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Intake of lead from game meat – a risk to consumers' health?

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Abstract Game meat may contain variable amounts of lead in the form of fine metallic residues originating from hunting ammunition. The effect of frequent game meat consumption on the blood lead levels of hunters, who are a high-risk lead exposure group, was studied. Blood lead levels of hunters and control subjects were measured using isotope dilution ICP-MS. Dietary information about game meat consumption was obtained from a questionnaire. The blood lead concentrations ranged from 21–171 ng/mL with a geometric mean of 57 ng/mL (n=25). However, the individual blood lead concentrations of the hunters did not correlate with the number of their weekly game meat meals (r=0.046). The blood lead levels were compared with a control group (n=21), which consisted of voluntary blood donors from the same region. Analysis of variance, adjusted for age, did not reveal a significant difference between the two populations (p=0.89). Thus, it was concluded that frequent consumption of wild game meat has no significant effect on blood lead levels.

Keywords Blood \cdot Lead \cdot Game \cdot Hunter \cdot Isotope dilution \cdot ICP-MS

Introduction

The primary lead contamination of game tissue is caused by intake of natural feed and habitat exposure to ambient lead [1]. Projectiles that are used to hunt animals, such as lead shot pellets, may cause secondary lead contamination in the meat that is used for human consumption. Therefore, certain portions of the meat may contain whole or discrete pieces of shot. In the close vicinity of the embedded shot residues, the highest tissue lead concentrations were found [2]. Normally, there is little risk of significant lead contamination in game meat because the area around the wound is cut away and discarded. However, even if the damaged part is cut away, some lead splinters or residues may still remain in the meat and small fragments have been detected up to 30 cm from the point of impact [3, 4].

The effects of lead are the same regardless of the route of exposure (inhalation or oral) and are correlated with internal exposure, as blood lead levels. The uptake of lead depends on a number of factors, e.g. concurrent ingestion of food, interaction with other substances, or the chemical form. The uptake of game meat lead may be less than dietary lead from other sources. However, the extent of absorbed lead is probably higher from previously marinated meat.

If lead shot or particles are swallowed it does not necessarily represent a health hazard. Ingestion of elemental lead as foreign bodies is generally believed to be a low risk with regard to clinically significant lead absorption, even though in certain cases, an increase of the blood lead concentration was observed [5, 6]. Prolonged retention of lead bodies may result in toxicity. Occasionally, retained lead shot in the appendix has been reported as a cause of lead poisoning [7, 8, 9]. The main target for lead toxicity is the nervous system, both in adults and in children. A primary symptom of chronic lead poisoning associated with exposure to lead is anemia. Children are particularly sensitive to the chronic effects of lead. Even low levels of lead exposure can affect a child's mental and physical growth [10, 11, 12].

Maximum lead concentrations in game meat were reported to be higher than 20 μ g/g [13, 14]. However, in bulk analysis this result will not be representative owing to the heterogeneous distribution of the lead shot residues. In a recent study, lead concentrations in meat samples (n=57) of deer collected during the hunting season ranged from 0.01–1.15 μ g/g with a mean value (± standard deviation) of 0.10±0.16 μ g/g [15]. In 1997, Swiss food control authorities found a mean lead concentration of 5.9±19 μ g/g (or a median of 0.2 μ g/g, n=64, 0.01–122 μ g/g) in retail big game meat samples [16].

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Thus, concerns have been raised regarding the consumption of game meat, inasmuch as regulations do not apply to game meat. The recent Swiss and European Union regulations set identical maximum levels for lead of 0.1 mg/kg wet weight only for the most important meat types, i.e. bovine animals, sheep, pig and poultry [17, 18].

To address these concerns, the main objective of the present study was to investigate whether meat from harvested game presents a risk to the health of hunters and their families who consume the edible muscle tissues on a regular basis. For this purpose, the lead blood levels of hunters were compared with those of a control group. The measurement of blood lead is a biomarker of human exposure and indicates the extent to which actual lead intake and uptake have occurred. Diet is currently considered the major contributing factor to blood lead levels due to decreasing lead concentrations in environmental sources, e.g. lead-free gasoline [19]. The blood samples were measured using an ICP-MS method, in which isotope dilution analysis was applied to compensate for non-spectroscopic interferences.

