Serum lithium as a compliance marker for food and supplement intake1–3

Nicole M de Roos, Jeanne HM de Vries, and Martijn B Katan

ABSTRACT

Background: Analyzing 24-h urine for lithium after consumption of lithium-tagged foods or supplements provides a validated compliance marker but is laborious. Objective: Most studies involve blood sampling; therefore, we tested whether serum lithium concentration could be used as a compliance marker. Design: We used serum lithium as a compliance marker in a dietary trial and an evaluation study. Results: In the dietary trial, 78 volunteers consumed 500 mL yogurt tagged with lithium (250 μmol/d) for 6 wk. Serum lithium increased from 0.9 ± 0.3 to 6.6 ± 1.5 μmol/L, which was close to the predicted concentration, indicating that the subjects were highly compliant. However, the interindividual variability in serum lithium concentration was large. To test whether this variability resulted from compliance differences or natural variability, we performed an evaluation study: 12 subjects took a lithium supplement (250 μmol/d) for 13 d under supervision. Serum lithium increased from 0.14 ± 0.03 to 3.9 ± 0.8 μmol/L (range: 2.6–5.4 μmol/L); thus, there was wide interindividual variation in serum lithium despite 100% compliance. However, within-subject variability was small, with a CV of 7% for serum lithium measured on 4 different days. We checked whether taking half the dose on each of 2 d (125 μmol lithium/d) would significantly lower serum lithium. Indeed, serum lithium dropped in all subjects, by a mean of 1.0 μmol/L on the second day (P < 0.0001) and by another 0.3 μmol/L on the second day (P = 0.0004). Thus, changes in serum lithium concentration of ≥1.0 μmol/L suggest altered compliance. Conclusion: Serum lithium concentrations after intake of lithium-tagged foods or supplements can be used to assess compliance in dietary trials. Am J Clin Nutr 2001;73:75–9.

KEY WORDS Lithium, compliance marker, dietary trials, compliance, noncompliance, nutrition research, dietary intervention, diet studies

INTRODUCTION

The results of dietary intervention studies are highly dependent on subject compliance. Poor compliance decreases the chances of finding an actual effect. Compliance can be measured objectively by tagging the experimental foods with a marker that appears in blood, feces, or urine. One such marker is lithium. This drug is a suitable marker because its concentration in the background diet is negligible (1). Lithium is better known for its use in treating manic-depressive disorders (2). The usual therapeutic dosage is 10–50 mmol/d (2–5), which is 40–200 times more than the marker dosage of 250 μmol/d in dietary trials. No adverse effects of lithium concentrations between baseline concentrations and the therapeutic range have been reported (5). Thus, use of lithium in a dosage of 250 μmol/d is safe. Lithium is almost completely excreted in the urine (2) and the 24-h urinary excretion of lithium reflects the daily dose. Thus, the intake of lithium-tagged foods can be estimated from the recovery of lithium in 24-h urine samples (6–9). However, this method has some drawbacks: incompletely collected urine or increased fluid and salt losses decrease the recovery of lithium from urine (2), which may lead to a false conclusion that the subject was noncompliant. Moreover, collecting 24-h urine samples is burdensome for volunteers, who may decline participation in a study for this reason. It would be easier if compliance could be assessed by measuring serum lithium concentrations because blood is collected in most studies anyway.

In a pilot study (NM de Roos, FJM Schouten, MB Katan, unpublished observations, 1996) in 6 volunteers, we found that consumption of 250 μmol lithium/d for 8 d increased serum lithium concentrations from 0.6 μmol/L at baseline (95% CI: 0.4, 0.9) to 5.3 μmol/L (95% CI: 4.3, 6.3). Because the rise in serum lithium concentrations was almost 10-fold, we concluded that serum lithium could be used to distinguish between intake and no intake of lithium-tagged foods. We then evaluated the feasibility of serum lithium as a compliance marker in a dietary trial. Because we found a wide range of serum lithium concentrations in that study, we performed an evaluation study to assess the variability of serum lithium after controlled feeding of trace doses. In the evaluation study, we also assessed the effect of missing half the daily dose of lithium.

