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The association between whole blood mercury and the risk of developing CVD among the Greenlandic population

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Preface

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Abstract

Introduction: The World Health Organization, estimated that 17.5 million people died from cardiovascular disease (CVD) in 2012 alone, accounting for 30% of global deaths and serving as the number one cause of death globally. Environmental pollutants, such as heavy metals (e.g. mercury) and persistent organic pollutants (POPs) (e.g. polychlorinated biphenyl (PCBs), may contribute to the burden of CVD, especially within the Arctic. The highest levels of whole blood mercury have been found in Greenland.

Objective: To explore the association between whole blood mercury and the risk of developing CVD among the Greenlandic population.

Methods: The continuous effects of whole blood mercury levels of incident CVD were investigated, among 3083 participants, from the population-based cohort study '*Inuit Health in Transition Greenland survey 2005-2010*' using cox regression. Both univariate and multivariate analyses were conducted, adjusting for a range of potential confounders. Whole blood mercury was measured at inclusion. Participants were followed in the National Patient registries for Denmark and Greenland and in the cause of death register for CVD events. The overall incidence rates and the hazard ratio of CVD events among participants for overall CVD were calculated. Potential interactions with sex were also investigated.

Results: The highest levels of whole blood mercury were found in men, who had a significantly higher median level of 19 μg/L, compared with women (15 μg/L ($p<0.001$)). The crude hazard ratio (HR) for developing overall CVD was 0.99 (95% CI 0.99-1.00) for any given level of whole blood mercury. After adjusting for several potential confounders, the HR remained 0.99 (95% CI 0.98-1.00).

Conclusion: The present study found no association between whole blood mercury and the risk of developing CVD among the Greenlandic population. Furture research on mercury should investigate genetic susceptibility of mercury among the Greenlandic population, as susceptibility to mercury, may be increased by genetic factors.

List of Abbreviations

AMAP	Arctic Monitoring and Assessment Programme
ALA	Alpha Lipoic Acid
BMI	Body Mass Index
BP	Blood Pressure
CHD	Coronary Heart Disease
CVD	Cardiovascular disease
GHDR	Greenlandic Hospital Discharge Register
GNPR	Greenland National Patient Register
HbA_{1c}	Glycated Haemoglobin A_{1c}
HDL	High-density lipoprotein
HR	Hazard Ratio
ICD-10	International Classification Diseases - tenth edition
IHD	Ischemic Heart Disease
IHIT	Inuit Health in Transition
IPAQ	International Physical Activity Questionnaire
IPAQ-L	International Physical Activity Questionnaire – Long version
LDL	Low-density Lipoprotein
MET	Metabolic Equivalents
NAC	N-Acetyl Cysteine
NPR	The Danish National Patient Register
OGTT	Oral Glucose Tolerance Test
PAEE	Physical Activity Energy Expenditure
PCB	Polychlorinated biphenyl
POPs	Persistent Organic Pollutants
RMR	Resting Metabolic Rate
WC	Waist Circumference
WHR	Waist-to Hip ratio
WHO	World Health Organization

1.0 Introduction:

Cardiovascular diseases:

Cardiovascular disease (CVD) is a non-communicable disease that encompasses a group of disorders of the heart and blood vessels including coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, and pulmonary embolism (1). The World Health Organization (WHO), estimates that 17.5 million people died from CVD in 2012 alone, accounting for 30% of global deaths and serving as the number one cause of death globally (2).

Important modifiable risk factors for CVD include unhealthy diet, overweight/obesity, elevated blood glucose, physical inactivity, smoking and excess alcohol consumption. Underlying determinants of CVD also include social, economic and cultural change, as well as poverty, stress and hereditary factors.

Environmental pollutants, such as heavy metals (e.g. mercury) and persistent organic pollutants (POPs) (e.g. polychlorinated biphenyl (PCBs), may contribute to the burden of CVD directly or indirectly via promotion of these and other risk factors and associated diseases. Considering the significant burden that CVD have on global mortality, it is imperative to explore and assess factors that may promote or exacerbate the pathogenesis of these diseases (2). Recently, more emphasis has been placed on the potential contribution of environmental factors on the risk of developing CVD. WHO recognized that deaths from CVD could be prevented by reducing or removing exposure to environmental pollutants such as heavy metals (2, 3).

Mercury in the Arctic:

Traditionally, it has been thought that the Inuit population, were protected against CVD through the traditional diet, mainly comprising fish and sea mammals, such as seals and whales. The diet containing of a high proportion of omega-3-fatty acids, proteins vitamins and minerals lead to the hypothesis that the Inuit were protected against ischemic heart disease (IHD) and CHD. In addition to the beneficial effects of the traditional diet, the diet also contributes to high intakes of lipophilic organic pollutants and mercury, which is reflected in blood levels.

The adverse health effects of the contaminants and the beneficial effects of n-3 fatty acids from the traditional Inuit diet, have led to the phenomenon known as ‘The Arctic Dilemma’ (4-8).

In 1991, the Arctic Monitoring and Assessment Programme (AMAP) was established to monitor identified pollutants and their effect on the Arctic ecosystem. The main objective of AMAP is to investigate the potential effects of contaminants on the health of the Arctic populations. AMAP has assisted in the development, establishment, continuity and evaluation of protocols for heavy metals and POPs for the United Nations Economic Commission for Europe and the Stockholm Convention on POPs.
AMAP has in Greenland and other Arctic countries, systematically monitored whole blood mercury levels, and within the Arctic, the highest levels of whole blood mercury have been found in Greenland (9-12).

Mercury:

Mercury is a globally pollutant, which has been ranked as the third most toxic environmental heavy metal after arsenic and lead (13, 14). It has been used for a variety of products including cosmetics and medical products; among others it has been used as a diuretic, antiseptic and as a treatment for syphilis, and has also been used as a poison (15, 16).
Mercury is a heavy metal highly toxic to humans (17), released into the environment through natural sources such as volcanoes, geothermal outgassing activities, and earthquakes or through anthropogenic sources as industrial point sources, chlor-alkali plants and coal-fired plants. Mercury exists in three different forms: Elemental mercury, Inorganic mercury and Organic mercury.
Elemental mercury is a liquid, but forms into vapour, when heated above room temperature. Elemental mercury oxidises to inorganic mercury in the air and settles in lakes, rivers or bays. Further inorganic mercury accumulates to organic mercury and is accumulated through the food chain through plankton, fish and large predators. Thus, populations with high intake of seafood are likely to be exposed to high levels of mercury, which has been reported to harm brain, lungs, kidneys and the heart (15-17). The harmful effects of exposure of mercury on human health were first discovered after accidents in Japan and Iraq. In the Japanese village of Minamata Bay in1953, industrial waste containing methylmercury was deposited in the bay. Mercury accumulated within the food chain, from plankton to fish and was ingested by the inhabitants. The inhabitants experienced neurological symptoms such as tremor, speech impairment, blindness, including occurrence of mental retardation, coma and death. Infants, whose mothers were affected by the deposit of industrial waste containing mercury, developed mental retardation, peripheral neuropathy and cerebral palsy (18). The potential association between mercury and CVD, was only discovered later after industrial workers in

the former Soviet Union exposed to prolonged mercury vapor, experienced an increase in the number of cardiac diseases with frequent hypertension (19).

The Mechanism of mercury:

The mechanism in which mercury affects the cardiovascular system is not fully clarified, but seems to involve mitochondrial dysfunction and an increase in oxidative stress (18, 20).
The mitochondrial dysfunction occurs because of an interaction of coenzymes, resulting in a depolarization and auto oxidation of the inner mitochondrial membrane with lipid peroxidation and severe mitochondrial dysfunction. The dysfunction increases the oxidant stress and decrease the oxidant defense (20).
Mercury has a high affinity for sulfhydryl groups, N-acetyl cysteine (NAC) and alpha lipoic acid (ALA). The Sulfhydryl group is one of the most reactive and universal molecules, and are involved in the cell membrane structure and function.
The interaction of organic mercury compounds with reactive sulfhydryl's results in changes in specific transport and permeability systems.
NAC and ALA are important for glutathione, which is the most potent intracellular antioxidant and protects against oxidative stress, inflammation and cardiovascular disease.
Selenium is a necessary cofactor for glutathione peroxidase activity, to break down hydrogen peroxides and other various toxic peroxidations, but mercury reduces the selenium availability, thus increasing the risk of CVD.
Mercury has shown to increase the oxidation of low-density lipoprotein (LDL), which is an important marker for the risk of developing CVD, and to also stimulate the proliferation of the vascular muscle cells and inactivate paraoxonase, an enzyme related to high-density lipoprotein (HDL). The high affinity for the groups above, compromises much of the antioxidant capacity of plasma, thus reduces the membrane and plasma oxidant defense, increasing the risk of CVD (15, 18-22).

Objective:

To explore the association between whole blood mercury and the risk of developing CVD among the Greenlandic population.

