



UIT

THE ARCTIC
UNIVERSITY
OF NORWAY

Faculty of Health Sciences / Department of Community Medicine

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/Nordlandssykehuset 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 grant 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).

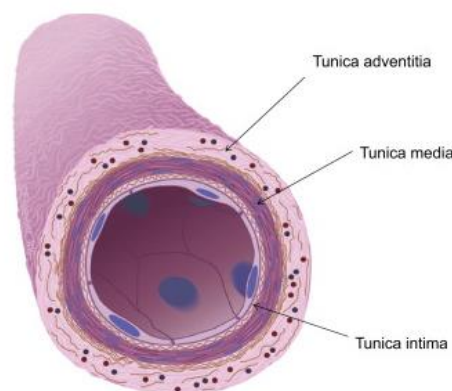


Figure 1 Cross-section of the artery wall.

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).

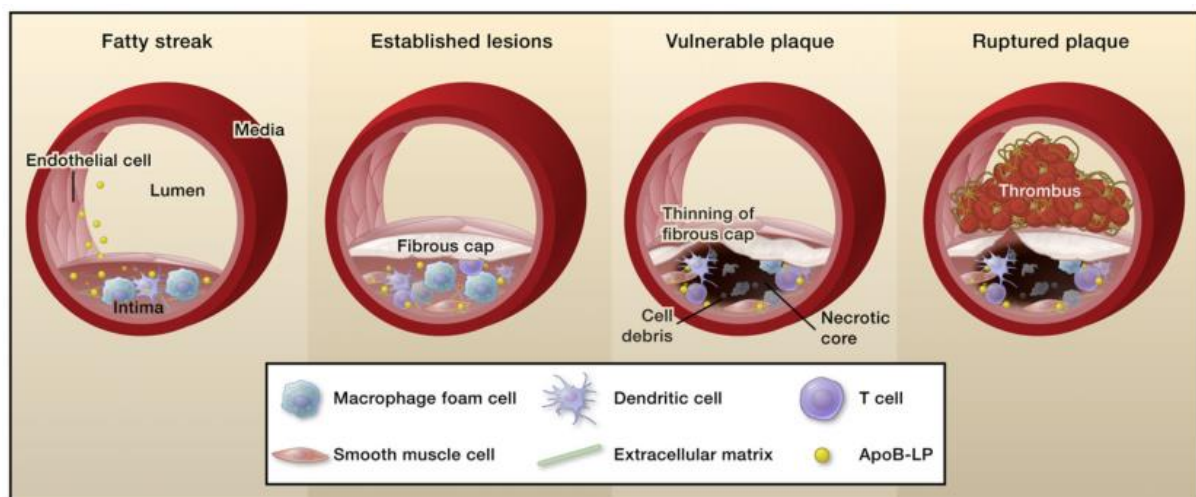


Figure 2 Progression of atherosclerotic plaque.

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 ≥ 140 mmHg or diastolic ≥ 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 $\mu\text{g/L}$ indicate that iron stores are present and higher levels reflect the amount of iron store whereas, low levels (<15 $\mu\text{g/L}$) indicate depleted iron stores (54). The production of transferrin is affected 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 acute 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).

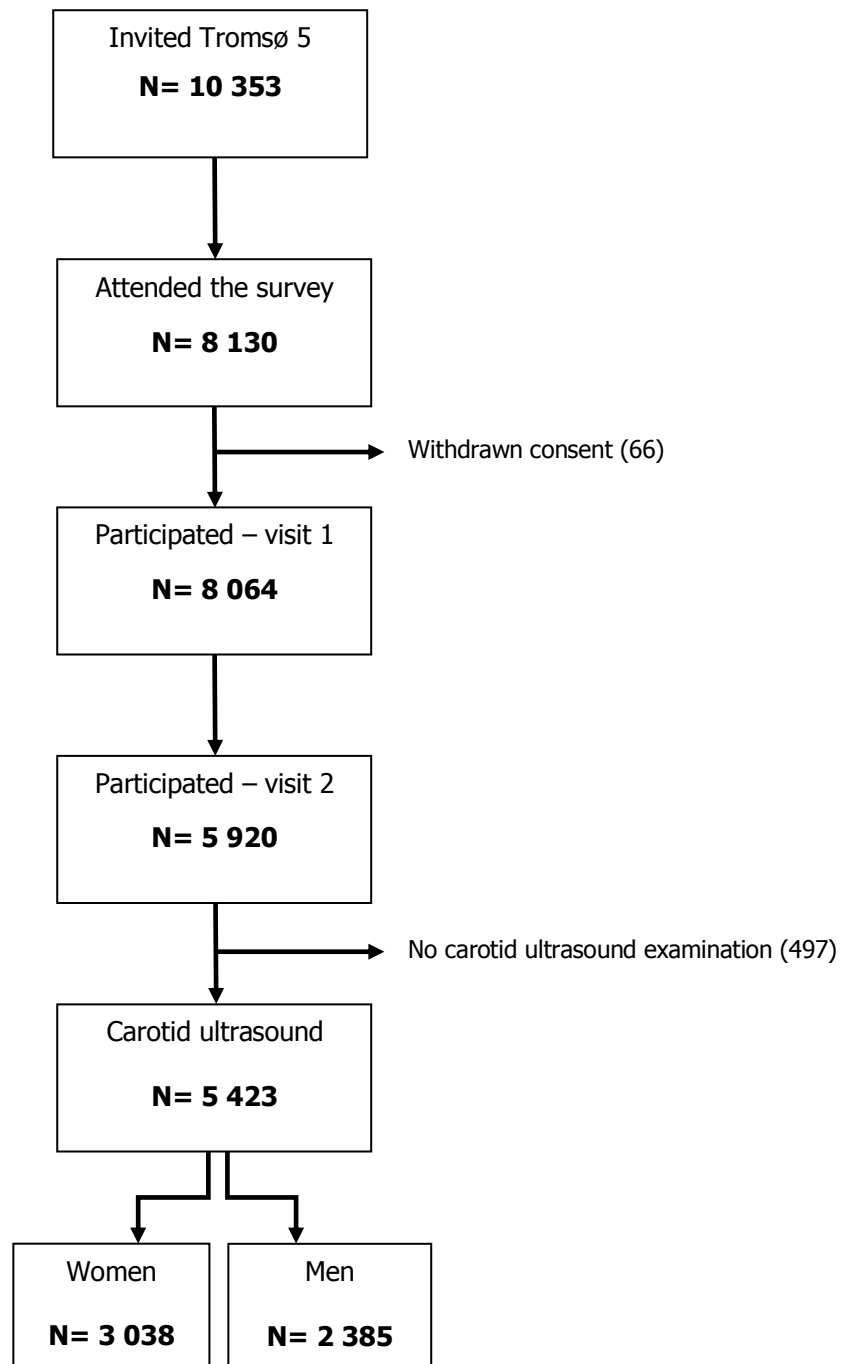


Figure 3 Flow chart of the study population.

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 $s\text{-TIBC } \mu\text{mol/L} = 25.1 \times s\text{-transferrin}$. Transferrin saturation (%) was calculated as $100 \times (s\text{-iron}/s\text{-TIBC})$ (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 $\text{HbA1c} \geq 6.5$.

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 reflect 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, mm ² [†]	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/m ²	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.

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

*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.

	Women		Men		p-value [‡]
	N	%	N	%	
Carotid plaque					<0.0001
No	1390	45.8%	770	32.3%	
Yes	1648	54.2%	1615	67.7%	
Total	3038	100%	2385	100%	
Number of plaques					<0.0001
1 plaque	830	50.4%	697	43.2%	
2 plaque	521	31.6%	515	31.9%	
≥ 3 plaques	297	18.0%	403	25.0%	
Total	1648	100%	1615	100%	

* 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.

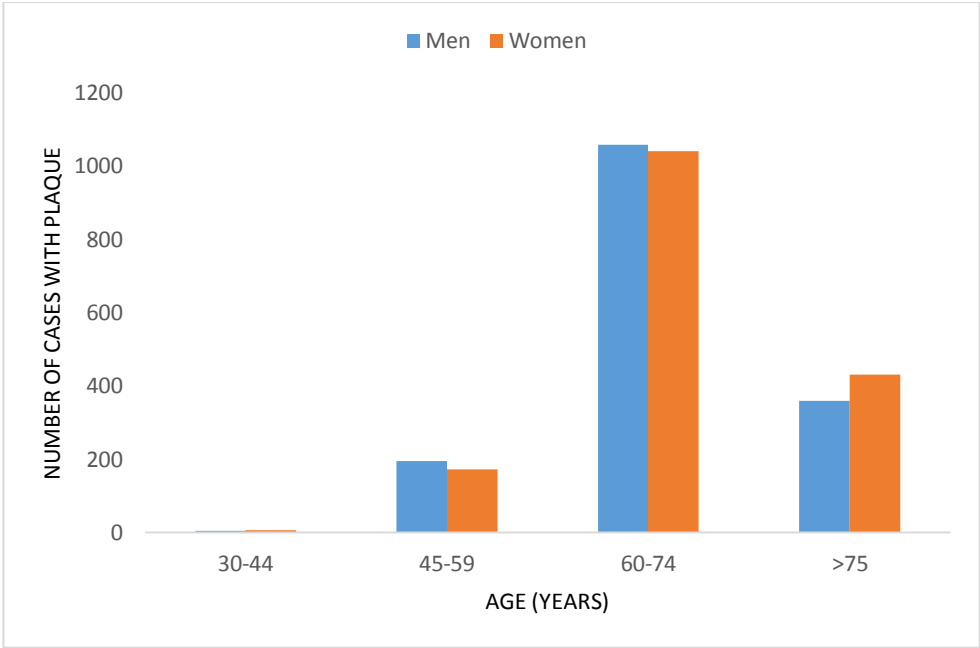


Figure 4 Histogram of number of cases with carotid plaque by age groups in men and women.

