Manuscript Details

Manuscript number	JTEMB_2019_166
Title	Cadmium, lead and mercury in Norwegian obese patients before and 12 months after bariatric surgery
Article type	Research Paper

Abstract

Purpose Previous studies have suggested a role for the toxic elements lead (Pb), mercury (Hg) and cadmium (Cd) in the development of insulin resistance and hypertension. Increased blood Pb levels have been reported after bariatric surgery and weight loss. As about 80% of patients undergoing bariatric surgery are women, most of them of childbearing age, there are concerns regarding fetal exposure to toxic trace elements. We measured whole blood Hg, Pb and Cd concentrations in morbidly obese patients before and 12 months after Roux-en-Y gastric bypass (RYGB). Patients and methods 46 patients eligible for bariatric surgery were recruited at Innlandet Hospital trust, Norway (2012 – 2014). A majority were women, of whom 54% were of reproductive age. Whole blood samples were collected prior to and 12 months after surgery. Trace element concentrations were measured using mass spectrometry (HR-ICP-MS). Results Median whole blood Pb concentrations increased by 73% during the 12 month study period while Hg and Cd decreased by 31 % and 27%, respectively. We found a negative correlation between Pb levels before surgery and BMI ($p=0.02$). Before surgery patients with hypertension had significantly higher median whole blood Hg levels compared to patients with normal blood pressure ($p<0.001$). Conclusion One year after bariatric surgery, the median whole blood Pb concentration was increased, while Hg and Cd were decreased. The majority of bariatric surgery patients are women of reproductive age and weight loss is associated with improved fertility. As even low dose Pb exposure during fetal life is associated with negative effects on the central nervous system the observed increase in whole blood Pb after weight loss causes concern. Further studies are needed to elucidate these observations.

Keywords	Obesity; Bariatric surgery; Lead; Cadmium; Mercury; Pregnancy
Taxonomy	Medical Biochemistry, Blood Biochemistry
Manuscript category	Biochemistry

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Oslo, Norway, March 13, 2019

Journal of Trace Elements in Medicine and Biology,

Editor-in-Chief: Dr. Dirk Schaumlöffel

Re: Manuscript "Cadmium, lead and mercury in Norwegian obese patients before and 12 months after bariatric surgery"

Dear Prof. Dirk Schaumlöffel

Please find enclosed our manuscript "**Cadmium, lead and mercury in Norwegian obese patients before and 12 months after bariatric surgery**" by Solveig Meyer Mikalsen, Anne-Lise Bjørke-Monsen, Trond Peder Flaten, Jon Elling Whist, and Jan Aaseth, to be taken into consideration for publication in **Journal of Trace Elements in Medicine and Biology.**

The paper takes into consideration the dysregulation of lead, cadmium and mercury in patients with morbid obesity, before and 12 months after bariatric surgery. In particular, the potential fetal neurotoxicity of the demonstrated postoperative increase in circulating lead is discussed.

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Cadmium, lead and mercury in Norwegian obese patients before and 12 months after bariatric surgery

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Short title: Toxic trace elements after bariatric surgery

Abstract

Purpose

Previous studies have suggested a role for the toxic elements lead (Pb), mercury (Hg) and cadmium (Cd) in the development of insulin resistance and hypertension. Increased blood Pb levels have been reported after bariatric surgery and weight loss. As about 80% of patients undergoing bariatric surgery are women, most of them of childbearing age, there are concerns regarding fetal exposure to toxic trace elements. We measured whole blood Hg, Pb and Cd concentrations in morbidly obese patients before and 12 months after Roux-en-Y gastric bypass (RYGB).

Patients and methods

46 patients eligible for bariatric surgery were recruited at Innlandet Hospital trust, Norway (2012 – 2014). A majority were women, of whom 54% were of reproductive age. Whole blood samples were collected prior to and 12 months after surgery. Trace element concentrations were measured using mass spectrometry (HR-ICP-MS).

Results

Median whole blood Pb concentrations increased by 73% during the 12 month study period while Hg and Cd decreased by 31 % and 27%, respectively. We found a negative correlation between Pb levels before surgery and BMI ($p=0.02$). Before surgery patients with hypertension had significantly higher median whole blood Hg levels compared to patients with normal blood pressure ($p<0.001$).

Conclusion

One year after bariatric surgery, the median whole blood Pb concentration was increased, while Hg and Cd were decreased. The majority of bariatric surgery patients are women of reproductive age and weight loss is associated with improved fertility. As even low dose Pb exposure during fetal life is associated with negative effects on the central nervous system the observed increase in whole blood Pb after weight loss causes concern. Further studies are needed to elucidate these observations.

Key-words: Obesity; Bariatric surgery; Weight loss; Lead; Cadmium; Mercury; Pregnancy

Introduction

Globally there is an increasing prevalence of obesity defined by a BMI ≥30 kg/m^2, which is known to predispose to comorbidities like type 2 diabetes, hypertension, dyslipidemia and coronary heart disease (1). Epidemiologic studies have suggested a role for the toxic metals lead (Pb), mercury (Hg) and cadmium (Cd) in the development of metabolic syndrome (2). All three elements have been shown to interact with obesity in various ways, like substituting for essential trace elements or increasing the risk for developing diabetes (3) and hypertension (2, 4, 5).

