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Population distribution of traditional and emerging cardiovascular risk factors. The REFINE-Reykjavik study with comparison with the Tromsø Study

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Abstract

Objectives Population statistics for carotid plaque and cardiovascular risk factors reported in scientific journals are usually presented as averages for the population or age and sex adjusted, rather than by sex and age-groups. Important population differences about atherosclerosis and cardiovascular risk factors may thus be missed.

Methods Here we present the carotid artery atherosclerotic plaque prevalence and risk factors levels for cardiovascular disease by sex in 5-year age-groups from the REFINE-Reykjavik study compared with data from the Tromsø 6 study.

Results The defined threshold of carotid plaque presence in the Tromsø 6 study fell between minimal and moderate plaque defined in the REFINE-Reykjavik study reflecting carotid plaque prevalence. The prevalence of minimal carotid plaque in the REFINE-Reykjavik study was 47% in men (40-69 years old) and 38% in women and 11% in men and 7% in women of moderate plaque. The prevalence of any plaque in the Tromsø 6 study was 35% in men and 27% in women. The mean CIMT was similar in both of the studies. In the Tromsø 6 study mean systolic blood pressure was 8 mmHg higher in men and 10 mmHg higher in women, mean level of LDL was 0.5 mmol/L higher in men and 0.3mmol/L higher in women and the prevalence of smoking was 4% higher in men and 9% higher in women. On the other hand, body mass index (BMI) was 0.8 kg/m^2 higher in men and 0.9 kg/m^2 higher in women in the REFINE-Reykjavik study.

Conclusion Comparison between Iceland and Norway revealed differences in the prevalence of carotid plaque, which was assumed to be due to different definition of plaque. However, clinically significant differences in conventional cardiovascular risk factors were seen. This underscores the importance of detailed comparison of population data across different populations.

Strengths and limitations of this study:

- The strength of this study is the random population design of both the REFINE-Reykjavik study and the Tromsø 6 study
- The rigid protocols of the two studies regarding data gathering and quality control and that the studies were conducted at similar time interval is also a strength.
- The main limitation of the study is some difference in carotid ultrasound protocols between the REFINE-Reykjavik study and Tromsø 6 study the study and it was done on only Caucasian participants.

Introduction

The value of comparing risk factors of cardiovascular disease between populations is undisputed. The Seven Countries Study and the World Health Organization led MONICA study are examples of studies that have monitored changes in risk factors and compared diets and lifestyles between countries. These studies contributed to knowledge, which led to changes in risk factor levels and the drop seen in the prevalence of coronary heart disease in the last decades of the 20th century (1, 2). However, detailed information on the presence of atherosclerotic plaque in the carotid arteries across different populations is not readily available in the current literature. Population statistics for carotid plaque and for cardiovascular risk factors reported in scientific journals are usually presented as an average for the population or adjusted for age and sex, rather than being reported by different sex and age groups. Thereby significant sex and age interaction in the development in atherosclerosis and/or in cardiovascular risk factors can be missed in comparison across different populations based on published data. For carotid plaque, which is one of the best-studied markers of subclinical atherosclerosis, different definitions of carotid plaque between studies also complicate the comparison.

We now publish results from the first phase of the Risk Evaluation For Infarct Estimates Reykjavik study (REFINE-Reykjavik study) started in December 2005 and completed in March 2011. The REFINE-Reykjavik study is a prospective cohort study on risk factors and aetiology of atherosclerotic disease in the population of the

Reykjavik area in Iceland. The study was performed on a large number (6661) of individuals (25-69 years of age) with ultrasound of the carotids and other measurements of both traditional risk factors and new risk factors for cardiovascular disease. The aim of REFINE-Reykjavik study was to demonstrate what characterizes individuals who develop atherosclerosis and to understand if carotid plaque or other factors measured in the study increase the accuracy of risk estimates for cardiovascular disease.

The population distribution of cardiovascular risk factors and the prevalence of atherosclerotic plaque in the common carotid arteries are presented in adults living in the greater Reykjavik area according to age groups and sex. We report the data in this detailed manner in order to make comparisons with other studies easier and more accessible. We also make direct comparison between results from the REFINE-Reykjavik study and the Tromsø 6 study and discuss the results in context with available outcomes from other population studies in Europe and USA.The two population studies, the REFINE-Reykjavik study and the Tromsø 6 study, were conducted within the similar time interval (REFINE-Reykjavik study 2006-2011, Tromsø 6 2007-2008), included both genders and both included middle age participants, mostly of Scandinavian origin and are therefore highly comparable.

Methods

Study population

The cohort in the first phase of the REFINE-Reykjavik study was a random sample of 9.480 men and women born in 1935-1985, living in Reykjavik in November in the year 2005 and with Icelandic citizenship. The cohort was divided into five year age groups from 25 to 69 years. The age distribution was designed to over-represent middle-aged individuals in order to concentrate the power of the study on the age span where development of atherosclerosis was to be most expected. In the age groups 25 to 34 the

number of individuals in each age group was 600, in the age groups from 35 to 64 the number was 1200 in each group and in the age group 65-69 the number of individuals was 480. The cohort in the REFINE-Reykjavik study was drawn from the same geographic area as the well-established Reykjavik study. The cohort in the Reykjavik-study included individuals born in 1907-1935(3). The birth year bracket in the REFINE-Reykjavik study (1935-1985) is therefore in continuation of the Reykjavik-study.

The cohort in REFINE-Reykjavik study is homogenous with the vast majority being of Scandinavian origin. Icelanders are genetically similar to other northern European countries (4) and risk of coronary heart disease and the contribution of the conventional risk factors to this risk is similar (5). In the final survey of the WHO MONICA Project conducted in 1992, of the 38 population investigated for coronary event rate in men, the Icelandic population was approximately in the middle. Twenty populations had higher coronary event rate and 17 populations had lower coronary event rate than the Icelandic population. (6) For comparison of both conventional risk factors for coronary heart disease, prevalence of carotid plaque and the level of CIMT in the population, data from Tromsø 6 study were used.

The Tromsø Study is an ongoing population-based cohort study in the municipality of Tromsø, Northern Norway, with a population of 72 000 inhabitants. The Tromsø 6 study was conducted in the years 2007–2008. The age span was 40-87 years. Invited to Tromsø 6 1st visit were all residents aged 40-42 and 60-87 (n=12,578), a 10% random sample of individuals aged 30-39 (n=1056), a 40% random sample of individuals aged 43-59 (n= 5787), and subjects who had attended the second visit of Tromsø 4, if not already included in the three groups above (n=341). The attendance rate was 66%.

Detailed description on recruitment methods, use of medication and supplements, clinic examination, blood analyses, ultrasound of the carotid arteries in the REFINE-Reykjavik study in supplementary text 1 and description of Tromsø 6 in supplementary text 2

Statistical methods

Age standardized means and proportions were presented and compared between the REFINE-Reykjavik and the Tromsø 6 study. The following risk factors were investigated; systolic blood pressure, low density lipoprotein, body mass index, prevalence of type 2 diabetes, cholesterol lowering medications (statins), hypertension medication, smoking, self-reported history of coronary heart disease, intima media thickness and plaque in the right carotids.

Age standardization was done according to the direct method, using the standard population age structure as defined by the European Standard Population (ESP) (7). Statistical significance between study summary estimates was investigated using linear regression for continuous variables and logistic regression for categorical variables. Regressions were run separately for each sex and adjusted for age.

Intima media thickness and prevalence of plaque in right carotids was presented visually by sex, age groups and study. Blood pressure measurements in the REFINE-Reykjavik study were done using arterial tonometry (8) whereas an electronic sphygmomanometer (Dinamap ProCare 300 monitor, GE Healthcare) was used in Tromsø 6 study. A set of approximately 400 available and concurrently measured sphygmomanometer readings in REFINE-Reykjavik study were compared to tonometry measurements using a linear mixed effects model, accounting for an inherent repeated measures aspect. Age-,sex- and method-specific predicted values were used to obtain a correction factor which was applied to the tonometry measurements in REFINE, in an effort to make them comparable to the Tromsø-6 study measurements.

Statistical analysis was done using Stata 14.1(9).

Results

Recruitment for the REFINE-Reykjavik study started in December 2005 and was completed in March 2011. The total number of individuals who participated in the study was 6661, 3277 men and 3384 women. The recruitment rate was 73%. The sex ratio was 49% men and 51% women. The mean age was 49.8 years (SD 11.2 years) and the age range was 25-69 years.

The mean BMI for men was 27.7 (SD 4.3) kg/m2 and 26.7 (SD 5.3) kg/m2 for women. Mean BMI was above 25kg/m2 in both sexes, which is the upper limit of ideal weight according to WHO expert committee report (10). BMI increased with increasing age (Supplement 3 table 1).

Both systolic and diastolic blood pressure rose with age but there was a decrease in the diastolic blood pressure in the oldest age group (65-69 year old) in both sexes. Average systolic blood pressure in men was 125.5 mmHg (SD 13.9) and 115.5 mmHg (SD 13.7) for women and average diastolic blood pressure was 70.7 mmHg (SD10.0) and 68.7 mmHg (SD 9.0) respectively. (Supplement 3 table 1)

A steady increase in total cholesterol (TC), low density lipoprotein (LDL) and triglycerides and was observed in women with increasing age. In men, TC, LDL and TG peaked in middle aged, decreasing again over the age of 60. HDL cholesterol increased with age in both sexes. (Supplement 3 table 1)

Family history of myocardial infarction increased with age and was somewhat higher in men than women (table 2). History of cardiovascular disease and history of coronary heart disease was rare in participants younger than 50 years old but increased sharply with age in men and it was 22.9% and 20.4% respectively in 65-69 years old men (table 2). The increase was more gradually in women, history of cardiovascular disease and history of coronary heart disease was 6.0% and 4.3 % respectively in 65-69 years old women (Supplement 3 table 2).

(Supplement 3table 2) Characteristics of the REFINE-Reykjavik study by age and sex, categorical parameters.

The prevalence of diabetes type 2 in the cohort is shown in Table 2. In men the prevalence on average was 6% and age adjusted 4.3%. The prevalence is lower in women or 3% and age adjusted 2.4%. The prevalence of diabetes increased with age in both sexes.

Hypertension was rare amongst young women (25-29 years) but 10% of young men had hypertension. With increasing age the prevalence of hypertension increased sharply so that in the oldest age group (65-69 years) the majority of men (71%) and more than half of the women were hypertensive (Supplement 3 table 2).

Use of blood pressure lowering drugs and cholesterol lowing drugs (statins) is shown in table 2. Drug treatment increased with age and was highest in the oldest age group. In the age group 65-69, 57.1% men and 51.4 % women were on treatment against high blood pressure and 33.8% of men and 18.4% of women were treated with statins (Table 2).

Current smoking was highest in the age group 25-29 years in both men (28.8 %) and women (27.4 %). The prevalence of current smoking decreases with increasing age. On average, 22% of men and 21% of women smoked Supplement 3 table 3).

Overweight, or BMI ≥25 (10) was very common in man (73 %) and in women (56%) and obesity, or BMI ≥30 was seen in 25% of men and 22% of women. More than half of men and women had cholesterol levels above 5mmol/L and 14% of men and women had cholesterol levels above 6.5 mmol/L (Supplement 3 table 3)

Physical activity was assessed by the following question in the health history questionnaire: “In the past 12 months, how often did you participate in moderate or vigorous physical activity (Examples of moderate or vigorous physical activity include badminton. golf (walking), biking, swimming, heavy gardening, weight lifting, hiking/ mountain climbing, fast walking/fast dancing/heavy housework, rowing, aerobics, jogging and running)” About 60-70% of men and women participated in at least moderate

physical activity for 1-3 hours a week and 30-40% were active 4-7 hours a week. No clear difference in physical activity was seen between men and women or different age groups (Supplement 3 table 3).

In supplement 3 table 4 mean common CIMT values are shown according to age and sex. The mean CIMT was 0.71mm (SD 0.10) in men and 0.67 mm (SD 0.08) in women. CIMT increased steadily with increasing age in both sexes, and was slightly higher in men than in women. For example, in the oldest age group (65-69 years) the mean CIMT was 0.91mm (SD 1.3) in men but 0.85 (SD 0.11) mm in women. Results from the maximum IMT thickness are also shown in table 3. Maximum IMT values increased similarly with age and the sex difference was similar.

The prevalence of carotid plaque increased with age in both sexes (Supplement 3 table 4). The prevalence was somewhat higher in men than women at all ages although the sex difference was small. For example 7.7% of 50-54 years old men had moderate plaque compared to 5.3% of women. One third of men in the oldest age group (65-69 years) had moderate or more carotid plaque but 27% of women. Severe plaque or semi occlusion was never detected in the younger participants but was detected in 4.4% and 4.8% in the oldest women and men respectively. In the youngest age group (25-29 years), 94.2% of men and 96.5% of the youngest women had no plaques, while this was seen in only 15.6% of the oldest men (65-69 years) and 21.6% of the oldest women

Table 1 shows the characteristics of the REFINE-Reykjavik study and the Tromsø 6 study in 40-69 years old men and women.

Table 1. Age standardized characteristic of participants in the REFINE-Reykjavik study and the Tromsø 6 study ¶.

	Men 40-69 years			Women 40-69 years		
	REFINE-Reykjavik (n=2629)	Tromsø 6 (n= 2214)	Difference	REFINE-Reykjavik (n=2719)	Tromsø 6 (n=2981)	Difference
Mean crude age (years) (SD)	54 (8.2)	59 (5.7)	-5	54 (8.2)	59 (6.1)	-5
Systolic BP∞ (mmHg) (SD)	130 (15.5)	138 (17.7)	-8**	123 (15.5)	133 (21.4)	-10**
LDL (mmol/L) (SD)	3.3 (0.9)	3.8 (0.9)	-0.5**	3.3 (0.9)	3.6 (0.9)	-0.3**
BMI (kg/m2) (SD)	28.4 (4.4)	27.6 (3.7)	0.8**	27.3 (5.3)	26.4 (4.7)	0.9**
CIMT¥ mean far wall (mm)(SD)	0.77 (0.12)	0.79 (0.15)	-0.02*	0.72 (0.10)	0.73 (0.12)	-0.01
Current smoker % (number)	21 (549)	25 (537)	-4**	20 (558)	29 (777)	-9**
HTMEDΨ users % (number)	30 (776)	18 (532)	12**	28 (759)	17 (690)	11**
Statin users (%) (number)	16 (410)	13 (401)	3*	7 (192)	9 (425)	-2*
Known heart attack and/or angina ǂ (%) (number)	8.4 (218)	7.7 (212)	0.7*	3.4 (93)	2.5 (119)	0.9
Type 2 diabetes (%) (number)	7.4 (196)	7.4 (204)	0.0	3.4 (90)	4.9 (186)	-1.5**
Plaque R- carotid: REF min , Tromsø-any (%) (number)	46.8 (1238)	35.3 (997)	11.5**	38.3 (1039)	26.6 (1042)	11.7**

¶ Values are mean (standard deviation) or percentage (number). Age standardized according the European Standard Population 2013

*p-value <0.05, **p-value <0.001, ∞ The REFNIE-Reykjavik study blood pressure measurement were adjusted for difference between measurements from arterial tonometry and sphygmomanometer measurements (see methods), ¥ Common carotid intima media thickness, ψ hypertensive medication, ł according to health questionnaire

The systolic blood pressure was 8 mmHg higher in men and 10 mmHg higher in women in the Tromsø 6 study than the REFINE- Reykjavik study. Participants in REFINE-Reykjavik study were more often taking antihypertensive medication than in the Tromsø 6 study (30% vs 18% in man and 28vs17% in women). Calculated LDL-cholesterol was somewhat higher in both men and women in the Tromsø 6 study than in REFINE- Reykjavik study (0.3mmol/L). The prevalence of statin use was similar in the two studies although somewhat more men were taking statins in REFINE-Reykjavik study than Tromsø 6 study (15% vs 13%) but less women (7.2% vs 9.4%). Smoking was less prevalent in REFINE-Reykjavik study than the Tromsø-6 study in both sexes but BMI was nearly one unit higher in the REFINE-Reykjavik study in both sexes. The prevalence of type2 diabetes was similar in men in both studies (7.4%) but was lower in women in the REFINE-Reykjavik study (3.9% vs 4.9%). The mean farwall CIMT in men was 0.02 mm thicker in the Tromsø 6 study than the REFINE-Reykjavik study (p-value < 0.05) and 0.01 mm thicker in women (not significant) (Table 1). The prevalence of minimal right site carotid plaque or more was higher in the REFINE-Reykjavik study than any right site plaque or more, in the Tromsø-6 study in both men and women. The difference in in cardiovascular risk factors between the studies was similar in both sexes and across age group as is shown in Supplement 4 figure1.

The mean farwall CIMT increased with age as can be seen in Fig 1. The CIMT was higher in the age group 40-49 in the Tromsø-6 study but was similar in the two studies after the age of fifty in both men and women (Fig1).

As shown in Fig 2 the prevalence curve for any right carotid plaques in the Tromsø 6 study lies between the prevalence curve for minimal or more carotid plaque and the prevalence curve for moderate or more carotid plaque in the REFINE-Reykjavik study

Discussion

In this paper we present the average prevalence of atherosclerotic plaque in the carotid arteries and average of cardiovascular risk factors in adult population of Reykjavik area in Iceland in the REFINE-Reykjavik study. We put the results in context with the results from the Tromsø 6 study. The main findings are that the evidence of manifest atherosclerosis. i.e. the prevalence of carotid plaques is similar in the two studies. Systolic blood pressure and LDL-cholesterol levels were higher in the Tromsø 6 study but the mean BMI was higher in the REFINE-Reykjavik study.

The two population studies, the REFINE-Reykjavik study and the Tromsø 6 study, were conducted within the similar time interval (REFINE-Reykjavik study 2006-2011, Tromsø 6 2007-2008), included both genders and both included middle age participants, mostly of Scandinavian origin and are therefore highly comparable. The prevalence curve for any carotid plaque by age and sex in the Tromsø 6 study lies in between the prevalence of minimal plaque and moderate plaque in the REFINE-Reykjavik study for both men and women. This can be seen in all age groups and the increase with age is similar. The difference in prevalence of carotid plaque in the two studies is most likely due to a different definition of carotid plaque. In the REFINE-Reykjavik study, minimal plaque was defined as a small isolated thickening uni- or multifocal, often with calcification approximately 2 times the adjacent normal CIMT. A moderate plaque was defined as a clear, reasonably easily visualized plaque with or without calcifications that may be located on both near and far wall in the segment causing some diameter reduction. The definition of plaque presence in the Tromsø 6 study was of a "… localized protrusion of the vessel wall into the lumen" (11). Focal calcifications without focal thickening or protrusion into the lumen were not regarded as atherosclerotic plaque in the Tromsø 6 study (11). Since both studies show similar increase in plaque prevalence with increasing age and the threshold for definition of carotid plaque in the Tromsø 6

study seems to lie in between the definition for minimal and moderate plaque in the REFINE-Reykjavik study, we assume that the differences in plaque prevalence are mainly due to different definitions of plaque although difference in prevalence of plaque cannot be excluded.

Comparison of the mean farwall CIMT between the REFINE-Reykjavik study and the Tromsø 6 study reviled close similarity between the two studies after the age of fifty. The mean CIMT was higher in the Tromsø 6 study in participants under the age of fifty than in the REFINE-Reykjavik study. However, the number of participants in this age group in the Tromsø 6 was relatively smaller compared to older age groups, and the confidence intervals for the CIMT measurements wider. We therefore concluded that the mean farwall CIMT was similar in the REFINE-Reykjavik study and the Tromsø 6.

