

## DETAILED INFORMATION ON STUDY MEASURES AND TESTS

### Visit 1 Vessels: Raine Study House and School of Human Sciences (Exercise and Sports Science) UWA 4 to 5 hours – parking provided

#### Fasting

Please arrive at the Raine Study assessment appointment fasted, which means having nothing to eat or drink (besides water) after 10pm the previous evening. This means no breakfast, coffee, tea, soft drink, alcohol or cigarettes. This is because certain blood tests (e.g. glucose and triglycerides) require a fasting blood sample.

Please do not have any recreational drugs in the 24 hours before the assessment as these can affect the vascular tests.

#### Dress

We ask that you wear a separate top and bottom, loose fitting shorts are preferred (we will have some available). Some of the tests require blood pressure cuffs on the skin of the upper leg, lifting your top up (waist measurements), and removing your top and putting on a gown (breast scan), this is easier to do if you are not wearing a dress or one-piece outfit. The visit will include an exercise test, so bring clothing and footwear suitable for exercise.

Testing Procedure	Testing Details	Estimated* Time Commitment
<b>Consent</b>	A researcher will provide details regarding the procedures included in the study and obtain your informed written consent.	10 mins
<b>Anthropometric Measures</b>	Obtain your height, weight, waist, and hip measurements.	7 mins
<b>Blood Pressure</b>	Whilst lying quietly on a bed, your blood pressure will be collected 10 times (once every 2 minutes over a 20 minute period).	20 mins
<b>Pulse Wave Velocity</b>	A blood pressure cuff and Doppler probe will be used simultaneously to measure your blood flow velocity.	15 mins
<b>Blood Test</b>	A blood sample will be collected from your arm.	5 mins
<b>Brain Blood Vessel Function (Cerebrovascular)</b>	Blood flow to your brain will be measured using Doppler probes placed on each temple. A variety of tests will be carried out.	70 mins
<b>Limb Blood Vessel Function (Peripheral Vascular Function)</b>	Blood vessels in your upper arm, thigh and neck will be scanned using ultrasound. A variety of tests will be carried out.	60 mins
<b>Heart Echocardiogram</b>	Your heart will be scanned using ultrasound	15-30 mins
<b>Breakfast</b>	A selection of breakfast food items will be available.	15-20 mins
<b>Dual Energy X-Ray Absorptiometry (DEXA)</b>	A whole body DEXA scan will measure your body composition.	10 mins
	An additional breast DEXA scan for females only.	10 mins
<b>Breast scan (TiBs)</b>	Optical Breast Spectroscopy-Light scan for females only.	15 mins
<b>Fitness (VO<sub>2</sub>max Test)</b>	An exercise test (exercise bike or treadmill) to measure your oxygen use with increasing exercise intensity.	10 mins
<b>Debrief</b>	Review of your results and provision of feedback.	5-10 mins

## Appendix 6 Detailed information on study measures and tests

### **Consent: 10 mins**

The Raine Study research assistant will explain the procedures and answer any questions you may have and obtain your signed informed consent. We will provide you with a copy of your signed consent form. We want you to understand that you can choose not to do any one or all of the tests, and that you can withdraw from the testing at any time.

### **Anthropometry: 5-7mins**

- Height: measured by a stadiometer, no shoes, and heels against the wall.
- Weight: measured by chair scales (remove shoes, heavy clothing, and contents of pockets).
- Waist: measured by tape measure across the belly button (over light clothing) on the skin at expiration. Two measures are taken and recorded in cm.
- Hip circumference: measured by tape measure around the body over the widest part of the buttocks (over light clothing) and recorded in cm.

### **Blood pressure and heart rate: (Dinamap): 20 mins**

- You will be sitting and the appropriate cuff size will be fitted to your right arm.
- After 5 minutes quiet rest 6 automatic recordings will be taken every 2 minutes.
- The average of the last 5 readings of blood pressure will be calculated.