As weight loss is associated with improvement in metabolic function (6), an increasing number of patients are referred to bariatric surgery (7). Increased blood Pb levels have been reported after bariatric surgery in women (8), and there are concerns regarding the redistribution of mercury (methylmercury) into blood after postoperative loss of fat tissue (9). About 80% of the patients undergoing bariatric surgery are women, many in their childbearing age (10). Weight loss is associated with improved fertility rates (11), however, bariatric surgery is also regarded as a risk factor for adverse pregnancy outcomes (12), particularly due to disturbances in micronutrient status (13). Exposure to toxic elements during fetal life and infancy may have serious long-term health consequences for the child, and cause damage to the central nervous system, the lungs and the kidneys (14-17).

We have studied changes in whole blood Hg, Pb and Cd concentrations before and 12 months after Roux-en-Y gastric bypass (RYGB) in a group of patients with a BMI ≥35, where the majority were women of reproductive age.

Materials and methods

Study population

Patients aged 18 to 60 years eligible for bariatric surgery due to a BMI $\geq$ 40 kg/m^2 or a BMI $\geq$ 35 with serious weight related comorbidities like type 2 diabetes or cardiovascular disease, were consecutively recruited at Innlandet Hospital Trust, Gjøvik, Norway, during 2012 to 2014. Exclusion criteria were major psychiatric disorders, serious somatic disorders not related to obesity, alcohol or drug addiction, former obesity surgery and other major abdominal surgery. The enrolled patients got an initial brief dietetic counselling six months before surgery. At the end of this period, they attended an eight week course on lifestyle changes, followed by bariatric surgery. The surgical technique used was laparascopic Roux-en-Y gastric bypass (RYGB), which involves transection of the upper part of the stomach leaving about 30 mL, which is anastomosed with the distal jejunum, resulting in bypass of the remaining major part of the stomach, duodenum and proximal jejunum (18). Reduced size of the functional stomach results in earlier satiety and thus restricted food intake. Furthermore, the food in this stomach pouch bypasses the duodenum and enters directly into the distal jejunum, leading to reduced absorption in the small intestine (18).

After surgery the patients were recommended daily supplementation with iron (100 mg), calcium (1000 mg) and vitamin D (20 µg) for 6 months, intramuscular vitamin B12 injections (1 mg) every third month, and lifelong daily multivitamin/mineral supplements.

Ethical approval of the protocol was obtained by the Regional Committee for Medical and Health Research Ethics (REK), Region South-East, Norway, ref. number 2012/1394. The study was conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all patients before enrolment.

Sample collection, preparation and analysis

Whole blood samples were collected immediately before surgery and 12 months after surgery. The samples were obtained from the cubital vein between 8:00 and 10:30 a.m. and collected in

Vacuette Trace Elements Sodium Heparin tubes (Greiner Bio-One) for trace element analyses. The samples were stored at –20 °C before analysis.

Approximately 0.7 mL blood was transferred to metal-free 18 mL teflon tubes. The exact weight of each sample was measured and converted back to volume by multiplying with 1.06 g/mL (the average density of whole blood). The samples were acidified with 1.0 mL 65% (V/V) ultrapure nitric acid, produced in-house from p.a. quality nitric acid (Merck, Darmstadt) using a sub-boiling distillation system (SubPur, Milestone, Shelton, CT). The samples were then digested using a high performance microwave reactor (UltraClave, Milestone). Digested samples were decanted into pre-cleaned 15 mL polypropylene vials (VWR, USA) and diluted with ultrapure water (Purelab Option-Q, Elga) to achieve a final acid concentration of 0.6 M.

Trace element concentrations were measured using high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS, Thermo Finnigan Element 2, Bremen). The sample introduction system consisted of an SC2-DX auto-sampler with ULPA filter, a prepFAST system, a concentric PFA-ST nebulizer coupled to a quartz cyclonic micro mist Scott spray chamber with auxiliary gas port, aluminium skimmer and sample cones, and an O-ring-free quartz torch and 2.5 mm injector (Elemental Scientific, Omaha, NE). The radio frequency power was set to 1350 W; nebulizer and T-connection sample gas flow were 0.75 L/min, and 0.55 L/min, respectively. Cooling gas flow was 15.5 L/min, auxiliary gas flow was 1.1 mL/min and additional gas consisted of 10% methane in argon with flow rate of 0.01 L/min.

Two multi-element stock solutions (Elemental Scientific, Omaha, NE) were used, one serving as a calibrating solution and the other as a quality control. These solutions were matrix matched with the blood samples for acid strength (0.6 M ultrapure nitric acid), and by adding 160 mg/L sodium and 115 mg/L potassium (Spectrapure Standards, Oslo). Corrections for instrumental drift were done by repeated measurements of one of the multielement standards.

The stability of the instrument was checked by inspection of the argon signal and measurements of 1 µg/L rhenium added as an internal standard through the prepFAST system. Repeated analysis of a certified reference material (Seronorm Level 1, Sero, Norway) was used to verify the accuracy of the instrument.

