Associations between vitamin D status and atherosclerosis among Inuit in Greenland

Camilla U. Gjødesen\textsuperscript{a}, Marit E. Jørgensen\textsuperscript{a,b,c}, Peter Bjerregaard\textsuperscript{a,c}, Inger K. Dahl-Petersen\textsuperscript{a}, Christina V. L. Larsen\textsuperscript{a}, Martin Noël\textsuperscript{d}, Mads Melbye\textsuperscript{e,f,g}, Arieh S. Cohen\textsuperscript{h}, Marika Lundqvist\textsuperscript{h}, David M. Hougaard\textsuperscript{h}, Jørn W. Helge\textsuperscript{i}, Nina O. Nielsen\textsuperscript{a,j}

\textsuperscript{a}National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark.
\textsuperscript{b}Steno Diabetes Centre Copenhagen, Gentofte, Denmark.
\textsuperscript{c}Greenland Centre for Health Research, University of Greenland, Nuuk, Greenland.
\textsuperscript{d}Université Laval, Quebec, Canada.
\textsuperscript{e}Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark.
\textsuperscript{f}Department of Clinical Medicine, University of Copenhagen, Denmark
\textsuperscript{g}Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
\textsuperscript{h}Department for Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark.
\textsuperscript{i}Center of Healthy Aging, Department of Biomedical Science, University of Copenhagen, Copenhagen, Denmark
\textsuperscript{j}Centre for Nutrition and Rehabilitation, University College Absalon, Næstved, Denmark (current address).

*Correspondence to: cugjoedesen@hotmail.com (C. U. Gjødesen)
Abstract

Background and aims: Low levels of vitamin D are suspected to be a risk factor for cardiovascular disease and atherosclerosis. The aim of this study was to assess the prevalence of subclinical atherosclerosis among Inuit in Greenland, and to evaluate the association with vitamin D status. We hypothesized that low vitamin D status could be associated with higher carotid intima-media thickness (IMT) as a marker of atherosclerosis.

Methods: 756 adults from the Inuit Health in Transition (IHIT) study carried out in Greenland in the period 2005-2010 were included. A blood sample donated in 1987 was available for a sub-sample of 102 individuals. Serum 25(OH)D3 from the IHIT study and the 1987 survey was used as a measure of vitamin D status. IMT measurements were conducted by ultrasound scanning. The prevalence of atherosclerosis was estimated, and the association between serum 25(OH)D3 and IMT measurements was examined by linear regression.

Results: The overall prevalence of subclinical atherosclerosis was 20.1% (n=152). The linear regression analyses indicated a weak positive association between serum 25(OH)D3 level and IMT measurements from the IHIT study, though not statistically significant after adjustment for potential confounders ($\beta=0.35\%$ per 10 nmol/l 25(OH)D3, $p=0.06$). Linear regression analyses of the association between serum 25(OH)D3 level in the 1987 survey and IMT measurements also indicated a positive, though not statistically significant, association after adjustment ($\beta=0.07\%$ per 10 nmol/l 25(OH)D3, $p=0.86$).

Conclusions: Our findings did not support the hypothesis of an association between low vitamin D levels and risk of atherosclerosis.
1. Introduction

Vitamin D is an essential fat-soluble vitamin with great importance for human health. There is an international consensus that vitamin D is important for maintenance of calcium homeostasis, bone health and prevention of fractures (1). Recent studies have identified low vitamin D as a new potential risk factor for atherosclerosis and cardiovascular disease in general (2, 3). Carotid atherosclerosis can be used as a clinical marker of systemic atherosclerosis due to early onset, location and easy detection by ultrasound (4). Additionally, the presence of carotid plaque and measurement of the carotid intima-media thickness (IMT) can be used as a surrogate marker of subclinical atherosclerosis (3).

In the Arctic, dermal production of vitamin D is low due to the large solar zenith angle (SZA), long periods with low sun exposure and the continuous need for protective outdoor clothing (5, 6). Additionally, the dark skin pigmentation in the Inuit population may reduce vitamin D synthesis in the skin (7). Previously, the high intake of traditional vitamin D rich diet containing fatty fish, seal and whale, probably compensated for the poor skin synthesis of vitamin D (5-7). During the last 50-60 years, the traditional diet of the Inuit has largely been replaced by imported foods (8), along with an observed lower vitamin D status in Arctic populations (6, 9, 10). A new study confirms that vitamin D deficiency is increasing in Greenland (10).