1 From the Division of Human Nutrition and Epidemiology, Wageningen University, Wageningen Centre for Food Sciences, Wageningen, Netherlands.
2 Supported by the Dutch Dairy Foundation on Nutrition and Health (dietary trial) and the Wageningen Centre for Food Sciences (evaluation study).
3 Address reprint requests to JHM de Vries, Division of Human Nutrition and Epidemiology, Wageningen University, Bomenweg 2, PO Box 8129, 6700 EV Wageningen, Netherlands.
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SUBJECTS AND METHODS

The protocols of the studies were approved by the Medical Ethical Committee of Wageningen University. We gave each volunteer a written and oral presentation of the purpose and execution of the studies. Each volunteer signed an informed consent form. We did not include pregnant or lactating women in the studies.

Dietary trial with lithium-tagged yogurt

Study purpose and subjects

The purpose of this study was to assess the feasibility of using serum lithium concentration as a marker of the intake of lithium-tagged yogurt. Seventy-eight healthy subjects (28 men, 50 women) aged 39.9 ± 8.5 y (range: 20–62 y) participated in a dietary study. The mean body mass index (in kg/m^2) of the subjects was 24.3 ± 3.

Study design

The dietary study was performed to investigate the effects of a *Lactobacillus acidophilus*-enriched yogurt and a control yogurt on blood lipid concentrations (10). The volunteers consumed their habitual Western-type diet supplemented with 500 mL control yogurt during a 2-wk run-in period. Then they consumed 500 mL enriched or control yogurt during a 6-wk treatment period. The yogurt was divided into a 200-mL morning portion and a 300-mL evening portion. During the 6-wk treatment period, the yogurt was tagged with 500 μmol lithium/L. Lithium chloride was added during the process of making the yogurt, to a mean concentration of 526 μmol/L.

We obtained 6 blood samples from every subject: 2 in the second week of the run-in period and 2 each in weeks 3 and 6 of the treatment period. The 2 samples from each week were pooled before analysis so that we obtained 3 lithium measurements per subject. The subjects did not consume the yogurt supplements under our supervision; they recorded missed portions of yogurt in a diary.

Measurement of lithium in serum

Lithium was measured by using electrothermal atomic absorption spectrometry (AAS) (11). Blood samples were allowed to clot at room temperature and were centrifuged at 2700 × g for 10 min at 4°C. Serum was stored at −20°C. Serum samples were deproteinized by adding 0.5 mL water and 1 mL trichloroacetic acid (20% wt:vol) to 1 mL serum. After we had mixed and centrifuged the samples, we mixed 20 mL supernate with 5 mL NH₄NO₃ (8% wt:vol) and atomized the mixture in a graphite furnace at 2000°C. The between-day error in the measurement of lithium in control serum tagged with lithium (4.32 μmol/L) was 1.7% (1 control serum, 4 measurement days). The variability between subjects was higher for the baseline concentrations than for the steady state concentrations. At baseline, we found a CV of 40% at a mean concentration of 0.8 μmol/L (11 subjects with 2 samples each), whereas at steady state we found a CV of 5.8% at a mean concentration of 7.2 μmol/L (9 subjects with 2 samples each).

Evaluation study

Study purpose and subjects

The purpose of the evaluation study was to assess the between- and within-subject variability in steady state serum lithium concentrations. We also studied the effect of missing half the lithium dose on 2 consecutive days.

We screened 15 volunteers for normal blood counts and absence of disease. Twelve of them (2 men, 10 women) were enrolled in the study. The volunteers were students or colleagues of the authors; all were fully aware of the aim of the study and were highly cooperative. Their mean (±SD) age was 21 ± 2 y (range: 19–26 y) and their average body mass index was 22.0 ± 1.8.