Statistical analysis

Results are presented as mean and standard deviation (SD), compared by Student's t-test or Anova, and median and interquartile range (IQR), compared by Mann-Whitney U test or Kruskal-Wallis test. Chi-square test was used for categorical data. Spearman correlations were used to explore relationships between data.

Graphical illustration of the relationship between age and whole blood Pb and Hg, alcohol intake and Pb, smoking history and Cd was obtained by generalized additive models (GAM).

The SPSS statistical program (version 23) and the packages "mgcv" in R®, version 3.3 (The R Foundation for Statistical Computing) were used for the statistical analyses. Two-sided p-values < 0.05 were considered statistically significant.

Results

The study population included 46 patients (age range 27 to 59 years) with baseline characteristics given in Table 1. The majority were women, of whom 21/39 (54%) were of reproductive age (<45 years).

At inclusion, both women and men tended to eat more meat than fish and seafood (Table 1). Nine of 39 women (23%) and one of six men (17%) were daily smokers (p=0.08) (Table 1), and the percentage declined from inclusion to 12 months after surgery (22% versus 3%). The number of patients who reported use of alcohol more than once a month, remained essentially unchanged from inclusion to 12 months after bariatric surgery (43% versus 41%).

Total mean weight loss from inclusion to 12 months after bariatric surgery was 42.5 (SD 11.9) kg, (range 13.4 to 68.7 kg). Approximately 25% of the weight loss was achieved by dieting and exercise before surgery (mean 10.3 (SD 4.6) kg), and 75% was achieved after surgery (mean 32.3 (SD 10.6) kg). There was a mean reduction of 33% in BMI from inclusion to 12 months postoperatively (mean BMI 27.8 (SD 3.4), with no gender difference (p=0.81).

Toxic element concentrations before and 12 months after bariatric surgery

Whole blood Pb, Hg and Cd concentrations changed significantly during the observation period of 12 months (Table 2, Table 4). Median Pb concentration increased by 73%, while median Hg and Cd concentrations decreased (by 31% and 27%, respectively).
BMI was significantly inversely correlated to whole blood Pb before surgery (rho=-0.34, p=0.02), while only weak, negative correlations were observed for BMI and Hg and Cd (p>0.12). Age was significantly correlated to whole blood concentrations of Pb (rho=0.39, p=0.007) and Hg (rho=0.53, p<0.001) before surgery (Figure 1), but not to Cd concentrations (rho=-0.04, p=0.8).

Reported alcohol intake at inclusion was positively related to whole blood Pb before surgery (rho=0.44, p=0.003), with a dose-response relationship between intake of alcohol in units and whole blood Pb, as shown by GAM corrected for age (Figure 2). Alcohol intake was also the strongest predictor for Pb in a multiple linear regression model, which additionally included gender, age, smoking history, fish intake, education, BMI at inclusion and weight reduction before surgery (Table 3).

Women had slightly higher median Hg levels 1.12 (IQR 0.73, 1.50) µg/L than men median Hg 0.94 (IQR 0.66, 1.01) µg/L before surgery, but this difference was not significant (p=0.14). However, in the multiple linear regression model, gender was a strong predictor for whole blood Hg followed by intake of fish and seafood (Table 3).

Before surgery, patients with hypertension (n=15) had significantly higher median whole blood Hg 1.76 (IQR 1.13, 2.01) µg/L compared to patients with normal blood pressure median Hg 1.00 (IQR 0.65, 1.17) µg/L, $p<0.001$. This difference was reduced after surgery and was no longer significant ($p=0.19$). No differences were observed for Pb and Cd concentrations in patients with a diagnosis of hypertension vs. those without this diagnosis.

Smoking history at inclusion was strongly correlated to whole blood Cd before surgery (rho=0.69, $p<0.001$) (Figure 3), and was the strongest predictor for Cd in the multiple linear regression model (Table 3).

At inclusion seven patients had a diagnosis of diabetes; no differences in Pb, Hg or Cd concentrations before or after surgery were seen in diabetic versus non-diabetic patients ($p>0.12$).

Discussion

In the present study, we found the whole blood Pb concentration to be negatively related to BMI in a population of obese Norwegian adults. The median whole blood Hg concentration was substantially higher in patients with hypertension, while no associations were found for any of these metals as related to a diagnosis of diabetes.

One year after bariatric surgery with a mean weight loss of 32.3 kg, median whole blood Pb increased by 73%, while median Hg and Cd concentrations declined by 31 and 27%, respectively. The majority of the patients were women of reproductive age (<45 years). As weight loss is associated with increased fertility, the increase in whole blood Pb concentration may be of concern for pregnancy outcome.