Formerly, it was a common notion that ischaemic heart disease is rare among Inuit in Greenland due to their marine diet with a high content of omega-3-fatty acids. However, the scientific evidence for this is weak since it is not based on systematic studies, but rather uncertain mortality statistics (11, 12). So far, only two studies have reported on atherosclerosis among Inuit in Greenland (13, 14). One of these reported that indigenous Greenlanders develop atherosclerosis to the same extent as Europeans (13), and the other concluded that the extent of advanced atherosclerosis in Greenlanders was similar to that reported among Alaska natives but less than
reported for Alaska non-natives (14). To our knowledge, no studies have investigated the association between vitamin D status and atherosclerosis in Arctic populations.

In this study, we hypothesized that low vitamin D levels were associated with higher risk of atherosclerosis among Inuit. We used serum 25-hydroxyvitamin D3 (25(OH)D3) concentration as a measure of vitamin D status and included blood samples collected in 2005-2010 and in 1987. Carotid intima-media thickness (IMT) was used as a surrogate marker of subclinical atherosclerosis. Subclinical atherosclerosis is hereafter referred to as atherosclerosis.

2. Materials and methods

2.1 Study population

The Inuit Health in Transition (IHIT) study, a general health study in Greenland and Canada that aimed to investigate health and diseases, lifestyle and life conditions in the Arctic regions, was carried out between 2005 and 2010 (15). One purpose of the IHIT study was to assess risk factors of cardiovascular disease to obtain a better understanding of the health effects of the transition from a traditional to a modern and industrialized lifestyle in the Arctic regions (15). Participants in the IHIT study in Greenland were selected through a stratified random sample of the adult population (+18 years) born in Greenland or Denmark. Greenland was divided into 10 regions based on geography and community size. From each region, one or more towns and villages were selected. The towns were selected as representative of the region in terms of living conditions (15). Villages were chosen randomly in the regions, and all adults living there were invited to participate (15). The total study sample consisted of 3108 Inuit (9.1% of the total adult population in 2005). The IHIT study population represented all age groups (+18 years), all geographical areas and all community sizes of Greenland. A valid serum 25(OH)D3 measurement was obtained from 2,877 individuals (93%) (10). 1,043 of the participants had an IMT measurement conducted (only a random selection
of individuals aged 40 years or above were examined), and 793 of these measurements were valid (individual mean IMT was based on a minimum of 6 segment measurements as described in section 2.4). Individuals with valid serum 25(OH)D3 and valid IMT measurements were included, which lead to the final number of 756 participants in the present study. Thus, due to invalid (less than 6 segment measurements available) data on IMT (n=250), missing data on IMT (due to random selection and cut-off at ≥ 40 years, n=1914), missing data on serum 25(OH)D3 (n=37) and ethnicity other than Greenlander (n=151), a total of 2352 individuals in the IHIT study were excluded from this study.

In 1987, due to a syphilis epidemic in western and southern Greenland, the health authorities in Greenland conducted a population-based serological examination. Among those that participated in the survey in 1987, we identified 102 who had a blood sample available for measurement of serum 25(OH)D3 and also had an IMT measurement conducted as part of the IHIT study in 2005-2010. The above described measurements allowed for an assessment of a cross-sectional association between 25(OH)D3 status and IMT in 2005-2010 and an association between 25(OH)D3 status in 1987 and development of IMT later in life (2005-2010) (fig. 1).

2.2 Collection of blood samples

Blood samples from 1987 were drawn from May to June, whereas samples from 2005-2010 were collected during all months of the year in the period from April 2005 to October 2010, except for July, November and December. Blood samples were handled and stored as described elsewhere (10).

2.3 Measurement of vitamin D
Serum 25(OH)D3 was used as a measure of vitamin D status. Samples were analysed after storage at -80 °C for 3-7 years (IHIT samples) and 26 years (1987-samples), respectively (10). Analyses were conducted by liquid chromatography-tandem mass spectrometry (LC-MSMS) using the “MSMS vitamin D” kit from Perkin Elmer (Waltham, MA) as described previously (16). This method measured both serum 25(OH)D2 and 25(OH)D3, but since concentrations of 25(OH)D2 were negligible, only 25(OH)D3 was included in the analysis.