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.

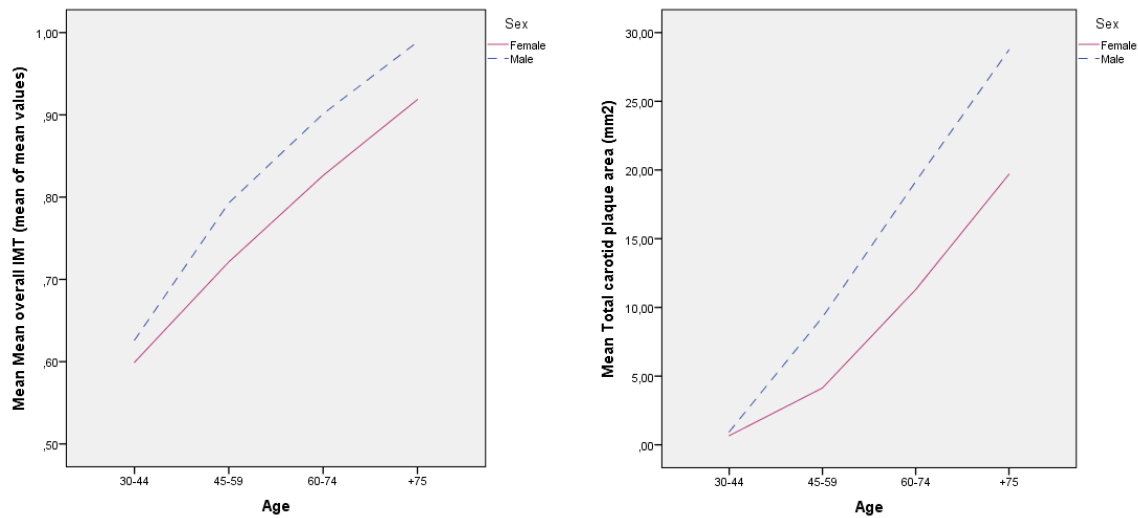


Figure 5 Line graphs of total mean IMT and TPA by age (years) groups in men and women.

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.

Plaque, yes versus no	Women				Men			
	N	OR	P	95%CI	N	OR	P	95%CI
Model 1								
Hemoglobin, g/L	2654	0.988	0.799	0.903 to 1.082	2135	1.052	0.289	0.958 to 1.155
Log-ferritin, µg/L	2886	0.904	0.064	0.813 to 1.006	2258	0.997	0.966	0.885 to 1.124
Quartiles s-ferritin, µg/L	2886				2258			
1.Q reference								
2.Q		0.876	0.261	0.695 to 1.104		0.922	0.555	0.705 to 1.207
3.Q		0.829	0.109	0.659 to 1.043		1.147	0.323	0.874 to 1.507
4.Q		0.841	0.139	0.668 to 1.058		0.879	0.343	0.674 to 1.147
Linear trend over Q	2886	0.945	0.126	0.879 to 1.016	2258	0.981	0.662	0.902 to 1.068
Transferrin saturation, %	2915	0.987	0.003	0.978 to 0.996	2289	0.997	0.582	0.988 to 1.007
S-TIBC, µmol/L	2916	1.014	0.001	1.005 to 1.022	2289	1.002	0.754	0.992 to 1.011
Model 2								
Hemoglobin, g/L	2551	0.891	0.024	0.806 to 0.985	2073	1.044	0.411	0.942 to 1.157
Log-ferritin, µg/L	2800	0.885	0.032	0.791 to 0.990	2207	0.996	0.944	0.879 to 1.128
Quartiles s-ferritin, µg/L	2800				2207			
1.Q reference								
2.Q		0.913	0.461	0.717 to 1.163		0.912	0.516	0.691 to 1.204
3.Q		0.808	0.082	0.635 to 1.028		1.135	0.380	0.855 to 1.506
4.Q		0.818	0.104	0.643 to 1.042		0.867	0.314	0.657 to 1.144
Linear trend over Q	2800	0.931	0.065	0.863 to 1.005	2207	0.977	0.613	0.895 to 1.068
Transferrin saturation, %	2828	0.988	0.011	0.979 to 0.997	2237	0.999	0.878	0.989 to 1.009
S-TIBC, µmol/L	2829	1.012	0.009	1.003 to 1.021	2237	0.999	0.812	0.989 to 1.009
Model 3								
Hemoglobin, g/L	2550	0.887	0.019	0.802 to 0.980	2072	1.036	0.498	0.935 to 1.149
Log-ferritin, µg/L	2451	0.833	0.033	0.739 to 0.940	1973	0.970	0.649	0.850 to 1.107
Quartiles s-ferritin, µg/L	2451				1973			
1.Q reference								
2.Q		0.865	0.271	0.667 to 1.120		0.864	0.330	0.645 to 1.159
3.Q		0.728	0.017	0.562 to 0.944		1.082	0.606	0.802 to 1.459
4.Q		0.724	0.015	0.558 to 0.939		0.820	0.186	0.612 to 1.100
Linear trend over Q	2451	0.893	0.007	0.823 to 0.970	1973	0.962	0.418	0.877 to 1.056
Transferrin saturation, %	2476	0.984	0.001	0.974 to 0.994	2001	0.998	0.682	0.987 to 1.009
S-TIBC, µmol/L	2477	1.015	0.002	1.005 to 1.024	2001	1.003	0.584	0.992 to 1.014

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.

Plaque-load	Women				Men			
	N	OR	P	95%CI	N	OR	P	95%CI
Model 1								
Hemoglobin, g/L	1467	0.997	0.969	0.846 to 1.174	1044	0.944	0.398	0.825 to 1.080
Log-ferritin, $\mu\text{g/L}$	1606	0.809	0.033	0.665 to 0.983	1115	0.902	0.251	0.757 to 1.076
Quartiles s-ferritin, $\mu\text{g/L}$	1606				1115			
1.Q reference								
2.Q		0.814	0.323	0.541 to 1.224		0.742	0.138	0.500 to 1.101
3.Q		0.670	0.060	0.441 to 1.017		1.072	0.730	0.723 to 1.590
4.Q		0.643	0.037	0.424 to 0.974		0.647	0.034	0.433 to 0.968
Linear trend over Q	1606	0.859	0.024	0.753 to 0.981	1115	0.911	0.150	0.803 to 1.034
Transferrin saturation, %	1621	0.986	0.085	0.969 to 1.002	1130	0.998	0.738	0.983 to 1.012
S-TIBC, $\mu\text{mol/L}$	1621	1.027	0.000	1.012 to 1.043	1130	1.005	0.493	0.991 to 1.018
Model 2								
Hemoglobin, g/L	1408	0.823	0.046	0.681 to 0.996	1012	0.893	0.157	0.763 to 1.045
Log-ferritin, $\mu\text{g/L}$	1559	0.754	0.010	0.608 to 0.935	1088	0.882	0.198	0.728 to 1.068
Quartiles s-ferritin, $\mu\text{g/L}$	1559				1088			
1.Q reference								
2.Q		0.787	0.297	0.501 to 1.235		0.674	0.066	0.442 to 1.027
3.Q		0.587	0.026	0.367 to 0.938		1.049	0.824	0.686 to 1.605
4.Q		0.562	0.014	0.355 to 0.889		0.603	0.024	0.388 to 0.937
Linear trend over Q	1559	0.818	0.007	0.707 to 0.947	1088	0.901	0.137	0.785 to 1.034
Transferrin saturation, %	1574	0.983	0.060	0.965 to 1.001	1103	0.997	0.664	0.982 to 1.012
S-TIBC, $\mu\text{mol/L}$	1574	1.030	0.000	1.014 to 1.047	1103	1.007	0.334	0.993 to 1.022
Model 3								
Hemoglobin, g/L	1408	0.817	0.038	0.674 to 0.989	1011	0.873	0.092	0.745 to 1.022
Log-ferritin, $\mu\text{g/L}$	1360	0.707	0.004	0.560 to 0.892	970	0.858	0.146	0.697 to 1.055
Quartiles s-ferritin, $\mu\text{g/L}$	1360				970			
1.Q reference								
2.Q		0.645	0.079	0.395 to 1.052		0.694	0.110	0.443 to 1.086
3.Q		0.454	0.003	0.272 to 0.758		1.101	0.678	0.699 to 1.734
4.Q		0.471	0.003	0.286 to 0.774		0.587	0.027	0.366 to 0.943
Linear trend over Q	1360	0.775	0.002	0.660 to 0.909	970	0.898	0.153	0.775 to 1.041
Transferrin saturation, %	1373	0.983	0.092	0.964 to 1.003	983	0.996	0.612	0.980 to 1.012
S-TIBC, $\mu\text{mol/L}$	1373	1.032	0.000	1.014 to 1.051	983	1.011	0.167	0.995 to 1.027

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.

