Differential Fat Accumulation in Early Adulthood According to Adolescent-BMI and Heavy Metal Exposure

Larissa Betanzos-Robledo[a], Martha M. Téllez-Rojo[a], Hector Lamadrid-Figueroa[b], Ernesto Roldan-Valadez[cd], Karen E. Peterson[e], Erica C. Jansen[e], Nil Basu[f], Alejandra Cantoral[g]

[a] *CONACYT, National Institute of Public Health, Center for Nutrition and Health Research, Cuernavaca, Mexico.*

[b] *Department of Perinatal Health, Reproductive Health Directorate, Center for Population Health Research, National Institute of Public Health, Cuernavaca, México.*

[c] *Directorate of Clinical Research; Hospital General de Mexico "Dr. Eduardo Liceaga", Mexico City, Mexico.*

[d] *Department of Radiology, I.M. Sechenov First Moscow State Medical University (Sechenov University); Moscow, Russia.*

[e] *Department of Nutritional Sciences, University of Michigan, Ann Arbor, MI, USA.*

[f] *Department of Natural Resource Sciences, McGill University, Montreal, Quebec, Canada.*

[g] *Department of Health, Universidad Iberoamericana, Mexico City, MX, Mexico.*

***Corresponding author:** Larissa Betanzos-Robledo. National Council of Science and Technology, National Institute of Public Health, Mexico City, MX. Direction: Avenida Universidad No. 655, Colonia Santa María Ahuacatitlan, Cerrada Los Pinos y Caminera. Cuernavaca, Morelos, Mexico. **Email address:** investigador36@insp.mx **Phone:** (+52) 777-267-4880. **ORCID:** https://orcid.org/0000-0001-9076-0078

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The authors have no conflicts to disclose, and certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-

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Author's contributions:

All listed authors have read and contributed to the manuscript substantially and have accepted the final submitted version. LBR: analyzed and interpreted of data, drafted the work and writing original draft and edit final work. MMTR: definition, conceptualization, editing the work and resources. HLF: formal analysis, and visualization. ERV: analysis, visualization, and resources. KEP: definition, conceptualization, and editing the work. ECJ: investigation, writing original draft, and edit final work. NB validation analysis and editing

Abstract

Introduction: Heavy metals such as Lead (Pb) and Mercury (Hg) can affect adipose tissue mass and function. Considering the high prevalence of exposure to heavy metals and obesity in Mexico, we aim to examine if exposure to Pb and Hg in adolescence can modify how fat is accumulated in early adulthood.

Methods: This study included 100 participants from the ELEMENT cohort in Mexico. Adolescent Pb and Hg blood levels were determined at 14-16 years. Age- and sex-specific adolescent BMI Z-scores were calculated. At early adulthood (21-22 years), fat accumulation measurements were performed (abdominal, subcutaneous, visceral, hepatic, and pancreatic fat). Linear regression models with an interaction between 7adolescent BMI Z-score and Pb

or Hg levels were run for each adulthood fat accumulation outcome with normal BMI as reference.

Results: In adolescents with obesity compared to normal BMI, as Pb exposure increased, subcutaneous (p-interaction= 0.088) and visceral (p-interaction <0.0001) fat accumulation increases. Meanwhile, Hg was associated with subcutaneous (p-interaction= 0.027) and abdominal (p-interaction= 0.022) fat deposition among adolescents with obesity.

Conclusions: Heavy metal exposure in adolescence may alter how fat is accumulated in later periods of life.

Key words: *heavy metals, obesogens, fat distribution, lead, mercury*

Introduction:

Heavy metals such as lead (Pb) and Mercury (Hg) are elements with a relatively high atomic weight and density compared to water [1]. These metals are considered priority metals of public health significance because of their degree of toxicity[2]. Heavy metals are cataloged as systemic toxicants since they induce multiple organ damage, even at lower levels of exposure, through their ability to accumulate in vital organs [3]. Also, they are widely dispersed in the environment, through soil, water, air, dust, human food, and manufacturing products, so people could be exposed to a myriad of metals throughout their lifetime [4].

The effects of heavy metals on children´s health have been extensively studied [5, 6]. Among the most recognized damages, Pb exposure has been associated with neurological effects such decreased neurological function, behavioral disturbance, and alterations in neuromotor and neurosensory function in children. Recently, Pb exposure has been associated with shorter sleep duration in adolescents [7] and with hepatic steatosis and hepatocellular damage in young Mexicans [8]. Similar to Pb, Hg exposure has also been associated with neurodevelopmental effects [9].

