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Swedish CArdioPulmonary bioImage Study (SCAPIS)

A National Resource for Present and Future Cardiopulmonary Research

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LIST OF ABBREVIATIONS

Abbreviation	Explanation
ABI	Ankle Brachial Index
Apo A1	Apolipoprotein A1
Apo B	Apolipoprotein B
AT	Adipose Tissue
ATS	American Thoracic Society
BBMRI	Swedish Biobanking and Biomolecular Resources Research Infrastructure
BMI	Body Mass Index
CACS	Coronary Artery Calcium Score
CCA	Common Carotid Artery
CCTA	Coronary Computed Tomography Angiography
CMIV	Center for Medical Image Science and Visualization
CO	Carbon monoxide
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CT	Computed Tomography
CVD	Cardiovascular Disease
DE	Dual energy
DL_{CO}	Carbon monoxide uptake
DNA	Deoxyribonucleic acid
EC	Ethics Committee
ECA	External Carotid Artery
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated glomerular filtration rate
EDTA	Ethylenediaminetetraacetic acid
ERS	European Respiratory Society
FEV_1	Forced Expiratory Volume in 1 second
FOV	Field of view
FVC	Forced Vital Capacity
GFR	Glomerular Filtration Rate
GIH	The Swedish school of sport and health sciences
GSM	Grey Scale Median
Hb	Hemoglobin
HbA1c	Glycated Hemoglobin
HDL	Hight Density Lipoprotein
hsCRP	High sensitivity C-reactive Protein
HU	Hounsfield Unit
ICA	Internal Carotid Artery

Abbreviation	Explanation
IMT	Intima-media Thickness
IPH	Intra plaque haemorrhage
LDL	Low Density Lipoprotein
LIMS	Laboratory Information Management System
LRNC	Lipid rich/necrotic core
MEF	Maximal Expiratory Flow
MI	Myocardial Infarction
MP-RAGE	Magnetization prepared rapid gradient echo
MRI	Magnetic Resonance Imaging
MT	Muscle Tissue
PW	Percentage White
RV	Residual volume
SCAPIS	Swedish cardiopulmonary bioimage study
SCORE	Systematic coronary risk evaluation
SES	Socioeconomic Status
SVC	Slow Vital Capacity
s-TG	Serum Triglycerides
TLC	Total lung capacity
TOF	Time of flight
VA	Alveolar volume
VA	Vertebral Artery
VC	Vital capacity
VCmax	Highest vital capacity (SVC or FVC)
VIN	Inspired vital capacity

1. BACKGROUND

Over the past few decades, the mortality and morbidity from myocardial infarction (MI) and stroke have declined in western societies [1]. This decline has been attributed mostly to reductions in smoking and cholesterol levels related to lifestyle changes in the population, but also to new treatments and improved management of acute MI [2]. However, this has also led to an increase in heart failure burden. These diseases still account for about 40% of all deaths in Sweden and remain a major threat to public health, with most fatal coronary events occurring outside the hospital [3] and thus mainly avoidable with preventive treatment.

Using modern imaging techniques (CT angiography, ultrasound and MRI), it is today feasible to directly visualize and identify diseased vascular segments both in the coronary and in the carotid arteries [4, 5]. Atherosclerosis (plaques) in these vascular segments can be morphologically characterized and the data used to identify plaque subtypes associated with future events (vulnerable plaques). Better tools to identify vulnerable plaques have the potential to greatly improve risk algorithms. Today, there are no large epidemiological studies that enable the investigation of the contribution of detailed vascular imaging to risk assessment.

Different algorithms, such as the Framingham risk score or SCORE, have been developed to calculate risk of MI or stroke based on age, gender and traditional risk factors (e.g. cholesterol, blood pressure). However, these algorithms lack precision with respect to timing of future events, have less precision for stroke [6], and are calibrated to the populations in which they were developed. Prevention guidelines recommend targeting persons identified as having high risk estimated using these scores, but a large proportion of all myocardial infarctions and stroke occur in people who are identified as intermediate risk individuals with these scores. A major goal of the SCAPIS study is to develop clinical prediction tools using advanced imaging technologies of atherosclerosis in the coronary and carotid arteries together with information obtained by proteomics/metabolomics/genomics technologies, to improve risk prediction. This is especially warranted in the intermediate risk group.

The prevalence and mortality of respiratory diseases, such as chronic obstructive pulmonary disease (COPD), are still on the rise, particularly among women [7]. In Sweden, 10% of individuals over 45 years of age have COPD [8], and it is the fourth most common cause of death in western societies. Many persons live with COPD for years before it is diagnosed, which often happens late in the disease process when the ventilatory capacity has been reduced by 50%. Although a large proportion of COPD patients are former smokers, a substantial number develop COPD without smoking [8], and a number of new important but poorly defined subgroups of COPD are emerging. Improved phenotyping of COPD is of great importance because clinical presentation, risk factor patterns, prognosis, and presumably optimal treatment differ between these clinical entities [9].

An increasing number of individuals worldwide have a common profile of overweight, insulin resistance, inflammation, atherosclerosis, and impaired lung function [10]. This is well recognized in the area of MI and stroke and it is likely that the increasing frequency of obesity in the future will offset the decline in these diseases seen in recent decades [10]. Several studies have also shown that obesity is associated with mild forms of COPD [11].

It is now recognized that body fat distribution has a major effect on disease development [12]. Importantly, ectopic fat accumulation in bodily compartments not designed to accommodate large lipid loads, such as the liver, heart and muscle, may trigger inflammatory responses and dyslipidemia. By using low-dose radiation CT imaging, fat distribution in all relevant compartments can be comprehensively mapped [13]. This type of imaging has never been combined with detailed imaging of vascular and pulmonary disease in a large population-based study and will for the first time give prospective information on the importance of fat deposition on disease outcome, and has potential for use in risk estimation.

2. RATIONALE

The aim of the Swedish CArdioPulmonary bioImage Study (SCAPIS) is to predict and prevent cardiovascular disease (CVD) and COPD. SCAPIS will recruit and investigate approximately 30,000 men and women aged 50 to 64 years with detailed imaging and functional analyses of the cardiovascular and pulmonary systems.

The risk factor patterns for MI, stroke and COPD have changed dramatically during the last two decades, from an environment with high levels of cholesterol, blood pressure and smoking, to a scenario dominated by obesity, hypertriglyceridemia and diabetes. The same era has seen an unparalleled development of imaging and proteomics/metabolomics/genomics ("-omics") technologies. As a consequence, strategies for diagnosis and prevention of cardiopulmonary diseases developed just a few decades ago lack evidence and perhaps also relevance in today's healthcare. These strategies have the potential to be dramatically improved using recently developed advanced imaging techniques that allow us to directly image the disease process rather than relying on the limited information provided by indirect risk factors, and by use of recent developments in large-scale -omics techniques, facilitating the identification of new biomarkers and mechanisms for disease. The only way to bridge this knowledge gap is to assemble a new large cohort study including examinations using the most recent and most promising techniques. The establishment of SCAPIS will yield a unique Swedish infrastructure for studies on MI, stroke and COPD at the highest international level. By extensively phenotyping 30,000 subjects with in-depth imaging techniques, functional tests, detailed questionnaires, and modern –omics technologies, the resulting database will allow unresolved scientific issues of critical importance to be addressed both today and for future generations.

Prevention of premature CVD and COPD are highly prioritized issues globally. Better risk discrimination will lead to more cost-effective preventive efforts. The bioimaging and -omics scenes have finally reached a stage where a giant leap forward in risk prediction would be possible. This research program is designed to set the stage for a new era of precise and cost-effective CVD and COPD prevention.

