**SUPPLEMENTARY MATERIAL**

**Biobanking and blood analyses**

This appendix shows an overview of the data collection and sampling (Table S1 and Figure S1). In addition, it describes the biobank and the analyses performed on blood samples (Tables S2 and S3).Glycated hemoglobin (HbA1c) and hemoglobin (Hb) were analyzed immediately on whole blood collected in a BD Vacutainer® K2 ethylene diamine tetraacetic acid (EDTA) 7.2 mg, 4 ml, REF# 368861). Remaining blood samples were sequentially processed into cryo-vials or pre-rinsed glass vials (serum PTS only): whole blood from one BD Vacutainer® (Trace element, K2 EDTA 10.8 mg, 4 ml, Ref# 368381; BD, Franklin Lakes, USA); and, serum and clot, both extracted from centrifuged (38 X for 10 minutes) 3 x BD Vacutainer® (SST™ II Advance, 10/8.5 ml, Ref# 367953).

Almost all laboratory analyses were performed at the Laboratory of the Department of Clinical Chemistry, University Hospital of North Norway (UNN), Tromsø from September 2014 to November (Table 2). Vitamin D was analyzed at the Department of Food and Environmental Sciences, University of Helsinki, Finland. Contaminants and toxic and essential elements were analyzed on parts of the sample at the Norwegian Institute for Air Research (NILU), Tromsø, Norway, and National Institute of Occupational Health (STAMI), Oslo, Norway, respectively.

Information about the different blood samples, dates of analysis and total numbers are included in Tables 1 and 2 of this appendix.

**Table S1.** Overview of the data collection. The SAMINOR 2 Clinical Survey (2012-2014).

|  |  |
| --- | --- |
|  | **Invitation materials** |
| **Municipalities** | **Collection period 1** | **Number of fieldworkers** | **Pamphlet/informational brochure** | **Questionnaire** | **Invitation letter** |
| Skånland/Evenes | 2012 Sept. 17th – Oct. 25 th | 11 | Norwegian | Norwegian | Norwegian |
| Karasjok | 2013 Jan. 28th – Febr. 21 th | 14 | Norwegian/Sami | Norwegian/Sami | Norwegian/Sami |
| Kautokeino | 2013 Febr. 25th – Mar. 21th | 10 | Norwegian/Sami | Norwegian/Sami | Norwegian/Sami |
| Porsanger | 2013 Apr. 15th – May. 30th | 9 | Norwegian/Sami/Kven | Norwegian 2 | Norwegian/Sami |
| Kåfjord | 2013 Sept. 16th – Oct. 11th | 9 | Norwegian/Sami | Norwegian 2 | Norwegian |
| Storfjord | 2013 Oct. 16th – Nov. 7th | 7 | Norwegian/Sami/Kven | Norwegian 2 | Norwegian |
| Nesseby | 2014 Febr. 12th – Febr. 25th | 6 | Norwegian/Sami | Norwegian/Sami | Norwegian/Sami |
| Tana | 2014 Febr. 27th – Apr. 3th | 11 | Norwegian/Sami | Norwegian/Sami | Norwegian/Sami |
| Lyngen | 2014 May. 7th – June. 12th | 10 | Norwegian/Sami | Norwegian 2 | Norwegian |

