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Consumption of lead-shot cervid meat and blood lead concentrations in a group of adult Norwegians

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ABSTRACT

Several recent investigations have reported high concentrations of lead in samples of minced cervid meat. This paper describes findings from a Norwegian study performed in 2012 among 147 adults with a wide range of cervid game consumption. The main aim was to assess whether high consumption of lead-shot cervid meat is associated with increased concentration of lead in blood. A second aim was to investigate to what extent factors apart from game consumption explain observed variability in blood lead levels.

Median (5 and 95 percentile) blood concentration of lead was 16.6 μg/L (7.5 and 39 μg/L). An optimal multivariate linear regression model for log-transformed blood lead indicated that cervid game meat consumption once a month or more was associated with approximately 31% increase in blood lead concentrations. The increase seemed to be mostly associated with consumption of minced cervid meat, particularly purchased minced meat. However, many participants with high and long-lasting game meat intake had low blood lead concentrations. Cervid meat together with number of bullet shots per year, years with game consumption, self-assembly of bullets, wine consumption and smoking jointly accounted for approximately 25% of the variation in blood lead concentrations, while age and sex accounted for 27% of the variance. Blood lead concentrations increased approximately 18% per decade of age, and men had on average 30% higher blood lead concentrations than women. Hunters who assembled their own ammunition had 52% higher blood lead concentrations than persons not making ammunition. In conjunction with minced cervid meat, wine intake was significantly associated with increased blood lead. Our results indicate that hunting practices such as use of lead-based ammunition, self-assembling of lead containing bullets and inclusion of lead-contaminated meat for mincing to a large extent determine the exposure to lead from cervid game consumption.

1. Introduction

Hunting is an important leisure time activity for many Norwegians: approximately 3% of the population participated in one or more hunting activities during the hunting season 2011/2012 (Statistics Norway, 2012). Game meat is a major food resource for families involved in hunting. Lead based bullet ammunition is permitted and is the ammunition of choice in cervid hunting. Recently, very high concentrations of lead in samples of minced moose meat were reported in a Norwegian study (Lindboe et al., 2012). In the wake of this publication there has been concern that consumption of lead-shot cervid meat may increase lead body burden. Studies from the USA and Europe have indeed found an association between cervid game consumption and blood lead (Hunt et al., 2009; Iqbal et al., 2009). The Federal Institute for Risk Assessment (BfR) in Germany performed investigations resulting in advice to vulnerable groups (i.e. women planning to get pregnant, pregnant women and children) to reduce their intake of game (BfR, 2011). The Swedish National Food Agency (SLV) published a risk management report in June 2012 based on samples of minced moose meat collected from the whole country. This report too concluded that vulnerable groups should be restrictive about their game consumption (SLV, 2012). The British Food Standards Agency published advice in October 2012 that frequent consumers of lead-shot game should reduce their intake, with special emphasis on vulnerable groups like toddlers, children and pregnant women. The advice is based on existing data on lead levels in these foods in the UK and a study of consumers of wild game, conducted by the Food Standard Agency UK (2012). Also EFSA considers high-consumers of game to be a vulnerable group for lead exposure, although foods consumed in larger quantities, such as grains and grain products, milk and dairy products,

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non-alcoholic beverages and vegetables, has the greatest impact on the exposure (EFSA, 2010, 2012).

After absorption, lead enters the bloodstream where it is predominantly bound to proteins in the erythrocytes. In whole blood, the average clearage half-time after a short-term limited exposure is approximately 35 days. Lead accumulates first and foremost in bone, in which the half-life is years to decades, depending on whether the bone is trabecular or cortical. In adults the skeleton contains about 90–95% of the lead body burden. Blood lead levels are an indicator of circulating lead that captures variation in recent external lead exposure as well as lead that has been mobilized from tissue stores (i.e. from soft tissues, but also to some extent bone). Thus, blood lead levels reflect a steady-state mixture of both external exposure and internal stores with almost no ability to distinguish between either (Hu et al., 2007).

It has been extensively documented that lead impairs neurodevelopment (Jakubowski, 2011; Sanders et al., 2009). It has also been shown to have cardiovascular, nephrotoxic, endocrine, gastrointestinal, haematological, musculoskeletal, reproductive and developmental effects (Landrigan et al., 2007). Since the late 1970s and early 1980s it has become increasingly clear that even low exposures to lead can impair neurodevelopment in the foetus and the child (Bellinger, 2004; Lanphear et al., 2005; Lucchini et al., 2012; Mazumdar et al., 2011). The health effects of lead have mainly been related to the concentration of lead in blood, and health based risk assessments and derivation of guidance values for lead in food have been derived from blood lead concentrations (WHO, 2011). However, the large inter-individual differences in the absorption of lead are a major challenge and there are indications that diet and nutritional status have an impact, for example iron and vitamin D status (EFSA, 2010; Meltzer et al., 2010; Moon, 1994; Rezende et al., 2010).

After the phasing out of lead-containing petrol around 1980, and of lead-soldering in canned foods some years later, there remain few substantial single sources of lead in the environment. EFSA considers cereals, tea, tap water, potatoes and potato products, fermented milk products and beer as important food contributors of lead exposure in the European population (EFSA, 2012). Public health concern has been expressed regarding potential high exposure to lead from consumption of cervid meat (Lindboe et al., 2012; SLV, 2012). To be able to assess the risks involved, more knowledge is needed about potential and actual lead exposure, and the relation between exposure and concentration of lead in blood.

This paper presents results from a study among Norwegian hunters and non-hunters with a wide range of cervid game consumption. The main aim of the investigation was to assess whether regular consumption of lead-shot cervid game increases the risk of elevated blood lead concentrations. A second aim was to relate age and other factors, including vitamin D and iron status, to assess their joint contribution to observed variability in blood lead concentrations.

2. Materials and methods

2.1. Study area and subjects

Based on geographical distribution and hunting statistics we contacted the forestry managers in five typical cervid game municipalities (Aurskog-Høland, Bygland, Bindal, Stor-Elvdal and Tingvoll). The forestry managers provided the names of hunting team leaders in their area. A random selection of the hunting team leaders were contacted and sent an information folder. In addition, the Norwegian Hunting and Fishing Organisation (NJFF) put us in contact with members who were leaders of hunting teams. In total we sent out 309 invitation letters to 37 different hunting team leaders who distributed them to their members. In addition, we recruited 48 participants at the Norwegian Institute of Public Health to ensure that we got a wide range of game meat intake among the participants. The inclusion period for this Norwegian Game and Lead Study lasted from primo April till mid October 2012. All together we received 224 participation consents, which is a participation rate of 63% of those invited. Of these, 195 donated a blood sample. In the final statistics there were 147 participants, i.e. the number when counting complete questionnaires and blood analyses. 103 of these were recruited through the hunting team leaders and 44 were in house participants.

As the invitation went out broadly to all consumers of cervid meat, not only moose and deer hunters, the final material included seven high-consumers of reindeer meat from Finnmark, the northernmost county in Norway.

