A 48-WEEK PHASE II, RANDOMISED, DOUBLE BLINDED PLACEBO CONTROLLED, PARALLEL-GROUP, MULTI-CENTRE TRIAL ON LIRAGLUTIDE'S SAFETY, EFFICACY AND ACTION ON LIVER HISTOLOGY AND METABOLISM IN OVERWEIGHT PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS, WITH OR WITHOUT TYPE II DIABETES

LIRAGLUTIDE EFFICACY AND ACTION IN NON-ALCOHOLIC STEATOHEPATITIS

LEAN

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AMENDMENTS

The following amendments and/or administrative changes have been made to this protocol since the date of preparation

Amendment No.	Date of Amendment	Version No.	Type of amendment? (e.g. substantial/non-substantial/administrative change)
1	**30th April 2010**	**2.0**	Non-substantial
2	**1st July 2010**	**3.0**	Substantial
3	**20th November 2010**	**4.0**	Substantial
4	**20th January 2011**	**5.0**	Substantial, administrative change
5	**28th April 2011**	**6.0**	Substantial + administrative change
6	**1st September 2011**	**7.0**	Substantial (additional site)

CHIEF INVESTIGATOR SIGNATURE PAGE

LEAN TRIAL

A 48-WEEK PHASE II, RANDOMISED, DOUBLE BLINDED PLACEBO CONTROLLED, PARALLEL-GROUP, MULTI-CENTRE TRIAL ON LIRAGLUTIDE'S SAFETY, EFFICACY AND ACTION ON LIVER HISTOLOGY AND METABOLISM IN OVERWEIGHT PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS, WITH OR WITHOUT TYPE II DIABETES

Version 7.0

This Protocol is approved by :

Dr Phil Newsome **Signature :** **Date :** 22/12/11

Chief Investigator

PROTOCOL SIGNATURE

I have thoroughly read and reviewed the study protocol:

A 48-WEEK PHASE II, RANDOMISED, DOUBLE BLINDED, PLACEBO CONTROLLED, PARALLEL-GROUP, MULTI-CENTRE TRIAL ON LIRAGLUTIDE'S SAFETY, EFFICACY AND ACTION ON LIVER HISTOLOGY AND METABOLISM IN OVERWEIGHT PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS (NASH), WITH OR WITHOUT TYPE II DIABETES

I have read and understood the requirements and conditions of the study protocol (Version 7.0 1st September 2011).

I am aware of my responsibilities as an Investigator under the guidelines of Good Clinical Practice (GCP), the Declaration of Helsinki, local regulations and the study protocol and I agree to conduct the study according to these guidelines and to appropriately direct and assist the staff under my control who will be involved in the study.

I agree to use the study material, including medication, only as specified in the protocol.

I understand that changes to the protocol must be made in form of an amendment, which has to be approved by the relevant Ethics Committee and regulatory health authorities prior to its implementation.

I understand that any violation of the protocol may lead to early termination of the study.

INVESTIGATOR'S NAME

……

SIGNATURE

……

DATE

……

TRIAL SYNOPSIS

Background

Non-alcoholic fatty liver disease (NAFLD) is responsible for an increasing prevalence of liver disease and is becoming the commonest cause of liver disease in the western world. NAFLD is recognised to be the hepatic manifestation of the metabolic syndrome, which is a cluster of metabolic abnormalities characterised by abdominal obesity, insulin resistance, impaired glucose metabolism, hypertension and dyslipidaemia. In its mildest form there is an accumulation of fat in the liver (steatosis) without any liver damage, however in many cases it progresses to non-alcoholic steatohepatitis (NASH), and cirrhosis.

Current treatment options for NASH are limited in efficacy, necessitating the development of more effective options. New agents such as Glucagon-like Peptide-1 (GLP-1) agonists that improve diabetic control and facilitate weight loss have been suggested as therapies in NASH.

No published studies to date have assessed the impact of the GLP-1 agonist, Liraglutide, on liver histology and metabolism in obese patients with NASH. This study hypothesises that treatment with liraglutide will result in a significant improvement in histological disease activity in obese patients with NASH, in the presence or absence of Type 2 Diabetes (T2DM).

Objectives

The primary objective is to investigate whether 48 weeks treatment with once-daily injections of liraglutide improves liver histology in overweight patients with NASH enough to warrant further investigation.

The secondary objectives are to investigate whether 48 weeks treatment with once-daily injections of liraglutide in overweight patients with NASH results in a clinically significant effect on the:

- Individual histological features of NASH including steatosis, hepatocyte inflammation and injury, and fibrosis.
- Non-invasive clinical markers of steatosis, steatohepatitis and fibrosis.
- Clinical components of metabolic syndrome
- Insulin resistance and hepatic lipogenesis
- Patients Quality of Life (QOL)
- Clinical safety profile

Entry Criteria

Main inclusion criteria:

- NASH criteria (all):
  - Liver biopsy must be performed within 6 months of screening for the trial.
  - 'Definite' diagnosis of NASH by two independent expert histopathologists from the central trial site (Birmingham, UK). The report by the central review of pathologists will be required in all cases prior to randomisation.
  - NAFLD Activity Score (NAS) ≥ 3 (1), comprising of a minimum of 1 point from each of the individual steatosis, lobular inflammation and hepatocyte ballooning scores
- Age ≥ 18 < 70 years old
- Body Mass Index (BMI) ≥ 25

- Patients with Type II Diabetes Mellitus at screening, must have;
    - o stable glycaemic control (HbA1c < 9.0%) and,
    - o be managed with one of the following:
        - ▪ diet-control alone
        - ▪ diet-control and metformin and/or sulphonylurea
- Non-Diabetic criteria (based on two separate fasting plasma glucose levels > 48 hours apart and/or Oral Glucose Tolerance Test (OGTT)):
    - o Impaired fasting glucose (IFG), defined using the European Criteria between 6.1 and 6.9 mmol/L

  and/or
    - o Impaired glucose tolerance (IGT), defined as two-hour plasma glucose levels between 7.8 and 11.0 mmol/ on the 75-g OGTT

  or
    - o Normal Fasting Plasma Glucose (FPG) < 6.1 mmol and Normal two-hour plasma glucose levels < 7.8 on the 75g OGTT.

Recruitment

This is an early phase multi-centre study based at the Liver Research Group/Units of the Queen Elizabeth University Hospitals NHS Foundation Trust (Birmingham, Lead Site UK). The Liver Research Group at Birmingham (UK) will act as the lead site for up to 5 UK-based recruitment centres, including Queen Medical Centre, Nottingham University Hospitals (Nottingham, UK), Southampton General Hospital (Southampton, UK), Hull Royal infirmary (Hull, UK) and St James University Hospital (Leeds, UK). The target recruitment is 50 patients (25 patients in each treatment arm).

Treatments

The patients will be randomised on a 1:1 basis to one of two groups:

Group 1 - Control group. Treatment with once-daily subcutaneous injection of inactive treatment (liraglutide placebo) (Supplied by Novo Nordisk Ltd, UK) for 48 weeks.

Group 2 - Experimental group. Treatment with once-daily subcutaneous injections 1.8mg active Liraglutide (Victoza ®) (Supplied by Novo Nordisk Ltd, UK) for 48 weeks.

Group 1 and 2 will contain approximately the same proportion of non-diabetics and Type II diabetics.

Outcome measures

The primary outcome measure is the proportion of patients with an improvement in liver histology after 48-weeks of treatment as defined by;

1. disappearance of Steatohepatitis (i.e. Disappearance of hepatocyte ballooning)

and

2. no worsening of the fibrosis score

The secondary outcome measures to be assessed at 48 weeks include:

- Change in the NAS pre and post-treatment on liver biopsy
- Steatosis, lobular inflammation, hepatocyte ballooning and liver fibrosis on liver biopsy

- Serological markers of steatosis, steatohepatitis and fibrosis using the Fibromax panel and CK-18
- NAFLD fibrosis score
- Transient Elastography (Fibroscan®)
- Change in weight (Kg), BMI (Kg/m^2) and waist:hip ratio
- Glycaemic control (HbA1c, Fasting plasma glucose)
- HOMA-IR, Triglycerides, HDL
- Total body, hepatic, muscle, and adipose insulin sensitivity using hyperinsulinaemic euglycaemic and adipose microdialysis studies.
- De-novo hepatic lipogensis (DNL) using stable isotope experiments
- Quality of life (SF-36v2) and Nutrition (Block Brief 2000 FFQ) questionnaires
- Safety measures (History and clinical examination, hypoglycaemia rates, routine bloods tests, TFTs and calcitonin levels, and liraglutide (Victoza ®) antibodies)

STUDY SCHEDULE

Study treatment (TD1) will start the day after **COMPLETION OF VISIT 2**

	Screening		Treatment					Follow-up
	Visit 1 (Max 14 days prior to TD1)	Visit 2 (1 day prior to TD1[6])	Visit 3 (TD 28)	Visit 4 (TD 84)	Visit 5 (TD 168)	Visit 6 (TD 252)	Visit 7 (1 Day + TD 336/ End of Treatment [EOT])	Visit 8 (12 weeks after EOT)
Informed consent	X							
Clinical assessment[1]	X		X	X	X	X	X	X
Vital Signs[2]	X		X	X	X	X	X	X
ECG/Urine Dipstix	X			X	X	X	X	X
Standard blood tests[3]	X		X	X	X	X	X	X
Screening blood tests[4]	X							
Lipid profile Serum insulin	X			X	X		X	X
OGTT (non-diabetics only)	X						X	
CK-18 & FibroMAX Panel[5]	X						X	X
Fibroscan®[11]	X						X	X
metabolic sub-studies[6]		X		X				
Questionnaires[7]	X						X	X
Liver biopsy	-[8]						X	
Adverse/ Clinical events[9]			X	X	X	X	X	X
Study medication dispensed		X[10]	X	X	X	X		

Key: TD – Treatment Day, EOT – End of treatment, CK-18 – Cytokeratin 18, ECG – Electrocardiogram, HBsAg – Hepatitis B surface antigen, HCV Ab – Hepatitis C Antibody, AMA – Antimitochondrial Antibody, ASA – Smooth muscle Antibody, Ig – Immunoglobulin, α1AT – Alpha 1 Anti-trypsin, AFP – Alpha Fetaprotein, HR-QOL – Health-Related Quality of Life

[1] Clinical assessment - consists of complete history and examination at screening and focussed history and relevant examination at subsequent visits. [2] Vital signs – Heart rate, blood pressure, weight (Kg), Height (cm), waist:hip circumference (cm), Body Temperature, Oxygen saturations (SaO_2), Respiartory Rate (RR). [3] Standard blood tests - Full Blood Count, Urea and Electrolytes, Liver Function Tests, International Normalised Ratio (INR), Thyroid function tests (TFTs), Fasting plasma glucose (FPG) and HbA_{1c} (except visit 3). [4] Screening blood tests – HBsAg, HCV Ab , AMA / ASA / Ig's, Ferritin/Transferrin saturation, Caeruloplasmin , α1AT, AFP. [5] FibroMAX panel – including FibroTest, SteatoTest, NashTest. [6] Overnight stay at research facility for metabolic studies – 2-step hyperinsulinaemic euglycaemic clamp, adipose microdialysis and stable isotope studies. [7] Questionnaires – AUDIT alcohol question, Block Brief 2000 Food frequency Quesionnaire, HR-QOL (SF-36v2). [8] Diagnostic liver biopsy performed as part of standard medical health care within 6 months of screening for the trial. Two independent liver histopathologists will review the liver biopsy to assess whether the patients meets the histological inclusion criteria. [9] Adverse Events/bloods and Clinical Events will be monitored continuously until completion of follow up. Calcitonin and AFP levels will be measured at visits 1, 5, 7 and 8. [10] If the study patient meets the eligibility criteria, he/she will be randomised at visit 2 to receive liraglutide (Victoza®) or inactive treatment (liraglutide placebo) and the allocated blinded study treatment will be dispensed on this visit. [11] The fibroscan requirement is optional and subject to individual centre availability. At site initiation participating sites will be required to confirm fibroscan availability and whether they will be able to perform fibroscan.

ABBREVIATIONS

α1AT	Alpha 1 Anti-trypsin deficiency
AASLD	American Association of the Study of Liver Disease
Ab	Antibody
ACE	Angiotensin Converting Enzyme
AE	Adverse Event
ARB	Angiotensin Receptor Blocker
AFP	Alpha Feto Protein
Ag	Antigen
ALD	Alcoholic Liver Disease
ALP	Alkaline Phosphatase
ALT	Alanine Transferase
AMA	Anit-Mitochondrail Antibody
ASA	anti-Smooth Muscle Antibody
AST	Aspartate Transferase
ATP	Adult Treatment Panel
AUROC	Area Under the Receiver Operating Characteristic Curve
BMI	Body Mass Index
BP	Blood Pressure
CK-18	Cytokeratin 18
CLD	Chronic Liver Disease
CRCTU	Cancer Research Clinical Trials Unit
CRF	Case Report Form
CVD	Cerebral Vascular Disease
DMC	Data Management Committee
DNL	De Novo Lipogenesis
DPP-4	Dipeptidyl Peptidase 4
DOB	Date of Birth
ECG	Electrocardiogram
EMA	European Medicines Agency
EOT	End of treatment
FBC	Full Blood Count
FDA	Food and Drug Administration
FFA	Free Fatty Acids
FFQ	Food Frequency Questionnaire
FPG	Fasting Plasma Glucose
GCP	Good Clinical Practice
GGT	Gamma Glutamyl Transferase
GLP	Glucagon-like Peptide
HbA_{1c}	Glycosylated Haemoglobin A1c
HBV	Hepatitis B Virus
HBsAg	Hepatitis B surface Antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HDAd	Helper-dependent Adenoviral Vector
HDL	High Density Lipoprotein
HEC	Hyperinsulinaemic Euglycaemic Clamp
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
HR	Heart Rate
IFG	Impaired Fasting Glucose
Ig	Immunoglobulin
IGT	Impaired Glucose Tolerance
IMP	Investigational Medicinal Product
INR	International Normalised Ratio
ISF	Investigator Site File
LEAD	Liraglutide Efficacy and Action in Diabetes
LEAN	Liraglutide Efficacyand Action in NASH
LFT	Liver Function Tests
MDT	Multi-disciplinary Team
MI	Myocardial Infarction
NAFLD	Non Alcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Non Alcoholic Steatohepatitis
NDH	Neutral Protamine Hagedorn
NPV	Negative Predictive Value
OLT	Orthotopic Liver Transplant
PI	Principal Investigator
PPAR	Peroxisome Proliferator Activated Receptor
PPG	Post Prandial Glucose
PPV	Positive Predictive Value
PVD	Peripheral Vascular Disease
OAD	Oral Anti-Diabetic Drug
QOL	Quality of Life
R&D	Research and Development
REC	Regulatory Authority and Ethics Committee
RR	Respiratory Rate
SAE	Serious Adverse Event
SaO_2	Oxygen saturations
SC	Subcutaneous
SCD-1	Steatoryl CoA Desaturase 1
SMPG	Self-monitored Plasma Glucose
SUSARS	Serious Unexpected Suspected Adverse Reaction
T2DM	Type 2 Diabetes Mellitus
TD	Treatment day
TE	Transient Elastography
TFTs	Thyroid Function tests
TMG	Trial Management Team
TZD	Thiazolinedione
TD	Treatment Day
U&E	Urea, creatinine and Electrolytes
UDCA	Ursodeoxycholic Acid
UK	United Kingdom
US	United States of America
WT	Wellcome Trust

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BACKGROUND AND RATIONALE

Background

Throughout the western world, the rates of liver-related morbidity and mortality continue to rise at a concerning rate [Figure 1]. In 2008, the Office for National statistics ranked liver disease as the fifth highest cause of death in the UK. Over the last 2 decades, liver-related mortality has continued to rise in comparison to the four highest ranked diseases, whose mortality rates continue to decrease with time (Office for National Statistics *Mortality statistics*: *Deaths registered in 2008,* DR_08).

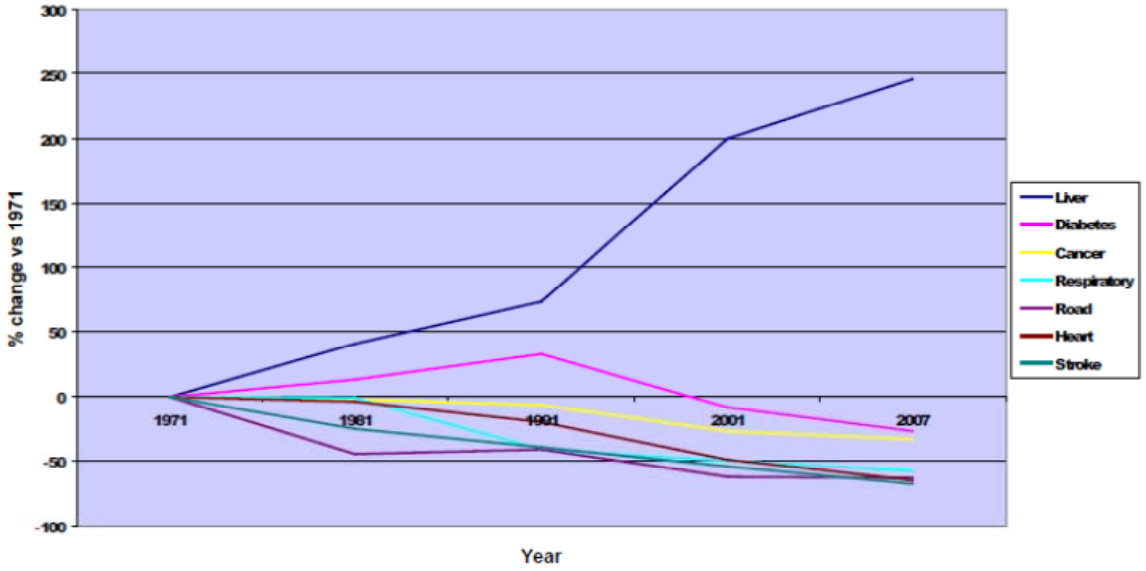


Figure 1. Movements in mortality 1971-2007 – Deaths per million of population (Office for National Statistics)

Non-alcoholic fatty liver disease (NAFLD) is now recognised to be the commonest cause of liver disease and is estimated to effect 20-30% of the western world population.(2;3)

In its mildest form fat accumulates within the liver (simple steatosis) without the presence of hepatocellular injury. However, in an estimated 2-3% of the population, it progresses to the clinically relevant stages of non-alcoholic steatohepatitis (NASH) and varying degrees of fibrosis.(2;4) The full extent of the severity of NASH has only been acknowledged in the last decade, as a result of a series of long-term follow-up studies, which alarmingly identified that 10-15% of biopsy proven NASH progressed to cirrhosis and its complications of liver failure and hepatocellular carcinoma (HCC).(5-7)

At present, liver transplantation remains the only curative treatment option for end-stage NASH cirrhosis and its complications. However, with the rising demand for transplantation outweighing the supply of donor organs and the high cardiovascular risk of NASH patients in general, transplantation will only be a realistic treatment option in the minority of these patients.

Several pharmaceutical interventions have been evaluated in recent years, but due to a lack of efficacy and side effect profile, none to date have been approved for clinical use. Therefore, despite an increasing awareness that NASH is rapidly becoming a public health problem, effective therapies are still urgently needed. Subsequently, developing treatments

which can reverse or prevent the more clinically relevant and advanced stages of NASH has rendered this a critical area of research.

Justification for patient population

The prevalence of NAFLD is as high as 80% and 90% in patients with Type 2 Diabetes Mellitus (T2DM) and morbid obesity, respectively.(4;8) The epidemiological trends and demographic features of NAFLD have paralleled the rise in obesity-related diseases, including T2DM, in recent years [Figure 2].(9) Indeed, NAFLD is recognised to be the hepatic manifestation of the metabolic syndrome,(10) which is a cluster of metabolic abnormalities characterised by abdominal obesity, insulin resistance, impaired glucose metabolism, hypertension and dyslipidaemia.

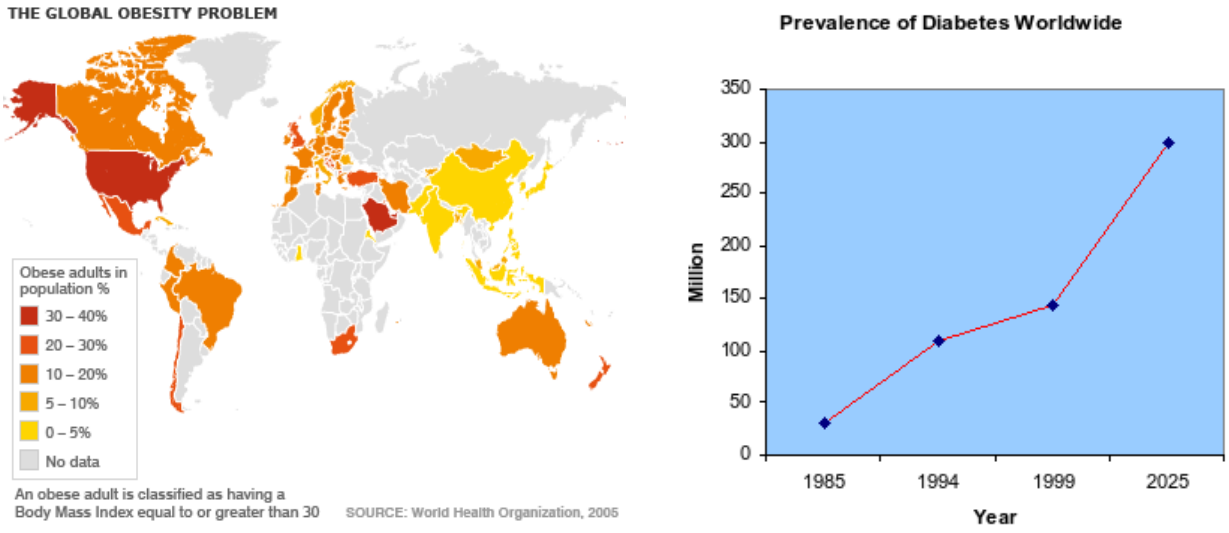


Figure 2 – Left: % of obese adults in the world population (WHO,2005). Right – Prevalence of diabetes world wide (WT Sanger ins.)