Study design

We provided the lithium as 10-mL solutions of lithium chloride dissolved in water in concentrations of 25 or 12.5 mmol/L. We made 2 batches of lithium solution before the study; the actual concentrations of the 25- and 12.5-mmol/L batches were 27.6 and 12.7 mmol/L, respectively.

The volunteers took 276 μmol lithium/d on days 1–12 and took 127 μmol/d on days 13 and 14. All supplements were taken between 0800 and 0900. On weekdays, the supplements were taken under our supervision. On weekend days, the volunteers telephoned us between 0800 and 0900 after they had taken their supplements. We measured steady state concentrations of lithium in serum on days 8, 9, 12, and 13 (days 10 and 11 were weekend days) and on days 14 and 15 to estimate the effects of missing half the dose. We obtained all blood samples in the morning, before the volunteers took their daily dose of lithium.

Measurement of lithium in serum

Because of the high measurement variability in the baseline samples of the dietary trial, we changed the method from electrothermal AAS to inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS is an accurate and precise method for measuring trace elements in biological samples such as serum and liver tissue (12). We used a Perkin-Elmer ELAN 6000 spectrometer (Perkin-Elmer, Norwalk, CT) with a beryllium internal standard (13). To prepare the samples, 250 μL serum was added to 4750 μL 0.1 mmol/L HNO₃ and 20 μL air. Recovery of lithium from a standard solution was 100% when 44.01 μg lithium/L was used and was 101.5% when 110.04 μg lithium/L was used. The between-day error in the measurement of lithium in control serum tagged with lithium (10.6 μmol/L) was 3.6% (1 control sample, 5 measurement days).

Statistical analysis

We report means, SDs, and 95% CIs. We estimated the between-subjects and within-subjects variability in serum lithium concentration from the evaluation-study data; we had 4 measurements of serum lithium while volunteers were taking the dose of 250 μmol/d. To estimate the variance components, we used an analysis of variance model with lithium concentration as the dependent variable and subject as the independent variable. The within-subjects variance was estimated from the mean square within subjects; the between-subjects variance was estimated from the difference between the mean squares within and between, divided by the number of repeated measurements (n = 4) (14). We used the SAS SYSTEM FOR WINDOWS, release 6.12 (SAS Institute Inc, Cary, NC) for the statistical analysis.

RESULTS

Dietary trial

Before the volunteers consumed the lithium-tagged yogurt, the average serum lithium concentration was 0.92 μmol/L (95% CI:
higher steady state concentrations of lithium in serum than did body weight, height, and serum lithium concentrations was 2.5 to 5.8. The range of concentrations at the dosage of 250 μmol/l was 0.26 μmol/L, with a corresponding CV of 7%. The variation between subjects was small: the SD within-subjects (\(\sqrt{\sigma^2}\)) was 0.26 μmol/L, with a corresponding CV of 7%. The variation between subjects was larger: the range of concentrations was wide, which meant that they could not be explained by a low lithium concentration of 1.7 μmol/L in week 6. One subject had consistently low lithium concentrations during the study (1.7 and 2.0 μmol/L in weeks 3 and 6, respectively), which suggested noncompliance. None of the subjects reported any adverse effects from consumption of the lithium solution or lithium-tagged yogurt.

**Evaluation study**

At baseline, the mean (±SD) serum lithium concentration was 0.14 ± 0.03 μmol/L (Figure 2). When the volunteers took 250 μmol lithium/d, serum lithium increased to a mean concentration of 3.9 ± 0.8 μmol/L (95% CI: 3.4, 4.4; mean of days 8, 9, 12, and 13). The day-to-day variation in lithium concentrations within subjects was small: the SD within-subjects (\(\sqrt{\sigma^2}\)) was 0.26 μmol/L, with a corresponding CV of 7%. The variation between subjects was larger: the range of steady state concentrations was 2.5 to 5.8 μmol/L, and the between-subject SD (\(\sqrt{\sigma^2}\)) was 0.77 μmol/L.

