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# Translating the potential of the urine steroid metabolome to stage non-alcoholic fatty liver disease

**Submission date**
15/12/2022

**Recruitment status**
No longer recruiting

**Registration date**
05/01/2023

**Overall study status**
Completed

**Last Edited**
03/10/2024

**Condition category**
Digestive System

[_] Prospectively registered
[X] Protocol
[_] Statistical analysis plan
[_] Results
[_] Individual participant data
[_] Record updated in last year

## Plain English summary of protocol

Background and study aims
Fat deposition in the liver (so called non-alcoholic fatty liver disease, NAFLD) is now the commonest chronic liver condition, affecting one-in-three individuals. It can lead to liver problems including cirrhosis and liver cancer, as well as increasing your risk of heart attacks and strokes. Simple blood tests often show normal results, and the current gold-standard test for assessing NAFLD severity is a liver biopsy which is an invasive procedure. We have developed a urine test that measures natural steroid hormones and we believe that this can provide an accurate reflection of how the liver is functioning in patients with NAFLD. Ultimately, in the future this test may be an alternative to performing a liver biopsy. If successful, our urine test could be used by GPs and hospitals and reduce the need for liver biopsies.

Who can participate?
Patients who have been diagnosed with NALFD and are scheduled to have a liver biopsy or have recently undergone a liver biopsy, and people who participate in the Oxford Biobank (https://www.oxfordbiobank.org.uk) and have been identified as being at very low risk of having NAFLD

What does the study involve?
NAFLD participant:
Once we have checked that you are happy to take part, one of the investigators running the study will go through it in detail with you again and answer any questions you may have. If you agree to take part and are happy to go ahead, you will be asked to sign a consent form.
As a patient with NAFLD, you will be having blood tests as part of your routine NHS clinic appointment. With your permission, we would like to take 2 extra tubes of blood (approximately 20 ml, equivalent to 4 teaspoons) and a urine sample (approximately, 10 ml or 2 teaspoons) for research purposes. As you will be having blood tests as part of your routine clinical care, this will not involve an extra needle. We will also confirm that you are happy for us to access your clinic records so that we can match our research test results with the results of the biopsy, scans and other tests that you have had as part of your NHS care. In total, obtaining the extra samples and clinical information will take no longer than 30 minutes. This would complete your involvement

in the study. Sometimes, you may be asked to attend a specific study visit outside of your routine NHS clinical care. We will schedule a specific research appointment to arrange to take the blood tests and obtain the urine sample.
We will also undertake a special scan of the liver (called transient hepatic elastography or Fibroscan) if this is not already being done as part of clinical care. This involves lying on an examination couch with your right arm above your head. Some cold jelly is applied to the skin a probe is gently placed on the skin over the liver. Painless ultrasound waves are then passed through the liver to produce a measure of liver stiffness which can provide an assessment of the severity of NAFLD. The investigation takes approximately 10 minutes and the scan will be made available to your clinical care team.

Healthy participant:
Once we have checked that you are happy to take part, one of the investigators running the study will go through it in detail with you again and answer any questions you may have. If you agree to take part and are happy to go ahead, you will be asked to sign a consent form. As you have been identified as being at very low risk of having NAFLD, if you are interested in participating in this study, we will arrange an appointment for you to come to our research facility in the Oxford Centre for Diabetes, Endocrinology & Metabolism (OCDEM) at the Churchill Hospital in Oxford for you to have a blood test (5 tubes of blood, approximately 35 ml or 7 teaspoons) and provide a urine sample (approximately 10 ml or 2 teaspoons). We will also undertake a special scan of the liver (called transient hepatic elastography or Fibroscan). This involves lying on an examination couch with your right arm above your head. Some cold jelly is applied to the skin a probe is gently placed on the skin over the liver. Painless ultrasound waves are then passed through the liver to produce a measure of liver stiffness which can provide an assessment of the severity of NAFLD. The investigation takes approximately 10 minutes.