SCAPIS will benefit the health care system both in the short- and the long-term. In the short-term, SCAPIS will improve: (1) understanding of new risk factor patterns and their aggregation in certain areas of society; (2) clinical evaluation of subclinical forms of disease; and (3) risk stratification based on information obtained using modern imaging techniques. Long-term effects include: (1) discovery of new mechanisms of diseases, potentially leading to new drug treatments; (2) health economic understanding of the cost-effectiveness of using imaging techniques in risk stratification.

The creation of an extensive blood and DNA biobank will greatly facilitate the search for new lipid, protein and genetic biomarkers of disease. Furthermore, the size and design of the study will ensure that age, gender and other confounding factors can be statistically accounted for, and provides the possibility to study sub-populations with high statistical power. Once established, the platform will be the basis for cutting-edge translational and reverse translational research in areas such as modern imaging techniques, gene-environment interaction studies, and case-control or case-cohort studies on metabolic, immunologic, and haemostatic mechanisms.

2.1 Advantages of SCAPIS over other similar cohorts

There are several current or planned large international cohort studies of MI, stroke and COPD including those from the Framingham Heart Study, the Copenhagen Heart Study, the Reykjavik Heart Study, PROCAM, FINRISK, the Rotterdam Heart Study, and EPIC-Heart. Worth mentioning are also the Dutch LifeLines cohort and the UK Biobank cohorts, as well as the Swedish LifeGene and EpiHealth cohorts. However, the strength of these studies is largely their great size. The novelty and significant contribution of SCAPIS is that it combines size with extensive and in-depth phenotyping and direct imaging of the disease process. Such extensive phenotyping is not a feature of these other studies.

A few large-scale international studies have performed extensive cardiovascular and pulmonary imaging: for example, the Multi-Ethnic Study of Atherosclerosis (MESA) [14], Dallas Heart Study [15] and High Risk Plaque (HRP) [16] initiative. The most important distinctions are:

1) The planned size of SCAPIS is almost three times that of the nearest competitor;
2) SCAPIS is the only study using coronary angiography, which allows direct visualization and quantification of plaques in the coronary arteries;
3) SCAPIS combines direct visualization of disease in lung and vessels with detailed metabolic imaging of fat depots on a scale not attempted in any other study;
4) SCAPIS is the only study with recruitment of a population sample; and
5) the Swedish identification number and registries give superior advantages in follow-up

3. STUDY OBJECTIVES

The overall objectives of the SCAPIS study are:

1. To improve risk prediction for cardiovascular disease by use of advanced imaging technologies of atherosclerosis in the coronary and carotid arteries together with information obtained by proteomics/metabolomics/genomics technologies. The clinical implications would be better-targeted intensive risk factor interventions, which will save both lives and medical costs.

2. To improve the understanding of underlying mechanisms of disease in CVD and COPD. An example is use of detailed metabolic imaging with computed tomography (CT) to elucidate the links between fat deposition patterns and risk of subclinical atherosclerosis and development of CVD in persons with and without COPD. This could lead to the discovery of new treatment targets and preventive strategies.

3. To improve the understanding of the epidemiology of CVD and COPD. An example is investigating why high morbidity and mortality rates from CVD and COPD persist in individuals with low socioeconomic status, despite an overall reduction in mortality from CVD and a leveling of age-adjusted mortality for COPD. This could lead to an improved care of COPD patients to prevent CVD, and better-targeted intervention in persons with a low socioeconomic status.

4. To evaluate the cost-effectiveness regarding the use of new imaging techniques and use of modern -omics technologies to target preventive efforts against CVD and COPD.

Each specific research question needs to be considered separately to evaluate the scientific value and to assure compliance with ethics approvals and informed consent.

4. STUDY POPULATION

SCAPIS will recruit approximately 30,000 subjects aged 50 to 64 years randomly selected from the Swedish population registry. The recruitment process will ensure that the final study population is representative for the Swedish population in general, with the exception of those who are unable to participate in study procedures due to language issues. The recruitment and examinations will be performed at six university hospitals in Sweden (Gothenburg, Linköping, Malmö/Lund, Stockholm, Umeå and Uppsala), each site recruiting around 5000 individuals. If other local studies may interfere with the SCAPIS recruitment, actions should be taken to assure that the selection process for SCAPIS is randomized and not biased.

4.1 Inclusion criteria

For inclusion in the study subjects must fulfil the following criteria

1. Signed informed consent to participate in the study and to be followed-up using national population registries. Consent for biobanking, data transfer to third country and contact for additional studies are optional
2. Men and women registered in one of the areas selected for the study at the time of selection from the population registry
3. 50 but not yet 65 years of age at the time of selection from the population registry
4. Received invitation to participate in the study (through random selection from the population registry)
5. Ability to understand instructions and complete questionnaires, as judged by the study staff

4.2 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled.

1. Previous selection and/or participation in the study at any of the study sites
2. Previous selection and/or participation in the SCAPIS pilot study

4.3 Restrictions

Restrictions are listed under each investigation, see section 5 Study Design And Procedures. A subject can be excluded from or decline to participate in one or more investigations without being excluded from the study.

4.4 Subject enrolment and randomization

Each Swedish citizen has a unique personal identification number, which allows for identification in the Swedish population registry. From this registry, random samples of men and women aged 50-64 years will be drawn from each recruitment area with the aim to include 5000 subjects from each area with an even gender and age distribution. The recruitment area definitions for each study site are specified in Appendix C.

4.4.1 Initial invitation

Randomly selected person records from each area will be imported in the booking system at regular intervals. An invitation containing a letter and a brochure will be sent to selected individuals. The invitation will briefly inform the subjects about the study. Phone number and e-mail address to a study contact centre, manned by study staff will be given as well as references to a study webpage.

4.4.2 Information call

Invited subjects will be encouraged to contact the study contact centre, otherwise they will be contacted by the study contact centre shortly after the invitation has been sent. During the contact the subject will be given further information and their interest in participating in the study will be evaluated. A subject will be defined as 'Non-reachable' if contact has not been made following 1 call attempt, or if a phone number cannot be found. A follow-up letter and sms (optional) will be sent to all subjects defined as 'Non-reachable' with a reminder about the study and an encouragement to contact the study contact centre if they are interested in study participation.

During all telephone contacts with subjects, the communication should be professional, neutral and free from any coercion while underlining the importance of further research in the field of cardiopulmonary diseases. It will be stressed that further information and time for additional questions will be the first procedure of Visit 1.

If the subject agrees to participate, visit dates for all visits are scheduled. The subject is tagged as 'Scheduled' in the booking system. If a subject chooses to decline participation, the subject is also asked for the reason. The call is ended and the subject is tagged as 'Declined' in the booking system and the reason for not participating is entered and categorized as one of the following; 'Illness', 'Language issues', 'No time', 'No reason given' or 'Other'.

Subjects who have reached the age of 65 years after the selection from the population registry should be scheduled for visit 1 within three months from the time of selection.

4.4.3 Re-scheduling

A scheduled subject can call the contact centre if, for any reason, it becomes impossible for them to attend at the agreed examination time. A new examination time will be agreed. Subjects can also call and resign from a scheduled examination if they change their mind.

During all scheduling activities, the strategy is to keep the near-time schedule as full as possible to optimize resource utilization and not fall behind in the overall study timelines.

4.4.4 Reminders of study visits

Use of a reminder system is encouraged, e.g. sms, e-mail or letter to subjects to confirm their appointment.

4.4.5 Subjects at the scheduled Visit 1 and informed consent

As the first procedure of Visit 1, informed consent will be collected from the subject. When the informed consent has been signed, the subject will be registered in the electronic Case Report Form (eCRF).