In our population of young and middle aged obese patients, age was significantly positively correlated to whole blood Pb and Hg, while BMI was negatively correlated to whole blood Pb before surgery. Weak negative, however not significant, correlations between BMI and Hg and Cd were observed. Published studies on the correlation between BMI and toxic metals have shown ambiguous results (3, 19-21). However, in large population studies including the US NHANES, an inverse correlation between BMI and whole blood Hg (19), whole blood and urine Pb (3, 20) and Cd (3, 21) have been reported. Pb and Cd accumulate progressively in the body with a long biological half-life of 15-30 years (22, 23), while Hg has a relatively short half-life of approximately 50 days (24). While both BMI and the levels of Hg, Pb and Cd tend to increase with age, these inverse relations have been difficult to explain, but seem to be independent of age and gender (3).

Lead

Compared to French adults (25), median whole blood Pb was lower in obese Norwegian adults both before and after surgery (Table 4). However, after bariatric surgery, obese Norwegian women of reproductive age had higher median whole blood Pb concentrations compared to age-matched Scandinavian women (26-28) (Table 4).

A substantial increase of more than 70% in median whole blood Pb concentration was observed after bariatric surgery, in parallel to the weight loss of about 30 kg. The strongest predictor for whole blood Pb at inclusion was use of alcohol, which is in accord with reports by others (27). Alcohol habits remained essentially unchanged from inclusion to 12 months after bariatric surgery.

More than 90% of Pb is stored in bone (22). Weight loss has been shown to increase bone turnover, thereby mobilizing Pb with resulting higher blood levels (8, 29). For example, in a small group of women (n=17) with a mean weight loss of 37.4, (SD 9.3) kg, whole blood Pb concentrations increased by 90% from mean 1.9 (SD 1.4) µg/dL to 3.9 (SD 3.4) µg/dL after six months (8), an increase comparable to what we observed.

As about 80% of the patients undergoing bariatric surgery are women, many of childbearing age (10), the increase in whole blood Pb concentrations observed one year after surgery is of concern. Weight loss is associated with improved fertility rates (11). There is no international consensus concerning the ideal time to conception after bariatric surgery, but the standard recommendation is to delay pregnancy for at least 1 to 1.5 years after surgery (30). During pregnancy, maternal blood Pb concentrations increase, and Pb is easily transferred across the placenta to the fetus (31). After birth, Pb is secreted into breast milk (31, 32). As even low levels of Pb exposure in children are associated with neurodevelopmental deficits (33, 34), the observed increase in whole blood Pb concentrations after bariatric surgery is worrying.

Mercury

Compared to French adults (25), median whole blood Hg concentrations were lower in obese Norwegian adults both before and after surgery (Table 4). Obese Norwegian women of reproductive age had lower median Hg concentrations both before and after bariatric surgery compared to age-matched Scandinavian women (26-28) (Table 4).

Before surgery, gender and seafood intake were the strongest determinants for Hg, relations also reported by others (26). We observed a higher median whole blood Hg concentration in patients with hypertension before surgery. Mercury has numerous negative vascular effects and the clinical consequences of mercury toxicity include, among others, hypertension (5). There are no published data on Hg levels after bariatric surgery. We observed a reduction in whole blood Hg after bariatric surgery induced weight loss. The reasons for this reduction is unknown, however, gastric bypass surgery reduces the size of the functional stomach and results in restricted food intake. As whole blood Hg has a relatively short half-life of approximately 50 days (24), a reduced intake of fish and seafood after surgery may be responsible for the reduced whole blood Hg concentrations.

Cadmium

Compared to French adults (25), median whole blood Cd concentrations were lower in obese Norwegian adults both before and after surgery (Table 4). Median Cd concentrations were higher in obese Norwegian women before surgery compared to other Scandinavian women (26-28) (Table 4). After surgery, median Cd levels were reduced in obese women resembling age-matched Danish women (28) (Table 4).

The strongest determinants for Cd concentrations before surgery were use of tobacco, as reported earlier (26, 27, 35). There are no published data on whole blood Cd concentrations after bariatric surgery. Whole blood Cd is an established marker of Cd exposure and is reported to also reflect short term fluctuations in exposure (23). As the percentage of daily smokers was substantially reduced one year after surgery, this may partially explain the observed reduction in whole blood Cd concentrations.

Conclusion

In this study of obese Norwegian adults whole blood concentrations of Pb, Hg and Cd were weakly negatively related to BMI. However, the weight loss induced by bariatric surgery increased median whole blood Pb significantly, whereas it reduced Hg and Cd concentrations as measured one year after surgery. Weight loss is known to increase fertility, and as the majority of bariatric surgery patients are women of reproductive age, this increase in whole blood PB concentrations is of concern.

Interestingly, patients with hypertension had significantly higher median whole blood Hg concentrations before surgery, but not postoperatively, while no differences in Pb, Hg or Cd concentrations were seen as related to a diagnosis of diabetes.

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Declarations of interests: The authors declare no conflicts of interest.