It is clear that the need for a standardized definition of plaque and CIMT is important both for clinical practice, in order to increase the availability of ultrasound laboratories that can perform high quality carotid plaque and CIMT evaluation, and to increase comparability between future studies. Both in Europe and the USA attempts have been made in that regard. In 2008 the American Society of Echocardiography Carotid Intima-Media Thickness Task Force published a consensus statement (12). There, carotid plaque was defined as "the presence of focal wall thickening that is at least 50% greater than that of the surrounding vessel wall or as a focal region with CIMT greater than 1.5 mm that protrudes into the lumen that is distinct from the adjacent boundary "(12). In 2012 the Mannheim carotid Intima-media thickness and plaque consensus (2004-2006-2011) was published where carotid plaque was "defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value or demonstrates a thickness> 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface" (13). These two consensus statements give very similar definitions of plaque and will hopefully reduce confusion that different definitions can cause.

In all age groups, except for 25-29 year old women, the mean BMI value was over 25 kg/m2 the upper limit of normal weight according to WHO definition (14) in the REFINE-Reykjavik study. The mean BMI value for women was 26.7 and for men 28.7 kg/m2. More than a third of men over the age of 50 were obese according to the WHO definition. We have previously analyzed the trend in BMI in Iceland. According to the Icelandic Heart Association study the mean BMI increased by 2 units in both

genders (45-64 year old) from 1967 to 2007 (15) . Comparison with the Tromsø 6 study shows, that the mean BMI was 0.9 units kg/m2 higher in women and 0.8 kg/m2 higher in men in the REFINE-Reykjavik study than in the Tromsø 6 study.

Almost a third of men aged 25-29 years smoked in the REFINE-Reykjavik study. This was somewhat lower than the average prevalence of smoking in developed countries according to a large international survey (16), where about 38% of men in this age group smoked in 2012. Smoking decreased with age and was down to 17% in the 65-69 year old group in the REFINE-Reykjavik study. Comparison between the REFINE-Reykjavik study and the Tromsø 6 study showed that smoking was somewhat more prevalent in both men and women in the Tromsø 6 study. In men the difference was 4% (21% REFINE-Reykjavik vs 25% Tromsø 6) and 9% in women (20% REFINE-Reykjavik vs 29% in Tromsø 6). Prevalence of women smokers in the REFINE-Reykjavik was similar to the prevalence of smoking amongst men in the same age groups (21%). This was similar as was seen in the Tromsö 6 study where smoking was even more prevalent amongst women (29.2%) than amongst men (25.4%). This is different from what was seen in many other developed countries where smoking amongst women is approximately half of the prevalence of smoking in men (16).

Blood pressure should be below 140/90 mmHg according to the European Society of Cardiology (ESC) 2012 guidelines. The mean values for blood pressure in the REFINE-Reykjavik study were well below the ESC targets for all age and gender groups. The mean blood pressure levels for men were 127/71 mmHg and 116/69 mmHg for women. We have previously shown that blood pressure levels have been dropping in Iceland from 1967 to 2007 in middle age men and women by approximately 20 mmgHg (17) and this drop has been seen in all age groups, indicating a population effect rather than an effect of treatment with blood pressure lowering drugs. However, the use of blood pressure lowering drugs was very common in the REFINE-Reykjavik study in the oldest age groups. More than half of men and women in the age group of 65-69 years were taking blood pressure lowering drugs. This high prevalence of drug use could lower the population mean in the oldest age groups. The blood pressure results in the REFINE-Reykjavik were 8 mmHg lower in men (aged 40-69 years) and 10 mmHg lower in women than in the Tromsø 6 study. The difference was similar in each age group. Difference in the use of blood pressure lowering drugs could add to this highly clinically significant difference. This difference is similar in magnitude as the decline in blood pressure in women from 1978-2008 in the Tromsø 6 study (18).

According to the 2016 ESC guidelines for the management of dyslipidemia, drug treatment should be considered if the 10-year risk of fatal cardiovascular disease exceeds 1% and LDL- cholesterol is between 2.6 to <4.0 mmol/L despite of lifestyle intervention (19). Mean LDL-cholesterol level in all age groups except in young women (25-29 years) was above this lower limit. The mean LDL-cholesterol level was highest in 55-59 years men (3.4mmol/L) and women 60-64 years (3.5mmol/l). Comparison with the Tromsø 6 study revealed that in 40-69 years old, the mean LDL-cholesterol was 0.5 mmol/L lower in men (3.3 vs 3.8) and 0.3 mmol/L lower in women (3.3 vs 3.6) in the REFINE-Reykjavik study than in the Tromsø 6 study. This difference was 15% in men and 11% in women. For comparison, the mean percentage lowering of LDL-cholesterol after administrating 20 mg of simvastatin has been shown to be on average 35% (20). In the REFINE-Reykjavik study the LDL-cholesterol was measured in participants after fasting from the evening before, whereas in the Tromsø 6 study the LDL-cholesterol was measured in non-fasting participants. In the Copenhagen General Population Study, the levels of LDL-cholesterol was 0.2mmol/L lower after- meal than after fasting so the difference in LDL-level between the REFINE-Reykjavik study and Tromsø 6 study could be even larger.(21)

We have previously published that total cholesterol (TC) levels in Iceland have been dropping as in other developed countries for the last decades (22). The drop has been similar in both genders and all age groups. The mean drop in TC in the Icelandic population from 1967 to 2008 was 1.5 mmol/L in males and 1.6 mmol/L in females (22).

The prevalence of diabetes has been historically been low in Iceland but the prevalence of diabetes in men in the REFINE-Reykjavik study was almost identical to the prevalence of diabetes in men according to a population based health care database in Sweden (23). Another recent Swedish study shows that prevalence of diabetes was 59.8 per 1000 (6%) for men and 38.4 per 1000 (4%) for women 40-64 years old in 2010 (24). Comparison on the prevalence of type 2 diabetes in men between the REFINE-Reykjavik study and Tromsø 6 study also showed very similar results (7.4%).The prevalence was low in both studies amongst women, (3.4% in REFINE-Reykjavik vs 4.9 in the Tromsø 6 study). The prevalence of diabetes in Iceland, Norway or Sweden has been, from a global viewpoint, relatively low. The prevalence of diabetes in USA in people older than twenty years was for example, according to the NHANES study, 13.4% in men and 10.2% in women in 2007-2010 (25).

In conclusion, the mean CIMT were similar in the REFINE-Reykjavik study and the Tromsø 6 study. The higher prevalence of carotid plaque in the REFINE-Reykjavik Study was probably due to differences in the definition of plaque between the two studies. However the mean for systolic blood pressure and mean LDL-cholesterol levels were higher and smoking more prevalent in the Tromsø-6 study but BMI was higher the REFINE-Reykjavik study.

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Conflict of Interest

None declared

Data sharing statement

Data are available through collaboration with the Icelandic Heart Association and the Tromsø study

Contributorship statement: Conception and design of study: BT, VG, TA, SS and GE; acquisition of data: BT, SS, EG ,KA, EM; analysis and/or interpretation of data: BT, GE, SS, EG, MB, TA, KA, Em ,VG; drafting the manuscript: BT, GE, SS, TA,VG; revising the manuscript critically for important intellectual content:GE ,SS,MB,TA, EM,VG All authors approved of the version of the manuscript to be published.

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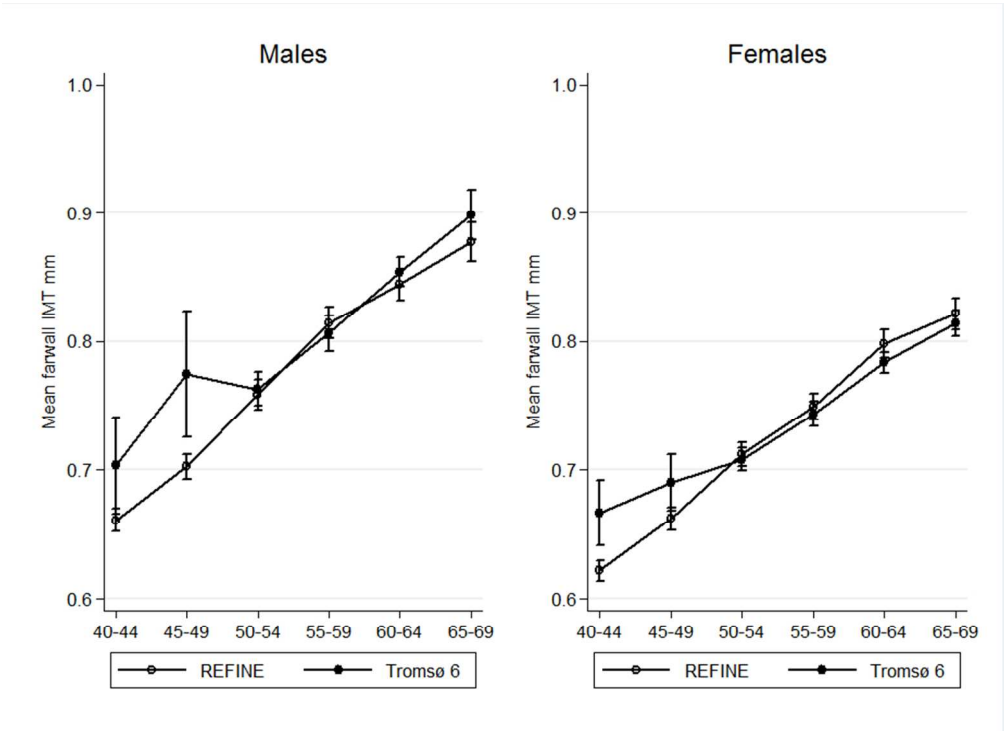

Fig 1. Mean farwall carotids intima media thickness in the REFINE-Reykjavik study and Tromsø 6 study by age and sex

325x236mm (72 x 72 DPI)

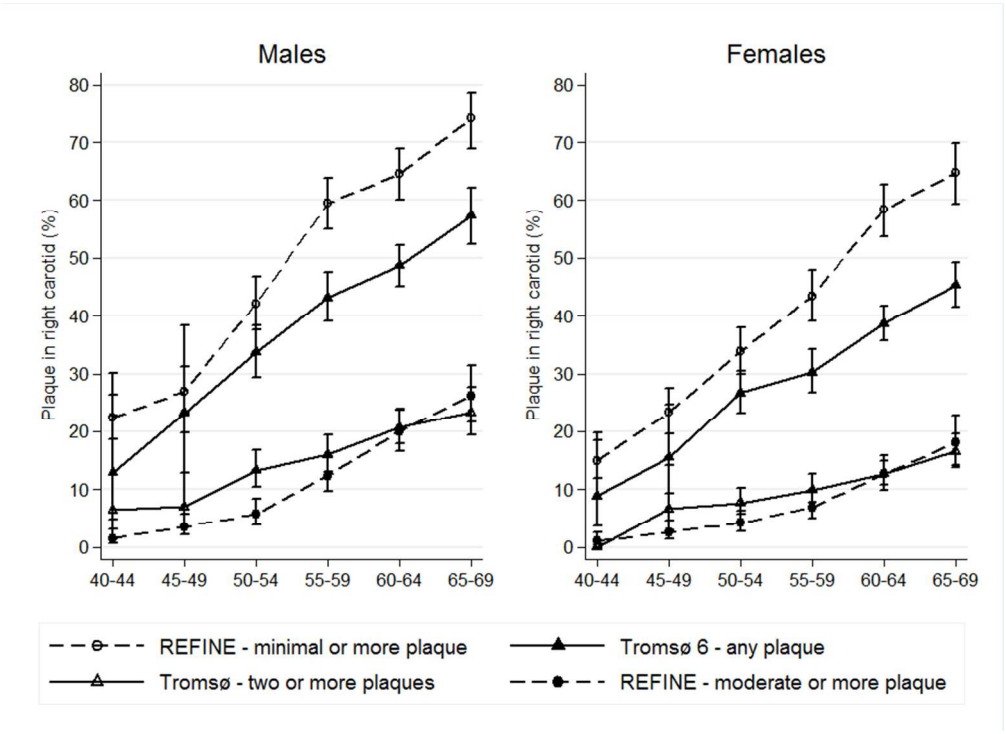

Fig 2. Prevalence of right carotids plaque in the REFINE-Reykjavik study and in the Tromsø 6 study by age and sex

325x236mm (72 x 72 DPI)

Supplement 1

Recruitment

In the REFINE-Reykjavik study, all individuals in the cohort receive an invitation letter by mail. Those who do not respond to the invitation letter are called by a trained telephone receptionist. Reasons for refusing participation were documented when possible. Recruitment started in December 2005 and was completed in March 2011. All participants were asked to fast from the evening before the clinic visit and give informed consent at arrival to the clinic. Participants got feedback regarding the blood test and examination results from a physician.

Use of medication and supplements

The invitation letter included instructions to bring all prescription and non-prescription medications and supplements taken regularly. A trained interviewer registered all medications and supplements taken within the last two-week period (14 days). All medications were classified according to the ATC (Anatomical-Therapeutical-Chemical Classification) codes and supplements by OCD (Over (7)the Counter Drugs Classification) codes when possible. In cases when the classification of drugs is ambiguous a board of two physicians and a nurse resolved the matter.

Clinic examination

Participants answered a health history questionnaire on the internet. Most participants answered at home through a secure web-site but those who had not answered answer on site when they arrive at the clinic. The questionnaire included both history and symptoms of coronary heart diseases (Rose chest pain questionnaire) (8), peripheral arterial diseases, history of vascular procedures, history of stroke, diabetes, high cholesterol, hypertension, chronic obstructive lung disease, sleeping habits, history of esophageal regurgitation, estrogen use in women, family history of CHD, education, profession, history of smoking and current smoking and former and current exercise.

History of cardiovascular disease and history of coronary heart disease were retrieved from the Landspitali- The National University Hospital of Iceland by gathering the ICD 10 and ICD 9 codes for all participants at arrival

into the study. Those participants that had been given the ICD 10 codes: I21-I25, I60-I64 and/or ICD 9 codes 410,411,414,429, 431-434, 436 were defined as having history of cardiovascular disease. Those participants that had been given the ICD 10 codes I21-I25 and/or the ICD 9 codes 410,411,414,429 were defined as having history of coronary heart disease. Diabetes type 2 was defined as history of diabetes due to health questioner or taking diabetes medication or fasting glucose ≥ 7 mmol/L and not taking insulin and not diagnosed younger than 30 years. Physical activity was assessed by the following question in the health history questionnaire: "In the past 12 months, how often did you participate in moderate or vigorous physical activity (Examples of moderate or vigorous physical activity include badminton. golf (walking), biking, swimming, heavy gardening, weight lifting, hiking/ mountain climbing,fast walking/fast dancing/heavy housework, rowing, aerobics, jogging and running)"

Blood pressure was measured semi-automatically during arterial tonometry measurements (Noninvasive Hemodynamics Workstation) according to a standardized protocol (9). Participants were in a supine position for 15-20 minutes before the blood pressure measurement. Hypertension was defined as systolic blood pressure above 140 and/or diastolic blood pressure above 90 or if the participants in the study were on blood pressure lowering drugs based on ATC codes.

An electrocardiography (ECG) was performed and stored digitally. Anthropometric measurements were measurements of body height, weight, hip and waist circumference and body composition by bio-impedance measurements. Certified staff members collected data according to rigid and standardized protocols. Regular quality assurance (QA) protocols were implemented to insure best quality of the data and to reduce inter- and intra-observer variability.

Blood analyses

All chemical measurements were carried out in the ISO accredited laboratory of the Icelandic Heart Association (IHA). The blood draw, handling, aliquoting, storing and measuring as well as switching Analyzers were performed according to the IHA Quality Manual documents. Hb, Hct, MCH, MCHC, MCV, RBC , WBC and platelets were measured in fasting whole blood on an automated cell counter, Coulter HmX AL Hematology Analyzer (Beckman Coulter, High Wycombe, England, UK) which was replaced in November 2011 with XT-2000i from Sysmex. Chemistry measurements were performed on Roche/Hitachi 912 which was updated in February 2010 to Roche/Cobas c311 using reagents from the respective manufacturers according to their

instructions. LDL-cholesterol was calculated using the Friedewald equation (total cholesterol-HDL cholesterol-(triglycerides/2,2)) when triglycerides < 4,5 mmol/L.

Ultrasound of the carotid arteries

Ultrasound of the carotid arteries is performed using a standardized scanning and analysis protocol for quantitative assessment of the common carotid intima-media thickness (IMT) and arterial stiffness. The protocol also included scans for semi-quantitative assessment of plaque presence/absence and plaque severity. The protocol was developed by experts from the Vascular Imaging Center, Julius Center for Health Care and Primary Care in the University of Utrecht in the Netherlands (MLB). The technicians that performed the ultrasound studies were trained by the same experts that developed the protocol.

The carotid arteries on both sides were imaged from 4 different interrogation angles with 30 degrees increments using a Sequoia C256, Acuson ultrasound system (Siemens Medical Systems, Erlangen, Germany) with an 8.0 MHz transducer. To standardize and control the interrogation angles, the Meijers Carotid Arc was used (10). The IMT measurements were quantified on a predefined segment in near and far wall of the carotid common arteries (11) using the Artery Measurement Software (AMS) II v1.131.

The REFINE-Reykjavik study uses strict quality control procedures for monitoring and testing consistency in image acquisition and image analysis. The quality control includes periodical tests of image analysis and acquisition reproducibility including re-reading of IMT every 6 months of the same 24 cases for assessment of inter-and intra-observer variability and consistency over time. There were typically 2 weeks between reading 1 and reading 2 for the intra-observer variability assessment. Inter-observer variability of carotid plaque presence and severity was tested by repeated acquisitions of up to 15 studies every year by each sonographer. In addition, intra-observer variability of IMT was further tested by the re-reading of 10 randomly selected studies by each observer every 6 months where there were typically 5 to 6 months between reading 1 and reading 2.

Mean intra-observer variability in IMT measurements for three observers (intra-class correlation and percent coefficient of variation respectively) based on the re-reading of the same 24 cases (n=24) over the course of the study ranged from 0.97 to 0.99 and 2.7% to 3.6% for the far wall of the carotid arteries and 0.96 to 0.97 and 3.6% to 4.9% for the near wall. Inter-observer variability for the same 24 cases and the same observers ranged from 0.91 to 0.94 and 4.7% to 6.0% for the far wall and 0.79 to 0.81 and 8.4% to 9.2% for the near wall. Intra-reliability assessment (kappa statistics) of carotid plaque presence and plaque severity between the observers

where the results by two observers were compared to the results of one observer that was considered a gold standard were 0.77 (n=68) ad 0.84 (n=60) demonstrating good to excellent agreement. The intra-observer variability in IMT measurements based on re-reading of a random selection of 10 cases every 6 months (intra-class correlation and percent coefficient of variation respectively) was 0.96 and 3.7% for the far wall and 0.91 and 5.6% for the near wall for observer 1 (accumulative total of re-readings, n=90) , 0.93 and 5.0% for the far wall and 0.92 and 6.3% for the near wall for observer 2 (accumulative total of re-readings, n=80) and 0.94 and 3.2% for the far wall and 0.96 and 3.5% for the near wall for observer 3 (accumulative total of re-readings, n=50).

Outcome parameters:

1. Common carotid intima-media thickness: B-mode images of the IMT are acquired for the predefined 10mm segment of each common carotid artery (11) (right and left) at defined interrogation angles using Meijers Arc. Standard images are obtained from 4 angles at each site. The mean intima-media thickness (IMT) of the near (shallower) and far (deeper) walls are determined from a single image at each interrogation angle for both the right and left common carotid arteries CCA. The average of all these IMT values comprised the mean IMT outcome parameter. The maximum IMT corresponded to the highest measured IMT value at the 4 angles.

2. Atherosclerotic plaque in the carotid bifurcation and internal carotid artery: Of the left and right carotid bifurcation and internal carotid artery the presence of atherosclerotic lesions is measured on line, i.e., during the ultrasound examination. The most severe lesion per segment is assessed in a semi-quantitative manner. The plaque image interpretation is based on the following 4 categories:

1. None: Complete absence of plaque, IMT thickening may be observed.

2. Minimal: small isolated thickening, uni- or multi focal, often with calcification approximately 2 times the adjacent normal IMT.

3. Moderate: clear, reasonably easy to visualize plaque with or without calcification. May be located on both near and far wall in the segment causing some diameter reduction.