In Mexico, the prevalence of children with higher blood lead levels (≥5 μg/dL) is 21.8% [10], far surpassing those reported in other countries, such United States where the prevalence of intoxication in 2012 was 2.5% [11]. Until now the principal non-occupational source of exposure to Pb in the general Mexican population is cooking or storing foods in lead-glazed ceramics [10, 12]. On the other hand, although Hg is a global contaminant of concern [13],

though little is known about exposures in the Mexican population. Though it is unclear the burden of disease imposed by Hg in Mexico, a study in Mexico City reported widespread exposures in children with a mean of blood Hg level of 1.8 ± 1.7 μg/L, with seafood considered the principal source of exposure [14].

Additionally to these effects, recently Pb and Hg have been considered obesogens since they have adipotropic effects in the adipose tissue [15]. In vivo and in vitro studies demonstrated that heavy metals can affect adipose tissue mass and function through modulation of adipogenesis via peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT/enhancer-binding protein (C/EBP) expression [16]. However, the effects of heavy metals on adipogenesis are biphasic and dose-dependent, varying from an increased adipogenesis at lower-dose exposure [17–21] to the inhibition of adipose tissue differentiation at higher doses, observed as an "anti-obesogenic" effect [22]. This anti-obesogenic effect has been demonstrated especially for Hg via the reduction of adipocyte size [22, 23], adipokine secretion, and the activation of apoptosis through induction of oxidative stress [24] and regulation of adipogenesis-related genes [25].

Aforementioned observations are important because there is evidence that early stages of life such as fetal life, infancy, childhood and adolescence are critical windows of time where environmental exposures can have a long-term phenotypic effect [26, 27] . Therefore, the weight gain in these stages could be an important predictive of later obesity risk [28]. Additionally, adolescence is one of the life periods where fat deposition peaks [27], a time of rapid growth where significant changes in the amount and distribution of adipose tissue occur [29]. It has been demonstrated that adolescents with obesity are 5 times more likely to become obese adults compared to adolescents without obesity [28].

Multiple environmental factors can affect obesity susceptibility and body fat accumulation [30]. Obesity is currently one of the leading public health problems since it is a major contributor to the global burden of chronic diseases including cardiovascular disease, non-alcoholic fatty liver disease, type 2 diabetes mellitus, and certain types of cancer [31]. In Mexico, all age groups are affected by obesity, with the prevalence in children and adolescents being one of the highest worldwide (17.5% and 14.6%,respectively) [32]. In addition, body fat distribution is a strong metabolic and cardiovascular risk factor [30]. Not all forms of fat distribution possess the same health risks and may have different physiological influences and pathological implications. Particularly, studies have shown that

visceral fat mass is associated with cardiometabolic risk, compared with other fat depots [33]. On the other hand, subcutaneous fat deposition has been associated with insulin resistance [34], and abdominal fat has been linked with changes in lipid profile, increased blood pressure and hyperinsulinemia [35]. According to the last National Health and Nutrition Survey in Mexico (ENSANUT, 2018), the prevalence of adults with obesity is 36.1% and the prevalence of abdominal obesity was 81.6% in all adults[36]. Hepatic and pancreatic fat are associated with the development of hepatic and pancreatic steatosis, respectively. These fat stores increase the risk of cirrhosis and hepatocellular carcinoma [37, 38]; chronic pancreatitis, pancreatic cancer and insulin resistance [39, 40].

Considering the high prevalence of exposure to heavy metals and obesity in Mexico, it will be important to understand the effects of this environmental exposure during the adolescent developmental period on adipose tissue mass [41]. In addition, we aim to explore how these exposures could interact with the BMI status on the distribution of fat accumulation in a future stage of life. Therefore, the objective of the present study was to examine if exposure to heavy metals (Pb and Hg) has a modification effect on the association of adolescent BMI status and fat accumulation during early adulthood.

Methods:

Study population

The present analysis includes a subsample from the first cohort of the Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) [42]. The cohort began in 1994 with the recruitment of 631 mother-child pairs from three maternity hospitals in Mexico City, Mexico. Details of the cohort are described elsewhere [43]. Then, during 2008 and 2011, a second follow-up of the cohort was implemented when the participants were between 14 and 16 years of age (n = 206). Finally, during 2016 and 2017, when subjects were between 21 and 22 years of age, they were invited to participate in a third follow-up of 100 subjects (Figure 1). Comparison of the original cohort and the analytical sample has been described elsewhere [44]; no significant differences were found except for the duration of maternal education (Supplementary Table 1). The analysis of the present study was restricted to 92 participants who had available data of blood Pb levels and 79 participants who had available data on blood Hg levels during adolescence (2008-2011), and measurements of fat accumulation at

early adulthood (waist circumference, visceral and subcutaneous fat, hepatic and pancreatic fat fraction).