Materials and methods

Study subjects

The study subjects included active hunters and voluntary blood donors as controls from the same region (Bern). After having given a detailed description of the project, hunters were recruited from a local hunting society. Subjects had to be either active hunters or family members who lived in the same household. Those who were willing to participate in the study gave their written agreement. Active male hunters (25) and females (6), who were either hunting by themselves or family members of hunters, enrolled in the study. The blood was sampled at the annual meeting of the hunting society on February 4, 2000. The venous blood samples were taken by two trained nurses from the regional blood bank service, Bern. A blood sample of approximately 5 mL was collected by venipuncture from each subject using the blood collection system S-Monovette (Sarstedt, Sevelen, Switzerland) that is particularly suitable for subsequent metal determinations in blood samples. The walls of the tubes were coated with lithium heparin.

Samples of voluntary blood donors (42) were received in early August 2000 (ZLB Bioplasma, Bern, Switzerland). The samples were small portions of about 10 mL of the donated blood that was intended for plasma processing. All blood samples were stored in a deep freezer (-20 °C) for several days up until the measurement.

Questionnaire

A questionnaire was sent to the participating hunters and their family members to obtain sociodemographic characteristics, information on hunting, and data on consumption of game meat, e.g. number of weekly meals, type of game or cooking preferences. Another important aspect was to reveal lifestyle or environmental factors that serve as potential pathways for lead uptake. The controls did not fill in the questionnaire, as they were anonymous blood donors. Thus, the information for this group was limited to age and sex.

Analytical method

For a 1:30 dilution of the blood samples, a solution of 0.1% ethylendaminetetraacetic acid diammonium salt (Micro Select, Fluka,

Switzerland) and 0.1% Triton X-100 (Merck, Darmstadt, Germany) was used. Triton X-100 allows for rapid washout and helps to protect the tubes and nebulizer tips from blockage. The enriched isotope spike was 95.9% ²⁰⁶Pb (Techsnabexport, former USSR); 138 µL of 10 µg/mL ²⁰⁶Pb was added to 1 L of the reagent mixture. The sample solutions consisted of 8.7 mL of the spiked reagent mixture and 300 µL blood that was pipetted and dispensed using an automatic dilution device (Gilson, Middleton, WI, USA). The resulting sample solution, containing 12 ng of the lead spike, was shaken vigorously to accomplish complete mixing of the lead isotopes. The measurements were carried out using a Perkin Elmer Elan 5000 ICP-MS equipped with a standard torch, nickel cones, and a micro auto sampler (CETAC ASX-100 Omaha, NE, USA). A mini-cyclonic spray chamber of reduced volume was used, with a Seaspray (AR30–1-FSS2) nebulizer, operated using an argon flow of 0.77 L/min and a sample uptake rate of 0.5 mL/min (both devices were obtained from Glass Expansion, Vevey, Switzerland). Outer and intermediate argon gas flow rates were 15 and 0.85 L/min, respectively. The applied plasma power was 1.1 kW. Signals of the spike and reference isotopes, respectively, were monitored at m/z 206 and 208 with 150 ms dwell time for each mass (10 sweeps with 10 replicates).

Quality control measures

The freeze-dried whole blood reference materials Seronorm level I and II were measured (Nycomed, Oslo, Norway). This material was reconstituted before use by adding 5 mL deionized water. The vials were closed and allowed to stand for 30 min. The accuracy of the ICP-MS method was additionally validated by comparison of methods. The reference method was graphite furnace atomic absorption spectrometry (GF-AAS). A Perkin Elmer model 4100 ZL Zeeman atomic absorption spectrometer, equipped with a PE AS-70 auto-sampler, was used for all experiments. The manufacturer's recommendations for wavelength, spectral bandwidth, and lamp current parameters were followed. An adapted temperature programme for Pb determination was used. Prior to the GF-AAS measurements, the blood samples were mineralized using a high-pressure asher-autoclave HPA (PAAR, Graz, Austria). Blood (0.5 mL) was mineralized with 0.5 mL of 30% hydrogen peroxide (suprapur, Merck), and 1 mL of 65% nitric acid (suprapur, Merck), using the following heating program: 80-110 °C over 30 min and then 90 min at 230 °C.

