Seasonal Changes in Lead Absorption in Laboratory Rats

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A retrospective study of the relationship of season to the absorption of radiolabeled lead in laboratory rats was performed using mass spectrometry data of 305 animals from 38 experiments over 6 calendar years. Male Wistar rats weighing 200 to 250 g were given 1 µg of radiolabeled lead in an aqueous solution, pH 4.0, in isolated small intestine, and absorption of the radiolabeled lead was quantified after a 4-hour interval using whole-body counting. Similar values of absorption occurred in the summer (June–August) and fall (September–November), 20.51 ± 1.11% (1 SEM) and 23.0 ± 1.23% of the test dose, respectively, but significantly lower values occurred in the winter (December–February) and spring (March–May): 16.51 ± 0.77%, p < 0.01, and 11.87 ± 0.99%, p < 0.01, respectively. Harmonic analysis yielded an excellent approximation of the mean quarterly absorption data. The resulting cosine function had a period of 4.08 ± 0.03 quarter-years with an amplitude of 7.32 ± 1.06%; predicted peak absorption values fell precisely between summer and fall. The relationships of these observations to possible mechanisms of lead absorption and to summertime epidemics of lead poisoning in children are discussed.

Introduction

The absorption of lead by the small intestine occurs through enterocytes by an active transport mechanism (1). Although many factors can significantly influence lead absorption (2–7), most cellular and physiological characteristics of this process remain unknown. Increased lead absorption has been blamed for the summertime outbreaks of acute lead poisoning among children, which have been documented for many years and in many localities (8–15), but there has been no direct evaluation of lead absorption in these epidemics. Because an informal review of our notebooks suggested that there might be seasonal fluctuation in lead absorption in laboratory rats, a complete review of our records was performed. Data were analyzed for a group of 305 rats, similarly reared and experimentally treated over a 6-year interval. The corresponding lead absorption data were closely fitted with a cosine curve having a periodicity of almost exactly 4 quarter-years and showed that there is significantly greater absorption of lead from rats isolated small intestine in the late summer and early fall. These results document for the first time in any species that there is seasonal variation of lead absorption and provide further insights into the causation of summertime epidemics of lead poisoning.

Materials and Methods

Selection of Animal Data

Records of all in vivo radiolabeled lead absorption experiments performed in rats by use of the isolated gut loop technique in our laboratory since the inception of a model system for the study of lead metabolism (2–7,16) were reviewed; previously unpublished results were also included. The data were categorized according to animal weight, dietary history, test dose (including quantity of elemental lead and volume and pH of dose), absorptive site tested, and duration of absorption of the test dose. The largest uniform group of data thus attainable was that concerning male rats weighing 200 to 250 g at the time radiolabeled lead was quantified. These rats had been fed since weaning on a standard food. Each rat received 1 µg of elemental lead as radiolabeled PbCl₂ in 1.0 mL at pH 4.0 via isolated small intestine from which absorption occurred over 4 hr. In this group there were 305 rats studied as normal subjects in 36 different experiments over 6 calendar years (1975–1981); the number of rats per experiment was 8.58 ± 0.36 (mean ± SEM; range 5–16). Animals were not purchased for experiments in September of any year because of laboratory and institutional fiscal policies.

Animal Care

Male albino rats of a pathogen-free Wistar strain (Southern Animal Farms, Prattville, AL) weighing 200 to 250 g at the time of absorption measurements were used in all experiments. The principles of laboratory
animal care as promulgated by the National Research Council were observed. All animals were housed after purchase for at least 48 hr in polypropylene cages (4 rats/cage) floored with stainless steel grids to prevent pica for absorbent bedding and feces. The animal compound contained no other animals and was provided with automatically controlled temperature (22 ± 0.5 °C, relative humidity 40–60%) and lighting (light 0700–1800 hr, cool white fluorescent bulbs). Some natural light entered the room through a small window with a western exposure located just beneath the ceiling, although cages were never placed directly opposite the window. Cages were systematically rotated on cage racks daily. After weaning, all animals received a standard pelleted food (Wayne Lab-Blox, Allied Mills, Chicago, IL) and tap water ad libitum. Identical food and a similar environment, but without natural light exposure, were provided for the rats prior to their shipment to the laboratory.

Measurement of Radiolead Absorption

Lead absorption and retention studies were performed in rats by the quantification of total body radioactivity in a small-animal, whole-body liquid scintillation detector (Packard-ARMAC, Packard Instrument Co., Downers Grove, IL) (2–7,17). Radioisotopes were lead-210 nitrate (specific activity 10 mCi/mg lead) or lead-203 acetate (10–50 mCi/mg lead), obtained from either New England Nuclear (Boston, MA) or Amersham Corp. (Arlington Heights, IL). All measurements of radioactivity were corrected for both radiodecay (by comparison to an appropriate standard after subtraction of background radioactivity) and for resolving time delay. The primary author conducted all experiments with five assistants; the services of individual laboratory technicians could not be correlated with the variations observed in lead absorption.

