. Group 1

The concentration of $^{97}\text{Mo}$ for 48 h following its infusion into Group 1 and its effect on total and natural intrinsic molybdenum in the plasma are shown in Table 3. All $^{97}\text{Mo}$ concentrations were the highest in the first sample collected following infusion and declined to near baseline by the 24 h collection. The administration of 33 $\mu$g of $^{97}\text{Mo}$ increased both the natural intrinsic plasma molybdenum and the total molybdenum content of the plasma.

4.2. Group 2

Plasma molybdenum was significantly lower during the 102-day depletion period than at beginning of the study, as shown in Table 4. It increased significantly when dietary molybdenum increased to 467 $\mu$g/day. During the depletion period, plasma molybdenum averaged 7.8, 6.9, 6.5, 5.5, 5.0, 3.9 and 4.0 nmol/L on days 1, 14, 25, 50, 76, 92 and 103, respectively, but these means were not significantly different from one another. Fig. 2 shows the patterns of total plasma molybdenum and $^{100}\text{Mo}$ concentrations during the course of the study in Group 2 and following the addition of $^{100}\text{Mo}$ to the diet during depletion and repletion. $^{100}\text{Mo}$ was higher on the day following each oral administration, but total molybdenum was not significantly higher on the days following the isotope feeding than on other days of the same dietary period.

4.3. Group 3

The effect of 5 increasing levels of dietary molybdenum on plasma molybdenum is shown in Table 4. Plasma molybdenum increased as dietary molybdenum increased, but the differences did not reach significance until the 467 $\mu$g/day level. Fig. 3 shows the patterns of total plasma molybdenum and $^{100}\text{Mo}$ concentrations during the course of the study and following the addition of $^{100}\text{Mo}$ to the diet at the 5 increasing levels of dietary molybdenum. The oral $^{100}\text{Mo}$, which replaced the usual molybdenum in the diet on the day of administration, increased the amount of $^{100}\text{Mo}$ in the blood on the day following administration, but the total molybdenum was not significantly higher ($P < 0.05$) following $^{100}\text{Mo}$ administration than at the end of the dietary period.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subjects</th>
<th>Dietary Mo $\mu$g/d</th>
<th>Day</th>
<th>Plasma Mo $\mu$g/L</th>
<th>SEM $\mu$g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4</td>
<td>1</td>
<td>22</td>
<td>7.8 ± 0.9</td>
<td>(0.74 ± 0.08)$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>5.4 ± 0.4</td>
<td>(0.52 ± 0.04)$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>467</td>
<td>16.5 ± 0.6</td>
<td>(1.59 ± 0.06)$^c$</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1</td>
<td>22</td>
<td>8.6 ± 3.0</td>
<td>(0.82 ± 0.30)$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>72</td>
<td>4.2 ± 2.1</td>
<td>(0.40 ± 0.21)$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>121</td>
<td>5.8 ± 2.5</td>
<td>(0.56 ± 0.25)$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>467</td>
<td>6.6 ± 2.3</td>
<td>(0.64 ± 0.23)$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1490</td>
<td>19.7 ± 2.1</td>
<td>(1.90 ± 0.21)$^a$</td>
</tr>
</tbody>
</table>

1 Plasma molybdenum at day 1 reflects prestudy dietary intake.
2 Mean ± SEM of all points for each dietary treatment period.
3 Different superscripts within a group indicate means are significantly different ($p < 0.05$).
period. $^{100}$Mo concentration declined by the end of the treatment period, but was consistently higher than it was at the end of the previous treatment period.

Table 5 shows the average mean plasma, urinary and fecal molybdenum of each dietary period for Groups 2 and 3. Plasma molybdenum concentration and mean daily urinary and fecal molybdenum all correlated significantly with dietary molybdenum ($P = 0.0001$).

5. Discussion

During the 24-day study with low dietary molybdenum, total plasma molybdenum declined significantly by d 14 demonstrating that the intake was lower than prestudy dietary molybdenum intake. The usual molybdenum intake of young men is about 120 μg/day [11] and we estimated, based on dietary records, that the intake of our subjects was somewhat above this. The infusion of $^{97}$Mo increased plasma levels of the isotope markedly. It was still higher than baseline after 60 min and it returned to approximately preinfusion levels by 24 hr. The infusion affected total plasma molybdenum similarly, increasing and remaining slightly higher after 60 min, then returning to near baseline by 24 h. This was primarily due to the amount of isotope administered and appearing in the sample and demonstrates that this amount perturbed molybdenum metabolism. The natural intrinsic molybdenum, which is the total plasma molybdenum less the $^{97}$Mo, showed a similar but less extreme pattern. The cause of this increase is not known, but the data could suggest that the load of $^{97}$Mo infused, which was higher than a physiological amount, was taken up by other tissues, which then released more natural intrinsic molybdenum along with the stable isotopes, increasing its level in the blood.

