Proton magnetic resonance imaging and spectroscopy: Assessment of abdominal fat and pancreas fat

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Proton magnetic resonance imaging and spectroscopy: Assessment of abdominal fat and pancreas fat

Contributed by: Nathan Johnson, University of Sydney

Study: Novel Exercise Therapies for Type 2 Diabetes (HIIT Diabetes)
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1 Introduction / Background

The location of adipose tissue, particularly in the liver, heart and skeletal muscles appears to be of greater importance than total body fat when determining clinical implications and treatment options for obesity-related disease (1). Whilst most of these “ectopic fat” depots have been extensively researched, the same cannot be said for fat found in the pancreas, which is situated underneath fat of the abdominal cavity, making in vivo studies difficult (2).

Recent advances in non-invasive imaging techniques have increased the capacity for human research on human pancreas fat (3). Emerging evidence suggests that “fatty pancreas” may contribute significantly to the underlying cardiovascular and metabolic dysfunction observed in obesity and related type 2 diabetes (4,5), including beta cell dysfunction (6,7), and that diet intervention alone or in combination with bariatric surgery, may reduce pancreas fat and improve pancreatic function (8-11).

This standard operating procedure (SOP) describes the instructions for MRI and proton magnetic resonance spectroscopy (1H-MRS) scanning for abdominal fat and pancreas fat determination for participants in the Novel Exercise Therapies for Type 2 Diabetes (HIIT Diabetes) research trial.

1.1 Terminology and abbreviations

- $^{1}$H-MRS – proton magnetic resonance spectroscopy
- T2 – spin-spin or transverse relaxation time
- CH$_2$ – methylene
- ppm – parts per million
- T – Tesla
- MRI – magnetic resonance imaging

2 Key papers / theoretical basis for the method

2.1.1 Theoretical background

The development of non-invasive proton magnetic resonance spectroscopy (1H-MRS) has led to significant advances in the measurement of fat content within organs. The described 1H-MRS is undertaken using a clinical MRI scanner (usually 1.5 or 3.0 Tesla), which involves using strong magnetic fields and radiowaves to detect signal from protons within a tissue. This is typically undertaken by a specialist “provider” i.e. private or hospital/research-based radiology clinic.

Together with protons from water within a tissue, methylene protons (-CH$_2$-) of triglyceride acyl chains resonating at ~ 1.3 ppm account for the majority of proton signal acquired from tissues such as the liver and pancreas by localized 1H-MRS at 1.5T (12). The triglyceride concentration within the tissue can be quantified as the ratio of methylene:water signal (13, 6).
Detailed information concerning the development of the method and reliability/validity can be found in relevant literature including: (12, 13). Whilst localised proton spectroscopy is increasingly recognised as the most reliable non-invasive method to quantify organ fat, the technique can be confounded by movement artefact (particularly in the pancreas), and T2 effects. Consideration of these issues can be found elsewhere in the scientific literature e.g. (14).

2.1.2 References


3 Ethical considerations

MRI involves exposure to high magnetic fields, but no ionising radiation. There are no known clinical risks associated with this technique. However, there is a risk of claustrophobia in the scanner and some people may be contraindicated from MRI scanning if they are pregnant or have any ‘ferrous’ metal materials within their body, in particular: a cardiac pacemaker, metal clips on a cerebral aneurysm, metal particles in the eye or joint replacements.

These issues should be discussed and informed consent obtained by the specialist radiography staff prior to scanning.

Please see Appendices 9.1 and 9.2 for the Participant Information Sheet and Participant Consent forms.

4 Facility and equipment

The described measurement technique requires a whole body MRI scanner and appropriate hardware, including surface coil, respiratory gating as necessary and software and specialist staff to acquire localised spectra.

The method described for determination of pancreatic fat for participants in the Novel Exercise Therapies for Type 2 Diabetes (HIIT Diabetes) research trial is for a 1.5T Philips Medical Systems Achieva scanner at Specialist MRI Suite G4-6 RPAH Medical Centre, 100 Carillon Avenue Newtown NSW Australia. Acquisition protocol and sequences may need to be modified to suit the scanner and clinic, as necessary. In all cases acquisition parameters should remain unchanged during the course of a research study.
4.1 Testing facility requirements

As required by the clinic to perform clinical MRI scanning.

