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# Methods used in the Northern Sweden Health and Disease study (NSHDS)

This document provides a summary of methods and algorithms designed for the optimal utilization of phenotypic data stored for participants in the NSHDS biobank. There are a few shifts in methodology used during the period in which samples and data were collected, which are relevant primarily for time-trend analyses. These shifts are related to blood pressure, blood glucose, and blood lipids and are described in detail later in this document.

Further information on the NSHDS cohorts can be found at the webpage: https://www.umu.se/en/brs/provsamlingar-och-register/nshds/ and in the **cohort profile published in 2025** (Späth F et al. Cohort Profile: The Northern Sweden Health and Disease Study (NSHDS). Int J Epidemiol. 2024 Dec 16;54(1):dyaf004. doi: 10.1093/ije/dyaf004. PMID: 39899988; PMCID: PMC11790227).

| | |
|---|---|
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| Scientific board: | Stefan Söderberg, Johan Hultdin & Patrik Wennberg. |
| Referees: | Veronica Hellström, Ulrika Morris, and Robert Johansson, at the Section of Biobank and Registry Support Research Unit, as well as Ingvar Bergdahl, Associate professor at Department of Epidemiology and Global Health, and Viktor Oskarsson, Department of Public Health and Clinical Medicine, who took part in the discussions and Elisabet Söderström, Clinical Chemistry at Sunderby Hospital provided who data on laboratory methods from Region Norrbotten. |
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# Västerbotten Intervention Program (VIP), Methods for CVD risk factors

| **Cohort [1, 2]** |
|---|
| 1985-1991: Health examinations could be performed either in the morning or after lunch, and hence the fasting period was at least 8 hours or at least 4 hours, respectively.<br><br>1992-2018: A fasting period of minimum of 8 hours before blood sampling was required. In practice, this implied an overnight fast and blood sampling was done in the morning.<br><br>From 2018: A fasting period of minimum of 9 hours before blood sampling is required. |
| **Blood pressure** |
| 1985-2000: Blood pressure was measured manually once, usually to the nearest 5 or 10 mm with a mercury sphygmomanometer after 5 min rest with the subject in a recumbent position.<br><br>2000-2008: Health care centres increasingly, and after their own decisions, shifted to automated digital blood pressure measurements using calibrated devices.<br><br>Sept. 2009-2020: Blood pressure was measured twice with a mercury sphygmomanometer (precision 2 mm) or automated digital blood pressure measurements using calibrated devices after 5 min rest with the subject in a sitting position. The mean of the two measurements of systolic and diastolic blood pressures was recorded.<br><br>From 2021: Blood pressure is measured in the right and left arms and recorded separately. Heart rate is also recorded.<br><br>Transformation algorithms to be used on blood pressure data before 1st September 2009 are available on page 12. |
| **Blood-glucose** |
| 1985-2021: Oral Glucose Tolerance Test performed according to WHO standards [3]. 1985-2004: Fasting and 2-hour glucose were analyzed on capillary plasma on a Reflotron Bench top analyzer (Roche Diagnostics).<br><br>2004-2021: Fasting and 2-hour glucose were analyzed on capillary plasma on a bench-top HemoCue® Glucose analyzer [1]).<br><br>Reflotron and Hemocue analyzers were regularly tested in a calibration scheme provided by EQUALIS, the External Quality Assurance in Laboratory Medicine in Sweden.<br><br>From 2021: HbA1c was implemented in 2021, and the oral glucose tolerance test was excluded from the protocol. In addition, local capillary fasting glucose analysis was replaced by venous glucose analysis using routine methods at Clinical Chemistry, Laboratory Medicine, Umeå University Hospital.<br><br>HbA1c is analyzed with an automated ion-exchange chromatography on a G8 or G11 HPLC analyzer, TOSOH Bioscience Inc., Grove City, Ohio, USA. The calibrators are traceable to the IFCC (International Federation of Clinical Chemistry) reference method for HbA1c. External quality insurance is provided by Equalis AB, Uppsala, Sverige. |

**Blood lipids**

All lipid analyses have been conducted in plasma or serum, where appropriate.