References

1. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA. 1999;282(16):1523-9.
2. Bulka CM, Persky VW, Daviglus ML, Durazo-Arvizu RA, Argos M. Multiple metal exposures and metabolic syndrome: A cross-sectional analysis of the National Health and Nutrition Examination Survey 2011-2014. Environ Res. 2019;168:397-405.
3. Padilla MA, Elobeid M, Ruden DM, Allison DB. An examination of the association of selected toxic metals with total and central obesity indices: NHANES 99-02. Int J Environ Res Public Health. 2010;7(9):3332-47.
4. Houston MC. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction. Altern Ther Health Med. 2007;13(2):S128-33.
5. Houston MC. Role of mercury toxicity in hypertension, cardiovascular disease, and stroke. J Clin Hypertens (Greenwich). 2011;13(8):621-7.
6. Magkos F, Fraterrigo G, Yoshino J, Luecking C, Kirbach K, Kelly SC, et al. Effects of Moderate and Subsequent Progressive Weight Loss on Metabolic Function and Adipose Tissue Biology in Humans with Obesity. Cell Metab. 2016;23(4):591-601.
7. Angrisani L, Santonicola A, Iovino P, Formisano G, Buchwald H, Scopinaro N. Bariatric Surgery Worldwide 2013. Obes Surg. 2015;25(10):1822-32.
8. Riedt CS, Buckley BT, Brolin RE, Ambia-Sobhan H, Rhoads GG, Shapses SA. Blood lead levels and bone turnover with weight reduction in women. J Expo Sci Environ Epidemiol. 2009;19(1):90-6.
9. Slater C, Morris L, Ellison J, Syed AA. Nutrition in Pregnancy Following Bariatric Surgery. Nutrients. 2017;9(12).
10. Kochkodan J, Telem DA, Ghaferi AA. Physiologic and psychological gender differences in bariatric surgery. Surg Endosc. 2018;32(3):1382-8.
11. Best D, Avenell A, Bhattacharya S. How effective are weight-loss interventions for improving fertility in women and men who are overweight or obese? A systematic review and meta-analysis of the evidence. Hum Reprod Update. 2017;23(6):681-705.
12. Falcone V, Stopp T, Feichtinger M, Kiss H, Eppel W, Husslein PW, et al. Pregnancy after bariatric surgery: a narrative literature review and discussion of impact on pregnancy management and outcome. BMC Pregnancy Childbirth. 2018;18(1):507.
13. Rottenstreich A, Elazary R, Goldenshluger A, Pikarsky AJ, Elchalal U, Ben-Porat T. Maternal nutritional status and related pregnancy outcomes following bariatric surgery: A systematic review. Surg Obes Relat Dis. 2018.
14. Counter SA, Buchanan LH. Mercury exposure in children: a review. Toxicol Appl Pharmacol. 2004;198(2):209-30.
15. Rodrigues EG, Bellinger DC, Valeri L, Hasan MO, Quamruzzaman Q, Golam M, et al. Neurodevelopmental outcomes among 2- to 3-year-old children in Bangladesh with elevated blood lead and exposure to arsenic and manganese in drinking water. Environ Health. 2016;15:44.
16. Sanders AP, Claus Henn B, Wright RO. Perinatal and Childhood Exposure to Cadmium, Manganese, and Metal Mixtures and Effects on Cognition and Behavior: A Review of Recent Literature. Curr Environ Health Rep. 2015;2(3):284-94.
17. Tang M, Xu C, Lin N, Liu K, Zhang Y, Yu X, et al. Lead, mercury, and cadmium in umbilical cord serum and birth outcomes in Chinese fish consumers. Chemosphere. 2016;148:270-5.
18. Higa KD, Ho T, Boone KB. Laparoscopic Roux-en-Y gastric bypass: technique and 3-year follow-up. J Laparoendosc Adv Surg Tech A. 2001;11(6):377-82.