4.2 Equipment

- 1.5T Philips Medical Systems Achieva scanner (Best Netherlands).
- Other equipment as required by the clinic performing clinical MRI scanning and localised spectroscopy.

4.2.1 Personal Protective Equipment (PPE)

As required by the clinic and radiography staff performing clinical MRI scanning.

5 Training/qualifications/competencies

Yes ☒ No ☐

Formal qualification required

If yes, please provide details: See Section 6 below.

6 Restricted access

The described method is to be implemented by radiography staff with appropriate training and qualifications in MRI.

7 Health and safety / Risk Assessment

All scanning should be preceded by appropriate risk assessment. This should be undertaken by radiography staff using site-specific approach/material.

For the research trial Novel Exercise Therapies for Type 2 Diabetes (HIIT Diabetes), refer to Risk Assessment and Management procedures of provider:

- Specialist Magnetic Resonance Imaging
  RPAH Medical Centre
  100 Carillon Ave
  Newtown NSW 2042
8 Workflow

8.1 Before the participant arrives

- The participant should follow any pre-scanning procedures instructed by the radiology clinic/provider.
- There is no definitive evidence that pre-scanning diet needs to be controlled for prior to pancreatic fat assessment but participants may be advised to refrain from eating for 2 h prior to scanning.

8.2 Procedures

1. Patient risk assessment and consent.

2. Following participant preparation and instruction, the participant should be escorted into the room and placed on the scanner. The standard operating procedure for the Novel Exercise Therapies for Type 2 Diabetes (HIIT Diabetes) research trial is detailed below. Radiographers may use this approach, with adjustment for site-specific needs/hardware/software differences as necessary:

3. File entry: patient with true name, description is “HIIT Diabetes (baseline or 12 weeks)”, comment line include visit number and study ID

4. Positioning (MRI): head first, supine, arms over head

5. Scanning 1: survey (Q body) centre between diaphragm and crests

6. T1 axial (Q body) (arms up breath held)
   6.1 ~ 20-30 slices, top liver to iliac crests (thickness 10mm, gap 10mm)
   6.2 multiple breath holds

7. Re-positioning: arms by side
   7.1 Surface coil (Flex-L) on abdomen
   7.2 Centre pt to coil
   7.3 Respiratory bellows on
   7.4 FOV optimised for anatomy (but consistent between visits for same pt)

8. Scanning 2: survey (arms down)

9. T1 axial (Q body) arms down breath-hold

10. Spectro (non-water suppressed) free-breathing triggered (32 NSA/measurements)
10.1 SV PRESS TR 3000 Te (40), respiratory trigger, samples 1024, spectral band width 1000

10.2 Voxel (10x20x10) position along head of pancreas

10.3 Volume shim (shim size larger than voxel e.g. 15x25x15) “Shim align” - ON

10.4 Visual inspection of voxel position (screen capture) and aim to replicate on subsequent visits

8.3 Post-test care

As per standard protocol for the clinic

8.4 Data handling and management

1. Post-processing: run basic scripts to confirm spectroscopy worked
   1.1 Select - Spectro view / script / select “RUN”
   1.2 Visually inspect 2 peaks and resolution

2. If ‘error’ in spectrum re-run steps 9.1-9.3, may need re-positioning of voxel

3. Copy imaging and spectro data to study external hard-drive

8.5 Reporting of information

The workflow describes the operating procedure for the provision of spectroscopy data for research purposes. An example of a de-identified report provided to a research patient can be found in Appendix 9.3. There is currently no accepted clinical application for this technique and thus reporting of the spectroscopy result is not required by a supervising physician (Radiologist). However, the Radiologist may report the abdominal examination on the basis of MRI images/localising images at the provider’s discretion.
9 Appendices
9.1 Participant Information Sheet

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NOVEL EXERCISE THERAPIES FOR TYPE 2 DIABETES

PARTICIPANT INFORMATION STATEMENT

(1) What is the study about?

You are invited to take part in research that will study the effects of different exercise therapies on your health and fitness. People with diabetes are at risk of developing damage to their blood vessels and tissues which can lead to increased risk of complications and heart disease. Some studies have shown that regular exercise can help to reduce tissue fat levels, improve glucose control, blood flow and fitness. However the optimal type and dose of exercise for health improvement in people with type 2 diabetes is unclear.