4. Severe: Significant plaque formation very easy to image with or without calcifications and visualized on several different scan projections in near and far wall causing clear diameter reduction.

Images of observed plaques were stored.

Supplement 2

The Tromsø Study

For comparison of both conventional risk factors for coronary heart disease, prevalence of carotid plaque and the level of CIMT in the population, data from Tromsø 6 study were used.

The Tromsø Study is an ongoing population-based cohort study in the municipality of Tromsø, Northern Norway, with a population of 72 000 inhabitants. The study design includes 7 surveys (Tromsø 1: 1974, Tromsø 2: 1979–1980, Tromsø 3: 1986–1987, Tromsø 4:1994–1995, Tromsø 5: 2001-2002, Tromsø 6: 2007–2008, and Tromsø 7: 2015-2016) to which total birth cohorts and representative samples of the population were invited. From Tromsø 4 and onwards, the study design has included two screening visits, with more extensive examinations at the second visit, including ultrasound examination of the carotid arteries.(Jacobsen BK et al, Int J Epidemiol 2012;41:961-7) Invited to Tromsø 6 1st visit were all residents aged 40-42 and 60-87 (n=12,578), a 10% random sample of individuals aged 30-39 (n=1056), a 40% random sample of individuals aged 43-59 (n= 5787), and subjects who had attended the second visit of Tromsø 4, if not already included in the three groups above (n=341). The attendance rate was 66%. Those eligible for the 2nd visit were all 1st visit eligible aged 50-62 and 75-84 years (n=7657), a 20% random sample of 1st visit eligible aged 63-74 (n=942), and subjects who had attended the second visit for Tromsø 4, if not already included in the two groups above (n=2885). Subjects had to attend the 1st visit in order to be invited to the 2nd visit. The attendance rate to the 2nd visit was 92%. The Population Registry of Norway (by September 12th 2007) was the source for the invitations. The invitation file was created from census data from Statistics Norway, where all citizens in Norway have a unique national identity number given after birth or immigration. The file was periodically updated for mortality and emigration throughout the study period. The Regional Committee of Medical and Health Research Ethics and the Norwegian Data Protection Authority has approved the Tromsø Study (1).

Information on angina pectoris, myocardial infarction, stroke, smoking habits, diabetes, use of antihypertensive and lipid-lowering drugs was obtained from self-administered questionnaires. Blood pressure was recorded three times at one-minute intervals after two minutes of seated resting with the use of an automatic device (Dinamap ProCare 300 monitor, GE Healthcare) by specially trained technicians. The mean of the last two recordings was

used for analyses. Height and weight were measured to the first decimal in participants wearing light clothing and no footwear on an automatic electronic scale (Jenix DS 102 stadiometer). BMI was calculated as weight divided by the square of height (kg/m2).

Analyses of non-fasting serum total cholesterol and triglycerides were done within 10 hours by an enzymatic colorimetric method. HDL and LDL cholesterol were analyzed by homogeneous enzymatic colorimetric methods. All analyses were performed at the Department of Laboratory Medicine, University Hospital of North Norway.

High-resolution B-mode ultrasonography was performed with GE Vivid 7 duplex scanners with linear 12 MHz transducers. The ultrasonographers were blinded to laboratory and clinical data. Subjects were examined in the supine position with the head slightly tilted to the left side. The sonographers were instructed to view the arteries from all possible angles, in order to find the optimal view for visualization of plaque and IMT in each subject. No fixed angle of insonation was used. Measurements of plaque and IMT were anlayzed offline with the semi-automated AMS software. A plaque was defined as a localized protrusion into the vessel lumen of more than 50% thickening compared to the adjacent IMT. Six locations were scanned for the presence of plaques, the far and near walls of the right common carotid artery, bifurcation and internal carotid artery. ECG-triggered uptakes of IMT were obtained from the distal 10 mm segment of the far and near wall of the common carotid artery and of the proximal 10 mm segment of the far wall of the carotid bifurcation. Plaques were included in the IMT measurements if present in the predefined location of interest. The mean IMT from the 3 pre-selected images was calculated for each location, and the average of the mean IMT from the three locations was used in the analyses. The inter- and intra-observer and inter-equipment reproducibility of IMT and plaque measurements was acceptable (2-4).

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Supplement 3

			Age		BMI		Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)		Total cholesterol (mmol/L)		High density lipoprotein (mmol/L)		Low density lipoprotein (mmol/L)		Triglycerides (mmol/L)	
	Age groups	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Men	25-29	175	27.2	1.39	26.6	5.2	123.1	10.9	62.5	9.4	4.56	0.78	1.25	0.27	2.77	0.65	1.17	0.63
	30-34	182	32.1	1.40	27.1	4.3	122.3	12.2	66.5	9.8	5.00	0.97	1.23	0.28	3.15	0.82	1.35	1.07
	35-39	291	37.2	1.39	26.8	3.5	121.8	12.0	66.9	9.4	5.08	1.01	1.32	0.30	3.21	0.91	1.23	0.97
	40-44	457	42.2	1.44	28.0	4.4	124.5	13.9	71.7	10.4	5.32	0.92	1.30	0.31	3.41	0.81	1.38	0.87
	45-49	453	47.0	1.43	27.8	4.2	123.3	13.7	72.9	10.2	5.38	0.93	1.33	0.34	3.44	0.89	1.35	0.79
	50-54	453	52.0	1.46	28.5	4.1	125.9	13.9	75.1	10.3	5.39	1.01	1.31	0.34	3.44	0.91	1.45	0.85
	55-59	477	57.0	1.42	28.4	4.4	129.2	16.9	75.8	10.6	5.37	0.98	1.33	0.36	3.40	0.91	1.44	0.87
	60-64	469	61.9	1.39	28.8	4.4	131.8	17.2	75.9	10.4	5.22	1.05	1.35	0.34	3.26	0.97	1.37	0.73
	65-69	320	66.6	1.39	28.7	4.7	134.9	17.9	74.0	9.9	5.05	1.07	1.37	0.38	3.10	0.95	1.27	0.64
	Total	3277	49.8	11.2	28.1	4.4	126.8	15.5	72.6	10.8	5.23	1.00	1.32	0.34	3.30	0.91	1.36	0.83
	Total *		44.8		27.7	4.3	125.5	13.9	70.7	10.0	5.14	0.96	1.30	0.32	3.24	0.86	1.33	0.84
Women	25-29	173	26.8	1.45	24.7	4.7	109.7	10.9	62.3	8.4	4.47	0.86	1.59	0.38	2.49	0.77	0.86	0.38
	30-34	190	32.0	1.45	26.6	6.0	109.4	11.4	64.7	9.7	4.61	0.86	1.50	0.35	2.68	0.76	0.94	0.56
	35-39	302	37.2	1.39	26.2	5.3	109.0	11.2	66.3	8.9	4.63	0.79	1.57	0.38	2.65	0.75	0.90	0.56
	40-44	475	42.1	1.43	26.7	5.8	112.1	11.6	68.2	8.9	4.89	0.86	1.58	0.37	2.86	0.78	0.97	0.61
	45-49	460	47.0	1.41	27.0	5.2	115.0	13.8	70.2	9.2	5.18	0.94	1.64	0.40	3.10	0.87	0.98	0.52
	50-54	525	52.0	1.44	27.2	5.3	117.7	15.6	72.2	9.0	5.54	0.97	1.70	0.45	3.36	0.93	1.09	0.76
	55-59	476	57.0	1.45	27.1	5.0	121.8	17.7	72.9	9.4	5.64	0.97	1.66	0.43	3.46	0.90	1.14	0.62
	60-64	468	62.0	1.40	28.2	5.1	126.8	17.5	73.0	8.7	5.74	1.06	1.67	0.46	3.50	0.99	1.24	0.67
	65-69	315	66.5	1.40	27.8	5.3	129.0	17.2	72.3	8.7	5.73	0.99	1.74	0.44	3.44	0.91	1.22	0.60
	Total	3384	49.6	11.2	27.0	5.3	117.8	16.3	70.1	9.5	5.27	1.04	1.64	0.42	3.15	0.94	1.06	0.63
	Total *		44.9		26.7	5.3	115.5	13.7	68.7	9.0	5.09	0.92	1.62	0.40	3.01	0.84	1.02	0.58

* Adjusted according to population age structure in 2010

table 1 Characteristics of the REFINE-Reykjavik study by age and sex. Continuous parameters.

			Family History of Myocardial Infraction		History of Cardiovascular Disease		History of Coronary Heart Disease		Diabetes Type II		Statins		Hypertension		Medication for Hypertension	
	Age groups	N	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Men	25-29	175	14	8.2	0	0	0	0	0	0	0	0	17	9.7	1	0.6
	30-34	182	24	13.6	0	0	0	0	0	0	0	0	18	9.9	6	3.3
	35-39	291	57	20.4	0	0	0	0	2	0.7	0	0	27	9.3	10	3.4
	40-44	457	122	27.3	8	1.8	4	0.9	11	2.4	15	3.3	87	19.0	25	5.5
	45-49	453	155	35.0	6	1.4	4	0.9	19	4.2	16	3.5	112	24.7	68	15.0
	50-54	453	193	43.7	21	4.9	19	4.4	22	4.9	62	13.7	181	40.0	121	26.9
	55-59	477	193	42.4	39	8.4	35	7.5	48	10.1	84	17.7	231	48.4	170	35.9
	60-64	469	189	41.9	51	11.1	40	8.7	50	10.7	125	26.7	272	58.0	210	44.9
	65-69	320	125	40.1	72	22.9	64	20.4	46	14.4	108	33.8	228	71.3	182	57.1
	Total	3277	1072	33.8	197	6.2	166	5.2	198	6.0	410	12.5	1173	35.8	793	24.3
	Total *			33.8		6.2		5.2	198	4.3	410	8.8		28.3	793	17.9
Women	25-29	173	19	11.4	0	0	0	0	1	0.6	0	0	3	1.7	1	0.6
	30-34	190	30	16.0	0	0	0	0	4	2.1	0	0	14	7.4	10	5.3
	35-39	302	71	24.1	3	1.0	1	0.3	3	1.0	1	0.3	20	6.6	10	3.3
	40-44	475	134	28.9	7	1.6	7	1.6	8	1.7	1	0.2	51	10.7	38	8.0
	45-49	460	188	42.7	4	1.0	3	0.7	5	1.1	5	1.1	87	18.9	63	13.8
	50-54	525	247	48.7	8	1.6	5	1.0	14	2.7	24	4.6	152	29.0	122	23.2
	55-59	476	243	53.1	14	3.1	10	2.2	14	2.9	45	9.5	188	39.5	155	32.6
	60-64	468	219	49.1	24	5.5	18	4.1	24	5.1	59	12.6	262	56.0	219	46.8
	65-69	315	146	48.2	18	6.0	13	4.3	25	7.9	58	18.4	201	63.8	162	51.4
	Total	3384	1297	39.7	78	2.4	57	1.8	98	2.9	193	5.7	978	28.9	780	23.1
	Total *			39.7		1.8		1.3	98	2.4	193	4.0		22.2	780	17.4

*Adjusted according to population age structure in 2010

table 2 Characteristics of the REFINE-Reykjavik study by age and sex, categorical parameters.

		Smoking						BMI ≥25		BMI ≥30		Cholesterol ≥5mmol/L		Cholesterol ≥6.2 mmol/L		Physical activity ≥ 1 hours/week		Physical activity ≥ 4 hours/week	
		Never		Former		Current													
	Age groups	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Men	25-29	87	51.2	34	20.0	49	28.8	100	57.1	28	16.0	43	24.6	4	2.3	124	72.9	63	37.1
	30-34	84	47.2	49	27.5	45	25.3	121	66.5	34	18.7	82	45.1	15	8.2	116	64.8	63	35.2
	35-39	171	60.0	57	20.0	57	20.0	197	67.7	42	14.4	147	50.5	39	13.4	196	68.3	110	38.3
	40-44	196	43.3	151	33.3	106	23.4	357	78.1	110	24.1	282	61.7	73	16.0	287	63.6	163	36.1
	45-49	170	37.8	170	37.8	110	24.4	334	73.7	115	25.4	303	66.9	68	15.0	269	60.4	148	33.3
	50-54	160	35.6	196	43.7	93	20.7	370	81.7	147	32.4	291	64.2	89	19.6	262	59.4	153	34.7
	55-59	153	32.7	221	47.2	94	20.1	382	80.1	155	32.5	307	64.4	95	19.9	287	62.1	150	32.5
	60-64	137	29.6	236	51.0	90	19.4	378	80.6	159	33.9	269	57.4	77	16.4	257	56.5	134	29.5
	65-69	89	28.3	169	53.8	56	17.8	259	80.9	105	32.8	168	52.5	45	14.1	187	59.6	116	36.9
	Total	1247	38.6	1283	39.7	700	21.7	2498	76.2	894	27.2	1892	57.7	505	15.4	1985	62.0	1100	34.3
	Total*	1247	42.1	1283	35.2	700	22.7		73		24.5		53.5		13.5		63.7		35.0
Women	25-29	102	60.7	20	11.9	46	27.4	66	38.2	21	12.1	42	24.3	6	3.5	116	67.8	53	31.0
	30-34	98	51.6	52	27.4	39	20.6	97	51.1	40	21.1	59	31.1	5	2.6	121	65.1	51	27.4
	35-39	156	52.2	81	27.1	62	20.7	147	48.7	61	20.2	95	31.5	10	3.3	208	68.9	98	32.5
	40-44	210	44.7	165	35.1	95	20.2	256	53.9	108	22.7	202	42.5	37	7.8	341	73.0	180	38.5
	45-49	167	37.0	178	39.5	106	23.5	273	59.3	99	21.5	251	54.6	60	13.0	317	70.3	177	39.2
	50-54	191	36.7	218	41.9	111	21.3	313	59.6	136	25.9	372	70.9	124	23.6	358	69.9	194	37.9
	55-59	182	38.7	188	40.0	100	21.3	305	64.1	110	23.1	351	73.7	126	26.5	295	64.0	157	34.1
	60-64	157	34.1	209	45.4	94	20.4	339	72.4	143	30.6	347	74.1	141	30.1	292	64.2	163	35.8
	65-69	134	43.1	125	40.2	52	16.7	214	67.9	92	29.2	246	78.1	95	30.2	192	62.5	100	32.6
	Total	1397	41.9	1236	37.0	706	21.1	2010	59.4	810	23.9	1965	58.1	604	17.8	2240	67.6	1173	35.4
	Total*	1397	45.1	1236	33.2	706	21.7		55.8		22.2		50.6		13.9		67.7		34.3

*Adjusted according to population age structure in 2010

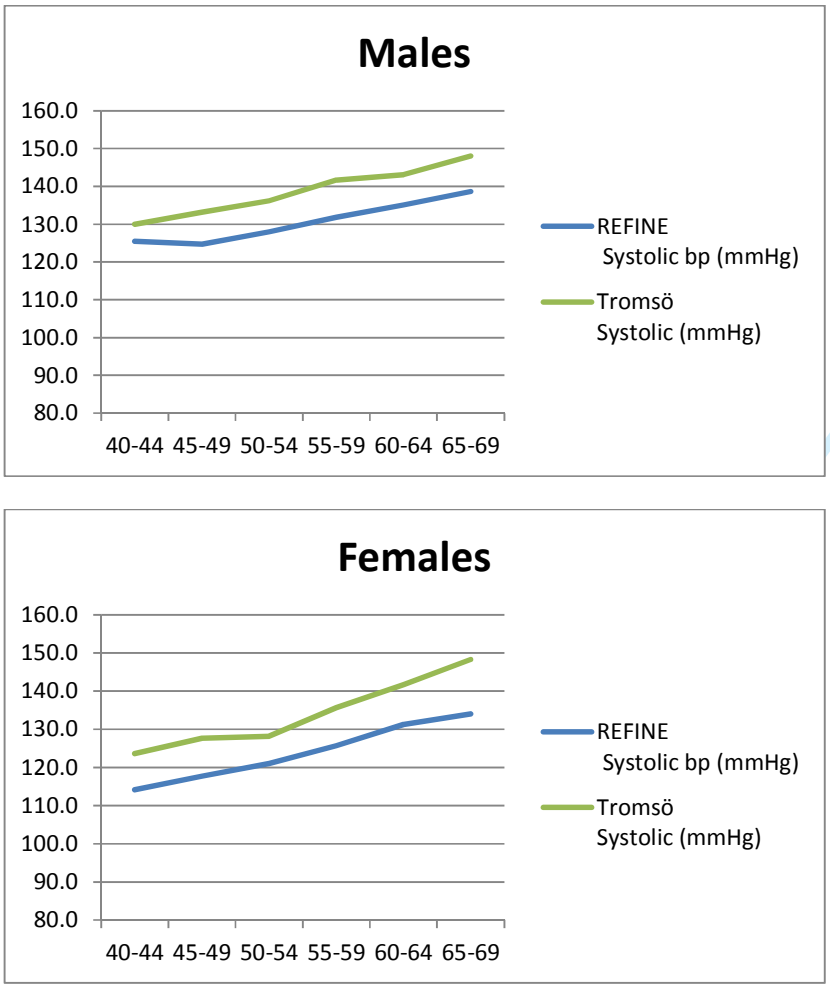
table 3 Characteristics of the REFINE-Reykjavik study by age and sex. Categorical parameters (continuous)

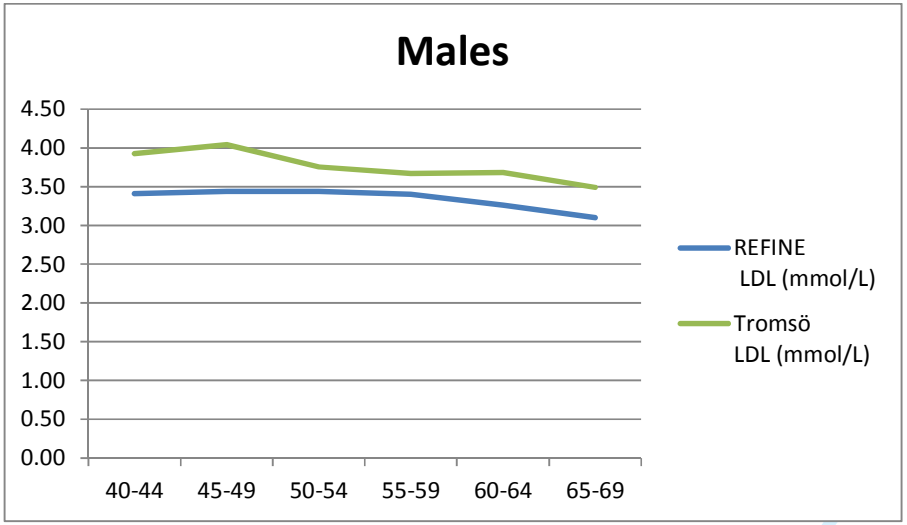
			Intima media thickness (mean)		Intima media thickness (max)		Moderate or more plaque in carotids		Plaque categories							
									None		Minimal		Moderate		Severe or more	
	Age groups	N	Mean	SD	Mean	SD	N	%	N	%	N	%	N	%	N	%
Men	25-29	175	0.57	0.07	0.67	0.08	0	0.0	163	94.2	10	5.8	0	0.0	0	0.0
	30-34	182	0.60	0.07	0.71	0.09	2	1.1	157	86.3	23	12.6	2	1.1	0	0.0
	35-39	291	0.63	0.09	0.74	0.10	2	0.7	257	88.3	32	11.0	2	0.7	0	0.0
	40-44	457	0.68	0.08	0.80	0.10	13	2.9	310	68.0	133	29.2	13	2.9	0	0.0
	45-49	453	0.72	0.09	0.85	0.11	21	4.7	260	57.6	170	37.7	20	4.4	1	0.2
	50-54	453	0.78	0.11	0.91	0.12	36	7.9	194	42.8	223	49.2	35	7.7	1	0.2
	55-59	477	0.84	0.12	0.98	0.14	87	18.3	141	29.6	248	52.1	80	16.8	7	1.5
	60-64	469	0.87	0.12	1.01	0.15	121	25.8	107	22.8	241	51.4	107	22.8	14	3.0
	65-69	320	0.91	0.13	1.06	0.15	107	33.4	50	15.6	163	50.9	91	28.4	16	5.0
	Total	3277	0.76	0.15	0.89	0.17	389	11.9	1639	50.1	1243	38.0	350	10.7	39	1.2
	Total *		0.71	0.10	0.83	0.11		8.3		60.9		30.8		7.6		0.8
Women	25-29	173	0.53	0.05	0.62	0.06	0	0.0	167	96.5	6	3.5	0	0.0	0	0.0
	30-34	190	0.57	0.06	0.67	0.08	1	0.5	176	92.6	13	6.8	1	0.5	0	0.0
	35-39	302	0.60	0.07	0.71	0.08	2	0.7	268	88.7	32	10.6	2	0.7	0	0.0
	40-44	475	0.64	0.07	0.75	0.08	7	1.5	365	76.8	103	21.7	7	1.5	0	0.0
	45-49	460	0.68	0.08	0.80	0.10	18	3.9	308	67.1	133	29.0	17	3.7	1	0.2
	50-54	525	0.74	0.09	0.86	0.11	29	5.5	292	55.7	203	38.7	28	5.3	1	0.2
	55-59	476	0.78	0.10	0.90	0.12	53	11.2	216	45.5	206	43.4	49	10.3	4	0.8
	60-64	468	0.83	0.11	0.96	0.13	82	17.5	157	33.5	229	48.9	70	15.0	12	2.6
	65-69	315	0.85	0.11	0.98	0.12	85	27.0	68	21.6	162	51.4	70	22.2	15	4.8
	Total	3384	0.71	0.13	0.83	0.15	277	8.2	2017	59.7	1087	32.2	244	7.2	33	1.0
	Total *		0.67	0.08	0.79	0.09		5.9		68.5		25.6		5.3		0.7