### **Pulse wave velocity: 15 mins**

This test measures aortic stiffness, arterial stiffness and central blood pressure. Lying down for approximately 20 minutes, blood pressure cuffs on your upper arm and thigh will periodically inflate (in the same way as we measure blood pressure or as normally performed at your doctors). A small non-invasive pressure sensitive probe will be placed against the skin at the location of the strongest pulse in your neck.

#### Potential Risks

*This assessment will feel similar to a typical clinical blood pressure assessment. A slight 'squeeze' will be felt when the blood pressure cuff inflates, and may cause temporary discomfort in your arm or leg. Also a slight discomfort and/or pressure may be felt when the probe is applied to your neck. There are no short term or long term side effects associated with this procedure.*

### **Blood test: 5 mins**

- Our Raine Study phlebotomist will take a blood sample from your arm. You will be lying down during this procedure. The total quantity of blood drawn will be 40 ml. This is a small amount relative to a 'unit' of blood given at a standard blood donation (~440 mL in a single sitting).
- We will test your blood for a full blood count, cholesterol, glucose, insulin, inflammatory markers, kidney and liver function and vitamin D. The results of some of these blood tests will be sent to you. If the results are outside of the normal range we will advise you to see your GP.

Potential Risks Generally, pain or discomfort associated with the taking of blood is very minor. A mild sting and there is a possibility of bruising near the puncture site afterwards. There is minimal risk of infection when performed under sterile conditions. There is a slight possibility that participants with needle phobia may experience a dizzy spell during the routine blood tests. You can lay down during blood collection. If this has happened to you before, or you have a particular aversion to needles, please inform the staff. There are no long-term adverse effects associated with dizzy spells.

### Urine sample:

When you arrive we will give you a little jar and ask that when you need to go to the toilet, you collect a urine sample in the jar, put the lid on tightly and then put it in our specimen fridge. Urinary sodium and potassium will be measured.

### Brain Blood Flow (Cerebrovascular) Function: 70 mins

Three different tests will be carried out: neurovascular coupling, cerebral flow mediated dilation (cFMD) and cerebro-autoregulation. During these tests, you will wear a head frame, similar to a bicycle helmet, which is mounted with two Doppler probes, one on each temple.

- In some instances, ultrasound will be used to simultaneously measure blood flow through the blood vessels in the neck. Ultrasound is the same technology used to monitor unborn babies and is considered completely safe with no known side effects.
- You will wear a facemask, similar to a scuba mask, and breathe a gas mixture containing specific concentrations of carbon dioxide.
- After a short rest time, an eye movement test and a memory test will be conducted.

### More details:

#### Test 1 - Neurovascular Coupling

Following 10 minutes of supine rest in a dimly lit room, you will be asked to stare eyes open at a handheld computer screen with a flashing checker board pattern for a 2 minute baseline period. Following the 2 minute eyes open baseline, you will then rest quietly with your eyes closed for 2 minutes. You will then open and close your eyes and stare at the checkerboard device for five 30-second cycles. The standardized visual stimulation protocol is expected to increase blood flow in the posterior circulation of the brain by approximately 10%. This test will be followed by a computer-based cognitive test (i.e., cog-state).

#### Test 2 - Cerebral Flow Mediated Dilation

This assessment provides an indication of the function and health of the internal carotid artery (ICA), specifically its dilator responsiveness to an imposed shear stress stimulus, the latter induced by carbon dioxide (CO<sub>2</sub>) inhalation and cerebrovascular hypercapnia (relatively high CO<sub>2</sub> levels). After 10 minutes of supine rest, you will breathe through a mouthpiece connected to a three way valve, first breathing room air for a 2 minute baseline period. Baseline ICA images will be collected prior to turning the three way valve. The valve will then be turned so that the participant will be breathing from a Douglas bag containing 21% O<sub>2</sub> oxygen, 6% CO<sub>2</sub> carbon dioxide, and balanced N<sub>2</sub> nitrogen, for 3 minutes (normal air composition is 21% O<sub>2</sub> oxygen, 0.03% CO<sub>2</sub> dioxide and 78 % N<sub>2</sub> nitrogen and mixture of other gases). Following the 3 minutes of breathing from the Douglas bag, the three-way-valve will be turned to room air again while ultrasound images are continuously collected for 3 minutes of recovery. This is a safe protocol and has been approved by the UWA's committee on multiple occasions.