The purpose of this research is to examine the effect of different exercise therapies on cardio-metabolic outcomes in individuals with diabetes. The results of this research will help to direct public health guidelines for exercise intervention in the management of diabetes and the associated health complications.

(2) Who is carrying out the study?

The study is being conducted by Dr Nathan Johnson, Dr Michael Baker, Dr Vivienne Chuter, Ms Shelley Keating, Mr James Gerofli, Mr Sean Lanting, Ms Kimberley Way, Professor Stephen Twigg, Mr Angelo Sabag and Ms Rachelle Sultana. The research will be undertaken at the Charles Perkins Centre, University of Sydney (Camperdown).

(3) What does the study involve?

Participant’s involvement requires:

- Willingness to participate for 3 months (12 weeks) in an exercise program and;
- Three assessments in total. These include: baseline, three months (at the completion of the training) and a follow up assessment at six months (three months after completion of the training [optional]);
- Participation may involve attending supervised exercise sessions (3 times per week) at the Charles Perkins Centre for 3 months.

In order to participate in this study you must:

- be 18-65 years of age and have been diagnosed with type 2 diabetes
- have a body mass index between 30-45
- not currently undertake exercise on 3 or more days per week, or >150 minutes of exercise per week
- be free of any medical condition which makes you unsuitable for exercise. These may include:
  - Chest pain
  - Unstable blood sugar levels
  - Unstable abnormal heart rhythms
  - Unstable hypertension
Assessment & Screening

If you agree to participate in this study, you will be asked to sign the Participant Consent Form and to complete questionnaires regarding your current health. You will first be examined by the study doctor and given a full physical examination to check that it is safe for you to undertake an exercise program. Blood will be collected to measure your glucose control and other routine blood measures. You will also be required to have an electrocardiograph (ECG) reading both at rest and during treadmill walking exercise (stress test). This involves placement of leads on your body to get a reading of the electrical activity of your heart. This should not cause any discomfort, but you will experience fatigue during the treadmill walking assessment. You will also undertake assessments of your body fat and muscle, and tests of your blood vessel function.

There is a small risk of experiencing Delayed Onset Muscle Soreness (DOMS) post-treadmill testing. This may be experienced as muscle discomfort 24-48 hours after the exercise bout. However, this is a common and transient response in previously inactive people which gradually lessens after this period, and this type of treadmill testing is routinely used in inactive individuals who perform stress testing. The regular training bouts (3 days per week for 12 weeks) will be performed on the stationary cycle which has negligible eccentric muscle activity. Risk of musculoskeletal injury will be further reduced by providing the participant with an individualized exercise prescription, tailored to their own exercise capacity.

An outline of the assessment and screening process is provided in the table below:

<table>
<thead>
<tr>
<th>Week: Day</th>
<th>Tests and exercise</th>
<th>Time Required</th>
<th>Location</th>
</tr>
</thead>
</table>
| 1: 1      | Baseline screening (including informed consent, physician screening)  
Fasting blood test  
DEXA scanning  
Vascular function test | 150 min       | Charles Perkins Centre        |
| 2         | MRI scanning  
Exercise test | 40 min  
45 min | SMRI at the RPA Medical Centre  
Charles Perkins Centre |
| 3         | Exercise training | 20- 60 min | Charles Perkins Centre        |
| 2-11: 1,2,3 | Exercise training | 20- 60 min | Charles Perkins Centre        |
| 12: 1     | Exercise training | 20- 60 min | Charles Perkins Centre        |
| 2         | Post intervention assessments  
Fasting blood test  
DEXA scanning  
Vascular function tests | 120 min       | Charles Perkins Centre        |
| 3         | MRI scanning  
Exercise test | 40 min  
45 min | SMRI at the RPA Medical Centre  
Charles Perkins Centre |
| 6 month follow up | Post intervention assessments  
Fasting blood test  
DEXA scanning  
Vascular function tests | 90 min       | Charles Perkins Centre        |
d. Blood Tests for Metabolic Control

- Blood sampling will be performed to measure blood insulin, glucose, fats, liver function and other markers of metabolic health.