Recent studies in the US have suggested that obesity is independently associated with NAFLD and HCC.(9;11) Similarly, several retrospective studies have shown that T2DM appears to be independently associated with advanced liver fibrosis and HCC, even after adjustment for viral hepatitis and alcohol consumption.(9;12) By evaluating a NAFLD cohort from the 1980s, Younossi and colleagues, identified that the mortality rate in the diabetic patients was twice that of the non-diabetics (56.8 vs 27.3%).(13) In fact, the Verona study suggested that the mortality rate from cirrhosis in diabetic patients was higher than that for cardiovascular disease.(14)

Significant advances have been made in recent years into our understanding of the epidemiology and pathogenesis of NASH. General consensus suggests that only patients with NASH, rather than simple steatosis, require treatment and only these treatment options should be the targets of future clinical trial.(15) To date clinical trials have shown that safe and effective treatment options remain limited for overweight patients with biopsy proven NASH.

Justification for design

Over the last decade numerous randomised pharmaceutical clinical trials, of variable subject size, have evaluated the effect of anti-diabetic medications,(16-23) weight reduction,(22;24) HMG-CoA reductase inhibitors,(25) Ursodeoxycholic acid (UDCA)(26) and anti-oxidant therapies(21;23;26) in patients with biopsy-confirmed NASH, with limited efficacy.

To the best of our knowledge, only two clinical trials to date are investigating the effects of GLP-1 agonsim (exenatide) on liver histology in patients with NAFLD.(27) Both of these trials are still recruiting and are non-randomized, open labelled studies in a single patient group of T2DM with NASH.(27) The LEAN trial will not only be the first randomised, double-blinded, placebo-controlled trial of GLP-1 agonists in NASH, but will be the first clinical trial to evaluate the efficacy of the once daily GLP-1 agonist, liraglutide, in diabetic and non-diabetic patients with NASH.

A placebo-control group will be recruited to provide a 'benchmark' reference of what results would be seen in an unbiased comparable untreated group. The placebo-control (supplied by Novo Nordisk A/S) will be an inactive injection substance that has no intended treatment value, but has exactly the same appearance and application as liraglutide (Victoza ®). Subsequently, the two groups of patients cannot be biased (i.e. placebo effect), because they won't know if they are receiving liraglutide (Victoza ®) or the inactive treatment (liraglutide placebo). Therefore the decision to subject the control group to once-daily subcutaneous injections for 48 weeks, with no expected treatment benefit, is to ensure that changes to the primary and secondary outcome measures will be solely attributed to treatment with liraglutide (Victoza ®), rather than placebo effect.

There is currently no standard treatment available for NASH so participants randomised to receive placebo injections are receiving the current standard of care had they not been enrolled in the trial.

Participants will be randomized to receive either liraglutide (Victoza ®) or inactive treatment (liraglutide placebo) for 48 weeks to minimise selection bias. Randomisation will be stratified by the presence of T2DM to ensure that there are approximately equal numbers of each in the liraglutide (Victoza ®) and placebo groups, as it is a potential confounding factor.

The Birmingham tertiary liver transplant unit, UK and centres in Nottingham and Southampton comprise national experts in the fields of NAFLD and GLP-1 agonism. Screening amongst these centres will enable rapid recruitment.

The histological primary end-point of the trial will be assessed after 48-weeks of treatment. Efficacy and safety of liraglutide (Victoza ®) will be known at the 48 week time-point. At this time-point investigators will also be able to assess the accuracy of a combination of clinical, serological and radiological non-invasive markers of NASH at predicting changes in liver histology in the trial patients.

Choice of treatment

The strong association of NASH with the metabolic syndrome, in particular T2DM and obesity, has meant that clinical trials to date have mainly focused on therapies that promoted weight loss [i.e. bariatric surgery, orlistat, sibutramine],(28-30) lipid-lowering [i.e. statins],(25;31) reduced insulin resistance and improved glycaemic control [i.e. metformin, thiazolidinediones].(17;18;20;22)

A novel group of agents, known as GLP-1 agonists, have recently been shown to have promising efficacy and safety in large randomised-control trials in T2DM [Table 1]. Therefore by promoting weight loss and improving insulin sensitivity long-acting GLP-1 based therapies may represent a novel strategy that impacts on the natural progression of NASH in obese patients.

Table 1. *Summary of Trials with Exenatide.*

Reference	Medication and Dose	n	Study Weeks	Additional Medications	HbA1c Δ (%)	Weight Δ (kg)
Fineman *et al.*	Exenatide, 0.08 μg BID	109	4	SU ± metformin	−1.1	No data
	Exenatide, 0.08 μg BID				−0.7	
	Exenatide, 0.08 μg BID				−1.0	
	Placebo				−0.3	
Buse *et al.*	Exenatide, 5 μg BID	377	30	SU	−0.46	−0.9
	Exenatide, 10 μg BID				−0.86	−1.6
	Placebo				+0.12	−0.6
DeFronzo *et al.*	Exenatide, 5 μg BID	336	30	Metformin	−0.4	−1.6
	Exenatide, 10 μg BID				−0.78	−2.8
	Placebo				+0.08	−0.3
Kendall *et al.*	Exenatide, 5 μg BID	733	30	Metformin + SU	−0.6	−1.6
	Exenatide, 10 μg BID				−0.8	−1.6
	Placebo				+0.2	−0.9
Zinman *et al.*	Exenatide, 10 μg BID	233	16	TZD + metformin	−0.9	−1.8
	Placebo				+0.1	−0.2
Heine *et al.*	Exenatide, 10 μg BID	551	26	Metformin + SU	−1.1	−2.3
	Glargine				−1.1	+1.8
Nauck *et al.*[a]	Exenatide, 10 μg BID	501	52	Metformin + SU	−1.0	−2.5
	Biphasic insulin				−0.9	+2.9
Kim *et al.*	Exenatide LAR, 0.8 mg/week	45	15	Metformin or diet	−1.4	0.0
	Exenatide LAR, 2.0 mg/week				−1.7	−3.8
	Placebo				+0.4	0.0

NOTE: All administration was via subcutaneous injection.
Abbreviations: BID, 2 times a day; HbA1c, hemoglobin A1c; LAR, long-acting release; n, number of patients in the trial; SU, sulfonylurea; TZD, thiazolidinedione.

Table 1 - Summary of Clinical Trials with the GLP-1 agonist, Exenatide, in Type 2 Diabetic Mellitus(32)

Glucagon-like peptide-1

GLP-1 is an incretin hormone secreted from the L-cells in the lower gut in response to meal ingestion, which in turn stimulates endogenous insulin secretion in a glucose-dependent manner. GLP-1 reduces excessive hepatic glucose production by suppressing glucagon secretion; delays gastric emptying resulting in lower postprandial glucose levels; and reduces food intake by central effects resulting, in weight loss. GLP-1 has also been shown to increase β-cell proliferation/differentiation and reduce β-cell apoptosis in pre-clinical in vitro and rodent models. There is also growing evidence that GLP-1 has beneficial cardiovascular effects.(33-37)

In the last decade, GLP-1 agonists have been targeted as promising treatments in T2DM, as a result of their potent glucose-dependent insulino-tropic effects.(38-40)The major drawback with endogenous GLP-1, with regards to administration as a medical treatment, is the short elimination half-life of 1.5 minutes ($t_{½}$ < 1.5 minutes after intravenous (iv) administration). This is due to GLP-1s rapid degradation by the capillary surface-membrane enzyme, dipeptidyl peptidase-4 (DPP-4).(41) From human studies it became clear that 24-hours infusion of native GLP-1 would be necessary to achieve satisfactory glycaemic control.(41) However this is both expensive and clinically impractical in T2DM patients. Treatment strategies circumventing this limitation, in the form of GLP-1 receptor agonists, have therefore been the main focus of recent research in T2DM.

Exenatide is the first of this group of agents to be approved, on both sides of the Atlantic, for clinical use in T2DM.(42) The fact that exenatide is administered twice daily by subcutaneous (SC) injection, in relation to meals, raised compliance concerns.

A once-daily human GLP-1 analogue, in the form of liraglutide, has now been manufactured by the Danish pharmaceutical company Novo Nordisk A/S. Liraglutide received marketing authorization by the European Medicines Agency (EMA) in Europe on July 3rd 2009, under the brand name Victoza ®.

Liraglutide (Victoza ®)

Liraglutide is a new once-daily human GLP-1 analogue developed by Novo Nordisk A/S (Bagsvaerd, Denmark). Liraglutide shares 97% sequence homology with human GLP-1, with the addition of a C16 fatty (palmitic) acid chain at position 26 (lysine) of the peptide and the lysine at position 34 replaced by arginine [Figure 3]. In contrast, exenatide, shares only 53% sequence homology with native GLP-1. In vitro receptor studies have shown that, despite these modifications, liraglutide retains selectivity, potency and affinity for the cloned human GLP-1 receptor.(43)

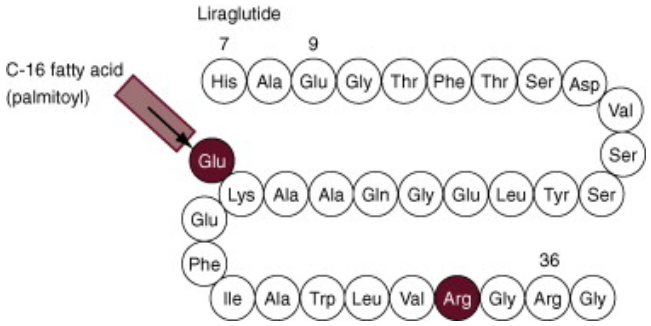


Figure 3 – Primary structure of liraglutide (shaded residues indicate differences from mammalian GLP-1). (44)

When administered subcutaneously, these structural modifications result in kinetic properties of the compound that produce stable elevated plasma levels of active GLP-1 after once daily administration. In rodent models of obesity and diabetes, liraglutide has been shown to lower blood glucose, stimulate insulin secretion, decrease plasma glucagon levels, inhibit gastric emptying, inhibit food intake, decrease body weight and improve ß-cell function when administered subcutaneously.(45-47) Therefore mirroring the incretin actions of native GLP-1 in animal models.

To date, 40 clinical trials, conducted world-wide, with liraglutide have been completed and are summarised in the EMA's European Public Assessment Report of 2009.(48) The 40

completed trials include 25 phase 1 trials, 8 phase 2 trials, and 7 phase 3 trials. Data from finalised trials have shown liraglutide to have a pharmacokinetic profile suitable for once daily administration, as evidenced by a relatively slow absorption ([t_{max}] =8-12 hours) with a terminal elimination half-life (t½) of approximately 13 hours. The pharmacokinetic profile is comparable between healthy subjects and subjects with type 2 diabetes.(48)

Mode-of-action trials in subjects with type 2 diabetes have demonstrated glucose lowering (FPG, postprandial glucose (PPG)), increased insulin secretion, restored ß-cell responsiveness to increasing glucose concentrations and delayed gastric emptying after a single SC dose of liraglutide. Importantly, during hypoglycaemia liraglutide did not impair glucagon action or the general counter-regulatory response, indicating a low risk of hypoglycaemia.(49;50) Subsequently, a comprehensive phase 3 evaluation consisting of six large randomized clinical trials of liraglutide in T2DM was started. The 'Liraglutide Effect and Action Diabetes (LEAD) program' involved 6500 participants from 41 countries world-wide and in total 4445 received liraglutide.(51) Results from these phase 3a trials in subjects with type 2 diabetes showed an improvement of glycaemic control after treatment with liraglutide.(52-56) A substantial and clinically relevant lowering of HbA_{1c} and fasting plasma glucose was observed after 26 weeks and 52 weeks of treatment with liraglutide. The various treatment regimens included in the trials were liraglutide doses of 0.6 mg, 1.2 mg or 1.8 mg per day as monotherapy or in combination with sulfonylurea, metformin or a thiazolidinedione. Based on the HbA_{1c} assessment it was concluded that treatment with liraglutide in monotherapy (1.2 or 1.8 mg) was superior to treatment with glimepiride 8 mg.(52) Furthermore, liraglutide in combination with one or two oral anti-diabetic drugs (OADs) was superior to treatment with the same OADs alone. Furthermore, the weight loss observed in earlier trials was confirmed by results from the phase 3a programme.(52-56) Interestingly, the recently published phase 3b trial showed that liraglutide was significantly superior to exenatide in glycaemic control and tolerability.(57)

Safety profile:

The safety profile of liraglutide exhibits the features expected for a GLP-1 analogue and is in accordance with observations from administration of both native GLP-1 and exenatide.(48) Gastrointestinal adverse events, including transient events of nausea, diarrhoea and vomiting were the most frequently reported events during the overall clinical development programme for liraglutide.(58) The highest reported frequency was seen during the initiation period of therapy. The gastrointestinal adverse events could however be mitigated by the use of a dose titration scheme.(44)

A small number of cases of acute pancreatitis in patients taking liraglutide monotherapy or in combination with OADs have been reported by Novo Nordisk A/S. However, the incidence rate is in the normal range for T2DM and to date, no cases of necrotizing or haemorrhagic pancreatitis have been reported.(48)

Benign and malignant thyroid C-cell tumours were seen in the 104-week carcinogenicity studies of liraglutide in rodents. By applying the Human Relevance Framework model,(59) studies concluded that the rodent thyroid C-cell tumours induced by dosing of liraglutide were caused by a non-genotoxic, specific receptor mediated mechanism to which rodents are particularly sensitive whereas non-human primates and humans are not.(60) In Novo Nordisk A/S's submission report to the Food and Drug Administration (FDA's) Endocrinologic and Metabolic Drugs Advisory Committee in April 2009, 6 cases of C-cell hyperplasia were reported in human clinical studies (with similar incidence rates between liraglutide and the comparator). In addition, papillary thyroid cancer cases (a more common type of thyroid cancer) were also reported at a rate of 1.6% versus 0.6% in patients treated with liraglutide and exenatide per 1000 patient-years of exposure, respectively.(60) A concern of the FDA committee was that it would be very difficult to rule out that liraglutide increases the risk of medullary thyroid cancer, due to the slow natural progression of the disease.(60) However, to date the phase 3 clinical programme (including phase 3b trials) has not shown any treatment-

related effect of liraglutide on the occurrence of medullary thyroid cancer.(61) In February 2010 Liraglutide received FDA approval for clinical use in the United States.

It is still not clear from the literature whether serum calcitonin levels should be routinely measured in patients with or at risk of thyroid nodular disease. Increased levels have been observed in rodent models exposed to liraglutide.(61) In contrast, the phase 3 clinical programme showed no treatment-related effect on calcitonin levels and in the phase 3b trial (liraglutide compared with exenatide) calcitonin levels actually decreased in the liraglutide treated patients.(57) Calcitonin screening for thyroid cancer has a high rate of false positives (e.g. inflammation) and has a low positive predictive value (PPV), especially for diagnosing medullary thyroid cancer (PPV < 10% for basal levels <100ng/L). Despite this, it is still recommended that clinical trials involving GLP-1 receptor agonists should monitor calcitonin levels and thyroid abnormalities closely.(61)

GLP-1 agonists in NAFLD

To date there has been no published human studies on GLP-1 analogues in biopsy-confirmed NASH. In a large open-labeled study (n=974) of obese, type 2 diabetic patients, 2 years of adjunctive exenatide treatment was shown to improve the liver injury biomarkers ALT and AST, to the extent that 39% of patients who entered the study with elevated ALT achieved a normal ALT (i.e. Female ≤19 IU/L; male ≤ 30 IU/L) by the end of treatment.(62) The studies main focus was the metabolic effects of exenatide in T2DM and was not specifically aimed at liver improvements in NAFLD patients. In fact, Bose and colleagues could not confirm the presence of NAFLD at baseline in the exenatide treated patients. They could only presume that the incidence of fatty liver disease in their study cohort was high, based on the knowledge that NAFLD is the most common cause of ALT elevations in patients with T2DM and obesity.(62)

Leptin deficient *Ob*/*Ob* mice have been extensively studied as naturally occurring models of hepatic steatosis.(63) The mutation of the *ob* gene prevents leptin transcription and subsequent biosynthesis. Utilizing the metabolic characteristics of *Ob*/*Ob* mice, both Ding et al and more recently Trevaskis JL, have identified that exenatide treatment is associated with reversed hepatic steatosis, lowered glucose and ALT levels, and attenuated weight gain.(64;65) Furthermore, exendin-4 (exenatide) treatment was seen in-vitro to significantly reduce mRNA expression of steatoryl-CoA desaturase 1 (SCD-1) and genes associated with de novo fatty acid synthesis in mice hepatocytes. The opposite effect was seen with genes (i.e. peroxisome proliferator-activated receptors (PPAR)) associated with fatty acid metabolism.(64) Of interest, Cohen's review in 2003 concluded that pharmaceutical manipulation of the SCD-1 enzyme, a recognized key component in metabolic syndrome, may have future implications in the treatment of obesity, T2DM and more specifically, excess fat accumulation in the liver.(66)

In-vivo gene therapy has also been used to characterize the long-term effects of elevated levels of GLP-1 analogues in diet-induced obesity mouse models. Samson and colleagues (67) constructed a helper dependent adenoviral (HDAd) vector for long-term expression of exendin-4 in-vivo. HDAd-Ex4 treatment reversed hepatic steatosis and reduced the expression of genes involved in de novo hepatic fatty acid synthesis in the obese mice models.(67) Similar findings have been recently reported in arthrogenic diet-induced obesity rat models exposed to sustained exendin-4 infusions.(65) Both studies recognized that the anti-NAFLD properties of exenatide were associated with significant body weight and adipose loss.(65;67) Whether these hepatic steatosis reversing properties of exenatide are due to associated weight loss or other specific GLP-1 receptor-mediated events, such as potential down-regulation of de novo hepatic fatty acid synthesis, remains to be determined.

Initial data presented by Anania and colleagues at AASLD November 2009, proposed that exenatide acts directly on human hepatocytes via a GLP-1 receptor, with a resultant insulin-

like response and reversal of triglyceride accumulation in human hepatocytes.(68) In contrast, the Tel Aviv liver research group failed to detect GLP-1 receptor expression in rat hepatocytes.(69) Despite this, however, Sholma et al agreed that GLP-1 is not solely acting via weight loss or indirect incretin effects, and maybe enhancing gluconeogenesis and suppressing lipogenesis by direct actions via a non-GLP-1 hepatocyte receptor (i.e. glucagon receptor).(69)

These studies highlight that human clinical studies designed to accurately assess changes in liver histology, lipid metabolism and metabolic components (i.e. weight) are required to elucidate GLP-1 agonist's direct and indirect effects in NASH.

AIMS, OBJECTIVES AND OUTCOME MEASURES

Aims and Objectives

This study will investigate the **efficacy** and **safety** of the long-acting GLP-1 agonist, Liraglutide in overweight patients with NASH, with or without type II diabetes.

In the type II diabetics, the study will evaluate standard lifestyle advice and the following therapeutic combinations against a placebo-control:

- Once-daily subcutaneous Liraglutide only
- Once-daily subcutaneous Liraglutide + oral metformin
- Once-daily subcutaneous Liraglutide + oral sulphonylurea
- Once-daily subcutaneous Liraglutide + oral sulphonylurea + oral metformin

In non-diabetics, the study will evaluate once-daily subcutaneous Liraglutide monotherapy or in combination with metformin against a placebo-control. The decision to have a non-diabetic with NASH on treatment with metformin will have been made by the patient's local liver specialist as part of the patient's standard healthcare prior to development of the current study. There is conflicting views in the literature on the efficacy of metformin in the treatment of NASH, however due to the metabolic overlap of NASH with 'impaired glucose tolerance' and insulin resistance the general consensus amongst liver specialists in the UK is that metformin (with its limited side effect profile) may have some benefit in NASH patients.

Primary Aim:

The primary aim of the study is to investigate whether 48-weeks treatment with Liraglutide improves liver histology in overweight patients with NASH enough to warrant further investigation.

Secondary Aims:

To investigate if any improvement in liver histology with 48-weeks treatment of Liraglutide in overweight patients with NASH is associated with:

- A reduction in global (hepatic, adipose and muscle) insulin resistance
- A reduction in hepatic de-novo lipogenesis (DNL)
- A reduction in BMI and weight
- A reduction in non-invasive inflammatory and fibrosis markers
- An improved QOL (SF-36v2)

To demonstrate that Liraglutide has a similar efficacy and safety in non-diabetics compared to diabetics in the treatment of NASH in overweight patients.

Primary objective:

The primary objective is to investigate whether 48 weeks treatment with once-daily injections of liraglutide improves liver histology in overweight patients with NASH enough to warrant further investigation.

BOTH of the following criteria MUST be met in order to report an improvement in liver histology after treatment;

- Disappearance of NASH (i.e. disappearance of hepatocyte ballooning)
- No worsening in fibrosis stage (as defined by Kleiner et al (1))

Secondary objectives:

Secondary objectives are to investigate whether 48 weeks treatment with once-daily injections of liraglutide in overweight patients with NASH;

(a) results in a significant change in:

- Mean NAS (as defined by Kleiner et al (1)) on liver histology
- Individual histological features of steatosis, hepatocyte inflammation and injury, and fibrosis.
- Non-invasive clinical markers of steatosis, steatohepatitis and fibrosis.
- Clinical components of metabolic syndrome (BMI, waist circumference, HDL)
- Insulin resistance and hepatic lipogenesis
- Patients QOL (SF-36)

AND

(b) does not compromise patient safety throughout the entirety of treatment and the washout (6-month) period.

Primary Outcome Measures

The primary outcome measure is the proportion of patients with an improvement in liver histology between biopsy at baseline and biopsy after 48-weeks of treatment.