When only half the lithium dose (125 μmol/d) was taken, serum lithium decreased from a mean steady state concentration of 3.9 μmol/L to 2.9 μmol/L on day 14 (\(P < 0.0001\) for difference between 3.9 and 2.9) and decreased further to 2.6 μmol/L on day 15 (\(P = 0.0004\) for difference between 2.9 and 2.6). The decrease was consistent in all subjects and ranged from 0.7 to 1.8 μmol/L. The range of serum lithium concentrations at the dosage of 125 μmol/d was 1.8 to 4.2 μmol/L and thus partly overlapped the range of concentrations at the dosage of 250 μmol/d.

**Body weight, height, and serum lithium**

Lighter and shorter subjects in the evaluation study reached higher steady state concentrations of lithium in serum than did heavier and taller subjects. The Pearson’s product-moment correlation coefficients were as follows: for the association between serum lithium and body weight, \(r = -0.61\) (\(P = 0.036\)); for the association between serum lithium and height, \(r = -0.43\) (\(P = 0.167\)).

In the dietary trial, we found significant but smaller negative correlations between serum lithium and body weight and height (Figures 3 and 4). When we split the group by sex, only the relationship between height and serum lithium in women remained significant (\(r = -0.39\); \(P = 0.003\); \(n = 56\)). In univariate regression models with all 78 subjects, height explained 10% and weight explained 7% of the variation in steady state concentrations. Serum lithium decreased by 0.57 μmol/L (95% CI: 0.19, 0.94) for every 10-cm increase in height or by 0.38 μmol/L (95% CI: 0.07, 0.7) for every 10-kg increase in weight. We also calculated the body surface area (15) and lean body mass (16) for each subject, but these variables were no better than height for explaining the variability in serum lithium concentrations.

**DISCUSSION**

We investigated whether serum lithium concentrations could be used to assess the intake of lithium-tagged supplements. We found that a daily dose of 250 μmol lithium, given for ≥7 d, increased serum lithium from values uniformly <0.5 μmol/L to values uniformly >2 μmol/L in 12 volunteers. Therefore, intake of a low, safe dose of lithium could be distinguished from no intake or noncompliance. However, the range of steady state concentrations was wide, which meant that they could not be used to distinguish between more-compliant and less-compliant volunteers. Within subjects, however, steady state concentrations of lithium in serum were stable.

The lithium marker method is limited to studies in which the food or supplement of interest is provided to the volunteers by the researchers. The usefulness of the lithium marker method for different situations in such studies is discussed below.

**Assessment of individual compliance**

Equal doses of lithium result in different steady state concentrations in different persons: we found a range of 2.5 to 5.8 μmol/L in the 12 subjects who participated in the evaluation study. When the daily dose was halved, some volunteers had serum lithium concent-

![FIGURE 1. Individual serum lithium concentrations of 78 volunteers who completed a dietary trial investigating the effect of an enriched and control yogurt on serum lipids. During the test period, both yogurts were tagged with lithium, providing a dosage of 250 μmol/l lithium/d. The mean serum lithium concentration at both week 3 and week 6 was 6.6 μmol/L, as shown by the horizontal lines.](https://academic.oup.com/ajcn/article/73/1/75/4729699)

![FIGURE 2. Individual serum lithium concentrations of the 12 volunteers who completed the evaluation study. The horizontal lines represent means.](https://academic.oup.com/ajcn/article/73/1/75/4729699)
trations that were as high as concentrations measured in other volunteers while taking the full dose. This means that serum concentrations $\geq$2.5 $\mu$mol/L do not distinguish between fully compliant subjects and subjects who occasionally miss a dose. Thus, serum lithium concentration does not quantitatively reflect compliance in different subjects. However, if we are looking for subjects who are grossly noncompliant in a study, these subjects will have low concentrations of serum lithium. Indeed, we found that a subject who stopped taking his supplements 2 days before the end of the dietary trial, serum lithium was 6.5 $\mu$mol/L. Another subject with low serum lithium concentrations did not report any missed supplements, but her values were so low that we considered her noncompliant. Thus, in a study involving lithium-tagged supplements or foods, subjects with very low concentrations of serum lithium are likely to have poor compliance.