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

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.

Table 7 Associations between IMT log-transformed and hemoglobin, s-ferritin log-transformed, transferrin saturation and s-TIBC in models 1, 2 and 3.

Log-mean IMT	Women				Men			
	N	B	P	95%CI	N	B	P	95%CI
Model 1								
Hemoglobin, g/L	2587	0.012	0.001	0.005 to 0.019	2099	0.002	0.535	-0.005 to 0.009
Log-ferritin, µg/L	2820	0.002	0.646	-0.006 to 0.01	2220	-0.002	0.722	-0.011 to 0.008
Transferrin saturation, %	2848	-0.001	0.005	-0.002 to -0.0003	2250	-0.001	0.184	-0.001 to 0.0002
S-TIBC, µmol/L	2849	0.001	0.006	0.0002 to 0.001	2250	0.0001	0.694	-0.001 to 0.001
Model 2								
Hemoglobin, g/L	2491	0.002	0.636	-0.005 to 0.009	2040	-0.006	0.113	-0.013 to 0.001
Log-ferritin, µg/L	2737	-0.003	0.514	-0.011 to 0.005	2173	-0.007	0.113	-0.016 to 0.002
Transferrin saturation, %	2765	-0.0005	0.151	-0.001 to 0.0002	2202	-0.0001	0.759	-0.001 to 0.001
S-TIBC, µmol/L	2766	0.0002	0.458	-0.0004 to 0.001	2202	-0.0005	0.196	-0.001 to 0.0002
Model 3								
Hemoglobin, g/L	2490	0.002	0.652	-0.005 to 0.009	2039	-0.007	0.083	-0.014 to 0.001
Log-ferritin, µg/L	2395	-0.002	0.642	-0.010 to 0.007	1943	-0.009	0.072	-0.018 to 0.001
Transferrin saturation, %	2420	-0.001	0.127	-0.001 to 0.0002	1970	0.0001	0.825	-0.001 to 0.001
S-TIBC, µmol/L	2421	0.0002	0.539	-0.0004 to 0.001	1970	-0.0004	0.303	-0.001 to 0.0004

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.

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 (β) in the log-linear model, expressed by the formula $\log(Y_i) = \alpha + \beta X_i + \varepsilon_i$, is that each one-unit increase in X multiplies the expected value of Y by e^β (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-analysis 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

1. Mathiesen EB, Bønaa KH, Joakimsen O. Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis the tromsø study. *Circulation*. 2001;103(17):2171-5.
2. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson Jr SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. *New England Journal of Medicine*. 1999;340(1):14-22.
3. Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicolaidis AN, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women the British Regional Heart Study. *Stroke*. 1999;30(4):841-50.
4. Organization WH. Health in 2015: from MDGs, Millennium Development Goals to SDGs. Sustainable Development Goals Switzerland: World Health Organization Available from: <http://www.who.int> [Accessed 09.10.2016].
5. Stocker R, Keaney JF. Role of oxidative modifications in atherosclerosis. *Physiological reviews*. 2004;84(4):1381-478.
6. Williams L. Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation*. 2002;106(25):3143-3143.
7. Robertson AM, Watton PN. *Mechanobiology of the arterial wall. Modeling of transport in biological media* Elsevier, New York. 2013:275-347.
8. Holzapfel GA, Gasser TC, Ogden RW. A new constitutive framework for arterial wall mechanics and a comparative study of material models. *Journal of elasticity and the physical science of solids*. 2000;61(1-3):1-48.
9. Golts M. Reference Module in Biomedical Sciences 2014 Available from: http://www.sciencedirect.com/topics/page/Tunica_media [Accessed 25.01.2017].
10. Newman III WP, Freedman DS, Voors AW, Gard PD, Srinivasan SR, Cresanta JL, et al. Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis. *New England Journal of Medicine*. 1986;314(3):138-44.
11. Nichols FT, Shaltoni HM, Yatsu FM. A cerebrovascular perspective of atherosclerosis. *Handbook of clinical neurology*. 2008;92:215-38.
12. Glagov S, Zarins C, Giddens D, Ku D. Hemodynamics and atherosclerosis. Insights and perspectives gained from studies of human arteries. *Archives of pathology & laboratory medicine*. 1988;112(10):1018-31.
13. Hegele RA. The pathogenesis of atherosclerosis. *Clinica Chimica Acta*. 1996;246(1):21-38.
14. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*. 1993;362(6423):801.
15. Ross R. Atherosclerosis—an inflammatory disease. *New England journal of medicine*. 1999;340(2):115-26.
16. Johnsen SH, Mathiesen EB. Ultrasound imaging of carotid atherosclerosis in a normal population. *The Tromsø Study*. 2009.
17. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell*. 2011;145(3):341-55.
18. Strong JP, Malcom GT, McMahan CA, Tracy RE, Newman III WP, Herderick EE, et al. Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. *Jama*. 1999;281(8):727-35.
19. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine*. 2005;352(16):1685-95.
20. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W, Rosenfeld ME, et al. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*. 1994;89(5):2462-78.

21. Dhingra R, Vasan RS. Age as a risk factor. *Medical Clinics of North America*. 2012;96(1):87-91.
22. Lakatta EG. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. *Circulation*. 2003;107(3):490-7.
23. Castelli W. Epidemiology of coronary heart disease: the Framingham study. *The American journal of medicine*. 1984;76(2):4-12.
24. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation*. 1998;97(18):1837-47.
25. Willeit J, Kiechl S. Prevalence and risk factors of asymptomatic extracranial carotid artery atherosclerosis. A population-based study. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 1993;13(5):661-8.
26. de Weerd M, Greving JP, Hedblad B, Lorenz MW, Mathiesen EB, O'leary DH, et al. Prevalence of asymptomatic carotid artery stenosis in the general population. *Stroke*. 2010;41(6):1294-7.
27. Barrett-Connor E, Grady D. Hormone replacement therapy, heart disease, and other considerations. *Annual review of public health*. 1998;19(1):55-72.
28. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *Jama*. 1998;280(7):605-13.
29. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL, et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *Jama*. 2003;289(19):2560-71.
30. Burt VL, Whelton P, Roccella EJ, Brown C, Cutler JA, Higgins M, et al. Prevalence of hypertension in the US adult population. *Hypertension*. 1995;25(3):305-13.
31. Wolf-Maier K, Cooper RS, Banegas JR, Giampaoli S, Hense H-W, Joffres M, et al. Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. *Jama*. 2003;289(18):2363-9.
32. van den Hoogen PC, Feskens EJ, Nagelkerke NJ, Menotti A, Nissinen A, Kromhout D. The relation between blood pressure and mortality due to coronary heart disease among men in different parts of the world. *New England Journal of Medicine*. 2000;342(1):1-8.
33. Collaboration BPLTT. Effects of ACE inhibitors, calcium antagonists, and other blood-pressure-lowering drugs: results of prospectively designed overviews of randomised trials. *The Lancet*. 2000;356(9246):1955-64.
34. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circulation research*. 2000;87(10):840-4.
35. Health UDo, Services H. Reducing the Health Consequences of Smoking, 25 years of progress. A report of the Surgeon General of the United States. 1989.
36. Mons U, Müezzinger A, Gellert C, Schöttker B, Abnet CC, Bobak M, et al. Impact of smoking and smoking cessation on cardiovascular events and mortality among older adults: meta-analysis of individual participant data from prospective cohort studies of the CHANCES consortium. *bmj*. 2015;350:h1551.
37. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. *Journal of the American college of cardiology*. 2004;43(10):1731-7.
38. Salonen R, Seppänen K, Rauramaa R, Salonen JT. Prevalence of carotid atherosclerosis and serum cholesterol levels in eastern Finland. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 1988;8(6):788-92.
39. Johnsen SH, Mathiesen EB, Fosse E, Joakimsen O, Stensland-Bugge E, Njølstad I, et al. Elevated high-density lipoprotein cholesterol levels are protective against plaque progression. *Circulation*. 2005;112(4):498-504.
40. Group SSSS. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *The Lancet*. 1994;344(8934):1383-9.
41. Gavin JR, Alberti KG, Davidson MB, DeFronzo RA, Drash A, Gabbe SG, et al. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes care*. 2003;26(SUPPL. 1).