2.4 Measurement of IMT by carotid ultrasonography

Measurements of the intimal to medial arterial wall thickness of the carotid arteries were performed using a high resolution B-mode ultrasound portable device (Model LogiqBook, GE Medical System, Milwaukee, WI, USA) with a linear 4–10 MHz probe (Model 10LB-Rs, GE Medical System, Milwaukee, WI, USA) by two experienced sonographers who conducted the ultrasound scanning according to a standard protocol (17, 18). A transverse scan was performed prior to a longitudinal scan in order to detect any hemodynamically relevant stenosis or presence of significant plaque defined by a focal echogenic structure protruded into the lumen vessel. Segments of 1 cm of the near and far walls of the Common carotid (1 cm below the flow divider), the Bifurcation and the Internal carotid (1 cm distal to the flow divider) arteries were digitally recorded and used for evaluation of the IMT. The segments had to be free of atherosclerotic plaque. All images were saved in digital imaging and communication in medicine (DICOM) database format. A single reader blinded to the clinical data performed off-line measurements. Analyses of 3 non-consecutive frames were completed using a dedicated semi-automatic edge detection software (Carotid analyser for research v5.5.6, Medical Imaging Application LLC, Coraville, IA, USA). When the analyser determined a region of interest (ROI) of 1 cm in length, the program automatically located the two interfaces defining walls (lumen-intima and media adventitia) using
algorithms based on grey level density and tissue recognition and drew two parallel lines. In case of visual discrepancies, the analyser had the possibility of editing the lines by fine-tuning the detection algorithms. IMT was measured every 10 µm of the length of the ROI (n = 100 successive local measures) for each frame. The measurements of each frame of the Common, Bifurcation and Internal carotid segments of the near and far wall on carotid artery in both right and left side of the neck were averaged and referenced as a mean IMT (17, 19). This study will refer to a mean IMT from each participant calculated as an average of available measured segments. However, to be a valid IMT, a minimum of 6 segment measurements were included in the mean IMT with at least one segment measurement from each frame (Common, Bifurcation and Internal) of both right and left carotid arteries. Atherosclerosis was categorized with the following cut-off values according to Riccioni et al. (20): No evidence of atherosclerosis (IMT < 0.8 mm), some atherosclerosis (0.8 mm ≤ IMT ≤ 1.2 mm) and extensive atherosclerosis (IMT > 1.2 mm).

2.5 Potential confounders

Information on age, sex and smoking was collected by interviews as part of the IHIT study. Hepatic insulin resistance (HOMA-IR) was calculated using the formula: fasting plasma glucose (mmol/L) x fasting plasma insulin (mU/L)/22.5 (21). Systolic BP was measured three times with an automatic measuring device (Kivex UA-779) with an appropriately sized cuff. The last two measurements were averaged and used for the analysis. Physical activity (PAEE) was recorded by the International Physical Activity Questionnaire (long version IPAQ), modified and validated for the living conditions of the Greenlandic population (22). Anthropometric measurements were used to calculate body mass index (BMI) as: weight in kg/(height in m²). HDL and triglycerides were measured in serum by automated enzymatic colorimetric methods. LDL cholesterol was calculated with the Friedewald formula. Methyl mercury was measured by inductively coupled mass spectrometry (ICP-MS) at the Centre de Toxicologie, Institut National de Santé Public, Québec,
Canada, as described previously (23). Information about genetic admixture was estimated from blood tests where the admixture proportion of Inuit and European ancestry was quantified. The analyses have accurately quantified the extent of European and Inuit ancestry in the participants included in the present study (24).

2.6 Ethics

Written informed consent was obtained from each participant included in the IHIT study, the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and it has been reviewed and approved by the Ethical Review Committee for Greenland.