1 In some municipalities the health examination site was closed from 1 up to 4 weekdays due to public holidays

2 The Sami questionnaire was available on request

**Original sample: 12,577**

Dead, moved or incorrect address: 118

Duplicates (invited twice): 4

Non-responders: 6,451

**Completed the questionnaire:5,983**

**Provided blood sample and completed questionnaire:**

**5,956**

**Provided blood sample:5,976**

**Eligible sample:12,455**

**Figure S1**. Sample description of the SAMINOR 2 Clinical Survey (2012 – 2014)

**Table S2.** Collected blood samples. The SAMINOR 2 Clinical Survey (2012-2014).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | n | Type of tube | Amount | Stored at |
| Total number of participants | 6004 |  |  |  |
| Agreed to blood sampling | 5998 |  |  |  |
| Blood sampling performed | 5996 |  |  |  |
| Whole blood designated for Hb | 5991 | EDTA | 4ml | – |
| Whole blood designated for HbA1c | 5982 | EDTA | 4ml | – |
| Whole blood designated for metal analyses | 5974 | Cryo | 2ml | -20°C/-35°C |
| Serum designated for lipid analyses | 5976 | Cryo | <2ml | -20°C/-70°C |
| Serum designated for Vitamin D analyses | 5954 | Cryo | 1ml | -20°C/-70°C |
| Serum designated for contaminant analyses | 5953 | Cryo | 2ml | -20°C/-70°C |
| Whole blood for storage in biobank | 5978 | Cryo | 2ml | -20°C/-35°C |
| Serum sample 1 for storage in biobank | 5921 | Cryo | 2ml | -20°C/-70°C |
| Serum sample 2 for storage in biobank | 5829 | Cryo | 2ml | -20°C/-70°C |
| Serum sample 3 for storage in biobank | 4039 | Cryo | 2ml | -20°C/-70°C |
| Clot (DNA) for storage in biobank | 5975 | SST | 10ml | -20°C/-70°C |

**Table S3.** Overview of the analyzed blood samples. The SAMINOR 2 Clinical Survey (2012-2014).

|  |  |  |
| --- | --- | --- |
| Table  | n | Date of analysis |
| At least one blood analysis available | 5996 |  |
| Hb | 5991 | 17 Sep 2012-12 Jun 2014 |
| HbA1c | 5982 | 17 Sep 2012-12 Jun 2014 |
| Serum analyzed UNN | 5975 | 6 Sep 2014-9 Nov 2014 |
|  | s-Ferritin | 5975 | 6 Sep 2014-9 Nov 2014 |
|  | s-Transferrin | 5972 | 6 Sep 2014-9 Nov 2014 |
|  | s-Iron | 5974 | 6 Sep 2014-9 Nov 2014 |
|  | Vitamin B12 | 5974 | 6 Sep 2014-9 Nov 2014 |
|  | Folate | 5866 | 6 Sep 2014-9 Nov 2014 |
|  | HS-CRP | 5972 | 6 Sep 2014-9 Nov 2014 |
|  | Random plasma glucose | 5974 | 6 Sep 2014-9 Nov 2014 |
|  | Apolipoprotein-A | 5974 | 6 Sep 2014-9 Nov 2014 |
|  | Apolipoprotein-B | 5973 | 6 Sep 2014-9 Nov 2014 |
|  | Total cholesterol | 5974 | 6 Sep 2014-9 Nov 2014 |
|  | LDL cholesterol | 5939 | 6 Sep 2014-9 Nov 2014 |
|  | HDL cholesterol | 5974 | 6 Sep 2014-9 Nov 2014 |
|  | Triglycerides | 5975 | 6 Sep 2014-9 Nov 2014 |
|  | Transferrin saturation | 5971 | 6 Sep 2014-9 Nov 2014 |
| 25-hydroxy-vitamin D analyzed at Helsinki University | 5953 | 2 Jun 2016 |
| Toxic and essential elements analyzed at STAMI | 470 | 27 Apr 2016 |
| Contaminants analyzed at NILU | 462 | 20 Apr 2017 |

***Description of blood analyses***

Reagents were purchased from the same company.

**Hemoglobin**

Hb was analyzed by the hemoglobincyanide (HiCN) method on a HemoCue Hb 201+ [1](#_ENREF_1). A drop of blood was placed on a hydrophobic surface, e.g., plastic fil, using a pipette, and a microcuvette was filled. The internal and external quality controls showed values within established control limits. Internal quality control was conducted daily with heamolysate.