The definition of an improvement in liver histology, as guided by the PIVENs(19) and Promrat et al.(30) trial designs and expert opinion from the American Association of the Study of Liver Disease (AASLD) 2009, requires BOTH of the following:

- Disappearance of NASH (defined as a disappearance of hepatocyte ballooning)

AND

- No worsening in Fibrosis

Hence, even in a case of NASH disappearance, a failure to meet the primary end point will be reported if the fibrosis stage has worsened on the post-treatment biopsy. Hepatocyte ballooning is widely recognised as the key lesion for distinguishing NASH from simple steatosis.

Liver biopsy remains the gold standard for diagnosing and assessing the extent of disease progression in NASH. Despite recent efforts to identify serological and radiological non-invasive markers of NASH,(70-76) histological evaluation remains the only definitive tool in determining the severity of NASH and distinguishing between 'simple steatosis' and steatosis with significant active hepatocyte injury and fibrosis.

The nomenclature and pathological features of NASH were first described in 1980.(10) It is now widely acknowledged that to confidently diagnose an individual as having NASH, the liver biopsy must contain a combination of;

- Steatosis (>5%)

AND

- Hepatocyte ballooning (+/- Mallory's Hyaline)*

AND

- Lobular inflammation (mixed infiltrate, related to foci of ballooning)**

* 'Definite' hepatocyte ballooning can be diagnosed on standard haemotoxylin and eosin (H&E) staining. For 'equivocal/uncertain' hepatocyte ballooning ubiquitin immune-histochemistry will be used to identify material compatible with Mallory's hyaline.

** Cases in which there is hepatocyte ballooning and pericellular fibrosis without conspicuous inflammation can also be classified as steatohepatitis ("steatofibrosis'').

Experts in the field of liver pathology regard hepatocyte ballooning as the most specific finding in NASH. To the extent that absence of hepatocyte ballooning in liver histology excludes a diagnosis of active NASH.

NAFLD activity score:

In the late nineties, Brunt (77) became the first histo-pathologist was to semi-quantitatively evaluate the histological features of NASH. Based on her criteria, Kleiner designed and validated the NAS for use in natural history studies of NAFLD (1). Once a histological diagnosis of NASH has been confirmed, NAS is the ideal histological scoring system for use in therapeutic interventional trials, as it specifically assesses only features of active liver injury, which are potentially reversible in the short term [Appendix I]. Fibrosis was excluded as

a component of the activity score, as it is less reversible and is felt to be as a result of disease activity.(1)

The total NAS is the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3) and hepatocyte ballooning (0-2), and ranges from 0 to 8. In the Kleiner study,(1) NAS scores of 0-2 and 5-8, were largely considered negative and positive for steatohepatitis respectively. Scores 3-4 were evenly distributed amongst cases not diagnostic, borderline, or positive for steatohepatitis [Figure 4].NAS is routinely used clinically at Birmingham's supra-regional liver unit for assessing NASH-confirmed individual's disease activity and progression.

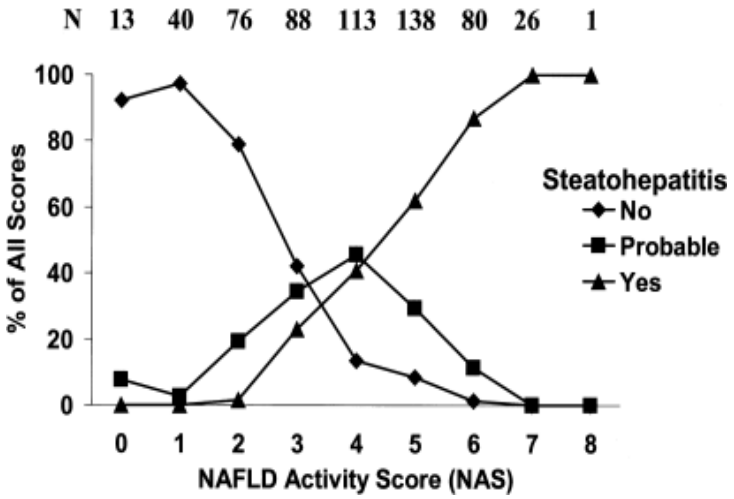


Figure 4. Fraction of adult cases with activity scores by diagnosis. For each activity score, the fraction of observations with a particular diagnostic categorization is shown. The total number of observations for each activity score is shown across the top of the graph.(1)

Justification for Primary outcome measure:

In recent years numerous therapeutic intervention trials in NASH, have utilised the Kleiner classification(1) of NAS as the international standard, to evaluate overall changes in histological disease activity in their subjects. To date, randomised-control trials have incorporated NAS as either the primary or secondary outcome measure. These studies highlight that NAS is a simple, reproducible scoring system that enables an accurate parallel evaluation of histological changes after therapeutic trials in NASH. Even though it is still unclear what prognostic implications the total NAS and its features have in the long-term, studies have commented that NAS is a more sensitive marker than fibrosis in assessing histological change in clinical trials of less than 24 months duration.(24)

Randomised placebo-control drug trials(22-24;30) have utilised the NAS as a primary end point by either;

- Calculating the difference between the treatment and the control groups in the MEAN change in NAS after treatment (see secondary outcome measures)

OR

- Describing histological criteria for what is 'a significant improvement in liver histology' and then calculating the percentage of each randomised trial arm that satisfied the criteria on the post-treatment liver biopsy.

Each of the trials, summarised in Appendix 2 define different 'Histological improvement' criteria. For the purpose of this study, a significant improvement in liver histology will be

defined as the disappearance of NASH and no worsening of fibrosis on the EOT liver biopsy (see above). In the absence of deterioration in fibrosis stage reversing a histological diagnosis of steatohepatitis to simple steatosis (+/- mild non-specific inflammation) would mean that liraglutide has had a significant clinical impact on the patient's liver disease activity.

The LEAN trial will state that the end-point will not have been met if the fibrosis stage has worsened after 48-weeks of treatment, in keeping with previously reported trials in NASH.(19)

Validation:

Two independent expert liver pathologists, from the trials lead centre (Queen Elizabeth Hospital, Birmingham, UK), will report the baseline biopsies (defined as liver biopsies performed as part of the patients standard healthcare within 6 months of trial screening) as either 'definitely NASH,' 'uncertain,' or 'not NASH.' .A patient will only be enrolled in the trial if the 2 independent pathologist reports agree that the biopsy indicates a diagnosis of 'definite NASH.'

Both the baseline (pre-study) and EOT liver biopsies will be analyzed by the two independent expert liver pathologists. All pathologists will be blinded to the assigned treatment, patients clinical and laboratory findings, and the liver biopsy sequence.

To validate the quality of the biopsy specimen per subject the core specimen length will be measured and the number of complete portal tracts will be recorded.

All the liver biopsies sampled in the trial will be assessed at the trials lead centre (Queen Elizabeth Hospital, Birmingham, UK). Therefore, prior to randomisation, the stained liver sections of potential trial participants in other UK trial centres will be sent to the lead trial centre in the UK. These sections will be pre-stained in other UK trial centres with H&E and specific connective tissue markers. A further 6 unstained sections will be sent to the lead trial centre, in the event that further staining is required by the two independent pathologists to accurately assess the parameters of steatosis, inflammation, hepatocyte ballooning and fibrosis.

The EOT liver biopsies (i.e. 48 weeks) collected from the trial participants enrolled at other UK trial centres will be sent over in the same manner to the lead trial centre in Birmingham, UK.

Secondary Outcome measures

The secondary outcome measures to be assessed at 48 weeks include:

Liver histology:

1.Calculation of the Mean Change in NAS on liver biopsy between baseline and 48 weeks (EOT). For each subject (n=1,2,3…):

Change in NAS (Δ NAS) = Baseline NAS - NAS after completion of treatment (TD 336)

Δ NAS will range from -8 to + 5 in each trial subject. A positive ΔNAS will indicate a histological deterioration and a negative ΔNAS will indicate a histological improvement in the individual trial subject with NASH.

2. Independent features of NAS:

Each of the independent features of the NAS (steatosis, lobular inflammation, hepatocyte ballooning) will be recorded by two independent pathologists (See section 2.2) on liver biopsies at baseline and 48 weeks. The scores for steatosis, lobular inflammation and hepatocyte ballooning are summarised in Appendix I.

3. Portal Tract Changes

There is increasing evidence in the field of liver pathology that portal tract changes are an intrinsic feature of NAFLD and may be important markers of disease progression in the future (Communication with Professor S Hübscher). For this reason histology sections will be scored on baseline histology and EOT histology for the following:

- Portal Inflammation: Score 0 – 4 (Ishak K, 1995)
- Interface Hepatitis: Score 0 – 4 (Ishak K, 1995)
- Ductular reaction: Score 0 - 3

4. Fibrosis stage

The liver biopsies will be staged according to the well-established Kleiner NAFLD Fibrosis Score (F0–F4).(1) The stages of fibrosis are shown in Table 2.

Fibrosis stage	Definition
0	None
1	Perisinusoidal or Periportal
• 1A	Mild, zone 3, perisinusoidal
• 1B	Moderate, zone 3, persinoidal
• 1C	Portal/periportal
2	Perisinusoidal and Portal/periportal
3	Bridging fibrosis
4	Cirrhosis

Table 2 – Fibrosis stages for liver disease.(1)

A 6-point modified Ishak score for fibrosis in NASH will also be retrospectively assessed in a blinded fashion after all the liver biopsies have been collected. The modified Ishak scoring system is marked out of 6, with 1-2 being fibrosis without bridging, 3-4 fibrosis with bridging and 5-6 representing fibrosis with nodular regeneration.

Well-established computerised morphometry will be utilised on each paired sample of stained liver sections to accurately analyse subtle differences in the extent of fibrosis that may not be detected by the scoring systems described above.

Glycaemic control

HbA_{1c}, FPG and Self-monitor Plasma Glucose (SMPG):

Serum HbA_{1c} is the clinical and research gold-standard for accurately measuring an individuals glycaemic control. Furthermore, it has been utilised as the primary end-point in numerous pharmaceutical clinical trials in T2DM, including the LEAD trials I-VI.(52-57;78) HbA_{1c}, FPG and SMPG will be recorded in the T2DM patients to monitor glycaemic control throughout the duration of the trial. The same will be carried out for the non-diabetics until visit 3, at which time they will discontinue SMPG sampling if they have experienced no symptomatic or recorded hypoglycaemic attacks in the preceding 4 weeks of treatment. The likelihood of an individual reporting a non-severe hypoglycaemic attack, if they have experienced no hypoglycaemic episodes during the first month of liraglutide treatment, is extremely unlikely.(79) However, if a non-diabetic reports a hypoglycaemic episode prior to visit 3, they will proceed with SMPG sampling until visit 4.

The LEAN trial will adopt the 4-pont SMPG profile. This involves the patient obtaining a droplet of capillary blood (via a finger-prick) and self measuring their glucose levels, using a Glucose monitor and test Strips, pre-breakfast and 90 minutes after breakfast, lunch and evening dinner. 4-pont SMPG profiles will be performed on two consecutive days in week 3 (prior to visit 3) and on two consecutive days every 12 weeks thereafter, until the end of treatment.

The LEAN trial has not adopted the 7-point SMPG profiles (i.e. before each meal, 90 min after breakfast, lunch, dinner and at bedtime) that were assessed in the large LEAD trials, of 26 weeks duration, as the investigators feel that the burden of having a new once daily injection and 7 finger pricks per day for plasma glucose monitoring in insulin naïve patients will be too much of a burden on the patients, for the purpose of a clinical trial. However, diabetic and non-diabetic patients will be given specialist nurse-led tuition on how to recognise symptoms of hypoglycaemia and subsequently record a SMPG in the event of this occurring.

Fasted serum samples will be collected for glucose and $HbA1_c$ at visits 1, 4, 5, 6, 7 and 8 (i.e. screening, treatment days 84,168, 252, 336 (EOT) and at 12 week follow-up post end of treatment (EOT).

Oral Glucose Tolerance Test (OGTT):

OGTT is the gold-standard for confirming a suspected new diagnosis of diabetes or impaired glucose tolerance. It is a simple, reproducible test that requires a fasted patient to consume 75g oral dose of glucose and have 2 hours post consumption blood glucose levels measured. Interpretation of the results is summarised in Table 3.

Non-diabetic patients prior to randomisation (i.e. visit 1) will undergo an OGTT to confirm whether they have normal or impaired glucose tolerance (IGT). To estimate whether treatment has had a significant impact on preventing the development of IGT or has actually

reverted them to a normal glucose tolerant state, will be assessed with a repeat OGTT at visit 7 (EOT).

Non-diabetics who have an OGTT and FPG result in keeping with a new diagnosis of T2DM on the screening visit will be counselled and referred to the diabetic specialist nurse. The results of the OGTT will be promptly forwarded to the primary care practitioner. Future management will be in concordance with the NICE guidelines for T2DM (i.e. lifestyle advice in keeping with standard health care).(42)

Type II Diabetic Patient	**FPB and OGTT in keeping with new diagnosis of diabetes**	• Fasting plasma venous glucose ≥7.0 mmol/l OR • 2-hour oral glucose tolerance test (OGTT) (with 75g glucose) plasma venous glucose ≥11.1 mmol/l
Non-Diabetic Patient	**Impaired glucose tolerance (IGT)**	• Fasting plasma venous glucose <7 mmol/l AND • 2-hour OGTT plasma venous glucose ≥7.8 mmol/l and <11.1 mmol/l
	Impaired fasting glucose (IFG)	• Fasting plasma venous glucose 6.1 to 6.9 mmol/L
	Normal Glucose Tolerance	• Fasting plasma venous glucose <6.1 mmol/l AND • 2-hour OGTT plasma venous glucose < 7.8 mmol/l

Table 3 – WHO/IDF Definition and Diagnosis of T2DM and IGT 2006(80)

Components of the metabolic syndrome

In recent years, NAFLD has been strongly associated with obesity, T2DM, hyperlipidaemia, hypertension and insulin resistance, which are the main features of the metabolic syndrome.(81) In 2002, the Adult Treatment Panel III (ATP III)(82) provided a working definition of the metabolic syndrome, based on a combination of central obesity, hypertension, hypertriglycerideaemia, low levels of high-density lipoprotein (HDL)-cholesterol, and hyperglycaemia. Approximately 33% of NAFLD patients have all 5 components of the metabolic syndrome.(81) Hepatic inflammation and fibrosis are associated with the presence and severity of the metabolic syndrome.(8;83) Furthermore, the presence of biopsy-confirmed steatohepatitis with fibrosis is associated with increased waist circumference, body mass index (BMI) and hypertriglycerideaemia.(84;85) Measuring the components of the metabolic syndrome will improve understanding of the incretin effect of liraglutide on these parameters in overweight patients with NASH. Correlations between changes in individual metabolic components (i.e. BMI) and liver histology may also be drawn in this cohort of patients.

Weight, BMI, waist:hip circumference:

Each participants weight (Kg), Height (cm), waist and hip circumferences will be measured at visits 1 to 8 (except visit 2), to calculate each participants BMI (Kg/m^2) and waist:hip circumference.

Weight will be measured with shoes and heavy clothing removed, using the same set of weighing scales for each participant and throughout the duration of the trial. Waist circumference will be recorded in keeping with ATP III recommendations of measuring the circumference immediately above the level of the iliac crests. The hip circumference will be recorded at the height of the greater trochanters. All measurements will be carried out by the same members of the trial team throughout the study.

Blood pressure:

Blood pressure will be measured using an established electronic blood pressure monitor on visits 1 to 8. The mean of two readings for each participant per visit will be recorded to reduce recording error. The pressure cuff size will be recorded.

Lipid profile:

Fasting blood samples will be collected on visits 1, 4, 5, 7 and 8 for total cholesterol, HDL-cholesterol and triglycerides. Concentrations will be recorded in mg/dl.

Non-invasive markers of liver inflammation and fibrosis

NAFLD fibrosis score

The NAFLD Fibrosis score was first described and validated by Angulo et al. in 2007.(74) It is a simple non-invasive scoring system that accurately seperates patients with NASH and advanced fibrosis (i.e. F≥3) from those without advanced fibrosis. The scoring system encompasses 6 independent indicators of advanced liver fibrosis;

NAFLD Fibrosis score = -1.675 + 0.037 X **age** (years) + 0.094 X **BMI** (Kg/m^2) + 1.13 X **IFG/diabetes** (yes = 1, no = 0) + 0.99 X **AST/ALT** ratio – 0.013 X **platelet** (x10^9/L) – 0.66 X **albumin** (g/dL)

A score of < - 1.455 has a 93% NPV of advanced fibrosis and a score of > + 0.676 has 90% PPV for advanced fibrosis (≥ F3). After retrospectively calculating the NAFLD fibrosis score Angulo and colleagues found that of their large cohort of 733 NASH patients, a liver biopsy could have been avoided in 75%. In patients with an indeterminate score of – 1.455 to 0.676, the study recommended that liver biopsy was deemed necessary in order to accurately assess the severity of NASH.(74) Subsequently, tertiary liver units throughout the UK, including the primary trial site, have adopted this scoring system as a means to determine if a biopsy is required in specific individuals.

The NAFLD Fibrosis score will recorded using a new web page calculator (86) at visits 1, 4, 5, 6, 7, and 8. The accuracy of the NAFLD Fibrosis scores from visits 1 and 7 in predicting the presence of advanced fibrosis on baseline and EOT liver biopsies will be assessed, respectively.

FibroMAX panel

FibroTest^{TM} (87), SteatoTest^{TM} (88) and NashTest^{TM} (89) are three simple blood test panels that have been developed to provide a non-invasive estimate of liver fibrosis and of its aggravating factors, steatosis and steatohepatitis, respectively.(71) The FibroMax ^{TM} (Biopredictive, Paris, France) is the combination of these three blood test panels on the same result sheet and provides researchers and physicians with a simultaneous and complete estimation of liver injury in NAFLD.(90) The FibroMax^{TM} combines 10 serum markers with the age, sex, height (m) and weight (kg) of each patient;

- Alpha 2 macroglobulin (g/L)

- Haptoglobulin (g/L)
- Apolipoprotein A1 (g/L)
- Total Bilirubin (µmol/L)
- GGT (IU/L)
- ALT (IU/L)
- AST (IU/L)
- Total cholesterol (mmol/L)
- Triglycerides (mmol/L)
- Fasting glucose (mmol/L)

The reliability of results has been shown to depend on local laboratory compliance with the pre-analytical and analytical conditions recommended by the quality chart of BioPredictive.(91) Analysis will be carried out at the BioPredictive laboratory at Hammersmith Hospital (London) to ensure maximum reliability of results in the LEAN trial.

Several large scale studies to date have highlighted the efficacy of FibroMax™ as a non-invasive biomarker for liver injury in T2DM and morbidly obese patients.(71;91) Furthermore, it has been shown to be a sensitive tool for detecting early histological changes in double-blinded pharmaceutical trials in NASH.(92) For a comprehensive summary of the FibroMax Panel refer to the BioPredictive Investigator's Brochure.(91)

The FibroMax panel will be performed on each participant on visits 1, 7 and 8.

Cytokeratin–18 (CK-18):

CK-18 (M30-CK18F) is generated by serum caspase 3 and is a product hepatocyte apoptosis in liver injury.(73) Recent research has evaluated the use of CK-18 assays as serum biomarkers of disease severity and progression in NASH.

Studies, encompassing large numbers of NAFLD patients, have stated that CK-18 fragment levels independently predict the presence of NASH and correlate with the magnitude of hepatocyte apoptosis and disease severity.(93;94) Furthermore, Fitzpatrick et al highlighted that CK-18 levels could distinguish between significant steatohepatitis (i.e. NAS ≥ 4 on biopsy) and simple steatosis with non-specific inflammation in paediatric NAFLD patients (median 334.5 IU/L vs 191 IU/L, respectively, p=0.007).(95) Area Under the Receiver Operating Characteristic Curve (AUROC) for NAS ≥ 4 was 0.75 and for fibrosis ≥ F2 was 0.69.(95) Ck-18 levels taken 6 months post bariatric surgery have been seen to significantly decrease in comparison to pre-surgical levels in obese patients.(96)

Overall, CK-18 appears to be one of the most promising serum predictors of histological NASH and severity of disease described in recent literature. For this reason, serum CK-18 fragment levels will be measured using an established specific immunoELISA (www.bioaxxess.com) at visits 1, 7 and 8. Analysis will be carried as per Bioaxxess Laboratory guidance at the centre for liver research (University of Birmingham) and concentrations will be recorded in IU/L.

Transient Elastography (TE) – Fibroscan® [optional depending on site availability]:

TE (Fibroscan®, Echosens, Paris, France) was first described in the literature in 2002.(97) It is a novel non-invasive imaging technique used to measure liver tissue stiffness and subsequently provide information on the severity of fibrosis. TE has been shown to be reliable in the assessment of liver fibrosis in numerous chronic liver diseases.(98;99)

Vibrations (Freq 50Hz) are transmitted through the liver tissue by an ultrasound transducer probe, which in turn induces an elastic shear wave that propagates through the underlying liver tissue. The velocity of the propagating wave, as measured by pulse-echo ultrasound acquisitions, is related directly to tissue stiffness.(70)

De Ledinghen and his French colleagues have recently presented TE data from 246 patients who had biopsy-proven NAFLD, with over 70% having a fibrosis score ≥ 3.(100) With a high negative predictive value (NPV) (i.e. value < 7.9 kPa, NPV 96%) and a modest PPV (i.e. value > 9.5 kPa, 72% PPV of biopsy proven ≥ F3), TE was stated to be a useful non-invasive screening test to exclude advanced fibrosis (i.e. ≥ F3). Most cases of discordance in this study were as a result of insufficient liver biopsy sample. However obese patients still contributed to discordance between the histology and the TE.(100) Using a new XL probe, the same centre increased the percentage of obese patients in which TE could give sufficient liver stiffness measurements by 60%.(101) For this reason, the new XL probe will be utilised in the LEAN trial for obese patients with a BMI ≥ 30Kg/m^2. De Ledinghen's group highlighted that the combination of NAFLD fibrosis score and TE resulted in an significant marker of biopsy-proven ≥F3 (i.e. AUROC = 0.92).