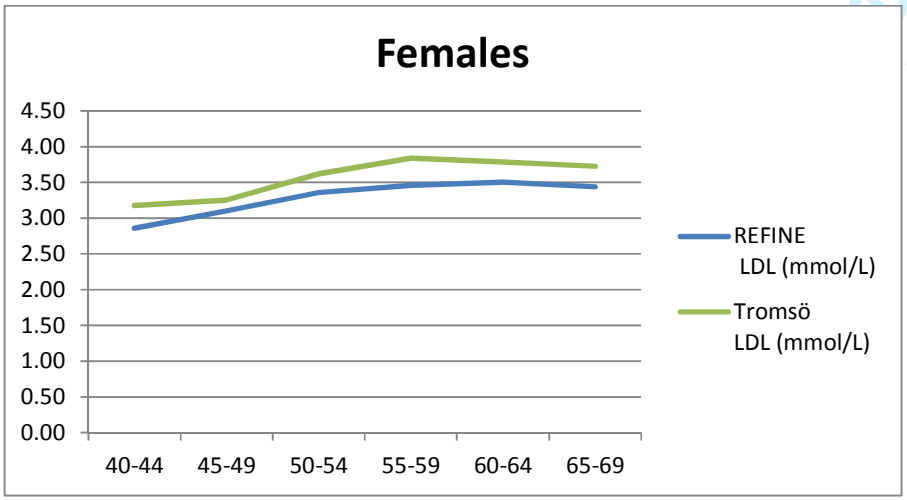
*Adjusted according to population age structure in 2010

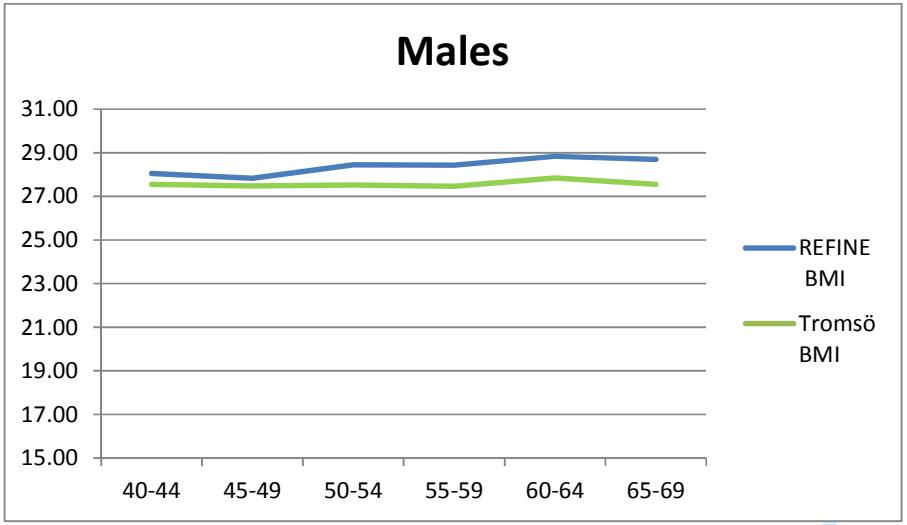
table 4. Characteristics of the REFINE-Reykjavik study by age and sex, image analysis.

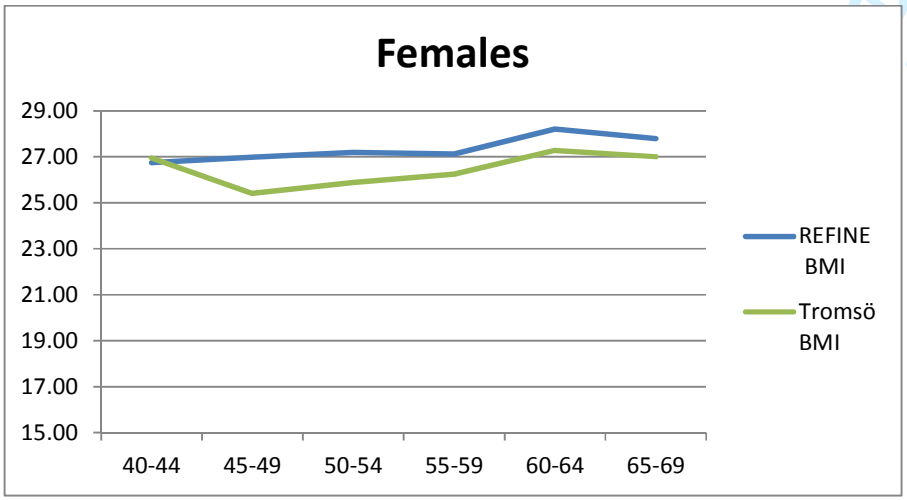
Supplement 4 figure 1. **Distribution of cardiovascular risk factors in the REFINE-Reykjavik study and Tromsø 6 study by age and sex.**

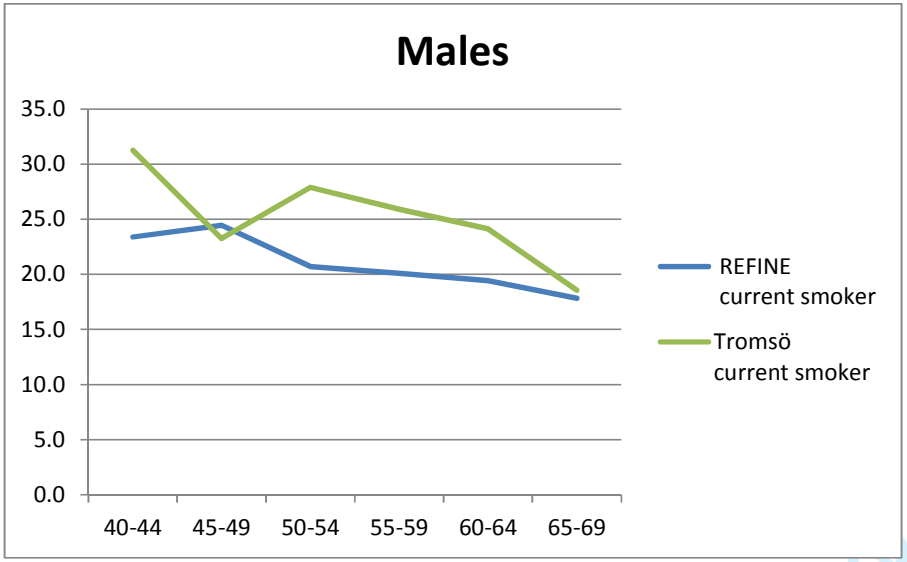


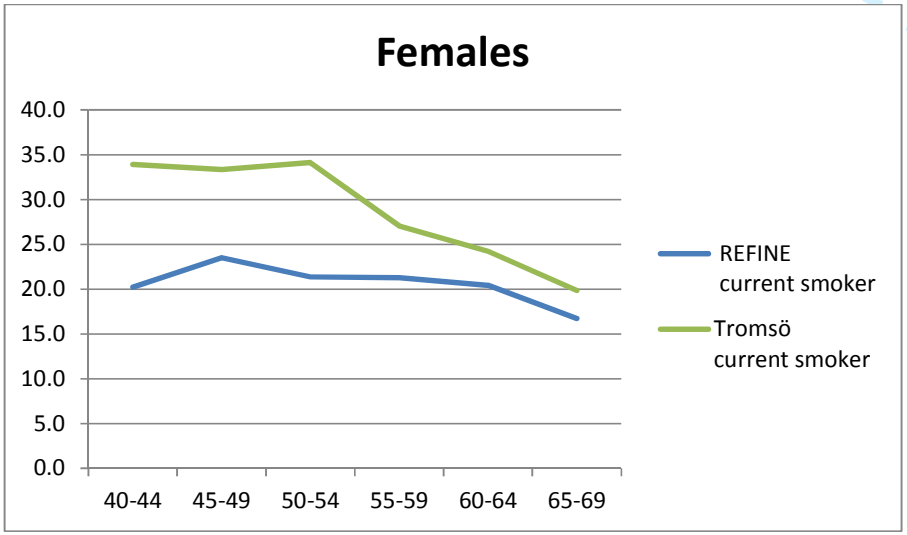


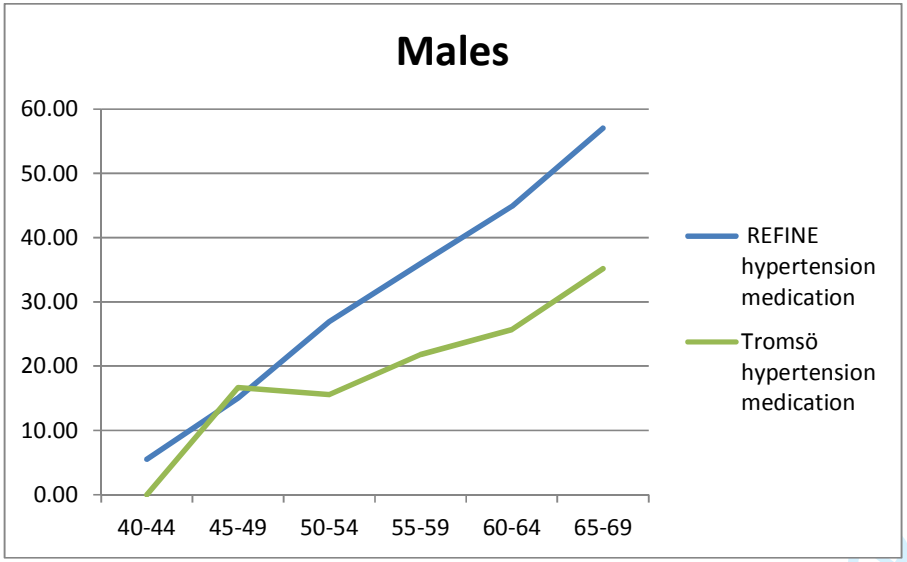


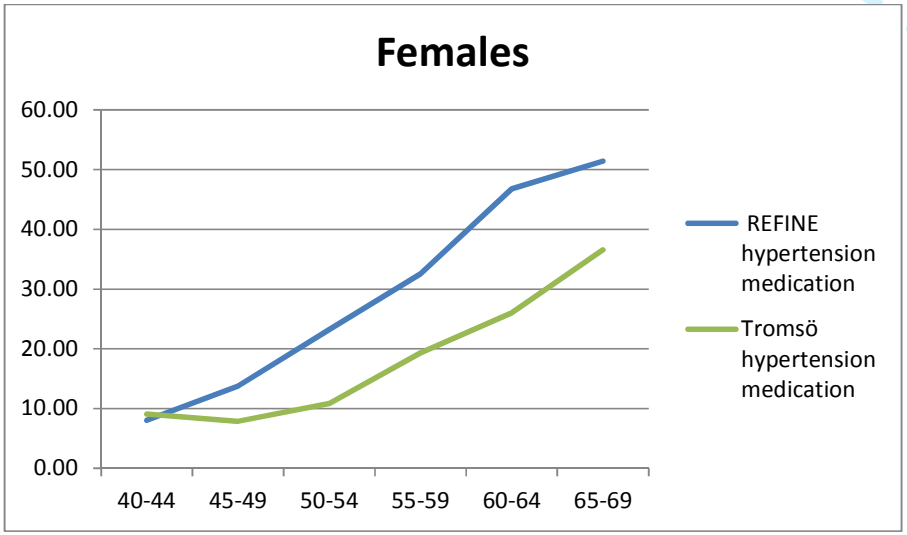


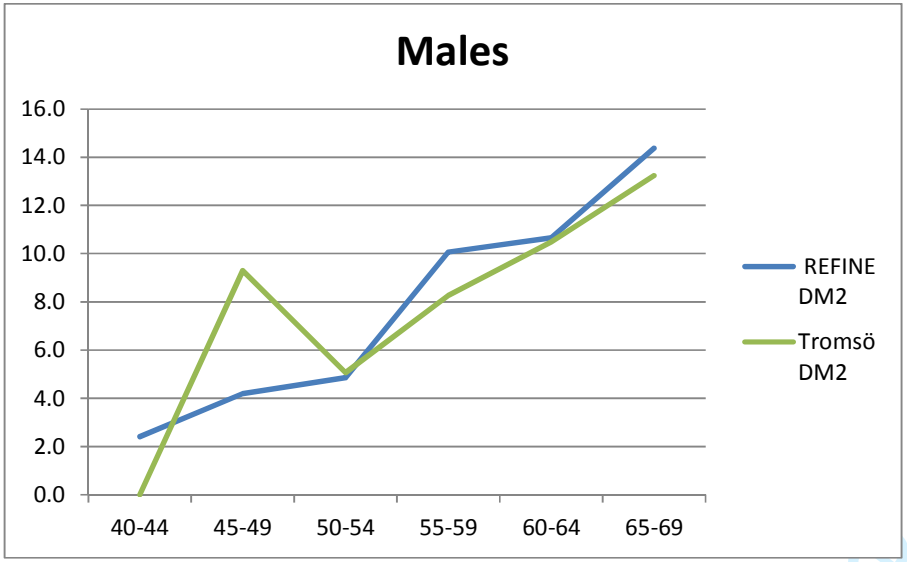


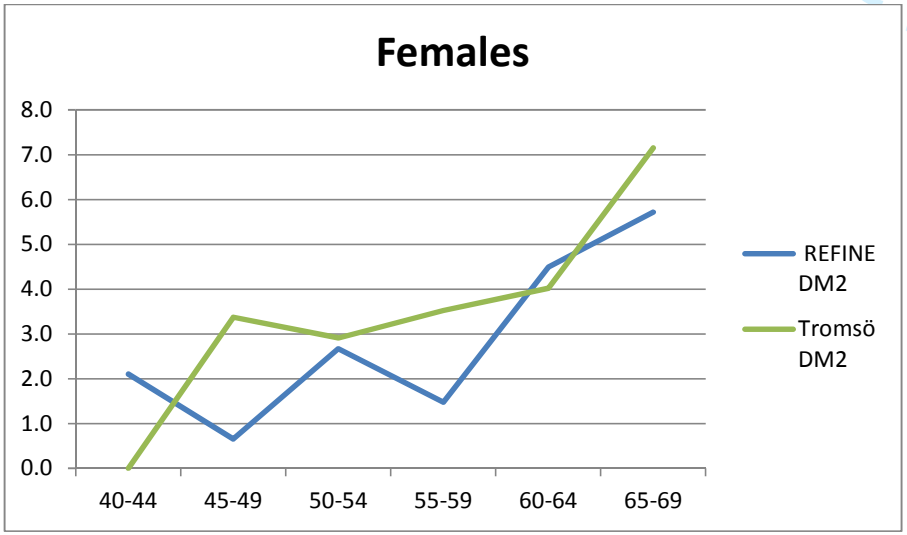












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Population distribution of traditional and the emerging cardiovascular risk factors carotid plaque and IMT: The REFINE-Reykjavik study with comparison to the Tromsø Study

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Abstract

Objectives: Population statistics for carotid plaque and cardiovascular risk factors reported in scientific journals are usually presented as averages for the population or age and sex adjusted, rather than sex and age-groups. Important population differences about atherosclerosis and cardiovascular risk factors may thus be missed. We compare the distribution of cardiovascular risk factors, carotids plaque and IMT in two population based studies.

Methods: Carotid artery atherosclerotic plaque prevalence and risk factors levels for cardiovascular disease by sex in 5-year age-groups from the REFINE-Reykjavik study were compared with data from the Tromsø 6 study.

Results: The threshold of carotid plaque presence in the Tromsø 6 study fell between minimal and moderate plaque defined in the REFINE-Reykjavik study reflecting carotid plaque prevalence. The prevalence of minimal carotid plaque in the REFINE-Reykjavik study was 47% in men (40-69 years old) and 38% in women and 11% in men and 7% in women of moderate plaque. The prevalence of any plaque in the Tromsø 6 study was 35% in men and 27% in women. The mean CIMT was similar in the studies. In the Tromsø 6 study mean systolic blood pressure was 8 mmHg higher in men and 10 mmHg higher in women, mean LDL was 0.5 mmol/L higher in men and 0.3mmol/L higher in women and the prevalence of smoking was 4% higher in men and 9% higher in women. On the other hand, body mass index (BMI) was 0.8 kg/m2 higher in men and 0.9 kg/m2 in women in the REFINE-Reykjavik study.

Conclusion: Comparison between Iceland and Norway revealed differences in the prevalence of carotid plaque, which was assumed to be due to different definition of plaque. However, clinically significant differences in conventional cardiovascular risk factors were seen. This underscores the importance of detailed comparison of population data across different populations.

Strengths and limitations of this study:

- The strength of this study is the random population design of both the REFINE-Reykjavik study and the Tromsø 6 study

- The rigid protocols of the two studies regarding data gathering and quality control and that the studies were conducted at similar time interval is also strength.
- The main limitation of the study is some difference in carotid ultrasound protocols between the REFINE-Reykjavik study and Tromsø 6 study the study and it was done on only Caucasian participants.

Introduction

The value of comparing risk factors of cardiovascular disease between populations is undisputed. The Seven Countries Study and the World Health Organization led MONICA study are examples of studies that have monitored changes in risk factors and compared diets and lifestyles between countries. These studies contributed to knowledge, which led to changes in risk factor levels and the drop seen in the prevalence of coronary heart disease in the last decades of the 20th century (1, 2). However, detailed information on the presence of atherosclerotic plaque in the carotid arteries across different populations is not readily available in the current literature. Population statistics for carotid plaque and for cardiovascular risk factors reported in scientific journals are usually presented as an average for the population or adjusted for age and sex, rather than being reported by different sex and age groups. Thereby significant sex and age interaction in the development in atherosclerosis and/or in cardiovascular risk factors can be missed in comparison across different populations based on published data. For carotid plaque, which is one of the best-studied markers of subclinical atherosclerosis, different definitions of carotid plaque between studies also complicate the comparison.

We now publish results from the first phase of the Risk Evaluation For Infarct Estimates Reykjavik study (REFINE-Reykjavik study) started in December 2005 and completed in March 2011. The REFINE-Reykjavik study is a prospective cohort study on risk factors and aetiology of atherosclerotic disease in the population of the Reykjavik area in Iceland. The study was performed on a large number (6661) of individuals (25-69 years of age) with ultrasound of the carotids and other measurements of both traditional risk factors and new risk factors for cardiovascular disease. The aim of REFINE-Reykjavik study was to demonstrate what characterizes individuals who develop atherosclerosis and to understand if carotid plaque or other factors measured in the study increase the accuracy of risk estimates for cardiovascular disease.