42. Barr EL, Zimmet PZ, Welborn TA, Jolley D, Magliano DJ, Dunstan DW, et al. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance. *Circulation*. 2007;116(2):151-7.
43. Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: the Framingham study. *Circulation*. 1979;59(1):8-13.
44. Langenfeld M, Forst T, Hohberg C, Kann P, Lübber G, Konrad T, et al. Pioglitazone decreases carotid intima-media thickness independently of glycemic control in patients with type 2 diabetes mellitus. *Circulation*. 2005;111(19):2525-31.
45. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation*. 1983;67(5):968-77.
46. Lavie CJ, Milani RV, Ventura HO. Obesity and cardiovascular disease. *Journal of the American College of Cardiology*. 2009;53(21):1925-32.
47. Barnett PA, Spence JD, Manuck SB, Jennings JR. Psychological stress and the progression of carotid artery disease. *Journal of hypertension*. 1997;15(1):49-55.
48. Spence JD. The importance of distinguishing between diffuse carotid intima-media thickening and focal plaque. *Canadian Journal of Cardiology*. 2008;24:61C-4C.
49. Sullivan J. Iron and the sex difference in heart disease risk. *The Lancet*. 1981;317(8233):1293-4.
50. Wang J, Pantopoulos K. Regulation of cellular iron metabolism. *Biochemical Journal*. 2011;434(3):365-81.
51. Andrews NC. Disorders of iron metabolism. *New England Journal of Medicine*. 1999;341(26):1986-95.
52. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of mammalian iron metabolism. *Cell*. 2010;142(1):24-38.
53. Jacobs A, Miller F, Worwood M, Beamish M, Wardrop C. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *Br med J*. 1972;4(5834):206-8.
54. Organization WH. Assessing the iron status of populations. World Health Organization 2007:108 Available from: <http://www.who.int> [Accessed 12.10.2016].
55. Huebers HA, Finch CA. The physiology of transferrin and transferrin receptors. *Physiological Reviews*. 1987;67(2):520-82.
56. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: past, present and future. *Biochimica et Biophysica Acta (BBA)-General Subjects*. 2010;1800(8):760-9.
57. Harman D. Aging: a theory based on free radical and radiation chemistry. 1955.
58. Galaris D, Pantopoulos K. Oxidative stress and iron homeostasis: mechanistic and health aspects. *Critical reviews in clinical laboratory sciences*. 2008;45(1):1-23.
59. Dröge W. Free radicals in the physiological control of cell function. *Physiological reviews*. 2002;82(1):47-95.
60. Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: an overview. *Free radical biology and medicine*. 2000;28(12):1815-26.
61. Steinberg D, Lewis A. Conner memorial lecture oxidative modification of LDL and atherogenesis. *Circulation*. 1997;95(4):1062-71.
62. Subbanagounder G, Watson AD, Berliner JA. Bioactive products of phospholipid oxidation: isolation, identification, measurement and activities. *Free Radical Biology and Medicine*. 2000;28(12):1751-61.
63. Witztum JL, Steinberg D. The oxidative modification hypothesis of atherosclerosis: does it hold for humans? *Trends in cardiovascular medicine*. 2001;11(3):93-102.
64. Suzuki H, Kurihara Y, Takeya M, Kamada N, Kataoka M, Jishage K, et al. A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. 1997.
65. Pang J, Jiang M-J, Chen Y-L, Wang F-W, Wang DL, Chu S-H, et al. Increased ferritin gene expression in atherosclerotic lesions. *Journal of Clinical Investigation*. 1996;97(10):2204.
66. Lee F-Y, Lee T-S, Pan C-C, Huang A-L, Chau L-Y. Colocalization of iron and ceroid in human atherosclerotic lesions. *Atherosclerosis*. 1998;138(2):281-8.

67. Lapenna D, Pierdomenico SD, Ciofani G, Uchino S, Neri M, Giamberardino MA, et al. Association of body iron stores with low molecular weight iron and oxidant damage of human atherosclerotic plaques. *Free Radical Biology and Medicine*. 2007;42(4):492-8.
68. von Hanno T, Bertelsen G, Broderstad AR, Wilsgaard T, Mathiesen EB. Serum Ferritin and Hemoglobin Are Independently Associated With Wider Retinal Venular Caliber: The Tromsø Study 2001–2008. *Investigative ophthalmology & visual science*. 2013;54(10):7053-60.
69. Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation*. 1992;86(3):803-11.
70. Kiechl S, Aichner F, Gerstenbrand F, Egger G, Mair A, Rungger G, et al. Body iron stores and presence of carotid atherosclerosis. Results from the Bruneck Study. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 1994;14(10):1625-30.
71. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis. *Circulation*. 1997;96(10):3300-7.
72. Ahluwalia N, Genoux A, Ferrieres J, Perret B, Carayol M, Drouet L, et al. Iron status is associated with carotid atherosclerotic plaques in middle-aged adults. *The Journal of nutrition*. 2010;140(4):812-6.
73. Wolff B, Völzke H, Lüdemann J, Robinson D, Vogelgesang D, Staudt A, et al. Association between high serum ferritin levels and carotid atherosclerosis in the study of health in Pomerania (SHIP). *Stroke*. 2004;35(2):453-7.
74. Ma H, Lin H, Hu Y, Li X, He W, Jin X, et al. Serum ferritin levels are associated with carotid atherosclerosis in Chinese postmenopausal women: the Shanghai Changfeng Study. *British Journal of Nutrition*. 2015;114(07):1064-71.
75. Grobbee DE, Roest M, Marx JJ, Voorbij HA, van der Schouw YT. Serum ferritin is a risk factor for stroke in postmenopausal women. *Stroke*. 2005;36(8):1637-41.
76. Danesh J, Appleby P. Coronary heart disease and iron status meta-analyses of prospective studies. *Circulation*. 1999;99(7):852-4.
77. Knuiman M, Divitini M, Olynyk J, Cullen D, Bartholomew H. Serum ferritin and cardiovascular disease: a 17-year follow-up study in Busselton, Western Australia. *American journal of epidemiology*. 2003;158(2):144-9.
78. Engberink MF, Geleijnse JM, Durga J, Swinkels DW, de Kort WL, Schouten EG, et al. Blood donation, body iron status and carotid intima-media thickness. *Atherosclerosis*. 2008;196(2):856-62.
79. Friedrich N, Milman N, Völzke H, Linneberg A, Jørgensen T. Is serum ferritin within the reference range a risk predictor of cardiovascular disease? A population-based, long-term study comprising 2874 subjects. *British journal of nutrition*. 2009;102(04):594-600.
80. Njølstad I, Mathiesen EB, Schirmer H, Thelle DS. The Tromsø study 1974–2016: 40 years of cardiovascular research. *Scandinavian Cardiovascular Journal*. 2016;50(5-6):276-81.
81. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I. Cohort profile: the Tromsø study. *International journal of epidemiology*. 2011;40:dyr049.
82. The Tromsø Study/Tromsøundersøkelsen Available from: www.tromsostudy.com [Accessed 15.04.2017].
83. Skjelbakken T, Dahl IMS, Løchen M-L. Changes in body mass index and smoking habits have a different impact on hemoglobin concentration in men and women: A longitudinal follow-up of the tromsø study, 1994–2002. *Gender medicine*. 2010;7(3):230-9.
84. Broderstad AR, Smith-Sivertsen T, Dahl IM, Ingebretsen OC, Lund E. Low prevalence of hereditary hemochromatosis in multiethnic populations in Northern Norway. 2011.
85. Lappegård J, Ellingsen T, Vik A, Skjelbakken T, Brox J, Mathiesen EB, et al. Red cell distribution width and carotid atherosclerosis progression: The Tromsø study. 2015.
86. Stensland-Bugge E, Bønaa KH, Joakimsen O. Reproducibility of ultrasonographically determined intima-media thickness is dependent on arterial wall thickness. *Stroke*. 1997;28(10):1972-80.
87. Wendelhag I, Liang Q, Gustavsson T, Wikstrand J. A new automated computerized analyzing system simplifies readings and reduces the variability in ultrasound measurement of intima-media thickness. *Stroke*. 1997;28(11):2195-200.

