Iron stores and hemoglobin are negatively related with carotid atherosclerosis: the Tromsø Study.

A cross-sectional study

Sara Margarida Santos Lousada
HEL-3950 Master's thesis in Public Health
May 2017

Supervisor: Therese von Hanno, MD, PhD, associate professor IKM, postdoc. research fellow IKM. University of Tromsø/Nordlandsykehuset Bodø.

Co-supervisor: Ellisiv B. Mathiesen, MD, PhD, professor IKM.
Acknowledgements

I would like to thank to the people that made this master’s thesis possible.

My gratitude to my main supervisor, Therese von Hanno, for her attention and guidance throughout the course of this study. I thank Ellisiv Mathiesen for her availability in being my co-supervisor.

To all professionals involved in the Tromsø Study, the data committee for granting the permission to use the data from Tromsø 5 and to all participants of the survey.

To the University of Tromsø for giving me the opportunity to participate in this great program of master in public health.

To my friends from the master for their friendship and sharing of wonderful moments over these two years.

To my boyfriend Joel for his love and support during my study years in Tromsø.

To my mom, sister and nephew for giving me strength and affection.

And to my father who taught me to follow the pathway that bring us happiness. Wherever he is I know that he has given me strength.

Thank you all!

Sara
Tromsø, May 2017
Abstract

Background: Cardiovascular diseases (CVD) remain the leading cause of mortality and morbidity globally. Iron has been suggested to have role in CVD though, epidemiological findings have been conflicting. The primary aim of this study was to investigate the possible role of body iron in carotid atherosclerosis in a population-based survey. Further, we wanted to analyze the prevalence of carotid plaques and differences in gender and age.

Material and methods: We performed a cross-sectional study based on data from the fifth survey of the Tromsø Study conducted in 2001-2002. Atherosclerosis was measured by ultrasound examination of the carotid artery in a total of 5423 subjects aged between 32 to 89 years. Iron status was obtained through laboratory measurements of hemoglobin, s-ferritin, s-transferrin, transferrin saturation, s-iron and s-TIBC.

Results: Iron stores and hemoglobin were negatively associated with carotid plaque. The third and the fourth quartiles of s-ferritin showed reduced odds by 54.6 % (OR=0.454, 95% CI=0.272 to 0.758) and by 52.9% (OR=0.471, 95% CI=0.286 to 0.774) respectively, compared with the lowest ferritin quartile towards high plaque load in women. Likewise, hemoglobin had a protective effect in plaque presence and in high plaque load in women, being reduced by 11.3% odds (OR=0.887, 95% CI=0.802 to 0.980) and by 18.3% odds (OR=0.817, 95% CI=0.674 to 0.989) respectively, per 1 unit increase in hemoglobin compared to men.

Conclusion: High levels of iron seem to be protective of plaque presence, high plaque load and plaque area with consistent findings in women, suggesting that they were associated with lower risk of disease.

Keywords: cardiovascular disease, atherosclerotic plaque, carotid, iron stores, hemoglobin, oxidation and epidemiology.
## Table of contents

Acknowledgements .................................................................................................................. iii
Abstract........................................................................................................................................ v
Table of contents ...................................................................................................................... vii
List of tables ............................................................................................................................ ix
List of figures ............................................................................................................................. x
List of abbreviations .................................................................................................................. xi

1 Introduction .............................................................................................................................. 1
  1.1 Atherosclerosis .................................................................................................................. 1
    1.1.1 Pathogenesis of atherosclerosis .................................................................................. 2
    1.1.2 Cardiovascular risk factors ....................................................................................... 5
    1.1.3 Ultrasonography of the carotid artery ....................................................................... 6
  1.2 Iron hypothesis .................................................................................................................... 7
  1.3 Metabolism of iron ............................................................................................................. 7
  1.4 Hypothetical pathway of iron, oxidative stress and vascular effect ............................... 8
  1.5 Aims of the thesis ............................................................................................................ 10

2 Material and methods ............................................................................................................. 12
  2.1 The Tromsø Study ............................................................................................................ 12
  2.2 Study population and design .......................................................................................... 13
  2.3 Measurements .................................................................................................................. 15
    2.3.1 Iron status, hemoglobin and risk factors measurements ......................................... 15
    2.3.2 Questionnaires ....................................................................................................... 16
    2.3.3 Carotid ultrasound examination .............................................................................. 16
  2.4 Data analyses .................................................................................................................... 17
  2.5 Ethical consideration and consent .................................................................................. 20

3 Results ...................................................................................................................................... 22
  3.1 Baseline characteristics ................................................................................................. 22
  3.2 Prevalence of carotid plaque ......................................................................................... 23
  3.3 Association between iron and carotid atherosclerosis ................................................. 25

4 Discussion .............................................................................................................................. 33
  4.1 Methodological considerations ...................................................................................... 33
4.1.1 Study design ............................................................................................................. 33
4.1.2 Internal validity ...................................................................................................... 33
4.1.3 External validity ..................................................................................................... 37
4.1.4 Statistical considerations ....................................................................................... 37
4.2 Findings ..................................................................................................................... 38
  4.2.1 Prevalence of carotid atherosclerosis ................................................................. 38
  4.2.2 Association between iron status and carotid atherosclerosis ............................ 38
5 Conclusion .................................................................................................................. 42
References ....................................................................................................................... 43
Appendices
Appendix 1: Invitation to the Tromsø 5
Appendix 2: Questionnaire 1
Appendix 3: Questionnaire 2
Appendix 4: Questionnaire 3
Appendix 5: Participation in the Tromsø 5
Appendix 6: Data transformation
List of tables

Table 1 Baseline characteristics of study subjects by sex ................................................................. 22
Table 2 Cardiovascular risk factors of study subjects with and without carotid plaque .................. 23
Table 3 Prevalence of carotid plaque and number of plaques in men and women .............................. 24
Table 4 Associations between plaque presence (yes versus no) and hemoglobin, s-ferritin log-
transformed, s-ferritin quartiles, transferrin saturation and s-TIBC in models 1, 2 and 3 ............... 27
Table 5 Associations between high plaque load (≥3 plaques versus no plaque) and hemoglobin, s-
ferritin log-transformed, s-ferritin quartiles, transferrin saturation and s-TIBC in models 1, 2 and 3 .. 28
Table 6 Associations between TPA log-transformed and hemoglobin, s-ferritin log-transformed,
transferrin saturation and s-TIBC in models 1, 2 and 3 ................................................................... 29
Table 7 Associations between IMT log-transformed and hemoglobin, s-ferritin log-transformed,
transferrin saturation and s-TIBC in models 1, 2 and 3 .................................................................... 30
List of figures

Figure 1 Cross-section of the artery wall .......................................................... 2
Figure 2 Progression of atherosclerotic plaque .............................................. 4
Figure 3 Flow chart of the study population ..................................................... 14
Figure 4 Histogram of number of cases with carotid plaque by age groups in men and women ........ 24
Figure 5 Line graphs of total mean IMT and TPA by age (years) groups in men and women .......... 25
List of abbreviations

BMI  Body mass index
BP   Blood pressure
CCA  Common carotid artery
CHD  Coronary heart disease
CRP  C-reactive protein
CVD  Cardiovascular disease
HbA1c Glycosylated hemoglobin
HDL  High-density lipoprotein
IMT  Intima-media thickness
LDL  Low-density lipoprotein
OR   Odds ratio
TIBC Total ironbinding capacity
TPA  Total plaque area
WBC  White blood cells
1 Introduction

The importance of body iron in the development of cardiovascular diseases (CVD) has been discussed in the scientific field during the last decades. However, the association between body iron status and atherosclerosis has not been extensively investigated, remaining a controversial topic with conflicting epidemiological findings. Beyond that, research in the topic has not been conducted in a population-based survey in Norway. The study of carotid atherosclerosis provides an important tool for the comprehensive of cardiovascular events particularly ischemic cerebrovascular events, due to atherosclerotic plaques and stenosis located in carotid bifurcation are considered to be major risk factors to stroke (1),(2),(3).

1.1 Atherosclerosis

The burden of noncommunicable diseases (NCD) caused an estimated 52% of total deaths under age 70 globally in 2012 (4). The CVD remain the major cause of mortality worldwide causing 17.5 million deaths in 2012. According with the same report, coronary heart disease (CHD) is the main cause of mortality leading to 7.4 million deaths, and stroke is the second major cause with an estimated 6.7 million deaths in 2012. Atherosclerosis is a chronic disease and the major cause of CVD events as ischemic stroke, myocardial infarction and peripheral arterial disease (5). It is characterized by the accumulation of cholesterol deposits and cell infiltration in the artery wall that compromises the lumen of the vessel, affecting the blood circulation and oxygen supply to the heart and the brain.

Cholesterol or lipid is a fat-like substance that is present in the blood circulation in different particles composed by both lipid and proteins, which are referred as lipoproteins (6). They include low density lipoproteins (LDL), high density lipoproteins (HDL) and very low density
lipoproteins (VLDL). The total serum cholesterol contains about 60% to 70% of LDL cholesterol and 20% to 30% of HDL cholesterol.

The arterial wall is divided in three cylindrical layers with different vascular cell types that are separated by macromolecules called elastin lamellae (7), (8). The innermost layer of the artery wall is tunica intima and is formed by endothelial cells, acting as an active barrier between the vessel wall and the blood circulation. The tunica media is the middle layer formed by smooth muscle cells within the extracellular matrix and provides the elastic function of the vessel. The outermost layer is the tunica adventitia that contains fibroblasts and the blood vessels that nourishes the cells of the arterial vessel. The medial layer is separated from the intima and the adventitia by elastin membranes. The figure 1 shows the structure of the arterial vessel wall (9).

![Figure 1 Cross-section of the artery wall.](image)

1.1.1 Pathogenesis of atherosclerosis

The pathological process of atherosclerosis may start in childhood (10) and the occurrence of the first cardiovascular event usually can last decades (11). The formation of atherosclerotic plaque tends to occur in sites with disturbed laminar flow as bifurcations and branchings of carotid and coronary arteries, that exhibit characteristics of lower flow velocity and wall shear
stress (12). The process of atherosclerosis development is called atherogenesis and it involves a cascade of events with different biological processes (13). The main hypothesis of the atherogenesis is known as “the response to injury” and it was postulated by Ross in the early 90’s (14). Multiple factors contribute to this complex and systemic disorder and they include endothelial and smooth muscle cells dysfunction, oxidative modifications and inflammatory response with intervention of immune cells due to injury of endothelial cells (15).

The known pathological stages of atherosclerosis can be classified as: 1. Intima-media thickening; 2. fatty streaks; 3. intermediate lesions; 4. fibrous plaques; 5. complicated plaques (13). The early development of atherosclerosis is characterized by the thickening of the subendothelial space that involves lipid deposition and cell infiltration, with later changes in the media layer (8). An increase in the intima-media thickness (IMT) includes both the widening of intima and the hypertrophy of media and so, the IMT reflects a hypertrophic response (16). Mechanical factors as wall shear stress and tensile stress have been implicated in the arterial wall thickness (12). Still in the early atherosclerosis, it occurs the fatty streak lesion that is defined as the accumulation of apolipoprotein B, the main protein component of LDL cholesterol, and the migration of monocytes and dendritic cells within the intima layer (17). In this pathological stage, it occurs the differentiation of the monocytes into macrophages, and unregulated scavenger receptors identify the oxidized LDL (13). This leads to the formation of foam cells that represents the building blocks of the plaque. The accumulation of foam cells leads to an increase of the intima thickness and the macrophages conduct a process of phagocytosis, ingesting the modified LDL (17). Fatty streak lesion can be found in adolescents and young adults (18) and usually it does not cause symptoms, though they may evolve to atherosclerotic plaque or disappear (19). The fatty streak lesion anticipates the intermediate lesion which is known as preatheroma, that contains lipid pools and multiple layers of macrophages and smooth muscle cells (20). The intermediate lesion can evolve to fibrous
plaque which is known as atheroma. This complex and occlusive lesion is composed by macrophages, smooth muscle cells, T lymphocytes and calcium deposits (14). The proliferation of smooth muscle cells and T cells in the intima layer form a cap over the lipid core and the retention of apolipoprotein B is expanded (13). The immune cells signal the activation and production of cytokines (19). Cytokines are defined as the mediators involved in the immunity and inflammation response, and the growth factors are implicated in cell proliferation (14). These two molecules seem to act very closely in the atherosclerosis. A complex process occurs in the formation of vulnerable plaque involving cell death of macrophage foam cells and smooth cells along with the degradation of the extracellular matrix (11). This leads to a necrotic core and thinning of the fibrous cap (17). In advanced fibrous plaques, it may occur fissures of the endothelial cells or ruptures causing hemorrhage, aggregation of platelet and thrombus formation, and consequently, the occlusion of the arterial lumen and ischemia (14). The development of the pathological steps of atherosclerosis is shown in the figure 2 (17).