The population distribution of cardiovascular risk factors and the prevalence of atherosclerotic plaque in the common carotid arteries are presented in adults living in the greater Reykjavik area according to age groups and sex. We report the data in this detailed manner in order to make comparisons with other studies easier and more accessible. We also make direct comparison between results from the REFINE-Reykjavik study and the Tromsø 6 study and discuss the results in context with available outcomes from other population studies in Europe and USA. The two population studies, the REFINE-Reykjavik study and the Tromsø 6 study, were conducted within the similar time interval (REFINE-Reykjavik study 2006-2011, Tromsø 6 2007-2008), included both genders and both included middle age participants, mostly of Scandinavian origin and are therefore highly comparable.

Methods

Study population

The cohort in the first phase of the REFINE-Reykjavik study was a random sample of 9.480 men and women born in 1935-1985, living in Reykjavik in November in the year 2005 and with Icelandic citizenship. The cohort was divided into five year age groups from 25 to 69 years. The age distribution was designed to over-represent middle-aged individuals in order to concentrate the power of the study on the age span where development of atherosclerosis was to be most expected. In the age groups 25 to 34 the number of individuals in each age group was 600, in the age groups from 35 to 64 the number was 1200 in each group and in the age group 65-69 the number of individuals was 480. The cohort in the REFINE-Reykjavik study was drawn from the same geographic area as the well-established Reykjavik study. The cohort in the Reykjavik-study included individuals born in 1907-1935(3). The birth year bracket in the REFINE-Reykjavik study (1935-1985) is therefore in continuation of the Reykjavik-study.

The cohort in REFINE-Reykjavik study is homogenous with the vast majority being of Scandinavian origin. Icelanders are genetically similar to other northern European countries (4) and risk of coronary heart disease and the contribution of the conventional risk factors to this risk is similar (5). In the final survey of the WHO

MONICA Project conducted in 1992, of the 38 population investigated for coronary event rate in men, the Icelandic population was approximately in the middle. Twenty populations had higher coronary event rate and 17 populations had lower coronary event rate than the Icelandic population. (6) For comparison of both conventional risk factors for coronary heart disease, prevalence of carotid plaque and the level of CIMT in the population, data from Tromsø 6 study were used.

The Tromsø Study is an ongoing population-based cohort study in the municipality of Tromsø, Northern Norway, with a population of 72 000 inhabitants. The Tromsø 6 study was conducted in the years 2007–2008. The age span was 40-87 years. Invited to Tromsø 6 1st visit were all residents aged 40-42 and 60-87 (n=12,578), a 10% random sample of individuals aged 30-39 (n=1056), a 40% random sample of individuals aged 43-59 (n= 5787), and subjects who had attended the second visit of Tromsø 4, if not already included in the three groups above (n=341). The attendance rate was 66%.

Detailed description on recruitment methods, use of medication and supplements, clinic examination, blood analyses, quality control of the ultrasound of the carotid arteries in the REFINE-Reykjavik study in supplementary text 1 and description of Tromsø 6 in supplementary text 2

Ultrasound of the carotid arteries

In the REFINE-Reykjavik study the Ultrasound of the carotid arteries was performed using a standardized scanning and analysis protocol for quantitative assessment of the common carotid intima-media thickness (IMT) and arterial stiffness. The protocol also included scans for semi-quantitative assessment of plaque presence/absence and plaque severity. The protocol was developed by experts from the Vascular Imaging Center, Julius Center for Health Care and Primary Care in the University of Utrecht in the Netherlands (MLB).

The technicians that performed the ultrasound studies were trained by the same experts that developed the protocol.

The carotid arteries on both sides were imaged from 4 different interrogation angles with 30 degrees increments using a Sequoia C256, Acuson ultrasound system (Siemens Medical Systems, Erlangen, Germany) with an 8.0 MHz transducer. To standardize and control the interrogation angles, the Meijers Carotid Arc was used (7). The IMT measurements were quantified on a predefined segment in near and far wall of the carotid common arteries (8) using the Artery Measurement Software (AMS) II v1.131.

Outcome parameters:

1. Common carotid intima-media thickness: B-mode images of the IMT are acquired for the predefined 10mm segment of each common carotid artery (8) (right and left) at defined interrogation angles using Meijers Arc. Standard images are obtained from 4 angles at each site. The mean intima-media thickness (IMT) of the near (shallower) and far (deeper) walls are determined from a single image at each interrogation angle for both the right and left common carotid arteries CCA. The average of all these IMT values comprised the mean IMT outcome parameter. The maximum IMT corresponded to the highest measured IMT value at the 4 angles.

2. Atherosclerotic plaque in the carotid bifurcation and internal carotid artery: Of the left and right carotid bifurcation and internal carotid artery the presence of atherosclerotic lesions is measured on line, i.e., during the ultrasound examination. The most severe lesion per segment is assessed in a semi-quantitative manner. The plaque image interpretation is based on the following 4 categories:

1. None: Complete absence of plaque, IMT thickening may be observed.

2. Minimal: small isolated thickening, uni- or multi focal, often with calcification approximately 2 times the adjacent normal IMT.

3. Moderate: clear, reasonably easy to visualize plaque with or without calcification. May be located on both near and far wall in the segment causing some diameter reduction.

4. Severe: Significant plaque formation very easy to image with or without calcifications and visualized on several different scan projections in near and far wall causing clear diameter reduction.

Images of observed plaques were stored.

In the Tromsø 6 study, high-resolution B-mode ultrasonography was performed with GE Vivid 7 duplex scanners with linear 12 MHz transducers. The ultrasonographers were blinded to laboratory and clinical data. Subjects were examined in the supine position with the head slightly tilted to the left side. The sonographers were instructed to view the arteries from all possible angles, in order to find the optimal view for visualization of plaque and IMT in each subject. No fixed angle of insonation was used. Measurements of plaque and IMT were anlayzed offline with the semi-automated AMS software. A plaque was defined as a localized protrusion into the vessel lumen of more than 50% thickening compared to the adjacent IMT. Six locations were scanned for the presence of plaques, the far and near walls of the right common carotid artery, bifurcation and internal carotid artery. ECG-triggered uptakes of IMT were obtained from the distal 10 mm segment of the far and near wall of the common carotid artery and of the proximal 10 mm segment of the far wall of the carotid bifurcation. Plaques were included in the IMT measurements if present in the predefined location of interest. The mean IMT from the 3 pre-selected images was calculated for each location, and the average of the mean IMT from the three locations was used in the analyses. The inter- and intra-observer and inter-equipment reproducibility of IMT and plaque measurements was acceptable (9-11).

Statistical methods

Age standardized means and proportions were presented and compared between the REFINE-Reykjavik and the Tromsø 6 study. The following risk factors were investigated; systolic blood pressure, low density lipoprotein, body mass index, prevalence of type 2 diabetes, cholesterol lowering medications (statins), hypertension medication, smoking, self-reported history of coronary heart disease, intima media thickness and plaque in the right carotids.

Age standardization was done according to the direct method, using the standard population age structure as defined by the European Standard Population (ESP) (12). Statistical significance between study summary

estimates was investigated using linear regression for continuous variables and logistic regression for categorical variables. Regressions were run separately for each sex and adjusted for age.

Intima media thickness and prevalence of plaque in right carotids was presented visually by sex, age groups and study. Blood pressure measurements in the REFINE-Reykjavik study were done using arterial tonometry (13) whereas an electronic sphygmomanometer (Dinamap ProCare 300 monitor, GE Healthcare) was used in Tromsø 6 study. A set of approximately 400 available and concurrently measured sphygmomanometer readings in REFINE-Reykjavik study were compared to tonometry measurements using a linear mixed effects model, accounting for an inherent repeated measures aspect. Age-,sex- and method-specific predicted values were used to obtain a correction factor which was applied to the tonometry measurements in REFINE, in an effort to make them comparable to the Tromsø-6 study measurements.

Statistical analysis was done using Stata 14.1(14).

Results

Recruitment for the REFINE-Reykjavik study started in December 2005 and was completed in March 2011. The total number of individuals who participated in the study was 6661, 3277 men and 3384 women. The recruitment rate was 73%. The sex ratio was 49% men and 51% women. The mean age was 49.8 years (SD 11.2 years) and the age range was 25-69 years.

The mean BMI for men was 27.7 (SD 4.3) kg/m2 and 26.7 (SD 5.3) kg/m2 for women. Mean BMI was above 25kg/m2 in both sexes, which is the upper limit of ideal weight according to WHO expert committee report (15). BMI increased with increasing age (Supplement 3 table 1a).

Both systolic and diastolic blood pressure rose with age but there was a decrease in the diastolic blood pressure in the oldest age group (65-69 year old) in both sexes. Average systolic blood pressure in men was 125.5 mmHg (SD 13.9) and 115.5 mmHg (SD 13.7) for women and average diastolic blood pressure was 70.7 mmHg (SD10.0) and 68.7 mmHg (SD 9.0) respectively. (Supplement 3 table 1a)

A steady increase in total cholesterol (TC), low density lipoprotein (LDL) and triglycerides and was observed in women with increasing age. In men, TC, LDL and TG peaked in middle aged, decreasing again over the age of 60. HDL cholesterol increased with age in both sexes. (Supplement 3 table 1b)

Family history of myocardial infarction increased with age and was somewhat higher in men than women (Supplement 3 table 2a). History of cardiovascular disease and history of coronary heart disease was rare in participants younger than 50 years old but increased sharply with age in men and it was 22.9% and 20.4% respectively in 65-69 years old men (Supplement 3 table 2a). The increase was more gradually in women, history of cardiovascular disease and history of coronary heart disease was 6.0% and 4.3 % respectively in 65-69 years old women (Supplement 3 table 2a).

The prevalence of diabetes type 2 in men on average was 6% and age adjusted 4.3%. The prevalence is lower in women or 3% and age adjusted 2.4%. The prevalence of diabetes increased with age in both sexes. (Supplement 3 table 2b).

Hypertension was rare amongst young women (25-29 years) but 10% of young men had hypertension. With increasing age the prevalence of hypertension increased sharply so that in the oldest age group (65-69 years) the majority of men (71%) and more than half of the women were hypertensive (Supplement 3 table 2b).

Use of blood pressure lowering drugs and cholesterol lowing drugs (statins) is shown in Supplement 3 table 2b. Drug treatment increased with age and was highest in the oldest age group. In the age group 65-69, 57.1% men and 51.4 % women were on treatment against high blood pressure and 33.8% of men and 18.4% of women were treated with statins (Supplement 3 table 2b).

Current smoking was highest in the age group 25-29 years in both men (28.8 %) and women (27.4 %). The prevalence of current smoking decreases with increasing age. On average, 22% of men and 21% of women smoked (Supplement 3 table 3a).

Overweight, or BMI ≥25 was very common in man (73 %) and in women (56%) and obesity, or BMI ≥30 was seen in 25% of men and 22% of women (Supplement 3 table 3a) More than half of men and women had cholesterol levels above 5mmol/L and 14% of men and women had cholesterol levels above 6.5 mmol/L (Supplement 3 table 3b).

Physical activity was assessed by the following question in the health history questionnaire: “In the past 12 months, how often did you participate in moderate or vigorous physical activity (Examples of moderate or vigorous physical activity include badminton. golf (walking), biking, swimming, heavy gardening, weight lifting, hiking/ mountain climbing, fast walking/fast dancing/heavy housework, rowing, aerobics, jogging and running)” About 60-70% of men and women participated in at least moderate physical activity for 1-3 hours a week and 30-40% were active 4-7 hours a week. No clear difference in physical activity was seen between men and women or different age groups (Supplement 3 table 3b).

In supplement 3 table 4a mean common CIMT values are shown according to age and sex. The mean CIMT was 0.71mm (SD 0.10) in men and 0.67 mm (SD 0.08) in women. CIMT increased steadily with increasing age in both sexes, and was slightly higher in men than in women. For example, in the oldest age group (65-69 years) the mean CIMT was 0.91mm (SD 1.3) in men but 0.85 (SD 0.11) mm in women. Results from the maximum IMT thickness are also shown in supplement 3 table 4a. Maximum IMT values increased similarly with age and the sex difference was similar.

The prevalence of carotid plaque increased with age in both sexes (Supplement 3 table 4b). The prevalence was somewhat higher in men than women at all ages although the sex difference was small. For example 7.7% of 50-54 years old men had moderate plaque compared to 5.3% of women. One third of men in the oldest age group (65-69 years) had moderate or more carotid plaque but 27% of women. Severe plaque or semi occlusion was never detected in the younger participants but was detected in 4.4% and 4.8% in the oldest women and men respectively. In the youngest age group (25-29 years), 94.2% of men and 96.5% of the youngest women had no plaques, while this was seen in only 15.6% of the oldest men (65-69 years) and 21.6% of the oldest women

Table 1 shows the characteristics of the REFINE-Reykjavik study and the Tromsø 6 study in 40-69 years old men and women.

Table 1. Age standardized characteristic of participants in the REFINE-Reykjavik study and the Tromsø 6 study ¶.

	Men 40-69 years			Women 40-69 years		
	REFINE-Reykjavik (n=2629)	Tromsø 6 (n= 2214)	Difference	REFINE-Reykjavik (n=2719)	Tromsø 6 (n=2981)	Difference
Mean crude age (years) (SD)	54 (8.2)	59 (5.7)	-5	54 (8.2)	59 (6.1)	-5
Systolic BP∞ (mmHg) (SD)	130 (15.5)	138 (17.7)	-8**	123 (15.5)	133 (21.4)	-10**
LDL (mmol/L) (SD)	3.3 (0.9)	3.8 (0.9)	-0.5**	3.3 (0.9)	3.6 (0.9)	-0.3**
BMI (kg/m2) (SD)	28.4 (4.4)	27.6 (3.7)	0.8**	27.3 (5.3)	26.4 (4.7)	0.9**
CIMT¥ mean far wall (mm)(SD)	0.77 (0.12)	0.79 (0.15)	-0.02*	0.72 (0.10)	0.73 (0.12)	-0.01
Current smoker % (number)	21 (549)	25 (537)	-4**	20 (558)	29 (777)	-9**
HTMEDψ users % (number)	30 (776)	18 (532)	12**	28 (759)	17 (690)	11**
Statin users (%) (number)	16 (410)	13 (401)	3*	7 (192)	9 (425)	-2*
Known heart attack and/or angina ł (%) (number)	8.4 (218)	7.7 (212)	0.7*	3.4 (93)	2.5 (119)	0.9
Type 2 diabetes (%) (number)	7.4 (196)	7.4 (204)	0.0	3.4 (90)	4.9 (186)	-1.5**
Plaque R- carotid: REF min , Tromsø-any (%) (number)	46.8 (1238)	35.3 (997)	11.5**	38.3 (1039)	26.6 (1042)	11.7**

¶ Values are mean (standard deviation) or percentage (number). Age standardized according the European Standard Population 2013

*p-value <0.05, **p-value <0.001, ∞ The REFNIE-Reykjavik study blood pressure measurement were adjusted for difference between measurements from arterial tonometry and sphygmomanometer measurements (see methods), ¥ Common carotid intima media thickness, ψ hypertensive medication, ł according to health questionnaire

The systolic blood pressure was 8 mmHg higher in men and 10 mmHg higher in women in the Tromsø 6 study than the REFINE- Reykjavik study. Participants in REFINE-Reykjavik study were more often taking antihypertensive medication than in the Tromsø 6 study (30% vs 18% in man and 28vs17% in women). Calculated LDL-cholesterol was somewhat higher in both men and women in the Tromsø 6 study than in

REFINE- Reykjavik study (0.3mmol/L). The prevalence of statin use was similar in the two studies although somewhat more men were taking statins in REFINE-Reykjavik study than Tromsø 6 study (15% vs 13%) but less women (7.2% vs 9.4%). Smoking was less prevalent in REFINE-Reykjavik study than the Tromsø-6 study in both sexes but BMI was nearly one unit higher in the REFINE-Reykjavik study in both sexes. The prevalence of type2 diabetes was similar in men in both studies (7.4%) but was lower in women in the REFINE-Reykjavik study (3.9% vs 4.9%). The mean farwall CIMT in men was 0.02 mm thicker in the Tromsø 6 study than the REFINE-Reykjavik study (p-value < 0.05) and 0.01 mm thicker in women (not significant) (Table 1). The prevalence of minimal right site carotid plaque or more was higher in the REFINE-Reykjavik study than any right site plaque or more, in the Tromsø-6 study in both men and women. The difference in in cardiovascular risk factors between the studies was similar in both sexes and across age group as is shown in Supplement 4 figure1.The mean farwall CIMT increased with age as can be seen in Fig 1. The CIMT was higher in the age group 40-49 in the Tromsø-6 study but was similar in the two studies after the age of fifty in both men and women (Fig1).

As shown in Fig 2 the prevalence curve for any right carotid plaques in the Tromsø 6 study lies between the prevalence curve for minimal or more carotid plaque and the prevalence curve for moderate or more carotid plaque in the REFINE-Reykjavik study (Fig2).

Discussion

In this paper we present the average prevalence of atherosclerotic plaque in the carotid arteries and average of cardiovascular risk factors in adult population of Reykjavik area in Iceland in the REFINE-Reykjavik study. We put the results in context with the results from the Tromsø 6 study. The main findings are that the evidence of manifest atherosclerosis. i.e. the prevalence of carotid plaques is similar in the two studies. Systolic blood pressure and LDL-cholesterol levels were higher in the Tromsø 6 study but the mean BMI was higher in the REFINE-Reykjavik study. The main limitation of the study is the methodological difference in the definition of atherosclerotic plaque in the carotids arteries as further discussed below.

The two population studies, the REFINE-Reykjavik study and the Tromsø 6 study, were conducted within the similar time interval (REFINE-Reykjavik study 2006-2011, Tromsø 6 2007-2008), included both genders and both included middle age participants, mostly of Scandinavian origin and are therefore highly comparable. The

prevalence curve for any carotid plaque by age and sex in the Tromsø 6 study lies in between the prevalence of minimal plaque and moderate plaque in the REFINE-Reykjavik study for both men and women. This can be seen in all age groups and the increase with age is similar. The difference in prevalence of carotid plaque in the two studies is most likely due to a different definition of carotid plaque. In the REFINE-Reykjavik study, minimal plaque was defined as a small isolated thickening uni- or multifocal, often with calcification approximately 2 times the adjacent normal CIMT. A moderate plaque was defined as a clear, reasonably easily visualized plaque with or without calcifications that may be located on both near and far wall in the segment causing some diameter reduction. The definition of plaque presence in the Tromsø 6 study was of a "… localized protrusion of the vessel wall into the lumen" (16). Focal calcifications without focal thickening or protrusion into the lumen were not regarded as atherosclerotic plaque in the Tromsø 6 study (16). Since both studies show similar increase in plaque prevalence with increasing age and the threshold for definition of carotid plaque in the Tromsø 6 study seems to lie in between the definition for minimal and moderate plaque in the REFINE-Reykjavik study, we assume that the differences in plaque prevalence are mainly due to different definitions of plaque although difference in prevalence of plaque cannot be excluded.

Comparison of the mean farwall CIMT between the REFINE-Reykjavik study and the Tromsø 6 study reviled close similarity between the two studies after the age of fifty. The mean CIMT was higher in the Tromsø 6 study in participants under the age of fifty than in the REFINE-Reykjavik study. However, the number of participants in this age group in the Tromsø 6 was relatively smaller compared to older age groups, and the confidence intervals for the CIMT measurements wider. We therefore concluded that the mean farwall CIMT was similar in the REFINE-Reykjavik study and the Tromsø 6.