TE is painless, easy and rapid (<5 min) to perform in the outpatients clinic. TE will be undertaken on each participant on visits 1 (screening), 7 (EOT), and 8 and the results will be expressed in kilopascals (kPa). The median value of 10 validated measurements will be recorded with an expected range from 2.5 to 75 kPa. Normal values are reported as being around 5.5 kPa.(70)

Insulin Resistance

Insulin resistance is almost universal in NAFLD and is recognised to play an important role in hepatic fat accumulation by promoting peripheral lipolysis and hepatic de novo lipogenesis. It is therefore no surprise that numerous clinical trials to date have assessed the efficacy of insulin sensitizing drugs (i.e. Metformin and thiazolinediones (TZD)) in non-diabetic and diabetic patients with NASH. A recent Cochrane review concluded that the insulin-sensitizing drugs trialled to date lack efficacy in patients with NASH and further randomised clinical trials are needed if the potential effectiveness of this group of drugs for NASH is to be evaluated.(102)

Improvements in FPG and beta-cell function were consistently reported throughout the LEAD I–VI Trials in the T2DM patients whom received liraglutide.(79) Thus, the concept of whether liraglutide improves insulin resistance in patients with biopsy-proven NASH and whether improved insulin sensitivity is associated with a histological improvement after treatment with liraglutide, will be assessed in the LEAN trial.
#

<u>Homeostasis Model Assessment for Insulin Resistance (HOMA-IR):</u>

The homeostasis model assessment (HOMA) is a simple and robust mathematical model that provides estimates of insulin sensitivity from fasting serum samples of glucose and insulin.(103) The optimal sample should be the mean of three results at 5 minute intervals because insulin secretion is pulsatile. However, for research purposes many investigators have used single fasted samples.

This well-established model is an appropriate method for assessing change in insulin resistance in individuals as a result of an intervention (i.e. lifestyle, pharmaceutical).(103) HOMA-IR has been utilised in numerous therapeutic trials, including the recent PIVENs and FLIRT trials, to estimate drug efficacy on insulin sensitivity.(19;92) T2DM patients who require glycaemic control with Insulin Determir (see section 7.1) during the trial, will discontinue from

having fasting insulin levels measured and the HOMI-IR calculated. For the reason that the HOMA-IR calculation is only accurate in insulin naïve patients.

HOMA-IR will be calculated as:

HOMA-IR = [Fasting serum Insulin (µIU/L) x fasting serum Glucose (mmol/L)] ÷ 22.5 (104)

The advantage of using HOMA-IR in the LEAN trial is that it gives an estimate of basal insulin resistance, whereas all other methods in the literature provide estimates of stimulated insulin resistance (103). A measure of < 2.5 is considered normal and >2.5 is a marker of insulin-resistance.

HOMA-IR will be calculated from fasted serum samples at visits 1, 4, 5, 7 and 8.

2-step hyperinsulinaemic euglycaemic clamp + adipose microdialysis (**Sub-group and Birmingham site ONLY**)

Hyperinsulinaemic Euglycaemic Clamps (HEC) remain the research gold-standard for investigating and quantifying insulin resistance in patients.(103) HEC measures the amount of glucose necessary to compensate for an increased level of insulin without causing hypoglycaemia. During Step-1, the rate of glucose infusion, which is required to maintain euglycaemia (i.e. 5 mmol/L) in response to a low-dose insulin infusion, is an accurate measure of the hepatic insulin sensitivity. During step 2, the rate of glucose infusion in response to a higher dose of insulin infusion is recorded and provides a good estimate of an individual's peripheral insulin sensitivity (i.e. muscle/adipose) (see Appendix 3).

To specifically assess the extent of insulin resistance in an individual's adipose tissue (i.e. measure of peripheral lipolysis in response to insulin) a stable isotope tracer will be measured throughout the clamp experiment using adipose microdialysis (see Appendix 3).

In summary, these state-of-the-art techniques will provide an accurate measure of the effect of 12-weeks trial treatment on hepatic, muscle and adipose insulin sensitivity in patients with NASH. A full description of these protocols is outlined in Appendices 4 and 5.

Fractional Hepatic DNL (Sub-group and Birmingham site ONLY):

Studies have demonstrated that the main contributors to excessive hepatic fat accumulation are increased free fatty acid (FFA) mobilization from adipose tissue and enhanced hepatic de novo lipogenesis.(105) To assess the fraction that hepatic DNL contributes to total fat content of the liver and to what extent trial treatment effects this fraction, a unique stable isotope experiment(106) will be undertaken at visits 2 and 4. A full description of this protocol is outlined in Appendix 3.

Standardized Questionnaires: QOL (UK only), Nutrition (UK only), Alcohol (All sites)

SF-36 (v2) Health-Related QOL questionnaire (UK sites only)

Patients with NAFLD have been shown to have a significant decrement in QOL.(107;108) Both studies surveyed QOL using the generic Short Form 36 (SF-36v2) QOL questionnaire. Impaired quality of life was most evident in physical health rather than mental health.(107) A BMI greater than 40, the presence of biopsy-confirmed NASH and type 2 diabetes were all associated with poorer physical health in NAFLD patients.(107) Therefore participants meeting the inclusion criteria for the LEAN trial are likely to have a poorer QOL than the European population without chronic illness. Numerous studies have highlighted that low QOL scores are associated with an increased mortality risk.(109) This emphasizes the importance of the impact a new experimental drug (i.e. liraglutide) may have on an individuals QOL. On this basis, the most recent randomised-controlled pharmaceutical clinical trial in NASH to be reported from the US, adopted the SF-36 HR QOL questionnaire as a secondary outcome measure.(19)

The SF-36v2 questionnaire is a practical, reliable, and valid measure of physical and mental health that can be completed in five to ten minutes. SF-36 version 2® Health Survey (110) asks 36 questions to measure functional health and well-being from the patient's point of view.(111;112) Scoring of the SF-36 questionnaire will based on the instructions provided in the SF-36 users manual.(110)

The questionnaire will be completed by each trial participant at visits 1, 7 and 8.

Block Food Frequency Questionnaire (FFQ) (UK sites only):

In 1982, Block and colleagues developed a food item questionnaire to estimate usual and customary intake of a wide array of nutrients and food groups.(113) The food list for this questionnaire was developed from the National Health and Nutrition Examination Survey (NHANES) III dietary recall data.(114;115) The main drawback of the original questionnaire for use in clinical trials is that it required the investigator to carry out a 40 minute interview on each patient. Block have now developed a self-administered questionnaire consisting of 70-food related questions, which takes 15 to 20 minutes to complete items.(116)

The Block brief 2000 FFQ (116) is now one of the most widely used questionnaire for metabolic and dietary intervention studies. It has been used in multi-ethnic and mixed gender study populations. It is a validated, self-administered questionnaire with pictures of standardized serving sizes to estimate the usual dietary intake. It ranks individuals along the distribution of food intake and is sensitive to changes in intake, making it appropriate for many research purposes.

The Block Brief 2000 FFQ will be filled in paper format out at visits 1, 7 and 8. The questionnaire will be reviewed by a member of the trials team at each of these visits to ensure that there are no omissions or foods that are not addressed in the survey. A crib sheet will be available for use with the questionnaire to ensure that the patient understands what the American brands referred to are. The completed questionnaires will be analysed by Block Dietary Data Systems in Berkley, CA, USA.

The Block FFQ was chosen as it has been successfully used for nutritional assessment in metabolic disease populations similar to our study.(19;117)

Alcohol Use Disorders Identification Test (AUDIT):

The Alcohol Use Disorders Identification Test (AUDIT) questionnaire is a 10-item questionnaire that takes about 2–5 minutes to complete. The AUDIT questionnaire addresses frequency of alcohol consumption, alcohol related problems, and dependence

symptoms.(118) Each response to each of the 10 questions is scored from 0 to 4. Therefore the overall score ranges from 0 to 40. The following cut-offs apply:

- A score of ≥ 8 (men) and ≥ 7 (women) indicates a strong likelihood of hazardous or harmful alcohol consumption.
- A score of 13 or more is indicative of significant alcohol-related harm/dependence, and further assessment is advisable.
- A positive questionnaire score (> 8) is a good indication of hazardous alcohol consumption, and a negative score (< 8) is a good indication of no alcohol.

The AUDIT questionnaire has a positive predictive value of 98% (95% CI 97 to 100) for hazardous drinking, and a negative predictive value of 97% (95% CI 94 to 100) for alcohol dependence.(119) The AUDIT questionnaire will be filled out on paper format at visits 1,7 and 8. The purpose of the questionnaire is to ensure that the NASH patients enrolled in the LEAN trial have no risk factors for alcohol induced steatohepatitis (ASH). On visit 1 a score of ≥ 8 (men) and ≥ 7 (women) will exclude patients from enrolling in the trial.

TRIAL DESIGN

This is a 48-week, randomised controlled, double-blind, parallel group, multi-centre, multi-national (Birmingham [UK], Nottingham [UK] and Southampton [UK]) trial to determine the safety and efficacy of the GLP-1 agonist, liraglutide, on liver histology, metabolic components, insulin sensitivity and hepatic DNL in overweight patients with NASH.

The study will consist of three stages:

Stage 1	Screening, enrolment, randomisation and baseline investigations (2 weeks)
Stage 2	Treatment up to and including Day 336 (48 weeks) from randomisation
Stage 3	Follow-up assessment 12 weeks after EOT

The maximum duration of the trial including screening, treatment and the follow up visit will be approximately 62 weeks per subject, with the maximum treatment duration being 336 days (48 weeks). Patients will be randomly assigned to one of two trial groups:

Group 1	Control group. Treatment with once-daily subcutaneous injection of inactive treatment (liraglutide placebo) (Supplied by Novo Nordisk Ltd, UK)
Group 2	Experimental group. Treatment with once-daily subcutaneous injections 1.8mg active Liraglutide (Victoza ®) (Supplied by Novo Nordisk Ltd, UK)

Stratified randomisation will ensure that there are equal numbers of the following in each of the treatment groups (i.e. experiment vs control):

- Enrolled patients with T2DM
- Lead trial centre (Birmingham) versus non-birmingham trial centres (Hull, Nottingham, Southampton, Leeds)

Sub-group Study:

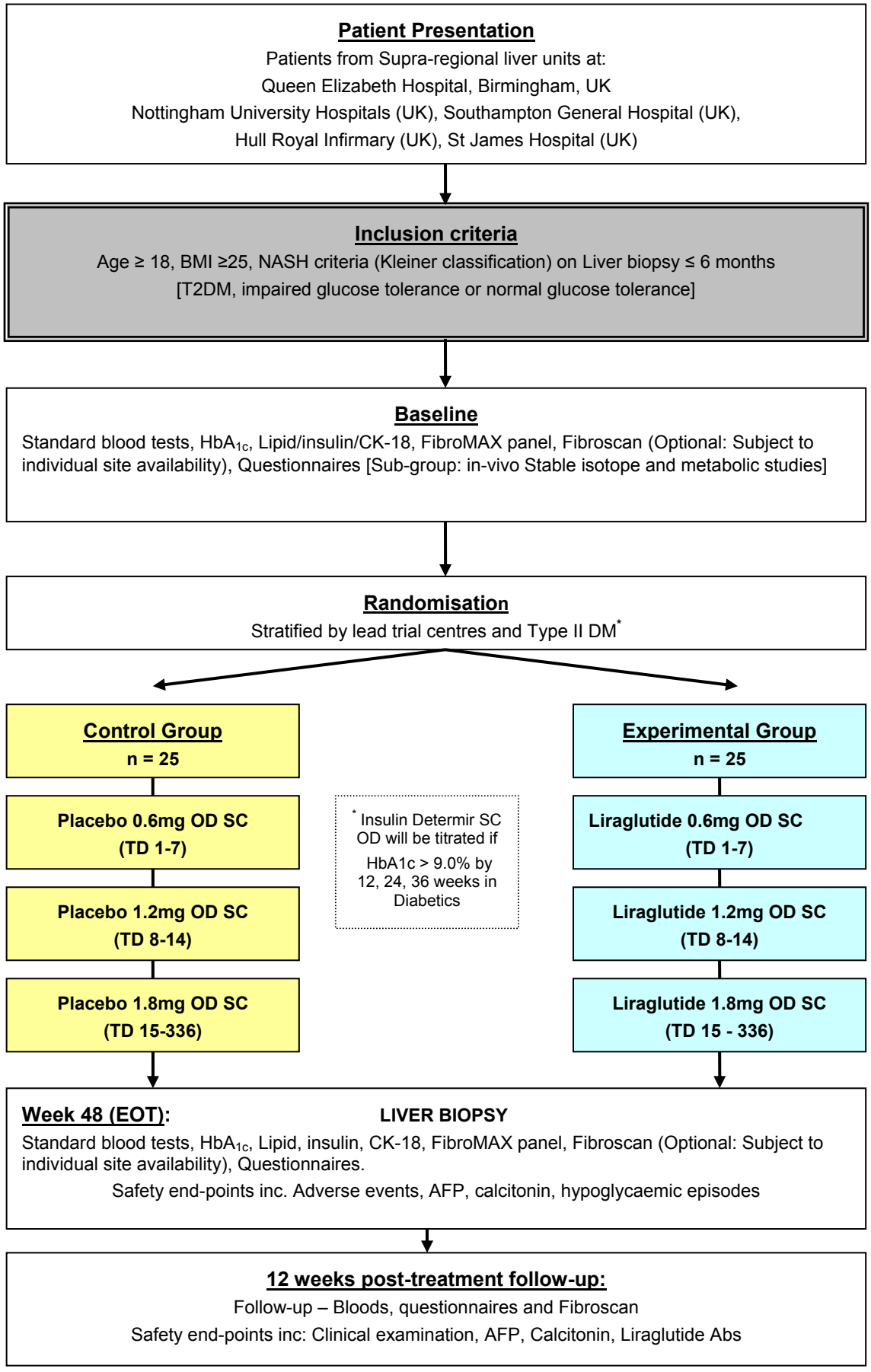
The sub-group study will consist of the specialist invasive metabolic studies. 2-step hyperinsulinaemic euglycaemic clamps and the adipose micro-dialysis are gold standard for measuring an individual's insulin sensitivity in the liver, muscle and adipose tissue respectively. Specialist stable invasive isotope studies will be utilised to measure the contribution of de-novo hepatic lipogenesis to total fat accumulation. Jeremy Tomlinson, a co-investigator at the Birmingham LEAN Trial site, has a wide knowledge and expertise in

invasive metabolic studies and has well established safety-proven protocols at the trial site (Appendix 3, 4 and 5). For these reasons the sub-group metabolic studies will only be undertaken on trial participants whom consent at a UK trial site, who can attend the highly specialised research facility (i.e. Wellcome Trust Clinical Research Facility, WTCRF) at Birmingham University Hospital. After randomisation to either the liraglutide or placebo arm, patients will undergo baseline invasive metabolic studies on a single visit prior to starting the treatment schedule (i.e. visit 2). Repeat invasive metabolic studies will then take place within 3 days of TD 94 (i.e. week 12 of treatment). The sub-study will involve an overnight stay at the well-equipped WTCRF on visits 2 and 4. The overnight stay will eliminate the inconvenience of having two trial visits on two consecutive days.

To enable enrolment into the LEAN trial, all patients must give consent to participation in the 48-week treatment period, follow-up appointments and compliance with investigations required for drug efficacy and safety monitoring. At any stage between randomisation and week 12, a patient may withdraw consent from being a participant in the sub-group metabolic studies, without necessarily giving a reason and without any personal disadvantage. The details of withdrawal will be clearly documented and communicated to the Trials Office. The date and reason the patient withdraws consent (state 'reason unknown' if no reason provided) will be clearly documented in the patient's medical notes. By withdrawing from the sub-study metabolic studies, unless specified, the patient will continue to be a participant for the remainder of the LEAN trial, as this will not impact on the primary outcome measure.

A full protocol of the invasive metabolic sub-studies is summarised in Appendix 3. The LEAN trial protocol is summarised in Figure 5.

Figure 5: **Outline of LEAN Trial**



ELIGIBILITY

Inclusion Criteria

- **NASH criteria at randomisation, all must have**:
  - Liver biopsy must be performed within 6 months of screening for the trial.
  - 'Definite' diagnosis of NASH by two independent expert histopathologists ['Definite' diagnosis of NASH defined as moderate macrovesicular steatosis, hepatocyte ballooning (+/- Mallory's hyaline), and lobular inflammation (mixed infiltrate, related to foci of ballooning) in the presence or absence of fibrosis]
  - NAFLD Activity Score (NAS) ≥ 3 (1), comprising of a minimum of 1 point from each of the individual steatosis, lobular inflammation and hepatocyte ballooning scores

- **Age ≥ 18 < 70 years old at randomisation**

- **Body Mass Index (BMI) ≥ 25 at randomisation.**

- **Patients with Type II Diabetes Mellitus at randomisation, must have;**
  - stable glycaemic control (HbA1c < 9.0%) and,
  - be managed with one of the following:
    - diet-control alone
    - diet-control and metformin and/or sulphonylurea

- **Patients with Non-Diabetes at randomisation, must be confirmed with the following:**
  - Impaired fasting glucose (IFG), defined using the European Criteria between 6.1 and 6.9 mmol/L

  and/or
  - Impaired glucose tolerance (IGT), defined as two-hour plasma glucose levels between 7.8 and 11.0 mmol/ on the 75-g OGTT

  or
  - Normal Fasting Plasma Glucose (FPG) < 6.1 mmol and Normal two-hour plasma glucose levels < 7.8 on the 75g OGTT.

Exclusion Criteria

Generic exclusion criteria:

- Refusal or lacks capacity to give informed consent to participate in the trial
- Participation in any clinical trial of an investigational therapy or agent within 3 months of randomisation
- Patient (or carer) deemed not competent at using the correct site and technique for subcutaneous injection of the trial treatment (containing dummy drug on practice) at visit 2
- NAS < 3
- Child's B or C cirrhosis
- Past medical history of multiple drug allergies (defined as anaphylactoid drug reactions in >2 drug groups)
- Presence of any acute/chronic infections or illness that at the discretion of the chief investigator might compromise the patient's health and safety in the trial
- Pregnancy or breastfeeding
- Women, of child-bearing age, who are not willing to practise effective contraception (i.e. barrier, oral contraceptive pill, impenon or PMHx hysterectomy) for the 48 week duration of the trial and for one-month after the last administration of the drug.
- Men, sexually active with women of child-bearing age, who are not willing to practise effective contraception for the 48 week duration of the trial and for one-month after the last administration of the drug.
- Liver disease of other aetiologies (i.e. drug-induced, viral hepatitis, autoimmune hepatitis, PBC, PSC, haemochromatosis, A1AT deficiency, Wilsons disease)
- Past medical/surgery history of;
    - Gastric bypass surgery
    - Orthotopic liver transplant (OLT) or listed for OLT
    - Hepatocellular, pancreatic, thyroid carcinoma (inc. Medullary thyroid carcinoma)
    - Multiple Endocrine Neoplasia syndrome type 2 (MEN 2)
    - Acute or chronic pancreatitis
    - Total Parenteral Nutrition within 6 months of randomisation
- Diagnosis of malignancy within the last 3 years (with the exception of treated skin malignancies)

- Hepatocellular Carcinoma – dysplastic or intermediate nodules to be excluded. Borderline cases to be discussed at Birmingham's tertiary hepato-biliary multidisciplinary team (MDT) meeting. Regenerative and other nodules to be included at the discretion of the chief investigator and the MDT.

- Family history of Medullary thyroid carcinoma

- Clinical evidence of decompensated chronic liver disease:
  - Radiological or clinical evidence of ascites
  - Current or previous hepatic encephalopathy
  - Evidence of portal hypertensive haemorrhage on endoscopy

- Abnormal clinical examination of thyroid (i.e. unexplained goitre or palpable nodules)

- ALT or AST > 10 x upper limit of normal

- Average alcohol consumption per week > 21 units (approx. 210g) male, >14 units (140g) female within the last 5 years.

- >5% weight loss since the diagnostic liver biopsy was obtained.

- Recent (within 3 months of the diagnostic liver biopsy or screening visit) or concomitant use of the following drugs;
  - Inducers of Hepatic steatosis – steroids (intravenous/oral), methotrexate, amiodarone
  - Weight-reducing therapies – Orlistat

- Recent (within 3 months of the diagnostic liver biopsy or screening visit) or significant change (as judged by the chief investigator) in dose of the following drugs;
  - Multi-vitamins/Vitamin E (containing > 200% recommended daily amount;>30mg/day)

- Known positivity for antibody to Human Immunodeficiency virus (HIV)

- Serum creatinine > 150 µmol/L or currently being treated with renal replacement therapy (i.e. Haemodialysis or Peritoneal Dialysis)

Subjects with Type II Diabetes exclusion criteria:

- Current or previous insulin therapy, with exception of previous short-term insulin treatment in connection with intercurrent illness is allowed (≥ 3 months prior to screening), at the discretion of the chief investigator.

- Subjects receiving Thiazolidinediones (TZDs), Dipeptidyl Peptidase (DPP) IV inhibitors and other GLP-1 agonists (i.e. Exenatide)

- $HbA_{1c} \geq 9.0\%$

- Recurrent major hypoglycaemia or hypoglycaemic unawareness as judged by the chief investigator

SCREENING AND CONSENT

Pre Screening

Potential trial subjects will be recruited from the NAFLD liver services at the supra-regional liver units in Birmingham's Queen Elizabeth Hospital (UK). Trial subjects may also be recruited from a further 5 liver specialist centres within the UK (including Nottingham, Southampton, Hull) to obtain the sample size required.

Potential trial subjects who are eligible for the trial (section 4.0 eligibility criteria) will be identified by the NAFLD specialist physicians (i.e. Hepatologist and Endocrinologist) who directly follow-up their care in the specialist NAFLD clinics (number of patients attending clinic per year > 650).