In Group 2, the depletion study, with a much longer time on a low molybdenum diet than the other groups, plasma molybdenum was significantly lower during depletion than repletion and the beginning of the study (Table 4). This confirms that plasma molybdenum reflects low dietary intake. It is possible that a longer period of time with 22 μg/day would result in molybdenum depletion. This is in agreement with the recommendations for dietary molybdenum that were recently established, the Dietary Reference Intakes [12]. The Estimated Average Requirement of adult males was set at 34 μg/day, based on a requirement of 25 μg/day with an added factor for bioavailability. Since the

Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary Mo μg/d</th>
<th>Plasma Mo nmol/L</th>
<th>Urine Mo μg/d</th>
<th>Fecal Mo μg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>22</td>
<td>5.4 ± 0.4</td>
<td>14.2 ± 1.7</td>
<td>10.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>467</td>
<td>16.5 ± 0.6</td>
<td>410.5 ± 4.7</td>
<td>39.2 ± 1.6</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>4.2 ± 2.2</td>
<td>21.3 ± 14.1</td>
<td>10.4 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>5.8 ± 2.6</td>
<td>59.8 ± 14.1</td>
<td>12.8 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>121</td>
<td>6.6 ± 2.3</td>
<td>100.6 ± 14.1</td>
<td>17.8 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>467</td>
<td>19.7 ± 2.2</td>
<td>418.6 ± 14.1</td>
<td>35.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>1490</td>
<td>439 ± 2.2</td>
<td>1316.3 ± 14.1</td>
<td>86.6 ± 1.9</td>
</tr>
</tbody>
</table>

1 Mean ± SEM of all points in treatment periods, excluding the first 6 days.
2 Different superscripts within a column and group indicate means are significantly different ($p < 0.05$).

Fig. 3. Plasma molybdenum and $^{100}$Mo concentrations in 4 subjects (mean + SD) at dietary molybdenum intakes shown. 24 μg $^{100}$Mo was added to the diet on the day with lowest intake and was substituted for most of the dietary molybdenum at the higher intakes. Arrows indicate days of oral isotope administration. Vertical lines indicate the day dietary molybdenum changed. Plasma molybdenum for the days within dietary periods did not differ from one another ($p < 0.05$). Means of days of last two dietary periods are significantly higher than those in the first three periods and are different from one another. Plasma $^{100}$Mo increased following each administration of the isotope, then declined. See text for further discussion of the $^{100}$Mo data.
molybdenum in our diet was highly available [8], the requirement of 25 μg/day would apply and is slightly above the amount in our depletion diet. This is consistent with the slight decline in plasma molybdenum observed in this group.

As shown in Fig. 2, plasma molybdenum increased after 13 days on the depletion diet, when the dietary intake increased to 467 μg/day, but was not significantly different between the two days of that dietary period. The administration of 100Mo increased the total dietary molybdenum on the day of isotope administration at the low level of molybdenum, but not the higher level. In contrast to the infused dose, it did not have a significant effect on the total plasma molybdenum at either level of intake.

In Group 3, plasma molybdenum increased as dietary molybdenum increased. The mean of each dietary period was higher, but not significantly different until the diet contained 467 μg/day (Table 4). As in Group 2, the change in dietary molybdenum was reflected in the plasma, but was not significantly different between the days of the dietary treatment period, as shown in Fig. 3. Also similar to group 2, the 100Mo added to the diet did not significantly influence total molybdenum in plasma. This would be expected since, except at the lowest level, the isotope replaced usual molybdenum in the diet and total dietary molybdenum did not increase. However, the amount of 100Mo in the plasma did increase significantly following the feeding. It returned to lower levels with time, but at the end of each feeding period, was slightly higher than it was at the end of the previous period (Fig. 3). This reflects the increased body pool of 100Mo following repeated feeding of the isotope. The highest levels of plasma molybdenum observed in this study are close to values observed in uremic patients and patients on hemodialysis mentioned in the introduction. The intake is close to, but slightly below the new Upper Level of Intake recommended in the new Dietary Reference Intakes [12].

We compared the effects of dietary molybdenum on plasma, urinary, and fecal molybdenum. All were strongly associated with dietary molybdenum (P = 0.0001). Table 5 shows that urinary molybdenum was close to, but slightly lower than dietary molybdenum.

These relationships contrast with the effect of dietary intake on plasma and urinary levels of most essential minerals. For example, dietary copper does not influence plasma copper over a broad range of intakes [13] and plasma copper only changes when the diet is so low in copper that individuals become depleted and symptoms of deficiency appear [14]. Urinary copper is extremely low, regardless of intake [15]. Similarly, plasma and urinary zinc levels do not change in response to dietary intake [16].

The results of this study demonstrate that, like urinary molybdenum, plasma molybdenum is an indicator of dietary intake. Urinary molybdenum is more directly related to intake. Blood samples are simpler to collect than a 24-hr urine collection, but more invasive. Both urine and plasma could be useful in establishing whether an individual or groups of individuals were at risk for deficiency by consuming too little molybdenum or toxicity by consuming excess molybdenum.

References