It is clear that the need for a standardized definition of plaque and CIMT is important both for clinical practice, in order to increase the availability of ultrasound laboratories that can perform high quality carotid plaque and CIMT evaluation, and to increase comparability between future studies. Both in Europe and the USA attempts have been made in that regard. In 2008 the American Society of Echocardiography Carotid Intima-Media Thickness Task Force published a consensus statement (17). There, carotid plaque was defined as "the presence of focal wall thickening that is at least 50% greater than that of the surrounding vessel wall or as a focal region with CIMT greater than 1.5 mm that protrudes into the lumen that is distinct from the adjacent boundary "(17). In 2012 the Mannheim carotid Intima-media thickness and plaque consensus (2004-2006-2011) was published where carotid plaque was "defined as a focal structure that encroaches into the arterial lumen of at least 0.5 mm

or 50% of the surrounding IMT value or demonstrates a thickness> 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface" (18). These two consensus statements give very similar definitions of plaque and will hopefully reduce confusion that different definitions can cause.

In all age groups, except for 25-29 year old women, the mean BMI value was over 25 kg/m2 the upper limit of normal weight according to WHO definition (19) in the REFINE-Reykjavik study. The mean BMI value for women was 26.7 and for men 28.7 kg/m2. More than a third of men over the age of 50 were obese according to the WHO definition. In the OECD report " Obesity update 2017" it is stated that on average one in five adults over 15 years of age is obese in the OECD countries. Iceland is near the OECD average in the same report.(20) We have previously analyzed the trend in BMI in Iceland. According to the Icelandic Heart Association study the mean BMI increased by 2 units in both genders (45-64 year old) from 1967 to 2007 (21) . However, in the OECD report it is revealed that obesity has stabilized in England, Italy, Korea and Spain(20). Comparison with the Tromsø 6 study shows, that the mean BMI was 0.9 units kg/m2 higher in women and 0.8 kg/m2 higher in men in the REFINE-Reykjavik study than in the Tromsø 6 study.

Almost a third of men aged 25-29 years smoked in the REFINE-Reykjavik study. This was somewhat lower than the average prevalence of smoking in developed countries according to a large international survey (22), where about 38% of men in this age group smoked in 2012. Smoking decreased with age and was down to 17% in the 65-69 year old group in the REFINE-Reykjavik study. Comparison between the REFINE-Reykjavik study and the Tromsø 6 study showed that smoking was somewhat more prevalent in both men and women in the Tromsø 6 study. In men the difference was 4% (21% REFINE-Reykjavik vs 25% Tromsø 6) and 9% in women (20% REFINE-Reykjavik vs 29% in Tromsø 6). Prevalence of women smokers in the REFINE-Reykjavik was similar to the prevalence of smoking amongst men in the same age groups (21%). This was similar as was seen in the Tromsö 6 study where smoking was even more prevalent amongst women (29.2%) than amongst men (25.4%). This is different from what was seen in many other developed countries where smoking amongst women is approximately half of the prevalence of smoking in men (22).

Blood pressure should be below 140/90 mmHg according to the European Society of Cardiology (ESC) 2012 guidelines. The mean values for blood pressure in the REFINE-Reykjavik study were well below the ESC targets for all age and gender groups. The mean blood pressure levels for men were 127/71 mmHg and 116/69 mmHg for women. We have previously shown that blood pressure levels have been dropping in Iceland from

1967 to 2007 in middle age men and women by approximately 20 mmgHg (23) and this drop has been seen in all age groups, indicating a population effect rather than an effect of treatment with blood pressure lowering drugs. However, the use of blood pressure lowering drugs was very common in the REFINE-Reykjavik study in the oldest age groups. More than half of men and women in the age group of 65-69 years were taking blood pressure lowering drugs. This high prevalence of drug use could lower the population mean in the oldest age groups. The blood pressure results in the REFINE-Reykjavik were 8 mmHg lower in men (aged 40-69 years) and 10 mmHg lower in women than in the Tromsø 6 study. The difference was similar in each age group. Difference in the use of blood pressure lowering drugs could add to this highly clinically significant difference. This difference is similar in magnitude as the decline in blood pressure in women from 1978-2008 in the Tromsø 6 study (24).

According to the 2016 ESC guidelines for the management of dyslipidemia, drug treatment should be considered if the 10-year risk of fatal cardiovascular disease exceeds 1% and LDL- cholesterol is between 2.6 to <4.0 mmol/L despite of lifestyle intervention (25). Mean LDL-cholesterol level in all age groups except in young women (25-29 years) was above this lower limit. The mean LDL-cholesterol level was highest in 55-59 years men (3.4mmol/L) and women 60-64 years (3.5mmol/l). Comparison with the Tromsø 6 study revealed that in 40-69 years old, the mean LDL-cholesterol was 0.5 mmol/L lower in men (3.3 vs 3.8) and 0.3 mmol/L lower in women (3.3 vs 3.6) in the REFINE-Reykjavik study than in the Tromsø 6 study. This difference was 15% in men and 11% in women. For comparison, the mean percentage lowering of LDL-cholesterol after administrating 20 mg of simvastatin has been shown to be on average 35% (26). In the REFINE-Reykjavik study the LDL-cholesterol was measured in participants after fasting from the evening before, whereas in the Tromsø 6 study the LDL-cholesterol was measured in non-fasting participants. In the Copenhagen General Population Study, the levels of LDL-cholesterol was 0.2mmol/L lower after meal than after fasting so the difference in LDL-level between the REFINE-Reykjavik study

and Tromsø 6 study could be even larger.(27)

We have previously published that total cholesterol (TC) levels in Iceland have been dropping as in other developed countries for the last decades (28). The drop has been similar in both genders and all age groups. The mean drop in TC in the Icelandic population from 1967 to 2008 was 1.5 mmol/L in males and 1.6 mmol/L in females (28).

The prevalence of diabetes has been historically been low in Iceland but the prevalence of diabetes in men in the REFINE-Reykjavik study was almost identical to the prevalence of diabetes in men according to a population based health care database in Sweden (29). Another recent Swedish study shows that prevalence of diabetes was 59.8 per 1000 (6%) for men and 38.4 per 1000 (4%) for women 40-64 years old in 2010 (30). Comparison on the prevalence of type 2 diabetes in men between the REFINE-Reykjavik study and Tromsø 6 study also showed very similar results (7.4%).The prevalence was low in both studies amongst women, (3.4% in REFINE-Reykjavik vs 4.9 in the Tromsø 6 study). The prevalence of diabetes in Iceland, Norway or Sweden has been, from a global viewpoint, relatively low. The prevalence of diabetes in USA in people older than twenty years was for example, according to the NHANES study, 13.4% in men and 10.2% in women in 2007-2010 (31).

In conclusion, the mean CIMT were similar in the REFINE-Reykjavik study and the Tromsø 6 study. The higher prevalence of carotid plaque in the REFINE-Reykjavik Study was probably due to differences in the definition of plaque between the two studies. However the mean for systolic blood pressure and mean LDL-cholesterol levels were higher and smoking more prevalent in the Tromsø-6 study but BMI was higher the REFINE-Reykjavik study.

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Conflict of Interest

None declared

Data sharing statement

Data are available through collaboration with the Icelandic Heart Association and the Tromsø study

Contributorship statement: Conception and design of study: BT, VG, TA, SS and GE; acquisition of data: BT, SS, EG ,KA, EM; analysis and/or interpretation of data: BT, GE, SS, EG, MB, TA, KA, EM ,VG; drafting the manuscript: BT, GE, SS, TA,VG; revising the manuscript critically for important intellectual content: GE, SS, MB, TA, EM, VG All authors approved of the version of the manuscript to be published.

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Figure legends:

Fig 1. Mean farwall carotids intima media thickness in the REFINE-Reykjavik study and Tromsø 6 study by age and sex

Fig 2. Prevalence of right carotids plaque in the REFINE-Reykjavik study and in the Tromsø 6 study by age and sex

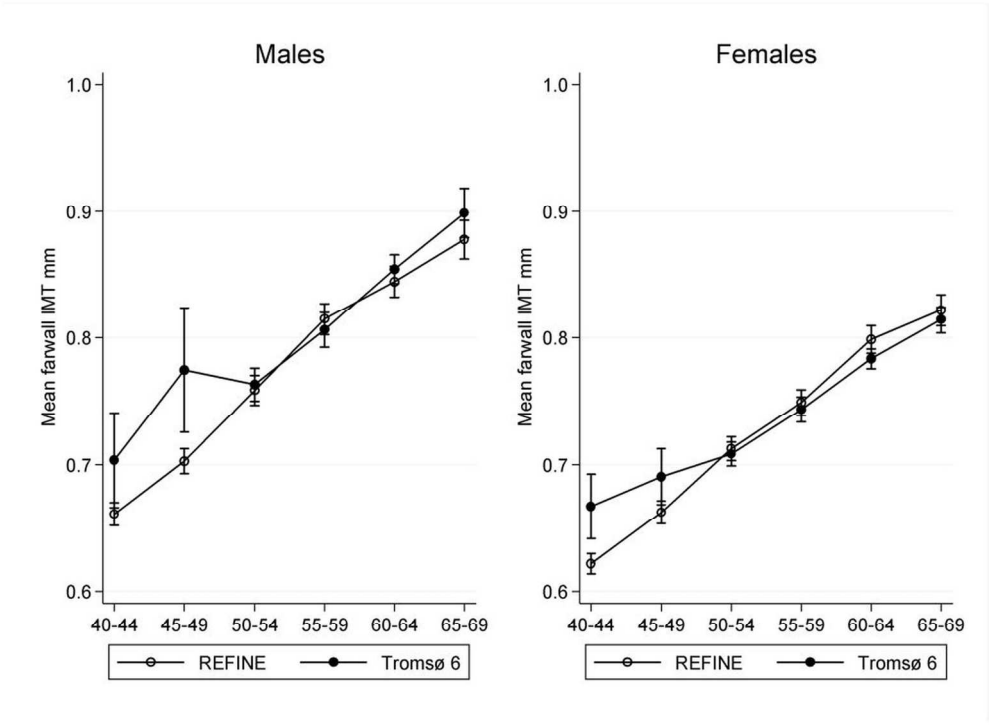


Fig 1. Mean farwall carotids intima media thickness in the REFINE-Reykjavik study and Tromsø 6 study by age and sex

162x119mm (300 x 300 DPI)

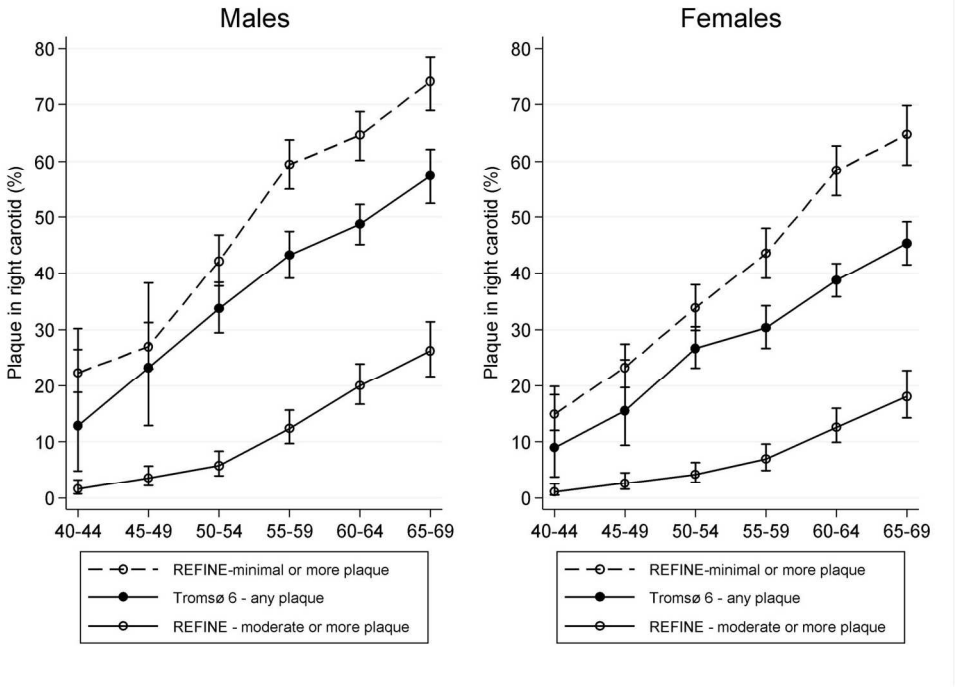


Fig 2. Prevalence of right carotids plaque in the REFINE-Reykjavik study and in the Tromsø 6 study by age and sex

162x119mm (300 x 300 DPI)

Supplement 1

Recruitment

In the REFINE-Reykjavik study, all individuals in the cohort receive an invitation letter by mail. Those who do not respond to the invitation letter are called by a trained telephone receptionist. Reasons for refusing participation were documented when possible. Recruitment started in December 2005 and was completed in March 2011. All participants were asked to fast from the evening before the clinic visit and give informed consent at arrival to the clinic. Participants got feedback regarding the blood test and examination results from a physician.

Use of medication and supplements

The invitation letter included instructions to bring all prescription and non-prescription medications and supplements taken regularly. A trained interviewer registered all medications and supplements taken within the last two-week period (14 days). All medications were classified according to the ATC (Anatomical-Therapeutical-Chemical Classification) codes and supplements by OCD (Over (7)the Counter Drugs Classification) codes when possible. In cases when the classification of drugs is ambiguous a board of two physicians and a nurse resolved the matter.

Clinic examination

Participants answered a health history questionnaire on the internet. Most participants answered at home through a secure web-site but those who had not answered answer on site when they arrive at the clinic. The questionnaire included both history and symptoms of coronary heart diseases (Rose chest pain questionnaire) (8), peripheral arterial diseases, history of vascular procedures, history of stroke, diabetes, high cholesterol, hypertension, chronic obstructive lung disease, sleeping habits, history of esophageal regurgitation, estrogen use in women, family history of CHD, education, profession, history of smoking and current smoking and former and current exercise.

History of cardiovascular disease and history of coronary heart disease were retrieved from the Landspitali- The National University Hospital of Iceland by gathering the ICD 10 and ICD 9 codes for all participants at arrival

into the study. Those participants that had been given the ICD 10 codes: I21-I25, I60-I64 and/or ICD 9 codes 410,411,414,429, 431-434, 436 were defined as having history of cardiovascular disease. Those participants that had been given the ICD 10 codes I21-I25 and/or the ICD 9 codes 410,411,414,429 were defined as having history of coronary heart disease. Diabetes type 2 was defined as history of diabetes due to health questioner or taking diabetes medication or fasting glucose ≥ 7 mmol/L and not taking insulin and not diagnosed younger than 30 years. Physical activity was assessed by the following question in the health history questionnaire: "In the past 12 months, how often did you participate in moderate or vigorous physical activity (Examples of moderate or vigorous physical activity include badminton. golf (walking), biking, swimming, heavy gardening, weight lifting, hiking/ mountain climbing,fast walking/fast dancing/heavy housework, rowing, aerobics, jogging and running)"

Blood pressure was measured semi-automatically during arterial tonometry measurements (Noninvasive Hemodynamics Workstation) according to a standardized protocol (9). Participants were in a supine position for 15-20 minutes before the blood pressure measurement. Hypertension was defined as systolic blood pressure above 140 and/or diastolic blood pressure above 90 or if the participants in the study were on blood pressure lowering drugs based on ATC codes.

An electrocardiography (ECG) was performed and stored digitally. Anthropometric measurements were measurements of body height, weight, hip and waist circumference and body composition by bio-impedance measurements. Certified staff members collected data according to rigid and standardized protocols. Regular quality assurance (QA) protocols were implemented to insure best quality of the data and to reduce inter- and intra-observer variability.

Blood analyses

All chemical measurements were carried out in the ISO accredited laboratory of the Icelandic Heart Association (IHA). The blood draw, handling, aliquoting, storing and measuring as well as switching Analyzers were performed according to the IHA Quality Manual documents. Hb, Hct, MCH, MCHC, MCV, RBC , WBC and platelets were measured in fasting whole blood on an automated cell counter, Coulter HmX AL Hematology Analyzer (Beckman Coulter, High Wycombe, England, UK) which was replaced in November 2011 with XT-2000i from Sysmex. Chemistry measurements were performed on Roche/Hitachi 912 which was updated in February 2010 to Roche/Cobas c311 using reagents from the respective manufacturers according to their

instructions. LDL-cholesterol was calculated using the Friedewald equation (total cholesterol-HDL cholesterol-(triglycerides/2,2)) when triglycerides < 4,5 mmol/L.

Quality control of ultrasound of the carotid arteries

The REFINE-Reykjavik study uses strict quality control procedures for monitoring and testing consistency in image acquisition and image analysis. The quality control includes periodical tests of image analysis and acquisition reproducibility including re-reading of IMT every 6 months of the same 24 cases for assessment of inter-and intra-observer variability and consistency over time. There were typically 2 weeks between reading 1 and reading 2 for the intra-observer variability assessment. Inter-observer variability of carotid plaque presence and severity was tested by repeated acquisitions of up to 15 studies every year by each sonographer. In addition, intra-observer variability of IMT was further tested by the re-reading of 10 randomly selected studies by each observer every 6 months where there were typically 5 to 6 months between reading 1 and reading 2.

Mean intra-observer variability in IMT measurements for three observers (intra-class correlation and percent coefficient of variation respectively) based on the re-reading of the same 24 cases (n=24) over the course of the study ranged from 0.97 to 0.99 and 2.7% to 3.6% for the far wall of the carotid arteries and 0.96 to 0.97 and 3.6% to 4.9% for the near wall. Inter-observer variability for the same 24 cases and the same observers ranged from 0.91 to 0.94 and 4.7% to 6.0% for the far wall and 0.79 to 0.81 and 8.4% to 9.2% for the near wall. Intra-reliability assessment (kappa statistics) of carotid plaque presence and plaque severity between the observers where the results by two observers were compared to the results of one observer that was considered a gold standard were 0.77 (n=68) ad 0.84 (n=60) demonstrating good to excellent agreement. The intra-observer variability in IMT measurements based on re-reading of a random selection of 10 cases every 6 months (intra-class correlation and percent coefficient of variation respectively) was 0.96 and 3.7% for the far wall and 0.91 and 5.6% for the near wall for observer 1 (accumulative total of re-readings, n=90) , 0.93 and 5.0% for the far wall and 0.92 and 6.3% for the near wall for observer 2 (accumulative total of re-readings, n=80) and 0.94 and 3.2% for the far wall and 0.96 and 3.5% for the near wall for observer 3 (accumulative total of re-readings, n=50).

Supplement 2

The Tromsø Study

For comparison of both conventional risk factors for coronary heart disease, prevalence of carotid plaque and the level of CIMT in the population, data from Tromsø 6 study were used.

The Tromsø Study is an ongoing population-based cohort study in the municipality of Tromsø, Northern Norway, with a population of 72 000 inhabitants. The study design includes 7 surveys (Tromsø 1: 1974, Tromsø 2: 1979–1980, Tromsø 3: 1986–1987, Tromsø 4:1994–1995, Tromsø 5: 2001-2002, Tromsø 6: 2007–2008, and Tromsø 7: 2015-2016) to which total birth cohorts and representative samples of the population were invited. From Tromsø 4 and onwards, the study design has included two screening visits, with more extensive examinations at the second visit, including ultrasound examination of the carotid arteries.(Jacobsen BK et al, Int J Epidemiol 2012;41:961-7) Invited to Tromsø 6 1st visit were all residents aged 40-42 and 60-87 (n=12,578), a 10% random sample of individuals aged 30-39 (n=1056), a 40% random sample of individuals aged 43-59 (n= 5787), and subjects who had attended the second visit of Tromsø 4, if not already included in the three groups above (n=341). The attendance rate was 66%. Those eligible for the 2nd visit were all 1st visit eligible aged 50-62 and 75-84 years (n=7657), a 20% random sample of 1st visit eligible aged 63-74 (n=942), and subjects who had attended the second visit for Tromsø 4, if not already included in the two groups above (n=2885). Subjects had to attend the 1st visit in order to be invited to the 2nd visit. The attendance rate to the 2nd visit was 92%. The Population Registry of Norway (by September 12th 2007) was the source for the invitations. The invitation file was created from census data from Statistics Norway, where all citizens in Norway have a unique national identity number given after birth or immigration. The file was periodically updated for mortality and emigration throughout the study period. The Regional Committee of Medical and Health Research Ethics and the Norwegian Data Protection Authority has approved the Tromsø Study (1).