1985-2009: Cholesterol and Triglycerides were analyzed on a Reflotron Benchtop analyzer (Roche Diagnostics).

From 2009-09-01: Lipids (cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol) are analysed with routine methods at Clinical Chemistry, Laboratory Medicine, Umeå University Hospital.

The differences in mean and median values for cholesterol and triglycerides measured with both methods were only 2-3%. The methods are thus comparable, and we recommend that no adjustments for method should be done.

However, for triglycerides, the minimum detectable value for the earlier method was 0.8 mmol/l, and 0.1 mmol/l for the later method.

**Clinical Chemistry, Laboratory Medicine, Umeå University Hospital**

Clinical Chemistry, Laboratory Medicine, Umeå University Hospital is accredited by the national accreditation body Swedac (https://www.swedac.se/) since 1995 with accreditation number 1397.

**Total cholesterol**

1985-01-01: Enzymatic method, wet chemistry, Hitachi-705, and from 1989 Hitachi 717.

1996-04-01: Ektachem 950 IRC (dry chemistry, Johnson & Johnson, same as Ortho Clinical Diagnostics).

2007-02-26: Changed to Vitros 5.1 FS.

2015-03-01: Cobas CHOL2 on c501/c502/c701.

2020-02-25: Changed from c701 to c503.

**Triglycerides**

1985-01-01: Analysed using a coupled enzymatic chromogenic reaction with Hitachi-705, and from 1989 Hitachi 717.

1996-04-01: Ektachem 950 IRC (dry chemistry, Johnson & Johnson, same as Ortho Clinical Diagnostics).

2007-02-26: Changed to Vitros 5.1 FS.

2015-03-01: TRIGL on c501/c502/c701.

2020-02-25: Changed from c701 to c503.

**HDL-Cholesterol**

1985-01-01: Enzymatic method, wet chemistry, Hitachi-705, and from 1989 Hitachi 717.

1996-04-01: Vitros 950 IRC (dry chemistry, Johnson & Johnson, same as Ortho Clinical Diagnostics).

2000-05-01: VITROS Magnetic HDL method.

2005-11-07: HDL-slides for direct HDL, VITROS slide method for direct HDL.

2007-02-26: Changed to Vitros 5.1 FS.

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| 2015-03-01: Cobas HDLC3 on c501/c502/c701.<br>2018-02-27: Changed of reagents to HDLC4.<br>2021-12-13: Non-HDL-cholesterol is calculated.<br>**LDL-cholesterol**<br>LDL-cholesterol calculated with Friedewald's formula:<br>LDL-cholesterol = total cholesterol - HDL-cholesterol – 0.45 × triglycerides<br>LDL-cholesterol is only calculated if the triglyceride level is ≤4.0 mmol/L. Triglyceride levels above 4.0 mmol/L will result in unreliable LDL-cholesterol values.<br>**non-HDL-Cholesterol**<br>Non-HDL-cholesterol is calculated as: non-HDL = Total cholesterol – HDL-cholesterol<br>**Lipid error "flag variable"**<br>To identify post-analytical manual clerical errors in lipid data entry, LDL cholesterol was recalculated. A discrepancy of >0.3 mmol/L between the LDL cholesterol value recorded in the database and the recalculated LDL cholesterol value suggests a potential error in one or more lipid measurements beyond what would be expected from rounding. A flag variable indicates that one or more of the entered lipid variables may be incorrect. |
| **Height** |
| Since 1985: Without shoes. Measured to the nearest cm using a calibrated wall-mounted height rod or a height rod that is mounted onto the scale. |
| **Weight** |
| Since 1985: Light indoor clothing. Measured to the nearest kg using a calibrated scale. |
| **Waist** |
| Since 2003: Measured with an inelastic tape, on the skin, to the nearest cm, in the midway between the lowest palpable rib and top of the iliac crest. The subject is in a standing position and after a calm exhalation. |

## References Västerbotten Intervention Program (VIP)

1. Norberg M, Wall S, Boman K, Weinehall L. The Västerbotten Intervention Programme: background, design and implications. Glob Health Action. 2010;3.