88. Boslaugh S. Encyclopedia of epidemiology: Sage Publications. 2007.
89. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105(9):1135-43.
90. Bhopal RS. Concepts of epidemiology: integrating the ideas, theories, principles, and methods of epidemiology: Oxford University Press. 2016.
91. Miquel P. A Dictionary of Epidemiology: Oxford University Press. 2014.
92. Grimes DA, Schulz KF. Bias and causal associations in observational research. *The Lancet*. 2002;359(9302):248-52.
93. Jacobsen BK, Eggen AE, Mathiesen EB, Wilsgaard T, Njølstad I. Cohort profile: the Tromsø study. *International journal of epidemiology*. 2012;41(4):961-7.
94. Melton LJ, Dyck PJ, Karnes JL, O'Brien PC, Service FJ. Non-response bias in studies of diabetic complications: the Rochester Diabetic Neuropathy Study. *Journal of clinical epidemiology*. 1993;46(4):341-8.
95. Adams GJ, Simoni DM, Bordelon CB, Vick GW, Kimball KT, Insull W, et al. Bilateral symmetry of human carotid artery atherosclerosis. *Stroke*. 2002;33(11):2575-80.
96. Fosse E, Johnsen SH, Stensland-Bugge E, Joakimsen O, Mathiesen EB, Arnesen E, et al. Repeated visual and computer-assisted carotid plaque characterization in a longitudinal population-based ultrasound study: the Tromsø study. *Ultrasound in medicine & biology*. 2006;32(1):3-11.
97. Punnonen K, Irjala K, Rajamäki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood*. 1997;89(3):1052-7.
98. Allison PD. *Missing Data*: SAGE Publications; 2001.
99. Bennett DA. How can I deal with missing data in my study? *Australian and New Zealand journal of public health*. 2001;25(5):464-9.
100. Feng C, Wang H, Lu N, Chen T, He H, Lu Y, et al. Log-transformation and its implications for data analysis. *Shanghai archives of psychiatry*. 2014;26(2):105-9.
101. Altman DG. *Practical statistics for medical research*: CRC press. 1990.
102. Tabachnick B, Fidell L. *Using Multivariate Statistics*. Pearson Education. Boston, MA. 2007.
103. De SD, Krishna S, Jethwa A. Iron status and its association with coronary heart disease: systematic review and meta-analysis of prospective studies. *Atherosclerosis*. 2015;238(2):296-303.
104. Dickersin K. The existence of publication bias and risk factors for its occurrence. *Jama*. 1990;263(10):1385-9.
105. Song F, Hooper L, Loke Y. Publication bias: what is it? How do we measure it? How do we avoid it? *Open Access Journal of Clinical Trials*. 2013;2013(5):71-81.
106. Begg CB, Berlin JA. Publication bias: a problem in interpreting medical data. *Journal of the Royal Statistical Society Series A (Statistics in Society)*. 1988:419-63.
107. Orino K, Lehman L, Tsuji Y, Ayaki H, Torti SV, Torti FM. Ferritin and the response to oxidative stress. *Biochemical Journal*. 2001;357(1):241-7.
108. Balla G, Jacob HS, Balla J, Rosenberg M, Nath K, Apple F, et al. Ferritin: a cytoprotective antioxidant strategem of endothelium. *Journal of Biological Chemistry*. 1992;267(25):18148-53.
109. Rashid KA, Hevi S, Chen Y, Le Cahérec F, Chuck SL. A proteomic approach identifies proteins in hepatocytes that bind nascent apolipoprotein B. *Journal of Biological Chemistry*. 2002;277(24):22010-7.
110. Hevi S, Chuck SL. Ferritins can regulate the secretion of apolipoprotein B. *Journal of Biological Chemistry*. 2003;278(34):31924-9.

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!*

Take the chance!

-a collaboration between:



**Department of Community Medicine,
University of Tromsø**

tf: 77 64 48 16 (kl. 9 - 11) Tromsous@ism.uit.no



National Health Screening Service

tf: 22 24 21 00 (kl. 9 - 15) post@shus.no

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

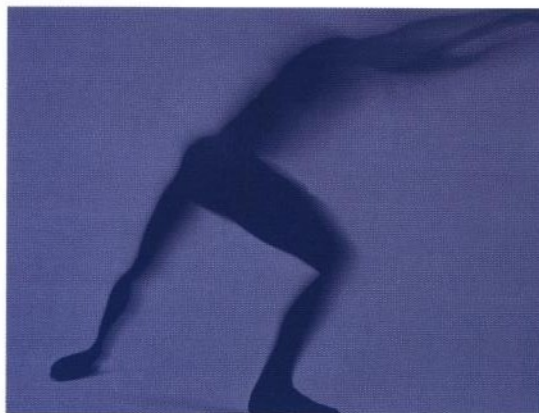
www.shus.no

**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.



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.

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.

Everyone who participated in the Special Study in 1994-95 is also offered to take part in another Special Study. This study provides information on the heart and the main arteries in neck and abdomen, and offers a more detailed analysis on tendency of osteoporosis. This survey is also located at the Elisabeth-center in Tromsø. A time will be scheduled for you and information is provided upon arrival.

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.

We need your consent

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.

When will you receive your results?

About four weeks after you participated in the study you will receive a letter wherein your recorded values for cholesterol, blood pressure and blood sugar are stated. You will also receive more information on the different risk factors.

People who are found to be at particularly high risk of developing cardiovascular diseases and diabetes will be recommended to seek further examination from their own doctor.



Appendix 2: Questionnaire 1

Health survey

Personal Invitation

Don't write here

5.3 (Municipality)

(County)

(Country)

↓

9.3 (Business)

9.4 (Occupation)

14.7 (Mark)

1. YOUR OWN HEALTH

1.1 What is your current state of health? (Tick one only)

Poor 1 Not so good 2 Good 3 Very good 4

1.2 Do you have, or have you had?:

	Yes	No	Age first time
Asthma.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> T
Hay fever	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Chronic bronchitis/emphysema	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Osteoporosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Fibromyalgia/chronic pain syndrome	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Psychological problems for which you have sought help	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
A heart attack	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Angina pectoris (heart cramp)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Cerebral stroke/brain haemorrhage	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>

1.3 Have you noticed attacks of sudden changes in your pulse or heart rhythm in the last year? Yes No

1.4 Do you get pain or discomfort in the chest when: Walking up hills, stairs or walking fast on level ground? Yes No

1.5 If you get such pain, do you usually:

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

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

1.7 Can such pain occur even if you are at rest?..... Yes No

2. MUSCULAR AND SKELETAL COMPLAINTS

2.1 Have you suffered from pain and/or stiffness in muscles and joints during the last 4 weeks?

(Give duration only if you have had problems)

	No complaint			Duration	
	Some complaint	Severe complaint	Up to 2 weeks	2 weeks or more	
Neck/shoulders	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Arms, hands	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Upper part of your back...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lumbar region	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hips, legs, feet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other places	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

1 2 3 1 2

2.2 Have you ever had:

	Yes	No	Age last time
Fracture in the wrist/forearm	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Hip fracture?.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>

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)

	No complaint	Little complaint	Pretty much	Very much
Sudden fear without reason	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Felt afraid or anxious	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Faintness or dizziness	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Felt tense or upset	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tend to blame yourself	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sleeping problems	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Depressed, sad	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Feeling of being useless, worthless	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Feeling that everything is a struggle	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Feeling of hopelessness with regard to the future	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

1 2 3 4

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)

	None	1-3 times	4 or more
General practitioner (GP)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Medical officer at work	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Psychologist or psychiatrist (private or out-patient clinic)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other specialist (private or out-patient clinic)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Emergency GP (private or public)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hospital admission	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Home nursing care	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Physiotherapist	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chiropractor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dentist	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Alternative practitioner	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

5. CHILDHOOD/YOUTH AND AFFILIATION

5.1 How long altogether have you lived in the county? year (Put 0 if less than half a year)

5.2 How long altogether have you lived in the municipality? year (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 1

Another municipality in the county 2 Which one: _____

Another county in Norway 3 Which one: _____

Outside Norway 4 Country:: _____

5.4 Have you moved within the last five years? 1 Yes, one time 2 Yes, more than once 3

6. BODY WEIGHT

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

7. FOOD AND BEVERAGES

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

	Rarely /never	1-3 times /month	1-3 times /week	4-6 times /week	1-2 times /day	3 times or more /day
Fruit, berries	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cheese (all types).....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Potatoes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Boiled vegetables	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fresh vegetables/salad	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fatty fish (e.g. salmon, trout, mackerel, herring)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	1	2	3	4	5	6

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

	Don't use	Butter	Hard margarine	Soft/light margarine	Oils	Other
On bread	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
For cooking	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	1	2	3	4	5	6

7.3 Do you use the following dietary supplements:

	Yes, daily	Sometimes	No
Cod liver oil, fish oil capsules	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Vitamins and/or mineral supplements?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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

	Rarely /never	1-6 glasses /week	1 glass /day	2-3 glasses /day	4 glasses or more /day
Full milk, full-fat curdled milk, yoghurt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Semi-skimmed milk, semi-skimmed curdled milk, low-fat yoghurt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Skimmed milk, skimmed curdled milk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Extra semi-skimmed milk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Juice	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Water	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Mineral water (e.g. Farris, Ramløsa etc)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cola-containing soft drink	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other soda/soft drink	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	1	2	3	4	5

7.5 Do you usually drink soft drink: with sugar 1 without sugar 2

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

Filtered coffee	<input type="text"/>
Boiled coffee/coarsely ground coffee for brewing	<input type="text"/>
Other type of coffee	<input type="text"/>
Tea	<input type="text"/>

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	Have not consumed alcohol last year	A few times last year	About 1 time a month
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4
2-3 times per month	About 1 time a week	2-3 times a week	4-7 times a week
<input type="checkbox"/> 5	<input type="checkbox"/> 6	<input type="checkbox"/> 7	<input type="checkbox"/> 8

To those who have consumed the last year:

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

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: (Tick one or more)

Beer	Wine	Spirits
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

8. SMOKING

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

8.2 Did any of the adults smoke at home while you were growing up? Yes No

8.3 Do you currently, or did you previously live together with a daily smoker after your 20th birthday? Yes, now Yes, previously Never

8.4 Do you/did you smoke daily? If NEVER: Go to question 9 : (EDUCATION AND WORK)

8.5 If you smoke daily now, do you smoke: Yes No

Cigarettes?.....