2.0 Methods

Setting:

Greenland is situated in the Arctic and is the world's largest island. The country has approximately 57,000 inhabitants spread out in 18 towns and approximately 60 villages, primarily on the South and central West coast. Towns and villages in Greenland often need to be self-sufficient and are very vulnerable to external factors because of their small size and isolation. Regarding lifestyle, living conditions, educational achievement and occupational structure, substantial differences are found between the larger towns and the more remote often sparsely populated towns and villages. The economic conditions in a village are often less favourable than those found in the nearest town (23).
In 1953, Greenland became a Danish colony, and in 1979 Greenland was granted self-government from the Danish parliament. Further in 2009, Greenland gained the right to self-governance, with the future goal to attain full independence. Nuuk is the capital with a population of 16.000 inhabitants, where the central hospital in Greenland, Queen Ingrid's Hospital, is located (24).

Since 1953, the population in Greenland has undergone profound demographic and socioeconomic changes. The changes included a reduction in the prevalence of infectious diseases such as tuberculosis, but an increase in chronic diseases such as cancer and CVD, as well as mental health problems, abuse of alcohol, youth suicides and violence (25). The traditional hunting economy has been replaced by wage earning and social welfare, and the changes have led to marked health changes in Greenland (26, 27). Smoking is prevalent among the population in the Arctic, not only among adults but also among pregnant women and adolescents. However surveys conducted in Greenland show a decrease in the prevalence of smoking. Physical activity patterns have changed along with the transition where a decline in physical activity has been observed(26). Abuse of alcohol is also a major health problem among the Inuit population (28, 29). In Greenland the alcohol consumption increased from low levels in the 1950s to high levels in the 1980s. Since then the consumption has declined to an average yearly consumption equivalent to 8.8 L pure alcohol per person aged 15 years and older. During the past century, the diet of the Inuit has become increasingly westernized. In Greenland, the traditional diet accounted for 82% of the energy intake around year 1900. However, over the past 60 years the consumption of the traditional diet has decreased to 21% of the total energy intake. The contemporary diet has increasingly been replaced by imported

food of low quality such as junk food, sweets and soda. The dietary changes have resulted in a population that is steadily becoming more obese (26). Metabolic and cardiovascular diseases are major public health issues among indigenous populations in the Arctic (26).

In 2011, the primary health care system was restructured from 16 districts to five regions with one leading local clinic in each region (30). Each region has a hospital staffed with doctors, nurses, physiotherapists and other professionals in the town and health clinics in every village. The village clinics are staffed according to population size. The largest clinics have a nurse as the responsible, while the smallest clinics have a health worker without formal health education responsible for the village. According to regulations, all village clinics are visited regularly by doctors, midwifes and other health care staff. In case of emergency patients can be admitted to larger hospitals by boat or plane, depending on the wheather conditions. Prescription of medicine and all services in the health care system in Greenland are free of charge (23).

2.1 Study Design & sample:

The present study is a prospective longitudinal cohort study, using data derived from the population-based cohort study '*Inuit Health in Transition Greenland survey 2005-2010*' (IHIT). IHIT was designed with the aim to study the interaction between environmental and genetic factors on health and disease patterns of the Inuit in Greenland, as well as contribute to a better understanding of transition from a traditional lifestyle to a modern industrialized life and its effects on health. One of the specific aims of the IHIT study was to explore risk factors for CVD and diabetes. The study comprised participants selected through a stratified random sample of adult (18+ years of age) inhabitants with residence in Greenland during 2005-2010. Greenland was divided into strata based on geography (Southwest coast, Central Westcoast, Northwest coast, East Greenland, North Greenland), and community size (towns with$\geq 2000, < 2000$ inhabitants and villages). From each of these strata one or more towns and 2-3 villages were selected for the study as being representative for the stratum with regard to living conditions.

The data collection took place from 2005-2010, information was collected in 9 towns and 13 villages, some towns were visited twice, in order to achieve the desired participation rate (31).

Inclusion and exclusion criteria:

Inclusion criteria for participation in the IHIT study was age of 18 or older born in either Greenland or Denmark.

Participants who had participated in the population survey, conducted in 1999-2001 were excluded, except in Qasigiannguit (*n =902*) where they were followed up.
Participants were contacted by letter, explaining the purpose of the study and examination procedures. If the recipients wanted to participate in the study, they informed the data collection team by letter or phone.

Validation of data:

The data was collected by a team consisting of a local person responsible for the recruitment of the participants, a supervisor, laboratory technicians, interviewers and clinical assistants. The data collection and the processing of data were done by a total of 81 people.
The data collected was double entered into EpiData. The data files were imported into SAS combined with the results of blood analyses and clinical examinations. The validity of the data was further checked against permitted values and logical errors (31).

Pilot study:

Prior to the IHIT study, a logistic pilot study was conducted in 2003 on three villages, in Ilulissat on the West coast of Greenland. The participation rate for the pilot testing was 49.7% and totaled 97 participants. Information was collected using questions designed for the 2005-2010 survey. After the pilot testing, new questions and procedures were included in the final survey (28).

Ethical Considerations:

The Committee for Research Ethics in Greenland granted ethical approval. After being informed about the study orally and in writing, participants gave their written consent (31).

2.2 Materials and Methods:

All participants in the IHIT and the pilot study underwent a questionnaire interview and clinical examinations.
Participants were instructed to show for the clinical examinations fasting, defined as at least 8 hours without eating and drinking.
After the fasting blood samples were drawn, a 2hr oral glucose tolerance test (OGTT), was performed. Participants underwent an interviewer-administrated questionnaire for approximately 40 minutes, and afterwards completed a self-reported questionnaire. Several clinical tests were also performed. At the end of the session, participants were informed of the results of the tests and 200 Danish kroner was paid to each participant.

Present study participants:

All participants from IHIT with a valid measurement of whole blood mercury and with no history of CVD at enrolment in IHIT were included in the present study.
If persons with a history of CVD were included, we could risk that the potential CVD event occurring after date of the whole blood mercury measurement was in fact a recurrent event and not a new event.

Blood samples:

In the present study, the following values of blood samples were included in the analyses: mercury, selenium, HDL-cholesterol, triglycerides, calculated LDL-cholesterol and glycated haemoglobin A_{1c} (HbA_{1c}).
All blood samples were drawn by venepuncture at normal venous pressure and stored for future analyses. Fasting and 120 min. plasma glucose, insulin and C-peptide after an OGTT, were also taken.
HbA_{1c}, total cholesterol, HDL-cholesterol, triglycerides, fatty acids in RBC membranes, mercury, selenium and organochlorines (PCB and pesticides) were taken in full blood.
LDL-cholesterol was calculated based on total cholesterol, HDL-cholesterol and triglycerides.
A spot urine sample was collected for each participant and the urine sample was stixed for albumine, nitrit and leucocytes and further analysed for micro albumin and creatinine.

Mercury and Selenium:

Levels of mercury and selenium was measured in full blood, stored at -20ºC and analysed using a inductively coupled mass spectrometry, in the laboratoire de Toxicologie/INSPQ, Sainte-Foy, Quebec, Canada. The detection limit for mercury was 0,5 nmol/l and 0,1 µmol/l for selenium (31).

Total cholesterol, HDL- cholesterol, triglycerides and calculated LDL-cholesterol:

Total cholesterol, HDL-cholesterol and triglycerides were measured with a minimum of 1.5 ml blood component, using a BD-vacutainer dry No. 367819. The samples were separated into a Nunc Cryo Tube 1.8 ml No 368632, and were allowed to rest for 30 minutes before they were centrifuged at 20ºC, 3000 rpm for 10 minutes. It was then stored frozen in -20ºC, and analysed in the laboratory at Steno Diabetes Centre, Gentofte, Denmark. Enzymatic colorimetric tests were performed using Hitachi 917 (12). Based on total cholesterol HDL-cholesterol and triglycerides, LDL-cholesterol was estimated using the following formula: LDL cholesterol = total cholesterol – (triglycerides (TG)/5 + HDL-cholesterol (32).

Haemoglobin A1c (HbA1c):

HbA_{1c} was measured using bioRAD sample preparation kit - Na-heparinised (5µl) capillary in Eppendorf tube with 1 ml EDTA and potassium cyanide solution (0.25 mmol/l).
The capillary was filled with blood stabilized with EDTA, and transferred into the sample preparation vial and shaken to rinse the blood from the capillary. The sample was stored at 4 ° C or at room temperature until shipment to Denmark. The samples were analysed at the laboratory at Steno Diabetes Centre, Gentofte, Denmark, using the Ion exchange HPLC method; measured by Tosoh G7 (26).

Other clinical measures:

In addition to blood sampling, the clinical procedures in the IHIT study also included anthropometric measurements such as height, weight, waist and hip circumference, as well as bio impedance, blood pressure (BP), electrocardiogram, ultrasound examination of subcutaneous and visceral abdominal fat and liver fat, ultrasound examination of carotid intima media thickness and a combined measurement of heart rate and body movement using Actiheart®.
For the present study body mass index (BMI), waist circumference (WC), waist-to hip ratio (WHR) and BP were included which will be elaborated below.