The specialist physician will then ask permission from the potential participant to be either contacted by the trial team or to be directly introduced to the trial team.

At the first meeting the trial team will introduce and explain the trial to the potential trial participant with oral and written information. At this stage the potential trial participant will have the opportunity to ask questions.

If the potential trial participant provisionally agrees to enrol in the trial, after reading the written information and discussing their potential participation with friends and family, a second visit will be scheduled (>24 hours after first meeting) to further discuss trial participation. Also at this stage, the LEAN dedicated histopathologists will be asked to review the Liver biopsy taken within the last 6 months (prior to screening) to decide whether or not the patient will be eligible for the trial with regards to the diagnosis of NASH.

Informed consent

The Investigator (or designated co-investigator as documented on the Signature and Delegation log) will obtain written informed consent for each patient prior to performing any trial related procedure. A Patient Information Sheet will be provided to facilitate this process. The Investigator will ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the patient. The Investigator should also stress that the patient is completely free to refuse to take part or withdraw from the trial at any time.

The patient will be given ample time (greater than 24 hours) to read the Patient Information Sheet and to discuss their participation with others outside of the site research team. The patient will be given an opportunity to ask questions which should be answered to their satisfaction. The right of the patient to refuse to participate in the trial without giving a reason will be respected.

If the patient expresses an interest in participating in the trial they should be asked to sign and date the latest version of the Informed Consent Form for the LEAN trial, inclusive of a well-established Ultrasound-guided liver biopsy consent form The Investigator (or designated representative) will then sign and date the form. A copy of the Informed Consent Form will be given to the patient, a copy should be filed in the hospital notes, and the original placed in the

Investigator Site File (ISF). Once the patient is entered into the trial the patient's trial number will be entered on the Informed Consent Form maintained in the ISF.

Details of the informed consent discussions will be recorded in the patient's medical notes, and will include date of and information regarding, the initial discussion, the date consent was given, with the name of the trial and the version number of the Patient Information Sheet and Informed Consent Form. Throughout the trial the patient will have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasions it may be necessary to re-consent the patient in which case the process above should be followed and the patient's right to withdraw from the trial respected. In addition to the LEAN trial consent form, the trial participant will be asked to consent for the ultrasound-guided liver biopsy prior to the procedure being undertaken at visit 7. This will be performed using standardised local NHS (for UK participants) healthcare consent form.

Details of all patients approached about the trial should be recorded on the Patient Screening/Enrolment Log and with the patient's prior consent their General Practitioner (GP) should also be informed that they are taking part in the trial. A GP Letter template will be provided to all participating sites for this purpose.

Only patients from the United Kingdom will be eligible to consent for the metabolic sub-group study due to the fact that the state-of-the-art facilities and local expertise required for the metabolic studies are based at the WTCRF, Birmingham, UK.

Screening

Visit 1 - Screening visit (Maximum of 14 Days prior TD 1)

Duration of visit estimated to be 2 ½ to 3 hours.

Prior to booking the screening visit the patient will be asked to fast from eating (water allowed) for 8 - 12 hours prior to screening blood samples being taken. The majority of the visits will start at 8 - 9am in the morning.

Patient consent:

- NO trial specific examinations, investigations or treatments, that do not involve part of the patient's routine standard healthcare, should be performed prior to obtaining written consent of the patient
- Discuss with patient all the relevant information, including aims, methods, risk and benefits of the trial, prior to obtaining consent.
- Once valid informed consent (i.e. written consent form signed and dated by the patient) the following screening data will be collected.

Demograhics + ID Number recorded:

- Patient's name, Age, DOB, sex, ethnicity
- Hospital trial site

Full medical history and examination, including:

- Current/recent symptoms, inc:
  - Weight loss/gain
  - Symptoms of hypoglycaemia (frequency, duration) if diabetic
- Past medical history:
  - Dates of diagnosis of:
    - NASH
    - Type II Diabetes
    - Overweight (i.e. duration (yrs) BMI ≥ 25)
    - Cardiovascular events (e.g. Myocardial Infarction (MI), Cerebrovascular Accident (CVA), Peripheral Vascular Disease (PVD)
    - Sleep apnoea, Polycystic Ovarian Syndrome (female only)
- Current/recent illnesses (3 months)
- Drug History:
  - Oral Anti-diabetic drugs (6 months) + dose changes
  - Anti-hypertensive
  - Lipid-lowering therapies
  - Multi-vitamins (containing vitamin E) + over-the-counter medications
- Drug Allergies
- Alcohol intake + history (confirmed by next of kin if possible)
- Full clinical examination (inc general, cardiovascular, respiratory, abdominal, neurological and thyroid)

Vital signs and observations:

- Record heart rate (beats per minute), blood pressure (mmHg), temperature (^{o}C), pulse oximetry (SaO_2) and Respiratory Rate (RR)
- Measure weight (kg), waist circumference (cm), hip circumference (cm) and height (cm) –see definitions
- Calculate BMI (kg/m^2) and Waist:Hip ratio

Blood samples:

- Baseline investigations:
  - FBC, PT, INR, U+E, LFT, AST, GGT, Amylase
  - Total cholesterol, HDL, TG
  - HbA_{1c}, TSH, Free Thyroxine (FT4)
  - Fasting samples of serum insulin and glucose
  - Calcitonin
  - CRP, AFP
  - FibroMAX panel, CK-18
- Liver Aetiology Screen (if no previous results available within < 6months)
  - HbsAg, HCV Ab
  - Ferritin, Transferrin Saturation, caeruloplasmin
  - AMA (+/-M2) , ASA, Ig's, serum electrophoresis
  - α1AT level (+/- phenotype if < 100mg/dL)

Calculate:

- NAFLD fibrosis score
- HOMA-IR score
- Total NAS for each liver biopsy (within 6 months)

Other investigations:

- 12-lead ECG
- Urine dipstix (store in -80^{0}C)
- Urinary pregnancy test (Females of child-bearing age)
- Transient Elastography (Fibroscan®)(Optional:Subject to individual site availability)
  - Median of 10 measurements (kPa) by expert single radiologist at the trial centre
  - Record Interquartile Range (IQR)
  - Probe used (M- or XL-probe)
- Oral Glucose Tolerance Test (Non-diabetics only)
  - Carbohydrate-rich meal (30–50 g) on night before test.
  - Overnight fast of 8–14 hours; drink only water.
  - Collect FPG
  - Timing of test (0 hours) starts at beginning of glucose drink.
  - Patient ingests 75 g glucose in 250–300 ml water over 5 minutes.
  - 2 hourly Plasma Glucose levels after 75g Oral Glucose will then be recorded
- Block Brief 2000 FFQ, and HR-QOL (SF-36v2) (see appendices)
- AUDIT alcohol questionnaire

If the results of FPG and OGTT in a patient, whom has no previous diagnosis of T2DM on screening, are in keeping with a new diagnosis of T2DM, the patient will be counselled accordingly at visit 2. The newly diagnosed T2DM patients will be managed as per NICE guidance and will still have the option to consent to the LEAN trial as a T2DM. On screening, a patient will be labelled as a non-diabetic if they have a normal FPG and OGTT, or IFG and/or IGT (Table 3).

Participants will be asked to read the patient information booklet and additional instructions of subcutaneous injections, as if they meet the above eligibility criteria, they will be trained in subcutaneous injections at visit 2 (prior to being started on the trial treatment).

TRIAL ENTRY

Confirmation of Eligibility

After the results of the screening visit (visit 1) are available, the following will be verified:

- Complete patient consent form

- Confirmation of all the inclusion criteria:
  - NASH criteria on Liver biopsy (within 6 months)
  - Age ≥ 18 years old
  - Body Mass Index (BMI) ≥ 25
  - Patients with Type II Diabetes Mellitus at randomisation must have a;
    - stable glycaemic control (HbA1c < 9.0%) and,
    - be managed with one of the following:
      - diet-control alone
      - diet-control and metformin and/or sulphonylurea
  - Non-Diabetic patients at randomisation, must have either:
    - Impaired fasting glucose (IFG), defined using the European Criteria between 6.1 and 6.9 mmol/L

    and/or
    - Impaired glucose tolerance (IGT), defined as two-hour plasma glucose levels between 7.8 and 11.0 mmol/ on the 75-g OGTT

    or
    - Normal Fasting Plasma Glucose (FPG) < 6.1 mmol and Normal two-hour plasma glucose levels < 7.8 on the 75g OGTT.

- Review of Exclusion Criteria

Randomisation

Visit 2 (1 to 2 days before starting study treatment – TD 1)

Patients will be randomized at the end of visit 2 when they have undergone all the baseline tests and have met all the eligibility criteria (including satisfactory competency at applying the study medication administration technique) to partake in the trial.

Patients will be randomly assigned to one of two treatment groups:

Group 1	Control group. Treatment with once-daily subcutaneous injection of inactive treatment (liraglutide placebo) (Supplied by Novo Nordisk Ltd, UK)
Group 2	Experimental group. Treatment with once-daily subcutaneous injections 1.8mg active liraglutide (Victoza ®) (Supplied by Novo Nordisk Ltd, UK)

Patients will be randomly assigned to either treatment on a 1:1 basis using computer generated randomisation. Stratified randomisation will ensure equal numbers of the following in each treatment group:

1. Type II Diabetes (vs non-diabetics)
2. Recruitment Trial Centre (Birmingham [lead site] vs non-Birmingham [Nottingham, Southampton, Hull, Leeds])

At randomisation patients will be allocated unique trial identification number to preserve patient confidentiality and to enable the study to be double-blinded. A 24 hour, 7 days a week unblinding service will be provided by the Emergency Scientific and Medical Services (eSMS) at Guy's and St Thomas' Hospital NHS Trust to ensure the safety of all trial participants (see section 8). The schedule for investigations and follow-up visits is summarised in section 7.

Contact details for 'Randomisation':

CRUK Clinical Trials unit
Vincent Drive
University of Birmingham
Birmingham
B15 2TT
Tel: 0800 371969 (9am-5pm, excluding weekends)
Fax: 0121 414828600

TREATMENT DETAILS

Medication preparation

Experimental Group:

Trade name: **Victoza ®** (Novo Nordisk Ltd, UK)

Active Substance: **Liraglutide** (human glucagon-like peptide-1 (GLP-1) analogue produced by recombinant DNA technology in *Saccharomyces cerevisiae*)

ATC Code: A10BX07

Liraglutide (Victoza ®), the Investigational Medicinal Product (IMP), will be supplied by Novo Nordisk Ltd, UK. This will be packaged and labeled in the standard manner by Novo Nordisk Ltd, to the extent that the receiving Trial Site will be blinded to the drug. Sealed envelopes will be sent with the drug packages to each of the trial sites pharmacy and coordinating departments. These envelopes will be opened by unblinded members of the study team (i.e. statistician and the database programmer in charge of randomization) to ensure firstly that each participant receives the correct study treatment and secondly that the participant and the remainder of the study team remain blinded to the allocated study treatment. The sponsor details and trial specific labels on each drug pack will meet with the requirements of the EU's Good Manufacturing Practice for Medicinal Products guidelines (Annex 13, Manufacture of investigational products, The Rules Governing Medicinal Products in The European Community, Volume IV). Liraglutide (Victoza ®) will be then stored in a refrigerator at 2 – 8 ^{o}c and dispensed by the hospital pharmacy at each trial site.

Liraglutide (Victoza ®) will be supplied in a cartridge contained in a pre-filled multi-dose disposable pen. Each pre-filled pen contains 18mg liraglutide (Victoza ®) in 3ml of clear, colourless, isotonic solution (inc. water for injections, disodium phosphate dehydrate, propylene glycol and phenol). Therefore each pre-filled pen delivers 30 doses of 0.6mg, 15 doses of 1.2mg or 10 doses of 1.8mg. Dosing with the pre-filled pen is controlled by turning the dose selector until the dose indicator lines up with the relevant dose.

Each patient will be trained to administer the study pre-filled pen subcutaneously, by a specialist nurse, in accordance with standard procedures in good clinical practice. Once the patient has been educated and is deemed competent he/she will be allowed to self-administer the trial medicine into their abdominal subcutaneous tissue.

The patient will be advised to discard the injection needle in accordance with local requirements after each injection and store the liraglutide (Victoza ®) pre-filled pen without an injection needle attached. This prevents contamination, infection, and leakage. It also ensures that the dosing is accurate.

The patients will be asked to administer liraglutide (Victoza ®) once daily at any time, independent of meals, and to inject subcutaneously, either in the abdomen, thigh or upper arm. The injection site and timing can be changed without dose adjustment. However, it is preferable that the patients inject liraglutide (Victoza ®) around the same time of the day, when the most convenient time of day for that individual has been chosen. Patients will be instructed to perform an air shot of 0.2µl (2 clicks) before the first use of a new pre-filled.

To improve gastro-intestinal tolerability the patients will be placed on 0.6mg liraglutide (Victoza ®) once daily for TD 1 - 7 of the trial. On TD 8 to 14, the dose will be increased to

1.2mg per day and by TD 15 the patient will be receiving the maximum daily dose of 1.8mg to the end of the trial. This 2 week dose escalation is in keeping with the recommendations in the European Public Assessment Report (EPARs) of liraglutide for authorised medicinal products for human use.(48)

In the T2DM patients, liraglutide (Victoza ®) will be administered as montherapy or dual therapy in combination with metformin or a sulphonylurea, or in triple therapy with metformin and a sulphonylurea. This combination of medications has previously been trialled in a phase 3 study(55), with only 2% of their 232 cohort experiencing a single hypoglycaemic event that required third party assistance. For this reason, the type II diabetics enrolled in the study will record self-measured plasma glucose (SMPG) when they are symptomatic or for two consecutive days (pre-breakfast, 1 hour post breakfast, post lunch and post evening meal) prior to each visit. In the event of recurrent hypoglycaemic (symptomatic, BM < 3.1 mmol/L) events the sulphonylurea dose will be reduced first by 50% (i.e. glimiperide from 4mg to 2mg OD) in keeping with European Public Assessment Report (EPARs) of liraglutide for authorised medicinal products for human use.(48)

The safety and efficacy of liraglutide (Victoza ®) 1.8mg once daily, in the treatment of type II diabetes, has been proven in a series of 6 large randomised-controlled trials containing in total over 3000 patients.(52-57) With the exception of hypoglycaemia the most common side effects of liraglutide (Victoza ®) used in combination with other anti-diabetes medicines, are nausea, vomiting, diarrhoea and headaches. The full list of all side effects reported with liraglutide (Victoza ®) are highlighted in the European Medicines Agency's (EMA) Package Leaflet and the patient information leaflet.(48)

The Committee for Medicinal Products for Human Use (CHMP) stated that Liraglutide's (Victoza ®) benefits in combination with one or two OADs were greater than its risks in achieving glycaemic control in T2DM. Subsequently, the European Commission granted a marketing authorisation valid throughout the European Union for liraglutide (Victoza ®) to Novo Nordisk A/S on 30 June 2009. The Marketing Authorisation Holder and Manufacturer, Novo Nordisk A/S, have supplied the investigators with a full summary of Liraglutide's (Victoza ®) characteristics and an EMA approved patient information leaflet. For further details please refer to the Summary of Product Characteristics (SmPC) for Liraglutide (Victoza ®) and Novo Nordisk A/S investigators brochure, last updated in May 2009.(48) In February 2010, the FDA granted a marketing authorisation valid throughout the United States of America for Liraglutide (Victoza ®) to Novo Nordisk.

Placebo-controlled group:

Name: **Liraglutide-placebo** (Novo Nordisk Ltd, UK)

Liraglutide placebo will be supplied by Novo Nordisk Ltd, UK. This will be packaged and labeled in the standard manner by Novo Nordisk to the extent that the receiving Trial Site will be blinded to the drug. Sealed envelopes will be sent with the drug packages to each of the trial sites pharmacy and coordinating departments. These envelopes will be opened by unblinded members of the study team (i.e. statistician and the database programmer in charge of randomization) to ensure firstly that each participant receives the correct study treatment and secondly that the participant and the remainder of the study team remain blinded to the allocated study treatment. Each drug pack will arrive at each trial site pre-labeled by Novo Nordisk with the trial specific labels (stating the sponsors, chief investigators name, and the name of the trial) to meet with the requirements of the EU's Good Manufacturing Practice for Medicinal Products guidelines (Annex 13, Manufacture of investigational products, The Rules Governing Medicinal Products in The European Community, Volume IV). Inactive treatment (liraglutide placebo) will be stored and dispensed by the hospital pharmacy at each trial site. Patient specific details (patient name and trial

number) will be added by the local pharmacy department AFTER the patient has been randomised into the clinical trial and prior to dispensing of the IMP.

Liraglutide-placebo will be supplied in a cartridge in a pre-filled multi-dose disposable pen. Each pre-filled pen will contain 3ml of clear, colourless, isotonic solution. The composition of the placebo solution for injection, apart from the active substance liraglutide, will be identical to the IMP. Each pen will deliver 30 doses of 0.6mg placebo, 15 doses of 1.2mg placebo and 10 doses of 1.8mg placebo. The amount of fluid injected will be escalated as per the liraglutide (experimental) group. The amount to be injected will be 0.6mg on treatment days 1 to 7, 1.2mg on days 8 to 14 and 1.8mg placebo on days 15 to 336 (i.e. equal amount of solution as per experimental liraglutide group, but without the active liraglutide substrate).

Instructions with regard to time, site, frequency and disposal of the liraglutide-placebo injection will be the same as with active liraglutide. This is to ensure, that with exception of the active liraglutide (Victoza ®) substance, both groups are treated identically to comply with the double-blinded nature of the study.

Insulin detemir [Recovery of glycaemic control in T2DM]:

Trade name: **Levemir** (Novo Nordisk A/S, Denmark)

Active Substance: **Insulin Determir** (produced by recombinant DNA technology in *Saccharomyces cerevisiae*)

Insulin Determir (Levemir®) will be prescribed by a study doctor (PI or co-investigator) and supplied by the patient's local hospital trust as the indication for prescription in the trial will be to ensure that a diabetic patient's glycaemic control is not compromised as a result of being a trial participant.

Insulin detemir (Levemir®) is a long-acting soluble insulin analogue, developed to enable subjects with diabetes to maintain more stable glucose levels with less day-to-day variation. Insulin detemir is a derivative of human insulin (Lys^{B29}(Nε-tetradecanoyl) des(B30) human insulin), in which the threonine residue at position B30 of the human insulin molecule has been removed and a C14 fatty acid side chain has been attached to position B29.(120)

Similar to native insulins, insulin detemir exists predominantly in the hexameric state in the presence of zinc and phenol. The protracted action of insulin detemir is mediated by the strong self-association of insulin detemir molecules at the injection site and albumin binding via the fatty acid side-chain. The rate of absorption is limited by the low concentration of insulin detemir available for diffusion through the tissue and passage across the capillary wall. More than 98% of insulin detemir in the bloodstream is albumin bound, and insulin detemir is distributed more slowly to peripheral target tissues compared to Neutral Protamine Hagedorn (NPH) insulin. These combined mechanisms of protraction provide a more reproducible absorption and action profile of insulin detemir compared to NPH insulin.(121)

In vitro studies have shown that insulin detemir is 98.8% albumin-bound (range 98.4-99.3%) in human plasma with no gender difference. No interaction on the plasma protein binding of insulin detemir was exerted by myristic acid or by palmitic acid. A recent review article(122) concluded that changes in plasma protein binding rarely affect the clinical exposure to a drug.

The insulin detemir preparation is clear, colourless and ready for use with no need for agitation or re-suspension.

Clinical trials have confirmed a sustained blood glucose lowering effect with insulin detemir in both healthy subjects, and in subjects with type 1 diabetes or type 2 diabetes. A less pronounced peak of action has been observed with insulin detemir compared with NPH insulin. In addition, lower within-subject variation has been observed compared with NPH insulin (123). Treatment with insulin detemir administered once-daily, in subjects with poorly controlled type 2 diabetes, has also been associated with a lower risk of hypoglycaemia and less body weight gain compared with NPH insulin. Insulin detemir is approved for treatment of diabetes mellitus in combination with oral antidiabetic agents and as part of a basal-bolus insulin regimen. For further details please refer to the Summary of Product Characteristics (SmPC) for insulin detemir (124) and the US Label Information.

Use in the study (T2DM only):

Treatment with insulin detemir will be open-labelled throughout the trial. **Insulin detemir will be offered to the T2DM patient enrolled in the trial if their HbA_{1c} > 9 % at visits 4 (12 week), 5 (24 weeks), and 6 (36 weeks)**. If the patient does not wish to start insulin treatment then their participation in the study will not be affected. The introduction of insulin at this stage is to ensure that the glycaemic control of the T2DM is not compromised by being part of the clinical trial. Experts in diabetes care (Prof S Gough) predict that the minority (if any) of diabetic trial participants will require the addition of insulin determir. For the reasons that the study exclusion cut-off for glycaemic control is a HbA_{1c} > 9 % at screening and that pre-study (as part of their standard healthcare) the diabetic patients will have been under strict glycaemic control in primary and secondary care. If prescribed insulin determir, the trial participant will be asked to perform one fasted pre-breakfast SMPG per day and record them in their trial booklet until the patient is on a stable dose of insulin determir. This will guide the trial investigator in titrating the insulin determir dose throughout the study. If the HbA1c is > 9.0 from the visits 4, 5 or 6 blood results, the patient will be contacted by telephone (by trial investigator) and an 'unscheduled' visit will be organised at the WTCRF (at the convenience of the patient) to give the participant guidelines of how to use insulin determir. A prescription for insulin determir will be given at this visit and the participants GP (and local, regular consultant diabetologist) will be informed by telephone and letter of the addition of insulin determir. Insulin detemir will be available in a concentration of 100 U/mL, as a 3 mL FlexPen®. Insulin detemir will be administered once-daily, with the evening meal or at bedtime, injected subcutaneously in the thigh, abdomen or upper arm. The injection area chosen should remain unchanged throughout the remainder of the trial, but rotation within the area is requested. Liraglutide (Victoza ®) and insulin detemir should not be injected in close proximity. Insulin detemir dose will be adjusted by the Investigator at site visits and telephone contacts, based upon the SMPG and the titration guidelines [Table 4].