Informed consent was obtained from each participant upon recruitment. The study was approved by the regional ethics committee (2012/100/REK). In addition to the name and address of the participant, the consent form included one global question about consumption of cervids: “How often do you eat cervid game meat?” There were four answering alternatives: “never”, “rarely during a year”, “one to three times per month” and “one or several times per week”. The two latter answer alternatives are in our text also referred to as regular (monthly) and often (weekly). The answers to this global question were used together with the more detailed consumption data described below.

2.2. The questionnaire

A self-administered four page questionnaire (see Supplementary material online) was sent to all who had delivered a consent form. The questionnaire had 32 main questions, and included questions about background (weight, height, occupation, household, and municipality), hunting habits (number of years hunting, assembling own ammunition, number of shots, type of hunting, etc.), consumption of game (moose, deer (both red and roe deer), reindeer, and small game) and factors which might modify lead levels in the body (dietary supplements, alcohol, smoking, etc.). The questionnaire also included questions to distinguish whether minced cervid meat originated from own hunting or was purchased. Since lead in blood has a mean residence time of approximately 30 days, we included questions on consumption of game meat both the last month and the last year. The questionnaire distinguished between game meat dishes comprising whole meat, minced meat and offal meat. The frequencies of consumption were from “never/rarely eaten”, number of meals per month or year to “number of meals per week”. In the latter category the options were 1, 2 or ≥ 3. From the questionnaire answers, consumption frequencies were calculated by computing expected frequencies per year: once per week translated to 52 per year, 2–3 per month became 30 per year etc. For figure and table presentations, the corresponding per month or per week figures were used, or condensed into tertiles of intake.

Since paint with pigments containing lead was phased out already in the 1920s in Norway, questions about building year of home, paint or renovation, etc. were not included. However, we did include questions about occupations and hobbies that might imply some lead exposure.

2.3. Blood sampling and analyses

Blood samples were drawn at the Medical Centre or doctor's office which the participant provided the name of on the consent form. These offices were sent an information letter including blood sample tubes and return envelopes. The blood sample tubes were sent by regular first-class mail directly to Fürsts Medical Laboratory in Oslo for analyses. On average, a letter posted within Norway is delivered to receiver within 24 h. We considered this procedure acceptable as neither lead, serum-ferritin nor vitamin D decomposes over such a short period (Drammeh et al., 2008). Upon arrival at the Laboratory, the samples were stored cool (4 °C) until analyses shortly afterwards.

Lead in blood was analysed with® Elan DRC™ II (PerkinElmer SCIEX, Ontario, Canada) ICP-MS (Inductively Coupled Plasma – Mass Spectrometry) instrument. Samples, standards and quality controls were diluted 1:20 with deionized water (> 18 M®, Millipore, Billerica, USA) and was added 0.1% (v/v) nitric acid (65% w/v, Suprapur®, Merck, Darmstadt, Germany) and 0.2% (v/v) Triton® X-100 (Ph Eur, NF, Merck, Darmstadt, Germany). As internal standard, 10 g/L Thulium (Tm) PerkinElmer Pure Atomic Spectroscopy Calibration Standard in 10% HCl, 1000 mg/mL (Shelton, USA) was added directly to the diluent and used. The sum of 206Pb, 207Pb and 208Pb was measured. External calibration with two point calibration curve (blank+-standard) was used and the standard was made with virtually the same matrix as the samples by adding Lead (Pb) PerkinElmer Pure Atomic Spectroscopy Calibration Standard in 2% HNO_3, 1000 mg/mL (Shelton, USA) to Auto Norm™ (Billingstad, Norway). The limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3SD and 10SD respectively of 10 replicates of a blood sample with lead concentration 16.1 μg/L. The LOD was 1.77 μg/L, and the LOQ was 5.89 μg/L. An internal quality control sample containing 18.72 μg/L Pb was analysed during 2012 ($n=111$) with a SD of 3.74 μg/L (RSD 20%). All the samples analysed in this study contained lead concentrations above the LOQ.

Serum vitamin D [25(OH)D] was measured by UPLC-MS (Ultra Performance Liquid Chromotography, Waters Acquity UPLC, Waters Quattro Micro MS, Milfort MA, USA). Standards were in-house prepared solutions, calibrated against serum controls (prod. no. 35080 and 35081) from Recipe (Recipe Chemicals+Instruments GmbH, München, Deutchland). Deuterized internal standard (IS) [25(OH)D3-d6] (Synthetica, Oslo Norway) was added to the samples. The samples were then liquid-liquid extracted, evaporated, reconstituted in mobile phase and analysed on the UPLC coupled to a tripple quadrupole MS (Quattro Micro, Waters Milford MA, USA). The limit of quantitation (LOQ) were 5.4 nmol/L (D2) and 7.6 nmol/L (D3)

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and coefficient of variation (CV%) were for D2 12.1 at 53.9 nmol/L and 9.9 at 198.3 nmol/L and for D3 it were 8.6 at 65.4 nmol/L and 6.8 at 218.8 nmol/L.

Ferritin was determined using a turbidimetric immunoassay (ADVIA Chemistry Ferritin (FRT); Siemens Healhtcare Diagnostics, Terrytown, US) analysed on an ADVIA 2400 (Siemens Healthcare Diagnostics, Terrytown, US),The LOD was 4.5 μg/L and the LOQ was 6.0 μg/L for %CV ≤ 20%.

2.4. *Study design and statistical analyses*

The aim of this study design was to collect data for assessment of possible associations between game consumption, several covariates and blood lead concentrations. Because it was a priori known that low doses of game meat normally contributed small amounts of lead, regression models were chosen as analysis tools.

The main study aim was to assess whether regular game consumption of lead-shot cervid increase the risk of elevated blood lead levels. This was assessed by linear regression models with game consumption, age and sex as basic independent variables and measured blood lead level as dependent variable.

Other covariates than game consumption, age and sex, were included for a second study aim, to predict the joint contribution of other factors to the variability of blood lead levels. We applied a multiple regression approach starting with all variables that reflected game consumption as well as several other covariates (20–30), including candidates for interaction terms in the model. We then applied variants of stepwise backward variable exclusion together with ANCOVA using Akaike's information criterion (AIC) for optimal variable selection in multivariate models (Claeskens and Hjort, 2008). ANOVA (analysis of variance) was used to assess differences in blood lead between groups. No correction for simultaneous tests of group differences was performed, as these multi-group tests were not used for inference. Model fit was checked by different plots, including standardized residuals and Cook's distance.

It should be noted that in an AIC-optimized model, the effect of all factors may not always be different from zero at the 0.05 level, typically potentially relevant factors with somewhat uncertain estimates may be included. 'Explained' variance is reported as R^2 adjusted for degrees of freedom in the model.

All data processing was done in the program R version 2.15.2 (R development core team). The geometric mean was not used for any variables in modelling or tabulation, but geometric means are occasionally reported for comparison purposes. For robustness, tertiles were used when appropriate. For tests of significance, the groups were either tested for linear trend or group differences.

Table 1
Characteristics of participants ($N=147$) in the Norwegian Game and Lead Study.