Cigars/cigarillos?.....

A pipe?.....

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

8.7 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

9. EDUCATION AND WORK

9.1 How many years of education have you completed? Number of years

(Include all the years you have attended school or studied)

9.2 Do you currently have paid work?

Yes, full-time 1 Yes, part-time 2 No 3 T

9.3 Describe the activity at the workplace where you had paid work for the longest period in the last 12 months. (e.g. Accountancy firm, school, paediatric department, carpentry workshop, garage, bank, grocery store, etc.)

Business: _____

If retired, enter the former business and occupation. Also applies to 9.4

9.4 Which occupation/title have or had you at this workplace? (e.g. Secretary, teacher, industrial worker, nurse, carpenter, manager, salesman, driver, etc.)

Occupation: _____

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

Self-employed Employee Family member

9.6 Do you believe that you are in danger of losing your current work or income within the next two years? Yes No

9.7 Do you receive any of the following benefits? Yes No

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.

	Hours per week			
	None	Less than 1	1-2	3 or more
Light activity (not sweating/out of breath).....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hard physical activity (sweating/out of breath).....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	1	2	3	4

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? 1

Walking, cycling or other forms of exercise at least 4 hours a week? 2
(Include walking or cycling to work, Sunday walk/stroll, etc.)

Participation in recreational sports, heavy gardening, etc.? 3
(Note: duration of activity at least 4 hours a week)

Participation in hard training or sports competitions, regularly several times a week? 4

11. FAMILY AND FRIENDS

11.1 Do you live with: Spouse/partner?.....

Yes No

11.2 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 other relatives.

Number of friends

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

Great interest 1 Some interest 2 Little interest 3 No interest 4 Uncertain 5

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

Number

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

Yes, a lot 1 Yes, some 2 Yes, a little 3 No 4 Never tried 5

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)

	Mother	Father	Brother	Sister	Child	None of these
Cerebral stroke or brain haemorrhage	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Heart attack before age of 60 years	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Asthma.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cancer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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):

Don't know, not applicable	Mother's age	Father's age	Brother's age	Sister's age	Child's age
<input type="checkbox"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

13. USE OF MEDICINES

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

13.1 Do you use:

	Now	Previously, but not now	Never used
Blood pressure lowering drugs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cholesterol-lowering drugs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

13.2 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
Painkillers non-prescription	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Painkillers on prescription	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sleeping pills	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tranquillizers	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Antidepressants	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other prescription medicines ...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	1	2	3	4

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)

Name of the medicine: (one name per line)	Reason for use of the medicine	How long have you used the medicine	
		Up to 1 year	1 year or more
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>

If there is not enough space here, you may continue on a separate sheet that you attach

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)

	Now	Before, but not now	Never
Oral contraceptive pills/mini pill/contraceptive injection	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hormonal intrauterine device (IUD) (not ordinary IUD) ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Estrogen (tablets or patches)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Estrogen (cream or suppositories)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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

Health survey

Personal invitation

Do not write here:

E13 (Municipality)

(County)

(Country)

E15 (Mark)

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?:

	T		Age first time	
	Yes	No		
Asthma.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
Chronic bronchitis/emphysema.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
Osteoporosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
Fibromyalgia/chronic pain syndrome	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
Psychological problems for which you have sought help	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
A heart attack	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
Angina pectoris (heart cramp)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>
Cerebral stroke/brain haemorrhage	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>	<input type="text"/>

Do you get pain or discomfort in the chest when: Yes No

Walking up hills, stairs, or walking fast on level ground?

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?.... Yes No

E2. ILLNESS IN THE FAMILY

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

A heart attack (heart wounds) or angina pectoris (heart cramp) Yes No Don't know

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

	Mother	Father	Brother	Sister	Child	None of these
Cerebral stroke or brain haemorrhage ...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Heart attack before age of 60 years	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Asthma	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cancer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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)

Don't know, not applicable Mother's age Father's age Brother's age Sister's age Child's age

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)

	No complaint	Little complaint	Pretty much	Very much
Sudden fear without reason	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Felt afraid or anxious	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Faintness or dizziness	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Felt tense or upset	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tend to blame yourself	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sleeping problems.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Depressed, sad	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Feeling of being useless, worthless ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Feeling that everything is a struggle	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Feeling of hopelessness with regard to the future.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

1 2 3 4

E4. TEETH, MUSCLE AND SKELETON

How many teeth have you lost/extracted? Number of teeth (disregard milk-teeth and wisdom teeth)

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

	No complaint	Little complaint	Severe complaint
Neck / shoulders	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Arms, hands.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Upper part of the back	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lumbar regions	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hips, legs, feet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other places.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Have you ever had:

	Yes	No	Age last time
Fracture in wrist/forearm?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>
Hip fracture?.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/>

Have you fallen down during the last year? (Tick once only)

No 1 Yes, 1-2 times 2 Yes, more than 2 times 3

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.

	Hours per week			
	None	Less than 1	1-2	3 or more
Light activity (not sweating/out of breath).....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hard physical activity (sweating/out of breath).....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

1 2 3 4

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)*

	Rarely /never	1-3 times /month	1-3 times /week	4-6 times /week	1-2 times /day	3 times or more /day
Fruit, berries	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cheese (all types) ...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Potatoes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Boiled vegetables ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fresh vegetables/salad	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fat fish (e.g. salmon, trout, mackerel, herring)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	1	2	3	4	5	6

Do you use dietary supplements: *Yes, daily* *Sometimes* *No*

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)*

	Rarely /never	1-6 glasses /week	1 glass /day	2-3 glasses /day	4 glasses or more /day
Full milk, full-fat curdled milk, yoghurt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Semi-skimmed milk, semi-skimmed curdled milk, low-fat yoghurt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Skimmed milk, skimmed curdled milk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Extra semi-skimmed milk	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Juice	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Water	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Soft drink, mineral water	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	1	2	3	4	5

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

Filtered coffee	<input type="text"/>	<input type="text"/>
Boiled coffee/coarsely ground coffee for brewing	<input type="text"/>	<input type="text"/>
Other type of coffee	<input type="text"/>	<input type="text"/>
Tea	<input type="text"/>	<input type="text"/>

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

Never consumed alcohol	Have not consumed alcohol last year	A few times last year	About 1 time a month
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4
2-3 times per month	About 1 time a week	2-3 times a week	4-7 times a week
<input type="checkbox"/> 5	<input type="checkbox"/> 6	<input type="checkbox"/> 7	<input type="checkbox"/> 8

To those who have consumed the last year:

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

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

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*

Do you/did you smoke daily? *Yes, now* *Yes, previously* *Never*

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

If you smoke daily now, do you smoke: *Yes* *No*

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: *(Tick once for each line)*

	Without problems	With some problems	With great problems	No
Take a 5 minute walk in fairly high pace?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Read ordinary text in newspaper, if necessary with glasses?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hear what is said in a normal conversation?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	1	2	3	4

Do you because of chronic health problems have difficulties with: *(Tick once for each line)* *No difficulties* *Some difficulties* *Great difficulties*

Move around in your home?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Get out of your home by yourself?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Participate in organization or other leisure time activities?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Use public transport?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Perform necessary daily shopping?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

E11. USE OF HEALTH SERVICES

How many times in the last 12 months

have you been to/used:

(Tick once for each line)

	None	1-3 times	4 or more
A general practitioner (GP)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Specialist (private or out-patient clinic)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Emergency GP (private or public).....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hospital admission	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Home nursing care	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Physiotherapist	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chiropractor	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Municipal home care	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dentist	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Alternative practitioner	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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

YES	NO	Don't know
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3

E12. FAMILY AND FRIENDS

Do you live: At home? 1 In an institution/shared apartment? 2

Do you live with:

	YES	NO
Spouse/ partner?.....	<input type="checkbox"/>	<input type="checkbox"/>
Other people?	<input type="checkbox"/>	<input type="checkbox"/>

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.....

Number of friends

--	--

How much interest do people show for what you do?

(Tick only once)

Great interest	Some interest	Little interest	No interest	Uncertain
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5

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

Number

--	--

E13. CHILDHOOD/YOUTH AND AFFILIATION

How long altogether have you lived in the county?

--	--

 years

How long altogether have you lived in the municipality?

--	--

 years

Where did you live most of the time before the age of 16? (Tick one option and specify)

Same municipality..... 1

Another municipality in the county..... 2 Which one: _____

Another county in Norway 3 Which one: _____

Outside Norway 4 Country: _____

Have you moved during the last five years?