Scheduled subjects not showing up at Visit 1 without any previous notice for change will be contacted by the contact centre to re-evaluate interest for participation and to re-schedule Visit 1. If the subject is no longer interested in participation or fails to show up a second time, no further contact will be attempted and will be tagged as 'No-shows'.

4.5 Discontinuation and withdrawal of subjects

A subject can at any time withdraw from any study procedure or the entire study. This will not influence the future care of the subject.

4.5.1 Premature termination of the study

The national Steering Committee may decide to stop the trial or part of the trial at any time. If the trial is prematurely terminated or suspended, the Ethics committee should be notified and provided a written explanation.

5. STUDY DESIGN AND PROCEDURES

The examinations will be performed over 2-3 days within approximately 2 weeks. Whenever possible, all visits should be performed within a period of 4 weeks. Examinations are divided into two categories: 1) core examinations that are common for all sites (listed in Table 1); and 2) optional examinations that are performed at one or more sites depending on the local research interest. The optional examinations should not interfere with the core examinations, with regards to e.g. interventions administered, examination burden for the subject etc.

The examination schedule can be locally adapted, but should follow all restrictions. Care should be taken that examinations including pharmaceuticals or other interventions that may affect the outcome of core examinations, are performed after those core examinations. All subjects will be fasting overnight (at least 8 hours) before the first visit. If a subject has not been fasting overnight, blood sampling should be rescheduled and performed preferably at an extra visit but no later than Visit 2. For subjects with elevated plasma

glucose levels, an overnight fasting glucose measurement will be repeated, see section 5.4.1.

Subjects should be instructed to take their regular medication as usual also when fasting. The only medication that should not be taken is medication for diabetes, per oral as well as injections. Subjects with diabetes should be fasting as all other subjects, and should bring their medication to take when having breakfast at the clinic.

To obtain standardized conditions for the imaging studies, subjects are recommended to be fasting for at least 4 hours before intake of a standardized meal. The standardized meal should be consumed 2 hours before the CT scan.

Table 1 List of core examinations

Core examinations	
Informed Consent	
Medical history	
Anthropometry	• Height • Weight • Waist and hip circumference
Biological sampling	• Clinical chemistry/hematology (Hb, p-Glucose, HbA1c, s-TG, s-Cholesterol, LDL, HDL, creatinine, hsCRP) • Biobank sampling (blood and urine)
Function tests relating to the lung	• Spirometry • CO uptake
Questionnaires	
Accelerometry	
Electrocardiogram (ECG)	
Heart rate	
Blood pressure	• Brachial arterial blood pressure • Ankle brachial index (ABI)
Ultrasound of carotid arteries	
Computed tomography (CT)	• Body composition • Lung tissue • Coronary artery calcium score (CACS) • Coronary computed tomography angiography (CCTA)
Magnetic Resonance Imaging (MRI)	

5.1 Informed consent

Upon arrival for the first study visit, the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and have sufficient time to

consider the information provided. The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study. The original informed consent form is stored at the study site and a copy is given to the subject. The informed consent procedure may be handled by a doctor, nurse (incl radiology nurse and nurse assistant) or biomedical scientist.

5.2 Medical history

The subject will be questioned about current and past cardiovascular and pulmonary diseases, allergies and current medication. Presence of any contraindications for spirometry or contrast media administration will be verified.

Subjects currently treated with metformin will be asked to discontinue treatment on the day of the CT scan examination. An s-creatinine sample should be taken 48 hours after contrast media administration. Metformin treatment is re-started when the creatinine value is within normal range. If a subject declines discontinuation of metformin treatment, CT will be performed without administration of contrast media.

Subjects will also be informed that in case their creatinine value indicates an estimated Glomerular Filtration Rate (eGFR) of 50-59 ml/min/1.73m^2, they will need to return for an additional s-creatinine sample at a minimum of 48 hours after the CCTA (see 5.10). If a subject declines this additional blood sample, CT will be performed without administration of contrast media.

5.3 Anthropometry

Height, weight, waist and hip circumference are measured as described below [17].

5.3.1 Height

The subject should be measured in indoor clothing to the nearest centimeter without shoes and hats, hair accessories etc that may affect the result, if applicable. Subjects should stand directly below the meter and keep their legs together, back straight and eyes straight ahead.

5.3.2 Weight

Weight should be measured on a balance beam or digital scale. Subjects should be dressed in light indoor clothing without shoes and be asked to empty pockets before weighing.

5.3.3 Waist circumference

All clothing except underwear should be removed to ensure correct positioning of the measuring tape. Subjects should stand erect with the abdomen relaxed, after exhalation, arms at the side, feet together, and weight equally divided over both legs. A non-stretchable tape should be placed at the waist midway between the palpated iliac crest and the palpated lowest rib margin in the left and right mid-axillary lines. The tape should be in a horizontal position (parallel to the floor), and it should not be twisted and the measurement scale should face outward. The tape shall just touch the skin but not compressing the soft tissue (it should allow one finger between the tape and the subject's body).

5.3.4 Hip circumference

The subject should stand erect with arms at the sides, feet together and weight equally divided over both legs, only dressed in underwear. The measurement should be taken at the maximum circumference over the buttocks with a non-stretchable tape. The tape should be kept horizontal, even, not twisted with the measurement scale facing outward. The assessor will be instructed to ensure that the tape is just touching the skin but not compressing the soft tissue.

5.4 Biological sampling

Samples for immediate laboratory analysis and for biobanking are obtained at the same time during Visit 1. Blood (max 100 ml) will be drawn from each subject.

Restrictions

- Subjects should be fasting overnight (at least 8 hours). Only water is allowed.

5.4.1 Clinical chemistry and hematology

A capillary blood sample is taken and analysed for p-Glucose using a HemoCue. The value is noted in the eCRF. Subjects with elevated p-glucose (≥7.0 mmol/L) will have the measurement repeated during a later visit. This does not apply for subjects previously diagnosed with diabetes.

Venous blood samples for immediate laboratory analysis of the following parameters are taken: Hb, p-Glucose, HbA1c, s-TG, s-Cholesterol, LDL, HDL, creatinine and hsCRP.

5.4.2 Biobanking

Venous blood and spot-urine for biobanking are processed and stored in collaboration with the local Biobank facility. The practical procedures have been set up in collaboration with the Swedish Biobanking and Biomolecular Resources Research Infrastructure (BBMRI.se) using their standardized routines for handling and storage. The procedure to access biobank samples from the national SCAPIS resource will be developed in collaboration with the Network for National Biobanking Services. A research project in order to ensure comparability and long-term sample quality will be issued in collaboration with SciLife Lab and the Network for National Biobanking Services.

As a quality marker, time from sampling to freezer is recorded i.e. time points for sampling and storage are noted/logged.

All biobank samples are labelled with a code linked to the donor's personal identification number. In order to ensure complete traceability of samples and related information, all codes and pre-analytical steps are controlled by the Biobank facility LIMS (Laboratory Information Management System).

Table 2 Number of tubes (4 ml) or aliquots (225 µl) to be delivered to the national biobank resource

Sample type	Volume
Whole blood for DNA extraction (EDTA)	1 x 4 ml
Plasma (EDTA)	12 x 225 µl
Plasma (Li-Hep)	12 x 225 µl
Plasma (Na-Citrate)	6 x 225 µl
Serum	12 x 225 µl
Urine	12 x 225 µl

Table 2 specifies the amount of samples to be used as a national resource. Additional samples may be stored locally to ensure that each site has access to samples from their own cohort for local projects. The total sampling volume of 100 ml blood should not be exceeded.