![Figure 2 Progression of atherosclerotic plaque.](Image)
1.1.2 Cardiovascular risk factors

The CVD risks factors have been widely investigated in the last decades and include age, gender, hypertension, cigarette smoking, hypercholesterolemia, diabetes mellitus and obesity (5). Age while not modifiable, is an important factor to predict incident CVD (21). Age reflects the accumulation of atherosclerosis over time and the increased exposure to risk factors. Besides, there are cardiovascular changes with age at structure and function levels of the artery, which increases the risk of CVD in the older people (22). The Framingham Heart Study showed that advancing age increased the incidence of CHD in men and women (23). According to the same study, men had higher rate of CHD than women. Differences in the incidence of CHD between genders is well documented (24). Premenopausal women have lower incidence of CVD although, the risk increases in postmenopausal women (25), (26). The use of estrogen therapy has been implicated in the protective effect of CHD in postmenopausal women (27) however, evidence has not revealed this. A randomized control trial did not show the risk reduction of disease in women with hormone replacement (28). Hypertension is defined as systolic blood pressure $\geq 140$ mmHg or diastolic $\geq 90$ mmHg (29) and increases with advancing age (30). It is considered to be highly correlated with stroke mortality (31) and a significant risk factor for death from CHD (32). The use of blood pressure lowering drugs have reduced substantially cardiovascular events in hypertensive patients (33). Moreover, hypertension seems to be related with endothelial dysfunction by oxidative stress that can contribute to the formation of atherosclerotic plaque (34). Cigarette smoking is a strong risk factor for atherosclerosis. It increases the risk by approximately 50% and doubles the incidence of CHD (35). A meta-analysis revealed that cigarette smoking is a strong predictor to CVD mortality and cardiovascular events and smoking cessation reduced the excess risk among former smokers in a dose-response form (36). Moreover, cigarette smoking leads to increased oxidation of LDL cholesterol, inflammation and thrombosis, affecting this way all stages of
atherosclerosis (37). Hypercholesterolemia or dyslipidemia refers to increased levels of serum cholesterol (6). Elevated LDL cholesterol is a major risk factor to atherosclerosis and in contrast, high levels of HDL cholesterol is considered to be a protective factor (38), (39). Cholesterol-lowering therapy contributes significantly to the reduction of the risk of death and morbidity in patients with CHD with an improvement in survival (40). Diabetes mellitus represents a major factor for atherosclerosis (41). A study showed that diabetes mellitus increased the risk of all-cause and CVD mortality (42). Another study showed that the incidence of ischemic stroke is twice in diabetic patients than non-diabetic people (43). Patients with diabetes type 2 may benefit from diabetic therapy in terms of reduction of carotid IMT (44). Obesity increases the risk for CVD (45), (46). However, its mechanism remains controversial and some risk factors of CVD frequently coexist in obesity (45). The genetic component seems to have a role in the development of atherosclerosis, where the occurrence of disease depends strongly on the interaction between genetic factors and the environment (13). The Framingham Heart Study was the first prospective study to suggest a role of family history to risk of CHD (23).

1.1.3 Ultrasonography of the carotid artery

High resolution B-mode ultrasonography is a non-invasive procedure that allows the detection of asymptomatic carotid atherosclerosis, providing measures of atherosclerotic plaques and IMT (16). The images obtained from the ultrasound are in high accordance with the true structure of the artery wall though, this method can not differentiate between the intima and the media layers. The IMT consists of approximately 20% of intima and 80% of media. The plaque growth occurs along the arterial wall two times faster than it thickens and so, measurements of
the IMT are considered to be relatively insensitive (47), while measurements of plaque volume and plaque area are considered to be more sensitive measures of atherosclerosis (48).

1.2 Iron hypothesis

Iron has been suggested to have role in CVD. A possible link between iron stores and risk of heart disease was firstly introduced by Sullivan in 1981, who claimed the “iron hypothesis” (49). There are gender differences in heart disease incidence where premenopausal women have lower incidence compared with men and postmenopausal women. Sullivan argued this might be due to lower iron stores through iron loss in menstruation, suggesting that iron depletion has a protective role against ischemic heart disease. In addition, this suggested protection in women decreases after menopause when body iron store levels increase.

1.3 Metabolism of iron

Iron is a vital mineral that is necessary in the majority of cells of the human body such as in the production of red blood cells, use in the muscle tissue and in the synthesis of DNA (50). It is essential to regulate the metabolism of iron in the body, both due to its function and its possible noxious effects, and it includes steps regarding the process of intake, absorption, transport, usage, storage and loss of iron. On average each day, the human body absorbs and loses 1 to 2 mg of iron (51). Iron is obtained from the diet, absorbed in duodenum wall and lost only passively through blood loss, sweat and loss of cells from the intestine, skin and urine (52). Iron is a fundamental functional component of hemoglobin, which is the molecule that transports the oxygen. The majority of the body iron is found in hemoglobin (1800 mg) present in circulating erythrocytes and the rest of the iron is distributed in the cells of the liver (1000 mg),
in myoglobin (300 mg) and a smaller amount in the plasma transferrin (3 mg) (51). Transferrin is the molecule that transports and releases iron in the plasma according to the needs of the body (52). Serum transferrin is usually about 30% saturated with iron. In the case of iron excess in the body, the ferritin protein stores the iron in the liver (50). Serum ferritin has been used in the diagnostic and assessment of iron storage status since 1970s (53). Levels of s-ferritin ≥15 µg/L indicate that iron stores are present and higher levels reflect the amount of iron store whereas, low levels (<15 µg/L) indicate depleted iron stores (54). The production of transferrin is affect by the levels of ferritin, increasing when ferritin levels in the liver are low and decreasing when cellular ferritin is high (55). Ferritin is an cute phase protein and levels of s-ferritin may be elevated in inflammation and infection processes (56). Despite the vast clinical use of s-ferritin, key issues related with its biology are yet not clear.

1.4 Hypothetical pathway of iron, oxidative stress and vascular effect

Free iron can be toxic due to its form in oxygen free radicals. The “free radical theory” was launched by Harman in 1956, who suggested that free radicals resulted from aerobic respiration (57). Iron among other metals can catalyze in vivo leading to oxidative reactions that along with aging effects is related with cellular damage. Oxidative stress refers to the increase of reactive oxygen species, which are superoxide anion, hydrogen peroxide and hydroxyl radicals (58). Elevated concentrations of these substances, in particular hydroxyl radicals, may cause cellular damage and vascular lesions, and have been involved in the pathogenesis of atherosclerosis (59). There is strong evidence that the oxidation of LDL cholesterol occurs in early stage of atherosclerosis. However, core issues remain unexplained as occurrence and location of LDL oxidation in vivo (60). Currently, the scientific support of atherogenesis
hypotheses process underlies in four issues. First, LDL oxidation contributes to atherogenesis process and is generated in vivo in arterial lesions (61). Second, oxidized LDL creates biological effects in vitro that may be critical in lesion development (62). Third, the use of antioxidants in different animals models show inhibition of LDL oxidation decreasing the atherogenesis process (63). And fourth, knockout experiments in mouse have supported the oxidation hypothesis (64).

An experimental study demonstrated a high expression of ferritin genes in atherosclerotic lesions of human and animal tissues (65). Other experimental study recorded iron deposits in atherosclerotic lesions of the aorta tissues (66). A study in patients with carotid stenosis removed by surgery, showed association between s-ferritin and the catalytic iron form, the low molecular weight iron (LMWI) and oxidant damage (67). A recent study of the possible role of iron in vascular damage showed association between high levels of s-ferritin and wider retinal venules caliber (68). Epidemiological studies have shown an association between iron and prediction of cardiovascular events. The first prospective cohort study, conducted by Salonen, showed that high s-ferritin was a significant risk factor for myocardial infarction (69). The association between carotid atherosclerosis and s-ferritin was shown for the first time in the Bruneck Study (70). Later, the same team investigated the topic in a prospective study that confirmed these relationship, showing that s-ferritin was a strong predictor in atherosclerosis progression (71). Other studies have shown that high levels of s-ferritin were associated with carotid atherosclerosis (72), (73), (74). And a study showed that s-ferritin was a risk factor for stroke in postmenopausal women (75). Still, results from different studies have been conflicting whether iron status, particularly ferritin, is a predictor of cardiovascular events. A meta-analyses did not show association between iron status and CHD (76). Moreover, findings from a later prospective study (17-year follow-up) showed a weak or no relationship between ferritin, stroke and CHD (77). Furthermore, a study including blood donation did not show association
between iron status and carotid atherosclerosis (78). And another study do not support the iron hypothesis in CVD incidence (79).

1.5 Aims of the thesis

The purpose of this thesis was to investigate whether iron status is associated with carotid atherosclerosis and determine the prevalence of disease in a population-based survey. This study may contribute to the clarification of this association and may add important knowledge to future studies. The aims of this master thesis are:

1. To investigate the relationship between iron status and carotid atherosclerosis.

2. To analyze the prevalence of carotid plaques and differences in gender and age.
2 Material and methods

2.1 The Tromsø Study

The Tromsø study is a population-based prospective study of the municipality of Tromsø, located in North Norway. The study includes a total of seven surveys (Tromsø 1-7) carried out from 1974 to 2016 with 5-7 years apart (80). The major aim of the first survey was to identify the causes of CVD related mortality in men in northern Norway, with focus on its prevention. Women were included from the second survey and a broader set of diseases as cancer, osteoporosis, mental and neurological diseases have been included throughout the surveys (81). According to the official population registry, varying samples of the residents of the municipality of Tromsø have been invited to participate in the survey. Personal invitations were sent by mail enclosed with a questionnaire and information about the survey and the examinations. The questionnaires included information about chronic diseases and health conditions. From Tromsø 1 to 7, a total of 160 427 subjects were invited to participate in the study and a total of 114 397 attended the surveys, with the majority having repeated measurements (82). Tromsø 4-7 includes two visits, the visit 1 with questionnaires and basic measures and visit 2 with extended examinations performed 2-4 weeks later (81). The selection for visit 2 was a predefined random selection of all participants, but only those attending visit 1 were invited to attend the visit 2. The surveys 1-5 had high attendance rates (over 75%), while somewhat lower in the Tromsø 6 (66%) and Tromsø 7 (65%) (82).
2.2 Study population and design

We performed a cross-sectional study based on data from the fifth survey of the Tromsø study (Tromsø 5) conducted in 2001-2002, as this survey has measurements of both iron status and ultrasound of the carotid artery. The iron status was measured in visit 1 and the measurement of hemoglobin and the ultrasound examination were performed in visit 2. The participants of Tromsø 5 were selected and invited if they had attended the second visit of Tromsø 4 (1994-95). In addition, a smaller group of subjects (N=1916) attended the survey as part of a national health study from the Norwegian Institute of Public Health (82). The survey had an attendance rate of 79% with 8130 attendees out of 10 353 invitees. Detailed information about the invited and attended subjects of the survey according with the age groups and sex is provided in the appendix of this thesis. A total of 5920 subjects attended the visit 2 and of these, a total of 5423 subjects performed the carotid examination, which represents the study population of this master thesis (Figure 3).
Invited Tromsø 5
N= 10 353

Attended the survey
N= 8 130

Withdrawn consent (66)

Participated – visit 1
N= 8 064

No carotid ultrasound examination (497)

Participated – visit 2
N= 5 920

Carotid ultrasound
N= 5 423

Women
N= 3 038

Men
N= 2 385

Figure 3 Flow chart of the study population.
2.3 Measurements

2.3.1 Iron status, hemoglobin and risk factors measurements

Non-fasting blood samples were performed in the cubital vein of the participants in sitting position. It was used an automated blood cell counter to measure the blood cell counts (Coulter Counter, Beckman Coulter, Inc., Brea, CA) (83). All blood samples were analyzed at the Department of Clinical Chemistry of the University Hospital of North Norway in Tromsø. The blood samples were analyzed within 24 hours. Before transportation to the laboratory, located at 1.4 km of distance, hematologic blood samples were stored at room temperature. The blood samples for the iron status were performed at visit 1 and include measurements of s-ferritin, transferrin saturation, s-iron, s-transferrin and s-total ironbinding capacity (TIBC). The blood samples for glycosylated hemoglobin (HbA1c), total and HDL cholesterol, triglycerides and glucose were also collected at visit 1. The samples for hemoglobin, c-reactive protein (CRP), white blood cells (WBC), fibrinogen and thrombocytes were drawn at visit 2.

Measurements of s-ferritin, s-iron and s-transferrin were conducted on a Hitachi 917 analyzer from Boehringer, Germany. The ferrozine method was used to measure the s-iron. S-ferritin and s-transferrin were measured by a turbidimetric assay. S-transferrin was reported in grams per liter (g/L), s-TIBC was calculated as s-TIBC µmol/L = 25.1 x s-transferrin. Transferrin saturation (%) was calculated as 100 x (s-iron/s-TIBC) (84). The analyses for triglycerides, total and HDL cholesterol were measured by standard enzymatic colorimetric methods and high sensitivity CRP was measured by a particle-enhanced immunoturbidimetric assay from Roche (Mannheim, Germany). The analyses for HbA1c were performed with Bayer DCA 2000 (Bayer AG, Leverkusen, Germany).

All physical examinations were performed by trained personnel. Electronic scales were used for height and weight measurements and the participants were wearing light clothing and no
footwear. The BMI was calculated from the body weight in kilograms divided by the square of height in meters (kg/m$^2$). The blood pressure was measured with an automatic device (Dinamap Vital Signs Monitor 1846 Criticon) three times at one minute intervals after two minutes of seated resting (85). The mean of the two last recordings was used in this study.

### 2.3.2 Questionnaires

Two different questionnaires were sent to citizens with age under 70 years and those with 70 years and above (82). Moreover, it was asked to the subjects that attended the survey to complete and send back another questionnaire with additional information. These questionnaires as well as the invitation are provided in the appendix of this thesis. Self-reported questionnaires included information about CVD, diabetes mellitus, smoking habits, alcohol consumption and use of medication. Self-reported myocardial infarction and/or stroke and/or angina pectoris were defined as CVD. Presence of diabetes was defined as self-reported diabetes and/or HbA1c \( \geq 6.5 \).