The Ethics, Biosafety, and Research Committees of the National Institute of Public Health, Mexico approved the study (approval number 1377). Participants provided written informed consent at each wave of the study. No incentives were given for the participation of the subjects, except that the result of the Magnetic Resonance Image (MRI) were delivered to the participants.

Outcomes: Fat accumulation assessment in early adulthood

At the early adulthood follow-up, measurements of different fat accumulation were performed:

Abdominal fat: Abdominal fat was determined by waist circumference (WC) measured twice to the nearest 0.1 cm with a SECA (model 201, Hamburg, Germany) measuring tape within the facilities of the research center. Elevated WC (abdominal obesity) was defined using the International Diabetes Federation (IDF) criteria for adults (men ≥94 cm, women ≥80 cm [45].

In order to estimate subcutaneous, visceral, hepatic and pancreatic fat, Magnetic Resonance Imaging (MRI) was carried out using a Philips Achieva 3.0 T MR-scanner (Achieva 3.0, Philips Healthcare, Best, The Netherlands). A certified radiologist and biomedical engineer who were "blinded" to each participant's BMI status and metal exposure implemented the MRI. A detailed description of imaging and post-processing procedures have been published elsewhere [46–48].

Subcutaneous and visceral fat: Subcutaneous adipose tissue area (SAA) and Visceral adipose tissue area (VAA) were quantified using abdominal MRI images with T1 and T2 sequences in the axial plane acquired. VAA was measured at the level of the L2-L3, L3-L4 vertebral superior endplate at the umbilicus level, with three slices obtained superior, and one slice inferior at 40 mm intervals (16 cm window); VAA was distinguished from SAA based on the abdominal wall muscle layer. Visceral and subcutaneous fat areas (cm^2) were measured separately, and images were analyzed using the Analize v14.0 software

(AnalyzeDirect Inc., Kansas, USA). Measurements of volumes from these regions have been recently reported [49].

Hepatic fat: Hepatic fat was determined by the quantification of fatty liver (% of hepatic triglyceride content) by MRI, a validated methodology considered the most accurate non-invasive method for measuring [50]. In order to estimate hepatic triglyceride content, we estimated proton density fat fraction (PDFF). We defined the presence of steatosis using the American Association for the Study of Liver Diseases (AASLD) cut-off point of 5% (hepatic triglyceride level of 55.6 mg/g) corresponding to the 95th percentile of the distribution of liver fat in healthy subjects [38].

Pancreatic fat: Pancreatic fat was determined by the estimation of pancreatic triglyceride content by MRI scanner, a validated methodology considered the most accurate non-invasive method for measuring [50]. We estimated proton density fat fraction (PDFF) and we defined the presence of pancreatic steatosis using cut-off point of 6.2% pancreatic fat [40].

Exposure: Adolescence-BMI Z-score

At the adolescent period (14-16 years of age), trained personnel performed the anthropometric measurements in duplicate. Weight and height were measured using a Tanita digital scale (Tanita Co. Tokyo, Japan) with a height rod (model WB-3000m). Weight and height were recorded to the nearest 0.1 kg and 0.5 cm, respectively. The observed values were averaged. BMI was calculated as weight in kg divided by height in meters squared (kg/m^2). To provide comparable indices with other international studies of weight status, age- and sex-specific BMI Z-scores were calculated using the 2007 World Health Organization (WHO) reference growth standard; BMI status was defined as underweight (<-2SD), normal (-2SD to +1SD), overweight (>+1SD to <+2SD) and obesity (>+2SD)
[51].

Effect modification variable: Adolescence heavy metal exposure

Adolescent whole blood samples were collected (at 14-16 years of age) into vials certified for trace metals analysis and stored at 4°C until analysis, using standardized protocols and after at

least 8 h of fasting. Pb were quantified using graphic-furnace atomic-absorption spectroscopy (model 3000; PerkinElmer, Chelmsford, Massachusetts, USA) at the research facilities of the American British Cowdray Hospital in Mexico City. All blood Pb samples were above the limit of detection and the precision of this instrument was within ±1 μg/dL [52]. Analytical measurement of total blood Hg content was carried out using a Direct Mercury Analyzer 80 (DMA-80, Milestone Inc., CT) as previously described [53]. The analytical detection limit was less than 1 μg/L per measure. Quality control measures included daily instrument calibration, procedural blanks, replicates, and several certified reference materials.