#### Test 3 - Cerebral-autoregulation

Following a 10 minute resting period, you will be asked to stand for a 1 minute baseline period prior to performing a series of half squat to stand manoeuvres (hand to knees and back up) at a frequency of 0.06 Hz for a total of 5 minutes.

### Potential Risks

During some of these tests, you may experience a mild breathlessness and/or light-headedness when breathing through the mouthpiece. However, this quickly resolves and is completely reversible. These procedures are safe and we will continuously monitor your blood pressure. You will be thoroughly briefed beforehand and reminded that you can remove the mask or cease the testing at any time. When you are breathing from a bag containing normal levels of oxygen but a higher than normal level of carbon dioxide this typically causes people to breathe deeper and faster than normal, but the effect goes away quickly following removal of the bag.

### **Limb Blood Vessel (Peripheral Vascular) Function: 60 mins**

- During these tests, we will perform three measures that will assess the properties (e.g. blood flow, diameter, wall thickness and function) of the brachial artery in the upper arm and femoral artery in the upper thigh. These include: resting blood vessel properties, flow mediated dilation (FMD) and glyceryl trinitrate (GTN).
- Images will also be obtained of the carotid arteries on both the left and right side of the neck.
- We will monitor your blood pressure using an automated machine periodically throughout these tests.

#### **More Details:**

##### Test 1 - Resting Blood Vessel Properties

During these tests we will simply hold an ultrasound probe on the skin of your arm and the leg. The resting scans on the neck (carotid arteries) will follow an identical procedure. This involves holding an ultrasound probe against the skin for a short duration, during which resting blood vessel properties will be measured. You should not feel any sensation other than slight pressure on the skin during this procedure.

##### Test 2 - Flow Mediated Dilation (FMD)

An ultrasound device will be used to capture images and blood flow of the large artery in the arm (brachial) and leg (femoral). Inflatable cuffs (similar to a blood pressure cuff) will be placed around the forearm and the thigh, and will be inflated simultaneously for exactly five minutes. The cuffs will then be deflated, causing a large increase in blood flow into the forearm and leg, allowing us to measure what is known as shear stress (frictional drag force of blood against the vessel wall). We will continue to observe the image of the artery for a further three minutes. The results of this test will be indicative of the health and function of the arteries.

##### Test 3 - Glyceryl Trinitrate (GTN)

A single dose of a drug called glyceryl trinitrate (GTN) will be sprayed under the tongue while the arteries are imaged. GTN causes blood vessels to relax and widen. It is a commonly used drug in people with heart disease. Throughout these tests we will monitor your blood pressure using an automated blood pressure machine.

### Potential Risks

The pressure induced by cuffs during the test may cause mild discomfort in the hand, fingers, forearm and/or thigh. You will feel a 'squeeze' similar to that of routine blood pressure assessment, and possible 'numbing' or 'tingling' sensation in the hand, fingers, forearm and/or lower leg. These resolve rapidly and spontaneously following cuff deflation. If this test is painful for you, please inform the investigators immediately and we will stop the test.

There is a small risk that the (glyceryl trinitrate) GTN spray may cause minor side effects such as mildly increased/decreased heart rate, headache, light-headedness and/or nausea. Around 1 in 20 people may experience some of these effects. These effects resolve rapidly and spontaneously, although any persistent headache can be managed using paracetamol. You cannot use GTN if you are using any of the blood pressure lowering medications and Viagra-type drugs.

We will ask questions about the medications you take when we book you in and during consent process.