Included in the blood tests will be an analysis of your DNA, which will allow us to investigate variations in known genes which may be associated with diabetes and exercise. The blood test will be used to look at your genotype to identify new genes or mutations in known genes as well as looking at genetic factors that may predict response to exercise therapy. Samples will be stored indefinitely in a -80°C freezer. Blood will be used immediately or stored for studies in the future. Blood will be used to extract DNA. The samples will be used for research and coded to protect your identity (although they are able to be re-identified by researchers so that we can alert people to the fact that they have a genetic disease if that situation arises. All medical information is stored in password protected databases.

Please note: Your provision of a DNA sample is for research purposes only, is optional, and does not constitute clinical genetic testing. You will be asked to provide an 18ml blood sample for this.

e. MRI SCANNING and DEXA

MRI scanning uses a magnetic field to take pictures of your body that can be used to measure levels of fat and muscle. The test involves lying in a scanner for 25-30 min.

DEXA involves the measurement of body composition by X-ray (lying on your back for 10-15 minutes).

f. Tests of Fitness (cycling)

We will test your cycling fitness, and your heart (including ECG) and lung response to 7-15 minutes of cycling. The test starts at a comfortable intensity and gradually increases to a maximal effort. You will experience transient fatigue during the cycle test.

g. Questionnaires

You will be asked to complete several questionnaires which will take approximately 30-60 minutes. The questionnaires are designed to find out how you feel about yourself, how well you sleep, your nutritional intake your activity level, and how you felt when completing the exercise sessions.

h. Activity monitors

You will be asked to wear a small activity monitor on your arm. This will measure your activity over the period of a week and will not interfere with your daily routine or pose any risk.

i. Gut Microbiota Analysis

Stool samples will be collected to analyse the composition of gut microbiota (bacteria). Your gut bacteria can affect the digestion, absorption and metabolism of food, immune system function, hormone regulation and brain function.

You will be asked to collect a stool specimen, record the date, and immediately freeze the sample after collection at your home. The frozen stool samples will be returned to the clinic at baseline, month 3 and month 6 (follow up). You will be provided with detailed instruction, a stool collection kit and hygiene materials. Please note: Your provision of a stool sample is optional.
j. Ambulatory Blood Pressure Monitoring

We request that you wear a portable blood pressure monitoring device for 24 hours on three separate occasions: i) before the intervention period; ii) after one exercise session; iii) and after the intervention period. A blood pressure cuff will be attached to your non-dominant arm, with a small measuring device strapped to your wrist.

The blood pressure cuff will inflate and take a recording every 15 mins during the day (6am-10pm), and every 30 mins during the evening (10pm-6am), which may disrupt your usual sleeping patterns. However, this will only occur on three separate occasions during the 12 week intervention, and is therefore similar to experiencing an occasional poor sleep. You will be advised on how to turn off the device once the 24 hours of monitoring has been completed.

You will not be able to undertake water-based activities (e.g. showering, swimming) while you are wearing the blood pressure monitor. It is recommended that you shower prior to having the blood pressure monitor attached at the Charles Perkins Centre.

Participation

During all study procedures, you will be monitored very closely by qualified and experienced health professionals. You are free to withdraw from the study at any stage, for whatever reason, without affecting your medical care. The doctor and/or investigators in charge of this study may stop the study or stop you taking part in the study, at any time, for any reason, without your consent.

Risks

EXERCISE TESTING AND TRAINING

As with any exercise program, there are possible risks of injury and a small risk of heart attack. Exercise training may also cause some muscle soreness and fatigue. To minimise these risks, we will carefully monitor you throughout your training, prescribe a training program that is in accordance with your physical capabilities and take care to set up the exercise equipment in a manner to maximise your safety. You will be closely supervised by trained and experienced health professionals during all testing procedures.

During each test procedure, and at regular intervals throughout the exercise training program, we will ask you to inform us of any side effects that you may experience. It is important that you contact the study staff immediately if there are any unusual health experiences, injury or bad effects. This notification should take place whether or not you believe that the problem is related to the exercise program or from some other cause. Prior to any testing, the study doctor will review your medical history to make sure that you are medically ready for the study procedures.