Average pre-breakfast SMPG (mmol/L)	**Insulin Determir dose adjustment** (Units)
>10.0	+ 8
9.1 – 10.0	+ 6
8.1 – 9.0	+ 4
7.1 – 8.0	+ 2
6.1 – 7.0	+ 2
If single SMPG measurement (mmol/L)	
3.1 – 4.0	- 2
< 3.1	- 4

Table 4 – Insulin Determir (Levemir) titration guidelines (48)

Titration target during the treatment period will be to reach the fasting SMPG target of 4.0-6.0 mmol/L (72 – 108 mg/dL). The frequency of site visits/telephone contacts will ensure correct titration of insulin detemir.

Treatment scheduling

The treatment schedule includes 336 days (48 weeks) of treatment (Liraglutide or Placebo) in total. The initial study visit will be scheduled for a mutually convenient date for both the patient and investigator.

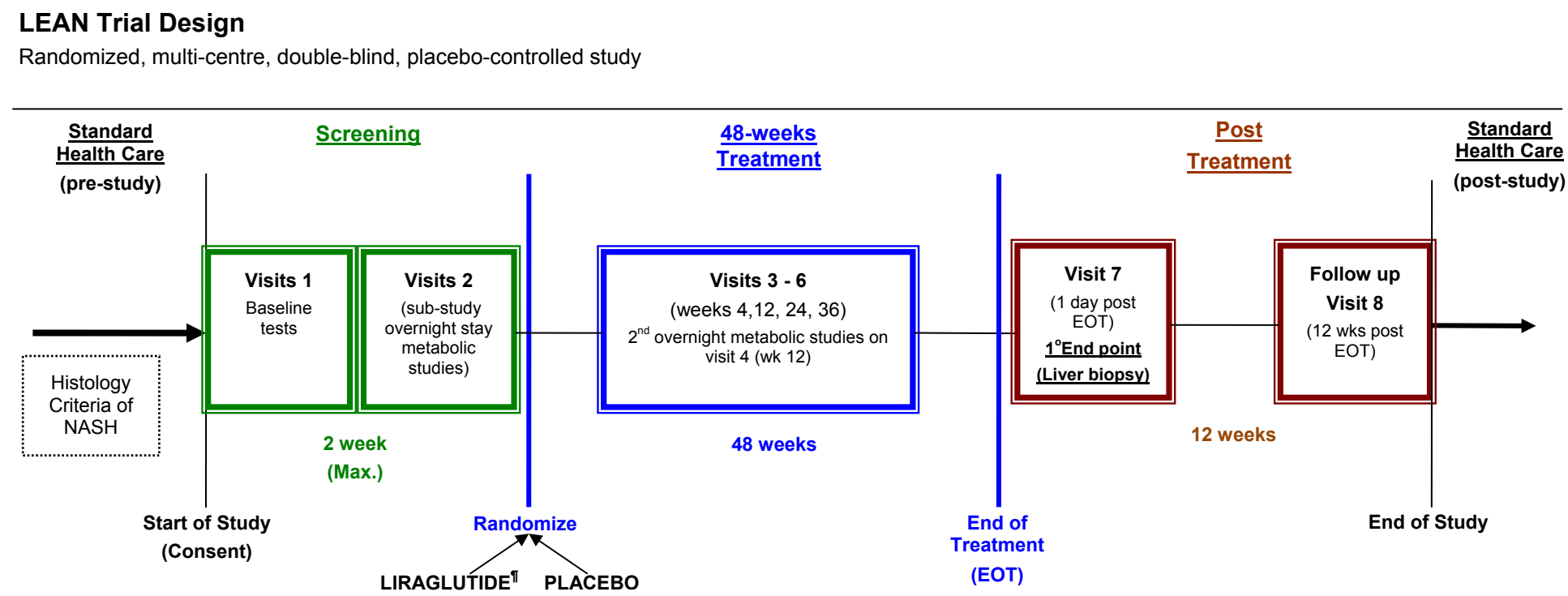
The time delay (maximum 14 days) between screening visit (visit 1) and the 1st day of treatment (TD 1) will be due to:

- Processing the screening blood samples [24 - 48 hours]
- Scheduling (overnight stay), preparation and procedures required for the baseline metabolic sub-group studies i.e. 2-step Hyperinsulinaemic Euglycaemic Clamp, Adipose Microdialysis and stable isotopes experiments [1 - 12 days]
- Training and competency assessment in subcutaneous injection of the pre-filled multi-dose disposable pen [24 – 48 hours]
- Process of randomization

The 1st day of treatment will be the morning after completion of visit 2. Patients will receive a treatment pack of 3 pharmacy-labelled treatment pre-filled pens, each containing 18mg/3 ml of either the placebo or liraglutide (Victoza ®). Pens will be supplied with 31G needles (by Novo Nordisk Ltd, UK) after randomisation at the end of visit 2. 1 pen containing 18mg/3ml will ensure a 10 day supply of either 1.8mg placebo per day or 1.8mg of liraglutide (Victoza ®) per day. The first pen prescribed will enable the two-week escalation course of the treatment, including a 0.2µl air shot, 7 days of 0.6 mg injections, 7 days of 1.2 mg injections and 3 days of 1.8mg injections. If the patient at any stage in the trial administers the wrong dose or loses a pen, the patient will be instructed to inform the investigating team by telephone conversation the same day. Prompt advice will either be given via telephone conversation or an unscheduled visit will be arranged depending on individual circumstances or degree of dose error. The treatment will only be un-blinded to the investigator in the case of an adverse event. A 24 hour un-blinding network will run throughout the duration of the trial.

The 1st day of treatment will be the day after completion of the baseline run of the hyperinsulinaemic euglycaemic clamp, adipose microdialysis and stable isotope experiments. The second run of the hyperinsulinaemic euglycaemic clamp and adipose microdialysis experiments will take place within 3 days of the TD 84 (12 weeks). If the trial site scheduling or patient availability does not permit this, then the second run of the hyperinsulinaemic euglycaemic clamp, adipose microdialysis and stable isotope experiments will commence as close to TD 84 as possible. The treatment will remain continuous and will not be prevented by a delay in experiment scheduling.

Figure 6 – Schematic diagram of LEAN Trial design



¶ Liraglutide or Placebo will be dose titrated from 0.6mg to 1.8mg OD over 2 weeks

Assessment Schedule

The trial is double-blinded, thus the following assessment schedule will apply to both the active liraglutide (Victoza ®) and non-active placebo-control groups.

Visit 1 (-14 days to - 3 days prior TD1)

Screening visit (section 5.3). A potential trial participant's liver biopsy will be reviewed and MUST be reported as a 'definite' diagnosis of NASH by two independent histopathologists, prior to undertaking in the blood sampling and radiological investigations required on the screening visit.

Visit 2 (-1 days to -2 days prior TD1) in the following order:

Total estimated duration = 30 to 60 minutes (without the metabolic sub-study) or 20 - 24 hours inclusive of the overnight stay (with the metabolic study).

- At the start of this visit each participant will be informed of any abnormal results from visit 1 that were unexpected and mean that they are not eligible to enter the study (i.e. HbA1c >9.0). If they are not eligible any concerns or questions they have regarding their results will be addressed by the trial investigators. The participants GP will be informed immediately by telephone and by written format of a result that may impact on the illegible patient's standard healthcare (i.e. HbA1c).

- Assessment of whether patient is competent at using the correct site and technique for the subcutaneous injection of the pre-filled multi-dose disposable pen (containing dummy drug on practice) will then take place if the patient has meet the eligibility criteria for the trial. This assessment will be carried out by either a trained diabetic specialist nurse, a Liver nurses or a co-investigator (listed above).

- When they are judged to be competent at correctly (and safely) administrating and storing the trial treatment, the eligibility criteria checklist will be complete.

- Patients will be provided with a 'Treatment and Clinical events booklet', in which they can record and sign off each administration of the treatment, dose and time of day. The booklet will also be used for them to document the time and frequency of symptoms, hypoglycaemic events and SMPG. A full list of their medications and changes to medications will also be documented in the booklet. A list of hypoglycaemic symptoms for the patients to be aware of will be documented.

- Patients will also be supplied with portable sharps bin that will be disposed of by the trial site. Both non-diabetic and diabetics will be supplied with a Blood Glucose monitor and test Strips.

- **At Birmingham's lead trial site only** - Perform protocols (Appendix 3,4 and 5) for:
    - 2-step hyperinsulinaemic euglycaemic clamp (HEC)
    - Adipose microdialysis
    - Stable isotope

- Randomization (section 6.2) will occur after completion and receipt of all of the screening investigations.

- Randomization will provide a unique trial identification number for each trial participant

- **The first participant's prescription of trial treatment will be dispensed by the sites main pharmacy on this visit. A 30 day supply will be dispensed (i.e. 3 pre-filled pens of 18mg/3ml of study medication)**

- Participant informed to be fasted from midnight the day before Visit 3

Visit 3 (2 days +/- TD 28/4th week of treatment)

Estimated duration of visit = 30 – 60 minutes

- Clinical assessment
    - Record clinical events
    - Record adverse events
    - Record new and changes to concomitant medications
    - Record of treatment compliance
    - Perform clinical examination (including injection sites and thyroid examination)
    - Review each patients 4-Point SMPG

- Measure Vital signs
    - HR, BP, weight (Kg), Height (cm), waist:hip circumference (cm), Temperature, SaO_2 and RR.

- Obtain blood for:
    - FBC, U+E, LFT, AST, GGT, PT, INR, TFTs, FPG, Amylase

- Prescribe 56 day (8 week) supply of treatment i.e. 6 x pharmacy-labelled treatment pre-filled pens (each containing either 3ml of placebo or 18mg/3ml of Liraglutide). This dose will last until the next visit.

- Schedule time and date of visit 4, which must be +/- 2 days from the 84th day of treatment

- Patient informed to be fasted from midnight the day before Visit 4

Visit 4 (2 days +/- TD 84/12th week of treatment)

Birmingham trial site: Metabolic Sub-group study only

Total estimated duration of visit = 20-24 hours (inclusive of an overnight stay, provided at the WTCRF)

A participant that has consented for the sub-group study will attend the evening (5-6pm) before visit 4, to initiate protocols for Metabolic Sub-study and overnight stay:

- 2-step hyperinsulinaemic euglycaemic clamp (HEC)
- Adipose microdialysis
- Invasive stable isotope study

All participants:

- Clinical assessment
    - Record clinical events
    - Record adverse events
    - Record new and changes to concomitant medications
    - Record of treatment compliance
    - Perform clinical examination (including injection sites and thyroid examination)
    - Review each patients 4-Point SMPG

- Perform 12-lead ECG
- Urinalysis
- Measure Vital signs
  - HR, BP, weight (Kg), Height (cm), waist:hip circumference (cm), Temperature, SaO_2 and RR.
- Obtain fasted blood samples (at 8 am, the day of the clamp study) for:
  - FBC, U+E, LFT, AST, GGT, INR, HbA_{1c}, TFTs, FPG, Amylase
  - Fasting Lipid profile and serum insulin
  - Calculate NAFLD Fibrosis Score and HOMA-IR
- After the clamp and adipose microdialysis study is complete, the investigator will prescribe 84 days (12 week) supply of treatment i.e. 9 x pharmacy-labelled treatment pre-filled pens (each containing either 3ml of placebo or 18mg/3ml of Liraglutide). This dose will last until the next visit.
- Schedule time and date of visit 5, which must be +/- 2 days from the 168th day of treatment
- Patient informed to be fasted from midnight the day preceding Visit 5
- In Type 2 Diabetic patients (only), If HbA_{1c} > 9.0 % on this visits blood results, the following will take place at unscheduled visit:
  - Provide patient information sheet on Insulin Determir (Levemir)
  - Contact details provided for diabetic specialist nurse
  - Patient will be offered treatment with Insulin Determir (Levemir) Sc once-daily, with the evening meal or at bedtime after completion of the invasive metabolic studies.
  - The addition of insulin to the patient's treatment regimen is recommended by national healthcare guidelines at this level of glucose control, but the patient will have the final decision of whether they chose to start insulin therapy.
  - Insulin detemir (Levemir) dose will be adjusted by the Investigator at site visits and telephone contacts, based upon the SMPG and the titration guidelines (see section 7.1).

Other UK trial sites: When sub-group study NOT included

Total estimated duration of visit = 1 hour

- Clinical assessment
  - Record clinical events
  - Record adverse events
  - Record new and changes to concomitant medications
  - Record of treatment compliance
  - Perform clinical examination (including injection sites and thyroid examination)
  - Review each patients 4-Point SMPG
- Perform 12-lead ECG
- Urinalysis
- Measure Vital signs
  - HR, BP, weight (Kg), Height (cm), waist:hip circumference (cm), Temperature, SaO_2 and RR.

- Obtain fasted blood samples (at 8 am) for:
  - FBC, U+E, LFT, AST, GGT, INR, HbA_{1c}, TFTs, FPG, Amylase
  - Fasting Lipid profile and serum insulin
  - Calculate NAFLD Fibrosis Score and HOMA-IR

- The investigator will prescribe 84 days (12 week) supply of treatment i.e. 9 x pharmacy-labelled treatment pre-filled pens (each containing either 3ml of placebo or 18mg/3ml of Liraglutide). This dose will last until the next visit.

- Schedule time and date of visit 5, which must be +/- 2 days from the 168th day of treatment

- Patient informed to be fasted from midnight the day preceding Visit 5

- In Type 2 Diabetic patients (only), If HbA_{1c} > 9.0 % on this visits blood results, the following will take place at unscheduled visit:
  - Provide patient information sheet on Insulin Determir (Levemir)
  - Contact details provided for diabetic specialist nurse
  - Patient will be given the option of being treated with Insulin Determir (Levemir) Sc once-daily, with the evening meal or at bedtime.
  - The addition of insulin to the patient's treatment regimen is recommended by national healthcare guidelines at this level of glucose control, but the patient will have the final decision of whether they chose to start insulin therapy.
  - Insulin detemir (Levemir) dose will be adjusted by the Investigator at site visits and telephone contacts, based upon the SMPG and the titration guidelines (see section 7.1).

Visit 5 (2 days +/- TD 168/ 24 weeks of treatment)

Estimated visit duration = 1 hour

- Clinical assessment
  - Record clinical events
  - Record adverse events
  - Record new and changes to concomitant medications
  - Record of treatment compliance
  - Perform clinical examination (including injection sites and thyroid examination)
  - Review each patients 4-Point SMPG

- Perform 12-lead ECG

- Urinalysis

- Measure Vital signs
  - HR, BP, weight (Kg), Height (cm), waist:hip circumference (cm), Temperature, SaO_2 and RR.

- Obtain blood for:
  - FBC, U+E, LFT, AST, GGT, PT, INR, HbA_{1c}, TFTs, FPG, Amylase
  - Fasting Lipid profile and serum insulin (ONLY insulin naïve patients)
  - Calcitonin, AFP
  - Calculate NAFLD Fibrosis Score and HOMA-IR

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- Prescribe 84 days (12 week) supply of treatment i.e. 9 x pharmacy-labelled treatment pre-filled pens (each containing either 3ml of placebo or 18mg/3ml of Liraglutide). This dose will last until the next visit.

- Schedule time and date of visit 6, which must be +/- 2 days from the 252nd day of treatment

- Patient informed to be fasted from midnight the day preceding Visit 6

- In Type 2 Diabetic patients (only), If HbA_{1c} > 9.0 on this visits blood results, the following will take place at unscheduled visit:
  - Provide patient information sheet on Insulin Determir (Levemir)
  - Contact details provided for diabetic specialist nurse
  - Patient will be offered treatment with Insulin Determir (Levemir) Sc once-daily, with the evening meal or at bedtime.
  - The addition of insulin to the patient's treatment regimen is recommended by national healthcare guidelines at this level of glucose control, but the patient will have the final decision of whether they chose to start insulin therapy.
  - Insulin detemir (Levemir) dose will be adjusted by the Investigator at site visits and telephone contacts, based upon the SMPG and the titration guidelines (see section 7.1).

Visit 6 (2 days +/- 252nd Day/36weeks of treatment)

Estimated visit duration = 1 to 1 ½ hours

- Clinical assessment
  - Record clinical events
  - Record adverse events
  - Record new and changes to concomitant medications
  - Record of treatment compliance
  - Perform clinical examination (including injection sites and thyroid examination)
  - Review each patients 4-Point SMPG

- Perform 12-lead ECG

- Urinalysis

- Measure Vital signs
  - HR, BP, weight (Kg), Height (cm), waist:hip circumference (cm), Temperature, SaO_2 and RR.

- Obtain blood for:
  - FBC, U+E, LFT, AST, GGT, PT, INR, HbA_{1c}, TFTs, FPG, AFP, Amylase
  - Calculate NAFLD Fibrosis Score

- Prescribe 84 days (12 week) supply of treatment i.e. 9 x pharmacy-labelled treatment pre-filled pens (each containing either 3ml of placebo or 18mg/3ml of Liraglutide). This dose will last until the next visit.

- Re-discuss with patient procedure for USS guided Liver Biopsy scheduled for visit 7. At this stage the participant will be asked to give written informed consent that they are still happy to proceed with the liver biopsy at visit 7.

- Schedule time and date of visit 7, which must be +/- 2 days from the 336nd day of treatment
- Patient informed to be fasted from midnight the day preceding Visit 7
- In Type 2 Diabetic patients (only), If HbA_{1c} > 9.0 on this visits blood results, the following will take place at unscheduled visit:
  - Provide patient information sheet on Insulin Determir (Levemir)
  - Contact details provided for diabetic specialist nurse
  - Patient will be offered treatment with Insulin Determir (Levemir) Sc once-daily, with the evening meal or at bedtime.
  - The addition of insulin to the patient's treatment regimen is recommended by national healthcare guidelines at this level of glucose control, but the patient will have the final decision of whether they chose to start insulin therapy.
  - Insulin detemir (Levemir) dose will be adjusted by the Investigator at site visits and telephone contacts, based upon the SMPG and the titration guidelines (see section 7.1).

Visit 7 - 1 day post the last day of treatment (i.e. 1 day + TD 336 / 48weeks of treatment)

- Clinical assessment
  - Record clinical events
  - Record adverse events
  - Record new and changes to concomitant medications
  - Record of treatment compliance
  - Perform clinical examination (including injection sites and thyroid examination)
  - Review each patients 4-point SMPG
- Perform 12-lead ECG
- Urinalysis (store in -80^0C)
- Measure Vital signs
  - HR, BP, weight (Kg), Height (cm), waist:hip circumference (cm), Temperature, SaO_2 and RR.
- Obtain blood for:
  - FBC, U+E, LFT, AST, GGT, PT, INR, HbA_{1c}, TFTs, FPG, Amylase
  - Fasting Lipid profile and serum insulin (ONLY insulin naïve patients)
  - FibroMAX panel, CK-18
  - Calcitonin, AFP, CRP
  - Calculate NAFLD Fibrosis Score and HOMA-IR
- OGTT in non-diabetics only
- Block Brief 2000 FFQ , HR-QOL (SF-36v2)
- AUDIT skinner alcohol questionnaire
- Perform Transient Elastography (Fibroscan ®)(Optional:Subject to individual site availability)
- Perform an ultrasound guided liver biopsy
  - Ideally the Liver Biopsy should be performed on this visit (especially as the patient is fasted), but if the trial site scheduling or patient availability does not

permit this, the biopsy will be performed at the next available date. This date MUST be within 14 days of the EOT.
    - Two independent pathologists will review the liver biopsies once all participants have completed the 48-week treatment.

- Schedule time and date of visit 8, which must be 12 weeks after visit 7
- Patient informed to be fasted from midnight the day preceding Visit 8

Participant follow-up

Visit 8 (2 days +/- 12 weeks post visit 7)

Estimated visit duration 1 ½ hours

- Clinical assessment
    - Record clinical events
    - Record adverse events
    - Record new and changes to concomitant medications
    - Perform clinical examination
- Perform 12-lead ECG
- Urinalysis
- Measure Vital signs
    - HR, BP, weight (Kg), Height (cm), waist:hip circumference (cm), Temperature (^{0}C), SaO_2 and RR.
- Obtain blood for:
    - FBC, U+E, LFT, AST, γGT, INR, HbA_{1c}, TFTs, FPG, AFP, Amylase
    - Fasting Lipid profile and serum insulin (ONLY insulin naïve patients)
    - FibroMAX panel, CK-18
    - Calcitonin, CRP
    - Calculate NAFLD Fibrosis Score and HOMA-IR
    - Serum antibodies against liraglutide (by radioimmunoprecipitation assay)
        - Due to the nature of the antibody assay, analysis of emergent antibodies against liraglutide cannot by completed until trial participants have been through a wash-out period from therapy.
- SF-36v2 Health-related Quality of Life, Block Brief 2000 FFQ
- AUDIT questionnaire
- Perform Transient Elastography (Fibroscan ®)

Unscheduled Visits

On enrolment, all participants will be provided with contact details (telephone, email) of the trial investigators at each trial site. The investigating team will provide prompt telephone or email (if available to the participant) advice.

If the investigating team or the patient suspects a case of a clinical or adverse event, an unscheduled visit will be arranged within 24 hours. The following will be assessed and recorded;

- Clinical assessment

- Record clinical events
- Record adverse events
- Record new and changes to concomitant medications
- Record of treatment compliance
- Perform clinical examination
- Review each patients 4-Point SMPG

- Perform 12-lead ECG

- Urinalysis

- Measure Vital signs
  - HR, BP, weight (Kg), Height (cm), waist:hip circumference (cm), Temperature, SaO_2 and RR.