Statistical analysis

Statistical analyses were carried out using the SAS program, mainly the module Insight (SAS system 8.02, SAS Institute Inc. Cary NC, USA) employing descriptive statistics and analysis of variance (ANOVA). Logarithmic data of the blood lead concentrations were used throughout to achieve normal distribution of the residuals. Residual analysis was performed by generating normal probability plots, which indicated near normality after logarithmic transformation.

Results and discussion

Accuracy of the ICP-MS method

The ICP-MS and GF-AAS measurements of lead in Seronorm whole blood standards gave quantitatively acceptable results (RSD <10%) that were in agreement with the reported values (Table 1). As expected, the between-run precision of isotope dilution ICP-MS measurements was better. In Fig. 1, intercomparison data are given for the concurrent determination of lead in se-



Fig. 1 Comparison of blood lead analysis by ICP-MS and AAS. A regression line with a slope of 0.92 (95%-CI: 0.79–1.05) and an intercept of 1.1 ng/g (95%-CI: -6.1–8.2 ng/g) is represented

Table 1 Measured lead concentrations in Seronorm whole blood(level 1 and 2) reference material compared with the reportedvalue

Method	Reported value (ng/mL)		Obtained value±sd (ng/mL)	RSD (%)
Isotope dilution-ICP-MS	41 ^a	6	38.7±1.6	4.1
GF-AAS	41 ^a	5	37.7±2.8	7.4
Isotope dilution-ICP-MS	401 (353–443) ^b	3	413±15	3.6

^a Lot 205052 (level 1), no confidence interval (CI) given ^b Lot 404107 (level 2) with 95% CI

lected blood samples that cover the range of assay values uniformly. The position of the linear regression line indicates a possible bias. The individual confidence intervals (CI) of the slope and the intercept included the ideal values 1 and 0, respectively, and thus there was no significant deviation between the two methods [20].

Outcome of the questionnaire

The summary of relevant results of the questionnaire is shown in Table 2. The most important outcome was the fact that the hunters regularly consumed game meat harvested with lead shot. In contrast, the portion of game meat that came from industrial producers was negligible. During the hunting season, the hunters consumed 2.2 (range 0.3–6) wild game meals per week on average, which amounts to a daily intake of approximately 50 g. Although food frequency questionnaires may be unreliable and inadequate for assessing absolute amounts of intake [21], this amount clearly exceeds the daily intake of ordinary consumers. The per capita daily intake of wild game meat in Switzerland is about 1 g [22]. However, this value is a gross estimate of what is actually



Fig. 2 Frequency distribution of the blood lead levels of the hunters and voluntary blood donors (controls)

Table 2 Outcome of the questionnaire completed by the hunters

Questions	Percentage or number of positive answers
Suspected occupational lead exposure Preference for marinated game meat ^a Game meat from retail outlets Habitual drinking (1–3 dL wine daily) Habitual smoking (10–20 cigarettes per day) Number of weekly game meat meals during the hunting season	24% 32% <5% 92% 28% >2

^a The bioavailability of absorbed lead may be higher if the meat has been previously marinated

consumed because it does not account for losses that occur between wholesale and fork level.

Excessive drinking of alcoholic beverages such as wine or habitual smoking may contribute to the lead status of an individual [23]. However, among the hunter group under study, only one subject was in the habit of drinking more than 1-3 dl wine per day and no subject was identified as a heavy smoker (Table 2). The questionnaire comprised other items that were not answered by the majority of the subjects and, therefore, not listed in Table 2, e.g. questions about the spare-time activities and use of special dishes such lead crystal or glazed pottery.