What are the possible benefits and risks of participating?
Whilst you may not directly benefit from this study, it will hopefully allow us to develop better tests to detect and assess the severity of NAFLD that may ultimately lead to patients not needing a liver biopsy.

NAFLD Participants:
You may experience minor discomfort and a minor bruise during the taking of blood samples. As a participant with NAFLD, the research blood samples will be taken at the same time as routine clinical blood samples (avoiding the need to have an extra needle) to minimise disruption and inconvenience. If the samples are taken at a separate visit, you will require an extra blood test. The blood that we take is a small volume and conveys no significant risk, neither does providing the urine sample. There are no risks associated with the scan.

Healthy Participants:
Participants without NAFLD will be required to travel to the OCDEM for a study visit.
You may experience minor discomfort and a minor bruise during the taking of blood samples. The blood that we take is a small volume and conveys no significant risk, neither does providing the urine sample. There are no risks associated with the scan.

Where is the study run from?
Oxford Centre for Diabetes, Endocrinology & Metabolism (OCDEM), University of Oxford (UK)

When is the study starting and how long is it expected to run for?
December 2020 to September 2024

Who is funding the study?
Wellcome Trust (UK)

Who is the main contact?
Prof. Jeremy Tomlinson (Chief Investigator), jeremy.tomlinson@ocdem.ox.ac.uk (UK)

# Contact information

### Type(s)
Principal investigator

### Contact name
Prof Jeremy Tomlinson

### ORCID ID
https://orcid.org/0000-0002-3170-8533

### Contact details
Oxford Centre for Diabetes, Endocrinology & Metabolism (OCDEM)
University of Oxford
Churchill Hospital
Headington
Oxford
United Kingdom
OX3 7LJ
+44 (0)1865 857359
jeremy.tomlinson@ocdem.ox.ac.uk

### Type(s)
Scientific

### Contact name
Prof Jeremy Tomlinson

### Contact details
Oxford Centre for Diabetes, Endocrinology & Metabolism (OCDEM)
University of Oxford
Churchill Hospital
Headington
Oxford
United Kingdom
OX3 7LJ
+44 (0)1865 857359
jeremy.tomlinson@ocdem.ox.ac.uk

### Type(s)
Public

### Contact name
Prof Jeremy Tomlinson

**Contact details**
Oxford Centre for Diabetes, Endocrinology & Metabolism (OCDEM)
University of Oxford
Churchill Hospital
Headington
Oxford
United Kingdom
OX3 7LJ
+44 (0)1865 857359
jeremy.tomlinson@ocdem.ox.ac.uk

# Additional identifiers

**Integrated Research Application System (IRAS)**
300260

**Central Portfolio Management System (CPMS)**
50044

# Study information

**Scientific Title**
TrUSt-NAFLD

**Acronym**
TrUSt-NAFLD

**Study objectives**
The urinary steroid metabolome, when combined with generalised matrix learning vector quantization (GMLVQ) analysis, provides an accurate method to diagnose and stage non-alcoholic fatty liver disease

**Ethics approval required**
Old ethics approval format

**Ethics approval(s)**
Approved 19/08/2021, a substantial amendment approved 20/12/2021, West Midlands - The Black Country Research Ethics Committee (Meeting held by video-conference via Zoom; +44 (0) 207 104 8010, (0)207 104 8141; blackcountry.rec@hra.nhs.uk), ref: 21/WM/0177, amendment TrUSt-NAFLD-SA1

**Primary study design**
Observational

**Study design**
Multi-centre prospective validation study

**Study type(s)**
Diagnostic

## Health condition(s) or problem(s) studied
Non-alcoholic fatty liver disease

## Interventions
The urinary steroid metabolome of patients with non-alcoholic fatty liver disease (NAFLD) and healthy controls will be analysed using mass spectroscopy followed by the application of generalized matrix learning vector quantization (GMLVQ) analysis to attempt to diagnose and stage NAFLD.

This test involves the development and refinement of an algorithm utilising the measurement of steroid hormones and their metabolites in a urine sample.