2.7 Statistical methods

All statistical analyses were performed using the statistical software STATA version 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). The associations between serum 25(OH)D3 and IMT as a measure of atherosclerosis were explored by linear regression analysis with serum 25(OH)D3 as the explanatory variable and IMT measures as the outcome variable. IMT measures did not follow a normal distribution and were therefore log-transformed to achieve normal distribution before entered into the linear regression model. β-estimates from linear regression models were back-transformed and presented as a percentage increase per 10 nmol/L increase in serum 25(OH)D3. The cross sectional association between IMT and serum 25(OH)D3 measured in the IHIT study was assessed in three linear regression models and adjusted for an increasing number of confounders. Model 1: Age, sex and genetic admixture. Model 2: As model 1 + BMI, smoking, HDL, triglycerides, systolic BP, HOMA-IR and PAEE. Model 3: As model 2 + methyl mercury. The association between serum 25(OH)D3 measured in the 1987 survey and IMT measured in IHIT was assessed in two linear regression models: Model 1:
3. Results

3.1 Characteristics of the study population

In the IHIT study, the median age was 46 years (interquartile range, IQR, 40-56 years), the median serum 25(OH)D3 concentration was 54 nmol/L (IQR 35-75 nmol/L) and the median IMT was 0.69 mm (IQR 0.61-0.77 mm). A sub-group of 485 participants from the IHIT study had previously participated in the 1987 survey, and among them, data on IMT and serum 25(OH)D3 was available from 102 individuals. Of the 102 individuals in the 1987 sample, 52 (51%) were men. The median age was 31 years (IQR 23-39) in 1987, median serum 25(OH)D3 concentration was 72 nmol/L (IQR 49-109 nmol/L) and median IMT was 0.70 mm (IQR 0.62-0.80 mm).

Table 1 gives characteristics of the study population in 1987 and 2005-2010 by sex. The prevalence of smokers was 69% among men and 72% among women. The degree of genetic admixture was 82% for both men and women. Median PAEE was higher among men than women (56 kj/kg/day vs. 46 kj/kg/day). IFG was more prevalent among men than women (23% vs. 16%).

3.2 Prevalence of atherosclerosis using IMT as a surrogate marker

Based on IMT measurements, three (0.4%) of the 756 individuals had extensive atherosclerosis and 149 (19.7%) had some atherosclerosis. Thus, according to IMT, the overall prevalence of atherosclerosis was 20.1% in the study population.

3.3 Associations between IMT and serum 25(OH)D3 in the IHIT sample

Unadjusted. Model 2: Adjusted for age, sex and genetic admixture. In all models, a quadratic term of age was included in order to take into account the non-linear effect of age on the association between serum 25(OH)D3 and IMT.
Table 2 shows the association between serum 25(OH)D3 level and IMT measurements in the IHIT sample. In model 1, the estimate indicated no association between serum 25(OH)D3 level and IMT measurements after adjustment for age, sex and genetic admixture. After additional adjustment for BMI, smoking, HDL, triglycerides, systolic BP, HOMA-IR and PAEE (model 2), the estimate indicated a positive association between serum 25(OH)D3 and IMT. Thus, increasing serum 25(OH)D3, expressed as 10 nmol/L, was associated with increasing IMT (0.41%, 95% confidence interval (CI): 0.07% – 0.75%, \( p=0.02 \)). However, after additional adjustment for methyl mercury (model 3), the association disappeared (0.35%, CI: -0.01% – 0.71%, \( p=0.06 \)).

3.4 Associations between IMT and serum 25(OH)D3 in the 1987 sample

A linear unadjusted regression analysis (n=102) of the association between serum 25(OH)D3 level in the 1987 sample and IMT measurements in the IHIT sample showed a positive association between serum 25(OH)D3 level and IMT (1.1%, CI: 0.4% -1.9%, \( p=0.003 \)). However, after adjustment for age, sex and admixture, the association disappeared (0.07%, CI: -0.68% – 0.82%, \( p=0.86 \)).