### 2.3.3 Carotid ultrasound examination

The ultrasound examination was performed on the right carotid by four different examiners. The equipment used was an duplex scanner (Acuson Xp10 128 ART-upgraded) with a linear array 7.5-MHz transducer (16). In order to obtain equal and standardized examination techniques, all examiners have participated in a pre-study training protocol during two months. The carotid examination was performed longitudinally in 6 locations, in the near and far walls of respectively the right common carotid artery (CCA), the bifurcation (bulb) and the internal carotid artery. An atherosclerotic plaque was defined as a localized protrusion into the lumen of the vessel wall of more than 50% compared to the adjacent IMT (38). A maximum of 6
plaques were registered in each subject. Plaques and IMT were recorded at angles which provided the best view of plaque size and IMT (16). All plaque measurements were recorded on videotapes and the images were digitized through a video grabber card (meteor II/Matrox Intellicam). The Adobe Photoshop image-processing program (version 7.0.1) was used to calculate the plaque area. The plaque area was assessed by outlining the plaque perimeter manually with a cursor and the plaque area was calculated (39). The total plaque area (TPA) was calculated as the sum of all plaque areas (16). For the IMT measurement, it was used an automated R-triggered assessing the near and far walls of the CCA and the far wall of the bulb (86). In addition, an automated computerized edge-detection program was used to measure the IMT and the lumen diameter of the CCA. The program was developed by the Wallenberg Laboratory of the Sahlgrenska University Hospital in Gothenburg, Sweden (87), and it provides estimates of the mean IMT and lumen diameter, from 100 measurements along a predefined 10 mm segment of the CCA and the bulb (16). The average of the mean IMT of the 3 locations was used in this study. Subjects with suspected carotid stenosis or presence of occlusion, were referred to the Department of Neurology at the University Hospital of North Norway.

2.4 Data analyses

The statistical analyses of this study was performed using the program of Statistical Package for the Social Sciences (SPSS) version 24.0 for Windows. The level of statistical significance was defined as double-sided p-value <0.05 for all analyses. All statistical analyses were based on cases with complete data on all variables in the respective models.
The data analyses included descriptive and analytical statistics. To test the mean difference of the baseline characteristics between the sexes (Table 1), it was performed the Student’s t-test (independent-samples T test) for continuous variables with parametric distribution, whereas continuous variables with non-parametric distribution were analyzed by Mann-Whitney U test. The Chi-square test was used to test differences between categorical variables. Categorical variables were presented as percentages and normal distributed continuous variables were presented as mean with standard deviation. Due to non-parametric distribution, the variables of age, TPA, triglycerides, s-ferritin and CRP were presented as median and interquartile range.

The dependent (outcome) variable, carotid atherosclerosis, was investigated in continuous and categorical variables that are: 1) total mean IMT; 2) TPA; 3) plaque presence (yes/no); and 4) high plaque load (≥3 plaques/no plaque). The independent (exposure) variables were hemoglobin, s-ferritin, transferrin saturation and s-TIBC. Dichotomized plaque outcomes were analyzed with multiple binary logistic regression models. Continuous carotid outcomes (total mean IMT and TPA) were analyzed with multiple linear regression models. Exposure variables were analyzed in separate models for each outcome to avoid multicollinearity.

Assumptions were checked for the dependent and independent variables in multiple linear regression and logistic regression. The histogram from the total mean IMT variable, showed slight skewness to the right with several outliers and thus, data was logarithm transformed to approximate normal distribution. Data from TPA variable was not normally distributed, being also logarithm transformed to approximate normal distribution. Due to non-parametric distribution, with skewness to the right and presence of outliers, s-ferritin was log-transformed and if significantly associated also presented as quartiles with the lowest quartile as the reference. The data transformations were performed using the natural log and are provided in the appendix of this thesis.
We choose a priori to perform stratification by sex in all multivariate analyses because the levels of iron status and hemoglobin were lower in women than in men. In addition, there was significant interaction between sex and hemoglobin and between sex and age.

The association between iron and carotid atherosclerosis was examined in three models. The model 1 was adjusted by age. The model 2 included adjustment for traditional cardiovascular risk factors (age, systolic and diastolic BP, smoking, total and HDL cholesterol, BMI and HbA1c). And the model 3 included adjustment for traditional cardiovascular risk factors and WBC. Daily smoking was categorized as never, previous and current smokers with never smokers as the reference.

The same predictors were entered for men and women for each dependent variable, in order to compare results from the models. The variables of systolic and diastolic BP, total and HDL cholesterol, smoking, BMI and HbA1c were included in the analyses of plaque presence, high plaque load and IMT. In the analyses of TPA, the variables included were systolic and diastolic BP, HDL cholesterol, smoking and HbA1c. It was checked that in the association between hemoglobin and TPA in men, adding total cholesterol and BMI to the model did not change the statistical significance (p=0.003).

In the preliminary assessment of possible confounders, it was performed correlation tests between the outcome and the factors of age, systolic and diastolic BP, smoking, total and HDL cholesterol, triglycerides, BMI, HbA1c, CRP and alcohol consumption. In addition, correlation tests between the same factors and the iron status and hemoglobin were conducted. Then the criteria to include a candidate confounder in the multivariable analyses was based on the variables that had p-value <0.1. We observed that the variables of age, systolic and diastolic BP, smoking, total and HDL cholesterol, BMI, HbA1c and WBC were strong predictors to carotid plaque. Despite triglycerides and alcohol consumption were significantly correlated
with iron status and hemoglobin, they were not considered to be candidate confounders due to the p-values >0.1 in the multivariate analyses.

Ferritin is an acute phase response protein and so, its levels can be elevated in the presence of inflammation and infection (56). The c-reactive protein (CRP) is an inflammatory marker commonly used in the clinical area. We observed that CRP was significantly correlated with iron status and hemoglobin, though in the multivariable analyses the p-value was above 0.1. In addition, the change effect of CRP on the risk estimate in terms of odds ratio (OR) was below 10% (88). Moreover, we checked that the adjustment for CRP did not change the estimates of the iron parameters association with carotid plaque. Therefore, CRP was not considered to be a candidate confounder. On the other hand, the WBC were included in a third model because it was a significant predictor of carotid plaque especially among men in almost all multivariate analyses. This may reflects the inflammation response in atherosclerosis (89).

### 2.5 Ethical consideration and consent

Confidentiality and professional secrecy of all information of the participants from the Tromsø study have been assured by the professionals involved. The study complies the Declaration of Helsinki. The Data Inspectorate of Norway and the Regional Committee for Medical Research Ethics (REK) have approved the Tromsø study. Informed consent was signed by all subjects for participation in the Tromsø study. Participants had the right to withdraw consent.
3 Results

3.1 Baseline characteristics

The characteristics of the study subjects at baseline are shown in the table 1. Men had higher TPA and IMT than women. Men had also higher levels of hemoglobin, s-ferritin and transferrin saturation than women. A considerable percentage of the participants were using BP lowering drugs, though the mean of systolic BP was high. Women had higher levels of total and HDL cholesterol than men, though the use of cholesterol lowering drugs was higher among men. The prevalence of diabetes was higher in men than in women and both sexes were similarly current smokers. The mean of BMI was equal in both sexes and the subjects were overweight. Subjects were aged between 32 to 89 years.

Table 1 Baseline characteristics of study subjects by sex.

|                                | N    | Women          | N    | Men          | p-value
|--------------------------------|------|----------------|------|--------------|--------
| Age, years†                  | 3038 | 66.0 (13.0)    | 2385 | 67.0 (10.0)  | 0.1    |
| Total plaque area, mm²†      | 3022 | 5.3 (16.8)     | 2371 | 11.9 (28.7)  | <0.0001|
| Intima-media thickness, mm   | 2964 | 0.8 (0.2)      | 2344 | 0.9 (0.2)    | <0.0001|
| Systolic BP, mmHg            | 3036 | 143.5 (22.9)   | 2384 | 143.5 (20.6) | 0.9    |
| Diastolic BP, mmHg           | 3036 | 80.7 (13.0)    | 2384 | 82.5 (11.9)  | <0.0001|
| Current BP lowering drugs    | 742  | 25.1%          | 609  | 26.2%        | 0.6    |
| Current smokers              | 776  | 25.8%          | 604  | 25.4%        | <0.0001|
| Diabetes mellitus            | 129  | 4.4%           | 117  | 5.0%         | 0.3    |
| BMI, kg/m²                   | 3024 | 26.8 (4.6)     | 2369 | 26.8 (3.5)   | 0.7    |
| Total cholesterol, mmol/L    | 3027 | 6.5 (1.2)      | 2378 | 6.1 (1.1)    | <0.0001|
| HDL cholesterol, mmol/L      | 3026 | 1.6 (0.4)      | 2378 | 1.4 (0.4)    | <0.0001|
| Triglycerides, mmol/L †      | 3027 | 1.3 (0.8)      | 2378 | 1.4 (1.0)    | <0.0001|
| Current cholesterol lowering drugs | 366  | 12.6%         | 418  | 18.2%        | <0.0001|
| Hemoglobin, g/dL             | 2654 | 13.5 (0.9)     | 2135 | 14.5 (1.1)   | <0.0001|
| HbA1c, %                     | 2951 | 5.4 (0.8)      | 2342 | 5.6 (0.8)    | <0.0001|
| Serum ferritin, µg/L†        | 2886 | 65.0 (63.0)    | 2258 | 96.0 (98.0)  | <0.0001|
| Serum transferrin, g/L       | 2916 | 2.7 (0.4)      | 2289 | 2.6 (0.4)    | 0.004  |
| Transferrin saturation, %    | 2915 | 25.4 (9.2)     | 2289 | 26.9 (9.6)   | <0.0001|
| Serum iron, µmol/L           | 2915 | 16.7 (5.5)     | 2291 | 17.5 (5.8)   | <0.0001|
| Serum TIBC, µmol/L           | 2916 | 67.2 (10.1)    | 2289 | 66.5 (10.0)  | 0.004  |
| C-reactive protein, mg/L †   | 2979 | 1.5 (2.4)      | 2360 | 1.6 (2.3)    | 0.045  |
| White blood cells, 10 e9/L   | 2653 | 6.3 (1.8)      | 2134 | 6.4 (1.9)    | 0.017  |

*Continuous variables presented as mean (standard deviation) and categorical variables as %.
† Median (interquartile range) due to non-parametric distribution.
‡ Test for difference between sex, analysed by Student’s t-test for continuous variables and parametric distribution. Continuous variables and non-parametric distribution analysed by Mann-Whitney U test. Categorical variables analysed by chi-square test.
The traditional cardiovascular risk factors of the study subjects with and without carotid plaque are shown in the table 2. The participants with plaque had higher systolic and diastolic BP than those without plaque. The frequency of current smokers was lower among the subjects without plaque than those with plaque. The participants with plaque had elevated history of stroke and higher prevalence of diabetes than those without plaque. The subjects without plaque had lower levels of total cholesterol and triglycerides than those with plaque and the mean of HDL cholesterol was equal across the two groups.

Table 2 Cardiovascular risk factors of study subjects with and without carotid plaque.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>With plaque</th>
<th>N</th>
<th>Without plaque</th>
<th>p-value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mmHg</td>
<td>3260</td>
<td>147.7 (21.8)</td>
<td>2160</td>
<td>137.1 (20.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>3260</td>
<td>82.4 (13.0)</td>
<td>2160</td>
<td>80.2 (11.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current smokers</td>
<td>889</td>
<td>64.4%</td>
<td>491</td>
<td>35.6%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>171</td>
<td>69.5%</td>
<td>75</td>
<td>30.5%</td>
<td>0.002</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>3180</td>
<td>5.6 (0.8)</td>
<td>2113</td>
<td>5.4 (0.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of stroke</td>
<td>180</td>
<td>78.6%</td>
<td>49</td>
<td>21.4%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>3244</td>
<td>26.8 (4.1)</td>
<td>2149</td>
<td>26.8 (4.3)</td>
<td>0.9</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3252</td>
<td>6.4 (1.2)</td>
<td>2153</td>
<td>6.2 (1.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>3252</td>
<td>1.5 (0.4)</td>
<td>2152</td>
<td>1.5 (0.4)</td>
<td>0.019</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>3252</td>
<td>1.4</td>
<td>2153</td>
<td>1.3</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Continuous variables presented as mean (standard deviation) and categorical variables as %.
† Median (interquartile range) due to non-parametric distribution.
‡ Test for difference between subjects with and without carotid plaque, analysed by Student’s t-test for continuous variables and parametric distribution. Continuous variables and non-parametric distribution analysed by Mann-Whitney U test. Categorical variables analysed by chi-square test.

3.2 Prevalence of carotid plaque

Atherosclerotic plaque was observed in 1648 women and in 1615 men (Table 3). The prevalence of carotid plaque was 54.2% in women and 67.7% in men with significant statistical difference between the two groups. Men had higher plaque load than women, where 25% had 3 or more plaques compared with 18% of the women. The presence of only one plaque was more frequent among women than in men.
Table 3 Prevalence of carotid plaque and number of plaques in men and women.

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
<th>p-value ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Carotid plaque</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1390</td>
<td>45.8%</td>
<td>770</td>
</tr>
<tr>
<td>Yes</td>
<td>1648</td>
<td>54.2%</td>
<td>1615</td>
</tr>
<tr>
<td>Total</td>
<td>3038</td>
<td>100%</td>
<td>2385</td>
</tr>
<tr>
<td>Number of plaques</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 plaque</td>
<td>830</td>
<td>50.4%</td>
<td>697</td>
</tr>
<tr>
<td>2 plaque</td>
<td>521</td>
<td>31.6%</td>
<td>515</td>
</tr>
<tr>
<td>≥ 3 plaques</td>
<td>297</td>
<td>18.0%</td>
<td>403</td>
</tr>
<tr>
<td>Total</td>
<td>1648</td>
<td>100%</td>
<td>1615</td>
</tr>
</tbody>
</table>

* Categorical variables presented as %.
‡ Test for difference between sex, analyzed by chi-square test.

The number of subjects with atherosclerotic plaque increased with age in both men and women, increasing rapidly from the age of 60 years and declining from the age of 75 years, where the plaque predominance was in women. The figure 4 illustrates the distribution of the number of subjects with carotid plaque by equal age groups in men and women.