19. Rothenberg SE, Korrick SA, Fayad R. The influence of obesity on blood mercury levels for U.S. non-pregnant adults and children: NHANES 2007-2010. Environ Res. 2015;138:173-80.
20. Scinicariello F, Buser MC, Mevissen M, Portier CJ. Blood lead level association with lower body weight in NHANES 1999-2006. Toxicol Appl Pharmacol. 2013;273(3):516-23.
21. Wang Q, Wei S. Cadmium affects blood pressure and negatively interacts with obesity: Findings from NHANES 1999-2014. Sci Total Environ. 2018;643:270-6.
22. Abadin H AA, Stevens YW, Llados F, Diamond G, Sage G, et al. US Department of Health and Human Services Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles. Atlanta (GA. Toxicological profile for lead. 2007.
23. Jarup L, Akesson A. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol. 2009;238(3):201-8.
24. Smith JC, Farris FF. Methyl mercury pharmacokinetics in man: a reevaluation. Toxicol Appl Pharmacol. 1996;137(2):245-52.
25. Nisse C, Tagne-Fotso R, Howsam M, Members of Health Examination Centres of the Nord - Pas-de-Calais region n, Richeval C, Labat L, et al. Blood and urinary levels of metals and metalloids in the general adult population of Northern France: The IMEPOGE study, 2008-2010. Int J Hyg Environ Health. 2017;220(2 Pt B):341-63.
26. Abass K, Koiranen M, Mazej D, Tratnik JS, Horvat M, Hakkola J, et al. Arsenic, cadmium, lead and mercury levels in blood of Finnish adults and their relation to diet, lifestyle habits and sociodemographic variables. Environ Sci Pollut Res Int. 2017;24(2):1347-62.
27. Flotre CH, Varsi K, Helm T, Bolann B, Bjorke-Monsen AL. Predictors of mercury, lead, cadmium and antimony status in Norwegian never-pregnant women of fertile age. PLoS One. 2017;12(12):e0189169.
28. Rosofsky A, Janulewicz P, Thayer KA, McClean M, Wise LA, Calafat AM, et al. Exposure to multiple chemicals in a cohort of reproductive-aged Danish women. Environ Res. 2017;154:73-85.
29. Ricci TA, Heymsfield SB, Pierson RN, Jr., Stahl T, Chowdhury HA, Shapses SA. Moderate energy restriction increases bone resorption in obese postmenopausal women. Am J Clin Nutr. 2001;73(2):347-52.
30. Busetto L, Dicker D, Azran C, Batterham RL, Farpour-Lambert N, Fried M, et al. Obesity Management Task Force of the European Association for the Study of Obesity Released "Practical Recommendations for the Post-Bariatric Surgery Medical Management". Obes Surg. 2018;28(7):2117-21.
31. Arbuckle TE, Liang CL, Morisset AS, Fisher M, Weiler H, Cirtiu CM, et al. Maternal and fetal exposure to cadmium, lead, manganese and mercury: The MIREC study. Chemosphere. 2016;163:270-82.
32. Rebelo FM, Caldas ED. Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants. Environ Res. 2016;151:671-88.
33. Bellinger DC. Very low lead exposures and children's neurodevelopment. Curr Opin Pediatr. 2008;20(2):172-7.
34. Grandjean P. Even low-dose lead exposure is hazardous. Lancet. 2010;376(9744):855-6.
35. Aoki Y, Yee J, Mortensen ME. Blood cadmium by race/hispanic origin: The role of smoking. Environ Res. 2017;155:193-8.

Figure legends

Figure 1.

The association of age with whole blood lead (Pb) and mercury (Hg) concentrations by a generalized additive model (GAM).

The solid line shows the fitted model and the area between the dotted lines indicate 95% confidence interval.

Figure 2.

The association of alcohol intake with whole blood lead (Pb) concentrations by generalized additive model (GAM), adjusted for age.

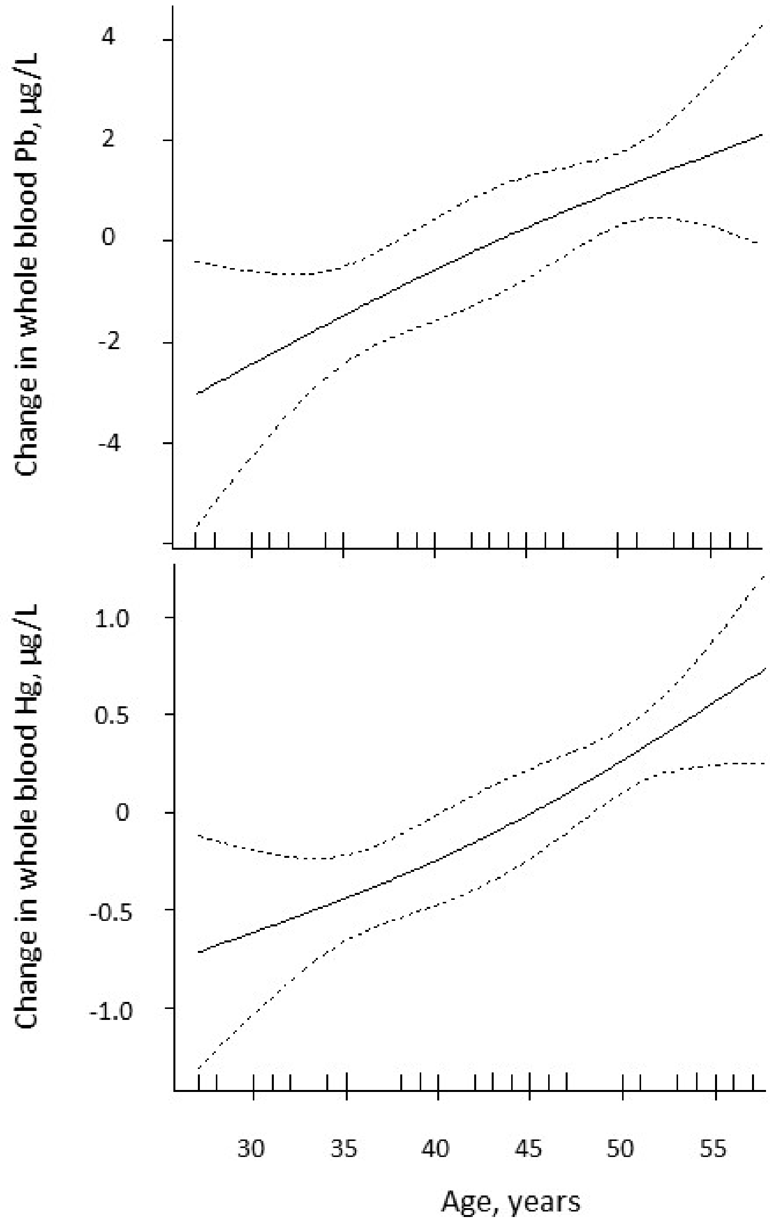
The solid line shows the fitted model and the area between the dotted lines indicate 95% confidence interval.