2. Späth F et al. Cohort Profile: The Northern Sweden Health and Disease Study (NSHDS). Int J Epidemiol. 2024 Dec 16;54(1):dyaf004. doi: 10.1093/ije/dyaf004. PMID: 39899988; PMCID: PMC11790227.

3. WHO and International Diabetes Federation Consultation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. *World Health Organisation* 2006.

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# MONICA, Methods for CVD risk factors

| **Cohort [1,2]** |
|---|
| The Northern Sweden MONICA study consists of eight population-based surveys (in 1986, 1990, 1994, 1999, 2004, 2009, 2014, and 2022) that used a random sampling design, stratified for sex and age (25 to 64 years in 1986 and 1990; 25 to 74 years in 1994 to 2022). Two thousand persons were invited in 1986 and 1990 and 2500 persons were invited in the subsequent surveys. In 1999, the participants who had been sampled in 1986, 1990 and 1994 were invited for follow-up measurements.<br><br>Participants were randomly assigned examination in the morning or after lunch and the fasting period was, therefore, at least 8 hours or at least 4 hours, respectively. In practice, this implies an overnight fast for those examined in the morning (approximately 60% of all participants). From 2004 and onwards, the actual fasting time (in hours) was reported. |
| **Blood pressure** |
| Blood pressure was measured in the right arm according to a standardized protocol by trained nurses. Two readings were taken in a seated position after five minutes of rest and the average value was recorded. The measurements were performed with a random-zero sphygmomanometer between 1986 and 2009 (Hawksley & Sons Ltd, Lancing, United Kingdom) and an Omron automated oscillometric device in 2014 and 2022 (M7 in 2014, M7 Intelli IT-AFIB in 2022; Omron Healthcare Europe BV, Hoofddorp, The Netherlands).<br><br>To approximate the blood pressure obtained from the sphygmomanometer, the oscillometric measurements in 2014 and 2022 can be corrected according to the equations below (as detailed by Eriksson *et al*. [3]). Alternatively, if data between 1986 and 2022 are pooled, categories based on survey-specific percentiles can be used for harmonization (thirds, quarters, etc.).<br><br>Systolic blood pressure (mmHg) = oscillometric value – (0.44-4.39[if women]+0.033 x age)<br>Diastolic blood pressure (mmHg) = oscillometric value + (2.68-1.33[if women]–0.126 x age) |
| **Blood glucose** |
| An oral glucose tolerance test (OGTT) was performed according to WHO standards in those randomly assigned a morning examination time [4, 5]. However, the sampling frame for the testing was unknown in 1986 and only the re-examined participants had an OGTT in 1999. Venous blood samples were used to calculate plasma (1986 to 1999) or plasma-equivalent glucose concentrations (2004 to 2022, derived from whole blood). Between 1986 and 1999, a hexokinase method was used in the laboratory at Boden Hospital (1986 to 1994) and Sunderby Hospital (1999) (BM/Hitachi system 717; Boehringer Mannheim GmbH, Mannheim, Germany). Since 2004, a HemoCue benchtop analyser was used on each study site in Norrbotten and Västerbotten (HemoCue AB, Ängelholm, Sweden). The benchtop analysers were factory-calibrated and traceable to the ID GC-MS method for glucose.<br><br>To approximate the glucose concentrations obtained from the Hemocue method, the hexokinase-estimated concentrations in 1986 to 1999 can be corrected according to the equations below (as detailed by Lilja *et al*. [5]; Nota bene: There is a typo in the original article, as the words “old |

method" and "new method" has been interchanged). Alternatively, if data between 1986 and 2022 are pooled, categories based on survey-specific percentiles can be used for harmonization (thirds, quarters, etc.). It is also important to stress that the shift in analytical method can introduce bias in the prevalence estimation of impaired fasting glucose, impaired glucose tolerance, and diabetes; that is, diagnoses that are based on absolute cut-off values of glucose concentrations. To limit such bias, one can restrict the analyses to the surveys in 2004 to 2022.