Other variables used in the analyses were collected from the cohort´s historical records such as child´s sex and mother´s education. Socioeconomic status (SES) was reported by the mothers at the recruitment through a validated questionnaire consisting of thirteen questions [54].

Statistical analysis:

First, bivariate correlations were used to test relationships among the interest variables. We conducted a descriptive analysis of the main characteristics of the study sample. The categorical variables are expressed as frequencies and proportions, and the continuous variables are presented as means or medians, according to their distribution. We estimated statistical differences between the general characteristics of the subjects by Adolescence-BMI Z-score status using Chi-square or Fisher's exact tests for the categorical variables; and ANOVA or Kruskal Wallis test for the continuous variables depending on their distribution. As Pb and Hg had a skewed distribution to the right, both were log-transformed.

The different fat accumulation at early adulthood by adolescent BMI Z-score statuses was assessed running a linear model to estimate the p-trend from the different BMI status. Additionally, we generated linear regression models with an interaction between BMI Z-score status (underweight, normal, overweight and obesity) and Pb or Hg levels (log Pb and log Hg levels) for each adulthood outcome (abdominal, subcutaneous, visceral, hepatic and pancreatic fat). Normal BMI status was considered as reference. Assumptions were tested after running the models (data not shown).

We considered a significance level $p<0.05$ for the tests and regressions and $p<0.1$ for the interaction term. All the analyses were performed in STATA 15 statistical software (StataCorp LLC, College Station, TX, USA).

Results:

General characteristics of the participants are described in Table 1. Of the total sample, 54% were male; the mean age at adolescence was 14 years old. The majority were low Socio Economic Status (SES) (52%), and 42% had mothers with a high school education level. The median of Pb levels was 3.1 µg/dL and 1.3 µg/L for Hg blood levels. The percentage of adolescents with higher blood Pb levels (≥5 µg/dL established as the current Mexican reference level in children) [55] was 13%. In addition, the percentage of adolescents with higher blood Hg levels (≥2 µg/L) [56] was 21%. Among 100 adolescents, 6% had underweight, 46% normal weight, 37% overweight, and 11% obesity according to the BMI Z-score cut points; male adolescents had higher overweight and obesity percentages. Measurements of blood Pb and Hg levels were not statistically significantly correlated. No correlations were found between BMI Z-score and heavy metals levels; in addition none of the fat deposition variables were correlated with Pb and Hg levels (Table 2).

Table 3 presents the mean values of variables measured for fat accumulation at early adulthood by adolescent BMI Z-score status. As expected, all means increased as BMI status changed from underweight/normal/overweight/obesity (p-trend = <0.05). When we introduced the interaction term (BMI status x log Pb or log Hg) into the model, we observed that adolescent BMI status modifies the association between adolescent heavy metal exposure and fat accumulation in early adulthood. In those who had obesity during adolescence, there was a different pattern of subcutaneous (p-interaction= 0.088) and visceral (p-interaction= <0.0001) fat accumulation, such that as Pb increased there was higher fat accumulation, whereas there was a null association in the normal BMI category (Supplementary Table 2 and Figure 2). Similarly, in the case of Hg, the accumulation pattern of abdominal (p-interaction= 0.022) and subcutaneous (p-interaction= 0.027) fat was different in those with obesity during adolescence, compared to the normal BMI category (Supplementary Table 3). Additionally, we observed (Figure 3) that in those who presented overweight during adolescence; there was an opposite pattern for all types of fat accumulation (this can be mainly observed in visceral, hepatic and pancreatic fat), such that as blood Hg levels increased, there was a lower fat accumulation. However, although these results are consistent, they are not statistically significant. Figure 2 and Figure 3 show the different early adulthood patterns of fat

accumulation (abdominal, subcutaneous, visceral, hepatic and pancreatic fat) by adolescent BMI as Pb or Hg levels increased.

Discussion:

We documented in this study population, which was nested in a cohort study, that heavy metal exposure in adolescence is associated with the way in which fat is accumulated within the body in later periods of life (early adulthood). Specifically, we observed that in adolescents with obesity, as Pb (p-interaction= 0.088) or Hg (p-interaction= 0.027) exposure increase, subcutaneous fat deposition increases, whereas there is no association in those with normal BMI. In the case of Pb, visceral fat (p-interaction= 0.000) also increases for those with obesity in adolescence; meanwhile, the relationship with Hg is also observed in the abdominal fat (p-interaction= 0.022). We did not observe any interaction effect in the hepatic or pancreatic fat accumulation. Considering the high prevalence of exposure to heavy metals and obesity in Mexico [27, 41], these results highlight that heavy metal exposures may increase, and modify the way that fat is accumulated within the body. In particular, the effects were more striking for visceral fat which is associated with increased cardiometabolic risk, compared with other fat depots [33].