Body size was inversely related to serum lithium concentration in that smaller and thinner subjects reached higher serum concentrations than did taller and heavier subjects. Thus, in a study in which all subjects take equal doses of lithium, low serum lithium concentrations are more likely to be the result of poor compliance in small, thin subjects than in tall, heavy subjects.

Comparison of compliance between treatment groups

Serum lithium concentrations can be used to check whether compliance is equal in the different treatment groups. In the dietary trial, serum lithium was 6.5 $\pm$ 1.5 $\mu$mol/L in the group that received enriched yogurt and was 6.7 $\pm$ 1.5 $\mu$mol/L in the group that received placebo yogurt ($P = 0.52$). Thus, we know that the outcome of the study could not have been affected by differences in compliance.

Monitoring compliance over time

Compliance with long-term dietary regimens is often better at the beginning of a study than after a few months or years (17–19). Serum lithium concentrations can be used to measure compliance over time because steady state concentrations within subjects are stable. Thus, changes in serum lithium concentrations indicate changes in compliance. When the lithium dosage was lowered from 250 to 125 $\mu$mol/d, serum lithium concentrations decreased in all subjects. In the dietary trial, we assessed the compliance of the volunteers at 3 and 6 wk and found serum lithium concentrations of 6.7 $\pm$ 1.5 and 6.5 $\pm$ 1.6 $\mu$mol/L, respectively ($P = 0.48$). Thus, compliance was stable over time.

Different measurement methods and dosage schedules yield different results for serum lithium

We found lower serum lithium concentrations at baseline and at steady state in the evaluation study than in the dietary study; the mean baseline lithium concentrations were 0.14 and 0.9 $\mu$mol/L, respectively. We ascribe this to differences in specificity between the ICP-MS method used for the evaluation study and the AAS method used for the dietary trial. With AAS, we found an absorption peak for lithium but also some smaller peaks (noise) that we could not account for. Thus, AAS appeared to be less specific than ICP-MS and therefore yielded higher values. During steady state, we found mean concentrations of 3.9 $\mu$mol/L in the evaluation study and 6.6 $\mu$mol/L in the dietary study. Compliance in the evaluation study was 100% because the subjects took the supplements under our supervision; therefore, lack of compliance could not explain the lower serum concentrations.

In addition to the different measurement techniques used in the 2 studies, the dosage schedule could also have accounted for the difference in steady state concentrations. Lithium was given in 1 daily dose in the evaluation study but in 2 split doses in the dietary trial. As a consequence, blood was sampled 24 h after the 250-$\mu$mol dose in the evaluation study and 12 h after the second split dose of 125 $\mu$mol in the dietary trial. Clinical studies showed that serum lithium is $\approx$20% lower when it is measured 24 h after a full daily dose than when it is measured 12 h after half a daily dose (2, 20). Thus, in assessing compliance, the dosage schedule and the time of blood sampling should be taken into account. However, when serum lithium concentrations are compared between groups or when compliance is followed over time, the absolute concentration is less important.

In conclusion, when lithium-tagged foods or supplements are used in a study, serum lithium concentrations can be measured to detect grossly noncompliant subjects, to compare compliance between treatment groups, and to follow compliance over time. However, because of the large variability between subjects, serum lithium cannot be used to quantify compliance in each individual participant. In this respect, serum lithium is similar to other markers of dietary intake (21–23). Lithium has the advantages that it can be added to most foods or supplements, it is stable and easy to measure, and natural serum lithium concentrations are so low that even trace doses will cause large increases in serum lithium. The cost of this method is comparable to the cost of measuring lithium in 24-h urine samples. Therefore, serum lithium is a valuable and practical marker of dietary intake.
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