Figure 4 Histogram of number of cases with carotid plaque by age groups in men and women.
The total mean IMT and TPA were higher in men than in women, increasing substantially with age in both sexes. There was a linear increase in IMT with age in women and in TPA in men. In women, the plaque area increased substantially after the age of 59 years. These relationships are presented in the figure 5.

![Figure 5 Line graphs of total mean IMT and TPA by age (years) groups in men and women.](image)

Interaction terms were performed and it was observed that sex*hemoglobin was statistically significant in the association with TPA (p=0.002). Likewise, the interaction sex*age was also statistically significant in the association between hemoglobin and plaque presence (p=0.028), and in the association between s-ferritin and high plaque load (p=0.023).

### 3.3 Association between iron and carotid atherosclerosis

It was found association between iron stores and hemoglobin and carotid plaque, which it was most consistent in women. High levels of iron seem to be protective of plaque presence, high plaque load and plaque area.
Hemoglobin was negatively associated with plaque presence and high plaque load in women in both multivariate adjustment models, as shown in Table 4 and Table 5. It seems that hemoglobin had a protective effect in plaque presence and in high plaque load in women, being reduced by 11.3% odds (OR=0.887, 95% CI=0.802 to 0.980) and by 18.3% odds (OR=0.817, 95% CI=0.674 to 0.989) respectively, per 1 unit increase in hemoglobin compared to men. In men, there was no association between hemoglobin and plaque presence (OR=1.0, 95% CI=0.935 to 1.149), and between hemoglobin and high plaque load (OR=0.873, 95% CI=0.745 to 1.022). However, there was a negative significant association between hemoglobin and plaque area in men, independent of adjustment models (Table 6). The significant association between hemoglobin and plaque area in women in the model adjusted for age, was explained by confounding of cardiovascular risk factors which were, high systolic BP, low HDL cholesterol, high HbA1c and smoking.

S-ferritin was negatively associated with plaque presence only in women and the association was strengthened with the adjustment models. Both s-ferritin log-transformed and s-ferritin quartiles had an inverse significant association with plaque presence. In model 3, the third (66-102 µg/L) and the fourth (>103 µg/L) quartiles of s-ferritin showed reduced odds by 27.2% (OR=0.728, 95% CI=0.562 to 0.944) and by 27.6% (OR=0.724, 95% CI=0.558 to 0.939) respectively, compared with the lowest s-ferritin quartile (≤39 µg/L).
Table 4: Associations between plaque presence (yes versus no) and hemoglobin, s-ferritin log-transformed, s-ferritin quartiles, transferrin saturation and s-TIBC in models 1, 2 and 3.

<table>
<thead>
<tr>
<th>Plaque, yes versus no</th>
<th>Women</th>
<th></th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>OR</td>
<td>P</td>
<td>95% CI</td>
<td>N</td>
<td>OR</td>
<td>P</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>2654</td>
<td>0.988</td>
<td>0.799</td>
<td>0.903 to 1.082</td>
<td>2135</td>
<td>1.052</td>
<td>0.289</td>
<td>0.958 to 1.155</td>
</tr>
<tr>
<td>Log-ferritin, µg/L</td>
<td>2886</td>
<td>0.904</td>
<td>0.064</td>
<td>0.813 to 1.006</td>
<td>2258</td>
<td>0.997</td>
<td>0.966</td>
<td>0.885 to 1.124</td>
</tr>
<tr>
<td>Quartiles s-ferritin, µg/L</td>
<td>2886</td>
<td>2258</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.Q reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear trend over Q</td>
<td>2886</td>
<td>0.945</td>
<td>0.126</td>
<td>0.879 to 1.016</td>
<td>2258</td>
<td>0.981</td>
<td>0.662</td>
<td>0.902 to 1.068</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>2915</td>
<td>2289</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s-TIBC, µmol/L</td>
<td>2916</td>
<td>1.014</td>
<td>0.001</td>
<td>1.005 to 1.022</td>
<td>2289</td>
<td>1.002</td>
<td>0.754</td>
<td>0.992 to 1.011</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>2551</td>
<td>0.891</td>
<td>0.024</td>
<td>0.806 to 0.985</td>
<td>2073</td>
<td>1.044</td>
<td>0.411</td>
<td>0.942 to 1.157</td>
</tr>
<tr>
<td>Log-ferritin, µg/L</td>
<td>2800</td>
<td>0.885</td>
<td>0.032</td>
<td>0.791 to 0.990</td>
<td>2207</td>
<td>0.996</td>
<td>0.944</td>
<td>0.879 to 1.128</td>
</tr>
<tr>
<td>Quartiles s-ferritin, µg/L</td>
<td>2800</td>
<td>2207</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.Q reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear trend over Q</td>
<td>2800</td>
<td>0.931</td>
<td>0.065</td>
<td>0.863 to 1.005</td>
<td>2207</td>
<td>0.977</td>
<td>0.613</td>
<td>0.895 to 1.068</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>2828</td>
<td>2237</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s-TIBC, µmol/L</td>
<td>2829</td>
<td>1.012</td>
<td>0.009</td>
<td>1.003 to 1.021</td>
<td>2237</td>
<td>0.999</td>
<td>0.812</td>
<td>0.989 to 1.009</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>2550</td>
<td>0.887</td>
<td>0.019</td>
<td>0.802 to 0.980</td>
<td>2072</td>
<td>1.036</td>
<td>0.498</td>
<td>0.935 to 1.149</td>
</tr>
<tr>
<td>Log-ferritin, µg/L</td>
<td>2451</td>
<td>0.833</td>
<td>0.033</td>
<td>0.739 to 0.940</td>
<td>1973</td>
<td>0.970</td>
<td>0.649</td>
<td>0.850 to 1.107</td>
</tr>
<tr>
<td>Quartiles s-ferritin, µg/L</td>
<td>2451</td>
<td>1973</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.Q reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear trend over Q</td>
<td>2451</td>
<td>0.893</td>
<td>0.007</td>
<td>0.823 to 0.970</td>
<td>1973</td>
<td>0.962</td>
<td>0.418</td>
<td>0.877 to 1.056</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>2476</td>
<td>2001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s-TIBC, µmol/L</td>
<td>2477</td>
<td>1.015</td>
<td>0.002</td>
<td>1.005 to 1.024</td>
<td>2001</td>
<td>1.003</td>
<td>0.584</td>
<td>0.992 to 1.014</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and stratified by sex.
Model 2: adjusted for age+systolic and diastolic BP+smoking (categorical)+BMI+total and HDL chol.+HbA1c and stratified by sex.
Model 3: adjusted for age+systolic and diastolic BP+smoking (categorical)+BMI+total and HDL chol.+HbA1c+WBC and stratified by sex.
Table 5 Associations between high plaque load (≥3 plaques versus no plaque) and hemoglobin, s-ferritin log-transformed, s-ferritin quartiles, transferrin saturation and s-TIBC in models 1, 2 and 3.

<table>
<thead>
<tr>
<th>Plaque-load</th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>OR</td>
<td>P</td>
<td>95%CI</td>
<td>N</td>
<td>OR</td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>1467</td>
<td>0.997</td>
<td>0.969</td>
<td>0.846 to 1.174</td>
<td>1044</td>
<td>0.944</td>
</tr>
<tr>
<td>Log-ferritin, µg/L</td>
<td>1606</td>
<td>0.809</td>
<td>0.033</td>
<td>0.665 to 0.983</td>
<td>1115</td>
<td>0.902</td>
</tr>
<tr>
<td>Quartiles s-ferritin, µg/L</td>
<td>1606</td>
<td></td>
<td></td>
<td></td>
<td>1115</td>
<td></td>
</tr>
<tr>
<td>1.Q reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.Q</td>
<td>0.814</td>
<td>0.323</td>
<td>0.541 to 1.224</td>
<td>0.742</td>
<td>0.138</td>
<td>0.500 to 1.101</td>
</tr>
<tr>
<td>3.Q</td>
<td>0.670</td>
<td>0.060</td>
<td>0.441 to 1.017</td>
<td>1.072</td>
<td>0.730</td>
<td>0.723 to 1.590</td>
</tr>
<tr>
<td>4.Q</td>
<td>0.643</td>
<td>0.037</td>
<td>0.424 to 0.974</td>
<td>0.647</td>
<td>0.034</td>
<td>0.433 to 0.968</td>
</tr>
<tr>
<td>Linear trend over Q</td>
<td>1606</td>
<td>0.859</td>
<td>0.024</td>
<td>0.753 to 0.981</td>
<td>1115</td>
<td>0.911</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>1621</td>
<td>0.986</td>
<td>0.085</td>
<td>0.969 to 1.002</td>
<td>1130</td>
<td>0.998</td>
</tr>
<tr>
<td>S-TIBC, µmol/L</td>
<td>1621</td>
<td>1.027</td>
<td>0.000</td>
<td>1.012 to 1.043</td>
<td>1130</td>
<td>1.005</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>1408</td>
<td>0.823</td>
<td>0.046</td>
<td>0.681 to 0.996</td>
<td>1012</td>
<td>0.893</td>
</tr>
<tr>
<td>Log-ferritin, µg/L</td>
<td>1559</td>
<td>0.754</td>
<td>0.010</td>
<td>0.608 to 0.935</td>
<td>1088</td>
<td>0.882</td>
</tr>
<tr>
<td>Quartiles s-ferritin, µg/L</td>
<td>1559</td>
<td></td>
<td></td>
<td></td>
<td>1088</td>
<td></td>
</tr>
<tr>
<td>1.Q reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.Q</td>
<td>0.787</td>
<td>0.297</td>
<td>0.501 to 1.235</td>
<td>0.674</td>
<td>0.066</td>
<td>0.442 to 1.027</td>
</tr>
<tr>
<td>3.Q</td>
<td>0.587</td>
<td>0.026</td>
<td>0.367 to 0.938</td>
<td>1.049</td>
<td>0.824</td>
<td>0.686 to 1.605</td>
</tr>
<tr>
<td>4.Q</td>
<td>0.562</td>
<td>0.014</td>
<td>0.355 to 0.889</td>
<td>0.603</td>
<td>0.024</td>
<td>0.388 to 0.937</td>
</tr>
<tr>
<td>Linear trend over Q</td>
<td>1559</td>
<td>0.818</td>
<td>0.007</td>
<td>0.707 to 0.947</td>
<td>1088</td>
<td>0.901</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>1574</td>
<td>0.983</td>
<td>0.060</td>
<td>0.965 to 1.001</td>
<td>1103</td>
<td>0.997</td>
</tr>
<tr>
<td>S-TIBC, µmol/L</td>
<td>1574</td>
<td>1.030</td>
<td>0.000</td>
<td>1.014 to 1.047</td>
<td>1103</td>
<td>1.007</td>
</tr>
<tr>
<td>Model 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>1408</td>
<td>0.817</td>
<td>0.038</td>
<td>0.674 to 0.989</td>
<td>1011</td>
<td>0.873</td>
</tr>
<tr>
<td>Log-ferritin, µg/L</td>
<td>1360</td>
<td>0.707</td>
<td>0.004</td>
<td>0.560 to 0.892</td>
<td>970</td>
<td>0.858</td>
</tr>
<tr>
<td>Quartiles s-ferritin, µg/L</td>
<td>1360</td>
<td></td>
<td></td>
<td></td>
<td>970</td>
<td></td>
</tr>
<tr>
<td>1.Q reference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.Q</td>
<td>0.645</td>
<td>0.079</td>
<td>0.395 to 1.052</td>
<td>0.694</td>
<td>0.110</td>
<td>0.443 to 1.086</td>
</tr>
<tr>
<td>3.Q</td>
<td>0.454</td>
<td>0.003</td>
<td>0.272 to 0.758</td>
<td>1.101</td>
<td>0.678</td>
<td>0.699 to 1.734</td>
</tr>
<tr>
<td>4.Q</td>
<td>0.471</td>
<td>0.003</td>
<td>0.286 to 0.774</td>
<td>0.587</td>
<td>0.027</td>
<td>0.366 to 0.943</td>
</tr>
<tr>
<td>Linear trend over Q</td>
<td>1360</td>
<td>0.775</td>
<td>0.002</td>
<td>0.660 to 0.909</td>
<td>970</td>
<td>0.898</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>1373</td>
<td>0.983</td>
<td>0.092</td>
<td>0.964 to 1.003</td>
<td>983</td>
<td>0.996</td>
</tr>
<tr>
<td>S-TIBC, µmol/L</td>
<td>1373</td>
<td>1.032</td>
<td>0.000</td>
<td>1.014 to 1.051</td>
<td>983</td>
<td>1.011</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and stratified by sex.
Model 2: adjusted for age+systolic and diastolic BP+smoking(categorical)+BMI+total and HDL chol.+HbA1c and stratified by sex.
Model 3: adjusted for age+systolic and diastolic BP+smoking(categorical)+BMI+total and HDL chol.+HbA1c+WBC and stratified by sex.
Table 6: Associations between TPA log-transformed and hemoglobin, s-ferritin log-transformed, transferrin saturation and s-TIBC in models 1, 2 and 3.