No	Yes, once	Yes, more than once
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3

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)

	Now	previously, but not now	Never used
Blood pressure lowering drugs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cholesterol-lowering drugs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Drugs for osteoporosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Insulin.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tablets for diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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
Painkillers non-prescription.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Painkillers on prescription	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Sleeping pills.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tranquillizers	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Antidepressants	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other prescription medicines	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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)

Name of the medicine: (one name per line):	Reason for use of the medicine:	How long have you used the medicine	
		Up to 1 year	One year or more
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>

If there is not enough space here, you may continue on a separate sheet that you attach.

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? Total number of years

	Never	Previously	Now			
Tablets or patches	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<table border="1" style="display: inline-table; vertical-align: middle;"><tr><td style="width: 30px; height: 20px;"></td><td style="width: 30px; height: 20px;"></td></tr></table>		
Cream or suppositories	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<table border="1" style="display: inline-table; vertical-align: middle;"><tr><td style="width: 30px; height: 20px;"></td><td style="width: 30px; height: 20px;"></td></tr></table>		

If you use estrogen, which brand you use now?

Have you ever used contraceptives pills? Yes No

Appendix 4: Questionnaire 3

Label

Additional questions to the health survey in Troms and Finnmark 2001-2002

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 would 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.

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.

I do not wish to answer the questionnaire

Date of completion:

Day Month Year

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
----------------------	----------------------	----------------------	----------------------	----------------------	----------------------

T

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..... 1
Farm 2
Flat/apartment 3
Terraced/semi-detached house 4
Institution/care home 5
Other 6

1.3 How big is your house? m² (gross)

1.4 Are you bothered by: (Tick once for each line)

- | | No complaint | Little complaint | Severe complaint |
|---|--------------------------|--------------------------|--------------------------|
| Moisture, drought or coldness in your home | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other forms of bad indoor climate | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Traffic noise (cars or aircraft) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Other noise (industrial, construction, etc.) | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Neighbour noise | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Drinking water quality | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Air pollution from traffic | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Air pollution from wood/oil heating, factory etc. | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

1.5 What home language did your grandparents have?
(Tick for one or more alternatives)

- | | Norwegian | Sami | Kven/
Finnish | Other
language |
|-----------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Mother's mother ... | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Mother's father | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Father's mother ... | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Father's father | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

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 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
1-3 times a month 2
Approximately once a week 3
More than once a week 4

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) 1
Work that requires a lot of walking?
(e.g. shop assistant, light industrial work, teaching) 2
Work that requires a lot of walking and lifting?
(e.g. Postman, nursing, construction) 3
Heavy manual labour?
(e.g. forestry, heavy farm-work, heavy construction) 4

2.2 Can you decide yourself how your work (paid or unpaid) should be organised? (Tick only once)

- No, not at all 1
To a small extent 2
Yes, to a large extent 3
Yes, I decide myself 4

2.3 Are you on call, do you work shifts or nights?

Yes No

T3. TOBACCO

3.1 Do you smoke?

Yes, daily 1 Yes, sometimes 2 No, never 3

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

T4. ALCOHOL

4.1 Are you a teetotaler?.....

Yes No

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

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

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 1 Yes, socially 2 Yes, both at work and social life 3 No, never 4

T5. FOOD AND DIETARY SUPPLEMENTS

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 1 Somewhat 2 Little 3 Not 4

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

T6. BODY WEIGHT

6.1 Do you currently try to change your body weight?

No 1 Yes, I try to gain weight 2 Yes, I try to lose weight 3

6.2 What weight would you be satisfied with (your "ideal weight")?..... kg

T7. ILLNESSES AND INJURIES

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

	Yes	No	Age last time
Severe injury requiring hospital admission	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> years
Ankle fracture	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> years
Peptic ulcer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> years
Peptic ulcer surgery	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> years
Neck surgery	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> years
Prostate surgery	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> years

7.2 Do you have, or have you ever had: (Tick once for each question)

	Yes	No
Cancer	<input type="checkbox"/>	<input type="checkbox"/>
Psoriasis.....	<input type="checkbox"/>	<input type="checkbox"/>
Thyroid disease	<input type="checkbox"/>	<input type="checkbox"/>
Glaucoma	<input type="checkbox"/>	<input type="checkbox"/>
Cataract	<input type="checkbox"/>	<input type="checkbox"/>
Osteoarthritis (arthrosis).....	<input type="checkbox"/>	<input type="checkbox"/>
Bent fingers	<input type="checkbox"/>	<input type="checkbox"/>
Skin contractions in your palms	<input type="checkbox"/>	<input type="checkbox"/>
Kidney stone	<input type="checkbox"/>	<input type="checkbox"/>
Appendectomy.....	<input type="checkbox"/>	<input type="checkbox"/>
Hernia surgery	<input type="checkbox"/>	<input type="checkbox"/>
Surgery/treatment for urine incontinence	<input type="checkbox"/>	<input type="checkbox"/>
Epilepsy.....	<input type="checkbox"/>	<input type="checkbox"/>
Poliomyelitis (polio)	<input type="checkbox"/>	<input type="checkbox"/>
Parkinson's disease.....	<input type="checkbox"/>	<input type="checkbox"/>
Migraine.....	<input type="checkbox"/>	<input type="checkbox"/>
Leg ulcer	<input type="checkbox"/>	<input type="checkbox"/>

Allergy and hypersensitivity:

	Yes	No
Atopic eczema (e.g. childhood eczema)	<input type="checkbox"/>	<input type="checkbox"/>
Hand eczema.....	<input type="checkbox"/>	<input type="checkbox"/>
Food allergy	<input type="checkbox"/>	<input type="checkbox"/>
Other hypersensitivity (not allergy).....	<input type="checkbox"/>	<input type="checkbox"/>

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 1-2 times 2 More than 2 times 3

T8. SYMPTOMS

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

	No	A Little	A lot	Very much
Nervous or worried	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bothered by anxiety.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Confident and calm	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Irritable.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Happy and optimistic	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Down/depressed	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lonely.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	1	2	3	4

8.2 Do you cough about daily for periods of the year? Yes No

If YES:

Is your cough productive? Yes No

Have you had this kind of cough for as long as 3 months in each of the last two years?..... Yes No

8.3 Have you had episodes with wheezing in the chest? Yes No

If YES:

Has this occurred: (Tick once for each question) Yes No

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 Yes No

If YES:

How long can you go before you notice the pain?..... meter

8.5 Do you get short-winded in the following situations?
(Tick once for each question)

While walking fast on level ground or slight up hills Yes No

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?... Yes No

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?..... Yes No

If YES:

Has the complaint reduced your leisure time activity? Yes No

For how long has the complaint endured in total?

approx. years and months

Has the complaint reduced your ability to work during the last year? (Also applies to domestic workers and pensioners) (Tick once)

No/insignificantly 1 To some extent 2 Significantly reduced 3 Do not know 4

Have you been on sick leave due to these complaints during the last year?..... Yes No Do not work

T8. SYMPTOMS (continue)

8.8 How often do you suffer from sleeplessness?
(Tick only once)

Never, or just a few times a year 1

1-3 times a month 2

Approximately once a week 3

More than once a week 4

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 1

Especially during the polar night 2

Especially during the midnight sun season 3

Especially in spring and autumn 4

8.10 Have you in the last year suffered from sleeplessness to the extent that it has affected your ability to work ? Yes No

8.11 Do you usually sleep during the day?..... Yes No

8.12 How often do you suffer from urinary incontinence?

Never 1

Not more than once a month 2

Two or more times a month 3

Once a week or more 4

8.13 Are you able to walk down 10 steps without holding on to something (e.g. a handrail) ... Yes No

8.14 Do you use glasses?..... Yes No

8.15 Do you use a hearing aid?..... Yes No

8.16 How is your memory?
(Tick once for each question)

Do you forget what you just have heard or read?..... Yes No

Do you forget where you have placed things?..... Yes No

Is it more difficult to remember now than earlier?.. Yes No

Do you more often write memos now than earlier? Yes No

If "YES" on one of these questions;
Is this a problem in your daily life?..... Yes No

T9. MEDICINES

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

	Now	Previously, but not now	Age when used 1 st time	Never used
Drugs for osteoporosis.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> years	<input type="checkbox"/>
Tablets for diabetes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> years	<input type="checkbox"/>
Drugs for hypothyroidism (thyroxine)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/> <input type="text"/> years	<input type="checkbox"/>

9.2 Do you use any medicines which you take as injections? Yes No

If YES:

Give the name of the medicines (for injection): T

(one name per line)

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)

	Mother	Father	Brother	Sister	Child	None of these
Heart attack (heart wound)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Angina pectoris (heart cramp)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
High blood pressure	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Aneurysm.....	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Gastric/duodenal ulcer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hip fracture	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Psychological problems ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Allergy	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Osteoarthritis (arthrosis) ..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dementia	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