Body mass index (BMI):

BMI was calculated as the participant's weight in kilograms divided by the square of the person's height in metres (33).
Height and weight was measured with the participant stripped to underwear and socks. Weight was measured on a standard electronic clinical scale (26).

Waist circumference (WC):

WC was measured midway between the rib cage and the iliac crest on the standing participant (31).

Waist-to-hip ratio (WHR):

WHR was calculated by measuring the waist and the hip circumference, at the widest diameter of the buttocks, and then dividing the waist measurement by the hip measurement (34).

Blood pressure (BP):

Blood pressure was measured on the right arm of the sitting participant after five minutes of initial rest, using an automatic measuring device (Kivex UA-779) with an appropriate size cuff. Blood pressure was measured three times with one-minute interval. The two last measurements were averaged for the analysis (26).

Questionnaire derived measures:

Two questionnaires were used in the IHIT study: an interviewer administered including questions on sociocultural and demographic factors, dietary habits, physical activity, smoking, self-rated health, and self-reported diseases as heart disease, diabetes and hypertension and a self-administrated questionnaire including questions related to private matters such as suicidal thoughts and attempts, alcohol and marihuana consumption, violence and sexual abuse and gambling habits.

When the participant had answered the questionnaire, the answers would be put in an envelope, which would be opened by the researcher in charge of the survey only, and the answers would be treated confidential and anonymously.

The questionnaires were translated from Danish into Greenlandic and translated back to Danish in order to validate the translation. For the present study, place of residence, educational level, physical activity and smoking were included.

Place of residence:

Each participant reported the name of the town/village where they were living. Based on the size of the town/village place of residence was categorized as town or village.

Education:

Participants were asked about the number of years in school and their highest attained education. This was then categorized into: still being a student, completed primary school or high school, a short vocational (post primary education less than three years) or longer vocational education (e.g an academic education).

Physical Activity:

Physical activity was measured using a modified version of the interviewer-administrated seven-day International Physical Activity Questionnaire (IPAQ) (long version) (IPAQ-L) (35). Since a wide variety of physical activities is undertaken by people throughout the world, IPAQ focus the cultural adaption of the questionnaire (36). The questionnaire was slightly modified to adjust to Arctic living conditions, by replacing some of the activity examples with

cultural examples, based on a pilot study. Participants reported time spent on physical activity in the previous seven days: how often (number of days per week) and for how long (average duration per day). Questions were asked separately for vigorous intensity, moderate intensity and walking in four domains: work, transportation, domestic and leisure time (31, 37). Physical activity energy expenditure (PAEE), was calculated by multiplying time reported (minutes/week) by the net metabolic cost of each activity, which was expressed in metabolic equivalents (METs). The net metabolic cost of each activity was assigned according to the PA Compendium's gross MET values subtracted by 1 MET to account for resting metabolic rate (RMR). An estimate of total daily sedentary time was calculated from time spent sitting, such as TV and computer use and reading. The time series of activity intensity were summarized into total PAEE kJ/kg/day (26).

Smoking:

Participants were asked whether they smoked, smoked but not daily or did not smoke. Participants, who did not smoke, were asked if they had formerly smoked. Former smokers would be asked approximately how much they used to smoke on average during a day, as well as the year the participant had stopped smoking.

2.3 Statistical analysis:

For the present study, the continuous effects of whole blood mercury levels among participants from the IHIT study and the pilot study, in both univariate and multivariate analyses were investigated. The overall incidence rates and the hazard ratio (HR) of CVD events among participants for overall CVD, using Cox regression were calculated. Potential interactions with sex, was also investigated.

Sensitivity analyses were conducted, where CVD was grouped in two groups, consisting of CVD related to the heart and CVD related to cerebrum.

Baseline characteristics:

The distributions of all baseline characteristics were assessed according to sex as there were sex-differences in both mercury levels and CVD incidence. Baseline characteristics were presented as mean and standard deviation for normally distributed variables and median and interquartile range for non-normally distributed variables. Normality and deviations hereof were assessed from histograms.

The normally distributed variables included: Cholesterol, HDL-cholesterol, calculated LDL-cholesterol, BMI, systolic/diastolic BP, WHR and HbA_{1c}.

Not normally distributed variables included: age, whole blood mercury, triglycerides, PAEE, WC and selenium.
All categorical variables: place of residence, education and smoking were presented as percentages. Using t-test for continuous variables and chi2 test for categorical variables, there was tested for difference by sex.
All statistical analyses were performed in Stata/MP 14.0.

Cox regression:

For the present study the association between whole blood mercury levels and the risk of CVD, both in a crude analysis and adjusting for several potential confounders was assessed. The analyses were performed using cox regression with calendar time as the underlying time-scale.

Cox regression makes a predictive model for time-to-event data, analysing the effect of several risk factors on survival or event of interest, in this case CVD.
Mathematically cox regression is written as following:
$h(t) = h_0(t) \times \exp\{b_1x_1+b_2x_2+\ldots+b_px_p\}$

To interpret the result the $\exp(b_1)$ was used referred to as hazard ratio (HR). HR can be regarded as a measure of relative risk. HR represents the probability that an individual would experience the event (CVD), at a particular given point in time after they entered the study, assuming that the individual has not experienced the event of interest till the end of follow up. A HR greater than one, indicates that the value of the covariate increases, thus the event hazard increases and the length of survival decreases (38-40).

Participants of the present study entered the analyses at date of examination and contributed risk time until date of CVD diagnosis/death, emigration, death from other causes or end of follow-up 30/09-2013.

Exposure variable:

The exposure variable in the present study was whole blood mercury, included as a continuous variable in the analyses.

Outcome variable:

The outcome in the present study was a composite endpoint comprising all fatal and non-fatal CVD diagnoses retrieved from the Greenland National Patient register (GNPR), the Danish National Patient Register (NPR) and in the causes of death register.
Based on the International Classification Diseases, tenth edition (ICD-10) coding, all deaths or hospital admissions from ischemic heart disease (ICD20-25), atrial fibrillation (ICD48), heart failure (ICD50), cerebrovascular diseases (ICD60-69) and vascular diseases (ICD70-79) were categorized as a CVD event.

Validated outcome:

All CVD diagnosis from the Greenlandic Hospital Discharge Register (GHDR) in the IHIT, were validated by a medical doctor. A second medical doctor double validated 48 medical records to assess inter observer disagreement. The second validation was in complete agreement with the first validation by the first primary doctor, thus the validity of cardiovascular diagnoses in GHDR is considered very good, with acceptable agreement between medical records and diagnoses in GHDR (41).

Linearity and inter-correlation:

In the regression analyses, all continuous variables were included as continuous. In the regression analyses, the effect of continuous variable is assumed to be linear. Therefor it was tested for deviations from linearity by also including the quadratic, cubic and logarithmic transformation of the continuous variable in the analyses. No deviation from linearity was found for whole blood mercury and it was kept as a continuous variable.
Selenium and triglycerides did deviate from linearity, but they were kept without transformations as they only were included as potential confounders.
The variables; total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides, were inter-correlated. So were WHR, WC and BMI. Multicollinearity is a situation in which two or more variables are very closely related and this can affect parameter estimation (18). Total cholesterol was therefore removed from the final analyses, as all the other measures of lipids were included in this variable. On obesity WHR and WC were removed, as BMI is generally a more common used measurement of obesity than WHR and WC (13).

Sensitivity analysis:

In the present study sensitivity analyses were conducted by grouping the outcome overall CVD into two groups, consisting of CVD related to the heart (ICD20-25, ICD48 and ICD50) and CVD related to cerebrum (ICD60-69 and ICD70-79).
This was to investigate if mercury may have different effects on CVD mainly related to the heart or CVD mainly related to the cerebrum (42, 43).
Persons with previous CVD were included in the sensitivity analyses, if the previous CVD was unrelated to the incident of CVD. Thus, persons who had experienced e.g. stroke before they were included in the analyses where CVD mainly related to the heart was the outcome and vice versa.

Effect modification:

Effect modification (or interaction) is a method to assess if the effect between the primary exposure of interest and the outcome differs among subgroups of individuals, in order to identify which subgroups of individuals may be at higher risk.
If the effects of the primary exposure varies by stratifying for subgroups there will be an effect modification (44, 45).
For the present study, whether the effect of whole mercury interacted with sex on the outcome CVD was assessed. Potential interactions with sex were investigated as there was found sex-differences in both the levels of mercury and in CVD incidence.

Confounder:

A confounder is a third factor that is both a risk factor for the outcome and is associated with the exposure in question. The confounder can affect the variable being studied, so the results do not reflect the actual relationship between the variables under the study (44, 46).
To control for confounding in the analyses, researcher adjusted the analyses for several potential confounding variables.