**Glycated haemoglobin**

HbA1c was analyzed with The DCA Vantage™ (Siemens Medical Solutions Diagnostics, Tarrytown, NY), which is based on latex agglutination inhibition immunoassay methodology and provides results in 6 minutes [2](#_ENREF_2). This is the successor of the DCA 2000™. Internal and external quality controls showed values within established control limits. The internal quality control was conducted daily or when new reagents were opened. The inter-assay coefficient for variations (CV) for HbA1c was <3% [3](#_ENREF_3).

**Serum ferritin**

Serum ferritin (s-ferritin) was measured on the Cobas 8000 system from Roche/Hitachi with an electrochemiluminescense immunoassay (ECLIA) [4](#_ENREF_4) using the sandwich principle. Ferritin ( REF 04491785) has been a standardized against the Ferritin assay (REF 11820982). The Ferritin assay (REF 11820982) has been standardized against the Enzymun – Test Ferritin method. This in turn has been standardized against the 1st International Standard (IS) National Institute for Biological Standards and Control (NIBSC) “Reagent for Ferritin (human liver)” 80/602 [5](#_ENREF_5). The analyzer automatically calculates the analyte concentration of each sample in µg/l.

**Serum transferrin**

Transferrin was measured on the Cobas 8000 system from Roche/Hitachi with a by immunoturbidimetric assay using human transferrin, which forms a precipitate with a specific antiserum [6](#_ENREF_6),[7](#_ENREF_7). This system automatically calculates the analyte concentration of each sample in mg/dlx 0,01=g/l. This method has been standardized against the reference preparation of the Institute for Reference Materials and Measurements (IRMM) BCR470/CRM470 (Reference Preparation for Proteins in Human Serum, RPPHS) [5](#_ENREF_5),[8](#_ENREF_8).

**Serum iron**

Serum iron was measured on the Cobas 8000 system from Roche/Hitachi with a colorimetric method. This method has been standardized against a primary reference material (SRM 937) [5](#_ENREF_5),[9](#_ENREF_9).

**Vitamin B12**

Vitamin B12 was measured on the Cobas 8000 system from Roche/Hitachi with by ECLIA [5](#_ENREF_5),[10](#_ENREF_10),[11](#_ENREF_11) using the competitive principle. Results were determined via a calibration curve, which is an instrument specifically generated by 2-point calibration and a master curve provided by the reagent barcode. This method has been standardized against the Vitamin B12 assay (REF 11820753) [5](#_ENREF_5). The analyzer automatically calculates the analyte concentration of each sample in pmol/l or pg/mL.

**Folate**

Folate was measured on the Cobas 8000 system from Roche/Hitachi with ECLIA [11](#_ENREF_11) using the competitive principle. The method has been standardized against World Health Organization International Standard NIBSC-code:03/178, where earlier generations are traceable to “Bio-Rad Quantaphase IIB12/Folat Radioassay [5](#_ENREF_5).

**Glucose**

Glucose was measured on the Cobas 8000 system from Roche/Hitachi using an *in vitro* test for the quantitative determination of glucose in human serum. The test principle is an ultraviolet test with enzymatic references method with hexokinase [12](#_ENREF_12). Glucose values for human serum obtained on the Roche/Hitachi c 701 analyzer (y) were compared with those determined using the same reagent on the Roche/Hitachi cobas 501 analyzer (x). This method has been standardized against **isotope dilution mass spectrometry reference measurement procedure** [5](#_ENREF_5). The analyzer automatically calculates the analyte concentration of each sample by conversion factor mg/dl x 0.0555= mmol/l.

[**High-Sensitivity C-Reactive Protein**](https://labtestsonline.org/tests/high-sensitivity-c-reactive-protein-hs-crp)

[High-Sensitivity C-Reactive Protein](https://labtestsonline.org/tests/high-sensitivity-c-reactive-protein-hs-crp) was measured on the Cobas 8000 system from Roche/Hitachi with an immunoturibidimetric assay [13](#_ENREF_13),[14](#_ENREF_14). The method has been standardized against the reference preparation of the IRMM BCR470/CRM470 (RPPHS)[5](#_ENREF_5),[15](#_ENREF_15),[16](#_ENREF_16). The analyzer automatically calculates the analyte concentration of each sample by conversion factor mg/Ll x 9.52= nmol/L.