4. Discussion

The overall prevalence of atherosclerosis was 20.1% according to the applied definitions (IMT ≥ 0.8). A Canadian study among Inuit in Nunavik found that 11.5 % of the study population had an IMT greater than 1 mm (19). That study referred to a higher risk of cardiovascular disease if IMT was greater than 1 mm, however, not linking IMT directly to atherosclerosis. The difference in cut-off between the Nunavik study and our study may at least in part explain the difference in the prevalence of atherosclerosis based on IMT. A study among Alaskan Eskimos reported that carotid atherosclerosis assessed by ultrasound was more commonly detected in that population compared to American populations (25), but the specific prevalence was not reported. A Canadian study
examining a multi-ethnic low-risk population found an overall prevalence of atherosclerosis of 23% (26). In that study, subclinical atherosclerosis was defined according to measures of mean maximum cIMT ≥ 75th percentile and is therefore difficult to compare directly to the present study. The Manfredonia Study, which examined an Italian population and used the same cut-off as used in the present study, found an atherosclerosis prevalence of 54% (27). However, a higher mean age in the Manfredonia Study may explain the higher prevalence. Most results are obviously not in line with the notion that Inuit may have lower risk of cardiovascular disease due to their intake of traditional Arctic diet with a high content of omega 3-fatty acids. As indicated above, there are challenges by comparing the prevalence of atherosclerosis among different populations due to methodological inconsistencies in the current literature.

The present study is, to our knowledge, the first to report on the association between serum 25(OH)D3 and atherosclerosis in an Inuit population. In contrast to our hypothesis, we found a weak positive association between 25(OH)D3 and IMT measures, though not statistically significant after adjustment for age, sex, genetic admixture, BMI, smoking, HDL, triglycerides, systolic BP, HOMA-IR, PAEE and methyl mercury. Previous studies in other populations evaluating the association between 25(OH)D levels and atherosclerosis assessed by IMT have shown conflicting results. In The Multi-Ethnic Study of Atherosclerosis including 3251 low risk participants, 25(OH)D were not significantly associated with IMT (28). Furthermore, in a multi-center study examining the association between serum 25(OH)D concentrations and IMT measures in 3430 middle-aged and elderly subjects with high CVD risk found no independent relationship between 25(OH)D and segment-specific or composite IMT measures (29). In contrast, several studies have found that low 25(OH)D levels were associated with higher IMT (3, 30-32) and low vitamin D seems also associated with adverse CV outcome (33). One study showed a positive association between serum 25(OH)D and IMT among patients with sufficient 25(OH)D levels (≥50
nmol/L) (34). Overall, most studies conducted in non-Inuit populations found that low vitamin D was associated with atherosclerosis. The present study indicates, however, that 25(OH)D3 plays a minor, if any, role in the development of atherosclerosis in an Arctic population. It is reasonable to consider if the inverse association found between 25(OH)D and atherosclerosis in some studies may be explained by residual confounding, e.g. predisposing cardiovascular risk factors, genetic factors, sun exposure and dietary factors. The lack of an association is in agreement with findings from Mendelian Randomization studies and a meta-analysis of eight RCTs suggesting that associations between 25(OH)D and atherosclerosis in epidemiological studies result from reverse causation or residual confounding (35, 36).

In the present study, methyl mercury was perceived as a classic potential confounder due to its association with both vitamin D and IMT. Environmental pollutants and methyl mercury have been shown to be associated with cardiovascular outcomes (23, 37-39) and IMT (40, 41). Of particular interest to Arctic populations, some studies suggest that Inuit who eat a traditional diet with a high intake of fatty fish, seal and whale, and therefore have a high intake of vitamin D (10), are at the same time highly exposed to environmental pollutants including methyl mercury (23, 42). Environmental pollutants and mercury bioaccumulate in marine mammals and fish and are transferred to humans through the diet. Mercury is present in the blood and organs of Greenlanders in concentrations well above international guidelines (39, 43). In the present study, the positive association between serum 25(OH)D3 and IMT was eliminated after adjustment for methyl mercury. A possible explanation could be that the association was “carried” by methyl mercury. Other environmental contaminants and heavy metals specific for the arctic area, such as polychlorinated biphenyls and cadmium (44), are reasonable to consider as factors with possible impacts on the findings.
The Inuit population is highly genetically differentiated from other populations, because of being isolated and affected by bottlenecks in population size (45), and diet and climate adaptation in the Arctic has favored genetic variants associated with severe insulin resistance and increased susceptibility to cardiometabolic disease (46, 47). In the present study, the population structure was taken into account by adjusting the association between 25(OH)D3 and IMT for genetic admixture, with the aim of minimizing the risk of confounding by this factor.