**If you are taking these, or any other forms of medication, please let us know**

## Heart Scan (Echocardiogram) 15-30

This assessment involves placing an ultrasound transducer (probe) onto the chest to visualise the heart, whilst you are lying on a bed. Your heart will be visualised from 2 or 3 positions to create an overall assessment of dimensions and function. These positions are on the skin mid-chest near the sternum and at the side of the chest, as well as from below the sternum looking up at the heart. The time taken to perform the assessment will vary from approximately 15-30 minutes. Upper body clothing will need to be removed we will provide you with a garment like a large netball bib will minimize chest exposure.

### Potential Risks

*This test uses ultrasound and is non-invasive.* You will however be required to partially remove clothing from the torso to allow imaging of the heart. At no time will you be fully exposed as we will provide a garment to help minimize chest exposure.

## DEXA scan: 10 to 20 mins

We are asking you to have a whole body DEXA scan and females an additional breast DEXA scan on the Lunar iDXA Advance Densitometer GE Healthcare DEXA Bone Densitometer - The iDXA is located in the upper level of the Exercise and Sport Science Building (near the CV Research Lab). You will receive approximately 0.1 micro Sieverts of radiation from the whole body scan and an additional low dose of micro Sieverts of radiation for the breast DEXA scan. To provide a comparison with other sources of radiation, an airline flight from Darwin to Perth results in exposure to 16 micro Sieverts and a normal chest x-ray exposes you to approximately 40 micro Sieverts.

- The whole body DEXA scanning process takes approximately 10 minutes. The scan will measure body composition (fat, bone and lean mass) and bone density. You will need to remove shoes and any metal objects (jewelry, belts, contents of pockets, piercings) and lie on the scanner bed for up to 10 mins.
- The Breast scan DEXA scan process takes an additional 10 mins (females only) with jewelry shoes etc already removed you will be asked to put on a hospital gown and remove your bra. You will lay on your side and a short scan will be completed of the upper torso on the right, left, then right again. The skin will be exposed and utmost care will be taken to support your modesty/privacy.

Anyone who is pregnant or thinks they may be pregnant will not be scanned. We will provide you with results from the whole body DEXA scan giving you details of your bone density and the percentage of body fat and lean mass (muscles, organs).

#### Potential Risks

The DEXA machine emits a very low dose of radiation, less than that experienced during high altitude airplane flight. The radiation exposure for whole body scan is equal to 0.1  $\mu$ Sv per scan and an additional low dose for the breast scan. This low dose x-ray is equivalent to about one thousandth of the background radiation received in one year of living in Perth.

#### **Optical Breast Scan (females only): 10-15 mins**

Breast density is usually measured using mammography which is not generally recommended to women under the age of 40 due to unnecessary radiation exposure. Some women have more dense tissue and others have more fatty tissue in their breasts.

We would like to scan the breasts of Raine Study females with a method called Transillumination Breast Spectroscopy (TiBS) to ascertain how much fatty tissue and how much dense tissue there is in breasts in this age group. This consists of shining an infra-red light or laser light through the breast.

It is not painful in any way and it does not cause any harm or any side effects. You need to change out of your bra and top into a hospital gown which we will provide. The Raine Study research assistant will help you select a special 'cup' which is the closest to your breast size. This has a device fitted to it, which shines a light from one side to the other. You hold the cup over your breast, and the light shines through your breast from one side to the other, and measures your breast density. This takes less than one minute. However, the whole procedure including changing, selecting the right size cup, holding it under the hospital gown takes about 10 to 15 minutes. The TiBS measurements are taken in a separate private assessment room in Raine Study House.

TiBS is not a diagnostic procedure; it just measures the density of the breast. If anything is seen on the scan that may look abnormal, arrangements will be made for you to see Professor Christobel Saunders, a breast specialist in Perth's multidisciplinary breast clinic at no personal expense. Anyone who has had breast enlargement, reduction or removal surgery should not perform this scan, so if you have had any other breast-related procedures, please let the Raine Study research assistant know.