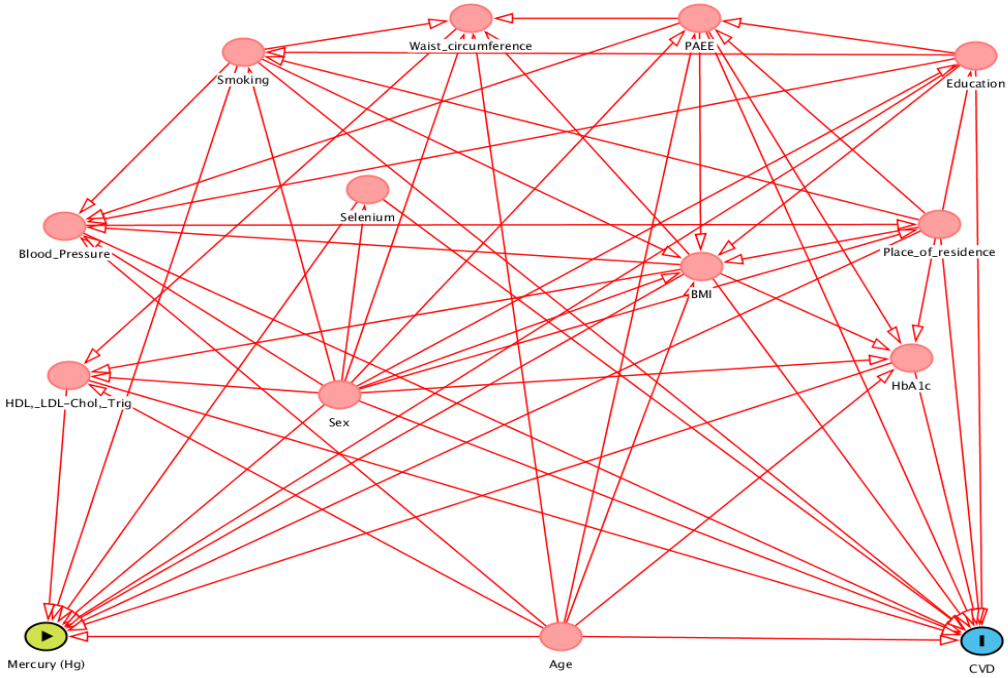
In order to identify potential confounders for the association between whole blood mercury and the risk of CVD, the theory of directed acyclic graphs (DAG) was applied. A DAG is a theoretical tool, which gives a visual representation of causal assumptions, and reveals the structure of associations that could be observed (47).

For the present study, following confounders, based on findings from previous studies, were included: age, sex, PAEE, selenium, place of residence, education, smoking, HDL and LDL-

cholesterol, triglycerides, BMI, WC, systolic and diastolic BP and HbA_{1c}. The assumed structure of whole blood mercury and the risk of developing CVD is visualized in figure 2. Researcher adjusted for confounders in the regression analyses using a forward stepwise approach.

In the crude model (model 1), only the crude association between whole blood mercury and CVD was assessed.
In model 2, researcher adjusted the association of whole blood mercury and CVD for age and sex. Age was included as a continuous variable, sex as a binary variable.
In model 3, there was adjusted for age and sex, as well as PAEE, selenium, place of residence and education. PAEE was included as a continuous variable along with selenium. Place of residence was included as a binary variable, while education was categorized into four groups.
In the final model (model 4), researcher adjusted the association of whole blood mercury and CVD for model 3 and additionally for smoking as a categorized variable and for HDL-cholesterol, calculated LDL-cholesterol, triglycerides, BMI, systolic/diastolic BP and HbA_{1c} as continuous variables.

Figure 2. DAG on the association between whole blood mercury and the risk of CVD



2.4 Previous studies on potential confounders:

Age and sex:

A study from Sweden, found that mercury levels increased with age, and that males had a higher blood mercury concentration than women (48). This was also seen in a study from America (49) Similarly a study conducted in Korea found that males had a higher concentrations of whole blood mercury than the women (50).
In Greenland, the total CHD prevalence was higher among men than among women (4). CVD is known to be lower among women than men and tends to increase with age. Worldwide on average, CHD mortality is up to five times higher among men than women (51).

BMI and PAEE:

Organic mercury compounds are very fat soluble: 90-100% of an oral dose is absorbed in the body (52). Inuit populations in the Arctic have high levels of obesity according to the international guidelines for BMI and WC (53).

A study consisting of participants from Korea, showed that blood mercury concentration increased according to BMI and WC, and obesity increased in relation to the blood mercury concentration. The study concluded that fat mass levels should be considered as a confounding factor when blood mercury is used as an indicator of exposure (54).
Physical activity plays a central role in the amount of adipose tissue. Since mercury accumulates in adipose tissue, the amount of adipose tissue may affect the concentration of mercury in the blood (54).

A study from Canada found a significant association between BMI and CVD, and concluded in their study that a BMI of 25-29.9 increased the risk of CVD with approximately 60% compared with lower BMI (55).
A study conducted in the United States, found both BMI and WC to strongly predict for the future risk of developing CHD (56).

Physical inactivity is a major contributor to CVD and a study conducted in the United States, showed that higher physical activity is associated with a lower risk of developing CVD compared to less physical activity (57). In a study conducted in Greenland, CHD was more common among the participants who were less physically active (4).

Selenium:

Mercury has a high affinity for sulfhydryl groups, which reduces the antioxidant defense. Selenium proteins are important components of antioxidant systems that actively protects against damage from free radicals (58). Mercury reduces the selenium availability, which is a necessary factor break down hydrogen peroxidase and other peroxidation products, thus increases the risk of CVD (59). A study conducted in Germany found that patients who had an acute coronary syndrome and low selenium concentrations, were at additional risk of future cardiovascular death (60).

Place of residence:

The highest measurements of mercury have been found in villages, compared to participants living in the towns. Thus, living in a village increases the level of mercury (61).
A study conducted in Greenland found the highest prevalence of CHD, in the least Westernized groups in Greenland, being in the villages (4).

Education:

In a study conducted in Korea, an association between education level and mercury was found (50). Participants with middle school as the reference level, for highest attained graduated school, had the highest levels of mercury (50). In a study conducted in Sweden, higher blood levels of mercury was associated with higher education levels (48).
In a study conducted in Norway, cardiovascular risk factors and high IHD mortality were more prevalent among the less educated participants, compared to highly educated participants (62).

Smoking:

Tobacco contains mercury. About 6 ng of mercury is released in smoke per unit length of cigarettes, and 80% of mercury in the smoke is retained in the human body.
Provided that 20 cigarettes are consumed per day, the daily amount of mercury will be 336 ng retained in the human body (63).
Tobacco smoking is a major cause of CVD, and beyond its status as an independent risk factor, smoking appears to have a multiplicative interaction with the other major risk factors for CHD such as high serum levels of lipids, untreated hypertension, and diabetes mellitus (64).

LDL, HDL-cholesterol and Triglycerides:

Mercury has shown to increase the oxidation of LDL-cholesterol, and inactivate paraoxonase, an antioxidative enzyme related to HDL. These mechanisms of mercury are thought to explain the increased risk of CVD found in some studies of the effect of mercury on CVD risk, including CHD, myocardial infarction, hypertension and arrhythmias (59).
High levels of lipids are risk factors for CVD. High levels of LDL cholesterol, can lead to atherosclerosis, increase the risk of heart attack and IHD and stroke. HDL cholesterol reduces the risk of CVD as it carries cholesterol away from the blood stream. High levels of LDL cholesterol and triglycerides combined, increases the risk of atherosclerosis and the risk of heart attack and stroke, thus increase the risk of CVD (65).

Blood pressure:

A study conducted in the Brazilian Amazon, found a significant dose-effect relation between mercury exposure and BP, leading to the conclusion high mercury levels could increase the BP (4).
Similar study conducted in Greenland found no relationship regarding mercury exposure and increased BP (32).
Increased BP, hypertension, defined as a systolic blood pressure at or above 140 mmHg and/or a diastolic BP at or above 90 mmHg is a risk factor for CHD, stroke and atherosclerosis, thus a risk factor for CVD (66).

HbA_{1c}:

Mercury exposure has been associated with the higher incidence of diabetes. Studies have shown that high mercury exposure may be associated with pancreatic β-cell dysfunction, suggesting that mercury may be a risk factor for diabetes (67). Exposure to mercury, decrease cell viability in the pancreatic β-cell line and cause pancreatic islet β-cell dysfunction, which may lead to the development of diabetes (24).
Several studies have been conducted regarding the association between high levels of HbA_{1c} and the risk of CVD, have found increasing risks of CVD and total mortality with high levels of HbA_{1c} (68-71).