All study visits for participants with NAFLD will coincide with routine NHS clinical care and no additional study visits will be scheduled for the patients. Patients who meet the inclusion criteria will be approached by members of the clinical team embedded within the respective clinics and will be provided with the patient information leaflet. In addition, if recruitment is slow, existing clinical databases will be scrutinised and information letters sent to patients to raise awareness of the study.

Once informed written consent has been obtained, additional blood samples and a urine sample will be collected from the patient. These will be analysed for steroid hormones and their metabolites. Very commonly, patients will be having blood tests as part of their routine NHS care and therefore the additional samples for the serum and plasma save will not require any additional venepuncture (simply the collection of extras samples). A Fibroscan (transient hepatic electrography, a form of ultrasound scan) is almost always performed as part of routine clinical care in patients with NAFLD. If this has not been performed, it will be performed as part of the research visit. Consent will be obtained to gather relevant anonymised information from their clinical records, including the formal reports of the histological grading of their liver biopsy.

Control (non-NAFLD) participants will be recruited from the Oxford Biobank (https://www.oxfordbiobank.org.uk) (OBB), a database of >9000 volunteers in Oxfordshire who have undergone extensive metabolic phenotyping and consented to be re-approached for clinical research. Individuals with a BMI between 20-35kg/m2 with a HOMA-IR in the bottom 25 percentiles of their respective BMI and a fasting triglyceride level <2mmol/l (already performed as part of the phenotyping at enrolment into the OBB) will be recalled to identify participants without NAFLD. In patients without NAFLD, we will take blood samples to look for liver disease (only participants with normal liver biochemistry and non-invasive serum markers indicating low risk of advanced NAFLD will be included in the study) as well as taking urine and blood research samples in which we will measure steroid hormones and their metabolites (as in the patients with NAFLD). Where possible we will also perform a Fibroscan in the non-NAFLD patients. Laboratory analysis will be undertaken on the urine and plasma/serum samples to measure metabolites including steroid hormones that will be used to stage the severity of the underlying NALFD. Identical analyses will also be performed in the samples from patients without NAFLD.

We will then analyse the area under the curve (AUC) of the receiver operator characteristic (ROC) curve following generalised matrix learning vector quantization (GMLVQ) analysis of urine steroid metabolome.

The primary outcome will determine the accuracy of the urine steroid metabolome test to distinguish early from advanced NAFLD (biopsy finding F0-2 versus F3-4). Secondary analyses include comparing the performance of the urine steroid metabolome algorithm to stage NAFLD against other non-invasive NALFD biomarkers (FIB-4, NAFLD fibrosis score, ELF, transient

elastography), the accuracy of the urine steroid metabolome test to diagnose NAFLD through comparison versus control participants without NAFLD, and the changes in serum/plasma metabolites (including steroid hormone measurements) that are associated with different stages of NAFLD.

### Intervention Type
Other

### Primary outcome(s)
The urine collected at the study visit will be analysed using mass spectroscopy to generate quantities of 32 steroid metabolites. An area under the curve (AUC) of the receiver operator characteristic (ROC) curve will be generated following the generalized matrix learning vector quantization (GMLVQ) analysis of the urinary steroid metabolome of each patient to aim to distinguish those with early fibrosis (F0-2) from those with advanced fibrosis (F3-4) at one timepoint

### Key secondary outcome(s)
1. Area under the curve (AUC) of the receiver operator characteristic (ROC) curve following generalized matrix learning vector quantization (GMLVQ) analysis of the urinary steroid metabolome (generated by mass spectroscopy of the urine samples provided at the study visit) compared with AUC ROC using FIB-4, NAFLD fibrosis score, ELF, transient elastography measured using patient details recorded on the case report form at the study visit
2. GMLVQ analysis of urinary steroids (measured using mass spectroscopy of the urine collected at the study visit) in patients with biopsy-proven NAFLD compared with control participants without NAFLD at one timepoint
3. The levels of steroid hormones and other metabolites in serum/plasma measured using laboratory analysis of the blood samples taken at the study visit