#### **VO<sub>2</sub> max Fitness Test: 10-15 mins**

- Your physical fitness will then be measured while you perform an incremental exercise test using a specialized machine that measures oxygen use.
- This will require you to breath into a mouthpiece similar to a snorkel, while the speed and gradient of the treadmill or exercise bike are increased.
- The test concludes when either you feel you cannot continue any more or you have reached the desired workload.

#### Potential Risks

During the fitness test, you may experience a certain level of discomfort such as leg fatigue, increased heart and breathing rate, which are all expected responses from this type of activity.

This is a test designed to assess your maximal tolerance for exercise. You will be closely monitored throughout the test, for measures such as heart rate and breathing. We will also ask you repeatedly if you are comfortable to continue the exercise. You can stop the test at any time, simply by telling us that you wish to stop.

## **Debrief: 5-10 mins**

At the end of the examination we will discuss your results with you and provide you with results from; whole body dxa scan, anthropometric measures, blood pressure and aortic stiffness tests. We will email your blood test results and any other reports if needed.

## **Questionnaires**

Questionnaires will be available online through the Qualtrics or at the appointment. We will email you unique links and request you to complete the questionnaires. All data is stored on secure UWA servers accessible only by the Raine Study data staff.

We can post or give you paper versions of the questionnaires and/or access to a computer at your appointment if you request it. Data entry of any paper questionnaires will be done by a Raine Study research assistant onto the Qualtrics

**Participant questionnaire** to provide background in relation to occupation, income, education, exercise, general health and wellbeing including stress and anxiety, general diet, alcohol, smoking and drug taking, can either be done online prior to coming in, online at the appointment or on paper by request. This questionnaire takes about **30-60 minutes** to complete.

**Participant medical questionnaire** to provide information relating to your medical history. This is short and takes 5 minutes to complete at the appointment.

**Reproductive health questionnaire (females only)** to provide information relating to female reproductive health. This is short and takes 5 minutes to complete at the appointment.

## Appendix 6 Detailed information on study measures and tests

### Visit 2: Lions Eye Institute, 2 Verdun Street, Nedlands, Western Australia 6009

#### 2 hours - parking provided

You will be asked to attend the Lions Eye Institute (LEI) clinic on one occasion to undertake the eye tests. Please bring along any prescription glasses and avoid wearing contact lenses to the session if possible. Please bring a pair of sunglasses to wear after the session as your pupils will be dilated, which will make you more sensitive to light.

Testing Procedure	Testing Details	Estimated* Time Commitment
<b>Consent</b>	We will provide details regarding the procedures included in the study and obtain your informed written consent.	5-7 mins
<b>Sun exposures measures on the skin</b>	Mole count of the right arm and skin impression of the back of your hand	5 min
<b>Autorefraction</b>	An automated machine will determine whether your refractive error (short/long-sighted/astigmatism).	1 min
<b>Spectacle lens measurement</b>	Measurement of the refractive power of your spectacles (if any)	1 min
<b>Visual acuity</b>	Measurement of vision by reading the letters on a chart.	4 min
<b>Axial length</b>	A machine will measure the length of your eye.	5 min
<b>Conjunctival autofluorescence</b>	Photographs of the front of the eye will be taken to look for early signs of sun (ultraviolet)-related damage.	4 min
<b>Iris colour photography</b>	Photographs of the front of your eyes will be taken.	4 min
<b>Intra ocular pressure (eye pressure)</b>	Measurement of the pressure of the fluid in your eye by applying a tiny probe that very gently touches the front of your eye.	1 min
<b>Pupil dilation</b>	Eye drops will be applied to the eye to dilate the pupils for better quality of scans of the inside of the eye.	2 min
<b>Wide-field fundus Photography</b>	A special camera will take a photograph of the inside of the eye, including the retina and optic nerve.	3 min
<b>Optical coherence tomography</b>	A machine will take high-resolution scans and thickness measurements of the layers of the retina and nerve fibres inside your eye.	10 min
<b>Anterior segment tomography</b>	A machine will perform scans of the front of your eyes to measure the shape and thickness of the cornea, and thickness of the lens inside the eye. The pupils need to be dilated.	5 min
<b>Auto-refraction (post dilation)</b>	A second auto-refraction will be done after the pupils are dilated.	2 min