In looking for potential environmental lead exposure, 24% of the subjects suspected that they might come in contact with lead as a consequence of their working activities. The occupational history showed that occasional contact may have occurred while working with metallic alloys. However, occasional contact of the skin with lead alloys is not usually considered as a source of exposure. Moreover, regular indoor firearm training was reported. However, it was assumed that the lead exposure was negligible because of well-ventilated indoor firing ranges.

Blood lead concentrations

The frequency distribution of the blood lead levels is shown in Fig. 2, stratified by the population groups un-

Subjects	Sex	Age (years)	n	Arithmetic mean±sd (ng/mL)	Median (ng/mL)	Geometric mean (ng/mL)	Range (ng/mL)
Hunters	Male	21–70	25	64±36	59	57	21–171
Family members ^a	Female	21–60	6	41±6.4	42.5	40	31–49
Blood donors	Male	30–66	21	62±31	58	57	24–156
Blood donors	Female	23–64	21	43±19	41	40	20–104

^a Two of which are female hunters

der study. The data from both groups, the hunters and the controls, resulted in right-skewed distributions. The Shapiro-Wilk test on the normality indicated that the distribution is not normal (p < 0.001). After logarithmic transformation the values followed approximately normal distribution (p=0.92). Most of the blood lead levels of the hunters and the control group fell in the two histogram classes between 25 and 75 ng/mL. Both distributions showed similar profiles with a tail reaching 150-200 ng/mL. Blood lead levels are presented in Table 3 for both sexes in terms of arithmetic mean, median, and geometric mean. The latter is more appropriate for comparisons as the results appear to follow a log normal distribution. The blood levels of lead expressed by mean and median values (Table 3) are probably not associated with adverse health effects, even though existing epidemiological studies do not provide evidence of a threshold level [24]. It appears that some effects, particularly changes in the levels of certain blood enzymes and in children's neurobehavioral development, may occur at blood lead levels so low as to be essentially without a threshold. At blood lead levels in excess of 100 ng/mL, discernible effects begin to be apparent [10, 25]. For example, slowed nerve conduction in peripheral nerves in adults occurs at blood lead levels of 300-400 ng/mL.

Other Swiss surveys were not available for comparison as the data were much older and a time trend attributed to the changeover from leaded to unleaded gasoline was observed [26].

No correlation was observed between the individual game meat frequency data (meals per week) and the corresponding logarithmic values of the blood lead concentrations (Pearson correlation coefficient r=0.046). Two-way ANOVA was used to uncover main and interaction effects of age and type of subject (hunters and controls) on the dependant variable blood lead. Female subjects were not included in the ANOVA model. The independent variable age was broken up into in three categories of young, middle-aged, and older subjects (20-39, 40-59 and >60 years of age). The variable *age* was conceived as a control variable because age effects on blood lead levels have been described [27]. The main factors, age (p=0.47) and sub*jects* (p=0.89) as well as the interaction of the combination (p=0.75) had no effect on the blood lead levels. Thus, there was no statistically significant difference in exposure to lead between the hunters and the controls.

Although the peak season for hunting was from mid-September to November 1999, game meat was con-

sumed throughout the winter. If a significant effect of game meat consumption on blood lead levels had existed, it would have been evident in early February 2000, when the blood samples of the hunters were taken. Blood lead levels resulting from a given dietary intake can be estimated from a simple model, where the range reflects uncertainty. In adults, blood lead levels between 23–70 ng/mL are associated with 100 µg dietary lead intake per day under steady state conditions [11, 28]. Accordingly, the consumption of roughly 50 g game meat per day (estimated from the questionnaire) containing $0.2 \ \mu g/g$ lead (median from ref. [16]) amount to a lead intake of 10 µg, which corresponds to a blood lead concentration of about 8 ng/mL. It is interesting to note that the conversion factor for children is greater than for adults [11].

In summary, the results in this study suggest that the uptake of lead from game meat was negligible, given that this type of food was a dietary source of high lead concentration.

Conclusion

Frequent consumption of harvested game meat did not cause blood lead concentrations that were higher than normal levels in the population under study. Accordingly, this type of food does not represent a relevant risk of exposure to lead to the consumer's health.

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