Fasting plasma glucose (mmol/L) = (hexokinase value x 0.863) + 0.886
Two-hour post-load plasma glucose (mmol/L) = hexokinase value x 1.027

HbA1c was analyzed in 1994, 1999, 2009, and 2022 with an automated ion-exchange chromatography on a G8 or G11 HPLC analyzer (TOSOH Bioscience Inc., Grove City, USA). The analyses were traceable to the IFCC (International Federation of Clinical Chemistry) reference method for HbA1c.

Between 1986 and 1994, insulin was analyzed with a radioimmunoassay from Pharmacia (Uppsala, Sweden).

**Blood lipids**

All lipid analyses have been conducted in plasma or serum, where appropriate.

All measurements of total cholesterol have been done at a central laboratory, at Boden Hospital in 1986-1994, at Sunderby Hospital in 1999-2014, and at Clinical Chemistry, Laboratory Medicine, Umeå University Hospital in 2022. The analytical quality was checked against standards from the WHO reference laboratory in Prague up until 1994. Laboratory medicine in Sunderbyn has been accredited by the national accreditation body Swedac (https://www.swedac.se/) since 2000 with accreditation number 1658. Clinical Chemistry, Laboratory Medicine, Umeå University Hospital has been accredited by the national accreditation body Swedac (https://www.swedac.se/) since 1995 with accreditation number 1397.

An enzymatic method was used between 1986 and 1994, a dry chemistry method between 1999 and 2014, and a wet chemistry method in 2022 (as detailed under "Lipid analysis in Region Norrbotten"). In 1986, the cohort did not provide satisfactory results for total cholesterol analyses. Therefore, a subsample was re-analysed: the concentrations were found to be too low and corrected accordingly [2].

To approximate the total cholesterol concentrations obtained from the enzymatic method, the dry chemistry-estimated concentrations in 1999 to 2014 can be corrected according to the equation below (as detailed by Stegmayr et al [2]). Please note that the wet chemistry method in 2022 has not been compared to neither the enzymatic method nor the dry chemistry method. As such, if data between 1986 and 2022 are pooled, it might be more suitable to use survey-specific percentiles for harmonization (thirds, quarters, etc.).

Total cholesterol (mmol/L) = (dry chemistry value x 1.014) + 0.010

HDL cholesterol concentrations were measured in 1986, 1990, 1994, and 2022; and triglyceride concentrations were measured in 1986, 1990, 1994, 1999, 2004, and 2022.

**Lipid analysis in Region Norrbotten**

**Total Cholesterol**

**Boden Hospital:**

1978-05-29: Automatic method, colorimetric at 620 nm. (Technicon).

1985-06-18: Multistat cholesterol: reagent Monotest Cholesterol CHOD-PAP method and triglycerides with reagents from Boehringer Mannheim.

1987-03-01: Corona (Clinicon AB). Same method principle as 1985. Reagents from Boehringer Mannheim.

1990-09-12: Hitachi 717 with reagents from Boehringer Mannheim.

1997-03-31: Vitros (dry chemistry, Johnson & Johnson, same as Ortho Clinical Diagnostics).

**Sunderbyn Hospital:**

2006-05-04, Vitros 5.1 Ortho Clinical Diagnostics.

2017-06-07 Architect c8000 Abbott Diagnostics.

**Clinical Chemistry, Laboratory Medicine, Umeå University Hospital:** see page 4.

**Triglycerides:**

**Boden Hospital:**

1978-05-29: Autoanalyzer, Technicon (Fluorescence at 510 nm).

1985-06-18: Reagents from Boehringer Mannheim.

1987-03-01: Corona (Clinicon AB). Same method principle as 1985. Reagents from Boehringer Mannheim.

1990-09-12: Hitachi 717 with reagents from Boehringer Mannheim.

1997-03-31: Vitros (dry chemistry, Johnson & Johnson, same as Ortho Clinical Diagnostics).

**Sunderbyn Hospital:**

2006-05-04: Vitros 5.1 Ortho Clinical Diagnostics.