There was a positive association between serum 25(OH)D3 level in the 1987 sample and IMT measurements from the IHIT sample, though not statistically significant after adjustment for age, sex and genetic admixture. Thus, the longitudinal findings confirmed the absence of an association in the cross-sectional analysis. According to the literature review, only the Multi-Ethnic Study of Atherosclerosis, with 3251 low-risk participants, examining serum 25(OH)D in relation to IMT, had a longitudinal design (10 years follow-up) (28), and the finding of no association is consistent with the findings in the present study. However, a limitation in the present study is the lack of information on IMT from the 1987 sample. Furthermore, adjustments for confounders were limited to age, sex and genetic admixture, which may have resulted in residual confounding. Additionally, the sample size of 102 participants in this part of the study may not provide sufficient power to detect a possible association. However, the finding of a similar association pattern with IMT when using 25(OH)D3 measurements from the 1987 sample and from the IHIT sample indicates some robustness of the findings.

Strengths of the study include the use of serum 25(OH)D3 measurements from a large population based and geographically representative study of Inuit in Greenland, who also had a carotid ultrasonography. Vitamin D status (serum 25(OH)D3) was measured in blood samples and not estimated from questionnaires, which minimizes the risk of misclassification. Another major strength is that detailed ultrasound measurements of IMT from both right and left carotid arteries
were performed and 6-12 measurements were included. The American Heart Association has endorsed the use of IMT in cardiovascular risk assessment (17) and B-mode ultrasound IMT measurements are seen as the best current method to provide objective information of the degree of subclinical atherosclerosis (26, 48). Thus, both exposure and outcome are objectively measured and not based on self-reports. Furthermore, methyl mercury, HDL, triglycerides, genetic admixture and HOMA-IR levels were obtained from measurements in serum samples, and detailed information on physical activity was available from the IPAQ validated questionnaire and used for adjustment of the analyses.

In conclusion, this study is the first to examine the association between vitamin D and atherosclerosis among Inuit in Greenland. No longitudinal or cross sectional association was found between serum 25(OH)D3 and IMT in the IHIT sample and the 1987 sample, and based on these results it seems reasonable to conclude that vitamin D has no impact on the development of atherosclerosis among Inuit in Greenland. Genetic status is likely overruling a possible role of vitamin D in the development of atherosclerosis in this population. Furthermore, the contribution of lifestyle related risk factors such as smoking, physical inactivity and a diet containing high levels of saturated fat and sugar might affect the development of atherosclerosis substantially more than vitamin D status.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.
Financial support

The study was supported by the Government of Greenland’s health department (2011-047012/2012-074239), Aase og Ejnar Danielsen’s Foundation (10-000444), Dagmar Marshalls Foundation, Karen Elise Jensens Foundation and Nuna Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

C.U.G., N.O.N., M.E.J and J.H. conceived and designed the study. C.U.G. analyzed the data and wrote the manuscript. N.O.N. assisted with data analysis. P.B., I.D.P, C.V.L.L, M.E.J. and M.N. collected the IHIT data. M.M., A.S.C., M.L. and D.M.H. contributed with study materials and analysis tools. All authors reviewed the manuscript and contributed with critical comments to the content. All authors approved the submitted version of the manuscript. C.U.G. is the guarantor of this work, and, as such, had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements

The authors thank the study participants for participating in the study. Mrs. Ingelise Olesen is thanked for her efforts in the practical organization of the IHIT study and participation in data collection, and the interviewers are thanked for their valuable assistance in data collection. The late Eric Dewailly is thanked for his contribution to the collection of the ultrasound measurements. Colleagues from the laboratories of the Steno Diabetes Centre Copenhagen, Gentofte, Denmark, in particular Mrs. Maja Lis Dybdahl Halkjær, are thanked for indispensable assistance with handling of the blood samples.
References


Fig. 1. Selection of study participants.
Flow chart of the IHIT study (2005-2010) and 1987 survey.
Table 1. Characteristics of the study populations in 1987 (n=102) and 2005-2010 (n=756) according to sex.

