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).