**Apolipoprotein A**

Apolipoprotein A was measured on the Cobas 8000 system from Roche/Hitachi with an immunoturibidimetric assay [17](#_ENREF_17),[18](#_ENREF_18). The method has been standardized against the IFCC SP1-01 reference standard ( WHO-IRP October 1992) [5](#_ENREF_5). The analyzer automatically calculates the analyte concentration of each sample in by conversion factor mg/dL x 0.01= g/L.

**Apolipoprotein B**

Apolipoprotein B was measured on the Cobas 8000 system from Roche/Hitachi with a immunoturibidimetric assay [17](#_ENREF_17),[18](#_ENREF_18). The method has been standardized against the IFCC SP3-07 reference standard ( World Health Organization-IRP October 1992) [5](#_ENREF_5). The analyzer automatically calculates the analyte concentration of each sample by conversion factor mg/dL x 0.01= g/L .

**Cholesterol**

Cholesterol was measured on the Cobas 8000 system from Roche/Hitachi with a homogeneous enzymatic colorimetric method [19](#_ENREF_19),[20](#_ENREF_20). The method has been standardized against the designated Centers for Disease Control reference method (designated comparison method). The standardization meets the requirements of the “HDL Cholesterol Method Evaluation Protocol for Manufactures” of the US national Reference System of Cholesterol, (Cholesterol Reference Method Laboratory Network, CRMLN), November 1994 [5](#_ENREF_5). The analyzer automatically calculates the analyte concentration of each sample by conversion factor mg/dL x 0.0259=mmol/L.

**Low-density lipoprotein Cholesterol**

Low-density lipoprotein(LDL) Cholesterol was measured on the Cobas 8000 system from Roche/Hitachi with a homogeneous enzymatic colorimetric method [20-22](#_ENREF_20). The method has been standardized against the beta quantification method as defined in the recommendations in the LDL Cholesterol Certification Protocol for Manufacturers [5](#_ENREF_5). The analyzer automatically calculates the analyte concentration of each sample by conversion factor mg/dL x 0.0259= mmol/L.

**High-density lipoprotein Cholesterol**

High-density lipoprotein(HDL) Cholesterol was measured on the Cobas 8000 system from Roche/Hitachi with a homogeneous enzymatic colorimetric method. The method has been standardized against the designated CDC reference method (designated comparison method) [23](#_ENREF_23). The standardization meets the requirements of the “HDL Cholesterol Method Evaluation Protocol for Manufacturers” of the US National Reference System of Cholesterol, Cholesterol Reference Method Laboratory Network (CRMLN), November 1994 [5](#_ENREF_5). The analyzer automatically calculates the analyte concentration of each sample by conversion factor mg/dL x 0.0259=mmol/L.

**Triglycerides**

Triglycerides were measured on the Cobas 8000 system from Roche/Hitachi with a homogeneous enzymatic colorimetric method. The method has been standardized against the designated ID/MS method [5](#_ENREF_5),[14](#_ENREF_14),[24](#_ENREF_24). The analyzer automatically calculates the analyte concentration of each sample by conversion factor mg/dL x 0.0113=mmol/L.

**25-hydroxy-vitamin D**

25-hydroxy-vitamin D [25(OH)D]was measured by the IDS-iSYS 25-Hydroxy Vitamin Dˢ assay on the IDS-iSYS analyzer (IDS Ltd., Boldon, UK).25(OH)D analysis in serum blood samples was performed at the Department of Food and Environmental Sciences, University of Helsinki. The laboratory method is standardized, validated and certified by “The vitamin D Standardization Program” (VDSP) <https://ods.od.nih.gov/Research/vdsp.aspx>.

**Contaminants**

For details of chemicals analyses of contaminants, we refer to former [25](#_ENREF_25) and up-coming publications.

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