The median Pb levels in our study were 3.1 µg /dL (IQR 2.2 - 4.5), higher than the current U.S. average of 1.2 ug/dL. Our results suggest that the Pb exposure differentially affects subcutaneous and visceral fat in obese adolescents. These results are consistent with previous epidemiological studies. A cross sectional study in 5558 adults reports a positive association between Pb blood levels with BMI and obesity in women [57]. In another birth cohort study in 442 mother-child pairs of US urban population, findings suggest that maternal elevated Pb exposure was associated with increased risk of intergenerational child body mass index [58]. However, in another birth cohort study with 299 young children, Pb blood levels were associated with smaller BMI at ages 2–3 years [59]. It is important to highlight to the best of our knowledge, no previous study has investigated the associations of Pb exposure with subcutaneous and visceral obesity.

On the other hand, the median Hg levels in this sample was 1.3 µg /L (IQR 0.9 - 2.1), which is lower than the last adolescents' geometric mean reported in the Korean National Health and Nutrition Examination Survey (KNHANES) of 1.93 µg/L [60], but higher than those reported for US adolescents of 0.73 µg/L (SD 0.91) [61]. In the case of Hg, our results suggest that the exposure affects abdominal and subcutaneous fat in obese adolescents. In support, a previous cross-sectional study with elderly Koreans living in coastal areas reported a positive association between blood mercury and waist-to-hip ratio [62]. However, in the KNHANES, blood Hg was positively associated with visceral adiposity but negatively associated with body fat percentage [18, 63]. According to the WHO data on background Hg levels in the general population are <5 ug/L[64]. However, the recent focus on the health impact of exposure to Hg is more on chronic, low or moderate grade exposure for the danger and effects of low grade Hg exposure [56].

To the best of our knowledge, this study is the first to assess if exposure to Pb and Hg in adolescence can modify how fat is accumulated in adulthood. While the underlying mechanisms are still not well understood, there is biological plausibility for heavy metals in obesity pathogenesis. There are a few mechanisms studied by in vivo and in vitro studies that may underlie the association between Pb exposure and the differential fat accumulation. It is known that Pb effectively accumulates in human adipose tissue [65]; in vivo and in vitro studies have demonstrated that Pb exposure resulted in a significant increase in bone marrow adiposity characterized by increased adipocyte size and number through upregulation of PPARγ gene expression [66] . The effects of mercury exposure on the adiposity have also been demonstrated. I*n vitro* studies have shown the impact of Hg exposure in adipokine secretion through the formation of a lower number of adipocytes and clumped lipid droplets as well as activation of apoptosis through induction of oxidative stress and systemic inflammation [24]. In addition, mercury exposure causes disturbances in carbohydrate and lipid metabolism. These effects, caused by mercury exposure, could lead to an increased risk of the development of obesity-related metabolic disorders [60, 67]. We hypothesize that the mechanism of interaction observed in this study, which was mainly restricted to obese adolescents, is due to the obesity-induced changes in adipose tissue microenvironment. Adipocyte hypertrophy leads to cell death, which contributes to increased production of proinflammatory cytokines, the development of chronic low-grade inflammation, metabolic dysfunction and oxidative stress [68, 69]. Such physiological changes may render adipocytes more vulnerable to subsequent chemical exposures, such as Pb or Hg.

It is important to mention that we observed a different pattern from what we expected to find in overweight adolescents, such that as Hg exposure increases, all fat type of deposition decrease; however, these results are not statistically significant but are consistent. This pattern does not necessarily imply that Hg protects against fat accumulation among overweight adolescents, though is consistent with what has been described by Tinkov A. et al, [REFERENCE # HERE] as a biphasic adipogenic response to heavy metal exposure. They postulated that with low-level exposures to heavy metals, individuals may up-regulate key adipogenic factors, such as PPARγ, thus promoting excessive adipogenesis and contributing to obesity. With higher exposures to metals, adipogenesis may be inhibited through the

down-regulation of C/EBPs and PPARγ, and these may be associated with ultimate toxic effects of the metals because of pro-inflammatory and pro-oxidant activities [15].