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Testing Procedure	Testing Details	Estimated* Time Commitment
<b>Non-invasive retinal blood vessels imaging</b>	A machine will capture images of your retinal blood vessels.	10 min
<b>High resolution retinal photography</b>	A machine will take high magnification images of the inside of your eye that will allow us to visualise the photo-sensitive cells and the thickness of the walls of the blood vessels.	20 min
<b>Debrief</b>	At the end of the examination results will be discussed and you will be provided with an eye report, and advised on results and whether they might need glasses.	10 min
<b>Total</b>		<b>100 min +</b>

These are estimates of time commitments. Testing may sometimes take longer than this due to transition between stations.

### During the appointment at LEI

We will ask you to do several different eye tests. The eye tests will be set up in a series of rooms at LEI, and the Raine Study research assistant will greet you and direct you, along with the LEI staff. The tests will be done by ophthalmologists and other trained eye specialists.

#### Consent: 5-7 min

- The Raine Study research assistant will meet you and explain the procedures for the vision assessment and answer any questions you may have and obtain your signed informed consent. You will be provided with a copy of your signed consent form. We want you to understand that you can choose not to do any or all of the tests, and that you can withdraw from the testing at any time.

#### Sun damage on the skin: 5 min

We have gathered these measurements before and they are an important part of your history relating to sun exposure and the research we are doing on sun damage. We last collected these measure the 22 year follow up.

- We will count the number of moles (dark spots that are visible when squinting) on your right arm, front and back, from hand to shoulder.
- We will apply a quick drying (2 mins) dental impression paste to the back of the right hand to gain an impression of the sun damage.

#### Potential risks

You might feel a slight pull on the hairs on the back of your hand as the skin impression is removed. There are no short or long term side effects associated with this test.

#### Auto-refraction (pre and post-dilation): 1 min

- This scan is commonly used in optometry and ophthalmology clinics to provide a quick summary of the non-refractive status and to obtain corneal curvature measurements, pre and post-dilation. You will be asked to be seated and place your head on a chin rest and look at a blurry image of an air balloon inside the instrument while the machine takes the scan of one eye at a time.

#### Potential risks

You might feel a little uncomfortable if the height of the instrument is too high or too low for you, but we will adjust the height of the instrument according to your preferred height. There are no short or long term side effects associated with this test.

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### Spectacle lens measurement: 1 min

- An automated machine will measure the optical correction type and strength of your glasses (if any). You will be asked to provide the operator with any prescription glasses that you wear for a quick measurement.

#### Potential risks

This test will cause no physical discomfort to you. There is a very slight chance that your glasses may be scratched during the process, similar to that when your optometrist or nurse handles glasses. The experienced operator will handle your glasses with maximal care.

### Visual acuity: 4 min

- This is the vision test that is used in all optometry and ophthalmology clinics, as well as during driver licensing testing, to determine your level of central vision. You will be asked to identify letters of decreasing size on a letter chart using one eye while the other eye is covered, wearing your own glasses for distant viewing (if any). Following which, you will be asked to read down the chart again but this time with pinholes placed in front of the tested eye. This procedure is then repeated for the other eye.

#### Potential risks

Your eyes might feel a little tired reading down the chart. However, this test will only take only a few minutes. There are no short or long term side effects associated with the test.

### Axial length: 5 min

- This is a measurement of the length of your eyes and corneal curvature. You will be asked to be seated and place your head on a chin rest and look at an orange/yellow light inside the machine. You will be asked to keep your eyes as still as possible, and not to blink while the scans are being taken, so as to maximize the quality of the scans and speed up the process of the measurement.