- Obtain blood for:
  - FBC, U+E, LFT, AST, γGT, INR, HbA_{1c}, TFTs, FPG

- Consider expert evaluation as needed

An unscheduled visit will also include those patients (T2DM only) who have a HbA1c > 9.0% on the most recent visits blood results, and thus as stated in the protocol require the administration of insulin determir to optimise glycaemic control. The participant will be asked to attend via telephone conversation and then on the visit will have their result explained to them and will be counselled with regards to the benefits, side effects, route of administration and storage of insulin determir. The patient will also be given the contact details of specialist diabetic nurse as per routine standard healthcare.

Treatment compliance

Accurate monitoring of treatment compliance within the trial is essential as each patient will self administer the trial treatment at home. On each visit, the following will be undertaken to assess compliance;

- The solution level remaining in the cartridge of the pre-filled pen and number of empty pens will be recorded. The cartridge level should never be used as a treatment dose guide, but will give an estimate of the amount of treatment remaining in the pen.
- Each patient's treatment and clinical events booklet will be reviewed, to witness written evidence of dose, time and date when the treatment was administered. This will also confirm whether the patient has been injecting the trial treatment at the same time each day.
- On clinical examination, patient's injection sites will be reviewed

The recent LEAD series of large randomised-control clinical trials with liraglutide (78) provide supporting evidence that lack of compliance is unlikely to have a significant impact on this trial [Table 5].

Trial	No. subjects exposed to 1.8mg liraglutide	Duration (weeks)	Combination therapy	No. non-compliance with protocol	% non-compliance (1dp)
LEAD-1 (53)	234	26	Sulphonylurea only	3	1.3%
LEAD-2 (54)	242	26	Metformin only	5	2.1%
LEAD-3 (52)	246	26	Metformin or sulphonylurea or thiazolidinediones	11	4.5%
LEAD-4 (56)	178	26	Metformin + thiazolidinediones	4	2.2%
LEAD-5 (55)	232	26	Metformin + sulphonylurea	1	0.4%
LEAD-6 (57)	235	26	Metformin +/- sulphonylurea	4	1.7%

Table 5 – Rate of non-compliance in the groups of subjects receiving 1.8mg liraglutide in the LEAD trials

In total, 1367 subjects were randomised to receive 1.8mg liraglutide in the LEAD trials I – VI. The mean rate of non-compliance was 2.0% (range 0.4 - 4.5%).

Dose reductions

No dose reduction of liraglutide (Victoza ®) or liraglutide-placebo will be allowed throughout the 48 weeks of the trial. Oral anti-diabetic therapy (i.e. metformin +/- sulphonylurea) will be maintained at pre-trial doses unless unacceptable hypoglycaemic events occur. In the event of recurrent major hypoglycaemic episodes, sulphonylurea doses will be reduced by 50%, at the discretion of the chief investigators.

The type 2 diabetic patients will be placed on once-daily injections of insulin Determir (levemir) if after 12 weeks of commencing the trial treatment their HbA_{1c} > 9.0 %. Insulin detemir dose will be adjusted by the investigators at site visits and telephone contacts, based upon the SMPG and the titration guidelines (section 7.1).

Treatment Discontinuation

Treatment with liraglutide (Victoza ®) will be discontinued immediately in the event of any of the following:

- Serious adverse event, defined as an adverse event that resulted in death, hospitalisation, disability, a birth-defect, was life-threatening, or that required medical or surgical intervention to prevent one of the other outcomes. Examples include;
  - Diagnosis of cancer (i.e. HCC, pancreatic, thyroid)
  - Evidence of decompensated cirrhosis (ascites, encephalopathy, variceal haemorrhage)

  - Severe, acute pancreatitis (requiring hospitalisation > 3 days)
  - Major hypoglycaemia (requiring hospitalisation > 3 days)
- Serious allergic (anaphylactoid) reaction to liraglutide (Victoza ®)
- Non-accidental overdose of liraglutide (Victoza ®), as defined by a dose of > 18 mg in a day
- An unacceptable rise in ALT or AST, as judged by the chief investigator

Concomitant therapy

All medication that each participant is taking at the time, or within 3 months, of enrolment will be recorded. New medications or changes to current medications during the trial will also be recorded.

The pharmaceutical/trade name, dose, route of administration, indication, start/stop date of each new medication within the trial will be recorded. Any drug that is licensed within the United Kingdom and Europe, that is deemed necessary for the participant's health-care, will be permitted at the discretion of the chief investigator. The exceptions to this include;

- The introduction of (as judged by the Chief Investigator);
  - Insulin therapy, with exception of insulin Determir (Levemir) in type 2 diabetic participants
  - Other GLP-1 agonists (e.g. exenatide), DPP IV inhibitors and TZDs
  - Steroids, methotrexate, amiodarone (>7days)
  - Orlistat
  - Multi-vitamins, containing vitamin E

Participants will be asked to comply with the Departmental of Health recommendations of alcohol consumption per week (Men ≤ 21 units, Women ≤ 14 units) and ideally remain abstinent from alcohol throughout the trial. Alcohol consumption at the EOT will be assessed by the AUDIT skinner alcohol questionnaire.

Participant withdrawal

A patient may terminate participation in the trial or withdraw consent at any time during the trial without necessarily giving a reason and without any personal disadvantage. The details of withdrawal should be clearly documented and communicated to the Trials Office. The date and reason the patient withdraws consent (state 'reason unknown' if no reason provided) should be clearly documented in the patient's medical notes.

The investigators can withdraw a participant from the trial, after consideration of the benefit:risk ratio, at any stage of the trial. Justifiable reasons for doing so include;

- Serious Adverse Events (SAE), requiring immediate discontinuation of treatment
- Non-compliance of the trial protocol
- Technical grounds (e.g. patient moves away from trial area and can no longer meet the requirements of the trial protocol)
- Pregnancy
- Withdrawal of patient consent
- Unpredictable events (non-clinical or clinical): any event which at the discretion of the investigator makes further treatment inadvisable (i.e. Non-accidental overdose

The published literature on clinical trials in NASH reports a mean 10 – 20% participant withdrawal rate. The sample size calculation for the LEAN was based on an estimated 20%

withdrawal rate and therefore an additional 8 participants will be randomised to the trial, above that which is required to reach statistical significance (i.e. n = 42).

All participants will be included in the analysis based on the intention to treat principle, either to the point of the end-point of the trial or to the point in which consent was withdrawn.

ADVERSE EVENT REPORTING

Reporting requirements

All adverse events (AEs), whether serious or not, will be recorded throughout the study. At each contact with the site, the patient will be asked about adverse events. All adverse events, either observed by the Investigator or reported by the subject, will be recorded by the Investigator and evaluated.

Collection, Recording and Reporting of Adverse Events:

In line with the Medicines for Human Use (Clinical Trials) Regulations 2004, an accurate and up to date record of all adverse events reported by investigators will be maintained throughout the trial. This record will include details of nature, onset, duration, severity, outcome and any relationship to the investigational product. The sponsor, appropriate regulatory authority and ethics committee will be informed as required by current regulations.

The NCI CTC AE Version 4.0 will be used to grade each AE. The worst grade for a particular event is to be documented. If an AE is not described in the above classification, it should be recorded as 'other' in the Case Report Form (CRF) [refer to the CTC guide for grading]. A non-leading, open question will be asked initially to evaluate for possible adverse events. Examples include;

At screening (visit 1) - 'Are you experiencing any symptoms?'

At subsequent visits (visits 2 – 8) – 'How have you been since your last visit?'

As a minimum requirement, all suspected adverse drug reactions will be reported to Novo Nordisk A/S. Events such as medication errors and suspected transmission of an infectious agent via a trial product will always be considered as medical events of special interest and will be reported to Novo Nordisk A/S irrespective of seriousness.

Pre-existing conditions

A pre-existing condition will not be reported as an AE unless the condition worsens by at least one CTC grade during the trial. The condition, however, must be reported in the CRF.

Reporting Period

The reporting period for AEs will commence at the screening visit (visit 1) and will continue until the follow-up visit (visit 8), scheduled to be 12 weeks after completing the 48-week treatment schedule. Serious Adverse Events (SAEs) will be reported until day 336 (week 48) of the trial treatment and for 30 days post end of treatment.

Definitions

European Directive 2001/20/EC

- **Adverse Event**

Any untoward medical occurrence in a participant administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An adverse event can be any unfavourable and unintended sign, symptom or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

- **Adverse Reaction**

All untoward and unintended responses to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator as having a reasonable causal relationship to a medicinal product qualify as adverse reactions.

- **Unexpected Adverse Reaction**

An unexpected adverse reaction, the nature, or severity of which is not consistent with the applicable product information (eg SmPC). When the outcome of the adverse reaction is not consistent with the applicable product information, this adverse reaction should be considered as unexpected.

- **Serious Adverse Event or Serious Adverse Reaction:**

Any untoward medical occurrence or affect that:

- Results in death
- Is life threatening*
- Requires hospitalisation or prolongation of an existing hospitalisation**
- Results in persistent or significant disability/incapacity or
- Is a congenital anomaly or birth defect, or
- Is otherwise considered medically significant by the Investigator***

*Life threatening in the definition of serious adverse event refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which might have caused death if it was more severe.

**Hospitalisation is defined as an unplanned, overnight, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. Thus hospitalisation for protocol treatment, elective procedures (unless brought forward due to worsening symptoms) or for social reasons are not regarded as a SAE.

***Medical judgement should be exercised in deciding whether AE is serious in other situations. AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the SAE definition above, should be considered serious.

If a patient dies as a result of a SAE, any post-mortem findings including histopathology must be provided.

Event Grade:

Mild Subject is aware of the event or symptom, but the event or symptom is easily tolerated.

Moderate Subject experiences sufficient discomfort to interfere with or reduce their usual level of activity.

Severe Significant impairment of functioning, subject is unable to carry out usual activities and/or the subject's life is at risk from the event..

Relationship:

The relationship of the AE to the study therapy/ investigation medicinal product (IMP) will be assessed using the following definitions:

Definitely:

- The AE starts a reasonable time after the study drug/IMP administration,
- The AE stops/ improves when the study drug/IMP has been stopped,
- The AE can reasonably be explained by known characteristics of the study drug/IMP

Probably:

- A causal relationship is clinically/biologically highly plausible
- There is a plausible time sequence between onset of the AE and administration of the study therapy/IMP
- There is a reasonable response on withdrawal of the study therapy/IMP
- It cannot be reasonably explained by known characteristics of the patient's clinical state

Possibly:

- A causal relationship is clinically/biologically plausible
- There is a plausible time sequence between onset of the AE and administration of the study therapy/IMP, however
- The AE could have been produced by the subject's clinical state or other modes of therapy administered to the patient

Unlikely to be related:

- The time association or the patient's clinical state is such that the study drug/IMP is not likely to have had an association with the observed effect.
- Another documented cause of the AE is most plausible

Unrelated:

- The AE is definitely not associated with the study drug administered.
- Another documented cause of the AE is most plausible

Assessment

All SAEs and adverse reactions will be evaluated by the investigator and recorded. This includes an evaluation of the seriousness and causality between treatment and the adverse event.

The trial office will keep detailed records of all adverse events recorded and perform an evaluation with respect to seriousness, causality and expectedness. The office is responsible for the prompt notification to all investigators and research ethics committee of findings that could adversely affect the health of subjects or impact on the conduct of the trial.

Reporting

All SAEs will be reported to the Cancer Research UK Clinical Trials Unit (CRCTU) and within 24 hours of the investigator becoming aware of the event. SAEs will be documented on the SAE Form, which will be faxed within 24 hours to CRCTU on the following numbers;

SAE FAX NUMBERS: 0121 414 8286 (Primary Number)
0121 414 2230 (Secondary Number)

In addition to notifying the appropriate regulatory agencies within the specified country, all SAE events that occur during this study and are related to Liraglutide VICTOZA ® (SAR and SUSAR's) will be reported to Novo Nordisk by the CRCTU LEAN study clinical trial team, within 24 hours of the team becoming aware of the event. SAEs will be reported at the country of occurrence to the respective affiliate. Therefore all UK cases will be reported to the UK affiliate Novo Nordisk Ltd on:

PHONE NUMBER: **0845 600 5055**
FAX NUMBER: **01293 611819**
EMAIL: **NNGB-safety@novonordisk.com**

The form will be completed and signed by the investigator and faxed to the trial office immediately. In signing of the SAE form the investigator is confirming the causality assessment.

All SAEs that are at least possibly related to the study treatment – Serious Adverse Reactions (SAR) - still present at the end of the study will be followed at least until the final outcome is determined. Even if it implies that the follow-up continues after the patients leave the trial and when appropriate until the end of the planned period of follow-up.

Within 90 days following the anniversary of the authorization date for the trial an Annual Safety Report will be sent by the Chief Investigator to the MHRA and the Main Research Ethics Committee. A copy of the report will also be sent to the sponsor and Novo Nordisk A/S.

Serious Unexpected Suspected Adverse Reactions (SUSARs)

In line with the Medicines for Human Use (Clinical Trials) Regulations 2004, all relevant information about adverse drug reactions to the investigational medicinal product (liraglutide, Victoza ®) in the LEAN trial, that are both serious and unexpected, will be subject to expedited recording to the appropriate Regulatory Authority and Ethics Committee (REC).

The sponsor will report all the relevant safety information previously described to the concerned competent authorities and to the Ethics Committee concerned. The sponsor will inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

A SUSAR that is fatal or life threatening will be reported to the relevant regulatory authority (MHRA) and main REC within **7 working days** after the sponsor becomes aware of the event. In each case relevant follow-up information will be sought and a report completed as soon as possible. It will be communicated to the MHRA and the Ethics Committee within an additional eight calendar days.

A SUSAR that is not fatal or life threatening will be reported to the relevant regulatory authority (MHRA) and main REC within **15 working days** after the sponsor becomes aware of the event. Further relevant follow-up information will be given as soon as possible.

Information on the final description and evaluation of an adverse reaction report may not be available within the required time frames for reporting. For regulatory purposes, initial expedited reports will be submitted within the time limits as soon as the minimum following criteria are met:

- a suspected investigational medicinal product,
- an identifiable subject (e.g. study subject code number),
- an adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship,
- an identifiable reporting source and, when available and applicable a unique clinical trial identification (EudraCT number)

In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality will be actively sought from the reporter or other available sources. The sponsor will report further relevant information after receipt as follow-up reports.

In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.

Electronic reporting will be the expected method for expedited reporting of SUSARs to the MHRA. In that case, the format and content as defined by the Guidance 1 will be adhered to.

The CIOMS-I form is a widely accepted standard for expedited adverse reactions reporting. However, no matter what the form or format used, it is important that the basic information/data elements described in annex 3 of the EU directive, when available, be included in any expedited report (some items may not be relevant, depending on the circumstances).

Follow-up of Adverse Events

During and following a patient's participation in the study, the Investigator will ensure that adequate medical care is provided to the subject for any adverse events, including clinically significant laboratory values related to the trial. The Investigator will inform the patient when medical care is needed for adverse event(s) of which the Investigator becomes aware.

The follow up information will only include new (updated and/or additional) information that reflects the situation at the time of the Investigator's signature.

All serious AE's and all non-serious AE's classified as severe or possibly/probably related to the trial product will be followed up until the subject has recovered, recovered with sequelae or fatal and until all queries have been resolved. For cases, of chronic conditions or if the subject dies from another event, follow-up until the outcome category is "recovered" is not required, as these cases can be closed with an outcome of "recovering" or "not recovered".

Queries or follow-up requests from Novo Nordisk Ltd will be responded to within 14 Calendar days.

Code breaks / Unblinding of study medication

Code Breaks / Unblinding for Medical Reasons Only (Site staff or other medical personnel located within the Hospital of admission)

When a patient taking part in the LEAN clinical trial is admitted to a hospital for an adverse event, careful consideration should be taken before a code break request is made. The patient should only be unblinded if the identity of the study drug is necessary for patient care. When considering if the patient should be unblinded, reference should be made to the current summary of product (SmPC) for Liraglutide (Victoza®) for drug contra-indications and known adverse event management. Where possible, prior discussion and approval for unblinding of study medication should be sought from one of the LEAN clinical coordinator(s) before a formal request is made. Contact details of the LEAN clinical coordinator(s) can be found in the Study Personnel section of the protocol (pages 2&3).

A 24 hour unblinding service will be provided by Guy's and St Thomas' Emergency Scientific & Medical Services (sSMS). Details of this service can be found in the pharmacy folder or on the individual patient cards. This will allow the local site Investigator, or other medically qualified person, to identify the study medication (Liraglutide VICTOZA® or matched placebo) for an individual patient in an emergency, 24 hours a day and 365 days of the year. For further information regarding code break procedures please refer to the pharmacy file and the local site investigator file, or contact the LEAN Trial Coordinator.

Code Breaks for serious adverse event clinical evaluation by Lean clinical coordinators (Lean Trial main coordinating centre staff only)

When a serious adverse event received by the central LEAN coordination office (Birmingham UK) is deemed unexpected and possibly, probably or definitely related to the IMP and classified as a Suspected Unexpected Serious Adverse Reaction at first clinical evaluation by one of the LEAN Clinical Coordinator(s), the treatment will be subsequently be unblinded by a member of the study team. The event will then be clinically re-evaluated taking the treatment medication (Liraglutide VICTOZA® or Placebo) information into consideration. The resultant classification of the individual event will be either Unrelated Serious Adverse Event (for patients who received placebo), or Suspected Unexpected Serious Adverse Reaction

(SUSAR) and will be reported as per the national clinical trial regulations of both the UK and Germany. Neither the patient nor the treating physician will be informed of the results of the code break and the patient will remain on study as per the clinical trial protocol. A record of the unblinding event will be stored along with the SAE and clinical evaluation in the main study Trial Master File. If it is subsequently deemed necessary to inform the treating clinician, study site staff or patient of the treatment allocation (for patient safety reasons only), then the patient will come off study treatment and be followed up as per protocol. All code break requests for this study will be made via the 24 hour unblinding service provided Guy's and St Thomas' emergency scientific & medical services.

Reporting of Pregnancy

Patients will be instructed to notify the Investigator immediately if they (female patients) or their partner (male patients) become pregnant. The Investigator will report any pregnancy reported during the trial to Novo Nordisk Ltd. Trial subjects will give consent on enrolment that the Investigator will report any pregnancy during the trial to Novo Nordisk Ltd and that she will be asked to provide information about her pregnancy, delivery and the health of her infant until age one month. The Investigator will report information on pregnancy and follow-up within 14 calendar days of obtaining the information using a Pregnancy Form and a Pregnancy Follow-up Form respectively. Pregnancy complications will be recorded as AEs. If the infant has a congenital anomaly/birth defect this will be reported and followed up as a SAE.

DATA HANDLING AND RECORD KEEPING

Data Collection

The Case Report Form (CRF) will comprise the following forms:

Form	Summary of data recorded
Eligibility checklist	Check of Inclusion and Exclusion Criteria
Registration and Randomisation	Patient demographics, Trial ID number and details of treatment group
Initial assessment	History and examination findings, vital signs, ECG/Urine dipstix, Baseline blood results, Fibroscan, invasive metabolic studies and Liver biopsy (baseline) results.
Treatment details	Dates, dosages and routes of administered traetments
336 day assessment	Relevant examination findings, vital signs. Investigational results for visits 1 – 7 (TD1 to TD 336). Primary and secondary outcomes (inc. Liver histology post treatment)
Follow assessment	Relevant Examination findings, vital signs and investigational results from visit 8
Concomitant Medications	Medications at randomisation. Changes to medications during study
Clinical Events	Record of Clinical events – Dates, severity, management and outcomes.
Adverse Effects	Record of Adverse effects – Dates, severity, management and outcomes.

Ad hoc forms

Serious Adverse Event form:

The CRF will be completed, signed/dated and returned to the LEAN Trials Office by the Investigator or an authorised member of the site research team (as delegated on the Site Signature and Delegation Log). The exception is the SAE Form which will be co-signed by the Investigator. See Adverse Event reporting *section 8* for further details.

Entries on the CRF will be made in ballpoint pen, in blue or black ink, and must be legible. Any errors will be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not obvious why a change has been made, an explanation will be written next to the change.

Data reported on each form will be consistent with the source data or the discrepancies will be explained. If information is not known, this will be clearly indicated on the form. All missing and ambiguous data will be queried. All sections will be completed before returning..

In all cases it remains the responsibility of the Investigator to ensure that the CRF has been completed correctly and that the data are accurate.

Completed CRFs submitted to the LEAN Trial Office will be reviewed by the Trial Co-ordinator who will enter the data into an electronic database. Any queries raised on the submitted data will be sent to the site and answered queries will be returned to the Trial Co-ordinator, who will update the database.

LEAN Trial forms may be amended by the Trials Office, as appropriate, throughout the duration of the trial. Whilst this will not constitute a protocol amendment, new versions of the form must be implemented by participating sites immediately on receipt.

Archiving

The Principal Investigator will ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, patients' hospital notes, copies of CRFs etc) at their site are securely retained for at least 5 years after the end of the LEAN trial. Participating sites in Birmingham, UK (and other UK sites) will be sent a letter specifying the permissible disposal date.

Regulatory authorities will have the right to audit such records in accordance with ICH GCP guidelines and EU directive 2001/20/EU.

QUALITY MANAGEMENT

The LEAN trial is being conducted under the auspices of the Cancer Research UK Clinical Trials Unit (CRCTU) according to the current guidelines for Good Clinical Practice (GCP). Participating sites will be monitored by CRCTU staff to confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki (Appendix 6).

Both the UK and Germany sites will be required to sign a Clinical Study Site Agreement prior to participation. In addition all participating investigators will be asked to sign the necessary agreements and supply a current CV to the LEAN Trial Office. All members of the site research team will also be required to sign the Site Signature and Delegation Log, which should be returned to the Trials Office.

Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial design, protocol procedures, adverse event reporting, collection and reporting of data and record keeping. Sites will be provided with an Investigator Site File and a Pharmacy File containing essential documentation, instructions, and other documentation required for the conduct of the trial. The Trials Office must be informed immediately of any change in the site research team.