BLOOD GLUCOSE (SUGAR) LEVELS

It is possible that during the study test procedures, your blood glucose levels may not be balanced at times. You may not experience any symptoms as a result of this. However, it is possible that you might experience symptoms of hypoglycaemia (low blood sugar levels), which may present as shaking, sweating, weakness and hunger. You will be fully supervised at all times during test procedures. Should you experience symptoms suggestive of hypoglycaemia, the study physician or research assistant will immediately test your blood sugar level with a finger-stick test, and administer an oral concentrated sugar-containing beverage that is rapidly absorbed. You will be monitored closely until it is clear that your blood sugar has returned to normal and any symptoms have resolved.
BLOOD COLLECTION

Blood collection is a very common procedure and causes little discomfort or risk. During the course of taking blood samples, mild pain and/or bruising may occur at the site of the needle entry. The risks and discomforts will be minimised, as this procedure will be performed under sterile conditions by highly experienced staff. The total amount of blood taken during the baseline, post-intervention and follow-up tests is small (four samples of approximately 9 ml each) and will not result in any harm.

OPTIONAL DNA COLLECTION

It is possible, although very unlikely, that someone could get access to your DNA data without permission. As part of this study we might coincidentally find a gene pattern or defect which increases your risk for a disease (e.g. breast cancer). If the condition is treatable or preventable, you can specify on the Consent Form if you want to be informed about such a finding. Learning this information may be upsetting. It could also affect your ability to get life insurance. The information could be used against you in the work setting. Therefore, although we think that there are benefits in having that information, individuals have to weigh up the risks and decide whether or not to receive that information. In the unlikely event that a risk for genetic condition is identified we will help to put you in contact with a local genetics service. If we identify that you could be at risk for a disorder, which is currently untreatable and unpreventable, we will not disclose this information. If you become upset or distressed as a result of your participation in the research, the researcher is able to refer you to counselling services or other appropriate support such as Beyond Blue (telephone: 1300 22 4636) or a study physician is able to refer you to your GP for appropriate management. In addition, you may prefer to suspend or end your participation in the research if distress occurs.

MRI SCANNING and DEXA

MRI involves exposure to high magnetic fields, but no ionising radiation. There are no known clinical risks associated with this technique. There is a risk of claustrophobia in the scanner, but you are free to exit the magnet at any time. You will not be permitted to undergo scans if you have any ‘ferrous’ metal materials within your body (such as a pacemaker or metal implants). However, a lot of metal pins used these days are ‘non-ferrous’ and are not affected by the magnetic field of the scanner. These issues will be discussed with you by the radiographer prior to scanning.

During the DEXA scan you will be exposed to radiation. However, the dose of radiation is very small and at this level no harmful effects of radiation have been demonstrated and the risks are very low. Each measurement involves a very small dose of radiation (~0.02 mrem), which is far lower than a typical radiation dose from a chest x-ray (30 mrem).

(4) How much time will the study take?

You will be assessed over 2 days on three occasions, at baseline and 3 months (end of the trial) and 3 months after completing the trial (follow-up). We expect each visit to take up to 3 hours. If you are allocated to a supervised exercise training group, you will be required to attend training sessions at the Charles Perkins Centre on 3 days each week (20-60 minutes for visit). If you are allocated to a physical activity advice group you will be asked to attend an educational session with a study researcher at the Charles Perkins Centre at the beginning and end of the program.
(5) Can I withdraw from the study?

Being in this study is completely voluntary - you are not under any obligation to consent and - if you do consent - you can withdraw at any time by informing one of the researchers, without affecting your relationship with the University of Sydney or with the researchers. You may choose to have your data to be removed from the study if you discontinue with the study.

If you choose to withdraw, your data will still be useful for research purposes and will be de-identified for use. However, if you wish to have your data removed, please inform the researcher and they will ensure your data are not used.

(6) Will anyone else know the results?

All aspects of the study, including results, will be strictly confidential and only the researchers will have access to information on participants.

A report of the study may be submitted for publication, but individual participants will not be identifiable in such a report.

(7) Will the study benefit me?

While we intend that this research study furthers medical knowledge and may improve management of diabetes it may not be of direct benefit to you.

(8) Can I tell other people about the study?

You are welcome to tell other people about the study.

(9) What if I require further information about the study or my involvement in it?

When you have read this information, Ms Way, Mr Lanting, Mr Sabag or Ms Sultana will discuss it with you further and answer any questions you may have. If you would like to know more at any stage, please feel free to contact them on 0407 252 422 (Kimberley Way), 0403 800 712 (Sean Lanting), 0410 232 582 (Angelo Sabag) or 0450 660 193 (Rachelle Sultana). Alternatively please feel free to contact Dr Johnson on 02 9351 9137.