	Min	10%	Median	90%	Max	Mean	SD
Age							
Women	19	28	46	63	70	46	14
Men	18	35	50	66	76	51	12
Body Mass Index							
Women	18.4	21.2	23.9	27.3	35.1	24.4	3.0
Men	19.4	22.7	25.9	31.1	37.0	26.4	3.4
Vitamin D (Serum 25(OH)D), nmol/L							
Women	39	58	73	117	173	82	27
Men	27	57	75	99	150	78	20
Serum ferritin, μg/L							
Women	4	18	47	171	295	73	66
Men	22	61	165	266	295	162	73
Bullet shots per year							
Women	0	0	5.5	14	18	6.5	7.4
Men	0	2	58	500	2500	204	372
Years with lead bullets							
Women	0	0	5	17	19	7	8
Men	0	12	31	50	62	30	14
Years with game consumption							
Women	0	0	20	40	50	22	16
Men	0	5.5	35	50	72	33	17
Game, meals/month							
Women	0	0.1	3.5	16	40	6.2	8
Men	0	0.5	5.5	16	56	7.8	8.5
Minced meat moose/deer, meals/month							
Women	0	0	1.2	8.3	13.0	2.6	3.5
Men	0	0	2.1	8.6	13.0	3.3	3.5
Non-minced game, meals/month							
Women	0	0	2.0	7.9	30.0	3.6	5.4
Men	0	0.5	2.9	8.9	44.0	4.4	6.2
Moose, meals/month							
Women	0	0	1.8	12.0	22.0	4.3	5.4
Men	0	0	4.5	13.0	27.0	5.1	5.4
Deer, meals/month							
Women	0	0	0.2	3.7	18.0	1.4	3.6
Men	0	0	0.4	5.1	19.0	1.8	3.4
Reindeer, meals/month							
Women	0	0	0	0.2	5.0	0.2	0.7
Men	0	0	0	0.7	8.3	0.4	1.2
Offal, meals/month							
Women	0	0	0	0.2	5.0	0.2	0.7
Men	0	0	0	0.2	13.0	0.3	1.4
Small game, meals/month							
Women	0	0	0	0.2	1.0	0.1	0.2
Men	0	0	0	0.7	5.0	0.3	0.8

Game: total game from all sources; Small game: hare/rabbit, fowl etc; Years with lead bullets: number of years using lead ammunition

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3. Results

3.1. The participants

Table 1 summarizes background data of the participants. We had more male than female participants, 63 and 37% respectively, of totally 147 persons. There were high- and low-consumers of cervid meat irrespective of recruitment pathway. There were many more participants in the age groups above 40 than below, 3/4 versus 1/4 respectively (Table 2).

The majority of the participants had college/university education (56%) and only 14% reported to have primary education only. Only 14% ($n=20$) reported occasional or daily smoking, while 20% reported use of “snus” which is a moistured version of snuff.

3.2. Game meat consumption

Cervid meat consumption reported upon recruitment (on the consent form) was consistent with the much more detailed frequencies calculated from the questionnaire, which is presented

Table 2
Blood lead concentration (μg/L) by participant subgroups in the Norwegian Game and Lead Study ($N=147$).

	N	%	Min	5%	Median	95%	Max	Mean	SD
Sex									
Women	55	37.4	6.2	6.8	12.9	29.0	35.4	14.7	7.0
Men	92	62.6	6.0	10.2	19.9	42.7	69.3	22.3	11.2***
All	147	100.0	6.0	7.5	16.6	39.0	69.3	19.4	10.5
Age, years									
< 40	35	23.8	6.2	7.1	13.1	30.2	44.1	15.1	8.2***a
40–60	73	49.7	6.0	7.4	16.4	36.5	55.1	18.7	9.3
> 60	39	26.5	9.6	10.6	22.0	45.4	69.3	24.7	12.3
Body Mass Index									
≤ 25	73	49.7	6.0	7.4	14.5	33.4	40.1	17.1	8.5***a
> 25–30	60	40.8	6.7	9.0	17.9	42.1	55.1	21.0	10.5
> 30	20	13.6	6.2	8.2	21.8	53.3	69.3	24.1	15.5
Education									
Primary	21	14.3	10.6	10.6	21.5	41.8	44.9	22.7	10.0
Upper secondary	46	31.3	7.3	7.7	17.7	43.1	55.1	22.1	11.4
Higher education	82	55.8	6.0	6.9	14.6	32.9	69.3	17.0	9.5*
Self-assembled lead ammunition									
No	134	91.2	6.0	7.4	15.6	34.1	69.3	18.1	9.4
Yes	13	8.8	20.0	21.0	31.4	49.0	55.1	33.5	10.7***
Smoking									
Never	85	57.8	6.0	7.3	14.4	33.4	69.3	17.9	11.1
Former	42	28.6	6.9	10.1	18.8	38.6	41.6	20.9	8.7
Occasionally	6	4.1	12.5	12.9	20.6	33.1	37.0	21.0	8.6
Daily	14	9.5	6.7	9.8	21.7	41.1	44.9	23.8	11.2
Cervid consumption reported upon recruitment									
Never/rarely	43	29.3	6.0	6.9	12.5	28.2	33.5	14.0	6.4
Regularly/often	104	70.7	6.2	8.7	20.1	41.4	69.3	21.7	11.0***
Game meat consumption reported in the detailed questionnaire									
Moose									
Never/rarely	54	36.7	6.0	6.8	13.0	29.2	33.5	14.9	6.6
Regularly	22	15.0	7.3	9.4	19.1	38.3	40.1	20.3	9.1*
Often	71	48.3	6.2	8.7	20.2	44.5	69.3	22.7	12.0***
Deer									
Never/rarely	105	71.4	6.0	7.3	14.6	39.0	49.3	17.6	9.5
Regularly	20	13.6	8.7	15.3	23.0	37.8	69.3	26.1	12.3***
Often	22	15.0	6.7	10.9	20.7	36.7	55.1	22.4	10.7*
Reindeer									
Never/rarely	140	95.2	6.0	7.5	16.5	38.8	69.3	19.2	10.4
Regularly/often	7	4.8	11.0	12.1	17.3	38.5	39.1	23.6	11.7
Small game and offal									
Never/rarely	138	93.9	6.0	7.5	15.9	38.9	69.3	19.0	10.5
Regularly/often	9	6.1	15.0	15.9	27.2	38.3	39.1	26.2	8.6
Wine									
Never	46	31.3	6.0	6.7	14.7	33.5	44.9	18.4	9.9
1-2 glass/week	91	61.9	6.9	7.7	15.8	39.8	69.3	19.4	11.2
> 2 glass/week	18	12.2	6.7	13.6	22.3	34.3	36.2	23.7	7.9
Serum-ferritin									
Below median	73	49.7	6.7	9.9	20.0	39.3	69.3	21.9	10.7
Above median	74	50.3	6.0	7.1	14.2	35.0	55.1	17.1	9.7**
Vitamin D									
Below median	73	49.7	6.9	8.2	15.6	35.7	40.1	17.8	8.1
Above median	74	50.3	6.0	7.1	17.8	44.4	69.3	21.1	12.2