5.5 Function tests relating to the lung

Equipment

- Jaeger Spirometer (spirometry)
- Jaeger MasterScreen PFT (CO uptake/spirometry)
- The gases used for single-breath determination of CO uptake are carbon monoxide 0.3%, oxygen and helium or methane/acetylene, according to ATS/ERS guidelines [20].

Pharmaceuticals

400 µg salbutamol (Ventoline Evohaler with spacer)

Restrictions

- Myocardial infarction within 1 month before examination

Conditions where suboptimal spirometry results are likely:

- Chest or abdominal pain of any cause
- Abdominal or thoracic surgery during the last month
- Oral or facial pain exacerbated by a mouthpiece
- Stress incontinence

5.5.1 Spirometry

Procedure

In total 400 µg salbutamol is administered via spacer, in four doses of 100 µg. After each dose of 100 µg has been administered in the spacer, the subject should be instructed to inhale deeply and then hold their breath for 5-10 s. The procedure is repeated four times.

Dynamic spirometry is performed at least 15 minutes after inhalation of salbutamol using a nose clamp with the subject in sitting position. Slow vital capacity (SVC), forced

expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and maximal expiratory flow (MEF) are measured. The procedures are performed according to ATS/ERS standards [18, 19].

The test procedure starts with evaluation of SVC. The test is begun by instructing the subject in the VC manoeuvre and demonstrating the appropriate technique. The manoeuvre is not forced; it is performed in a relaxed manner, except near end-inspiration and end-expiration. The subject is instructed to breathe normally (stable tidal breathing, 3-4 breaths), then exhales completely to residual volume (RV), then inhales to total lung capacity (TLC), and finally exhales to RV again.

Three acceptable measurements are required out of maximum 8 attempts. The resulting value is the maximum value from the accepted attempts. Acceptable reproducibility is defined as a difference between the highest and second highest value of ≤150 ml.

After a period of normal breathing (stable tidal breathing, 3-4 breaths), the FVC manoeuvre is started with a full inhalation to TLC, immediately followed by a forced expiration until no more air can be expelled while maintaining an upright posture.

Three acceptable measurements are required out of maximum 8 attempts. For each variable, the resulting value is the maximum value from the accepted attempts. Acceptable reproducibility is defined as a difference between the highest and second highest value of ≤150 mL (or ≤100 ml if FVC is ≤1 L).

5.5.2 Single-breath determination of carbon monoxide uptake

Procedure
Carbon monoxide (CO) uptake (DL_{CO}) is measured using the single Breath Method according to the ATS/ERS standards [20]. The primary measured variable is DL_{CO}/VA and the CO uptake (DL_{CO}) is obtained by multiplication with the alveolar volume (VA). The subject will be in a sitting position using a nose clamp. The subject starts with normal breathing (stable tidal breathing, 3-4 breaths), followed by unforced, maximal expiration to RV and then a rapid inhalation to TLC (<2.5 s) and breath holding followed by a full exhalation. The breath holding must be in the interval 8-10 s. The inspired vital capacity (VIN) should be at least 85% of the highest VC.

In case VIN is only 80-84% of VCmax, the measurement can still be accepted but a comment should be entered in the eCRF. Measurements with VIN <80% of Vmax should be discarded.

In case the inhalation is ≥2.5 s but <4 s, the measurement can still be accepted but a comment should be entered in the eCRF. Measurements with inhalation ≥4 s should be discarded.

Two acceptable measurements are required out of maximum 5 attempts. The resulting value is the mean of these two. Acceptable reproducibility is defined as a difference of <10% between the measurements. At least 4 min should be allowed between tests to allow an adequate elimination of test gas from the lungs.

5.6 Questionnaire

Equipment
Device with internet access.

Restrictions
- Not applicable

Procedure
An extensive questionnaire separated in validated sets relating to dimensions central to the research aims. Detailed information on diet, environmental and lifestyle factors as well as data on psychosocial well-being, socioeconomic status (SES) and other social determinants is collected.

The questionnaire is completed in an electronic system. All subjects receive unique login details. The questionnaire can be completed during the visit, or afterwards at home.

5.7 Accelerometry

Equipment
Actigraph Activity Monitor GT3X-BT accelerometer.

Restrictions
- Not applicable

Procedure
To get detailed information on physical activity pattern and estimates of daily energy expenditure each subject will be equipped with an accelerometer that picks up movements in all directions and also information on sedentary bouts. The accelerometer is worn for 7 days on the right side of the hip, attached to an elastic belt.

To use the most refined analysis methods, accelerometry data from all sites will be analyzed at the same time at the end of the study by accelerometry expertise at the Swedish School of Sport and Health Sciences (GIH). Therefore, each site will regularly send complete raw-data (.gt3x-files) to GIH for quality control. GIH will store the data, with a copy at a repository at Gothenburg University.

5.8 ECG

Equipment
Standard 12-lead ECG equipment with possibility to store data electronically

Restrictions
- Should be performed before administration of β-stimulants (spirometry) and β-blockers (CT evaluation) or on a different day.

Procedure
The subject should be instructed to remove all clothes from the upper body. Electrode patches are positioned on the chest cage and extremities (distal placement). Three minutes of supine rest with eyes closed should then be allowed for heart rate stabilization and

standardization. In all, subjects will be rested in supine position for a total of approximately 5 minutes before the recording is commenced.

The ECG recording is stored electronically at each site. Heart rate recorded during the ECG measurement is noted in the eCRF.

5.9 Blood pressure

5.9.1 Brachial arterial blood pressure

Equipment
Omron M10-IT

Restrictions

- Should be performed before administration of β-stimulants (spirometry) and β-blockers (CT evaluation) or on a different day

Procedure
Brachial arterial blood pressure should be obtained by automatic measurement in both arms. Systolic and diastolic pressure is registered in supine position and after 5 minutes rest. Cuff size should be adjusted according to arm circumference. The cuff should be in level with the heart. Registration should be repeated with at least one minute between measurements, i.e. after the cuff has been emptied until the next registration is started. If the two results differ (>10 mmHg) (in the same arm, either for systolic or diastolic pressure), the measurement should be repeated until two subsequent results are within ±10 mmHg with a maximum of four attempts. If the difference is still >10 mmHg, the last two measurements should be used. Subjects will receive no information about the results until all measurements are completed. From the two stable registrations the mean value is calculated automatically by the eCRF system.

5.9.2 Ankle brachial index

Equipment
Hadeco Bidop ES-100V3

Restrictions

- Should be performed before administration of β-stimulants (spirometry) and β-blockers (CT evaluation) or on a different day
- Painful leg ulcers

Procedure
Manual measurement of brachial arterial blood pressure should be performed in the arm with the highest automatic systolic pressure. The measurement is performed twice using a Doppler pulse sensor with the subject in supine position. The blood pressure cuff is inflated until the pulse in the artery measured by the sensor ceases. The cuff is then slowly deflated. When the pulse is re-detected through the sensor the pressure in the cuff at that moment indicates the systolic pressure. If the two results differ (>10 mmHg), the measurement should be repeated until two subsequent results are within ±10 mmHg with a maximum of four attempts. If the difference is still >10 mmHg, the last two measurements should be used. Subjects will receive no information about the results until all

measurements are completed. From the two stable registrations the mean value is calculated automatically by the eCRF system.