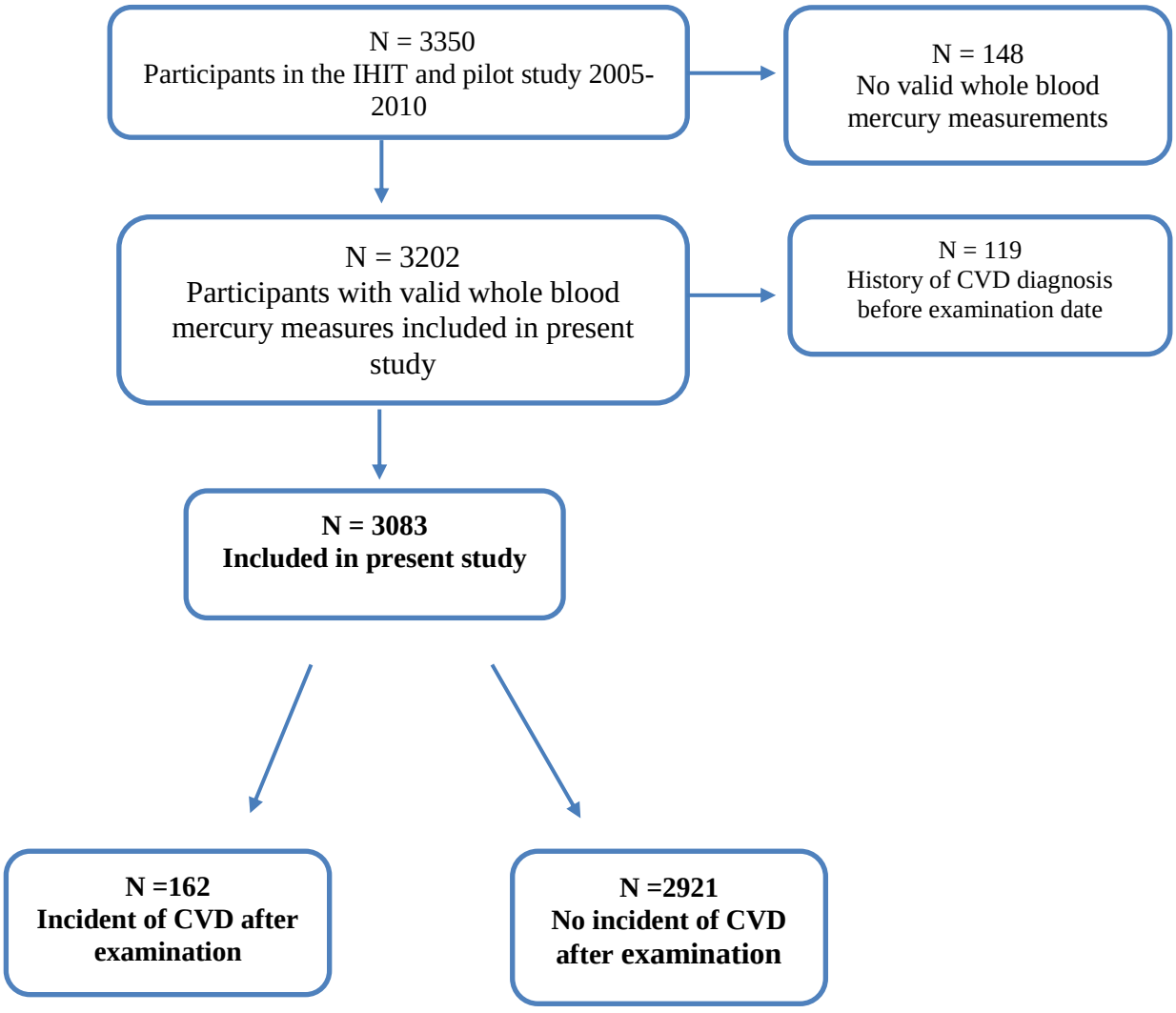
3.0 Results

Characteristics of study population:

All participants in IHIT (3.253) were included as well as the 97 persons, who had participated in the pilot study, leading to a sample of 3350 participants.

148 participants were excluded from the analyses as they did not have a valid measurement of whole blood mercury (Figure 1). Further, 119 persons with a past history of CVD were excluded, as researcher only was interested in looking at incidence of CVD, after mercury levels assessment. Thus, a total of 267 persons were excluded, leading to the final sample size consisting of 3083 participants. A total of 162 participants experienced CVD event during follow-up.

Figure 1. Flowchart - Inuit Health in Transition – Greenland Survey 2005-2010



All the baseline characteristics for the present study, stratified by sex can be seen in table 1. Of the final sample comprising 3083 participants, 43.4% (n=1338) participants were men and 56.6% (n=1745) were women.

Men had a significantly lower level of HDL-cholesterol ($p<0.001$) and a higher level of LDL-cholesterol compared to women (although not statistically significant). Women had a significantly higher BMI compared with men, whereas men had higher levels of systolic and diastolic BP as well as HbA_{1c}.

The median age for the men was 44 years and 43 years for women. The level of whole blood mercury was not equally distributed among men and women. The highest levels of whole blood mercury were found in men, who had a significantly higher median level of 19 μg/L, while women had a lower level of whole blood mercury with a median at 15 μg/L ($p<0.001$). PAEE was significantly higher among the men with a median of 54.0 KJ/kg/day compared to women with a median of 45.8 KJ/kg/day ($p<0.001$). Blood selenium was equally distributed among both sexes with a median level at 240 μmol/L ($p=0.24$).

Looking at the categorical variables, women had a higher tendency of daily smoking by (64.1 %) compared to men, were 60.5% reported daily smoking ($p=0.09$).
Education was more or less equally distributed among men and women. The highest and most frequent attained educational level for both sexes was ‘short vocational education (less than three years), where 54.4 % of the men had attained that level compared to 56.9% of the women. Of the women 7.1% had a longer vocational education/academic education, compared to 6.6% of the men.

Looking at the distribution of the place of residence, approximately 2/3 of both men and women lived in towns.

Table 1 – Baseline characteristics stratified by sex

Characteristics	Men *(n = 1338)*	Women *(n = 1745)*	P-value
Mean (SD) Normally Distributed			
HDL-cholesterol (mmol/L)	1.6 (0.6)	1.7 (0.5)	≤0.001
LDL-cholesterol (mmol/L)[1]	3.7 (1.1)	3.6 (1.0)	0.15
BMI (kg/m^2)	25.8 (4.6)	26.6 (5.5)	≤0.001
Systolic bloodpressure (mmHg)[2]	137.3 (23.0)	129.6 (22.0)	≤0.001
Diastolic blood pressure (mmHg)	82.7 (17.0)	78.8 (14.0)	0.04
HbA_{1c} (mmol/L)[3]	5.7 (0.5)	5.6 (0.4)	≤0.001
Median (first quartile, third quartile) Not normally distributed			
Age (years)	44 (18-54)	43 (18-52)	0.02
Whole blood mercury (µg/L)	19 (0.9-44)	15 (0.8-32)	≤0.001
Triglycerides (mmol/L)	0.99 (0.4-1.4)	1.0 (0.4-1.4)	0.64
PAEE (KJ/kg/day)[4]	54 (0-99)	45.8 (0.7-69)	≤0.001
Blood selenium (µmol/L)[5]	240 (94.8-440)	240 (100-410)	0.25
Percentages (%) Categorized			
Smoking:[6]			0.09
- Daily	808 (60.5%)	1118 (64.1%)	
- Yes, but not daily	76 (5.7%)	94 (5.4%)	
- No	452 (33.8%)	529 (30.4)	
Education:[7]			0.29
- Still student	14 (1%)	21 (1.2%)	
- Primary school/High school	504 (38%)	599 (34.6%)	
- Short vocational education (less than three years)	723 (54.4%)	984 (56.9%)	
- Longer vocational education/ academic education	87 (6.6%)	124 (7.1%)	
Place of residence			0.52
Town	977 (73.0%)	1292 (74%)	
Village	362 (27.0%)	453 (26%)	

[1] Missing (M) = 25
[2] M = 10 on both systolic and diastolic BP
[3] M = 11
[4] M = 99
[5] M = 17
[6] M = 6
[7] M = 27

Association between whole blood mercury and the risk of CVD

Table 2 shows the regression analyses of whole blood mercury and the risk of developing CVD.

In the crude analysis (model 1), the HR for developing overall CVD was 0.99 (95% CI 0.99-1.00) for any given level of whole blood mercury. Thus, there was no crude association between whole blood mercury and the risk of developing CVD in the study population.
The HR did not change after adjusting for age and sex in model 2. Age was however, a risk factor for developing CVD (HR: 1.08, 95% CI 1.07-1.09), furthermore women had a lower risk of developing CVD, compared with men (HR 0.55, 95% CI 0.40-0.76).

In model 3, additionally adjusted for physical activity energy expenditure (PAEE), selenium, place of residence and education, there were no effects of whole blood mercury on the risk of developing CVD (HR 0.99, 95% CI 0.98-1.00). PAEE and selenium was not associated with the risk of developing CVD. However, living in a village was associated with a lower risk of developing CVD (HR 0.58 95% CI 0.36-0.92).
HR for short vocational education level was 0.88 (95% CI 0.20-3.75), while the HR for longer vocational level was 0.24 (95% CI 0.03-1.81), compared with still being a student.

In model 4, researcher adjusted for all variables. Again, no associations between whole blood mercury and the risk of developing CVD was seen (HR 0.99 95% CI 0.98-1.00).
Smoking was associated with a lower risk of developing CVD (HR 0.83 95% CI 0.57-1.20), compared with non-smokers. Higher levels of LDL-cholesterol (HR 1.08 95 % CI 0.93-1.26) and HbA_{1c} (HR 1.11 95% CI 0.82-1.50) levels tended to be associated with higher risk of developing CVD.