Information on angina pectoris, myocardial infarction, stroke, smoking habits, diabetes, use of antihypertensive and lipid-lowering drugs was obtained from self-administered questionnaires. Blood pressure was recorded three times at one-minute intervals after two minutes of seated resting with the use of an automatic device (Dinamap ProCare 300 monitor, GE Healthcare) by specially trained technicians. The mean of the last two recordings was

used for analyses. Height and weight were measured to the first decimal in participants wearing light clothing and no footwear on an automatic electronic scale (Jenix DS 102 stadiometer). BMI was calculated as weight divided by the square of height (kg/m2).

Analyses of non-fasting serum total cholesterol and triglycerides were done within 10 hours by an enzymatic colorimetric method. HDL and LDL cholesterol were analyzed by homogeneous enzymatic colorimetric methods. All analyses were performed at the Department of Laboratory Medicine, University Hospital of North Norway.

1. Eggen A, Mathiesen E, Wilsgaard T, Jacobsen B, Njølstad I. The sixth survey of the Tromso Study (Tromso 6) in 2007-08: collaborative research in the interface between clinical medicine and epidemiology: study objectives, design, data collection procedures, and attendance in a multipurpose population-based health survey. Scand J Public Health. 2013;41(1):65*80.

Supplement 3

			Age		BMI		Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)	
	Age groups	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Men	25-29	175	27.2	1.39	26.6	5.2	123.1	10.9	62.5	9.4
	30-34	182	32.1	1.40	27.1	4.3	122.3	12.2	66.5	9.8
	35-39	291	37.2	1.39	26.8	3.5	121.8	12.0	66.9	9.4
	40-44	457	42.2	1.44	28.0	4.4	124.5	13.9	71.7	10.4
	45-49	453	47.0	1.43	27.8	4.2	123.3	13.7	72.9	10.2
	50-54	453	52.0	1.46	28.5	4.1	125.9	13.9	75.1	10.3
	55-59	477	57.0	1.42	28.4	4.4	129.2	16.9	75.8	10.6
	60-64	469	61.9	1.39	28.8	4.4	131.8	17.2	75.9	10.4
	65-69	320	66.6	1.39	28.7	4.7	134.9	17.9	74.0	9.9
	Total	3277	49.8	11.2	28.1	4.4	126.8	15.5	72.6	10.8
	Total *		44.8		27.7	4.3	125.5	13.9	70.7	10.0
Women	25-29	173	26.8	1.45	24.7	4.7	109.7	10.9	62.3	8.4
	30-34	190	32.0	1.45	26.6	6.0	109.4	11.4	64.7	9.7
	35-39	302	37.2	1.39	26.2	5.3	109.0	11.2	66.3	8.9
	40-44	475	42.1	1.43	26.7	5.8	112.1	11.6	68.2	8.9
	45-49	460	47.0	1.41	27.0	5.2	115.0	13.8	70.2	9.2
	50-54	525	52.0	1.44	27.2	5.3	117.7	15.6	72.2	9.0
	55-59	476	57.0	1.45	27.1	5.0	121.8	17.7	72.9	9.4
	60-64	468	62.0	1.40	28.2	5.1	126.8	17.5	73.0	8.7
	65-69	315	66.5	1.40	27.8	5.3	129.0	17.2	72.3	8.7
	Total	3384	49.6	11.2	27.0	5.3	117.8	16.3	70.1	9.5
	Total *		44.9		26.7	5.3	115.5	13.7	68.7	9.0

* Adjusted according to population age structure in 2010

table 1a Characteristics of the REFINE-Reykjavik study by age and sex. Continuous parameters.

			Age		Total cholesterol (mmol/L)		High density lipoprotein (mmol/L)		Low density lipoprotein (mmol/L)		Triglycerides (mmol/L)	
	Age groups	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Men	25-29	175	27.2	1.39	4.56	0.78	1.25	0.27	2.77	0.65	1.17	0.63
	30-34	182	32.1	1.40	5.00	0.97	1.23	0.28	3.15	0.82	1.35	1.07
	35-39	291	37.2	1.39	5.08	1.01	1.32	0.30	3.21	0.91	1.23	0.97
	40-44	457	42.2	1.44	5.32	0.92	1.30	0.31	3.41	0.81	1.38	0.87
	45-49	453	47.0	1.43	5.38	0.93	1.33	0.34	3.44	0.89	1.35	0.79
	50-54	453	52.0	1.46	5.39	1.01	1.31	0.34	3.44	0.91	1.45	0.85
	55-59	477	57.0	1.42	5.37	0.98	1.33	0.36	3.40	0.91	1.44	0.87
	60-64	469	61.9	1.39	5.22	1.05	1.35	0.34	3.26	0.97	1.37	0.73
	65-69	320	66.6	1.39	5.05	1.07	1.37	0.38	3.10	0.95	1.27	0.64
	Total	3277	49.8	11.2	5.23	1.00	1.32	0.34	3.30	0.91	1.36	0.83
	Total *		44.8		5.14	0.96	1.30	0.32	3.24	0.86	1.33	0.84
Women	25-29	173	26.8	1.45	4.47	0.86	1.59	0.38	2.49	0.77	0.86	0.38
	30-34	190	32.0	1.45	4.61	0.86	1.50	0.35	2.68	0.76	0.94	0.56
	35-39	302	37.2	1.39	4.63	0.79	1.57	0.38	2.65	0.75	0.90	0.56
	40-44	475	42.1	1.43	4.89	0.86	1.58	0.37	2.86	0.78	0.97	0.61
	45-49	460	47.0	1.41	5.18	0.94	1.64	0.40	3.10	0.87	0.98	0.52
	50-54	525	52.0	1.44	5.54	0.97	1.70	0.45	3.36	0.93	1.09	0.76
	55-59	476	57.0	1.45	5.64	0.97	1.66	0.43	3.46	0.90	1.14	0.62
	60-64	468	62.0	1.40	5.74	1.06	1.67	0.46	3.50	0.99	1.24	0.67
	65-69	315	66.5	1.40	5.73	0.99	1.74	0.44	3.44	0.91	1.22	0.60
	Total	3384	49.6	11.2	5.27	1.04	1.64	0.42	3.15	0.94	1.06	0.63
	Total *		44.9		5.09	0.92	1.62	0.40	3.01	0.84	1.02	0.58

* Adjusted according to population age structure in 2010

table 1b Characteristics of the REFINE-Reykjavik study by age and sex. Continuous parameters.

			Family History of Myocardial Infraction		History of Cardiovascular Disease		History of Coronary Heart Disease	
	Age groups	N	N	%	N	%	N	%
Men	25-29	175	14	8.2	0	0	0	0
	30-34	182	24	13.6	0	0	0	0
	35-39	291	57	20.4	0	0	0	0
	40-44	457	122	27.3	8	1.8	4	0.9
	45-49	453	155	35.0	6	1.4	4	0.9
	50-54	453	193	43.7	21	4.9	19	4.4
	55-59	477	193	42.4	39	8.4	35	7.5
	60-64	469	189	41.9	51	11.1	40	8.7
	65-69	320	125	40.1	72	22.9	64	20.4
	Total	3277	1072	33.8	197	6.2	166	5.2
	Total *			33.8		6.2		5.2
Women	25-29	173	19	11.4	0	0	0	0
	30-34	190	30	16.0	0	0	0	0
	35-39	302	71	24.1	3	1.0	1	0.3
	40-44	475	134	28.9	7	1.6	7	1.6
	45-49	460	188	42.7	4	1.0	3	0.7
	50-54	525	247	48.7	8	1.6	5	1.0
	55-59	476	243	53.1	14	3.1	10	2.2
	60-64	468	219	49.1	24	5.5	18	4.1
	65-69	315	146	48.2	18	6.0	13	4.3
	Total	3384	1297	39.7	78	2.4	57	1.8
	Total *			39.7		1.8		1.3

*Adjusted according to population age structure in 2010

table 2a Characteristics of the REFINE-Reykjavik study by age and sex, categorical parameters.

			Diabetes Type II		Statins		Hypertension		Medication for Hypertension	
	Age groups	N	N	%	N	%	N	%	N	%
Men	25-29	175	0	0	0	0	17	9.7	1	0.6
	30-34	182	0	0	0	0	18	9.9	6	3.3
	35-39	291	2	0.7	0	0	27	9.3	10	3.4
	40-44	457	11	2.4	15	3.3	87	19.0	25	5.5
	45-49	453	19	4.2	16	3.5	112	24.7	68	15.0
	50-54	453	22	4.9	62	13.7	181	40.0	121	26.9
	55-59	477	48	10.1	84	17.7	231	48.4	170	35.9
	60-64	469	50	10.7	125	26.7	272	58.0	210	44.9
	65-69	320	46	14.4	108	33.8	228	71.3	182	57.1
	Total	3277	198	6.0	410	12.5	1173	35.8	793	24.3
	Total *		198	4.3	410	8.8		28.3	793	17.9
Women	25-29	173	1	0.6	0	0	3	1.7	1	0.6
	30-34	190	4	2.1	0	0	14	7.4	10	5.3
	35-39	302	3	1.0	1	0.3	20	6.6	10	3.3
	40-44	475	8	1.7	1	0.2	51	10.7	38	8.0
	45-49	460	5	1.1	5	1.1	87	18.9	63	13.8
	50-54	525	14	2.7	24	4.6	152	29.0	122	23.2
	55-59	476	14	2.9	45	9.5	188	39.5	155	32.6
	60-64	468	24	5.1	59	12.6	262	56.0	219	46.8
	65-69	315	25	7.9	58	18.4	201	63.8	162	51.4
	Total	3384	98	2.9	193	5.7	978	28.9	780	23.1
	Total *		98	2.4	193	4.0		22.2	780	17.4

*Adjusted according to population age structure in 2010

table 2b Characteristics of the REFINE-Reykjavik study by age and sex, categorical parameters.

		Smoking						BMI ≥25		BMI ≥30	
		Never		Former		Current					
	Age groups	N	%	N	%	N	%	N	%	N	%
Men	25-29	87	51.2	34	20.0	49	28.8	100	57.1	28	16.0
	30-34	84	47.2	49	27.5	45	25.3	121	66.5	34	18.7
	35-39	171	60.0	57	20.0	57	20.0	197	67.7	42	14.4
	40-44	196	43.3	151	33.3	106	23.4	357	78.1	110	24.1
	45-49	170	37.8	170	37.8	110	24.4	334	73.7	115	25.4
	50-54	160	35.6	196	43.7	93	20.7	370	81.7	147	32.4
	55-59	153	32.7	221	47.2	94	20.1	382	80.1	155	32.5
	60-64	137	29.6	236	51.0	90	19.4	378	80.6	159	33.9
	65-69	89	28.3	169	53.8	56	17.8	259	80.9	105	32.8
	Total	1247	38.6	1283	39.7	700	21.7	2498	76.2	894	27.2
	Total*	1247	42.1	1283	35.2	700	22.7		73		24.5
Women	25-29	102	60.7	20	11.9	46	27.4	66	38.2	21	12.1
	30-34	98	51.6	52	27.4	39	20.6	97	51.1	40	21.1
	35-39	156	52.2	81	27.1	62	20.7	147	48.7	61	20.2
	40-44	210	44.7	165	35.1	95	20.2	256	53.9	108	22.7
	45-49	167	37.0	178	39.5	106	23.5	273	59.3	99	21.5
	50-54	191	36.7	218	41.9	111	21.3	313	59.6	136	25.9
	55-59	182	38.7	188	40.0	100	21.3	305	64.1	110	23.1
	60-64	157	34.1	209	45.4	94	20.4	339	72.4	143	30.6
	65-69	134	43.1	125	40.2	52	16.7	214	67.9	92	29.2
	Total	1397	41.9	1236	37.0	706	21.1	2010	59.4	810	23.9
	Total*	1397	45.1	1236	33.2	706	21.7		55.8		22.2

*Adjusted according to population age structure in 2010

table 3a Characteristics of the REFINE-Reykjavik study by age and sex. Categorical parameters (continuous)

		Cholesterol ≥5mmol/L		Cholesterol ≥6.2 mmol/L		Physical activity ≥ 1 hours/week		Physical activity ≥ 4 hours/week	
	Age groups	N	%	N	%	N	%	N	%
Men	25-29	43	24.6	4	2.3	124	72.9	63	37.1
	30-34	82	45.1	15	8.2	116	64.8	63	35.2
	35-39	147	50.5	39	13.4	196	68.3	110	38.3
	40-44	282	61.7	73	16.0	287	63.6	163	36.1
	45-49	303	66.9	68	15.0	269	60.4	148	33.3
	50-54	291	64.2	89	19.6	262	59.4	153	34.7
	55-59	307	64.4	95	19.9	287	62.1	150	32.5
	60-64	269	57.4	77	16.4	257	56.5	134	29.5
	65-69	168	52.5	45	14.1	187	59.6	116	36.9
	Total	1892	57.7	505	15.4	1985	62.0	1100	34.3
	Total*		53.5		13.5		63.7		35.0
Women	25-29	42	24.3	6	3.5	116	67.8	53	31.0
	30-34	59	31.1	5	2.6	121	65.1	51	27.4
	35-39	95	31.5	10	3.3	208	68.9	98	32.5
	40-44	202	42.5	37	7.8	341	73.0	180	38.5
	45-49	251	54.6	60	13.0	317	70.3	177	39.2
	50-54	372	70.9	124	23.6	358	69.9	194	37.9
	55-59	351	73.7	126	26.5	295	64.0	157	34.1
	60-64	347	74.1	141	30.1	292	64.2	163	35.8
	65-69	246	78.1	95	30.2	192	62.5	100	32.6
	Total	1965	58.1	604	17.8	2240	67.6	1173	35.4
	Total*		50.6		13.9		67.7		34.3

*Adjusted according to population age structure in 2010

table 3b Characteristics of the REFINE-Reykjavik study by age and sex. Categorical parameters (continuous)

			Intima media thickness (mean)		Intima media thickness (max)		Moderate or more plaque in carotids	
	Age groups	N	Mean	SD	Mean	SD	N	%
Men	25-29	175	0.57	0.07	0.67	0.08	0	0.0
	30-34	182	0.60	0.07	0.71	0.09	2	1.1
	35-39	291	0.63	0.09	0.74	0.10	2	0.7
	40-44	457	0.68	0.08	0.80	0.10	13	2.9
	45-49	453	0.72	0.09	0.85	0.11	21	4.7
	50-54	453	0.78	0.11	0.91	0.12	36	7.9
	55-59	477	0.84	0.12	0.98	0.14	87	18.3
	60-64	469	0.87	0.12	1.01	0.15	121	25.8
	65-69	320	0.91	0.13	1.06	0.15	107	33.4
	Total	3277	0.76	0.15	0.89	0.17	389	11.9
	Total *		0.71	0.10	0.83	0.11		8.3
Women	25-29	173	0.53	0.05	0.62	0.06	0	0.0
	30-34	190	0.57	0.06	0.67	0.08	1	0.5
	35-39	302	0.60	0.07	0.71	0.08	2	0.7
	40-44	475	0.64	0.07	0.75	0.08	7	1.5
	45-49	460	0.68	0.08	0.80	0.10	18	3.9
	50-54	525	0.74	0.09	0.86	0.11	29	5.5
	55-59	476	0.78	0.10	0.90	0.12	53	11.2
	60-64	468	0.83	0.11	0.96	0.13	82	17.5
	65-69	315	0.85	0.11	0.98	0.12	85	27.0
	Total	3384	0.71	0.13	0.83	0.15	277	8.2
	Total *		0.67	0.08	0.79	0.09		5.9

*Adjusted according to population age structure in 2010

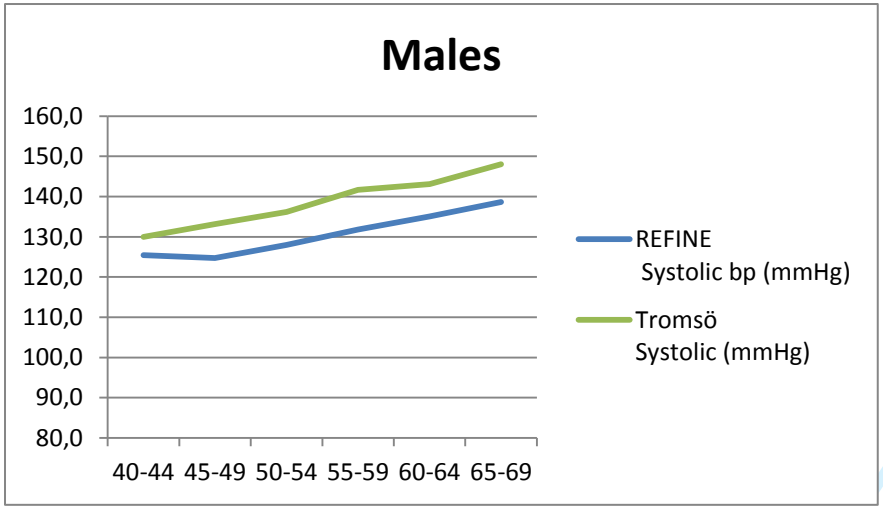
table 4a. Characteristics of the REFINE-Reykjavik study by age and sex, image analysis.

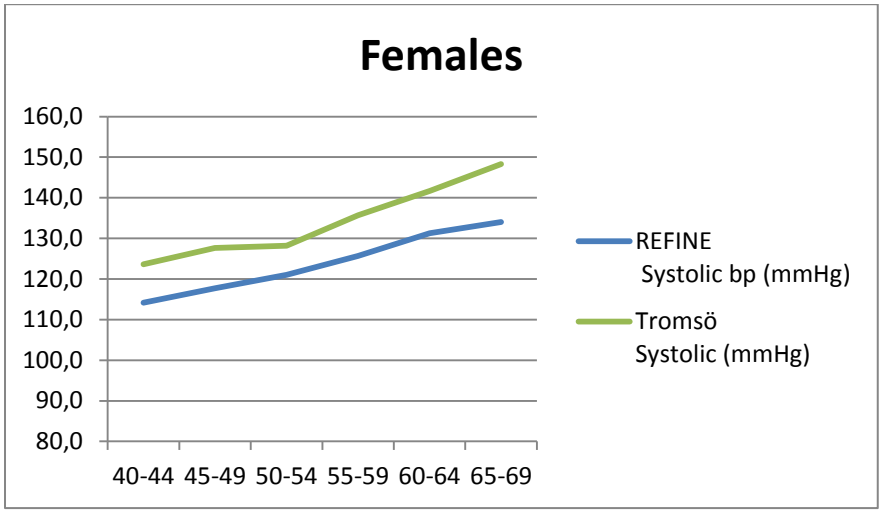
			Plaque categories							
			None		Minimal		Moderate		Severe or more	
	Age groups	N	N	%	N	%	N	%	N	%
Men	25-29	175	163	94.2	10	5.8	0	0.0	0	0.0
	30-34	182	157	86.3	23	12.6	2	1.1	0	0.0
	35-39	291	257	88.3	32	11.0	2	0.7	0	0.0
	40-44	457	310	68.0	133	29.2	13	2.9	0	0.0
	45-49	453	260	57.6	170	37.7	20	4.4	1	0.2
	50-54	453	194	42.8	223	49.2	35	7.7	1	0.2
	55-59	477	141	29.6	248	52.1	80	16.8	7	1.5
	60-64	469	107	22.8	241	51.4	107	22.8	14	3.0
	65-69	320	50	15.6	163	50.9	91	28.4	16	5.0
	Total	3277	1639	50.1	1243	38.0	350	10.7	39	1.2
	Total *			60.9		30.8		7.6		0.8
Women	25-29	173	167	96.5	6	3.5	0	0.0	0	0.0
	30-34	190	176	92.6	13	6.8	1	0.5	0	0.0
	35-39	302	268	88.7	32	10.6	2	0.7	0	0.0
	40-44	475	365	76.8	103	21.7	7	1.5	0	0.0
	45-49	460	308	67.1	133	29.0	17	3.7	1	0.2
	50-54	525	292	55.7	203	38.7	28	5.3	1	0.2
	55-59	476	216	45.5	206	43.4	49	10.3	4	0.8
	60-64	468	157	33.5	229	48.9	70	15.0	12	2.6
	65-69	315	68	21.6	162	51.4	70	22.2	15	4.8
	Total	3384	2017	59.7	1087	32.2	244	7.2	33	1.0
	Total *			68.5		25.6		5.3		0.7

*Adjusted according to population age structure in 2010

table 4b. Characteristics of the REFINE-Reykjavik study by age and sex, image analysis.