(10) What if I have a complaint or any concerns?

Any person with concerns or complaints about the conduct of a research study can contact The Manager, Human Ethics Administration, University of Sydney on +61 2 8627 8176 (Telephone); +61 2 8627 8177 (Facsimile) or r.o.humanethics@sydney.edu.au (Email).

This information sheet is for you to keep
9.2 Participant consent form

PARTICIPANT CONSENT FORM

I, ..........................................................[PRINT NAME], give consent to my participation in the research project.

TITLE: NOVEL EXERCISE THERAPIES FOR TYPE 2 DIABETES

In giving my consent I acknowledge that:

1. The procedures required for the project and the time involved have been explained to me, including any inconvenience, risk, discomfort or side effect, and their implications, and any questions I have about the project have been answered to my satisfaction.

2. I have read the Participant Information Statement and have been given the opportunity to discuss the information and my involvement in the project with the researcher(s).

3. I understand that being in this study is completely voluntary – I am not under any obligation to consent.

4. I understand that my involvement is strictly confidential. I understand that any research data gathered from the results of the study may be published however no information about me will be used in any way that is identifiable.

5. I understand that I can withdraw from the study at any time, without affecting my relationship with the researcher(s) or the University of Sydney, University of Newcastle or Australian Catholic University now or in the future.

6. I consent to:
   - Receiving Feedback [ ] YES [ ] NO
   - DNA [ ] YES [ ] NO
   - Stool sample [ ] YES [ ] NO

Novel Exercise Therapies for Type 2 Diabetes
Version 2, 16/03/2015

Author: Nathan Johnson
If you answered YES to the "DNA" question, do you wish to be contacted by the researchers if a DNA finding for an untreatable or unpreventable condition becomes treatable during the period of your involvement in the study or in the following 5 years?

YES ☐ NO ☐

If you answered YES to the "Receiving Feedback" question, please provide your details i.e. mailing address, email address.

Feedback Option

Address: ______________________________________________________
___________________________________________________________

Email: ______________________________________________________

___________________________________________________________

Signature

___________________________________________________________

Please PRINT name

___________________________________________________________

Date
9.3 Participant report example

THE UNIVERSITY OF SYDNEY
Faculty of Health Sciences & Charles Perkins Centre
Dr. Nathan Johnson, Discipline of Exercise and Sport Science,
East Street (P.O. Box 170), Lidcombe, NSW, Australia 2141
Tel: 9351-9137, Fax: 9351-9264, Email: nathan.johnson@sydney.edu.au

June xx, 2016

Doctor xxxxx
xxx xxxxx Road,
xxxxx, NSW, 20xx

Dear Dr. xxxxx,

Re: Mrs xxx xxx (DOB: xx/xx/19xx)

Mrs xxxxx has volunteered to participate in an exercise trial through the Boden Institute of Obesity, Nutrition and Exercise at the University of Sydney entitled: “Novel Exercise Strategies for the Management of Type 2 Diabetes” (USyd Ethics Ref No: 2014/961).

Mrs xxxxx participated in a light exercise training program, involving flexibility and core exercises, once a fortnight, for the past 3 months.

Follow up tests were conducted on Thursday xxth of xxx, 2016. These tests indicated alarmingly high readings for Fasting glucose (8.2 mmol/L), Cholesterol (5.9 mmol/L), LDL Cholesterol (3.9 mmol/L), HbA1c (IFCC) (67 mmol/mol), and HbA1c (8.2%). Biochemistry and CRP were normal. Pancreas fat as determined by proton magnetic resonance spectroscopy, was estimated at 2.6% (5.5% is considered to be consistent with non-alcoholic fatty pancreas disease). In the setting of abnormal results, please investigate and manage as you feel necessary.

Dr. Namson Lau and Dr. Kathryn Williams are the overseeing physicians for the exercise trial and have reviewed Mrs xxxxx’s results. A copy of the pathology results is attached to this letter for your reference. She is identified on these documents with the patient code: xxxxx.

Mrs xxxxx has been encouraged to make an appointment with you to discuss these results.

Sincerely,

[Nathan Johnson]