Table 2 – Association between exposure to mercury and the risk of developing CVD events for overall CVD *(n=3083)*

Model 1 - Crude			
Exposure	**Hazard Ratio**	**95% CI**	
Whole blood mercury (µg/L)	**0.99**	**0.99**	**1.00**
Model 2 – Adjusted for age and sex			
Whole blood mercury (µg/L)	**0.99**	**0 .99**	**1.00**
Age (Years)	1.08	1.07	1.09
Sex			
- Male	1.00 (ref)		
- Female	0.55	0.40	0.76
Model 3 – Adjusted for age, sex, PAEE, selenium, place of residence, education			
Whole blood mercury (µg/L)	**0.99**	**0.98**	**1.00**
Age (Years)	1.07	1.05	1.08
Sex			
- Male	1.00 (ref)		
- Female	0.53	0.38	0.73
Physical Activity Energy Expenditure (KJ/kg/day)	0.99	0.99	1.00
Blood selenium (µmol/l)	1.00	0.99	1.00
Place of residence			
- Town	1.00 (ref)		
- Village	0.58	0.36	0.92
Education			
- Still Student	1.00 (ref)		
- Primary school/ high school	1.23	0.30	5.04
- Short vocational education (less than 3 years)	0.88	0.20	3.75
- Longer vocational education/academic	0.24	0.03	1.81
Model 4 -Adjusted for age, sex, PAEE, selenium, place of residence, education, smoking, HDL/LDL-chol, triglycerides, BMI, WC, Sys/Dia BP, HbA1c			
Whole blood mercury (µg/L)	**0.99**	**0.98**	**1.00**
Age (Years)	1.07	1.05	1.09
Sex			
- Male	1.00 (ref)		
- Female	0.50	0.33	0.73
Physical Activity Energy Expenditure (KJ/kg/day)	0.99	0.99	1.00
Blood selenium (µmol/l)	1.00	0.99	1.00
Place of residence			
- Town	1.00 (ref)		
- Village	0.53	0.32	0.86
Education			
- Still Student	1.00 (ref)		
- Primary school/ high school	1.15	0.27	4.77
- Short vocational education (less than 3 years)	0.78	0.18	3.40
- Longer vocational education/academic	0.21	0.02	1.62
Smoking			
- Yes	0.83	0.57	1.20
- Yes, not daily	0.81	0.34	1.91
- No	1.00 (ref)		
Hdlc (mmol/L)	0.92	0.26	4.55
Ldlc (mmol/L)	1.08	0 .93	1.26
Triglycerides (mmol/L)	0.92	0.67	1.26
Body mass index (BMI)	0.98	0 .89	1.08
Waist circumference (cm)	1.03	0.98	1.08
Systolic blood pressure (mmHg)	1.00	0.99	1.01
Diastolic blood pressure (mmHg)	0.99	0.98	1.00
HbA_{1c} (mmol/L)	1.11	0.82	1.50

Sensitivity analysis:

The effects of whole blood mercury on CVD mainly related to the heart (IHD, atrial fibrillation and heart failure) are shown in Table 3.

In the crude analysis (model 1), the HR for developing CVD mainly related to the heart was 1.00 (95% CI 0.99-1.00) for any given level of whole blood mercury. Thus, there was no crude association between whole blood mercury and the risk of developing CVD mainly related to the heart in the study population.

After stepwise adjusting for potential confounders in the same manner as the main analyses, researcher still found no effect of whole blood mercury on incident CVD mainly related to the heart (HR 0.99 95% CI 0.99-1.00).

Sensitivity analyses where cerebrum related CVD (stroke and vascular diseases) is the outcome can be seen in Table 4.

In the crude analyses (model 1) there was no crude association between whole blood mercury and the risk of developing CVD related to cerebrum. The HR for developing cerebrum related CVD was 1.00 (95% CI .99-1.00) for any given level of whole blood mercury.

Further analyses adjusted for potential confounders again showed no association on whole blood mercury and the risk of developing CVD mainly related to cerebrum HR 0.99 (95% CI 0.98-1.00).

Table 3 – Association between whole blood mercury and the risk of developing CVD mainly related to the heart (IHD, atrial fibrillation and heart failure).

Model 1 - Crude	**Hazard Ratio**	**95% CI**
Whole blood mercury (µg/L)	**1.00**	**0.99 1.00**
Model 2 – Adjusted for age and sex		
Whole blood mercury (µg/L)	**0.99**	**0 .99 1.00**
Age (Years)	1.09	1.07 1.10
Sex		
- Male	1.00 (ref)	
- Female	0.45	0.30 0.67
Model 3 – Adjusted for age, sex, PAEE, selenium, place of residence, education		
Whole blood mercury (µg/L)	**0 .99**	**0 .99 1.00**
Age (Years)	1.08	1.06 1.10
Sex		
- Male	1.00 (ref)	
- Female	0.53	0.38 0 .73
Physical Activity Energy Expenditure (KJ/kg/day)	1.00	0 .99 1.00
Blood selenium (µmol/L)	1.00	0.99 1.00
Place of residence		
- Town	1.00 (ref)	
- Village	0.54	0.31 0.95
Education		
- Still Student	1.00 (ref)	
- Primary school/ high school	2.11	0.28 15.50
- Short vocational education (less than 3 years)	1.92	0.24 14.93
- Longer vocational education/academic	0.33	0.02 5.63
Model 4 -Adjusted for age, sex, PAEE, selenium, place of residence, education, smoking, HDL/ LDL -chol, triglycerides, BMI, WC, Sys/Dia BP, HbA1c		
Whole blood mercury (µg/L)	**0.99**	**0.99 1.00**
Age (Years)	1.08	1.06 1.10
Sex		
- Male	1.00 (ref)	
- Female	0.49	0.30 0.78
Physical Activity Energy Expenditure (KJ/kg/day)	1.00	0.99 1.00
Blood selenium (µmol/L)	1.00	0.99 1.00
Place of residence		
- Town	1.00 (ref)	
- Village	0.49	0.27 0.89
Education		
- Still Student	1.00 (ref)	
- Primary school/ high school	1.69	0.22 12.77
- Short vocational education (less than 3 years)	1.46	0.18 11.80
- Longer vocational education/academic	0.27	0.01 4.75
Smoking		
- Yes	0.63	0.40 0.98
- Yes, not daily	0.81	0.34 1.91
- No	1.00 (ref)	
Hdlc (mmol/L)	0.89	0.58 1.38
Ldlc (mmol/L)	1.16	0.98 1.38
Triglycerides (mmol/L)	0.94	0.65 1.35
Body mass index (BMI)	0.94	0.84 1.05
Waist circumference (cm)	1.03	0.99 1.07
Systolic blood pressure (mmHg)	1.00	0.99 1.01
Diastolic blood pressure (mmHg)	0.99	0.98 1.01
HbA_{1c} (mmol/L)	1.16	0.82 1.64

Table 4 – Association between whole blood mercury and the risk of cerebrum related CVD (stroke and vascular diseases).

Model 1 - Crude	**Hazard Ratio**	**95% CI**	
Whole blood mercury (µg/L)	**1.00**	**0.99**	**1.00**
Model 2 – Adjusted for age and sex			
Whole blood mercury (µg/L)	**0.99**	**0.98**	**1.00**
Age (Years)	1.07	1.05	1.08
Sex			
- Male	1.00 (ref)		
- Female	0.69	0.44	1.08
Model 3 – Adjusted for age, sex, PAEE, selenium, place of residence, education			
Whole blood mercury (µg/L)	**0.99**	**0 .98**	**1.00**
Age (Years)	1.06	1.03	1.08
Sex			
- Male	1.00 (ref)		
- Female	0.68	0.43	1.07
Physical Activity Energy Expenditure (KJ/kg/day)	0.99	0.99	1.00
Blood selenium (µmol/L)	1.00	0.99	1.00
Place of residence			
- Town	1.00 (ref)		
- Village	0.55	0.28	1.08
Education			
- Still Student	1.00 (ref)		
- Primary school/ high school	1.32	0.18	9.70
- Short vocational education (less than 3 years)	0.86	0.11	6.74
- Longer vocational education/academic	0.52	0.04	6.06
Model 4 -Adjusted for age, sex, PAEE, selenium, place of residence, education, smoking, HDL/LDL-chol triglycerides, BMI, WC, Sys/Dia BP, HbA1c			
Whole blood mercury (µg/L)	**0 .99**	**0.98**	**1.00**
Age (Years)	1.06	1.03	1.08
Sex			
- Male	1.00 (ref)		
- Female	0.61	0.37	1.02
Physical Activity Energy Expenditure (KJ/kg/day)	0.99	0.99	1.00
Blood selenium (µmol/L)	1.00	0.99	1.00
Place of residence			
- Town	1.00 (ref)		
- Village	0.50	0.25	0.98
Education			
- Still Student	1.00 (ref)		
- Primary school/ high school	1.44	0.19	10.71
- Short vocational education (less than 3 years)	0.98	0.12	7.81
- Longer vocational education/academic	0.61	0.05	7.27
Smoking			
- Yes	0.82	0.49	1.38
- Yes, not daily	0.96	0.33	2.79
- No	1.00 (ref)		
Hdlc (mmol/L)	1.08	0.69	1.68
Ldlc (mmol/L)	1.13	0.93	1.39
Triglycerides (mmol/L)	0.80	0.50	1.29
Body mass index (BMI)	1.02	0.90	1.15
Waist circumference (cm)	0.98	0.93	1.02
Systolic blood pressure (mmHg)	0.99	0.98	1.01
Diastolic blood pressure (mmHg)	1.09	0.68	1.74
HbA_{1c} (mmol/L)	1.16	0.82	1.64

Effect modification:

Whole blood mercury's potential interactions with sex was assessed by including an interaction term between sex and whole blood mercury in both crude and fully adjusted models. No statistically significant associations was found with sex in either the crude analyses (p=0.73) or in the fully adjusted analyses (p=0.44) (data not shown).