Ankle arterial blood pressure should be measured bilaterally using a Doppler pulse sensor with the subject in supine position. Systolic blood pressure is measured manually in the dorsalis pedis and posterior tibial arteries. A blood pressure cuff is positioned low down on the calf and inflated until the pulse in the artery measured by the sensor ceases. The cuff is then slowly deflated. When the pulse is re-detected through the sensor the pressure in the cuff at that moment indicates the systolic pressure of the artery. After the cuff has been completely deflated, the measurement is repeated. If the two results (from the same point of measurement) differ >10 mmHg, the measurement should be repeated until two subsequent results are within ±10 mmHg with a maximum of four attempts. If the difference is still >10 mmHg, the last two measurements should be used. Subjects will receive no information about the results until all measurements are completed. From the two stable registrations the mean value is calculated automatically by the eCRF system.

Ankle brachial index (ABI) is used to evaluate peripheral artery disease and is calculated as the ratio between ankle and brachial systolic pressures. This is done in the eCRF when the ankle and brachial pressures are entered.

5.10 Imaging tests using computed tomography (CT)

CT will be performed using a state-of-the-art multi-slice scanner that allows the acquisition of high-resolution volume data with low radiation dose. CT imaging will be used for evaluation of body composition, lung tissue, coronary artery calcium score (CACS) and coronary computed tomography angiography (CCTA). Images will be obtained in the following order:

- Lateral and frontal reference images
- Scans for body composition
- Thorax scan
- Dual Energy scan over the liver
- CACS over the heart
- CCTA scan

The radiation dose should be kept as low as possible and adhere to the approved decision by the local radiation committee.

Equipment
Siemens Definition Flash 2x128 slice, stellar-detector.
4D-Care dose, Care-kV and SAFIRE.

Restrictions

- Subjects will be fasting 4 hours prior to intake of a standardized meal, which should be consumed 2 hours before the examination.
- CACS will not be measured in case of:
  - Presence of cardiac stent
  - Previous by-pass surgery
- Restrictions for administration of pharmaceuticals are listed below (5.10.4).

5.10.1 Body composition

Vascular and lung imaging will be combined with CT imaging of epicardial, intra-abdominal, intramuscular and intrahepatic fat deposits. In addition, the liver will be imaged using dual-energy CT; this approach allows for identification of excess iron stores in the liver, a sign of steatosis.

Procedures
Using the CT reference image, three cross-sectional images will be selected. The first slice will be positioned at the mid-thigh level, measured between the lateral point of the acetabulum and the lateral knee joint, the second at the fourth lumbar vertebra level (L4), and the third is a DE-scan at the level of right and left liver lobe including lung and spleen tissue. A fourth slice at the same liver position will be calculated from the lung volume scan.

Image interpretation and analyses of outcome variables
Liver density will be measured as the mean CT number within three circular regions placed in the dorsal aspects of the liver and attempts will be made to avoid blood vessels, artefacts, and areas of inhomogeneity. Tissue areas of the abdomen and of the thigh will be determined as the area of all pixels within specific CT number intervals, e.g. for adipose tissue (AT) -190 to -30 HU and for muscle tissue (MT) -29 to +151 HU.

5.10.2 Lung tissue

Early structural changes in lung tissue will be imaged using a low-dose spiral CT scan over the full lung volume. This provides information on airway wall thickness and emphysema and thereby essential information in the phenotyping of COPD.

Procedures
The subject should be coached to achieve and hold full inspiration, with several practice attempts prior to scan. CT scanning will be performed during a breath hold. For the lung characterization, it is important that all subjects achieve similar inspiratory and expiratory levels. No contrast media will be used.

Scanning will be performed from apex to base of lungs. Field of view should be selected to fit the entire lung without any part of the lung being cut off in inspiration or exspiration.

Image interpretation and analyses of outcome variables
Images will be analyzed using the Syngovia CT Pulmo 3D software to calculate the percentage of parenchyma with an attenuation below -950 HU. In addition to the automatic quantitative characterization of the lung, manual visual scoring will be performed. Visual scoring of the images will be performed to assess emphysema and airways according to score sheet used in the COPD Gene study (www.copdgene.org).

5.10.3 Coronary artery calcium score (CACS)

Procedures
The CACS scan is planned using the lung image. Two different CACS protocols are available. A flash spiral protocol will be used for subjects with a body weight ≤90 kg and a regular heart rate (CACS 1). For all others a sequential protocol (CACS 2) will be used.

Image interpretation and analyses of outcome variables
Calcium content in each artery will be measured with the scoring system previously described by Agatston et al [21].

5.10.4 Coronary computed tomography angiography (CCTA)

The coronary circulation will be assessed directly with CCTA using contrast media injection and ECG-gated imaging.

Procedures
In order to increase image quality, metoprolol will be administered to the subject to lower the pulse rate, striving for a rate ≤60 beats/min. Pulse rate should be stable. The stability is evaluated using the automated planning system for ECG triggering (flash test). Metoprolol is administered per orally 1.5-2 hours prior to the examination (up to 100 mg) and/or intravenously just prior to the examination (up to 15 mg). Dosing schedule is decided by local clinical routines.

Suggested dosing schedule:

- Oral metoprolol 1.5-2 hrs. prior to examination
  - 25 mg p.o if systolic BP >110mm Hg and HR 50-59 bpm
  - 50 mg p.o if systolic BP >110mm Hg and HR ≥60 bpm
  - 100 mg p.o if systolic BP >110mm Hg and HR >65 bpm (depending on local clinical routine)
- Intravenous metoprolol 1 mg/ml
  - 2.5 mg i.v. if systolic BP >110mm Hg and HR 50-59 bpm
  - 5 mg i.v. if systolic BP >110mm Hg and HR ≥60 bpm

Administration with caution if systolic BP <110 mm Hg.
In case the participant is already on beta-receptor blocking therapy the above will still be applied.

Metoprolol will not be administered in case of:

- symptomatic bradycardia with HR < 45 bpm
- asthma (treated)
- AV block; 2nd and 3rd degree, without bradycardia protection with pacemaker device
- allergy to metoprolol or other beta-receptor blocking drugs

Glyceryl nitrate will be administered in order to achieve dilatation of the vessels. If additional metoprolol is considered according to the above, caution should be taken that BP does not contraindicate administration of glyceryl nitrate.

Dosing schedule:

- Glyceryl nitrate 0.4 mg/dose:
  - Two doses sublingually if systolic BP >110 mm Hg
  - Administration with caution in case of systolic BP <110 mm Hg

  - Glyceryl nitrate will not be given if the subject has been treated with sildenafil/tadalafil/vardenafil within the last 48 hours
  - An interval of 4 min should be allowed between administration and CCTA scan

Contrast media will be administered according to the following (summarized in Figure 1):

- Omnipaque 350 mg I/ml:
  - 325 mg I/kg body weight, injection rate 12 sec
  - Contrast media will not be given to subjects if they have:
    - Previous allergy to contrast media
    - Untreated hyperthyroidism or untreated thyroid cancer
    - Impaired renal function as indicated by estimated glomerular filtration rate (eGFR, Lund-Malmö equation [22]) <50 ml/min/1.73m^2
    - eGFR 50-59 ml/min/1.73m^2 and at least one of the following risk factors:
      - Diabetes mellitus
      - BP >150/95
      - Ongoing hypertensive treatment
      - Ongoing diuretic treatment
  - Subjects with an eGFR value of 50-59 ml/min/1.73m^2 receiving contrast media should return for additional blood sampling and analysis of s-creatinine between 48 hours and 5 days after administration. In case of a >5% increase in s-creatinine, further follow-up will be decided by a study physician on an individual basis. If the subject declines this additional blood sample, CT will be performed without administration of contrast media.
  - Subjects currently treated with metformin, for whom contrast media administration is not contraindicated, will be asked to discontinue treatment on the day of the CT scan examination. An s-creatinine sample should be taken 48 hours after contrast media administration. Metformin treatment is re-started if the s-creatinine value is within <5% increase. In case of >5% increase, further follow-up will be decided by a study physician on an individual basis. If a subject declines discontinuation of metformin treatment or additional blood sampling, CT will be performed without administration of contrast media.