Some limitations of our study mainly related to our limited sample size should be mentioned. Fist, the modest sample size could have decreased the precision of our associations and limited statistical power. Nevertheless, we were able to detect statistically significant associations that were consistent across the different fat accumulation biomarkers we used and consistent with findings from other studies. Second, we decided to raise P value for interaction to $p<0.1$, therefore results from this study should be interpreted with caution. Third, we were not able to perform stratified analysis by sex. Considering that this variable is a determinant in the way fat is stored during adolescence [27, 41], we recommend that this work be a starting point to future analyses in larger analytic samples. An important strength of the present study includes that we used multiple biomarkers of fat accumulation. Not all forms of fat distribution possess the same health risks; for example, higher concentrations of visceral relative to subcutaneous adipose tissue are associated with greater metabolic risks [33]. Childhood and adolescent obesity are strongly correlated with adult obesity; however, our results demonstrate how the increased exposure to heavy metals may increase and can modify the way that fat is accumulated within the body in later periods of life. Additionally, our study sample in Mexico City represents a particularly vulnerable segment of the population not well-represented in the literature.

Many obesogens appear to induce a variety of effects and, therefore, may be acting through multiple mechanisms [16]. However, despite the evidence, obesogens exposure (including heavy metals) are not commonly included as an obesity prevention intervention [70]. Thus, there is an urgent need to understand how these environmental factors may be determinants contributing to obesity and the capacity for fat deposition to best implement effective prevention and therapeutic approaches [16]. In any case, early detection of heavy metal exposures in critical stages of life, policy regulations that limit the production and release of heavy metals into the environment, as well as an epidemiological surveillance system may aid in the development of preventive public health obesity strategies.

Conclusion:

These findings may be indicative of an important role of heavy metal exposure in alterations of energy homeostasis and excessive adiposity. Quantifying the impact of exposure to metals is crucial for identifying risk factors with environmental origins for obesity and its comorbidities and developing more targeted public health interventions.

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Table 1. Characteristics of participants by age- and sex-specific adolescent BMI Z-score status during adolescence (n=100)

		Adolescent BMI Z-score[a]				
	Overall	Underweight	Normal	Overweight	Obesity	
	(n=100)	(n=6)	(n=46)	(n=37)	(n=11)	p-value
Age (years), mean (SD)	13.7 (0.6)	13.7 (0.5)	13.7 (0.6)	13.6 (0.6)	13.5 (0.5)	0.8787
Sex, n (%)						0.3000
Male	54 (54.0)	5 (83.3)	21 (46.7)	21 (56.7)	7 (63.3)	
Female	46 (46.0)	1 (16.7)	25 (54.3)	16 (43.2)	4 (36.4)	
Maternal education, n (%)						0.1350
<8 years (secondary or primary)	24 (24.0)	1 (16.7)	16 (34.8)	7 (18.9)	1 (9.1)	
9-11 years (some high school)	42 (42.0)	1 (16.7)	22 (47.8)	14 (37.8)	5 (45.5)	
12 years (completed high school)	25 (25.0)	4 (66.6)	6 (13.0)	11 (29.7)	4 (36.4)	
>12 years (more than high school)	9 (9.0)	0 (0)	3 (4.4)	5 (13.5)	1 (9.1)	
Socioeconomic status						0.5760
Low	52 (52.0)	2 (33.3)	22 (47.8)	21 (56.7)	7 (63.6)	
Medium	48 (48.0)	4 (66.6)	24 (51.2)	16 (43.3)	4 (36.4)	
Pb blood levels, median (IQR)	3.1 (2.2-4.5)	3.3 (2.6-5.1)	3.0 (2.0-4.8)	3.2 (2.3-3.9)	4.2 (2.3-4.9)	0.6199
% ≥5 µg/dL (n=92, %)	13 (14.4)	1 (16.6)	7 (15.0)	4 (10.9)	1 (9.0)	0.6250
Hg blood levels, median (IQR)	1.3 (0.9-2.1)	1.2 (1.1-1.3)	1.3 (0.9-1.9)	1.4 (0.9-2.2)	1.3 (1.0-2.3)	0.9824
% ≥2 µg/L (n=79, %)	21 (26.7)	1 (16.6)	10 (21.7)	7 (18.9)	3 (27.3)	0.9490

Statistical significance was assessed using ANOVA or Kruskal Wallis tests for continuous variables and Chi2 or Fisher's Exact tests for the categorical variables according to their distribution.

Abbreviations: BMI: Body Mass Index; Pb: lead; Hg: Mercury; SD: Standard Deviation; IQR: Interquartile Range

[a] Age- and sex-specific BMI Z-scores were calculated using the 2007 World Health Organization (WHO) reference growth standard; BMI status was defined as underweight (<-2SD), normal (-2SD to +1SD), overweight (>+1SD to <+2SD) and obesity (>+2SD).