<table>
<thead>
<tr>
<th></th>
<th><strong>Log-TPA</strong></th>
<th><strong>Women</strong></th>
<th></th>
<th></th>
<th><strong>Men</strong></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>B</td>
<td>P</td>
<td>95%CI</td>
<td>N</td>
<td>B</td>
<td>P</td>
</tr>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>1424</td>
<td>0.047</td>
<td>0.018</td>
<td>0.008 to 0.085</td>
<td>1433</td>
<td>-0.038</td>
<td>0.033</td>
<td>-0.074 to -0.003</td>
</tr>
<tr>
<td>Log-ferritin, µg/L</td>
<td>1548</td>
<td>0.023</td>
<td>0.336</td>
<td>-0.023 to 0.069</td>
<td>1506</td>
<td>-0.023</td>
<td>0.343</td>
<td>-0.07 to 0.024</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>1566</td>
<td>-0.001</td>
<td>0.763</td>
<td>-0.005 to 0.003</td>
<td>1526</td>
<td>-0.002</td>
<td>0.416</td>
<td>-0.005 to 0.002</td>
</tr>
<tr>
<td>S-TIBC, µmol/L</td>
<td>1567</td>
<td>0.003</td>
<td>0.138</td>
<td>-0.001 to 0.006</td>
<td>1526</td>
<td>-0.0004</td>
<td>0.842</td>
<td>-0.004 to 0.003</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>1374</td>
<td>0.013</td>
<td>0.516</td>
<td>-0.026 to 0.053</td>
<td>1398</td>
<td>-0.056</td>
<td>0.002</td>
<td>-0.092 to -0.021</td>
</tr>
<tr>
<td>Log-ferritin, µg/L</td>
<td>1505</td>
<td>0.014</td>
<td>0.537</td>
<td>-0.031 to 0.060</td>
<td>1481</td>
<td>-0.024</td>
<td>0.310</td>
<td>-0.071 to 0.023</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>1522</td>
<td>-0.0003</td>
<td>0.888</td>
<td>-0.004 to 0.004</td>
<td>1500</td>
<td>0.0002</td>
<td>0.918</td>
<td>-0.004 to 0.004</td>
</tr>
<tr>
<td>S-TIBC, µmol/L</td>
<td>1523</td>
<td>0.003</td>
<td>0.157</td>
<td>-0.001 to 0.006</td>
<td>1500</td>
<td>-0.001</td>
<td>0.574</td>
<td>-0.005 to 0.003</td>
</tr>
<tr>
<td><strong>Model 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>1373</td>
<td>0.013</td>
<td>0.533</td>
<td>-0.027 to 0.052</td>
<td>1397</td>
<td>-0.059</td>
<td>0.001</td>
<td>-0.095 to -0.024</td>
</tr>
<tr>
<td>Log-ferritin, µg/L</td>
<td>1316</td>
<td>0.017</td>
<td>0.497</td>
<td>-0.032 to 0.066</td>
<td>1323</td>
<td>-0.028</td>
<td>0.260</td>
<td>-0.077 to 0.021</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>1331</td>
<td>0.001</td>
<td>0.772</td>
<td>-0.004 to 0.005</td>
<td>1342</td>
<td>0.001</td>
<td>0.778</td>
<td>-0.004 to 0.005</td>
</tr>
<tr>
<td>S-TIBC, µmol/L</td>
<td>1332</td>
<td>0.002</td>
<td>0.388</td>
<td>-0.002 to 0.005</td>
<td>1342</td>
<td>-0.001</td>
<td>0.498</td>
<td>-0.005 to 0.003</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and stratified by sex.
Model 2: adjusted for age+systolic and diastolic BP+smoking(categorical)+HDL chol.+HbA1c and stratified by sex.
Model 3: adjusted for age+systolic and diastolic BP+smoking(categorical)+HDL chol.+HbA1c+WBC and stratified by sex.

Furthermore, we found a negative consistent association between s-ferritin and plaque load in women in both analyses of log-transformed s-ferritin and quartiles, being strengthened with multivariate adjustment models. In model 3, the third and the fourth s-ferritin quartiles showed reduced odds by 54.6 % (OR=0.454, 95% CI= 0.272 to 0.758) and by 52.9% (OR=0.471, 95% CI=0.286 to 0.774) respectively, compared with the lowest s-ferritin quartile. In men, there was a significant negative association between the highest (>153 µg/L) compared to lowest (≤54 µg/L) s-ferritin quartile and plaque load that was independent of adjustment models. Although, this association was not as consistent as in women, as there was no linear trend over quartiles and no significant association in s-ferritin log-transformed. Moreover, there was no association between s-ferritin and plaque area neither in women nor in men.

Transferrin saturation was negatively associated with plaque presence only in women, that was strengthened by the adjustment models, suggesting reduced odds by 1.6% (OR=0.984, 95%
CI=0.974 to 0.994) per 1 unit increase in transferrin saturation compared to men. There was no association between transferrin saturation and high plaque load and plaque area neither in women nor in men. Furthermore, there was no association between iron stores and hemoglobin and IMT (Table 7). The significant association between hemoglobin and transferrin saturation and IMT among women in the model adjusted for age, was confounded by the factors of high systolic BP, low diastolic BP, smoking, high total cholesterol, low HDL cholesterol and high BMI. There was no association between s-TIBC and carotid atherosclerosis.

The association between s-ferritin and hemoglobin and plaque presence in women, was strengthened by age, systolic BP, smoking, high total cholesterol and low HDL cholesterol. In addition to these factors, high HbA1c was an important factor to strength the association between s-ferritin and high plaque load in women, whereas in the association between
hemoglobin and high plaque load, the important factors were age, systolic BP, smoking, low HDL cholesterol and high HbA1c. In men, the important factors that strengthened the association between s-ferritin and plaque presence were high age and systolic BP, low diastolic BP, smoking and WBC.

We used the coefficient of determination, the $R^2$, to assess the amount of variance in the outcome explained by the model in multivariate regression models. The higher values of the $R^2$ were observed when the cardiovascular risk factors were entered. The highest $R^2$ values in terms of Cox & Snell $R^2$ and Nagelkerke $R^2$ was that 30.4% and 42.2% respectively, of the variance in high plaque load among men was explained by s-ferritin, age, systolic and diastolic BP, smoking, BMI, total and HDL cholesterol, HbA1c and WBC. In this model, the strongest predictors assessed by the highest Wald test values were age (85.4), smoking (36.0), systolic BP (25.8) and s-ferritin (8.9), followed by WBC (7.3), diastolic BP (7.1) and HbA1c (2.4), total cholesterol (1.7) and BMI (1.3).

In all analyses, the strongest predictors to plaque presence and high plaque load were high age, high systolic BP and smoking. Smoking was a strong predictor to carotid atherosclerosis and its highest value in terms of OR was observed in the analyses of s-ferritin quartiles. In men, current smokers had 6 times increased odds (95% CI=3.857 to 10.806) of high plaque load compared to never smokers. And in women, current smokers had 5 times higher odds (95% CI=3.653 to 8.609) of high plaque load compared to never smokers.
4 Discussion

4.1 Methodological considerations

4.1.1 Study design

We performed a cross-sectional study which is an observational study that allows the investigation of the relationship between the exposure and the outcome, and the comparison of different groups in a representative sample at a single point in time (90). And so, it may well be used to measure the prevalence of carotid atherosclerosis and to examine the association between the iron status and disease. However, a cross-sectional study can not determine the disease etiology or causality of an association neither the relationship between risk factors and disease throughout the time. Our study was conducted in a representative sample and geographically defined, allowing sex-specific analyses that with the high attendance rates of the survey represent the major strengths of our study.

4.1.2 Internal validity

Validity is an important issue in epidemiological research and it can be defined as the extent of valid inferences taken from a study (91). There are two types of validity, the internal and the external validity. The internal validity is related with the extent of presence of bias or systematic errors in a study, and it depends on study methodology. Bias can be defined as a systematic deviation from the truth that weakens the internal validity of a study, leading to differential errors that may be different between groups (92). Usually, bias may occur in observational studies in three different categories that are selection bias, information bias and confounding, which it will be discussed separately.
Selection bias

The selection of the study subjects can lead to bias of the results. In the Tromsø 5 there was 2223 persons (21%) that did not attend the survey (93). In addition, 497 subjects were excluded from the analyses because they did not perform the carotid examination for some reason, and 66 persons have withdrawn their consent. There may be systematic differences between participants and non-participants. This phenomenon is called non-response bias and it may lead to differences in the relation of the exposure and the outcome between the two groups (92). Moreover, non-respondents of health surveys tend to be older, sicker and less educated. Thus, participants may possibly be healthier than the non-participants and so, it may occur the healthy participant effect (94). Nevertheless, the use of the official population registry in the Tromsø study to select the participants and the high attendance rate (79%) of the 5th survey, are important aspects to avoid selection bias.

Information bias

Information bias refers to the collection of the relevant information and the accuracy of the measurements (exposure, covariate or outcome), that can affect the quality of results (91). The carotid atherosclerosis was accurately measured through the ultrasound examination and other computer-assisted methods. Likewise, body iron status and potential confounders were accurately measured in blood samples and physical examinations. The objective and reliable measurements may minimize the information bias, which it can be considered as a strength of our study. However, it was used questionnaires to collect information about current diseases, history of CVD, smoking habits and alcohol consumption, among others. We can not completely exclude information bias, once self-reported information may affect the accuracy of the data collection and chance of misclassification. A further limitation is that the ultrasonography was performed only on the right carotid artery and it is known that carotid
atherosclerosis is a bilateral symmetrical disease (95). Thus, the examination of only one side of the artery may lead to loss of cases. Moreover, the ultrasound examination was performed on-line by four different examiners, which it may increase the chance to occur variability in the ultrasound reading (16). The overall reproducibility of plaque area was good with small inter-observer differences in the mean values (arithmetic and absolute values). Although, the process of plaque outlining counted on systematic difference between two readers, making the computer-assisted on-line method prone to systematic bias (96). Furthermore, other limitation is the measurement of the iron status only at baseline and as it is known, the concentrations of the iron parameters have a considerable individual variability throughout time (97). Thus, repeated measurements of the iron indicators would have been more accurate.

**Confounding**

Confounding is considered to be a problem in epidemiological studies. It is defined as a third variable that is related with both the exposure and the outcome, which it can lead to an error in the estimate of the measure (90). In our study, control for confounding included the use of multivariable regression analyses with adjustment for the factors of age, sex, systolic and diastolic BP, total and HDL cholesterol, smoking, BMI, HbA1c and WBC. Likewise, it was used the stratification by sex to handle confounding.

Despite, the multivariate models were adjusted for several confounders, we cannot exclude other candidate confounding factors such as the use of antioxidants like vitamins C and E and pharmacological antioxidants as aspirin. The use of antioxidants may have a role in the atherogenesis through the inhibition of LDL oxidation (63).
Missing data

Epidemiological and medical research are prone to missing data. It refers to incomplete information in some variables of some study subjects that may lead to systematic differences between the missing and the observed data (91). We performed complete case analyses that refers to a case with any missing data for any of the variables is excluded from the analyses. This approach may lead to a reduction of number of cases included in the analyses (98). Moreover, it may decrease statistical power and biased estimates, if the assumption of missing completely at random (MCAR) is not meet. This assumption considers that there are no systematic differences between the observed and the missing values, though it is difficult to meet this assumption in practice (99).

We observed a low amount of missing data in our study. In regard to our predictors of interest, we observed 4% of missing data for transferrin saturation (N=219) and for s-TIBC (N=218). S-ferritin data was missing in 5% (N=279) and about 12% of the participants had missing values for hemoglobin (N=634). Missing data for the hemoglobin samples was higher due to transportation logistic reasons, where the blood samples drawn on the day before the weekend (after 1:00 pm) were not analyzed leading to a loss of 12% (N=667) of the blood samples (83). Regarding the covariates, there was no missing data for the variables of age and sex. Low percentages of missing data were observed in the variables of systolic and diastolic BP (0.1%), total and HDL cholesterol (0.3%, 0.4%), BMI (0.6%) and HbA1c (2.4%). Likewise, as in hemoglobin, about 12% (N=636) of the participants had missing values for WBC. The fact that about 12% of the participants had missing data for hemoglobin and WBC, may not have been substantial to bias the study population. In addition, the statistical power of our study may not have been affected because our sample is large and representative.
4.1.3 External validity

The external validity is related with the extent that results from a study can be generalized to other populations in addition to the study subjects (91). Generalizability of the findings need to be cautious. There is need to ensure that populations are similar regarding socio-demographic characteristics such as age, sex, ethnicity, education and socioeconomic level (90). The results from our study may be generalized in Western populations with Caucasian ethnicity.

4.1.4 Statistical considerations

Effect modification (interaction)

The effect modification or interaction occurs when the relation between the exposure and the outcome is modified by other factor, affecting the effect estimate (90). We choose a priori the stratification by sex in all analyses because the levels of iron status and hemoglobin vary considerably across sex, where women have lower levels compared to men of the same age, before and after the menopause (54). In addition, this strategy was chosen to handle the interaction effect. Various interaction terms were performed and it was observed that sex*hemoglobin and sex*age were statistically significant. Sex seems to act as a moderator with different effect in men and in women, modifying the relationships of hemoglobin and age with carotid plaque.

Data transformation

Continuous s-ferritin variable and carotid outcomes were logarithm transformed to approximate normal distribution. Data transformation is an useful tool however, it implies some challenges in terms of making inferences about the original data (100). Log data involves mathematical transformation of the mean represented by the geometric mean, which is defined as the antilog...
of the arithmetic mean of log-transformed values (101). The logarithm of a number Y is defined as the power in terms of exponent, that a base number must be raised to obtain the original number. This can be expressed as, \( \log(y) = e^{\log(y)} = Y \). The interpretation of the estimated coefficient (\( \beta \)) in the log-linear model, expressed by the formula \( \log(Y_i) = \alpha + \beta X_i + \epsilon_i \), is that each one-unit increase in \( X \) multiplies the expected value of \( Y \) by \( e^\beta \) (102).

4.2 Findings

4.2.1 Prevalence of carotid atherosclerosis

The prevalence of carotid plaque tends to be higher among men than in women, increasing with aging in both sexes (26). Our study showed significant gender differences in the prevalence of atherosclerotic plaque, being higher in men (67.7%) than in women (54.2%). Men had also larger TPA and thicker intima media layer. Furthermore, the prevalence of carotid plaque increased with age in both sexes similarly although, women had a substantial increase of TPA after the age of 59 years that may be related with the establishment of menopause. Indeed, age and sex were strong and independent predictors of atherosclerotic plaque, which is in line with previous studies (26), (25).