Figure 3.

The association of smoking history with whole blood lead (Pb) concentrations by generalized additive model (GAM), adjusted for age.

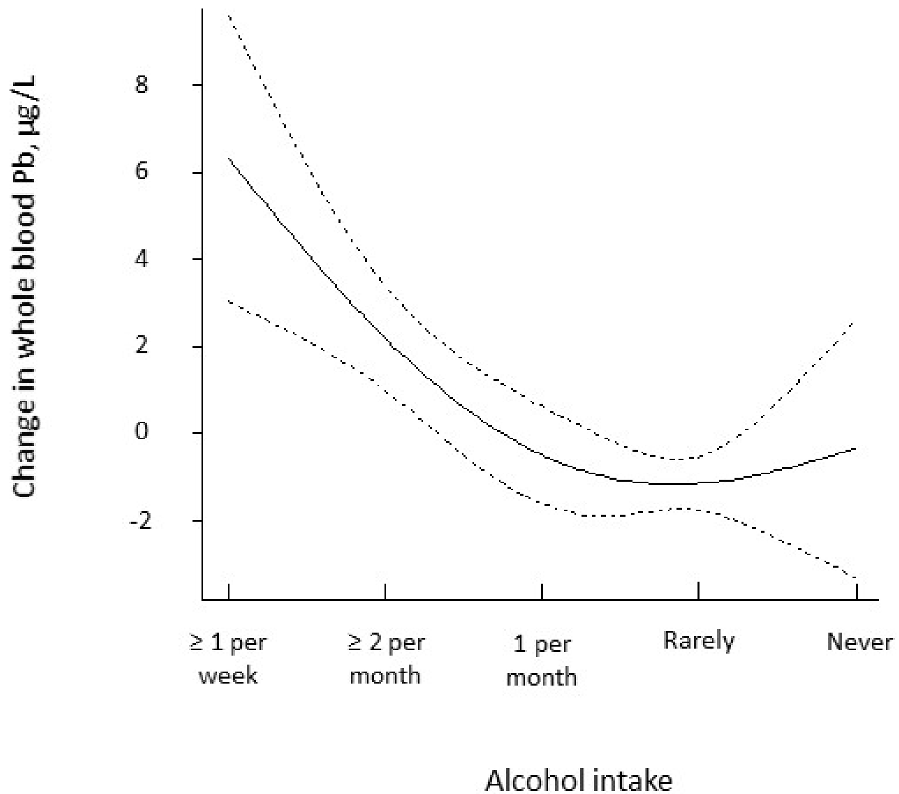
The solid line shows the fitted model and the area between the dotted lines indicate 95% confidence interval.

Figure 1.



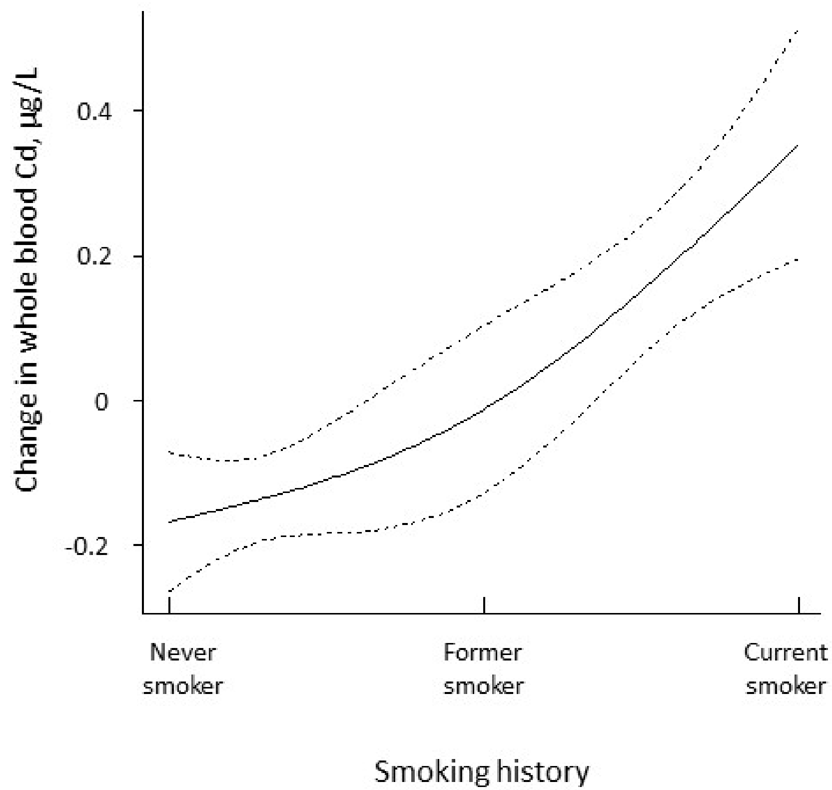
Change in whole blood Pb, µg/L
4
2
0
-2
-4
Change in whole blood Hg, µg/L
1.0
0.5
0
-0.5
-1.0
30
35
40
45
50
55
Age, years

Figure 2.