10.2 How many siblings and children do you have?

	Brothers	Sisters	Children
Number	<input type="text"/>	<input type="text"/>	<input type="text"/>

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

Yes, daily/almost daily	Yes, sometimes	No
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3

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

Yes No

10.5 Is your mother alive?

Yes	No	Age at death
<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>

10.6 Is your father alive?

Yes	No	Age at death
<input type="checkbox"/>	<input type="checkbox"/>	<input type="text"/>

T11. MOBILE TELEPHONE

11.1 Do you have (own, rent, etc.) a mobile telephone?

Yes, always	Yes, sometimes	No
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3

If Yes:

What do you use your mobile telephone for, and how often do you use it? (Tick once for each line)

	Number of times per day				
	30 or more	10-29	2-9	1 or less	Never
Conversations..	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Text messaging	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

12345

T12. THE REST IS TO BE ANSWERED BY WOMEN ONLY

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:
1 st child	<input type="text"/>	<input type="text"/>
2 nd child	<input type="text"/>	<input type="text"/>
3 rd child	<input type="text"/>	<input type="text"/>
4 th child	<input type="text"/>	<input type="text"/>
5 th child	<input type="text"/>	<input type="text"/>
6 th child	<input type="text"/>	<input type="text"/>

(If more children, use additional sheet)

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
<input type="text"/>	<input type="text"/>	<input type="text"/>

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

It stopped by itself	<input type="checkbox"/> 1
Uterus surgery	<input type="checkbox"/> 2
Surgically removed both ovaries	<input type="checkbox"/> 3
Other reason (e.g. radiation, chemotherapy) ...	<input type="checkbox"/> 4

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

Yes No

If YES:

How old were you when you started taking estrogen ?

years

If you stopped using estrogen,

How old were you when you stopped taking estrogen?.....

years

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

Yes No

If YES:

How old were you when you started taking the pill?.....

years

How many years in total have you taken the pills?....

Number of years

If you have given birth:

How many years did you take the pill before your first delivery?....

Number of years

If you stopped taking the pill:

How old were you when you stopped?....

years

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

Yes No

If YES:

How many times?.....

times

12.7 How is your current menstruation status?

I have not had menstruation in the last year	<input type="checkbox"/> 1
I have regular menstruation	<input type="checkbox"/> 2
I have irregular menstruation	<input type="checkbox"/> 3

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

Minimum	Maximum	Do not know
<input type="text"/> days	<input type="text"/> days	<input type="checkbox"/>

The periods were of approximately equal length every time?.....

Yes No

How many days did a typical menstrual bleeding period last?...

days

**Thank you for the help!
Remember to mail the form today!**

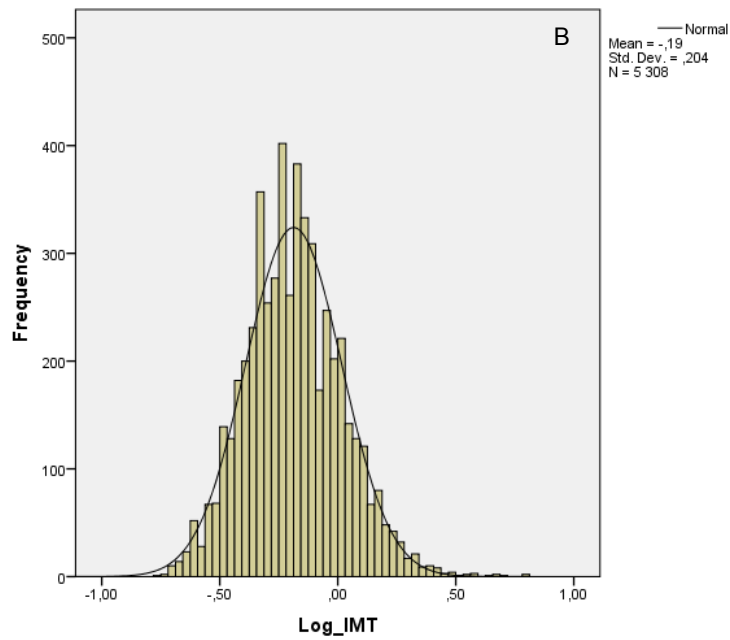
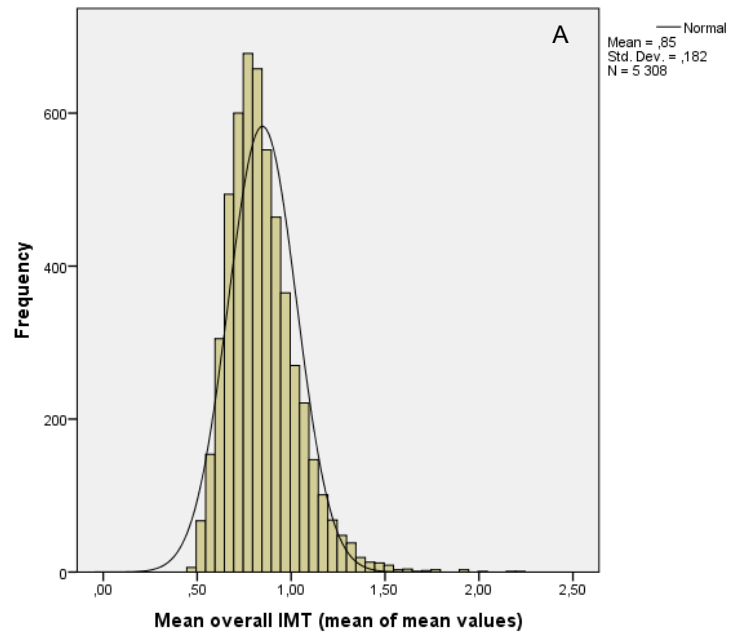
Appendix 5: Participation in the Tromsø 5

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

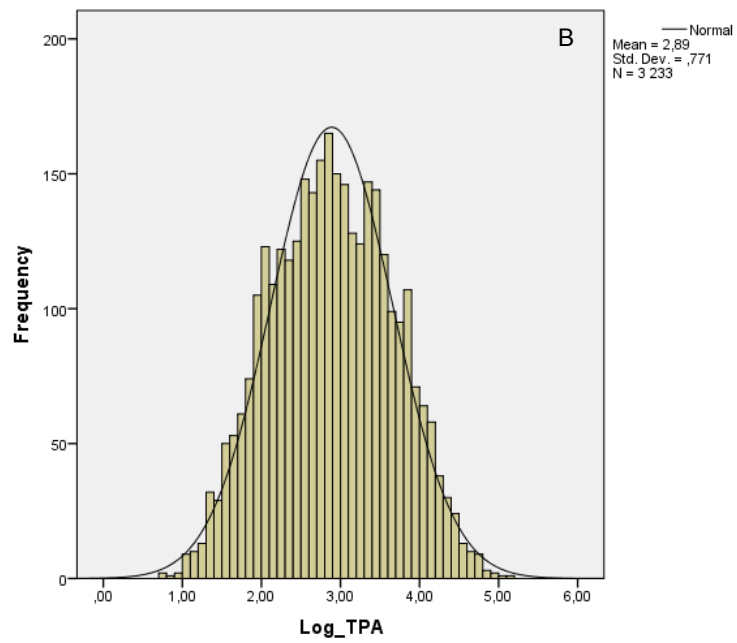
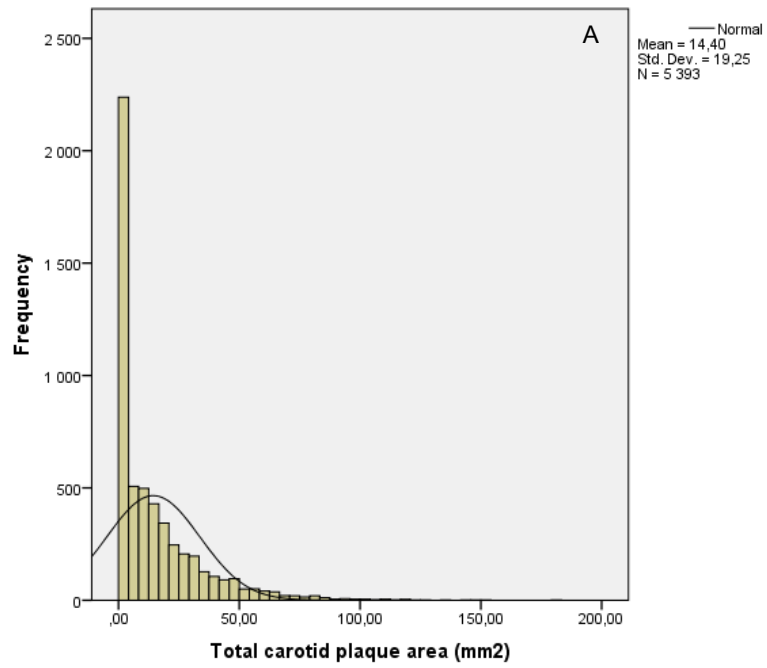
Attendance rate by sex and age groups of the 5th Tromsø Study (2001-2002).

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).