Supplement 4 figure 1. **Distribution of cardiovascular risk factors in the REFINE-Reykjavik study and Tromsø 6 study by age and sex.**





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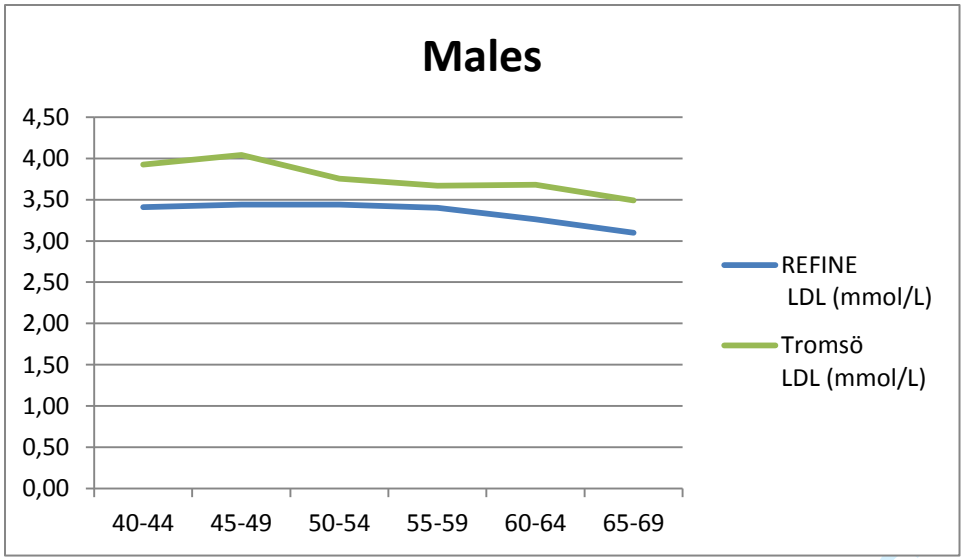

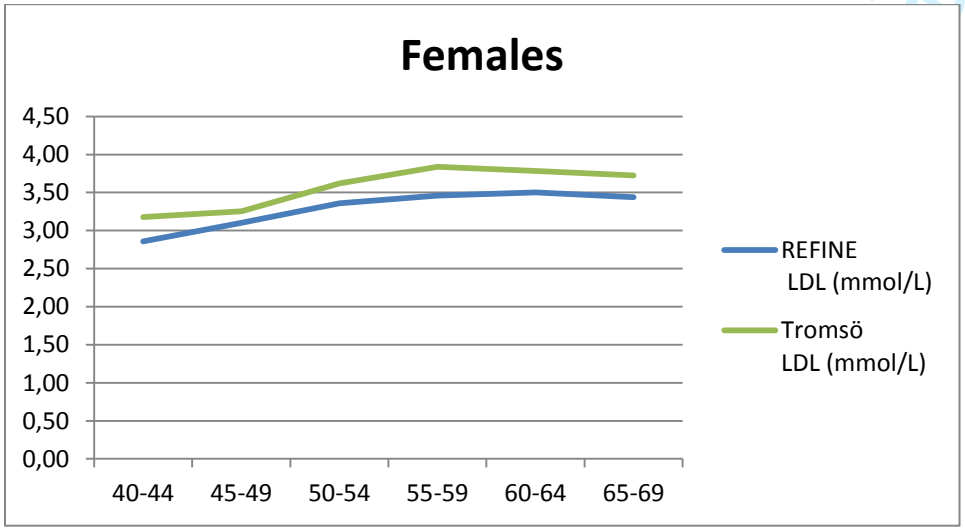

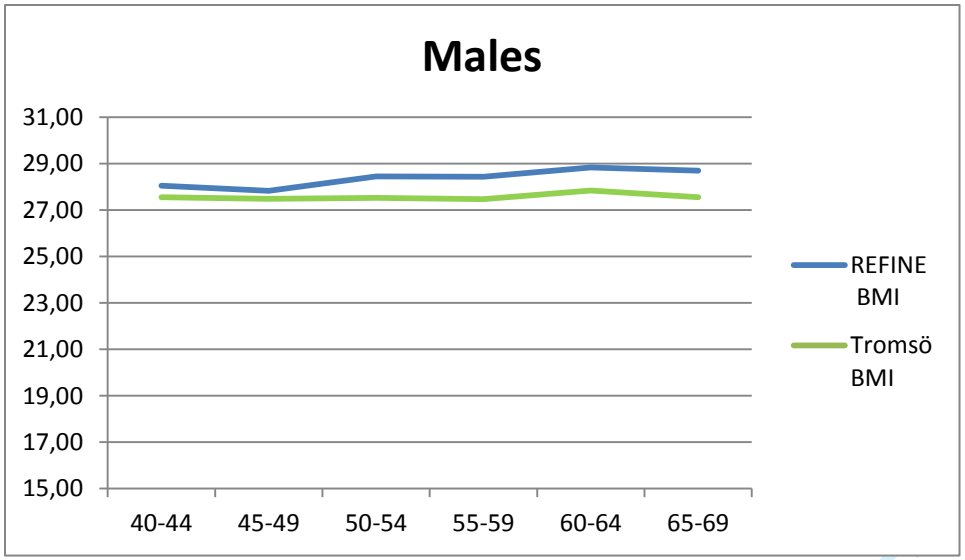

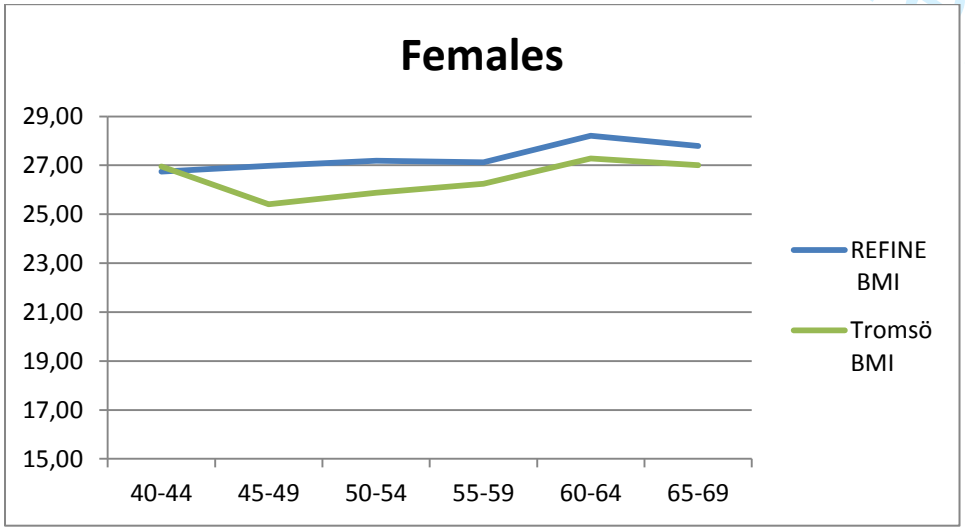

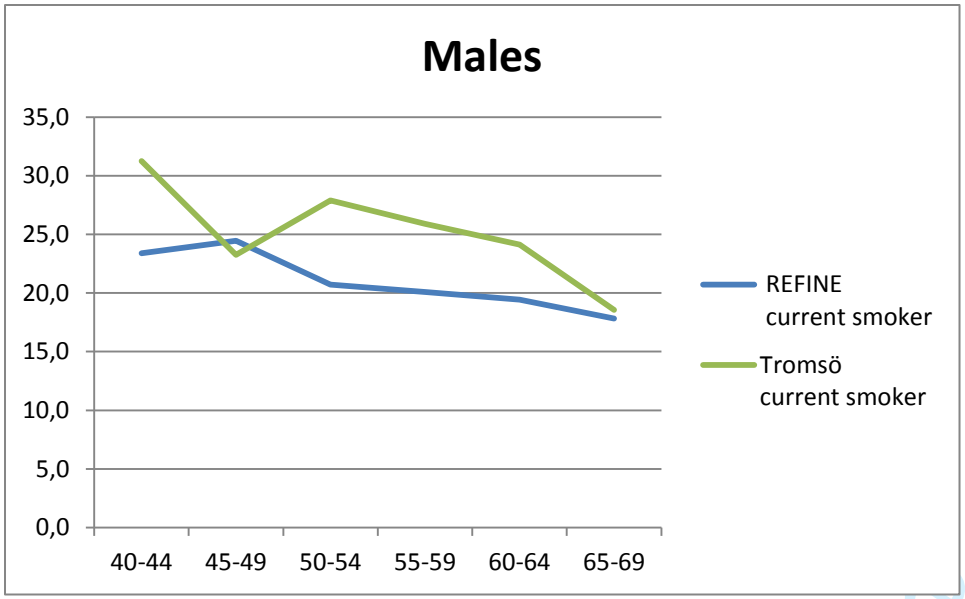

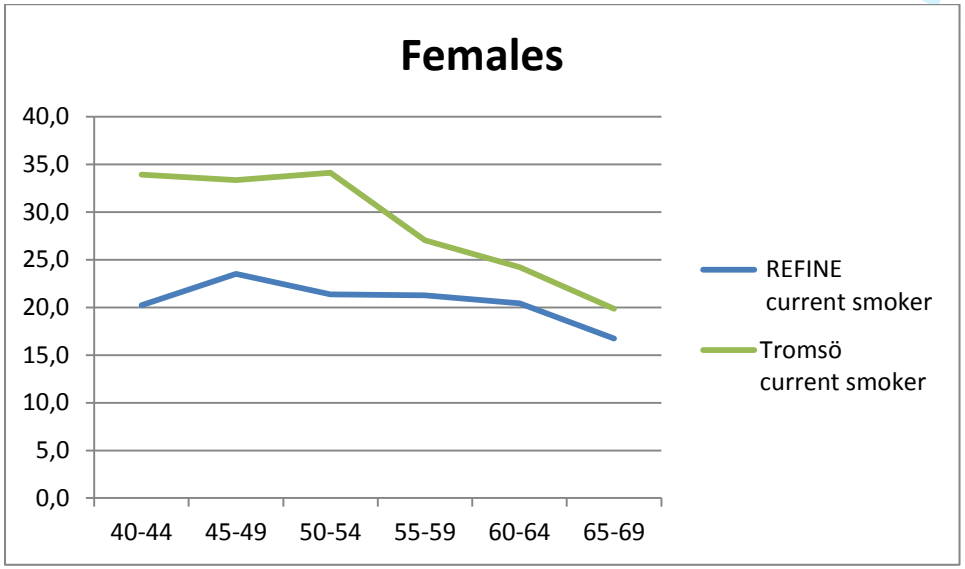

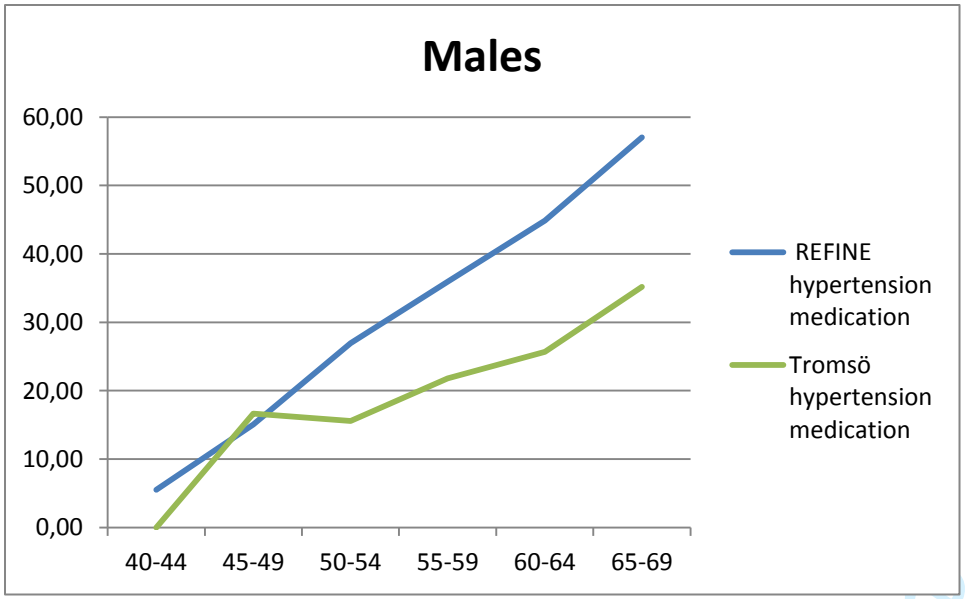

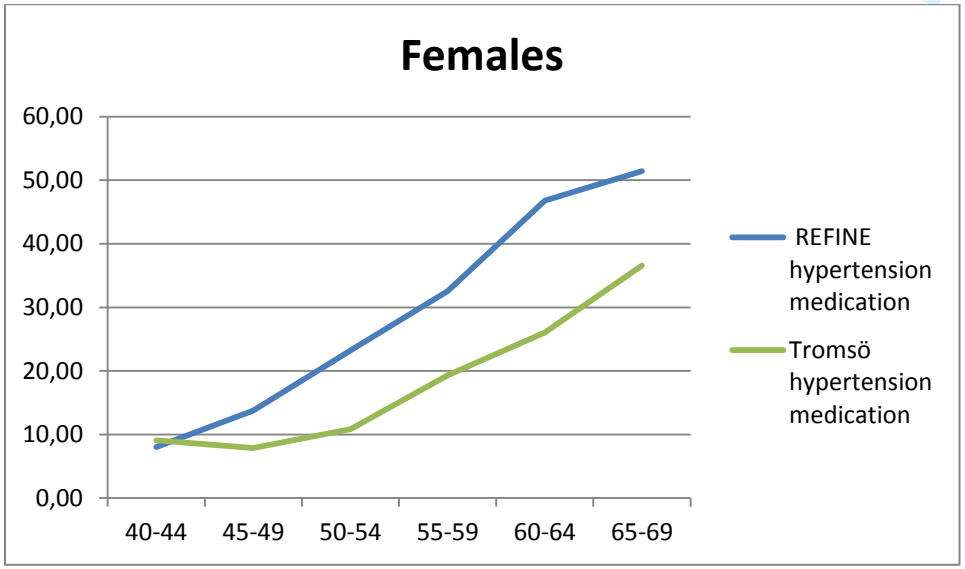

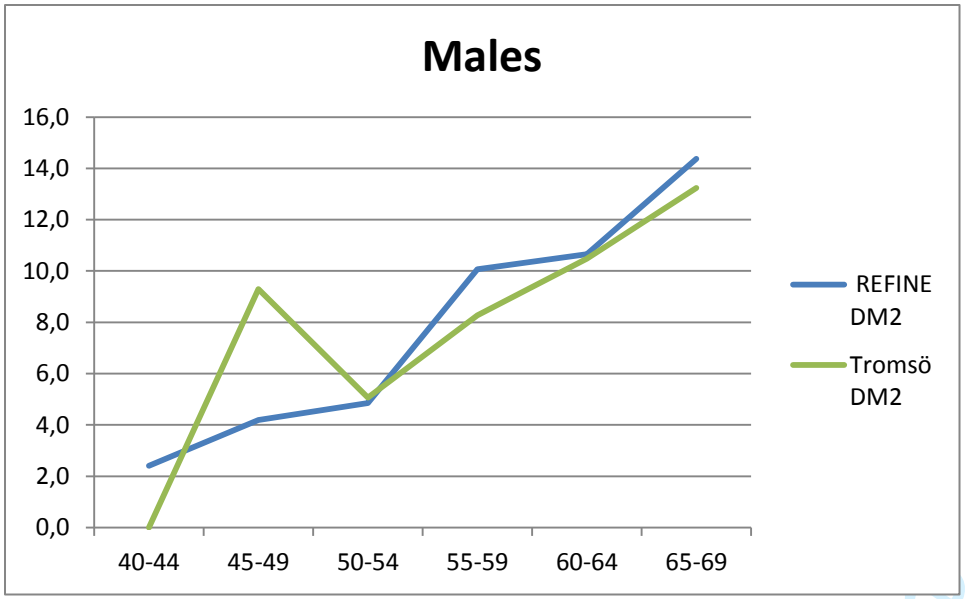

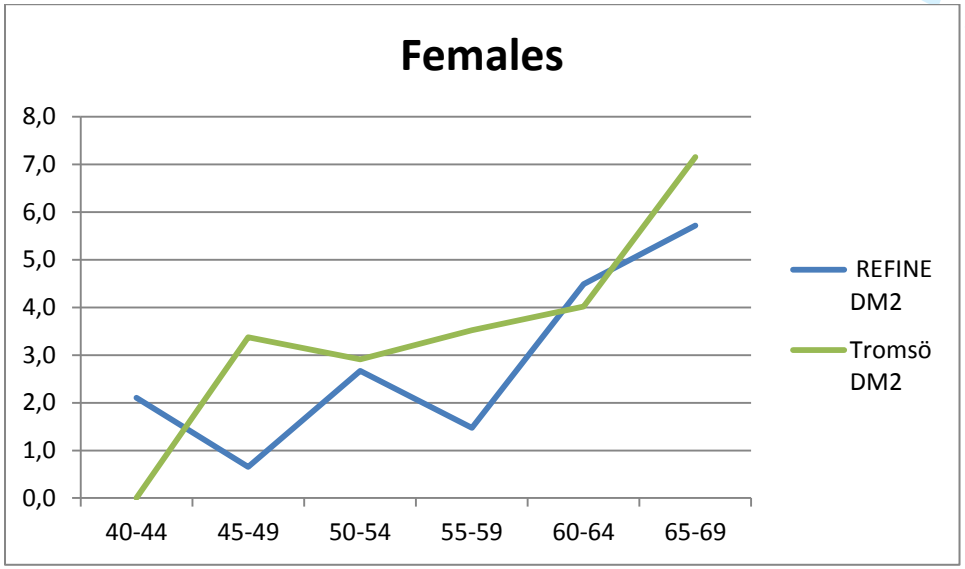

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No: bmjopen-2017-019385	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract : **Page 1**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found: **Page 2**
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **Page 2**
Objectives	3	State specific objectives, including any prespecified hypotheses **Page 2**
Methods		
Study design	4	Present key elements of study design early in the paper **Page 4**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Page 4**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **Page 4**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed **Not applicable**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Page 5-7 and supplement 1, 2**
Bias	9	Describe any efforts to address potential sources of bias **Page 4**
Study size	10	Explain how the study size was arrived at **Page 4**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **Page 7**
		(*b*) Describe any methods used to examine subgroups and interactions **Page 7**
		(*c*) Explain how missing data were addressed **Not applicable**
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses **Not applicable**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **Page 8**
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **Page 8**
		(b) Indicate number of participants with missing data for each variable of interest **Page 8 and in tables**
		(c) Summarise follow-up time (eg, average and total amount) **Not applicable**

Outcome data	15*	Report numbers of outcome events or summary measures over time **Not applicable**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **Page 8**
		(*b*) Report category boundaries when continuous variables were categorized **Supplementary text 1,2**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives **Page 15**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **Page 16**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **Page 16-18**
Generalisability	21	Discuss the generalisability (external validity) of the study results **Page 16-18**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **Page 19**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.