Change in whole blood Pb, µg/L
8
6
4
2
0
-2
≥ 1 per week
≥ 2 per month
1 per month
Rarely
Never
Alcohol intake

Figure 3.



Change in whole blood Cd, μg/L
0.4
0.2
0
-0.2
Never smoker
Former smoker
Current smoker
Smoking history

Table 1. Baseline characteristics in patients admitted for gastric bypass (n=46)	
Female gender, n (%)	39/46 (85)
Age, years, mean (SD)	43.9 (9.1)
Body mass index, mean (SD)	42.4 (3.6)
Education, years, mean (SD)	13 (3)
Full time occupation, n (%)	23/45 (51)
Married/cohabitant, n (%)	38/44 (86)
Daily intake of meat, grams, mean (SD)	164 (60)
Daily intake of fish/seafood, grams, mean (SD)	80 (49)
Daily smoker, n (%)	10/45 (22)
Alcohol intake ≥ 1 per month, n (%)	20/46 (43)
Current diagnosis of hypertension, n (%)	15/44 (34)
Current diagnosis of diabetes, n (%)	7/44 (16)

Table 2. Whole blood lead, mercury and cadmium concentrations before and 12 months after bariatric surgery (n=46)

Parameters	Before surgery N=46	12 months after surgery N=46	P value*
Whole blood lead, µg/L, median (IQR) Range	6.73 (4.13, 9.48) 1.58 – 17.78	11.66 (7.96, 14.91) 4.73 – 22.94	<0.001
Whole blood mercury, µg/L, median (IQR) Range	1.08 (0.72, 1.46) 0.38 – 3.97	0.74 (0.57, 1.31) 0.26 – 3.60	0.001
Whole blood cadmium, µg/L, median (IQR) Range	0.33 (0.21, 0.56) 0.12 – 1.96	0.24 (0.16, 0.58) 0.08 – 1.07	0.003

*Compared by Wilcoxon Signed Rank Test.

Table 3. Determinants of whole blood lead, mercury and cadmium before bariatric surgery (n=46) by multiple linear regression

Variables included in the model	Whole blood lead		Whole blood mercury		Whole blood cadmium	
	Beta	P	*Beta*	P	*Beta*	P
Gender[a]	-0.06	0.77	-0.43	0.03	0.04	0.80
Age, years	0.17	0.36	0.27	0.14	-0.05	0.74
Alcohol intake[b]	0.47	0.006	0.23	0.16	0.01	0.95
Smoking history [c]	0.04	0.80	-0.30	0.09	0.49	0.02
Intake of fish and seafood, grams/week	0.03	0.88	0.31	0.08	0.01	0.96
Education, years	0.31	0.05	-0.20	0.21	0.06	0.71
BMI at inclusion	-0.22	0.23	-0.15	0.41	-0.28	0.18

Weight reduction, after dieting, kg	0.21	0.25	0.09	0.62	0.05	0.80

Sex and the initial weight reduction after dieting were additionally included in the model

[a] Gender: Female, male

[b] Alcohol intake: Never, rarely, 1/month, ≥2/month and ≥1/week

[c] Smoking: Never smoker, former smoker and current smoker

Table 4. **Concentrations of whole blood lead, mercury and cadmium in different populations**

Parameters[a]	Obese Norwegian adults in 2012-14 Median (10, 90 percentile)				Norwegian women, 18-40 years in 2011-15 (n=158) Median (2.5, 97.5 percentile)	Finnish women, 31 years in 1997 (n=123) Geometrical mean (min, max)	Danish women, 18-40 years in 2011-12 (n=73) Geometrical mean (10, 95 percentile)	French adults, 20-59 years in 2008-10 (n=1992) Median (10, 90 percentile)
	All adults, 27-59 years (n=46)		Women, in reproductive age 27-45 years (n=21)					
	Before surgery	After surgery	Before surgery	After surgery				
Whole blood lead, µg/L	6.73 (2.58, 12.83)	11.66 (6.31, 18.46)	6.17 (2.10, 10.65)	9.53 (5.24, 15.19)	8.29 (4.14, 22.79)	9.06 (0.80, 91.9)	8.1 (5.3, 15.8)	18.3 (8.86, 38.7)
Whole blood mercury, µg/L	1.08 (0.50, 2.06)	0.74 (0.39, 1.87)	1.00 (0.41, 1.49)	0.64 (0.33, 1.44)	0.99 (0, 3.88)	1.85 (0.33, 11.00)	1.59 (0.69, 5.2)	1.64 (0.47, 3.91)
Whole blood cadmium, µg/L	0.33 (0.18, 1.11)	0.24 (0.11, 0.83)	0.33 (0.18, 1.37)	0.21 (0.16, 1.02)	0.15 (0.08 – 0.87)	0.12 (0.05 – 3.37)	0.2 (0.07, 0.76)	0.37 (0.17 – 1.22)
References	Mikalsen et al				Flotre et al	Abass et al.	Rosofsky et al	Nisse et al

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