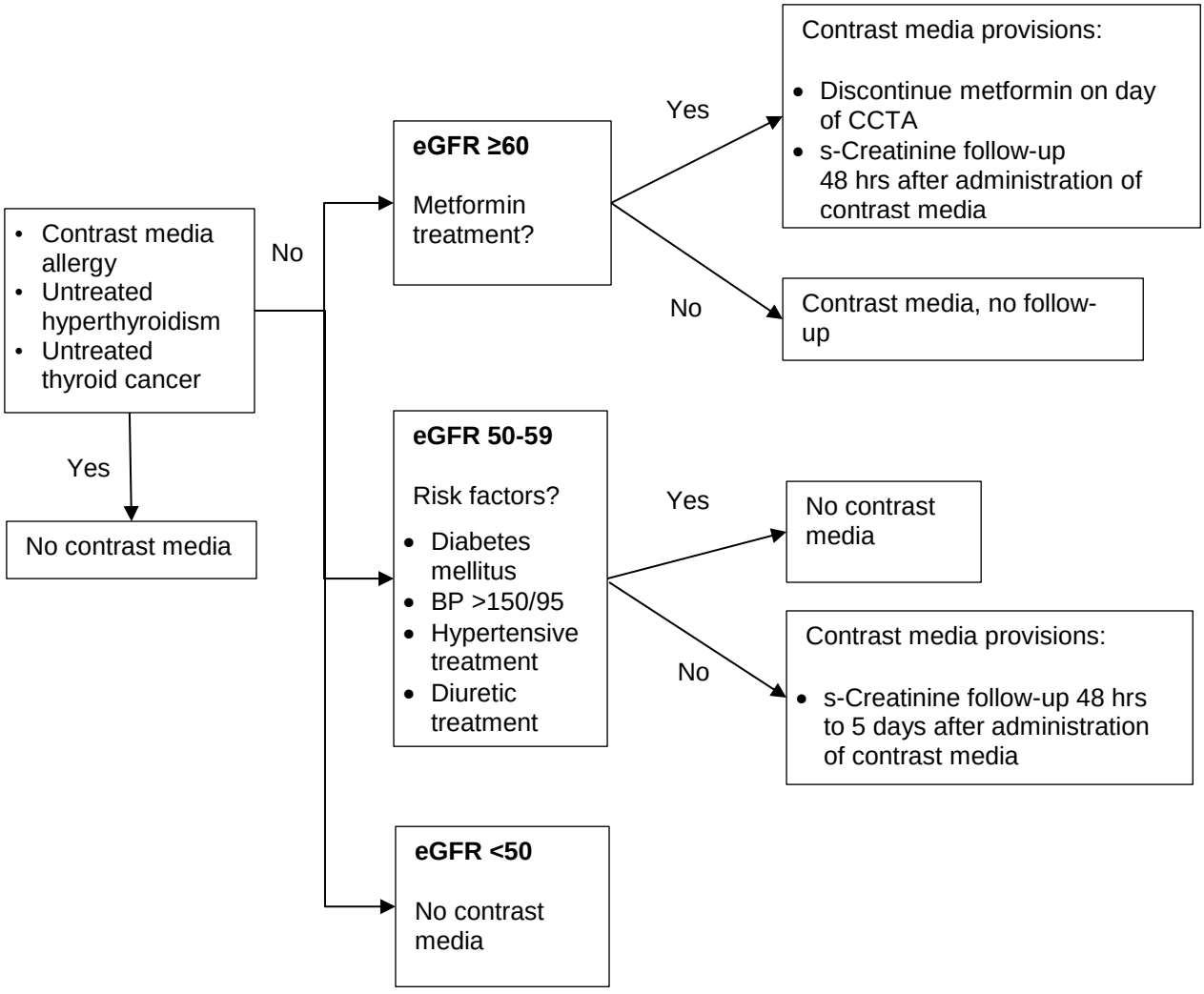

Figure 1 Flow-chart for administration of contrast media for CCTA

CT scanning will be performed during a breath hold. Five CCTA protocols are used:

CCTA 1: A flash scan will be used if body weight is ≤85 kg, no calcification is present at CACS and heart rate is steady and ≤60 beats/min. Scan length should be ≤12 cm.

If the conditions for CCTA 1 are not fulfilled, the following alternatives will be used:

CCTA 2: A sequence mode will be chosen if heart rate is stable, ≤70 beats/min and if the CT system can deliver a sufficient radiation dose for desired image quality.

CCTA 3: A sequence mode with padding will be used if the heart rate is slightly irregular (e.g. premature ventricular contractions) and/or >70 beats/min. The protocol is chosen if the CT system can deliver a sufficient radiation dose for desired image quality.

CCTA 4: If the dose marker indicates that dose limits are reached, either a) a sequence mode with prolonged rotation time (0.35 s) or b) a spiral CT with dual source function for higher scan dose will be used.

CCTA 5: A full spiral CT with dose modulation will be used if subject has an irregular heartbeat (e.g. atrial fibrillation).

Image interpretation and analyses of outcome variables
Each coronary vessel will be identified with a multiplanar reconstruction technique. Plaque characteristics will be analyzed on a per-segment basis according to the modified American Heart Association classification. Plaques will be defined as structures within and/or adjacent to the vessel lumen, which are clearly distinguished from the lumen and surrounding pericardial tissue.

Plaques are defined as: calcified if the plaque area is occupied by calcified tissue (density 130 HU in native scans); mixed if the plaque area is both calcified and without calcium; and non-calcified if they are completely without calcium.

5.11 Imaging tests using ultrasound examination

Ultrasound [5, 23] is the best validated non-invasive technique to detect, quantify and stage subclinical atherosclerosis in the carotid arteries. In addition to plaque size and number, the grey-scale of the 2D plaque image and its distribution can be used to differentiate homogenous vs. heterogeneous structures and low vs. high echogenicity.

Equipment
Siemens Acuson S2000.

Restrictions
- Should be performed before administration of β-stimulants (spirometry) or on a different day

Procedure
The carotid arteries are scanned bilaterally as follows:

Velocity of the blood is measured by Doppler (angle 60°, or if not possible, the angle should be below 45°) in the common carotid artery (CCA), the internal carotid artery (ICA), the external carotid artery (ECA) and the vertebral artery (VA). Images demonstrating the maximal velocities from each vessel are saved.

For measurements of intima-media thickness (IMT) in the CCA, two frozen images captured from two separate approximately 10s motion cine-loops are saved. One 5s cine-loop should also be saved, as identical as possible with the frozen images. An optimal image should show the vessel horizontally with distinct intima-media echoes from both the near and the far walls, in at least one centimetre just proximal to the beginning of the bulb. Approximately 2 cm of the distal CCA and 1 cm of the proximal bulb should be visualized in the image.

For measurements of IMT in the bulb, two frozen images captured from two separate approximately 10s motion cine-loops are saved. One 5s cine-loop should also be saved, as identical as possible with the frozen images. An optimal image should show the vessel

horizontally with a distinct intima-media echo from the far wall, in at least one centimetre just distal to the beginning of the bulb. Approximately 1 cm of the distal CCA as well as the flow divider between the ICA and ECA should be visualized in the image. Note that both vessels do not need to be visualized. One 5 s motion loop from the ICA is saved.