Table 2. Bivariate correlation matrix

	1	2	3	4	5	6	7	8
1 Lead	1.0000							
2 Mercury	-0.0418	1.0000						
3 BMI Z-score	0.0418	0.0088	1.0000					
4 Abdominal fat	0.0508	-0.0513	0.6094*	1.0000				
5 Subcutaneous fat	0.0402	-0.0793	0.5148*	0.8554*	1.0000			
6 Visceral fat	-0.0005	-0.1560	0.3683*	0.7002*	0.6633*	1.0000		
7 Hepatic fat	0.0995	-0.0618	0.2865*	0.5252*	0.4604*	0.5112*	1.0000	
8 Pancreatic fat	-0.1633	-0.0143	0.1922	0.3180*	0.2849*	0.3076*	0.5443*	1.0000

Note: Spearman´s rho
*p < 0.05

Table 3. Descriptive statistics (Mean (SD) and Median (IQR) of fat accumulation in early adulthood by adolescent BMI Z-score status (n=100)

	Fat accumulation at early adulthood				
	Abdominal (cm) mean (SD)	Subcutaneous (cc) median (IQR)	Visceral (cc) median (IQR)	Hepatic (%) median (IQR)	Pancreatic (%) median (IQR)
Overall	88.5 (12.1)	228 (176-317)	47.1 (32-66.4)	1.4 (0.7-3.7)	3.1 (1.7-5.8)
Adolescent BMI Z-score					
Underweight	76.2 (12.5)	111 (45-224)	35.4 (12.2-50.0)	0.9 (0.7-3.6)	1.8 (1.6-8.0)
Normal	82.7 (7.7)	189 (154-252)	39.4 (27.7-53.8)	1.2 (0.6-1.7)	2.8 (1.7-4.7)
Overweight	93.3 (8.9)	258 (214-329)	52.5(44.0-76.0)	1.9 (0.8-3.7)	5.6 (1.9-13.8)
Obesity	103.4 (15.9)	381.5 (195-539)	64.5 (34.3-90.7)	5.6 (1.9-13.8)	5.3 (2.6-9.6)
*p-trend**	0.000	0.000	0.015	0.005	0.052

*Statistical significance was assessed using linear regression models.
Abbreviations: BMI: Body Mass Index; Pb: lead; Hg: Mercury; SD: Standard Deviation; IQR: Interquartile Range