4.2.2 Association between iron status and carotid atherosclerosis

All iron parameters were significantly associated with carotid plaque in both categorized and linear models. Our findings revealed that s-ferritin, transferrin saturation and hemoglobin were negatively associated with carotid plaque with consistent findings in women, suggesting that they were associated with lower risk of atherosclerotic plaque. High levels of s-ferritin were
associated with greater protective effect towards high plaque load, where the third and the fourth s-ferritin quartiles showed reduced odds by 54.6% (OR=0.454, 95% CI=0.272 to 0.758) and by 52.9% (OR=0.471, 95% CI=0.286 to 0.774) respectively, compared with the lowest s-ferritin quartile. Hemoglobin had a protective effect in plaque presence and high plaque load in women, being reduced by 11.3% odds (OR=0.887, 95% CI=0.802 to 0.980) and by 18.3% odds (OR=0.817, 95% CI=0.674 to 0.989) respectively, per 1 unit increase in hemoglobin compared to men. We found no association between iron stores and hemoglobin and IMT.

The risk factors of CVD have been extensively investigated in the past and it includes age, gender, hypertension, cigarette smoking, hypercholesterolemia, diabetes mellitus and obesity (5). Beyond these traditional risk factors, iron has been suggested to have a role in CVD (49). The Sullivan hypothesis and other studies showed a positive relation between iron stores and risk of CVD (72), (75), (73), while other studies were opposed. A meta-analyses showed an inverse association between transferrin saturation and CHD, and no significant association for other iron markers (103).

Our surprising finding of high iron levels may confer a protective effect in atherosclerotic plaque led to a need to search for alternative explanations. There are few studies that suggest a negative association between iron status and CVD and this may be related with publication bias. Publication bias refers to systematic differences of results between the published and unpublished studies. Its occurrence is related when the publication of a study depends on the direction of its results, where there is a tendency to publish studies that suggested positive results than the studies that showed negative results (104), (105). The consequences of publication bias may be problematic due to difficulties in the interpretation of literature (106). Furthermore, the negative association between iron status and atherosclerosis found in our study may possibly be related with some biological mechanisms. The atherogenesis involves oxidative reactions, inflammatory responses and intervention of immune cells due to injury of
endothelial cells (13). Iron can catalyze in vivo leading to oxidative reactions that are related
cellular damage, which has been involved in the atherogenesis (59). S-ferritin is considered to
be the major indicator of total body iron stores (53). Ferritin may have a protection role from
oxidative damage through the sequestration of iron (5). This is supported by experimental
evidence that suggests that oxidative cell damage may be protected by ferritin (107), (108).
Beyond that, there is strong evidence that in early atherogenesis, it occurs the accumulation of
apolipoprotein B in the subendothelial space of the artery (17). Experimental studies suggested
that ferritin may interact with various plasma proteins particularly, apolipoprotein B. The ability
of ferritin binding with apolipoprotein B may lead to a decrease of apolipoprotein B secretion,
suggesting a possible intersection of the metabolic pathways of iron storage and intercellular
cholesterol (109), (110). This mechanism may also be related with our findings of high levels
of s-ferritin associated with a protection effect on carotid plaque.
5 Conclusion

Our findings suggest that in this population, s-ferritin, transferrin saturation and hemoglobin were negatively associated with carotid atherosclerosis with consistent findings among women, suggesting a protective effect of iron in atherosclerosis. This way our results seem to be opposed to the Sullivan hypothesis. We also conclude that the prevalence of carotid atherosclerosis increased with age and it was higher in men than in women.

The CVD represent a burden in public health as they are the main cause of mortality globally. It is necessary further epidemiological research on the possible role of iron in the CVD as this relationship has not been extensively explored, in order to achieve a better understanding of this issue. As this study is an observational study it is not possible to determine temporal and causal relations. We recommend future studies in prospective designs to assess the effect of the exposure by iron in atherosclerosis over time in a target population.
References

Appendices

Appendix 1: Invitation to the Tromsø 5
Appendix 2: Questionnaire 1
Appendix 3: Questionnaire 2
Appendix 4: Questionnaire 3
Appendix 5: Participation in the Tromsø 5
Appendix 6: Data transformation
Appendix 1: Invitation to the Tromsø 5
Welcome to the fifth round of the Tromsø Study!

-a collaboration between:

Department of Community Medicine, University of Tromsø
Tel: 77 60 48 10 (Mon.-Fri.) tromsoes@turkm.uio.no

National Health Screening Service
Tel: 22 24 21 00 (Mon.-Fri.) post@shus.no

You will find more information about the health survey on the homepage of the National Health Screening Service

www.shus.no

Take the chance!

INVITATION TO A HEALTH STUDY
Would you like to participate in the fifth survey of the Tromsø study?

Why a new round of the Tromsø study?
Large health studies were conducted in Tromsø in 1974, 1979-80, 1986-87, and 1994-95. These surveys have given us important knowledge concerning cardiovascular epidemiology and other serious diseases, such as cancer.

The main purpose of another Tromsø study is to monitor any changes in the health of the population since last survey. We will analyze the information we have about a person, both personal data and results from analysis of frozen blood, and see if there are relationships to diseases that occur. This way we learn more on how cardiovascular diseases, cancer and other major diseases develop and how they can be prevented.

Why are we asking you to participate?
We ask everyone who participated in the Special Study in the Tromsø study in 1994-95 and a selection of others older than 29 years.
What does the study include?
The Tromsø study is first and foremost a research project. Through following up as many as possible from the study of 1994-95, we gain valuable information of health and disease in the population of Tromsø.

Participants’ general health status will be examined with regard to certain diseases and risk conditions. If you have a high risk of developing cardiovascular diseases you will be notified of this.

On the day of the examination you will be guided through the survey and there will be an opportunity to ask questions. Your height, weight and waist circumference is measured, as well as blood pressure, and a blood sample is taken. Your lung capacity is determined, in addition to simple tests of vision and strength. Tests to determine osteoporosis is are also conducted.
The blood sample may later be analyzed for fatty substances, blood sugar, indicators of infections, diet, hormones, liver- and kidney function, and bone markers.

Where are you going to meet?
The survey will for the vast majority take place in Elizabeth Center in Tromsø. For some of the outer places in the municipality, the survey will take place locally. Those concerned are notified in this letter.

On the front page of the questionnaire that you receive with this letter are the opening hours for the health survey and when you have to attend the survey. If you cannot attend at that time, you are welcome any another time during the opening hours of the survey. There is no need to tell us about this — just show up when we are open.

The Questionnaires
With this letter a questionnaire is attached. We kindly ask you to complete this form at home and bring it on the day of the examination.

If you are unsure of how to answer a question, leave it blank. You will be aided at the examination.

Everyone who participates in the study will be given an additional questionnaire of other factors which might affect your health. The questionnaire is to be completed at home and sent to the National Health Screening Service in the enclosed envelope.

Future analysis of blood
The blood which is frozen will be used for medical research only, in order to find factors influencing disease. In most cases this means that data from people with a disease is compared to data from those without it. The comparison is done on already collected data and the new analysis from the frozen blood.

We might want to analyze parts of the DNA from the frozen blood cells. Because DNA is important for the regulating and development in human being, we need knowledge on DNA to understand why diseases evolve. Analysis of this kind are only conducted after the Data Inspectorate has given a permission and if The Regional Committee for Research Ethics has no objections to the analysis.
When you attend the study, you will be asked to sign a consent form where you agree to the following six points:

- That we may contact you with recommendations of follow ups, treatment or prevention of disease.
- That we may ask you to participate in similar studies in the future.
- That we may use the results for medical research.
- That the results, after legal approval from the Data Inspectorate, may be linked with information about you in other registries, to be used for research purposes. This might be registries including information on health, pension and disease, and also data on income, education and occupation, in addition to information from previous health studies in Tromsø. Examples of such registries are the Cancer Registry, the Cause of Death registry and population censuses. In these cases your name and social security number are removed when data is analyzed.
- That the blood sample may be stored and used for medical research. All use of this sample will only take place after approval from the Data Inspectorate and if The Regional Committees for Research Ethics has no objections.
- That the blood sample may also be used for analysis of DNA.

Even if you approve to this now, you are entitled to change your opinion later and also ask to have your profile deleted from the registry. You may also decline to consent to one or more of the points above. The Data Inspectorate has given consent to this fifth survey of the Tromsø Study, and the Regional Committee for Research Ethics has no objections. We keep your results confidential and safe. Everyone employed in the Tromsø Study has signed a confidentiality agreement.
Appendix 2: Questionnaire 1
<table>
<thead>
<tr>
<th></th>
<th>5.3 (Municipality)</th>
<th>(County)</th>
<th>(Country)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.3 (Business)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.4 (Occupation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.7 (Mark)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 1. YOUR OWN HEALTH

**1.1 What is your current state of health?**

<table>
<thead>
<tr>
<th></th>
<th>Poor</th>
<th>Not so good</th>
<th>Good</th>
<th>Very good</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

**1.2 Do you have, or have you had?:**

- Asthma
- Hay fever
- Chronic bronchitis/emphysema
- Diabetes
- Osteoporosis
- Fibromyalgia/chronic pain syndrome
- Psychological problems for which you have sought help
- A heart attack
- Angina pectoris (heart cramp)
- Cerebral stroke/brain haemorrhage

**1.3 Have you noticed attacks of sudden changes in your pulse or heart rhythm in the last year?**

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

**1.4 Do you get pain or discomfort in the chest when:**

- Walking up hills, stairs or walking fast on level ground?
- If you stop, does the pain disappear within 10 minutes?
- Can such pain occur even if you are at rest?

**1.5 If you get such pain, do you usually:**

- Stop?
- Slow down?
- Carry on at the same pace?

**1.6 If you stop, does the pain disappear within 10 minutes?**

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

**1.7 Can such pain occur even if you are at rest?**

### 3. OTHER COMPLAINTS

**3.1 Below is a list of various problems. Have you experienced any of this during the last week (including today)?**

(Tick once for each complaint)

<table>
<thead>
<tr>
<th>Problem</th>
<th>No complaint</th>
<th>Little complaint</th>
<th>Pretty much</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudden fear without reason</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Felt afraid or anxious</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faintness or dizziness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Felt tense or upset</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tend to blame yourself</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleeping problems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressed, sad</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeling of being useless, worthless</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeling that everything is a struggle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeling of hopelessness with regard to the future</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 4. USE OF HEALTH SERVICES

**4.1 How many times in the last 12 months have you been to/used:**

(Tick once for each line)

- General practitioner (GP)
- Medical officer at work
- Psychologist or psychiatrist (private or out-patient clinic)
- Other specialist (private or out-patient clinic)
- Emergency GP (private or public)
- Hospital admission
- Home nursing care
- Physiotherapist
- Chiropractor
- Dentist
-Alternative practitioner

**4.2 How many times in the last 12 months have you been to/used:**

(Tick once for each line)

- General practitioner (GP)
- Medical officer at work
- Psychologist or psychiatrist (private or out-patient clinic)
- Other specialist (private or out-patient clinic)
- Emergency GP (private or public)
- Hospital admission
- Home nursing care
- Physiotherapist
- Chiropractor
- Dentist
-Alternative practitioner

### 5. CHILDHOOD/YOUTH AND AFFILIATION

**5.1 How long altogether have you lived in the county?**

(Put 0 if less than half a year)

**5.2 How long altogether have you lived in the municipality?**

(Put 0 if less than half a year)

**5.3 Where did you live most of the time before the age of 16?**

(Tick one option and specify)

- Same municipality
- Another municipality in the county
- Another county in Norway
- Outside Norway

**5.4 Have you moved within the last five years?**

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Yes, one time</th>
<th>Yes, more than once</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

### 6. BODY WEIGHT

**6.1 Estimate your body weight when you were 25 years old:**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg</td>
</tr>
</tbody>
</table>
7. FOOD AND BEVERAGES

7.1 How often do you usually eat these foods? (Tick once per line)

Fruit, berries
Cheese (all types)
Potatoes
Boiled vegetables
Fresh vegetables/salad
Fatty fish (e.g. salmon, trout, mackerel, herring)

7.2 What type of fat do you usually use? (Tick once per line)

On bread
For cooking

7.3 Do you use the following dietary supplements:

Cod liver oil, fish oil capsules

7.4 How much of the following do you usually drink? (Tick once per line)

Full milk, full-fat curdled milk, yoghurt
Semi-skinned milk, semi-skinned curdled milk, low-fat yoghurt
Skimmed milk, skimmed curdled milk
Extra semi-skinned milk
Juice
Water
Mineral water (e.g. Farris, Ramlesa etc)
Cola-containing soft drink
Other soda/soft drink

7.5 Do you usually drink soft drink: with sugar

7.6 How many cups of coffee and tea do you drink daily? (Put 0 for the types you don't drink daily)

Filtered coffee
Boiled coffee/coarsely ground coffee for brewing
Other type of coffee
Tea

7.7 Approximately how often have you during the last year consumed alcohol? (Do not count low-alcohol and alcohol-free beer)

Never consumed alcohol last year
About 1 time a week
About 1 time a month

To those who have consumed the last year:

7.8 When you drink alcohol, how many glasses or drinks do you normally drink?

7.9 Approximately how many times during the last year have you consumed alcohol equivalent to 5 glasses or drinks within 24 hours?