If the subject has bradycardia or arrhythmia, the motion cine-loops are prolonged to 10 s.

If plaque is present (CCA, bulb or ICA), one frozen image captured from an approximately 10s motion cine-loop is saved for each plaque, where the plaque is clearly visible with a clear echo from the opposite wall. Further, the area, height and length of each plaque are measured. These measurements are done on the frozen image, and the images are saved.

Also for each plaque, a transversal, frozen image captured from an approximately 10s motion cine-loop is saved with plaque height measured.

Occurrence of shadowing due to calcification should be assessed. If part of a plaque is invisible due to acoustic shadowing, the degree of the shadowing should be assessed (< 25%, 25-50%, 50-75% or >75% of the plaque not visible).

Occurrence of ulceration should be assessed. An ulceration is defined as a rupture in the plaque surface, at least one millimetre deep, with turbulent flow detected with pulsed doppler. If plaque/plaques larger than 10 mm^2 is present, two frozen images with pre-defined GSM setting are to be captured from an approximately 10s motion cine-loop from at least one plaque on each side. One image should be with area marking and one image without marking. The image should be of amble quality so one can perform a marking around the plaque.

For the GSM-images the pre-set program (GSM) stored in the device should be used: TGC shall be straight and 2D gain shall be set as low as possible, but with the plaque visible.

If a plaque produces a stenosis, the grade should be assessed for further process. This should be done according to local criteria.

Image interpretation and analyses of outcome variables
An initial image analysis is performed offline, where IMT is measured bilaterally in the CCA and bulb, respectively.

An additional more in-depth image analysis is performed offline, where area, percentage white (PW) and grey scale median (GSM) of the plaque are assessed.

5.12 Imaging tests using Magnetic Resonance Imaging (MRI)

Subjects with at least one carotid plaque in Bulb or ICA with a plaque height of ≥2.7 mm, detected during the ultrasound examination, are asked to participate in an additional visit for MRI. The Carotid MRI examination is designed to answer the following questions:

1. Is there an association between the occurrence of morphological vulnerable plaques on MRI in the carotid arteries and plaques detected on CT in the coronary arteries?
2. What are the morphological plaque characteristics on MRI examination of those individuals showing the highest expected risk, estimated from survey data, biometrics and biochemical profile?

3. Is the expected risk higher (based on survey data, biometrics, biochemical profile) in those individuals having the most vulnerable plaques on MRI, based on current knowledge in plaque vulnerability using MRI?
4. What is the natural course for individuals with large carotid plaques in a cohort study?
5. What is the prospective value of carotid MRI in an asymptomatic cohort?

Equipment
A 3T MRI scanner with a dedicated surface coil for carotid examinations should be used. All examinations within one site should be performed with the same scanner and coil. Dedicated analysis software will be adapted to the different vendors of scanners and coils to produce similar results across sites.

Eligibility criteria
A list of subjects suitable for MRI will be automatically generated from the SCAPIS study database weekly according to the below criteria. The list is available for down-load by dedicated SCAPIS staff from the SCAPIS DataMart (https://scapisdata.wlab.gu.se). Subjects should be consecutively recruited from the list after considering the additional restrictions for MRI listed below. If not all subjects eligible to participate can be scheduled for MRI, a local procedure for random selection of subjects should be in place.

The following inclusion criteria are used from eCRF data:

1. Plaque of height ≥2.7 mm in bulb or ICA

The following exclusion criteria are used from eCRF data:

1. Need for Swedish interpreter
2. CTA has not been performed in SCAPIS
3. Reaction to contrast agent at CTA in SCAPIS
4. Estimated GFR from creatinine <60 ml/min

Restrictions
Additional restrictions before recruitment that need to be considered

- Previous reaction to gadolinium
- Claustrophobia
- Metallic foreign bodies
- Battery driven devices

The local site (local physician in charge of the MR scanner) has the final responsibility for the safe use of MRI.

Pharmaceuticals
Gadolinium contrast (Gadoteric Acid)

Procedures
The examination should be scheduled 2-8 weeks after the CTA examination has been performed.

MRI examination is performed in a supine position with the neck/head fixed in the coil. The coil elements are carefully placed over the bifurcation with minimal air-gap. The subjects are informed to lie still during the examination.

Each site is responsible for adaptation of their examination protocol, in order to generate optimal images for the analysis of the variables described below.

To enable segmentation of plaque components, the following general imaging concept should be implemented at each site:

- Imaging is centered over the flow divider on the index side (with the largest plaque as verified by US examination) and FOV is placed giving an even distribution over bulb and proximal ICA (covering at least ±18 mm from flow divider).
- Axial images should be perpendicular to the longitudinal direction of the table. Axial images in 2D are used for analysis.
- A bolus dose of gadolinium contrast is injected in a large brachial vein followed by 10 ml saline. Images are taken at 3 minutes after injection.

The following sequences should be included:

- A time of flight (TOF) angiography that can be viewed/reconstructed in ≤2 mm slices is used for visualization of the lumen and any ulcerations communicating with the lumen.
- To analyze plaque components, a T1-weighted, black blood sequence before and after gadolinium contrast is used. The sequence should generate 2 mm axial slices. Coverage should be at least ±18 mm from flow divider.
- To achieve maximum sensitivity for detection of intra-plaque hemorrhage (IPH, methemoglobin) a T1-weighted inversion recovery (IR-) based gradient echo sequence with a very rapid echo time is used. Coverage should be at least ±18 mm from flow divider.

Additional sequences for analyses of other plaque components can be run as local optional examinations at each site but must not interfere with the core protocol, which is to be run first not to tire the subject.

The on-line documentation in the eCRF serves to monitor the flow of subjects at each site. To do this the following data should be entered.

- Subject selected from eCRF (yes/no)
- Subject has contraindications for MRI (yes/no)
- Subject consented to participate in MRI examination (yes/no)
- MRI examination finalized (yes/no)
- Date
- Acceptable image quality for plaque analyses (yes/no)

Image interpretation and analyses of outcome variables
The examination results should be reviewed for safety within 4 weeks to find any pathology outside the artery vessels.

Image analysis will be performed at a central core laboratory. The following variables will be analyzed:

Maximum plaque height	[mm]

For every MRI slice:

Outer wall area	[mm2]
Lumen area	[mm2]
LRNC area	[mm2]
Calcification area	[mm2]
IPH area	[mm2]
Ruptured/invisible cap	[1/0]
Ulceration	[1/0]
Normal vessel wall without plaque	[1/0]

Calculated:

Normalized plaque volume ((outer wall – inner wall)/outer wall)
%LRNC
%Calcifications
%IPH
Minimum lumen area

6. STUDY FOLLOW-UP USING NATIONAL POPULATION REGISTRIES

Endpoints (MI or cardiac interventions, stroke and exacerbation of COPD) will be identified by linking the unique Swedish personal identification numbers to the Swedish National Hospital Discharge Register and the Swedish Cause of Death Register and other national registers such as the Pharmaceutical Register of Prescribed Drugs. The external and internal validity of these registers is high [24]. Data from national quality registers (e.g. SWEDHEART, Riks-STROKE) will be used to get more detailed information on the type of event and interventions used.

Information on home addresses over several decades will be combined with historical emission databases and dispersion models to obtain annual exposure levels for traffic pollution and from heating.