4.0 Discussion:

Main findings:

No association between whole blood mercury and the risk of developing overall CVD in the Greenlandic population was found. The conclusion was the same after adjustments for a range of potential confounding factors. Furthermore, no association between whole blood mercury and the risk of developing CVD related to the heart or to CVD related to the cerebrum was found in the Greenlandic population.

Strengths:

This is the first prospective longitudinal cohort study, which assessed the association between whole blood mercury and the risk of CVD in the Greenlandic population. Researcher was able to follow the study participants in several registers: GNPR, NPR and the causes of death register. Thus, all CVD events leading to hospital admissions or death are captured in our data. Furthermore, all CVD diagnoses in the study were validated by a medical doctor, thus the validity of CVD for this study is considered very good.

Whole blood mercury was used as a continuous measure, thus categorizing mercury into arbitrary categories was avoided. When using categorical measures, much information is lost, so the statistical power to detect a relation between the variable and the outcome is reduced. Secondly, there is a potential risk to underestimate the extent of variation in outcome between groups, such as the risk of some event, and considerable variability may be subsumed within each group. Individuals close to but on opposite sides of the cut point are characterized as being very different rather than very similar (72).

All Participants for the study were selected through a stratified random sample. Greenland was divided into strata based on geography, and from each of these strata one or more towns and 2-3 villages were selected for the study as being representative for the stratum with regard

to living conditions. The study had a participation rate of 66.7%, and surveyed a large proportion of the adult, Greenland born population (9.2%). Thus, it is believed that the persons included in the present study are more or less representative of the Greenlandic population.
In the present study, several potential confounders were assessed based on literature and previous studies.

Limitations:

Participants entered the analyses at date of examination, and contributed risk time until date of CVD diagnosis/death, emigration and death from other causes or end of follow-up 30/09-2013. The maximum follow-up time for the present study is eight years, and 162 participants experienced the event CVD. The short follow-up time and few events is a potential limitation for the present study. Nonetheless, the confidence intervals for the effects estimate of mercury on the risk of developing CVD were narrow. Thus, the results do not seem to be subject to lack of power.

The CVD events, derived from the patient registers and causes of death registers, only comprise diagnoses among patients hospitalized or dead from the CVD event. Thus, researcher may have underestimated the number of CVD events in the present study, as patients with a CVD event not admitted to the hospital were not captured by the registries. Nonetheless, as CVD is often a serious condition, it is believed that the majority of CVD events were referred to hospital or listed as cause of death.

Selection bias:

The participation rate varied across community size. In Nuuk the participation rate was 61.4%, in other larger towns the rate for participating was 65.1%, whereas the small towns had a participation rate of 69.9% and 68.5%.
A potential explanation for higher rates of non-participation in larger towns compared to villages could be because of a more busy daily life in towns, since the duration of the health examination took approximately two hours. In Nuuk in particular, many of the invited participants indicated lack of time as the reason for not wanting to participate (17% of the non-participant compared with 2% in the rest of the communities).

Persons with serious illness or disability and socially marginalized persons such as alcohol abusers, persons who frequently go in and out of jobs and the unemployed were over

represented among the non-participants. In addition, there was a distinct downwards participation trend from the beginning to the end of data collection in the towns. In some towns during the first week of the study 10% of those who had an appointment did not show up, whereas during the last week of the study as many as 26% failed to show up ($p<0.001$). Analyses of register-based income showed that the personal income was higher among participants compared with non-participants in the study, confirming a possible social bias (31, 73).

In this study there is a potential risk that hunters and fishermen are underrepresented, as they, in longer periods of time, are away from home and therefore not able to be present at the health examinations (73). Studies concerning dietary patterns in modern Greenland, has shown that hunters and fishermen were most likely to consume the traditional diet (74, 75). Thus, this underreporting could mean that the proportion of persons consuming the traditional diet is underrepresented in the present study.

Consistency with other studies:

The findings of no associations between mercury and incident CVD are consistent with studies conducted in America.

Using a nested case-control design, researchers investigated the association between mercury levels in toenails and the risk of incident CHD among male health professionals including dentists, nurses, veterinarians and pharmacists, with no previous history of CVD, at the age of 40-75 years in 1986. Researchers had 934 participants, three years of follow-up and categorized toenail mercury in quartiles. They found no association between toenail mercury and the risk of CHD (Relative risk 0.97, 95% CI 0.63-1.50) (76).

The results from the study is based only on male health professionals, thus the results might not be a true representative for a whole population, since underlying determinants of CVD also include social, economic and cultural change, as well as poverty and stress (2). In the present study we found the risk of CVD was lowest among participants with longer vocational education (HR 0.21, 95% CI .02-1.62). Thus, there is a potential risk participants at greater risk of CVD are being undetected in the study above.

However, a study conducted in Canada, found that Canadians with higher education and income tended to have higher blood levels of mercury (77). In the study the results are based on toenail mercury, categorized into quartiles.

The highest quartile had a median level of 1.34 µg/g mercury (range 0.87-14.56). The median level of mercury in the highest quartile is lower, compared to the present study, where the median level of mercury for men is 19µg/L. The study is conducted in America, a Westernized population, where most of the consumption of mercury is from fish. According to a study, the lack of an association between whole blood mercury and fish consumption, can be because of a lower average content of mercury in the various fish species versus seal and whale, which has a higher content of mercury (78). However, the findings of no associations between mercury and incident CVD are consistent with the present study, the differences in the study population e.g. lower levels of mercury in the diet, can explain the finding of no association. Nonetheless, fish are rich in long-chain n-3 polyunsaturated fatty acid (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The n-3 PUFA may reduce the risk of CVD. The beneficial effects of the fish can affect lipid and lipoprotein metabolism, blood pressure, platelet function, arterial cholesterol delivery, vascular function, and inflammatory responses (79). A study found the risk of a myocardial infarct was reduced in participants with high levels of PUFA (80). Thus, fish intake can have reduced the risk of CVD in the studies, explaining why they did not find an association.

Further, using the same study population, another nested case-control study was conducted on both men and women. Toenail mercury levels were categorized into quartiles. The highest quartile had a mean mercury level of 0.95 µg/g. The study consisted of 3427 participants with CVD, matched with 3427 controls that did not have a CVD event during the same period. The quartiles of mercury were sex-specific and the relative risks (RR) for each were combined. The researchers found no association between mercury exposure and an increased risk of CVD among men or women (RR= 0.85, 95% CI 0.72-1.01) (81).

On the contrary, a study conducted in the Amazon Basin Brazil, found a strong correlation between exposure to mercury compounds from consumption of fish, and increased arterial blood pressure measured in levels of mercury in the hair. Hair mercury $\geq$ 10 µ/g showed an odds ratio of 2.91 (95% CI 1.26-7.28) for elevated systolic BP (82). The study had a small sample size (259 participants), and the BP in the study was only measured once. It is well known that the predictive power of multiple blood pressure determinations is much greater than a single measure. A minimum of 2 measurements of the BP should be taken at intervals of at least 1 minute, and the average of those should be used to represent the patient's blood pressure (83).

Furthermore, studies conducted in Finland and Sweden, found high content of mercury in the hair to be a risk factor for acute coronary events, CVD and CHD (84-86). Both Finland and Sweden are known to be a population low on serum selenium (87-89). Selenium deficiency has been associated with increased risk of CVD and other chronic diseases (90-93), which might explain the findings mercury in the hair to be a risk factor for CVD. In the present study both men and women had the same high median level of blood selenium (240 µmol/L), according to a study conducted in Canada, high selenium exposure lowers the odds of CVD (94). Selenium is an essential mineral that is a component of major antioxidant, which protects against oxidative stress (89). Oxidative stress may play a crucial role in different types of CVD (95). However, there was no association between blood selenium and the risk of CVD in the present study (HR 1.00, 95% CI 0.99-1.00).