Potential risks you might feel a little tired from the scans as you will need to keep still during most of the test. The best effort will be taken to ensure that you are comfortable in your seating position to minimize discomfort. There are no short or long term side effects associated with this test

### Conjunctival autofluorescence: 4 min

- A photograph of the front of your eye will be taken with a camera fitted with a UV filter, which will allow us to detect areas of sun damage at the front of the eye (the 'whites of the eye'). In the photo, the sun-damaged cells in that area will appear as brighter (more fluorescent) patches on a blue background relative to the undamaged parts. You will be asked to be seated and place your head on a chin rest, looking straight ahead at the camera lens. The pictures need to be taken in the dark; we will therefore need to switch off the lights just before the photos are taken. There will be blue flashes from the camera while the photos are being captured.

#### Potential risks

You might feel a little uncomfortable when the lights are switched off for the photos to be taken in the dark. Please inform the operator if you do not feel comfortable when lights are switched off. The procedure will only take a few minutes and the lights will be switched back on as soon as all the pictures are taken. You might perceive some after-images and blurry vision from the camera flash, but those will fade in a few minutes. There are no short or long term side effects associated with this test.

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### Iris colour photography: 4 min

- Three close-up photographs of the front of each eye will be taken with a colour camera to document your eye colour, as well as any sun-related changes at the front of the eye, such as (yellow/white deposits) or pterygia (benign growth of transparent tissue of the conjunctiva). You will be asked to be seated and place

your head on a chin rest. While the photos are being captured, there will be flashes from the camera like the type you see from a normal camera.

#### Potential risks

You might perceive some after-images and blurry vision from the camera flash, which is at a similar intensity to those of a normal digital camera. Any of these visual symptoms will fade in a few minutes. There are no short or long term side effects associated with this test.

### Intra ocular pressure (eye pressure): 1 min

- Eye pressures are routinely measured in eye clinics and are an important aspect in the evaluation of eye health, particularly for screening for glaucoma. You will be asked to be seated while the operator will gently pull your upper lid up for the measurement. Using a handheld device, a probe will gently touch the surface of your eyes. You might feel a slight ticklish feeling during the quick measurement but it will not hurt. It is important to keep your eyes and head still during the measurement.

#### Potential risks

You might feel a little uncomfortable while the operator pulls your eyelids up and there is a very slight chance that the front of your eyes may be mildly scratched during the assessment. The experienced operators will make every effort to minimize any discomfort and risk of scratches. There are no short or long term side effects associated with this test.

### Pupil dilation - *One drop of tropicamide 1% will be administered*: 2 min

Eye drops will be instilled in your eyes (one drop per eye, once) to dilate your pupils (the opening in the centre of the eyes). This will allow us to scan and take images of the inside of your eyes as part of the eye health examination. These dilating eye drops are routinely used by optometrists and ophthalmologists for the same purpose.

#### Potential risks

There will be a slight stinging pain when the eye drops are instilled which will last for about 20 seconds. It will feel like opening your eyes in the ocean and salty water going into your eyes. Your pupils will remain dilated for a few hours, during which you will be more sensitive to light and it will be harder to read magazines and books, and use your mobile phone. These effects should fade within 3 to 4 hours. We advise that you bring sunglasses to wear when you step outdoors after the examination. Sunglasses will be offered if you did not bring your own. You should NOT drive until your vision is completely cleared.

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### Wide-field fundus Photography: 3 min

- A photograph of the inside of your eyes will be taken as part of the eye health examination. You will be asked to be seated and place your head on a chin and forehead rest with your face pressing against a soft face pad, and with one eye looking through an opening in the face pad. Once the operator gets the alignment and focus of the camera right, the operator will take the photo and there will be camera flash accompanying it.