Immediately prior to absorption measurements, the rats were fasted 12 hr overnight from food, but were given demineralized deionized water ad libitum. Under IP pentobarbital anesthesia (4 mg/100 g), the urethra was tied to prevent loss of absorbed lead. A laparotomy was performed, the small intestine isolated proximally (pylorus) and distally (ileocecal valve) with umbilical tape, and the bile duct was ligated with silk suture. Although the presence of bile in the small intestine enhances lead absorption (2,18), the ligation prevented the enterohepatic circulation of the metal during the experiments. One milliliter of an aqueous test solution containing radiolabeled lead (1 μg of elemental lead as PbCl₂ including 1 μCi of radiolabeled lead), pH 4.0, was injected into the isolated small intestine. This was accomplished by entering the gut lumen proximal to the proximal ligature with a 21-gauge hypodermic needle, passing it intraluminally through the ligature loop, tightening the ligature, and then injecting the test dose into the isolated whole small intestine. The abdomen was closed with stainless steel clips, and the rats were placed in vented 1-quart cardboard ice cream containers. Total body radioactivity was measured in the whole-body detector and compared to a 250-mL water-filled plastic bottle containing a test dose equal to that injected into the animals. Four hr after administration of the test dose, each animal was killed by cervical dislocation. The isolated intestine was excised from the carcass, and whole-body radioactivity was again measured and compared to the original whole-body radioactivity.

Statistical Methods

All absorption data represent the percentage of radiolabeled lead absorbed from the standard dose as described above. The raw data (mean results of individual experiments) suggested trends from low values of absorption in December through May to high values in June through November. Therefore, the data were summarized by seasons (quarters), defined as winter (December–February), spring (March–May), summer (June–August), and fall (September–November). These seasonal data were analyzed with the F test and with Duncan's multiple range procedure (19) to test all paired comparisons. The cosinor method of analysis (20) was used to fit the quarterly absorption data. This consists of fitting the cosine curve

\[ y = \mu + \alpha \cos(\omega t + \phi) \]

to the data where:

- \( y \) = value of absorption (percentage of dose);
- \( t \) = time in quarters;
- \( \mu \) = rhythm-adjusted mean absorption (also called the mesor);
- \( \alpha \) = amplitude (half the peak-to-trough distance);
- \( \omega \) = \( 2\pi p \), where \( p \) is the period; and
- \( \phi \) = acrophase (related to the time of the peak \( t_p = -\phi/\omega \)).

As previously noted (20), it is possible to fit a single cosinor to data such as these, even though in the present study there were serially independent points, missing data, and unequal time intervals.

Results

The summary of radiolead absorption data demonstrated that very significant differences among the seasonal means were present (\( p < 0.0001 \)). Paired comparisons showed that each of the seasonal means were different from the others at a confidence level of < 0.01, except for the values obtained for summer and fall, which did not differ significantly. Hence, the trends of low values of absorption observed in the winter and spring to higher values obtained in the summer and fall are not likely explained by chance.

The results of the fit of the quarterly absorption data by cosinor analysis are shown in Table 1. The 95% confidence interval of the period is (3.98, 4.18) and includes one year (4.00 quarters). The timing of the peak falls
Table 1. Results of cosinor analysis of the fit of quarterly absorption data.

| Parameter | Value | SE |
|-----------|-------|----|
| \( \mu \) | 18.62 | 0.69 |
| \( \alpha \) | 7.32 | 1.06 |
| \( \omega \) | 1.54 | 0.02 |
| \( \phi \) | -2.36 | 0.25 |
| \( t_p = \phi/\omega \) | 1.53 | 0.16 |
| \( p = 2\pi/\omega \) | 4.08 | 0.05 |

The fitted curve is described as

\[
\frac{\sin(\omega t + \phi)}{\sqrt{\pi \omega}} \exp\left(-\frac{t^2}{\omega^2}\right)
\]

The results for the parameters are as follows:

1. The percent of variability in the data accounted for by the fitted curve is \( R^2 = 82.8\% \).
2. The residuals were normally distributed; \( p = 0.976 \) by the Kolmogorov-Smirnov D-statistic (21), and showed no time trend \( p = 0.879 \).
3. The 95% confidence interval for each quarterly mean value of absorption is plotted (Fig. 1); because 13 of the 14 confidence intervals overlap the curve, one may consider this a good fit.
4. When fitting was performed using quarterly means, tests of normality about each mean yielded satisfactory results, except for the fourth, fifth, and eighth quarters analyzed.
5. When fitting was performed using medians as the center of the distribution, the results did not reveal any significant differences.
6. The fit was recalculated for seven consecutive quarters (Fig. 2), the longest such sequence, and yielded results similar to those obtained above.