Number of times

7.10 When you drink, do you normally drink:

Beer
Wine
Spirits

8. SMOKING

8.1 How many hours a day do you normally spend in smoke-filled rooms?

8.2 Did any of the adults smoke at home while you were growing up?

8.3 Do you currently, or did you previously live together with a daily smoker after your 20th birthday?

8.4 Do you/did you smoke daily?

8.5 If you smoke daily, do you smoke now:

8.6 If you previously smoked daily, how long is it since you quit?

8.7 If you currently smoke, or have smoked previously:

8.8 How many cigarettes do you or did you normally smoke per day?

8.9 How old were you when you began daily smoking?

8.10 How many years in all have you smoked daily?

9. EDUCATION AND WORK

9.1 How many years of education have you completed?

9.2 Do you/your current work or income within the next two years?

9.3 Describe the activity at the workplace

Business:

9.4 Which occupation/title have or had you at this workplace?

Occupation:

9.5 In your main occupation, do you work as self-employed, as an employee or family member without regular salary?

9.6 Do you believe that you are in danger of losing your current work or income within the next two years?

9.7 Do you receive any of the following benefits?

Sickness benefit (are on sick leave)
Old age pension, early retirement (AFP) or survivor pension
Rehabilitation/reintegration benefit
Disability pension (full or partial)
Unemployment benefits during unemployment
Social welfare benefits
Transition benefit for single parents
10. EXERCISE AND PHYSICAL ACTIVITY

10.1 How has your physical activity in leisure time been during this last year?  
Think of a weekly average for the year.  
Time spent going to work is count as leisure time. Answer both questions.  

<table>
<thead>
<tr>
<th>Light activity (not sweating/out of breath)</th>
<th>None</th>
<th>Hours per week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Less than 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 or more</td>
</tr>
</tbody>
</table>

10.2 Describe exercise and physical exertion in your leisure time. If your activity varies much e.g. between summer and winter, then give an average. The question refers only to the last year.  
(Tick the most appropriate box)  

- Reading, watching TV or other sedentary activity?  
- Walking, cycling or other forms of exercise at least 4 hours a week?  
- Participation in recreational sports, heavy gardening, etc.?  
- Participation in hard training or sports competitions, regularly several times a week?  

10.3 How has your physical activity in your local community where you live?  
(Include walking or cycling to work, Sunday walk/stroll, etc.)  

10.4 How much interest do people show for what you do?  

10.5 Do you feel that you can influence what happens in your local community where you live?  
(Tick only once)  

11. FAMILY AND FRIENDS

11.1 Do you live with:  
Spouse/partner?  
Yes  No  

11.2 How many good friends do you have?  
Number of friends  

11.3 How much interest do people show for what you do?  
(Tick only once)  

11.4 How many associations, sport clubs, groups, religious communities or similar do you take part in?  
(Write 0 if none)  

11.5 Do you feel that you can influence what happens in your local community where you live?  
(Tick only once)  
Yes, a lot  Yes, some  Yes, a little  No  Never tried  

12. ILLNESS IN THE FAMILY

12.1 Have one or more of your parents or siblings had a heart attack (heart wound) or angina pectoris (heart cramp)?  
Yes  No  Don't know  

12.2 Tick for the relatives who have or have had any of the illnesses:  
(Tick for each line)  

12.3 If any relatives have diabetes, at what age did they get diabetes? (if for e.g. many siblings, consider the one who got it earliest in life):  
Mother's age  Father's age  Brother's age  Sister's age  Child's age  

13. USE OF MEDICINES

With medicines, we mean drugs purchased at pharmacies. Supplements and vitamins are not considered here.  

13.1 Do you use:  
Blood pressure lowering drugs  
Cholesterol-lowering drugs  

13.2 How often have you during the last 4 weeks used the following medicines?  
(Tick once for each line)  

13.3 For those medicines you have checked in points 13.1 and 13.2, and that you've used during the last 4 weeks:  
State the name and the reason that you are taking/have taken these (disease or symptom):  
(Tick for each duration you have used the medicine)  

14. THE REST OF THE FORM IS TO BE ANSWERED BY WOMEN ONLY

14.1 How old were you when you started menstruating?  
Age in years  

14.2 If you no longer menstruating, how old were you when you stopped menstruating?  
Age in years  

14.3 Are you pregnant at the moment?  
Yes  No  Uncertain  Above fertile age  

14.4 How many children have you given birth to?  
Number of children  

14.5 Do you use, or have you ever used?  
(Tick once for each line)  

14.6 If you use/have used prescription estrogen:  
How long have you used it?  
Number of years  

14.7 If you use contraceptive pills, mini pill, contraceptive injection, hormonal IUD or estrogen, what brand do you use?
Appendix 3: Questionnaire 2
<table>
<thead>
<tr>
<th>E13 (Municipality)</th>
<th>(County)</th>
<th>(Country)</th>
<th>E15 (Mark)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### E1. YOUR OWN HEALTH

**What is your current state of health?** *(Tick only once)*

- Poor ........................................... 1
- Not so good ................................... 2
- Good ........................................... 3
- Very good ..................................... 4

**Do you have, or have you had?:**

- **Asthma** ........................................
- **Chronic bronchitis/emphysema** ........
- **Diabetes** ......................................
- **Osteoporosis** ..................................
- **Fibromyalgia/chronic pain syndrome** ...
- **Psychological problems for which you have sought help** ...
- **A heart attack** .............................
- **Angina pectoris** (heart cramp) ...........
- **Cerebral stroke**/brain haemorrhage ...  

**Do you get pain or discomfort in the chest when:**

- Walking up hills, stairs, or walking fast on level ground? Yes No

**If you get such pain, do you usually:**

- Stop? ........................................... 1
- Slow down? ................................. 2
- Carry on at the same pace? ............ 3

**If you stop, does the pain disappear within 10 minutes?** Yes No

**Can such pain occur even if you are at rest?**

### E2. ILLNESS IN THE FAMILY

**Have one or more of your parents or siblings had:**

- **A heart attack** (heart wounds) or
- **angina pectoris** (heart cramp) ........

**Tick for the relatives who have or have had any of the illnesses:** *(Tick for each line)*

- Cerebral stroke or brain haemorrhage ...
- Heart attack before age of 60 years
- Asthma ...........................................
- Cancer ...........................................
- Diabetes ........................................

**If any relatives have diabetes, at what age did they get diabetes?** *(if for e.g. many siblings, consider the one who got it earliest in life)*

### E3. COMPLAINTS

**Below is a list of various problems. Have you experienced any of this during the last week (including today)?** *(Tick once for each line)*

- Sudden fear without reason ............
- Felt afraid or anxious ....................
- Faintness or dizziness ....................
- Felt tense or upset ........................
- Tend to blame yourself ..................
- Sleeping problems ........................
- Depressed, sad ............................
- Feeling of being useless, worthless ..
- Feeling of hopelessness with regard to the future.

### E4. TEETH, MUSCLE AND SKELETON

**How many teeth have you lost/extracted?** *(number of teeth)*

**Have you been bothered by pain and/or stiffness in muscles and joints during the last 4 weeks?**

- Neck / shoulders ..........................
- Arms, hands ..............................
- Upper part of the back ..................
- Lumbar regions ............................
- Hips, legs, feet ............................
- Other places ..............................

### E5. EXERCISE AND PHYSICAL ACTIVITY

**How has your physical activity been during this last year?** *Think of a weekly average for the year. Answer both questions.*

- **Light activity** *(not sweating/out of breath)*
- **Hard physical activity** *(sweating/out of breath)*

### E6. BODY WEIGHT

**Estimate your body weight when you were 25 years old:** *(kg)*
E7.  EDUCATION

How many years of education have you completed?  Number of years  
(include all the years you have attended school or studied)

E8.  FOOD AND BEVERAGES

How often do you usually eat these foods?  
(Tick once for each line)

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Rarely/never</th>
<th>1-3 times/month</th>
<th>1-3 times/week</th>
<th>4-6 times/week</th>
<th>1-2 times/day</th>
<th>3 times or more/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit, berries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese (all types)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potatoes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh vegetables/salad</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat fish (e.g. salmon, trout, mackerel, herring)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

Do you use dietary supplements:  
Cod liver oil, fish oil capsules  
Vitamins and/or mineral supplements  

How much of the following do you usually drink?  
(Tick once for each line)

<table>
<thead>
<tr>
<th>Drink</th>
<th>Rarely/never</th>
<th>1-6 glasses/week</th>
<th>1 glass/day</th>
<th>2-3 glasses/day</th>
<th>4 glasses or more/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full milk, full-fat curdled milk, yoghurt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-skinned milk, semi-skinned curdled milk, low-fat yoghurt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skimmed milk, skimmed curdled milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra semi-skinned milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft drink, mineral water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How many cups of coffee and tea do you drink daily?  
(Put 0 for the types you do not drink daily)  Number of cups

<table>
<thead>
<tr>
<th>Drink</th>
<th>Rarely/never</th>
<th>1-2 glasses/day</th>
<th>3 glasses/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered coffee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled coffee/coarsely ground coffee for brewing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other type of coffee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How many years of education have you completed?  Number of years  
(include all the years you have attended school or studied)

E9.  SMOKING

How many hours a day do you normally spend in smoke-filled rooms?  Number of total hours  
Did any of the adults smoke at home while you were growing up?  
Yes  No

Do you currently, or did you previously live together with a daily smoker after your 20th birthday?  
Yes  No

If you have NEVER smoked daily:  
Go to question E11 (BODILY FUNCTIONS AND SAFETY)

If you smoke currently, or did you previously smoke daily:  

- Cigarettes?  
- Cigars/cigarillos?  
- A pipe?

If you previously smoked daily, how long is it since you quit?  Number of years

If you currently smoke, or have smoked previously:

- How many cigarettes do you or did you normally smoke per day?  Number of cigarettes  
- How old were you when you began daily smoking?  Age in years  
- How many years in all have you smoked daily?  Number of years

E10.  BODILY FUNCTIONS AND SAFETY

Would you feel safe by walking alone in the evening in the area where you live?  
Yes  A little unsafe  Very unsafe

When it comes to mobility, sight and hearing, can you:

- Take a 5 minute walk in fairly high pace?  
- Read ordinary text in newspaper, if necessary with glasses?  
- Hear what is said in a normal conversation?

Do you because of chronic health problems have difficulties with:  
(Tick once for each line)

- Move around in your home?  
- Get out of your home by yourself?  
- Participate in organization or other leisure time activities?  
- Use public transport?  
- Perform necessary daily shopping?
E11. USE OF HEALTH SERVICES

How many times in the last 12 months have you been to/used:
(Tick once for each line)

- A general practitioner (GP)
- Specialist (private or out-patient clinic)
- Emergency GP (private or public)
- Hospital admission
- Home nursing care
- Physiotherapist
- Chiropractor
- Municipal home care
- Dentist
- Alternative practitioner

Are you confident that you will receive health care and home assistance if you need it?

YES ☑ NO ☐ Don’t know ☐

E12. FAMILY AND FRIENDS

Do you live:

- At home ☑
- In an institution/shared apartment ☐

Do you live with:

- Spouse/partner ☑
- Other people ☐

How many good friends do you have?

Count the ones you can talk confidentially with and who can give you help when you need it. Do not count people you live with, but do include your children and other relatives.

How much interest do people show for what you do?

(Tick only once)

- Great interest
- Some interest
- Little interest
- No interest
- Uncertain

E13. CHILDHOOD/YOUTH AND AFFILIATION

How long altogether have you lived in the county?

- Years ☑
- Years ☐

How long altogether have you lived in the municipality?

- Years ☑
- Years ☐

Where did you live most of the time before the age of 16?

(Tick one option and specify)

- Same municipality ☑
- Another municipality ☐
- Which one:
- Another county in Norway ☐
- Which one:
- Outside Norway ☐
- Country:

Have you moved during the last five years?

- No ☑
- Yes, once ☐
- Yes, more than once ☐

E14. USE OF MEDICINES

With medicines, we mean drugs purchased at pharmacies. Supplements and vitamins are not considered here.

Do you use?
(Tick once for each line)

- Blood pressure lowering drugs
- Cholesterol-lowering drugs
- Drugs for osteoporosis
- Insulin
- Tablets for diabetes
- Painkillers non-prescription
- Painkillers on prescription
- Sleeping pills
- Tranquillizers
- Antidepressants
- Other prescription medicines
- Tablets or patches
- Cream or suppositories

How often have you during the last 4 weeks used the following medicines?
(Tick once for each line)

- Not used in the last 4 weeks
- Less than every week
- Every week, but not daily
- Daily

State the name of the medicines you are using now and the reason you are taking the medicines (disease or symptom):
(Tick for each duration you have used the medicine)

E15. THE REST OF THE FORM IS TO BE ANSWERED BY WOMEN ONLY

How old were you when you started menstruating?

Age in years ☑

How old were you when you stopped menstruating?

Age in years ☑

How many children have you given birth to?

Number of children ☑

Do you use, or have you ever used estrogen?

- Never
- Previously
- Now
- Never
- Previously
- Now

If you use estrogen, which brand you use now?

- Yes
- No

Have you ever used contraceptives pills?

- Yes
- No
Appendix 4: Questionnaire 3
## T1. NEIGHBORHOOD AND HOME (cont.)

### 1.6 What do you consider yourself as?  
*(Tick for one or more alternatives)*  
- [ ] Norwegian  
- [ ] Sami  
- [ ] Kven/Finnish  
- [ ] Other  

### 1.7 Do you feel that you have enough good friends?  
- [ ] Yes  
- [X] No  

### 1.8 How often do you normally take part in organised gatherings, e.g. sewing circles, sports clubs, political meetings or other associations?  
*(Tick only once)*  
- [ ] Never, or just a few times a year  
- [ ] 1-3 times a month  
- [ ] Approximately once a week  
- [ ] More than once a week  

## T2. PAID AND UNPAID WORK

### 2.1 If you have paid or unpaid work, how would you describe your work?  
*(Tick only once)*  
- [ ] Mostly sedentary work?  
  *(e.g. office work, mounting)*  
- [ ] Work that requires a lot of walking?  
  *(e.g. shop assistant, light industrial work, teaching)*  
- [ ] Work that requires a lot of walking and lifting?  
  *(e.g. Postman, nursing, construction)*  
- [ ] Heavy manual labour?  
  *(e.g. forestry, heavy farm-work, heavy construction)*  

### 2.2 Can you decide yourself how your work (paid or unpaid) should be organised?  
*(Tick only once)*  
- [ ] No, not at all  
- [ ] To a small extent  
- [ ] Yes, to a large extent  
- [ ] Yes, I decide myself  

### 2.3 Are you on call, do you work shifts or nights?  
- [ ] Yes  
- [ ] No  

---

**Label**

---

The information you give us may later be linked with information from other public health registers in accordance with the rules laid down by the Data Inspectorate and the Regional Board of Research Ethics.