7. ETHICAL CONSIDERATIONS

SCAPIS has been evaluated and approved by the ethics committee as a multi-centre study (Umeå, February 21, 2011). The local radiation committees approve the use of radiation. The Data Inspection Board has given advice on data handling. In addition to these formal

approvals SCAPIS strives to broaden the knowledge base on ethical aspects of performing large scale epidemiological studies with detailed phenotyping. SCAPIS has therefore initiated a scientific program addressing these issues. Some specific considerations regarding ethical aspects of SCAPIS are discussed below:

Potential adverse effects of the radiation dose: Radiation has the potential to cause adverse events if large groups of patients are screened. The negative effects of radiation dose have to be balanced with the possibly life-saving clinical findings that can be made and the potential of new scientific discoveries. The dose of radiation used in the pilot trial was a median of 4.4 mSv for all imaging in each subject.

Management of pathological and incidental findings: The examinations will identify cases of diseases that are the focus of this study, cases of other diseases (incidental findings) and cases with unclear findings. This issue requires balancing of benefits, risks and costs to the subject, the health care system and the SCAPIS organization.

An evaluation process of all subjects found to have serious disease or those in need of risk factor intervention is required to avoid unnecessary, potentially dangerous clinical evaluations of subjects (e.g. invasive angiography) based on false positive findings as well as assure expedient handling of life-threatening disease. Details are found in section 8 Clinical Follow-up.

Adverse drug reactions: The subjects in SCAPIS will be exposed to contrast agents for CCTA as well as β-blockers and nitroglycerine before imaging. Bronchodilator will be administered in connection with the lung function tests. In the pilot study, adverse reactions occurred in 2% (n=22) of subjects, and those were mostly minor in character and all resolved.

8. CLINICAL FOLLOW-UP

Site specific instructions on handling of clinical findings should be agreed upon and documented at each site based on agreement by the national study physician committee. The general recommendations for clinical follow-up are as follows:

- Findings such as elevated plasma glucose levels, blood pressure elevation and ECG findings will be assessed by the study physician and if needed, communicated to the subjects.
- A first review of results from all investigations should be performed within 2 weeks.
- In case of pathological findings needing urgent clinical management (e.g. lung cancer, advanced cardiac disease on CCTA), subjects should be immediately informed and referred as appropriate.
- SCAPIS specific recommendations for handling of coronary findings, abnormal lung function and findings of pulmonary nodules have been issued by advisory expert groups and are found in Appendices E-G.
- Subjects who have isolated risk factors or deviating blood samples should be informed within 8 weeks of their last visit, according to guidelines agreed upon by the study physician committee.

- Moderate elevations in risk factors will be assessed by the study physician and, as a rule, be managed by asking the subject to contact his or her primary care physician.
- All subjects should receive a summary report on weight, BMI, blood pressure, pulse, accelerometry data, blood glucose, serum lipids and creatinine within 8 weeks of their last visit.

9. DATA MANAGEMENT

9.1 Recording of data

The investigator will ensure that all data collected in the study, are recorded in a timely manner according to any instructions provided.

Study data will be entered into a central database at the study site through electronic case report forms (eCRF) using the Trial-on-Line system (Replior). The database will be hosted in a secure professional hosting facility with audited appropriate physical and logical security levels for the stored data.

In case of database/eCRF failure, the data may be recorded on a back-up paper CRF. CRF data will be entered by study staff. The person entering the data will also sign the completed page electronically. The electronic questionnaires, will be completed on a device with internet access by study subjects.

The following safety measures will be taken to secure data quality:

- The electronic questionnaires, to be completed on computer by study subjects at the examination visits, will be designed to make it as easy as possible for the subject to reply to all questions.
- If subjects or study staff forget or intentionally try to skip a question, the program will give a signal and ask for completion. As the subject is free to refuse to answer any specific question, there will be a possibility for the subject to tick the following answer: 'I do not wish to or am unable to answer the question'.
- For all continuous variables, probability ranges will be used. If, at data entry, the value is outside this range, the program will give a signal and ask for verification.
- During the course of the study, the presence and distribution of all eCRF variables will be checked and compared intra-individually to detect any extreme outliers or obvious errors. Questionnaire data entered by the subjects themselves will not be analysed/cleaned/excluded from the database through any similar procedure.

9.1.1 Source data

Source data could be medical records, working sheets or eCRF. This might differ between sites, and will be defined in a source data document.

9.2 Data storage and distribution

Data generated within SCAPIS need to be available both to health care providers for clinical follow-up and to research groups. SCAPIS is designed to allow documentation of investigations and sharing of data across different research organizations. Therefore common storage areas for data from SCAPIS (eCRF data, laboratory reports, ECG and

lung function results, clinical CT data, CT raw data, carotis ultrasound and MRI data) will be set-up. The clinical data will be stored to easily be available to health care providers, while raw data, used only for research purpose, will be stored with less accessibility. However, the storage systems will follow national guidelines for patient security and integrity for all data.

9.2.1 Core Image Analysis Laboratories

The design of the study will need extensive image analysis, which will be done in two stages:

The **local site** will be responsible for immediate (within two weeks) image analyses of clinically relevant findings and writing of reports to the study physician. Quality assured semi-quantitative image evaluation for research purposes will be done on-line.

To develop procedures for in-depth analysis of the image material, SCAPIS is collaborating with a number of Swedish research networks and laboratories dedicated to this process.

10. STATISTICS

10.1 Sample calculation

The current size of the cohort is based on calculations of critical number of events needed for prospective analysis. Based on available Swedish statistics (www.socialstyrelsen.se/statistik), approximately 500 endpoints (i.e. 200 nonfatal or fatal MIs, 80 nonfatal and fatal ischemic strokes and 220 fatal or severe non-fatal COPD exacerbations) will occur in our cohort of 30,000 subjects within a period of 2 years.

11. QUALITY MANAGEMENT

11.1 Qualifications

All investigations will be performed by dedicated study staff. Study staff should be qualified to perform delegated tasks and be trained in study procedures.

11.2 Procedures for quality management

A quality management structure should be set up at each study site to ensure compliance with the study protocol and adequate training of study personnel. Detailed written instructions should be available for each investigation. All documentation related to study conduct should be clearly identifiable by the following information: document title, date and version number.

Investigations should always be performed according to the latest version of the appropriate instruction. Adherence to the instructions should be followed-up with regular intervals (2-3 times/year) and updates ensured whenever needed. Quality control activities should be documented.

All informed consents will be checked and the identity of the subject who signed the consent form will be verified. In addition, completion of critical variables will be confirmed.

12. INSURANCE

Subjects participating in SCAPIS are managed within the health care system and thus covered by the Patient Insurance according to the Swedish Patient Injury Act.

13. PROJECT ORGANISATION AND INFRASTRUCTURE

13.1 National organisation

SCAPIS is led by a Steering Committee comprising representatives from the six participating universities: Lund, Gothenburg, Linköping, Stockholm, Uppsala, and Umeå. The universities have endorsed the members from different specialities relevant to the study. The Steering Committee has the overall scientific and fiscal responsibility for SCAPIS.

13.2 Local organisations

Each university representative will organize a local steering group with competence in cardiology, pulmonary medicine and radiology.

13.3 Report and publications

SCAPIS will be a national resource for decades of future research. The scientific analysis to achieve the main objectives of SCAPIS will be organized by working groups. The working groups will develop publication plans within their research area to ensure timely delivery of high quality research. SCAPIS will also be open to national and international researchers after application to and subsequent approval by a scientific committee. The committee will evaluate proposals for new studies using the SCAPIS biobank and data repository, in order to keep track of and prevent overlap between presentations, publications and reports emanating from the study. The framework for the publication work is further described in a separate publication policy.

14. STUDY TIMETABLE

The study started in Gothenburg in November 2013. Each site will have 3 years to recruit approximately 5000 subjects and the study is expected to be completed by the end of 2018.

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