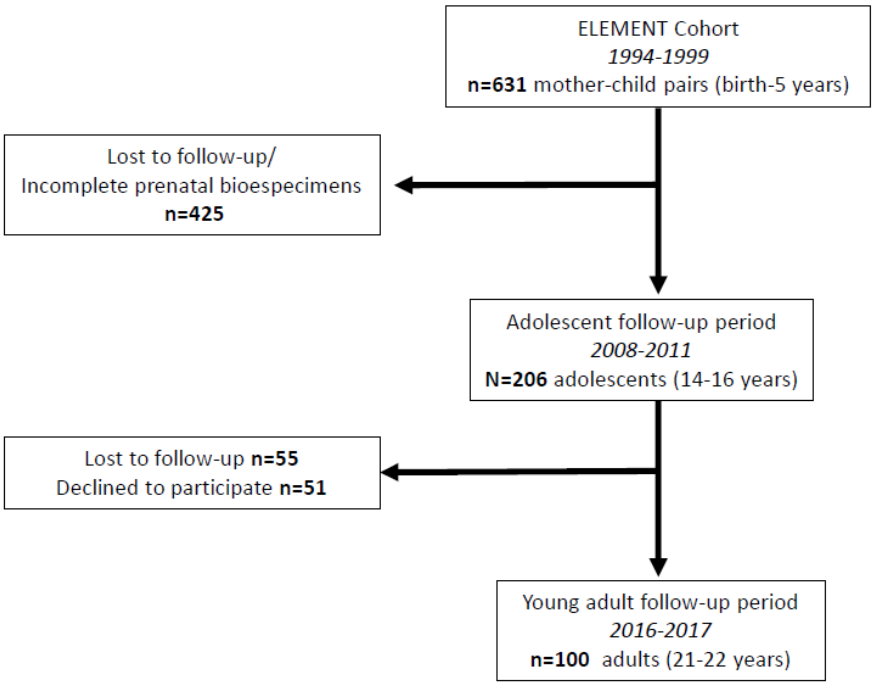

Figure 1. Flowchart of the study population

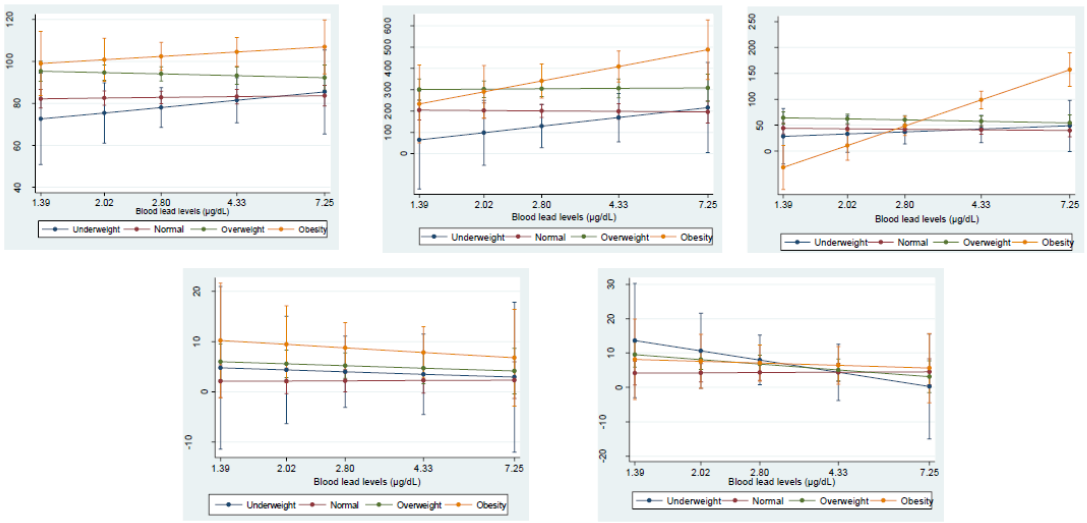

Figure 2. *Early* adulthood fat accumulation by adolescent BMI category and blood lead levels. The β estimates, 95% CIs and p-values were determined using linear regression models of blood lead levels (log transformed) in adolescence and fat accumulation in early adulthood with the effect modification of adolescent BMI status. Normal BMI z-score status was the reference. P-value <0.1 for interaction were found with subcutaneous and visceral fat. Blood lead levels were run as logarithmic values, in the graphic the values are presented as their normal measurement value (µg/L).

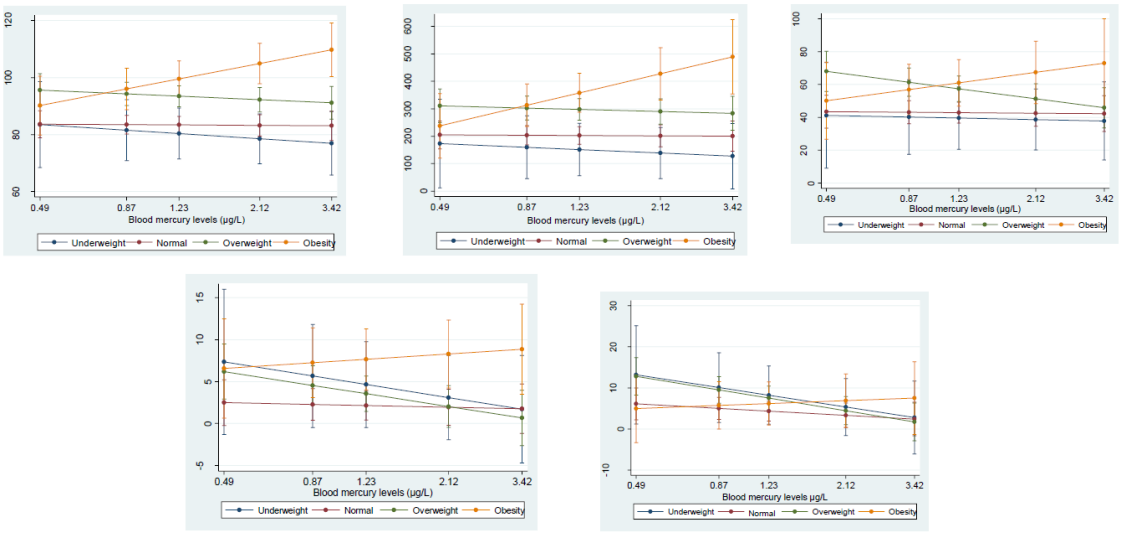

Figure 3. Early adulthood fat accumulation by adolescent BMI category and blood mercury levels. The β estimates, 95% CIs and p-values were determined using linear regression models of blood mercury levels (log transformed) in adolescence and fat accumulation in early adulthood with the effect modification of adolescent BMI status. Normal BMI z-score status was the reference. P-value <0.1 for interaction were found with abdominal and subcutaneous fat. Blood mercury levels were run as logarithmic values, in the graphic the values are presented as their normal measurement value (µg/L).