P-values for differences vs. group with highest or lowest mean. Variables with *P*-values for group trend < 0.05 are marked with ‘a’

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

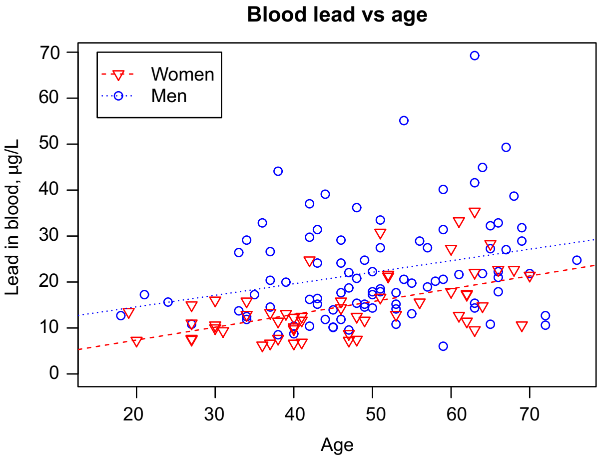

Fig. 1. Blood lead vs. age, separately for men and women. Regression lines, with age x in decades: $y_{\text{men}}=9.6+2.5x$, $y_{\text{women}}=1.8+2.8x$. The variance in blood lead increased with age for both sexes.

in Table 1. Forty-eight per cent ($n=71$) of the participants ate moose meat once a week or more, while 15% ($n=22$) reported eating deer meat once a week or more. Forty-three participants (29%) ate moose and deer never or rarely, thus the participants represented a wide range of cervid meat consumption. Few reported regular or frequent consumption of small game (hare and bird) or offal (small game, $n=4$; offal, $n=5$) (Table 2).

At all levels of game consumption, a considerable portion of intake was in the form of minced meat from moose or deer. Among those with highest consumption, there was a tendency for this proportion to decrease slightly, but at lower intakes there was a positive association between total game consumption and fraction eaten as minced meat, the fractions being 25%, 38% and 46% in the three tertiles of game consumption.

The majority of game consumers, 89 persons (69%), reported that their source of game was their own hunting or hunting party, while 46 participants had purchased the game meat they consumed.

3.3. Blood lead concentrations in relation to background variables

As shown in Table 2, median blood concentration of lead was 16.6 µg/L, 5 and 95 percentiles were 7.5 and 39 µg/L, range 6–69 µg/L. Arithmetic and geometric means were 19.4 and 17.0 µg/L, respectively. Table 2 also shows the lead concentration in blood by different characteristics of the participants. Lead concentrations in blood increased significantly with age, and there was also a sex-specific difference, medians among women and men being 12.9 and 19.9 µg/L, respectively (Table 2 and Fig. 1).

There was a significant positive correlation between unadjusted blood lead concentrations and BMI, but there was no significant difference in age-adjusted lead between educational groups. Furthermore, participants reporting self-assembling of lead-based bullet ammunition had higher blood lead concentrations (Table 2). There were, however, no significant bivariate associations between number of bullet shots fired per year or years with lead ammunition and blood lead (Table 2). Smokers (all levels pooled) had significantly ($P=0.04$) higher blood lead concentrations than non-smokers (mean value 23.8 vs. 17.9 µg/L). This difference persisted upon age adjustment, while there was no similar association for snus.

Wine consumption was associated with higher blood lead concentrations (mean value 18.4 vs. 23.7 µg/L in the lowest and highest intake groups, respectively); the bivariate association was not significant, but there were few high consumers (Table 2). Beer and spirits did not have the same kind of association with lead as wine (data not shown).

Table 3
Number of the participants's ($N=147$) blood concentrations of lead above EFSA's newly established BMDL_{01} and BMDL_{10} of 15 and 36 µg/L (per cent in parenthesis).

	Increased blood pressure BMDL_{01} 15 µg/L Above n (%)	Chronic kidney disease BMDL_{10} 36 µg/L Above n (%)
All	86 (59)	11 (7.5)
Cervid meat consumption		
Never/rarely ($n=43$)	15 (35)	0 (0)
Regularly/often ($n=104$)	71 (68)	11 (11)

There was a weak, but significant positive association between age-adjusted blood lead and serum ferritin in men ($P<0.03$), but not in women. Serum Vitamin D was negatively associated with age-adjusted blood lead in women ($P<0.02$), but not in men (data not shown.)

Fifty-nine per cent of the participants had blood lead concentrations above 15 µg/L, the BMDL_{10} for chronic kidney disease (EFSA, 2010) and seven per cent had concentrations above 36 µg/L blood, the BMDL_{01} for risk of higher blood pressure (EFSA, 2010), and the fraction exceeding these values were higher among regular consumers of cervid meat (Table 3).

3.4. Cervid meat consumption versus blood lead concentrations

In both crude and age- or age/sex-adjusted analyses, blood lead concentrations reflected the consumption of cervid meat intake; Fig. 2A shows results for age-adjusted blood lead concentrations. Participants who reported never or rare consumption of cervid meat had median (unadjusted) blood concentrations of 15.0 and 13.5 µg/L, while those who reported regular (monthly) or often (weekly) consumption had 22.0 and 21.4 µg/L, respectively. Thus, while there was no significant difference in blood lead between those who reported monthly and weekly cervid meat consumption, combined they had about 50% higher unadjusted mean blood lead concentrations than those who never or only rarely ate cervid meat: 21.7 vs. 14.0 µg/L ($P<10^{-4}$, Table 2).

The difference in blood lead between consumers and non-consumers of cervid meat seemed for a large part to be related to consumption of minced meat from moose or deer, as shown in Fig. 2B and C. Whilst age-adjusted blood lead concentrations increased with tertiles of cervid minced meat consumptions (Fig. 2B), this was less evident with consumption of non-minced cervid meat (Fig. 2C).

When sorted by tertiles, men and women in the upper tertile of non-minced-meat consumption category did not differ significantly in blood lead concentration from that of persons in the middle tertile (Fig. 2C). Thus, those who ate minced moose/deer meat more than twice a week apparently had no tendency to increased lead blood concentrations at higher intake of non-minced cervid meat (Fig. 2B and C). Those who had purchased their cervid meat consumed a somewhat larger fraction as minced (46% vs. 39%, $P=0.08$).

3.5. Contribution of other factors than game consumption to blood lead levels

Fig. 3A and B shows scatterplots of the association between the number of meals with minced meat of moose/deer and age-adjusted blood lead concentration, by purchased or self-procured (Fig. 3A) and wine consumption (Fig. 3B, here blood lead is also adjusted for sex).