#### Potential risks

You might feel a little uncomfortable if the height of the instrument is too high or too low for you and from pressing your face against the soft face pad. The height of the instrument will be adjusted to your preferred position and the operators will make every effort to minimize discomfort. You might perceive some after-images and blurry vision from the camera flash, which is at a similar intensity to those of a normal digital camera. Any of these visual symptoms will fade in a few minutes. There are no short or long term side effects associated with this test.

### Optical coherence tomography: 10 min

- High resolution scans of the layers of your retina and optic nerve (the inside of the eye) will be taken. You will be asked to be seated and to rest your head on a chin and forehead rest, with one eye looking at a blue light inside the instrument. The instrument will take multiple scans which it can combine to form three-dimensional images. It is important you keep your eyes and head still during the procedure. It will also be helpful if you do not blink while the scans are being taken to quicken the process.

#### Potential risks

You might feel tired from keeping your eyes and head still during the scan. The experienced operator will make every effort to keep the testing procedure as short as possible. Your eyes might feel dry or watery from not blinking while the scans are being taken. The operator will inform you when you can blink in between scans and lubricating eye drops will be available for your use. There are no short or long term side effects associated with this test.

### Anterior segment tomography: 5 min

- This scan will capture images which will show the shape of your cornea in detail, as well as the thickness of the lens in your eye. You will be asked to be seated and to rest your head on a chin and forehead rest, with one eye looking at an orange light inside the instrument. The instrument will start the scan automatically as soon as your eyes are aligned properly, and the light inside the instrument will start to spin. It is important you keep your eyes and head still, and not to blink during the scan.

#### Potential risks

You might feel a little uncomfortable if the height of the instrument is too high or too low for you, but we will adjust the height of the instrument according to your preferred height. Your eyes might feel dry or watery from not blinking while the scans are being taken. There are no short or long term side effects associated with this test.

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### Non-invasive retinal blood vessels imaging: 10 min

- This scan will allow us to visualize the structures of the blood vessels inside the eye (retina and choroid). You will be asked to be seated and to rest your head on a chin and forehead rest, with one eye looking at a blue light inside the instrument. It is important you keep your eyes and head still during the procedure. It will also be helpful if you do not blink while the scans are being taken to quicken the process.

#### Potential risks

You might feel tired from keeping your eyes and head still during the scan. The experienced operator will make every effort to keep the testing procedure as short as possible. Your eyes might feel dry or watery while the scans are being taken. Lubricating eye drops will be available for your use. There are no short or long term side effects associated with this test.

### High resolution retinal photography: 20 min

- This scan will take high magnification images of the inside of the eye that will allow us to visualise the light-sensitive cells and the thickness of the walls of the blood vessels. You will be asked to be seated and to rest your head on a chin and forehead rest, with one eye looking at a faint white light inside the instrument. The fixation light will change in position about every minute so as to allow the instrument to capture a total of 14 images at different parts of the inside of the eye. It is important you keep your eyes and head still, and do not blink during the scan.

#### Potential risks

You might feel tired from keeping your eyes and head still during the scan. The experienced operator will make every effort to keep the testing procedure as short as possible. Your eyes might feel dry or watery from not blinking while the scans are being taken. The operator will inform you when you can blink in between scans and lubricating eye drops will be available for your use. There are no short or long term side effects associated with this test.

## Appendix 6 Detailed information on study measures and tests

### Contacts

If you would like discuss any aspect of this study please feel free to contact

The Raine Study Gen2: 28 year Vision and Vessels Follow-up Coordinator Diane Wood  
Email: [diane.wood@uwa.edu.au](mailto:diane.wood@uwa.edu.au), Ph: +61 8 6488 6952 or Mob: 0447 863 944.

Vessels Chief Investigator Prof Daniel Green  
Email: [danny.green@uwa.edu.au](mailto:danny.green@uwa.edu.au), Ph:+61 6488 2361

Vision Chief Investigator Prof David Mackey  
Email: [DavidMackey@lei.org.au](mailto:DavidMackey@lei.org.au), Ph:+61 9381 0779