2017-06-07: Architect c8000 Abbott Diagnostics.

**Region Västerbotten, Clinical Chemistry, Umeå:** see page 4.

**HDL-Cholesterol**

**Boden in routine since 1978:**

1986-01-21: Multistat III Plus, reagents from Boehringer Mannheim.

1990-10-08: Hitachi 717, reagents from Boehringer Mannheim.

1997-03-31: Vitros (dry chemistry, Johnson & Johnson, same as Ortho Clinical Diagnostics).

1998-01-09: Hitachi 717, reagents Boehringer Mannheim

**Sunderbyn:**

2001-01-22: Hitachi 917 Instrument and reagents; Roche Diagnostica Scandinavia AB.

2006-02-22: Vitros 950 Ortho Clinical Diagnostics.

**Region Västerbotten, Clinical Chemistry, Umeå:** see page 4.

**LDL-Cholesterol**

LDL-cholesterol calculated with Friedewald's formula:

LDL-cholesterol = total cholesterol - HDL-cholesterol – 0.45 × triglycerides

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|---|
| LDL-cholesterol is only calculated if the triglyceride level is ≤4.0 mmol/L. Triglyceride levels above 4.0 mmol/L will result in unreliable LDL-cholesterol values.<br><br>**non-HDL-Cholesterol**<br><br>Non-HDL-cholesterol is calculated as: non-HDL = Total cholesterol – HDL-cholesterol<br><br>**Lipid error "flag variable"**<br><br>To identify post-analytical manual clerical errors in lipid data entry, LDL cholesterol was recalculated. A discrepancy of >0.3 mmol/L between the LDL cholesterol value recorded in the database and the recalculated LDL cholesterol value suggests a potential error in one or more lipid measurements beyond what would be expected from rounding. A flag variable indicates that one or more of the entered lipid variables may be incorrect. |
| **Height** |
| Height was measured without shoes to the nearest cm using a calibrated wall-mounted height rod or a height rod that is mounted onto the scale. |
| **Weight** |
| The subjects wore light clothes and no shoes, and weight was measured to the nearest 0.1 kg on a balance scale (calibrated daily) from 1986 to 1994, and on an electronic scale from 1999 onwards. |
| **Waist** |
| The waist and hip circumferences were measured in standing position with the feet fairly close together (12−15 cm apart). Tight undergarments were released or taken off before measuring the waist circumference, which was measured midway between the lower rib margin and the iliac crest. At the time of measuring, the subject was asked to exhale gently. The hip circumference was measured at the maximum circumference over the buttocks to the nearest 0.5 cm. |

# References MONICA

1. Eriksson M, Holmgren L, Janlert U, Jansson JH, Lundblad D, Stegmayr B, et al. Large improvements in major cardiovascular risk factors in the population of northern Sweden: the MONICA study 1986-2009. Journal of Internal Medicine. 2011;269(2):219-31.

2. Stegmayr B, Lundberg V, Asplund K. The events registration and survey procedures in the Northern Sweden MONICA Project. Scand J Public Health Suppl. 2003;61:9-17.

3. Eriksson M, Carlberg B, Jansson JH. Comparison of blood pressure measurements between an automated oscillometric device and a Hawksley random-zero sphygmomanometer in the northern Sweden MONICA study. Blood Press Monit. 2012;17(4):164-70.

4. WHO and International Diabetes Federation Consultation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia. *World Health Organisation* 2006.

5. Lilja M, Eliasson M, Eriksson M, Söderberg S. A rightward shift of the distribution of fasting and post-load glucose in northern Sweden between 1990 and 2009 and its predictors. Data from the Northern Sweden MONICA study. Diabet Med. 2013;30(9):1054-62.