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With subjects categorized according to high/low minced cervid meat consumption (above or below the median, 2.5 meals/month) and wine drinking, the low minced meat/low wine is a natural base category. Relative to this, age- and sex-adjusted blood lead was about 2 μg/L higher in the low minced meat/high wine group (NS), while it was 5.6 μg/L higher in the high minced meat/low wine group ($P<0.005$) and 12.2 μg/L higher in the high minced meat/high wine group ($P<10^{-6}$)

We present two AIC-optimal models for blood lead concentrations, models A and B. In model A, only dietary and demographic variables are used, constituting a subset of the variable set of model B, where ln-transformed blood lead is used as dependent variable. Ln-transformation improved the model fit significantly for demographic, but not for dietary, variables.

3.5.1. Model A: demographic and dietary variables

Age, sex, regular intake (yes or no) of game meat in general and minced cervid meat (meals per month) in particular were the

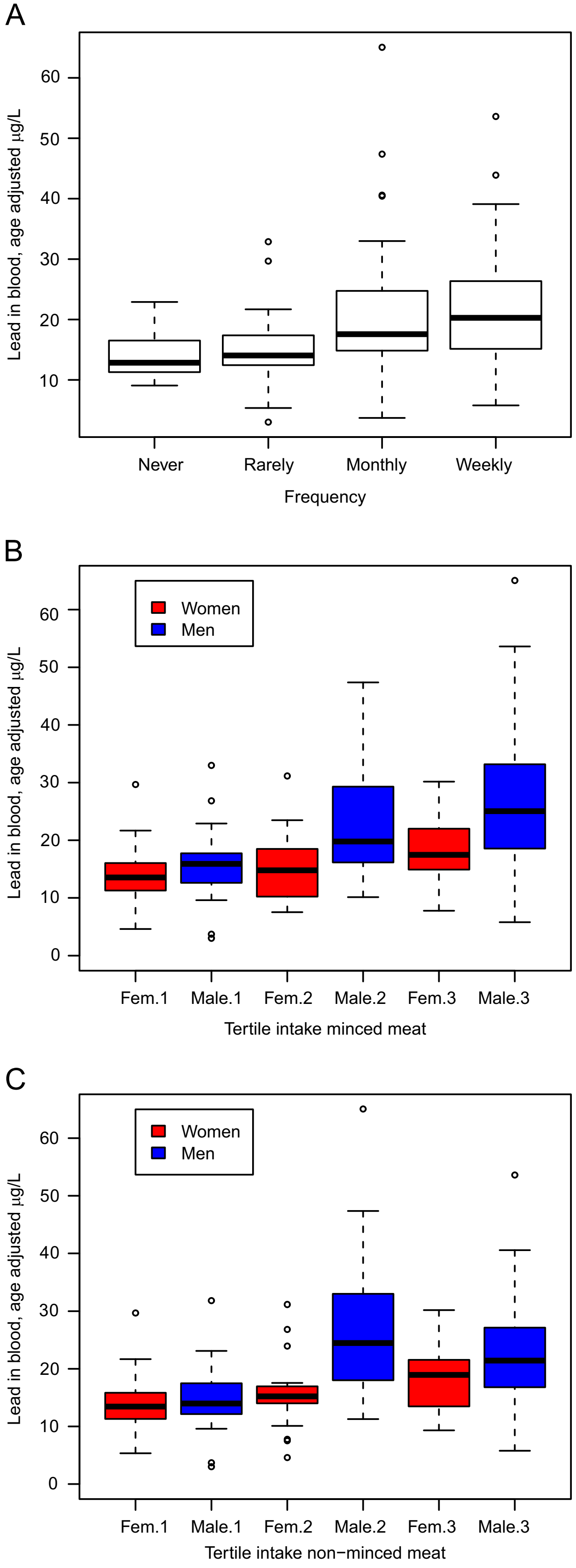

Fig. 2. (A) Age-adjusted blood lead by frequency of game consumption. (B) Age-adjusted blood lead by tertiles of intake of minced meat from moose or deer, separately for men and women. Blood lead means in the three men tertiles were 15.5, 23.0 and 26.7 μg/L, while the corresponding means for women were 13.7, 15.7 and 18.4 μg/L, respectively. Minced cervid meat intake was associated with total game intake at low and moderate game intakes, not but at the highest intakes. (C) Age-adjusted blood lead by tertiles of intake of meat from game, except for minced meat from moose or deer, men and women. No corrections have been made for intake of minced meat. Men in the highest tertile had somewhat lower blood lead than men in the middle tertile, 26.7 vs. 24.7 μg/L, but the difference did not reach significance ($0.1<P<0.2$).

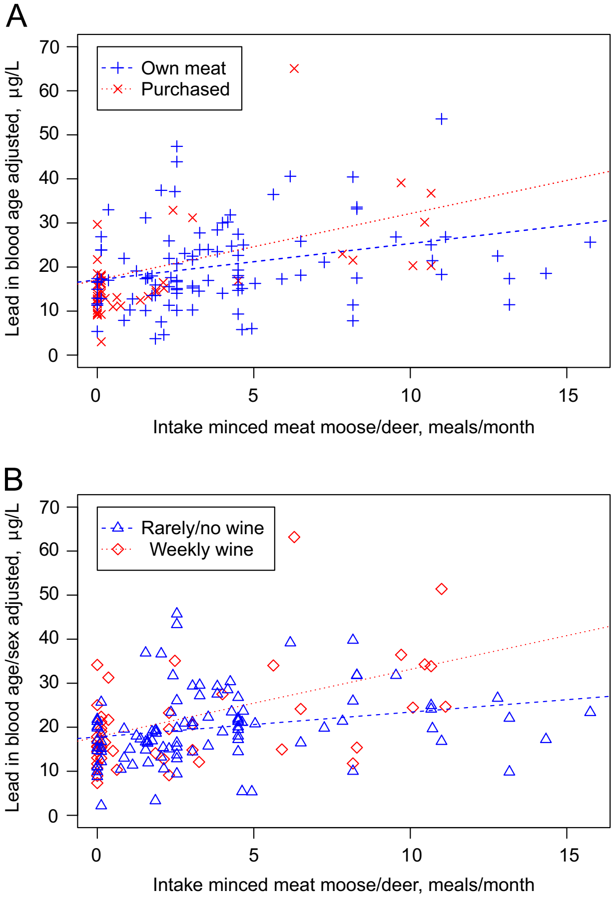

Fig. 3. (A) Age-adjusted blood lead vs. consumption of minced meat from moose/deer, by source of meat. Regression lines for own and purchased meat. Slope for minced meat from own hunting party: 0.83 ± 0.22, purchased meat: 1.51 ± 0.35. $P<0.05$ for equal slopes. (B) Age and sex adjusted blood lead vs. consumption of minced meat from moose/deer, by wine consumption. Regression lines for never and weekly wine intake. Slope for minced meat with no or low wine consumption: 0.56 ± 0.21, wine at least weekly: 1.53 ± 0.29. $P<0.05$ for equal slopes.

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Table 4
Optimal multivariate linear regression model for ln(blood lead) ($N=147$).