<table>
<thead>
<tr>
<th></th>
<th>1987 sample</th>
<th></th>
<th></th>
<th>IHIT sample (2005-2010)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Men</td>
<td>n</td>
<td>Women</td>
<td>n</td>
<td>Women</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52</td>
<td>33 (25-40)</td>
<td>50</td>
<td>27 (21-36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25(OH)D3 (nmol/L)</td>
<td>52</td>
<td>77 (48-120)</td>
<td>50</td>
<td>66 (50-92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>52</td>
<td>0.72 (0.65-0.86)</td>
<td>50</td>
<td>0.66 (0.59-0.77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Men</td>
<td>n</td>
<td>Women</td>
<td>n</td>
<td>Women</td>
</tr>
<tr>
<td>Age (years)</td>
<td>376</td>
<td>47 (40-57)</td>
<td>380</td>
<td>45 (40-59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>373</td>
<td>24 (22-28)</td>
<td>379</td>
<td>25 (22-29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D3 (nmol/L)</td>
<td>376</td>
<td>55 (35-78)</td>
<td>380</td>
<td>53 (34-72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>376</td>
<td>0.71 (0.62-0.81)</td>
<td>380</td>
<td>0.67 (0.60-0.74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity energy expenditure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(kJ/kg/day)</td>
<td>375</td>
<td>56 (23-104)</td>
<td>380</td>
<td>46 (24-73)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>376</td>
<td>1.6 (1.3-2.0)</td>
<td>380</td>
<td>1.7 (1.4-2.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>376</td>
<td>3.5 (2.9-4.1)</td>
<td>380</td>
<td>3.5 (2.9-4.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>376</td>
<td>0.9 (0.7-1.3)</td>
<td>380</td>
<td>1.0 (0.8-1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>376</td>
<td>5.8 (5.4-6.2)</td>
<td>380</td>
<td>5.6 (5.2-6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2h plasma glucose (mmol/L)</td>
<td>369</td>
<td>5.2 (4.1-6.5)</td>
<td>376</td>
<td>5.7 (4.7-6.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>376</td>
<td>28 (20-44.5)</td>
<td>380</td>
<td>40.5 (27-57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2h insulin (pmol/L)</td>
<td>369</td>
<td>63 (32-142)</td>
<td>375</td>
<td>139 (77-231)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>376</td>
<td>7.3 (4.9-12.1)</td>
<td>380</td>
<td>10.3 (6.6-14.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>376</td>
<td>130 (121-145)</td>
<td>380</td>
<td>122 (111-136)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>376</td>
<td>79 (70-89)</td>
<td>380</td>
<td>75 (68-83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mercury (µg/L)</td>
<td>376</td>
<td>24 (10-49)</td>
<td>379</td>
<td>19 (9-37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genetic admixture (%)</td>
<td>374</td>
<td>82 (63-99)</td>
<td>379</td>
<td>82 (66-99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>375</td>
<td>69</td>
<td>380</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFG (%)</td>
<td>314</td>
<td>23</td>
<td>332</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>Count</td>
<td>Min</td>
<td>Max</td>
<td>Proportion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-------</td>
<td>-----</td>
<td>-----</td>
<td>------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGT (%)</td>
<td>252</td>
<td>4</td>
<td>294</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2DM (%)</td>
<td>376</td>
<td>11</td>
<td>380</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Median with interquartile range and proportions are presented.
Table 2. Linear regression analysis of the cross sectional associations between serum 25(OH)D3 level and IMT measurements in the IHIT sample (n=753).

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted for age, sex and genetic admixture</td>
<td>Additionally adjusted for BMI, smoking, HDL, triglycerides, systolic BP, HOMA-IR and physical activity</td>
<td>Additionally adjusted for methyl mercury</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>n</th>
<th>Change (95% CI)</th>
<th>p-value</th>
<th>n</th>
<th>Change (95% CI)</th>
<th>p-value</th>
<th>n</th>
<th>Change (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>753</td>
<td>0.51% (-0.39-1.43)</td>
<td>p=0.27</td>
<td>665</td>
<td>0.41% (0.07-0.75)</td>
<td>p=0.0</td>
<td>664</td>
<td>0.35% (-0.01-0.71)</td>
<td>p=0.06</td>
</tr>
</tbody>
</table>

Percentage change in IMT per 10 nmol/L increase in serum 25(OH)D3
Fig. 1. Selection of study participants. Flow chart of the IHIT study (2005-2010) and 1987 survey.
Highlights

- The prevalence of atherosclerosis based on intima media thickness (IMT) was 20.1%.
- No association was found between serum vitamin D (25(OH)D3) and IMT.
- Genetic status and lifestyle factors may overrule the role of vitamin D.