If you are unsure about what to answer, tick the box that you feel fits best.

The completed form should be sent to us in the enclosed prepaid envelope. Thank you in advance for helping us.

Yours sincerely  
Department of Community Medicine  
University of Tromsø  
National Health Screening Service  

---

If you do not wish to answer the questionnaire, tick the box below and return the form. Then you will not receive reminders.

---

Date of completion:

---

T1. NEIGHBORHOOD AND HOME

### 1.1 In which municipality did you live at the age of 1 year?  
*(If you have not lived in Norway, state country of residence instead of the municipality)*

### 1.2 What type of house do you live in?  
*(Tick only once)*  
- [ ] Detached house/villa  
- [ ] Farm  
- [ ] Flat/apartment  
- [ ] Terraced/semi-detached house  
- [ ] Institution/care home  
- [ ] Other  

### 1.3 How big is your house?  

### 1.4 Are you bothered by:  
*(Tick once for each line)*  
- [ ] Moisture, drought or coldness in your home  
- [ ] Other forms of bad indoor climate  
- [ ] Traffic noise (cars or aircraft)  
- [ ] Other noise (industrial, construction, etc.)  
- [ ] Neighbour noise  
- [ ] Drinking water quality  
- [ ] Air pollution from traffic  
- [ ] Air pollution from wood/oil heating, factory etc.  

### 1.5 What home language did your grandparents have?  
*(Tick for one or more alternatives)*  
- [ ] Norwegian  
- [ ] Sami  
- [ ] Kven/Finnish  
- [ ] Other  

---

The main aim of the Tromsø Study is to improve our knowledge about cardiovascular diseases in order to aid prevention. The study is also intended to improve our knowledge of cancer and other general conditions, such as allergies, muscle pains and mental conditions. We therefore like you to answer some questions about factors that may be relevant for your risk of getting these and other illnesses. This form is part of the Health Survey, which has been approved by the Norwegian Data Inspectorate and the Regional Board of Research Ethics. The answers will only be used for research purposes and will be treated strictly confidential.

---

Additional questions to the health survey in Troms and Finnmark 2001-2002
3.1 Do you smoke?

Yes, daily ☐

Yes, sometimes ☐

No, never ☐

If "Yes, sometimes"

What do you smoke?

☐ Cigarettes

☐ Pipe

☐ Cigar/cigarillos

3.2 Have you used or do you use snuff daily?

Yes, now ☐

Yes, previously ☐

Never ☐

If YES:

How many years altogether have you used snuff?

☐ years

4.1 Are you a teetotaller?

Yes ☐

No ☐

4.2 How many times a month do you normally drink alcohol?

(Do not count low-alcohol beer. Put 0 if less than once a month)

☐ times

4.3 How many glasses of beer, wine or spirits do you normally drink in a fortnight?

(Do not count low-alcohol beer. Put 0 if you do not drink alcohol)

Beer ☐

Wine ☐

Spirits ☐

4.4 For approximately how many years has your alcohol consumption been at the same level you described above?

☐ years

4.5 Have you, in one or more periods in the last 5 years consumed so much alcohol that it has inhibited your work or social life?

Yes, at work ☐

Yes, socially ☐

Yes, both at work and socially ☐

No, never ☐

5.1 Do you usually eat breakfast every day?

Yes ☐

No ☐

5.2 How many times a week do you eat a warm dinner?

☐ times

5.3 How important is it for you to have a healthy diet?

Very ☐

Somewhat ☐

Little ☐

Not ☐

5.4 Do you use the following dietary supplements?

Yes, daily ☐

Sometimes ☐

No ☐

Iron tablets ☐

Calcium tablets or bonemeal ☐

Vitamin D supplements ☐

Cod liver oil ☐

6.1 Do you currently try to change your body weight?

No ☐

Yes, I try to gain weight ☐

Yes, I try to lose weight ☐

6.2 What weight would you be satisfied with (your “ideal weight”)?

☐ kg

7.1 Have you ever had:

Tick once for each question. Also give the age at the time. If you have had the condition several times, how old were you the last time

Severe injury requiring hospital admission ☐

Ankle fracture ☐

Peptic ulcer ☐

Peptic ulcer surgery ☐

Neck surgery ☐

Prostate surgery ☐

7.2 Do you have, or have you ever had:

(Tick once for each question)

Cancer ☐

Psoriasis ☐

Thyroid disease ☐

Glaucosa ☐

Cataract ☐

Osteoarthritis (arthrosis) ☐

Bent fingers ☐

Skin contractions in your palms ☐

Kidney stone ☐

Appendectomy ☐

Hernia surgery ☐

Surgery/treatment for urine incontinence ☐

Epilepsy ☐

Poliomyelitis (polio) ☐

Parkinson’s disease ☐

Migraine ☐

Leg ulcer ☐

Allergy and hypersensitivity:

Atopic eczema (e.g. childhood eczema) ☐

Hand eczema ☐

Food allergy ☐

Other hypersensitivity (not allergy) ☐

7.3 Have you had common cold, influenza, gastroenteritis, etc. during the last 14 days?

Yes ☐

No ☐

7.4 Have you during the last 3 weeks had common cold, influenza, bronchitis, pneumonia, sinusitis, or other respiratory infection?

Yes ☐

No ☐

7.5 Have you ever had bronchitis or pneumonia?

Yes ☐

No ☐

7.6 Have you during the last 2 years had bronchitis or pneumonia?

(Tick only once)

No ☐

1-2 times ☐

More than 2 times ☐
T8. SYMPTOMS

8.1 Have you in the last two weeks felt:
(Tick once for each question)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No</th>
<th>A Little</th>
<th>A lot</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nervous or worried</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bothered by anxiety</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confident and calm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Happy and optimistic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Down/depressed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lonely</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8.2 Do you cough about daily for periods of the year?

- If YES:
  - Is your cough productive?
  - Have you had this kind of cough for as long as 3 months in each of the last two years?

8.3 Have you had episodes with wheezing in the chest?

- If YES:
  - Has this occurred:
  - At night
  - In connection with respiratory infections
  - In connection with physical exertion
  - In connection with very cold weather

8.4 Do you get pain in the calf while walking?

- If YES:
  - How long can you go before you notice the pain?
  - How is your memory?

8.5 Do you get short-winded in the following situations?

- While walking fast on level ground
- While walking calmly on level ground
- While washing or dressing yourself
- While resting

8.6 Do you have to stop because of short-windedness while walking in your own pace on level ground?

8.7 Have you during the last year suffered from pain and/or stiffness in muscles and joints that have lasted continuously for at least 3 months?

- If YES:
  - Has the complaint reduced your leisure time activity?

8.8 How often do you suffer from sleeplessness?

- Never, or just a few times a year
- 1-3 times a month
- Approximately once a week
- More than once a week

8.9 If you suffer from sleeplessness monthly or more frequently, what time of the year does it affect you most?

- No particular time of the year
- Especially during the polar night
- Especially during the midnight sun season
- Especially in spring and autumn

8.10 Have you in the last year suffered from sleeplessness to the extent that it has affected your ability to work?

8.11 Do you usually sleep during the day?

8.12 How often do you suffer from urinary incontinence?

- Never
- 1-3 times a month
- More than once a month
- Once a week or more

8.13 Are you able to walk down 10 steps without holding on to something (e.g. a handrail)?

8.14 Do you use glasses?

8.15 Do you use a hearing aid?

8.16 How is your memory?

- Do you forget what you just have heard or read?
- Do you forget where you have placed things?
- Is it more difficult to remember now than earlier?
- Do you more often write memos now than earlier?

9.1 Do you use, or have you used any of the following medicines:

- Drugs for osteoporosis
- Tablets for diabetes

9.2 Do you use any medicines which you take as injections?

T9. MEDICINES

9.1 Do you use, or have you used any of the following medicines:

- Drugs for osteoporosis
- Tablets for diabetes

9.2 Do you use any medicines which you take as injections?
T10. ILLNESS IN THE FAMILY

10.1 Tick for the relatives who have or have ever had any of the diseases: (Tick for each line)

- Heart attack (heart wound)
- Angina pectoris (heart cramp)
- High blood pressure
- Aneurysm
- Gastric/duodenal ulcer
- Hip fracture
- Psychological problems
- Allergy
- Osteoarthritis (arthrosis)
- Dementia

Mother Brother Father Sister Child None of these

10.2 How many siblings and children do you have?

Brothers Sisters Children

Number

10.3 Do you usually do extra caring work because of illness etc. in your close family?

Yes, daily/almost daily
Yes, sometimes
No

1
2
3

10.4 Do you or your family receive home aid or home nursing care?

Yes
No

10.5 Is your mother alive? ......

10.6 Is your father alive? ......

10.7 How is your current menstruation status?

I have not had menstruation in the last year
I have regular menstruation
I have irregular menstruation

12.1 If you have given birth, fill in each child’s birth year and how many months you breastfed after delivery.
(If you did not breastfeed, write 0)

Child: Birth year: Number of months breastfed:

1st child 2nd child 3rd child 4th child 5th child 6th child

T12. THE REST IS TO BE ANSWERED BY WOMEN ONLY

12.2 If you still have menstruate or are pregnant: What date did your last menstruation start?

Day Month Year

12.3 If you no longer menstruate; why did your periods stop? (Tick once)

- It stopped by itself
- Uterus surgery
- Surgically removed both ovaries
- Other reason (e.g. radiation, chemotherapy)

12.4 Do you use or have you used prescribed estrogen (tablets or patches)?

If YES:
How old were you when you started taking estrogen?

If you stopped using estrogen,
How old were you when you stopped taking estrogen?

12.5 Do you use or have you used oral contraceptive pills?

If YES:
How old were you when you started taking the pill?

If you stopped taking the pill,
How many years did you take the pill before your first delivery?

If you stopped taking the pill,
How old were you when you stopped?

12.6 Apart from pregnancy and after giving birth, have you ever stopped having menstruation for 6 months or more?

If YES:
How many times?

12.7 How is your current menstruation status?

12.8 When you were 25-29 years old, how many days usually passed between the start of two periods?

The periods were of approximately equal length every time?

How many days did a typical menstrual bleeding period last?

Thank you for the help!
Remember to mail the form today!
Appendix 5: Participation in the Tromsø 5
<table>
<thead>
<tr>
<th>Age</th>
<th>Invited women</th>
<th>Attendees women</th>
<th>% Women</th>
<th>Invited men</th>
<th>Attendees men</th>
<th>% Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-34</td>
<td>654</td>
<td>348</td>
<td>53.2</td>
<td>628</td>
<td>237</td>
<td>37.7</td>
</tr>
<tr>
<td>35-39</td>
<td>87</td>
<td>75</td>
<td>86.2</td>
<td>55</td>
<td>46</td>
<td>83.6</td>
</tr>
<tr>
<td>40-44</td>
<td>559</td>
<td>389</td>
<td>69.6</td>
<td>533</td>
<td>315</td>
<td>59.1</td>
</tr>
<tr>
<td>45-49</td>
<td>520</td>
<td>373</td>
<td>71.7</td>
<td>473</td>
<td>299</td>
<td>63.2</td>
</tr>
<tr>
<td>50-54</td>
<td>96</td>
<td>92</td>
<td>95.8</td>
<td>160</td>
<td>144</td>
<td>90.0</td>
</tr>
<tr>
<td>55-59</td>
<td>674</td>
<td>630</td>
<td>93.5</td>
<td>232</td>
<td>219</td>
<td>94.4</td>
</tr>
<tr>
<td>60-64</td>
<td>916</td>
<td>827</td>
<td>90.3</td>
<td>744</td>
<td>657</td>
<td>88.3</td>
</tr>
<tr>
<td>65-69</td>
<td>687</td>
<td>636</td>
<td>92.6</td>
<td>637</td>
<td>591</td>
<td>92.8</td>
</tr>
<tr>
<td>70-74</td>
<td>683</td>
<td>604</td>
<td>88.4</td>
<td>542</td>
<td>495</td>
<td>91.3</td>
</tr>
<tr>
<td>75-79</td>
<td>627</td>
<td>495</td>
<td>78.9</td>
<td>470</td>
<td>390</td>
<td>83.0</td>
</tr>
<tr>
<td>80-84</td>
<td>202</td>
<td>145</td>
<td>71.8</td>
<td>156</td>
<td>114</td>
<td>73.1</td>
</tr>
<tr>
<td>+85</td>
<td>12</td>
<td>5</td>
<td>62.5</td>
<td>6</td>
<td>4</td>
<td>66.7</td>
</tr>
<tr>
<td>Total</td>
<td>5717</td>
<td>4619</td>
<td>80.8</td>
<td>4636</td>
<td>3511</td>
<td>75.7</td>
</tr>
</tbody>
</table>

Attendance rate by sex and age groups of the 5th Tromsø Study (2001-2002).
Appendix 6: Data transformation
Histograms of untransformed total mean IMT (A) and log-transformed total mean IMT (B).
Histograms of untransformed TPA (A) and log-transformed TPA (B).
Histograms of untransformed s-ferritin (A) and log-transformed s-ferritin (B).