	Estimate	Std. Error	t value	Pr(β > |t|)	Effect, % change in blood Pb (95% CI)
Intercept	1.033	0.187	5.52	$<10^{-6}$	
Cervid meat consumption					
Never/rarely	Reference				
Regularly/often	0.267	0.094	2.83	0.005**	
	31 (9,57)				
Sex					
Woman	Reference				
Men	0.26	0.067	3.91	0.0001***	30 (14,48)
Wine consumption	0.090	0.031	2.88	0.005**	9 (3,16)
Making own bullets					
No	Reference				
Yes	0.420	0.110	3.81	0.0002***	52 (23,89)
Age in 10 years	0.166	0.025	6.64	$<10^{-6}$***	18 (12,24)
Shots per year. 100	0.02	0.011	1.98	0.049*	2 (0,4)
Years with game consumption	−0.005	0.002	−2.57	0.011*	−1 (−1,0)
Smoking					
No	Reference				
Yes	0.159	0.090	1.78	0.08	17 (−2,40)
Minced meat moose/deer, meals per month: purchased	0.044	0.011	2.82	0.006**	4 (1,8)
Minced meat moose/deer, meals per month: own	0.019	0.016	1.77	0.08	2 (0,4)

Model summary: multiple R-squared: 0.55, adjusted R-squared: 0.52, residual standard error: 0.347 on 136 degrees of freedom, F-statistic: 16.68 on 10 and 136 DF. P-value: $<2.2e-16$

* $P<0.05$
** $P<0.01$
*** $P<0.001$

main demographic and dietary factors associated with lead concentrations in blood. In the optimized model, separate regression lines were applied according to source of meat (purchased vs. self-procured) and wine intake. Thus, when smoking and lead exposure from hunting was not included, a model for blood lead with sex ($P<0.001$), age ($P<0.0001$), general game meat intake ($P=0.1$) and intake of minced meat with meat source ($P<0.05$) and wine ($P<0.05$) as grouping covariates was AIC-optimal (adjusted $R^2=0.35$, with ln-transformed blood lead $R^2=0.41$). Age and sex jointly accounted for 21% of the variation. The standard error in this model was 8.4, which is about 30% of typical adult game consumers' blood lead. Model A (coefficients ± SD):

$$\begin{aligned}\text{Bloodlead}(\mu g/L) = {} & -1.8 \pm 3.1 + 5.4 \pm 1.5 \times \text{sex male} + 2.8 \pm 0.6 \\ & \times \text{Age in 10 yrs} + 3.2 \pm 1.9 \times \text{game monthly} \\ & + 0.29 \pm 0.27 \times \text{minced own/month} \\ & + 0.87 \pm 0.44 \times \text{minced purchased/month} \\ & + 0.78 \pm 0.35 \times \text{minced and wine/month}\end{aligned}$$

Thus, by this model, one extra meal with minced cervid meat per month is associated with an increase in blood lead (estimate and range of 95% CI) of about 0.3 (−0.2,0.8) μg/L when the meat is self-procured, and 0.9 (0.2,1.6) μg/L when it is purchased. Drinking wine weekly or more added an estimated 0.8 (0.1,1.5) μg/L extra per meal, resulting in an increase of 1.7 μg/L per extra monthly meal with purchased minced cervid meat.

3.5.2. *Model B: all relevant variables*

In the optimal model (Table 4), R^2 adjusted for the number of explanatory variables was 0.52. In this model with blood lead on the ln-scale, regular (monthly/weekly) consumption of cervid game meat was associated with 31% higher blood lead concentrations than never or rare consumption. In addition to the variable denoting regular cervid meat consumption (yes or no), the variables reflecting weekly meal frequencies of self-procured as well as purchased minced meat from moose and/or deer were significantly associated with blood lead concentrations. It was especially the purchased minced meat that had an impact, with 4% higher blood lead concentrations per monthly meal vs. 2% for subjects consuming self-procured minced meet, see also Fig. 3A. Self-assembling of ammunition was associated with 52% higher blood lead concentration. The number of years with game meat consumption was associated with a small but significant reduction in blood lead. Number of bullet shots fired per year was associated with 2% higher blood lead concentrations per 100 shots. Men had 30% higher blood lead concentrations than women. Age was positively correlated with blood lead, with 18% higher blood lead concentrations per 10 years increase in age. Wine consumption (but not beer or spirits) was apparently associated with 9% increase per increasing category of wine consumption. Finally, smokers had 17% higher blood lead concentrations than non-smokers.

Estimated sigma (model standard deviation) was 0.35. This corresponds to about 42% uncertainty if the model is used to estimate an unknown blood lead concentration.

Among the variables that were eliminated in the optimization were BMI, serum vitamin D, serum ferritin, education, region, a finer subdivision of levels of total game intake and different measures of consumption of non-minced game meat.

Variables coding for the most relevant interactions, such as age × sex, age × game intake, sex × game intake, age × minced moose/deer meat, wine × game or minced meat were eliminated by the optimization procedure, leaving no interaction terms in the optimal model.

4. Discussion

4.1. *Main findings*

In this study we investigated whether consumption of cervid game may be associated with lead body burden as expressed by

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blood lead concentrations. In multivariate analysis, cervid meat consumption was an important explanatory variable, with regular cervid meat consumers having about 31% higher blood lead concentrations than non-consumers. The increase was mostly associated with consumption of minced cervid meat, particularly purchased minced meat. An optimal multivariate model explained about 52% of the variance in ln-transformed blood lead, but as several among the ten factors in the optimal model for blood lead were strongly correlated, it is problematic to ascribe explained variance to a single factor. Cervid meat consumption together with number of bullet shots per year, years with game consumption, self-assembly of bullets, wine intake and smoking jointly accounting for approximately 25% of the variation in ln-transformed blood lead concentrations, while age and sex accounted for 27% of the variance.

4.2. Comparison of the reported game consumption with other studies

As the invitation to participate in this study was directed towards hunters in particular, the level and pattern of game consumption in the study is by no means representative of the general population. In the Norwegian nationwide survey Norkost III which comprised 1789 adults and was published in 2012, the average game consumption was approximately 3 g per day.[1] This corresponds to 5–7 meals of game per year with portion size of 150 g to 200 g. Dietary intakes were assessed by two 24-h recalls and the number of game meat consumers was only around 5%. In the Norwegian Fish and Game Study from 1999 with 6050 nationally representative participants, the mean intake of game meat was 6 g per day (Meltzer et al., 2002). This is equivalent to 9–12 meals a year. In the present study, about half of the participants ate game meat once a week or more, which is equivalent to an average consumption of >20 g per day. The large percentage of high consumers shows that we succeeded in recruiting the group we aimed for. Because the average game consumption and hunting activity in the general population is much lower than in our subjects, the percentage of the variation in blood lead concentration explained by parameters of hunting and game consumption would probably be much smaller in a random sample of the population than observed here.

4.3. Comparison of the measured lead concentrations with other studies

In spite of the large proportion of high consumers of cervids in this study, we found lower concentrations of lead in blood than those reported in most European or Norwegian studies over the past 10–20 years. With the exception of the recent study from Sweden with a median of 13.4 μg/L (Bjermo et al., 2013), the geometric mean or median concentrations of lead in blood in most European studies (including Norway) have been around 20 to 30 μg/L (Table 5). The U.S. NHANES from 2002–2003 showed an average of 15 μg/L (Centers for Disease Control and Prevention, 2005). A study from northern Sweden showed annual decrease of 5–6% in erythrocyte lead concentrations between 1990 and 1999 (Wennberg et al., 2006). Clearly, actions against lead pollution during recent decades have caused a remarkable decrease in exposure.