Studies regarding the adverse health effects of mercury have also been conducted within the Arctic. Studies conducted in Nunavik (Northern Quebec, Canada) and in Faroe Islands, found high levels of whole blood mercury to have adverse effects on blood pressure (BP) and heart rate variability (HRV) among Inuit adults (94, 96-98).
On the contrary, a study conducted in 2012 on the association between BP and whole blood mercury was assessed among the Greenlandic population. Among the highest quartile of mercury for men (HR: 0.97, 95% CI 0.93-1.01) or women (HR: 0.99, 95% CI 0.95-1.02) on the systolic BP, there was no association. Surprisingly, the diastolic BP and the risk of hypertension decreased with increasing whole blood mercury concentrations among men (HR: 0.93, 95% CI 0.89-0.98) (99).
The explanation to different findings can be because of differences in the study populations. On the studies conducted in the Faroe Islands, the study population only comprised men. Differences in dietary habit among the similar study population of Inuit in Canada and in Greenland, may explain the differences in the findings.
In the present study we assessed BP as continuous variables. Men had a higher systolic and diastolic BP than women (137 mmHg v 129 mmHg $p \leq 0.001$ - 82 mmHg v 78 mmHg $P<0.04$). However, systolic (HR: 1.00, 95% CI 0.99-1.01) or diastolic BP (HR: 0.99, 95% CI 0.98-1.00) was not associated with the increased risk of CVD in the present study, adjusted for a range of other exposures.

In a recent study, the association between whole blood mercury and glucose intolerance among adult Inuit in Greenland was investigated. There was a weak but significant

association for a 5µg/L increase in whole blood mercury with higher fasting plasma glucose (β=0.25, 95% CI 0.20-0.30 p-value <0.001) and type 2 diabetes (β=0.23, 95% CI 0.05-0.40 p-value 0.01) (100). The study is consistent with a study conducted in America, which found high levels of toenail mercury to increase the risk of diabetes later in life (HR: 1.65, 95% CI 1.07-2.56) (67).
In the present study, men had a mean HbA_{1c} of 5.7 mmol/L, and the women a mean of 5.6 mmol/L. Nonetheless, higher levels of HbA_{1c}, tended to increase the risk of CVD (HR: 1.11, 95% CI .82-1.50).

Implication for research:

Most environmental research on the effect of chemicals, focus on single exposures. However, people are often exposed to a mixture of chemicals and heavy metals, which can affect the same organ and induce similar effects. Nonetheless, studies that investigate effects of chemical mixtures in humans are limited (12).
In the present study our focus was mainly on whole blood mercury, and included blood selenium as a potential confounder.

Studies concerning mercury and adverse health effects of the BP, diabetes and now CVD, among the Greenlandic population has been conducted. Further studies, concerning the association between selenium and CVD could be investigated in order to assess if the finding of no association could be explained by the protective effects of selenium.

Biomonitoring levels of contaminants in Greenland have shown that the population has some of the highest concentrations of POPs and heavy metals in the arctic. In the IHIT study, high concentrations of PCBs was found in both men and women (12).
A study conducted on a Native American population, investigated if high serum PCBs were associated with lipids and CVD. They concluded that PCBs are directly responsible for increased levels of cholesterol and triglycerides, whereas it will increase the risk of CVD (101). The findings were consistent with another study conducted in Anniston, Alabama. They found increased concentrations of PCBs and organochlorine pesticides were associated with elevations in total serum lipids, total cholesterol and triglycerides, but the patterns were different for different groups of PCBs and different pesticides. They concluded that the elevation in concentrations of serum lipids could be the basis for the increased incidence of CVD found in persons with elevated exposures to PCBs and chlorinated pesticides (102).

Another study conducted in America, using data from the National Health and Nutrition Examination Survey from 1999-2004, found that PCBs were significantly associated with the increased risk of hypertension (103).
Although time series data indicate a current decline in concentrations of most heavy metals and POPs in the Arctic (9), biomonitoring contaminants in the Arctic should be continued. According to a recent study, climate changes causes glaciers to melt, which can increase the amount of mercury in the ocean in Greenland (104).

Studies regarding PCBs and their effect on the risk of CVD have not yet been conducted among Inuit in Greenland. Further research concerning the adverse effects of PCBs on the health of Inuit, should be conducted to assess if there is a potential association between PCBs and the risk of CVD, further studies regarding PCBs and their effects on lipids and BP should be assessed.

Concerning future research on mercury, it is suggested to investigate if there is an association between mercury and increased levels of lipids. According to a study, mercury increases LDL oxidation. High levels of LDL-cholesterol is associated with atherosclerosis (15).
However, studying genetic susceptibility of mercury could also contribute to a larger knowledge, since susceptibility to mercury may be increased by genetic factors (105).

Implication for policy

The traditional Greenlandic diet consists of a high intake of fish and marine mammals such as seal, whale and sea mammals. Fish and sea mammals are among the food items that contain the highest levels of environmental contaminants, exposing the Greenlandic population to a high intake of mercury. Greenland is the country within the Arctic, that have the highest levels of whole blood mercury (11, 106).

Among the present study participants, the geometric median level of whole blood mercury among men was 19 µg/L (IQR 0.9-44) and 15 µg/L (IQR 0.8-32) among women. The levels are just below the Health Canada guidelines for mercury blood concentrations of 20 µg/L, which is the level considered acceptable for the general adult population in relation to health (107).

The traditional Greenlandic diet also contains important trace elements and antioxidants such as selenium and unsaturated fatty acids which have shown to have favorable effects on health (94, 108, 109). The adverse health effects of the contaminants and the beneficial effects of selenium and n-3 fatty acids have led to the phenomenon known as the Arctic Dilemma (5, 108).

In 1997, The Greenland Board of Nutrition was established by the Ministry of Health (of the Greenlandic Government), with the purpose of collecting and documenting information about diet and health and assist the government in, establishing strategic plans for diet and nutrition. A major challenge to the board has been to advise the community about the intake of the traditional Greenlandic diet.
Through the years the consumption of the traditional diet consumed at least once per week (figure 3), has decreased (110). Younger people eat less traditional food compared to the elder generations and on average, traditional food only contributes to approximately 20% of the dietary intake (9, 110).

Figure 3.

Table 1. Difference in the amount of people in Greenland who consumed various traditional foods at least once per week in 1993 and in 2005–09. (1993: N=1,356–71; 2005–09: N=2,525–34). The model is adjusted for age and sex.

Food item	1993 (95% CI)	2005-09 (95% CI)	p-value
Seal	63% (61; 66)	37% (35; 39)	<0.0001
Whale	28% (25; 30)	27% (25; 28)	0.6
Fish	65% (63; 68)	58% (57; 60)	<0.0001
Reindeer	11% (9; 13)	21% (20; 22)	<0.0001
Game birds	42% (40; 45)	33% (31; 35)	<0.0001

Overall scientists in environmental health have pointed to the potential negative effects on health of the mercury in the meat, organs and blubber of seals and whales, willing to convey the alarming message to the public (111), in order to reduce the intake of mercury. However, The Greenlandic Board of Nutrition, encourage the population to continue eating the traditional diet (111, 112).

The association between whole blood mercury and the risk of CVD was assessed, and no association was found. The important modifiable risk factors for CVD, being unhealthy diet,

obesity, elevated blood glucose, physical inactivity and the underlying determinants of CVD including social, economic and cultural change (2, 27, 113) can explain why, whole blood mercury showed to have no effect on the risk of CVD in the present study. The lifestyle of the Inuit is becoming more sedentary and as a consequence of rapid changes in lifestyle the prevalence of obesity and diabetes has increased dramatically. In Greenland more than half of the Inuit population is overweight or obese when using body mass index as a measurement of obesity.

The consequences of the increasing levels of obesity and diabetes are serious in Greenland. The substantial changes which have taken place in Greenland the past 50 years, may have contributed to an increased incidence of CVD.
Early studies from Greenland indicated that CHD was infrequent as well as a low prevalence of IHD. Analyses indicated that mortality from stroke and cerebrovascular disease was high. Further it was concluded that mortality from CVD was higher among the Inuit than in white comparison populations (26, 114).
Studies of the Inuit in Greenland before the 1980s found a low prevalence of diabetes compared to Danes and other western populations. Recent studies of diabetes among the Greenlandic population found a high prevalence of 9.2% in the adult population (115, 116). Studies has concluded obesity in Greenland plays, a greater role for public health than the exposure to contaminants from the traditional diet (110, 111).
Thus, it is preferable that the Inuit in Greenland continue to consume the traditional diet, as it can have beneficial effects on health as mentioned above.
For future prevention of CVD in Greenland, further health care planning should focus on behavioral risk factors as physical activity and diet as well as biological risk factors such as obesity and diabetes.

5.0 Conclusion

The present study comprised 3083 participants with valid measures of whole blood mercury, and is, to the best of researchers knowledge, the first study to explore the association between whole blood mercury and the risk of CVD among the Greenlandic population.
Researcher found no association between whole blood mercury and the risk of CVD among the Greenlandic population.

Sensitivity analyses were conducted grouping the outcome overall CVD, into two groups consisting of CVD related to the heart CVD related to cerebrum, as well as assess potential interactions with sex. No association between whole blood mercury was found in both groups and potential interactions with sex were not significant.

Future research concerning mercury among the Greenlandic population, should focus on investigating genetic susceptibility of mercury, as susceptibility to mercury, may be increased by genetic factors.

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