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# Mammographic Screening Project

| **Cohort** |
| --- |
| Samples (mostly non-fasting) and data are collected in connection with mammography screenings 1995-2006. |
| **Height** |
| All years, self-reported height. |
| **Weight** |
| All years, self-reported weight. |

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# Recommendations for the interpretation of outlier values

Values within the indicated levels below are suggested to be handled as missing values. All outliers have been checked for possible mistakes in the data handling.

| **Parameter** | **Value** |
|---|---|
| Height | <130 or >210 cm |
| Weight | <35 kg |
| Body Mass Index | <15 or >70 kg/m² |
| Waist circumference | <60 cm |
| Skol | <0,5 or >15 mmol/L |
| HDL | <0,15 or >7 mmol/L |
| LDL | <0,0 mmol/L |
| Non-HDL | <0,0 mmol/L |
| Triglycerides | <0.15 mmol/L or >20 mmol/L |
| Blods0 | <1 or >25 mmol/L |
| Blods2 | <1 or >35 mmol/L |
| Sbt | <20 or >300 mmHg |
| Dbt | <20 or >250 mmHg |

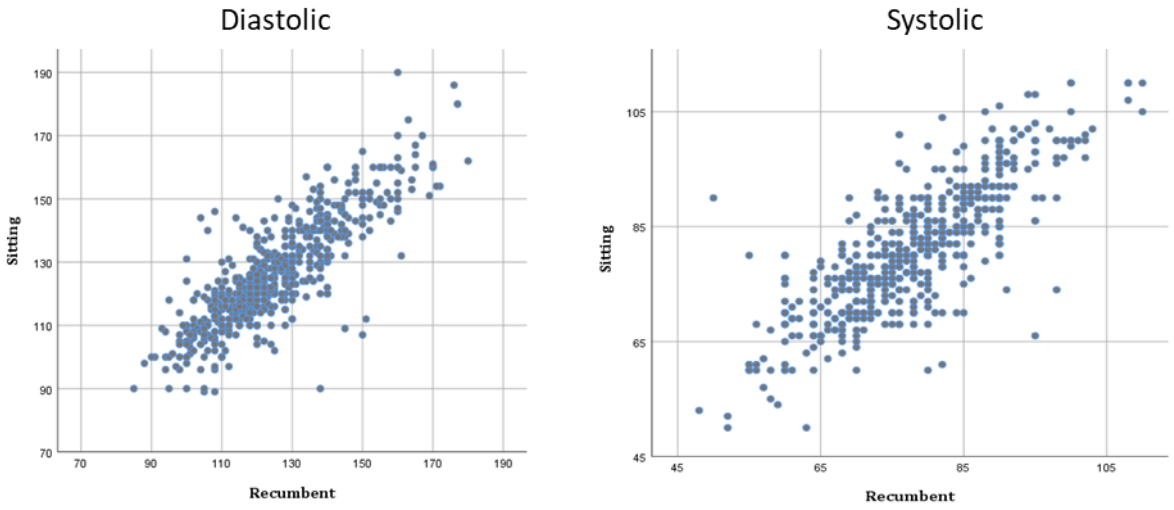
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# Conversion of sitting to recumbent blood pressure values and vice versa (VIP)

In the VIP-cohort, blood pressure was measured with subjects in recumbent (supine) position until September 1st 2009 and from that date with subjects in sitting position.

The differences in blood pressure values between measurements in the two various positions, recumbent or sitting, are negligible in a clinical point of view. However, in a dataset from the VIP-cohort where blood pressure was measured on the same individuals in the two different positions, a significant difference for both the systolic and diastolic values was found. Based on this calculation the diastolic value was in average 2.5 mm higher measured at sitting position and the systolic value 0.8 mm higher in this position. This is in line with previous findings on different populations [1, 2]. In these studies, the diastolic values were higher when measured in sitting position, while the effect on the systolic blood pressure showed a slight decrease. We therefore recommend an addition with 2.5 mm for conversion of the diastolic blood pressure from recumbent to sitting position, and no need for conversion for the systolic measurements.

A data set with blood pressure values from 653 VIP-participants at age 40, 50 or 60 years and measured in both positions is available after contacting BRS.