4.4. The impact of game consumption on blood lead concentrations

Several studies have indicated an association between cervid game consumption and lead levels in the body (Bjermo et al., 2013; Hunt et al., 2009; Iqbal et al., 2009). Elevated blood lead levels were found in pigs fed with meat from lead-hunted roe deer. The body burden of lead and other metals was examined in Swedish adults in a subgroup ($n=273$) of the national dietary survey Riksmaten 2010–2011. Total meat intake was not related to blood lead, but when examining categories of meat consumed, frequency of game intake (but not reindeer, sheep, horse, bovine, pig, bird, or fish) was associated with blood lead levels (Bjermo et al., 2013). Among 736 participants from six cities in North Dakota (USA), 80% reported consuming game meat (venison, moose, and birds harvested by hunting). After adjusting for age, sex, current and previous lead-related occupations and/or hobbies and other potential confounding variables, persons who consumed any such game meat had significantly higher blood lead concentrations than non-consumers. The results also showed that recent game meat consumption (<1 month ago) and lager serving size was associated with increased blood lead concentrations (Iqbal et al., 2009). In the present study, high cervid game intake generally appeared to be associated with increased concentrations of lead in blood, yet there were numerous individual exceptions, as illustrated in Fig. 3A and B. Conversely, game was only one of many sources of lead exposure.

To the best of our knowledge, no one has earlier had data to distinguish between the possible impact on blood lead concentrations of minced cervid meat versus cervid meat in general. As shown in Table 4, one extra meal with minced meat per month gave an expected increase in blood lead concentrations of approximately 2% if the meat came from the *participant's own hunting party*, and 4% if it was *purchased*. As described in the Results section, the proportional consumption of minced game meat was reduced among those with a high game meat intake. This could be indicative of a greater relative consumption of meat taken far from the bullet path, reducing the relative lead exposure in the high consumer group. This is consistent with the regression model selection choosing a variable with only two levels of game meat intake (never/rarely vs. regular/often), together with the variable frequency of minced moose/deer meat.

As model A, with only demographic and parameters related to game meat consumption demonstrates, blood lead may be even more strongly associated with minced meat intake than the optimal model may seem to suggest. Varying time spans between cervid meat intake and blood sampling adds to the variability of the association, but as the association between short and long term intake records and blood lead was about the same, there is no indication of systematic bias introduced by this. Properly controlled for such phenomena, the association could turn out to be stronger than estimated.

Fig. 2C illustrates that high intake of non-minced game meat seemed to have limited influence on blood lead concentrations, and a significant portion of the overall higher lead concentrations in blood (31%) in regular game eaters might thus be primarily due to minced meat. The higher concentration of blood lead among consumers of purchased meat could be interpreted in the same direction, but we have too few observations to clarify this.

The lead in meat comes mainly, though not solely, from lead ammunition dispersed into the meat as bullet fragments or deposited through tissue contact with the bullet or fragments (Mateo et al., 2007; Taggart et al., 2011). The meat with highest lead contamination is normally cut away and discarded, but bullet fragments may prevail, and the distance away from the bullet path which is removed varies between the hunting teams. Remaining fragments will usually be small, go unnoticed and they may end up

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[1] Personal information, Inger Therese Lillegaard at The Norwegian Scientific Committee for Food Safety

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Table 5
Concentration of lead in blood in European adults including the current population group.

	Age, years	Year/s sampled	*n*	Median, μg/L	Geometric mean, μg/L
The Norwegian Game and Lead Study[a]	18–79	2012	147	19.4	17
The Norwegian Fish and Game Study[a]	21–80	2003	185	25	24
The Lake Mjøsa Study[a –]	31–88	2004–2005	64	26	27
Pregnant women in the Norwegian Mother and Child Cohort Study validation study sample[a]	23–44	2003–2004	119	11	12
HUNT II study, women[b]	20–55	1996–1997	448	17.5	18
France[c]	18–74	2006–2007	2029	27	26
Germany[c]	18–70	2005	130	20	19
Portugal[c]	18–65	2006	180	28	23
The Netherlands[c]	Adults	2001	1482	26	–
Czech Republic[c]	Adult women	2005–2007	335	25	–
Italy[c]	Adult women	2004	36	24	–
Slovakia[c]	Adult women	2001–2005	98	28	–
Sweden[c]	Adult women	2003–2004	47	15	–
Sweden [d]	Adults	2010–2011	273	13	

[a] Results from populations studies carried out by the Norwegian Institute of Public Health and included in Reference: Norwegian Scientific Committee for Food Safety (2013).

[b] Reference: Meltzer et al. (2010).

[c] Reference Smolders et al. (2010).

[d] Reference Bjermo et al. (2013).

finely dispersed after the mincing process. Fragments of lead ammunition have been retrieved in samples of minced meat, and this appears to be a major source of lead in cervid game meat (Hunt et al., 2009; Lindboe et al., 2012; Tsuji et al., 2009). Although it can be expected that meat originating close to the bullet path is used for mincing, also meat distant from the bullet hole is used for the same purpose – this might contribute to the large variation of lead found in minced meat. There is uncertainty about how much lead is present in the meat, how much of these particular parts are consumed, and how much is subsequently absorbed and reaches the bloodstream and eventually accumulates in humans (BfR, 2011; Landrigan et al., 2007).

4.5. *The impact of hunting practices, age, sex and lifestyle factors on blood lead concentrations*

The ten factors in the AIC-optimal model (model B) for logarithmic blood lead were in part strongly correlated, but apparently, the parameters associated with lead exposure from hunting and game consumption jointly accounted for about 25% of the variation in ln(blood lead).

Despite few subjects practising it, *self-assembling of ammunition* was a very significant factor in the model and associated with approximately 52% increase in expected blood lead concentration, and it is reasonable to interpret this practice as an additional source of lead exposure. Shooting or hunting activity could not explain the effect. We have not seen this variable discussed in any other studies in this field, and it is not known whether the exposure might be dermal, oral (by hand to mouth activity) or inhalational, or both.

Age is a natural variable, as there is an accumulation of lead in bone through life (EFSA, 2010), and the relationship shown in Fig. 1 shows an increase in lead concentration in blood with age, but with very large spread. Combined with higher environmental lead exposure in the past, higher blood lead concentrations with age might also be due to higher bone turnover when getting older, which is accompanied with release of lead from bone (EFSA, 2010).

Sex was a very significant factor in the model, with about 30% higher blood lead among men than among women, but the interpretation of this is difficult. Many relevant explanatory variables are highly correlated with sex, so sex can be expected to act as proxy for variables that are not included in the model, as a correction term for the variables involved, and as a separate explanatory variable. This is in agreement with several other studies where women generally had significantly lower concentrations of metals in blood than men (Vahter et al., 2007).