### Completion date
30/09/2024

## Eligibility

### Key inclusion criteria
NAFLD Participants:
1. Participant is willing and able to give informed consent for participation in the study
2. Patients with a diagnosis of NAFLD who are scheduled for a liver biopsy OR patients who have had a liver biopsy with a confirmed diagnosis of NAFLD within the last 12 months
3. Aged ≥18 years

Healthy participants (recruited via the Oxford Biobank https://www.oxfordbiobank.org.uk):
1. BMI 20-35kg/m2
2. HOMA-IR in the bottom 25 percentiles of their respective BMI
3. Fasting triglyceride level <2 mmol/l

Only individuals with normal liver biochemistry, and non-invasive serum markers (including the enhanced liver fibrosis panel, ELF) indicating low-risk of advanced NAFLD will be included in the study

### Participant type(s)
Mixed

### Healthy volunteers allowed
No

### Age group
Adult

### Lower age limit
18 Years

### Sex
All

### Key exclusion criteria
1. Insufficient understanding of written and verbal English
2. The participant has not had sufficient time to read the Patient Information Leaflet (PIL) and understand the study requirements
3. Hepatic steatosis, inflammation or fibrosis with a primary aetiology other than NAFLD

### Date of first enrolment
01/10/2021

### Date of final enrolment
31/12/2023

## Locations

### Countries of recruitment
United Kingdom

England

### Study participating centre
**NIHR Nottingham Biomedical Research Centre**
Queens Medical Centre
Derby Road
Nottingham
United Kingdom
NG7 2UH

### Study participating centre
**Barts and the London NHS Trust**
Alexandra House
The Royal London Hospital
Whitechapel

London
United Kingdom
E1 1BB

**Study participating centre**
**Oxford University Hospitals NHS Foundation Trust**
John Radcliffe Hospital
Headley Way
Headington
Oxford
United Kingdom
OX3 9DU

**Study participating centre**
**Royal Berkshire NHS Foundation Trust**
Royal Berkshire Hospital
London Road
Reading
United Kingdom
RG1 5AN

**Study participating centre**
**Imperial College Healthcare NHS Trust**
The Bays
St Marys Hospital
South Wharf Road
London
United Kingdom
W2 1BL

**Study participating centre**
**University Hospitals Birmingham NHS Foundation Trust**
Queen Elizabeth Hospital
Mindelsohn Way
Edgbaston
Birmingham
United Kingdom
B15 2GW

**Study participating centre**

**Leeds Teaching Hospitals NHS Trust**
St. James's University Hospital
Beckett Street
Leeds
United Kingdom
LS9 7TF

**Study participating centre**
**University Hospitals Plymouth NHS Trust**
Derriford Hospital
Derriford Road
Derriford
Plymouth
United Kingdom
PL6 8DH

**Study participating centre**
**Churchill Hospital**
Churchill Hospital
Old Road
Headington
Oxford
United Kingdom
OX3 7LE

# Sponsor information

**Organisation**
University of Oxford

**ROR**
https://ror.org/052gg0110

# Funder(s)

**Funder type**
Research council

**Funder Name**
Wellcome Trust

### Alternative Name(s)
Wellcome, WT

### Funding Body Type
Private sector organisation

### Funding Body Subtype
Trusts, charities, foundations (both public and private)

### Location
United Kingdom

## Results and Publications

### Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study will be stored in a non-pubicly available repository.

### IPD sharing plan summary
Stored in non-publicly available repository

### Study outputs

| Output type | Details | Date created | Date added | Peer reviewed? | Patient-facing? |
|---|---|---|---|---|---|
| Protocol article | | 18/01/2024 | 19/01/2024 | Yes | No |
| HRA research summary | | | 28/06/2023 | No | No |
| Participant information sheet | version 1.2 | 18/08/2021 | 21/12/2022 | No | Yes |
| Participant information sheet | version 1.2 | 22/11/2021 | 21/12/2022 | No | Yes |