*The figures show correlation between the blood pressure values measured in two various positions, recumbent or sitting. The illustrations are based on a data set with 653 VIP-participants at age 40, 50 or 60 years, measured at both positions.*

**References VIP**:

1. Kahan T. Rätt mätt blodtryck tack! Läkartidningen 2009; 106: 1349-1350.
2. Kuwabaraa,M, Haradab K, Hishikib Y, Karioa K. Validation of an automatic device for the self-measurement of blood pressure in sitting and supine positions according to the ANSI/AAMI/ISO81060-2:2013 guidelines: the Omron HEM-9700T. Blood Pressure Monitoring 2019; 24: 146–150.

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# Cholesterol and triglycerides in the VIP-cohort, correlation between two different methods for measurement

Various methods have been used to calculate cholesterol and triglyceride values before and after 2009-09-01. We have previously recommended a certain algorithm to be able to compare older data with newer ones, where different methods have been used.

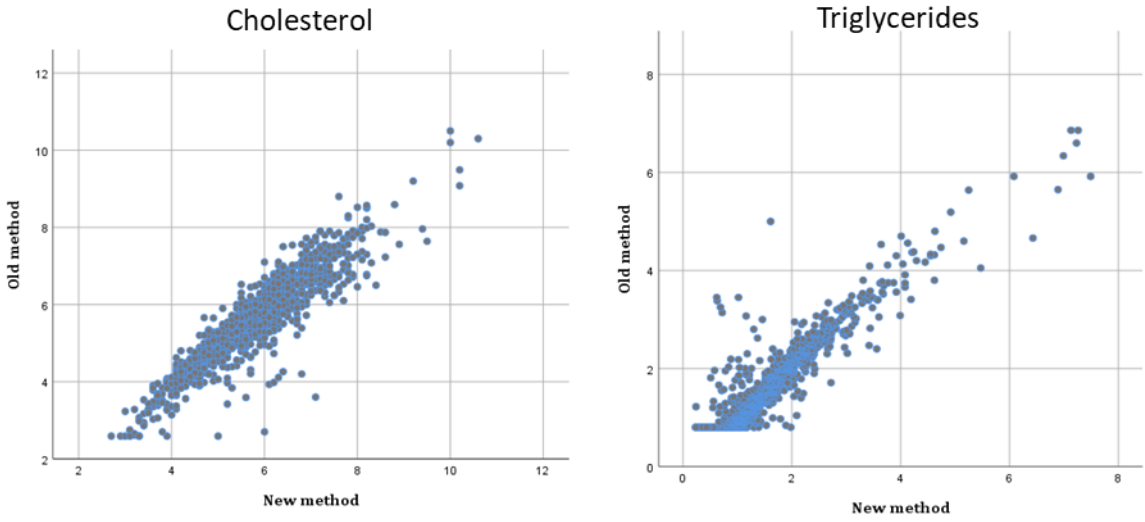
The algorithm has been calculated by linear regression but since there are random errors in both parameters, this method is not always optimal.

We have examined the material where there are measurement values from both methods and the difference in mean and median values is only 2-3%. The previous recommendation introduced a larger error, 7-10%, than the real difference.

Therefore, we recommend not to use any algorithm for VIP data. The methods are comparable. However, it should be kept in mind that for triglycerides, the minimum value with the earlier method is = 0.8.

Blood lipids have been measured by two methods during 1985 – 2009-08-31, on a Reflotron at local health centres. Samples with high cholesterol (>6.5 mmol/l) and/or triglycerides values were re-analysed at Clinical Chemistry, Laboratory Medicine, Umeå University Hospital for cholesterol, triglycerides and HDL. The reference value for high cholesterol in individuals with known cardiovascular disease was set at >5.2 mmol/l. From 2009-09-01 blood lipids were analysed by an enzymatic method at Clinical Chemistry, Laboratory Medicine, Umeå University Hospital.

A dataset with samples that are analysed with both methods is available. Regarding triglycerides there are data from 838 VIP-participants and regarding cholesterol from 1197 VIP-participants. This dataset is stored at BRS and available after contacting the unit.




*The figures show the correlation between the two methods used for blood lipid analyses. The illustrations are based on a data set with 1197 VIP-participants for cholesterol and 837 VIP-participants for triglycerides (old method = Reflotron, new method = enzymatic method).*