The quarters were defined in other ways (e.g., spring as April–June) and resulted in a very poor fit. A fit of

The monthly data was done also, but resulted in a poor fit; however, 67% of the data points were missing. Thus, there is uniqueness about the condensation of the data into seasons as defined above.

**Discussion**

This retrospective study relates direct measurements of lead absorption with season for the first time in any species and demonstrates that significant seasonal changes in the absorption of trace quantities of radio-labeled lead from rat small intestine occur. Because most conditions in our animal compound were controlled, either internal factors or the minimal exposure of the rats to daylight (the length of days) could have modulated lead absorption. Under constant experimental conditions, other behavioral, physiologic, and biochemical parameters (including properties of the digestive organs) in laboratory animals also fluctuate with circannual rhythmicity (22–28), as do the appetite, food preferences, and spontaneous intake of major categories of nutrients in humans (29).

Early investigators believed that “the lead stream follows the calcium stream” (30) and demonstrated that vitamin D administration exacerbated clinical and experimental lead poisoning (31,32). Later, it was documented that significant seasonal fluctuations (with peak levels in late summer or early fall) in the serum concentrations of vitamin D metabolites (primarily 25-hydroxyvitamin D) (33–37) occur in humans in relation to the stimulation of vitamin D synthesis by sunlight. These seasonal changes may account for synchronous, parallel changes in calcium absorption (38). Therefore, summer outbreaks of lead poisoning (8–15) have often been attributed to the enhancement of lead absorption by increased sunlight exposure and vitamin D synthesis. Vitamin D does stimulate intestinal mucosal synthesis of calcium-binding protein, which promotes calcium absorption (39,40) and binds lead (3). However, no positive correlation between blood concentrations of lead and vitamin D in either humans or rats has been found (41–45). Blood vitamin D was not quantified in our rats, but their lighting exposure alone appears to be an inadequate explanation for seasonal variation in vitamin D metabolites. 1,25-dihydroxyvitamin D administration increased the retention of orally administered radiolead in mice and rats, and the absorption of radiolead in chicks (17,46,47); in rats, however, this was an effect of prolonged gastrointestinal transit time rather than stimulation of the mucosal phase of radiolead absorption (17). Further, the active transport of calcium (48), but not lead (49), occurs with circadian rhythmicity in rat intestine in vitro. Therefore, significant seasonal changes in human vitamin D metabolism and calcium absorption occur, but vitamin D metabolites may not be important regulators of lead absorption. In addition, there may be significant interspecies differences in the importance of vitamin D and/or vitamin D-dependent calcium-binding protein to intestinal lead transport.

The state of body iron repletion (particularly iron de-

**Figure 1.** Superimposition of cosinor function on mean quarterly values (with 95% confidence intervals) of radiolead absorption. Summers are indicated by numerals on the abscissa.
ficiency) and dietary iron content exert important influences on lead absorption and metabolism in animals, because (1) iron deficiency significantly enhances lead absorption (4,50–52); (2) a variety of other factors similarly affect the absorption of iron and lead (5); (3) an iron-deficient diet decreases the competition of iron with lead for shared intestinal metal binding sites necessary for absorption (1,4); and (4) iron deficiency enhances morphologic and biochemical parameters of lead poisoning (53). In accordance with these observations, iron-deficient human adults absorb significantly increased quantities of lead (54), and there is a frequent association of iron deficiency and lead poisoning among children (55–57). The iron-binding protein transferrin possibly shuttles iron across the absorptive cell and thus regulates iron absorption (58), and lead binds to transferrin and other intestinal mucosal iron-binding proteins (4,59). Unfortunately, there is no description of the influence of season on iron absorption, although one report suggests that weather (and season) affect iron transport by rat intestine in vitro (60). Plasma or serum iron concentration in humans does not vary significantly according to season (61–64), but this parameter has no direct influence on iron absorption (65).

Our rats had significantly increased lead absorption in the summer and fall that occurred with minimal or subtle environmental variations. This result suggests that lead absorption among children could also be increased in the summer, but this latter phenomenon is not necessarily a result of the same mechanisms operant in laboratory rats. The seasonal maxima in blood concentrations of vitamin D metabolites may not stimulate lead absorption, but vitamin D could precipitate or exacerbate plumbism by accelerating the release of lead from storage sites in bone (17,32). Although body iron repletion, dietary iron content, and intestinal iron transport mechanism(s) are significant regulator(s) of lead absorption and important epidemiologic factors in childhood plumbism, the effect of season on iron absorption and metabolism is virtually unstudied. Lastly, a complete understanding of seasonal epidemics of lead poisoning requires that consideration be given not only to lead intake, absorption, metabolism, and excretion, but also to nutritional, environmental, racial, and socioeconomic factors important to the etiology of this summertime disease (66,67).

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