The models suggest that there may be a very significant effect of *wine intake* on blood lead. Model A provides a strong indication that wine might influence lead absorption from minced game meat, as does the simpler model illustrated in Fig. 3B. After adjustment for age and sex, subjects drinking wine with below median consumption of minced game meat had only slightly elevated blood lead compared to subjects not drinking wine. Wine drinkers with above median minced meat intake had about 6 μg/L higher blood lead than non-drinkers with similar game intake, and this difference can therefore not be explained solely by the lead from wine. While it may be of less significance today, historically both lead bottle-top covering of wine bottles and lead-containing pesticides have caused increased lead exposure in wine drinkers (Hense et al., 1992; Probst-Hensch et al., 1993). It is unclear to what degree the observed relationships primarily reflect historical conditions, how much wine drinking per se still is associated to higher lead exposure (or might be correlated with exposure to other sources of lead), and how much wine can influence lead absorption. In a recent publication from Spain it was shown that lead from ammunition in game meat is more bioaccessible after cooking, especially when recipes included vinegar and wine (Mateo et al., 2011). Thus, lead absorption could be influenced by organic acids in wine. Since neither beer nor liquor were significant factors by themselves, and combined indexes of alcohol intake gave poorer model fit than wine alone, there appears to be no specific effect of alcohol in our study.

Smoking can apparently be associated with increased exposure to lead, and we got a borderline significant, but apparently (see Table 2) not dose-dependent effect in that current smoking is associated with about 20% increase in lead levels. Neither former smoking nor use of snus was associated with lead. Cigarettes smoke is well established sources of lead, although the impact on blood lead concentrations have varied between studies (Ashraf, 2012; Chiba and Masironi, 1992).

It may be reasonable to interpret the *number of bullet shots fired per year* as one of several indicators of lead exposure through shooting activity, and this indicator was included in most model variants resulting from AIC-optimization. *P*-values were usually between $P=0.05$ and 0.08, so this was an example of the fact that not all the

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factors in the AIC-optimal model need be significantly different from zero at the 0.05-level. The estimated effect was modest: 100 shots more per year gave an expected increase in blood lead concentration of just above 2%.

4.6. The impact of iron and vitamin D status on blood lead concentrations

It is well established that low iron status in children increases lead absorption (Watson et al., 1986). Similar results have been observed in adolescents (Barany et al., 2005). In the present study there was a positive association between age-adjusted blood lead and serum ferritin in men, but not in women. The mean serum-ferritin concentration in women was 72.6 μg/L, approximately twice the average level of women of fertile age in Norway (Borch-Iohnsen et al., 2005) and indicating that most women in this study had more than adequate iron status. Only 10% had serum-ferritin below 18 μg/L which indicates empty iron stores. As cervid game meat is an excellent source of heme-iron, a form of iron with high bioavailability, one can speculate if the women participating in this study had higher than average intake of high-quality iron, protecting them from the inverse correlation between iron status and blood lead concentrations seen in other studies. Similarly, the men in this study had excellent iron stores as measured by serum-ferritin concentrations, indicating an iron-rich diet which may counterbalance increased absorption of divalent metals like lead. Thus, the positive association found between serum-ferritin and blood lead concentrations in men is not surprising, both metals in this case having the same main dietary source.

There are indications that lead uptake is influenced by vitamin D status via the calcium absorption mechanisms (Cortina-Ramirez et al., 2006; Fullmer, 1997; Rezende et al., 2008). In this study there was no association between blood lead concentrations and 25(OH) D in serum, a marker of vitamin D status when other factors associated with blood lead were taken into account. The participants had exceptionally good vitamin D status (Table 1), with very few having concentrations below the lower threshold of 50 μg/L serum (NNR, 2004). Furthermore, Norwegians in general are high-consumers of dairy products and have an accompanying high calcium intake (Norwegian Directorate of Health, 2012) which might explain the lack of any associations between vitamin D status and blood lead in the current study.

4.7. Blood lead concentrations vs. BMDL

The concentrations of lead in blood in the present study, which includes high consumers of cervid meat, are in the same range as what is commonly found in the Norwegian and other European populations (Table 5). These concentrations are also in the regions of the BMDL_{01} and BMDL_{10} for an increased risk of increased systolic blood pressure and chronic kidney disease, respectively. Although the risk for significant clinical effects for single individuals at blood lead concentrations at, or slightly above, these BMDLs is low or negligible, lead exposure needs to be lowered at the population level. Meat from lead-shot cervids has been identified as a major lead source in high consumers of such meat, and this exposure can be reduced by altering hunting practice and/or practices at partition of the carcass.

4.8. Strengths and limitations of the study

We consider the major strength of this study to be that we simultaneously record a large number of possible factors for influencing blood lead, and have a wide range of hunting and game meat consuming practices represented. The optimal model (Table 4) explains more than half of the variation in ln-transformed blood lead, indicating that we may have captured several of the most important factors influencing blood lead in our study population. It should, however, not be extrapolated to the general population, as there may be sources of lead exposure not adequately represented in our material.

The optimal model's standard deviation of 0.35 indicates that it can be used to make a rough estimate of lead levels in game consumers, with an expected estimation error of approximately 42%. In this type of analysis, small modifications in the model formulation can result in significant changes in the interrelationship between the explanatory variables, while overall explained variance, as measured by R^2, may be only marginally affected by such modifications. Therefore, even though the established model seems to be fairly robust, one must be cautious interpreting it.

We consider the limited number of participants relative to the considerable number of factors checked to be a weakness of the study. While we had suspected several of the significant associations we found, we did not design the study to test for them with reasonable power, the initial main objective being to assess the general association between game meat intake and blood lead, and provide estimates about the variability.

It may also be considered a weakness that we do not have lead analyses of samples of the meat consumed. Based on reports from Norway and Sweden, the lead concentrations in meat are expected to be highly variable. More data on lead concentration in Norwegian game meat, and in particular commercially available minced meat, are needed. Furthermore, the results of this study apply to adults only and may be seen as a weakness, not least in the wake of the very recent paper from Russia indicting that lead might influence growth negatively (Fleisch et al., 2013) in addition to the well-documented cognitive impairment. The exposure of children may be more important for public health than adult exposure, but our results provide strong indications that this has to be investigated in separate studies, carefully controlling for a range of confounding factors.

5. Conclusion

This study showed that high consumption of cervid meat was significantly associated with higher concentrations of lead in blood. This association seemed to be strongly influenced by consumption of minced game meat, especially purchased minced meat. Other factors contributing to explaining the variance in blood lead concentrations were age, sex, smoking, self-production of ammunition, years with game consumption, number of bullets shot per year, and wine drinking. In this study group, with relatively many hunters and high consumers of game meat, the parameters associated with hunting and game consumption jointly accounted for about 25% of the variance in logarithmic blood lead concentrations. Whilst the blood concentrations are in the region of reference values for low increased risk of higher blood pressure and chronic kidney disease at a population level, the risk at an individual level is low or negligible.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2013.08.007.

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