On-site Monitoring

Monitoring will be carried out as required following a risk assessment and as documented in the LEAN Trial Quality Management Plan. Additional on-site monitoring visits may be triggered by poor CRF return, poor data quality, excess toxicity, excessive number of patient withdrawals or deviations. If a monitoring visit is required the Trials Office will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the LEAN trial staff access to source documents as requested.

Central Monitoring

Trials staff will be in regular contact with the site research team to check on progress and address any queries that they may have. Trials staff will check incoming Case Report Forms for compliance with the protocol, data consistency, missing data and timing. Sites will be sent Data Clarification Forms requesting missing data or clarification of inconsistencies or discrepancies.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to LEAN Trial Management Group, Novo Nordisk A/S and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the trial protocol to the main REC and the Medicines for Health Care products Regulatory Agency (MHRA).

RECRUITMENT PERIOD

Recruitment in the LEAN trial is estimated to take place over an approximate 24 month period between the 1st Sept 2010 and concluding 1st Sept 2012. Due to the total number of included patients being set at 50 in total, the maximum recruitment rate required per country will be one patient fortnightly. With an estimated 5 new referrals per week at each site, the perception is that this target will be met without difficulty. The Principal Investigator is currently liaising with other Liver Units within the UK's Primary Trial Site (Birmingham) region with regards to trial involvement in UK.

STUDY TIMELINES (estimates)

- First Patient First Visit (FPFV) = 1st October 2010
- First Patient First Treatment (FPFT) = 3rd Oct – 14th Oct 2010 *(depending on consent for metabolic sub-groups study)*
- Last Patient Last Treatment (LPLT) = 1st August 2013
- Last Patient Last Visit (LPLV) = 1st November 2013

END OF TRIAL DEFINITION

The primary end-point will be analysed when the final participant has completed visit 7 (after 48 weeks study treatment). The LEAN Trial will end when the final participant has completed their follow-up visit 8 (i.e. after 12 weeks washout of the study treatment).

STATISTICAL CONSIDERATIONS

Power Calculations

This is an early phase trial randomising patients equally between two treatment arms; one experimental and one control. The aim is not to determine efficacy of liraglutide compared to placebo but to assess whether the efficacy and safety profile of liraglutide is worthy of further investigation. Recruiting patients into a no treatment control group provides simultaneous unbiased assessment of comparable patient groups.

The primary outcome measure is the proportion of patients with an improvement in liver histology on liver biopsy at baseline and 48 weeks (EOT). At the time of the study design there was no available data to estimate histological response with 48-weeks treatment of liraglutide (victoza®). Based on local clinical experience, it is assumed that 15-20% of patients undergoing current standard of care will have an improvement in NASH by week 48. The assumption that as many as 20% of the placebo-control arm will achieve an improvement in liver histology in the LEAN trial, in comparison to 14% and 17% in other pharmaceutical trials in NASH,(26;92) is that we estimate that the route of placebo administration, subcutaneous versus oral, will result in a greater placebo effect.

To justify further investigation of liraglutide treatment, a clinically relevant improvement in liver histology would be needed in at least 50% of patients. Using A'Hern's single stage early phase trial design methodology, with a significance level of 0.05 and power level of 0.9, requires a minimum of 21 patients to be randomised to each group. To indicate an effective treatment worthy of further investigation would require an improvement in at least 8 patients in the experimental treatment group. With 21 patients recruited to each group, the minimum required level of efficacy for liraglutide could be reduced to 45% with a reduction in power of 0.80.

The recruitment target is a minimum of 21 patients in each treatment group completing 48 weeks of treatment and an end of treatment biopsy to achieve the primary end-point. 20% of randomised patients are not expected to complete treatment and as such the recruitment target is inflated from 21 to 25 patients per treatment group to account for this. As such the total recruitment target is 50 patients.

The number of participants lost to follow-up, or withdrawn consent prior to initial treatment is expected to be minimal. The Data Monitoring Committee (DMC) may advise recruiting additional patients if the number of patients not completing treatment (and end of treatment biopsy) is higher than the anticipated 20% rate. The first DMC review will be 6 months after the first patient has been recruited into the clinical trial.

Analysis of Outcome Measures

All randomised patients will be analysed on the intention to treat principle.

The primary outcome measure is the proportion of patients with an improvement in liver histology on liver biopsy at baseline and 48 weeks (EOT) defined as both disappearance of steatohepatitis and no worsening of the fibrosis score. Patients will be categorised as achieving an improvement in liver histology or not. The proportion of patients with a reported improvement in liver histology will be presented and compared across treatments descriptively with 95% confidence intervals.

The decision criteria indicating an effective treatment worthy of further investigation is based on reporting an improvement in liver histology in at least 8 patients in the experimental treatment group.

Secondary measures collected as categorical data will be presented and compared descriptively across treatments using proportions and 95% confidence intervals.

Secondary measures collected as continuous data will be presented and compared descriptively across treatments using medians and ranges.

Secondary measures collected as longitudinal data (including quality of life data scored as per the questionnaire specific scoring manuals) will be presented as changes over time or quantified as area under the curve values and compared descriptively across treatment groups.

Planned Subgroup Analysis

Analyses will be presented for the Type 2 Diabetes and non-diabetic subgroups.

Final Analysis

Final analyses will be carried when all participants have completed the 48-week treatment schedule and have had the EOT biopsy.

Data Management Committee

An independent Data Monitoring Committee (DMC) will be supplied with the confidential data analyses and will advise as to whether the accumulated data from the LEAN trial, together with the results from other relevant research, justifies continuation of participant recruitment during the allocated 24 month recruitment period of the LEAN trial. The DMC will operate in accordance with a trial specific charter based upon the template created by the Damocles Group.

During the recruitment phase of the trial the DMC will meet after a certain number of patients have been enrolled in the trial and yearly thereafter. Additional meetings may be organised if recruitment rate is faster than anticipated and the DMC, at their discretion, request to meet more frequently or to continue to meet following completion of recruitment. If a safety issue arises then an emergency meeting will be scheduled.

The DMC will report directly to the LEAN Trial Management Group (TMG) who will convey the findings of the DMC to the sponsors, Novo Nordisk A/S, MHRA and the Ethics Committee. The DMC have the right to recommend closure of the trial if the recruitment rate or if any issues are identified which may compromise patient safety.

TRIAL ORGANISATIONAL STRUCTURE

Single Sponsor

UK LEAN Trial Sites only:

University of Birmingham **(confirmed)**
Edgbaston
Birmingham
B15 2TT
United Kingdom

The University of Birmingham will act as single sponsor for all UK sites on receipt of written evidence of local national ethics and regulatory authority approval. To date, the lead site in the UK, the Queen Elizabeth University Hospital Birmingham, has received full national ethics, MHRA, and local R&D approval. The addition of other UK trial sites will require local R&D approval prior to site initiation. All serious adverse events occurring in the UK will be reported initially to the Central Trials Office (Birmingham, UK) for clinical evaluation and review, prior to reporting to the National ethics committees and Competent authorities in the UK in accordance with country specific regulations.

Finance

The LEAN trial is partly funded by an educational grant from Novo Nordisk Ltd, UK. Blinded liraglutide (Victoza ®) and inactive treatment (liraglutide placebo), and the relevant packaging (inc. of pens and injection needles) for all trial participants will be provided free of charge by Novo Nordisk Ltd (UK) until the end of the trial.

The LEAN trial is also funded by the Wellcome Trust Clinical Research Fellow Grant awarded to Dr M.J.Armstrong, the co-investigator of the LEAN trial.

Trial Management Group (TMG)

Membership:

- Chief Investigators
- Research Physicians
- Senior Trial Co-ordinator
- Trial Statistician
- Liver Histo-pathologists
- NIHR BRU Management Board Representatives

Responsibilities

- Design and Conduct of Trial
- Preparation of Protocol and Amendments
- Preparation of Patient information sheets and Consent Forms
- Preparation of CRFs
- Reviewing progress of Trial and if necessary agreeing to changes in the protocol

- Providing Annual Report to MHRA and Ethics Committee
- SUSAR Reporting to MHRA
- Data Verification
- Data analysis
- Preparation of Trial Reports including DMC Reports
- Publication and Presentation of Results

Delegation

The Chief Investigator (based at the lead UK Trial site - Dr Philip Newsome, Birmingham) is solely responsible for the design of the LEAN trial protocol.

The Principal Investigator at each Trial Centre (Dr Phil Newsome, Birmingham; Dr Guru Aithal, Nottingham; Dr Kathyrn Nash, Southampton; Dr George Abouda; Dr Mark Aldersley, Leeds) will be ultimately responsible for;

- Patient identification
- Recruitment
- Data Collection
- Completion of CRFs
- Follow-up of trial participants
- Adherence to study protocol

These duties may be appropriately delegated to medical or nursing staff as detailed in the Site Signature and Delegation Log.

ETHICAL CONSIDERATIONS

The trial will be carried out in accordance with the recommendations guiding the physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland (June 1964), amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa, October 1996 (125).

The LEAN trial will be carried out under a Clinical Trial Authorisation and will be carried out in accordance with the principals of Good Clinical Practice (GCP) according to the EU directive 2005/28/EC and UK legislation.

The UK arm of the LEAN trial has been approved by **Leicestershire, Northamptonshire & Rutland Research Ethics Committee (13th May 2010), UK**. It will be the responsibility of each Principal Investigator to obtain approval from their respective Trust Research & Development (R&D) department. The UK trial sites will not be permitted to enrol participants until written confirmation of ethical and R&D approval is received by the Trials Office. It will be the responsibility of the Principal Investigator to ensure that all subsequent amendments gain necessary approval. However, this will not delay the individual clinicians' responsibility to take immediate action if though necessary to protect the health and safety of the each patient.

Informed written consent will be obtained from the patients prior to inclusion in the trial. The right of an eligible patient to refuse participation of the LEAN trial, without giving reasons, will be respected at all times. Informed written consent will confirm that the participant;

- Understands their right to withdraw from the trial, at any time, without prejudicing their further treatment.
- Understands that they are being invited to take part in a research study.
- Is not taking part in any other research study at this time and have not received any other investigational drug within 3 months prior to the screening biopsy and randomisation for the current trial.
- Understands the risks and benefits
- Understands that sections from their medical notes may be examined by responsible individuals from the Trial Management Team, NHS Trusts, or from national and international regulatory authorities where it is relevant to them taking part in the trial.
- Understand that serum samples and liver tissue obtained from them during the trial period may be stored and used for future research over the next five years. Throughout the storage period strict patient confidentiality will be maintained at all times. Samples will be stored in keeping with the Human Tissue Act 2004.
- Gives consent to their General Practitioner (GP)/Primary care physician and specialist doctors involved in their healthcare being contacted and access given to their medical notes held by their GP and at other hospitals.
- Agrees to take part in the LEAN trial.

CONFIDENTIALITY AND DATA PROTECTION

The personal demographics and trial data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the 1998 Data Protection Act. Patients will only be identified by their unique trial identification number, initials, hospital number and date of birth on all CRFs and any correspondence between the Study Office and the participating sites.

All documents not for submission to the Trial office will be maintained by the Principal Investigator in strict confidence. Patient confidentiality will be protected in the case of special problems and/or governmental queries, when it will be necessary to have access to complete patient study records.

The University of Birmingham's CRCTU will maintain the confidentiality of all patient data and will not disclose information by which patients may be identified to any third party, other than those directly involved in the treatment of the patient's health care. Representatives of the trial team may be required to have access to patient notes for quality assurance purposes, but patients should be reassured that their confidentiality will be respected at all times.

INSURANCE AND INDEMNITY

This study is a principal investigator-initiated and investigator-led study with grants provided by Wellcome Trust (WT) and Novo Nordisk Ltd (UK). The WT grant was activated in October 2009 and will provide funding for the study until completion. The UK-site study is being run by the CRCTU, Liver Research Group and the University of Birmingham.

The University of Birmingham will act as a single sponsor for all the UK trial sites **(confirmed)**. As sponsor the University is responsible for the general conduct of the study and shall indemnify the Centre against any claims in the UK **(confirmed)** arising from any negligent act or omission by the University in fulfilling the sponsor role in respect of the study. The university is under no obligation to indemnify the Centre against any claims arising from the conduct of the study centre.

The University of Birmingham will act as sponsors for additional trial sites, after the additional sites have satisfied the sponsor's (University of Birmingham) requirements. It is the responsibility of the PI's to ensure that Insurance and indemnity for the all arms of the LEAN trial will be obtained prior to commencing the trial in the respective countries.

In terms of liability, NHS Hospitals (UK) and Non-Trust Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical trial. Compensation will only be available in the event of clinical negligence being proven. There are no specific arrangements for compensation made in respect of any SAEs occurring through participation in the trial, whether from side effects listed, or others yet unforeseen.

PUBLICATION POLICY

The main trials results will be published in the name of the trial in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group appointed from amongst the TMG and high accruing Investigators. The CRTCTU and all participating centres and Investigators will be acknowledged in this publication. All presentations and publications relating to the trial must be authorised by the study TMG.

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APPENDIX 1

COMPONENTS OF NAFLD ACTIVITY SCORE (NAS)(1)

Component	Score	Extent	Explanation
Steatosis	0	<5%	Refers to amount of surface area involved by steatosis as evaluated on low to medium power examination; minimal steatosis (<5%) receives a score of 0 to avoid giving excess weight to biopsies with very little fatty change
	1	5-33%	
	2	>33-66%	
	3	>66%	
Lobular Inflammation	0	No foci	Acidophil bodies are not included in this assessment, nor is portal inflammation
	1	<2 foci/200x	
	2	2-4 foci/200x	
	3	>4 foci/200x	
Hepatocyte Ballooning	0	None	
	1	Few Balloon cells	The term "few" means rare but definite ballooned hepatocytes as well as cases that are diagnostically borderline
	2	Many cells/prominent ballooning	Most cases with prominent ballooning also had Mallory's hyaline, but Mallory's hyaline is not scored separately for the NAS

APPENDIX 2

Study	Duration	Histological Inclusion Criteria	N	Treatment drug	NAS criteria – definition of histological improvement	End Point	Power calc	% Placebo meeting end-point	% Treatment meeting end-point	Significant difference in mean NAS
Promrat K, Hepatology 2009	48 wks	- steatosis - lobular inflammation - ballooning or acinar zone 3 hepatocell injury	31	Lifestyle changes	1. at least 3 point fall in NAS **OR** 2. Post-treatment NAS of 2 or less	Change in NAS after 48 weeks (see criteria)	Based on weight	30	72 (p =0.05) Therefore **42%** difference in change	-2.4 vs -1.4 (p<0.001) Baselines were 4.4 vs 4.9. Therefore **26%** difference in change
PIVENs, 2009	96 wks	- NAS ≥ 5* + NASH** **OR** - NAS = 4* + definite NASH (2 out 3 pathologists)	247	Pioglitazone Vitamin E	**ALL 3 of** 1. EITHER at least 2 point fall in NAS or post-treatment NAS ≤ 3 2. at least 1 point fall in score for ballooning degeneration 3. No worsening of fibrosis score	Change in NAS after 96 weeks (see criteria)	NAS criteria – Expected proportion improvement: Placebo 0.14 Pioglitazone 0.40 (based on pioglitazone pilot study and placebo in URSO trial 2004)	awaited	awaited	
Nobili V, Hepatology 2009 (paediatric)	24 months	Dx NAFLD on biopsy + persistently raised ALT (Median NAS at inclusion was 4)	90 (53 had 2nd biopsy)	Anti-oxidant (alpha-tocopherol + ascorbic acid)	At least 2 point fall in NAS (in which 1 point must be due to inflammation or ballooning)	Change in NAS after 24 months (see criteria)	NAS criteria -To detect 40% difference between groups (i.e. 80% anti-oxidant vs 40% in placebo)	68% (Nb significant in mean NAS	68%	No significance
Vilar Gomez E, Aliment Pharmacol Ther 2009	6 months	All patients >3 on NAS (n=33 for 3-4, n=27 for >5)	60 (42 had 2nd Biopsy)	Viusid-diet	No pre-trial definition of change in NAS to define histological improvement	Mean change in NAS	Based on % expected to have histological improvement. To detect 39% difference (i.e. 69% Viusid vs 30% in placebo)	Nb placebo still had hypocaloric diet and exercise		-3.64 vs –2.25 (p<0.001) Nb baselines were 4.18 vs 4.45. Therefore **36%** diff. in change

APPENDIX 3

Sub-group: Overview of the state-of-the-art invasive metabolic studies

The day prior to this study subjects will visit the research facility in the evening for a blood sample to be taken [Free Fatty Acid (FFA), Very Low Density Lipoprotein (VLDL), Triglycerides (TG)]. They will then be given a standardized evening meal, after which they will drink half a loading dose of ^2H_2O (3g/kg body water) and be asked to have the remainder at 10pm at home and to drink only water enriched with ^2H_2O (4.5g ^2H_2O/litre drinking water).

Patients will then return to the research facility the next morning in the fasting state (08.00). A blood sample will be taken to measure;

- VLDL and TG concentrations and
- enrichment with ^2H, and plasma water enrichment from which de novo lipogenesis will be calculated.

Following this test the following 2 step clamp protocol will be performed;

a) 2 step hyperinsulinaemic-euglycaemic clamp (see also Appendix 4)

In the fasted state, a constant rate deuterated glucose infusion is started (bolus: 2mg/kg; continuous: 20 µg/kg/min). At t=90,105 and 120 minutes, samples are taken for assessment of basal, whole body glucose turnover. At t=120min, a soluble insulin infusion is commenced (0.2mU/kg/min), together with an infusion of 20% glucose, beginning at 2 mg/kg/min through the same line.

Blood is sampled through a second cannula in a contra-lateral hand vein, with the sampling hand warmed in a heated box or blanket to arterialize the blood and so minimize glucose extraction. Blood is sampled through the cannula every 5 minutes. Blood glucose is measured immediately at the bedside, and the readings are used to adjust the glucose infusion rates according to established formulae to maintain blood glucose in the euglycaemic target range, which is calculated based on fasting plasma glucose. Steady state samples are taken at 210-240 minutes (including insulin measurements at t=210, 225 and 240min) to provide a measurement of endogenous glucose production rate. At t=240, the insulin infusion rate is increased to 1.0 mU/kg/min and the glucose infusion adjusted accordingly (5 minutely measurements) to maintain euglycaemia. Steady state samples are then taken at t=330, 345 and 360min including insulin levels and used to calculate glucose disposal.

b) Subcutaneous adipose tissue microdialysis performed synchronously with the 2 step hyperinsulinaemic euglycaemic clamp (see also Appendix 4)

Adipose tissue microdialysis will be established once the patient has been admitted in the fasting state (Appendix 5). Microdialysis samples will be taken at 30 minutely intervals for the duration of the 2-step clamp and analyzed for glucose, lactate, pyruvate and glycerol. Importantly the insulin-mediated suppression of adipose tissue lipolysis (as measured by interstitial fluid glycerol) provides an index of adipose tissue insulin sensitivity.

APPENDIX 4

2-step Hyperinsulinaemic Euglycaemic Clamp

Requirements

IVAC infusion pump, syringe infusion pump, 50 ml syringes, Hot box / electric blanket, YSI glucose analyser, 20% dextrose, human insulin (Actrapid), stopwatch, cannulation apparatus and glucagon / 50% glucose (in case of emergency).

Method

1. Turn on Hot Box / electric blanket, calibrate YSI glucose analyser.
2. Weigh the subject
3. Lie subject on bed and place left hand in Hot Box.
4. Cannulate antecubital area on right arm.
5. Choose a straight non-valved vein on dorsum of left hand. Cannulate in the retrograde direction and take a 5 ml blood sample and place hand back in Hot Box.
6. Determine the resting glucose concentration using YSI.
7. Commence the deuterated glucose infusion (bolus and then continuous - bolus: 2mg/kg; continuous: 20 µg/kg/min)
8. Monitor blood glucose 15minutely, and at t=90, 105 and 120 take samples for measurement of insulin and assessment of whole body basal glucose turnover
9. Make up the insulin infusate aseptically into a 50 ml syringe: Add 15 IU human insulin to 48ml 0.9% NaCl saline + 2ml of subjects blood (= 300 mU/ml).
10. Attach the infusion pumps with the 300 mU / ml insulin infusate and 20% dextrose (= 200 mg/ml) to the antecubital cannula after running through any visible air pockets
11. Begin the insulin infusion (t=120) at the desired rate (0.2mU/kg/min)
12. At t=124mins begin the glucose (dextrose) infusion at 2 mg/kg/min.
13. At t=130 min take an arterialised 2ml blood sample and adjust the dextrose infusion rate accordingly and continue to take 5minutely samples for glucose analysis. At t=210, 225 and 240 taken additional samples for measurement of insulin and endogenous glucose production rate.
14. At t=240 increase the insulin infusion rate to 1.0mU/kg/min.
15. Continue sampling every 5 minutes and adjust the glucose infusion rate accordingly.
16. At t=330, 345 and 360 take addition samples for measurement of insulin and glucose disposal (Gd)
17. At t=360 hours the test is completed. At this point stop the insulin infusion whilst maintaining the glucose infusion. The total amount of blood taken during the test is approximately 200ml (less than 2/3 of a single unit of blood)
18. Feed the subject a high mixed carbohydrate meal. Continue to sample the blood every 10 mins whilst gradually reducing the glucose infusion rate.
19. When the glucose concentration is being maintained at a negligible infusion rate and following the meal, cease the infusion.
20. Warn the subject of possible symptoms of hypoglycaemia and how to overcome them.

APPENDIX 5

Adipose tissue microdialysis

Adipose tissue microdialysis allows the sampling of interstitial fluid intermediary metabolites including glycerol as a marker of adipose tissue lipolysis. The suppression of glycerol release by insulin provides an assessment of adipose tissue insulin sensitivity.

Requirements:

CMA microdialysis pump (CMA 106/107), miscrodialysis vials, microdialysate solution, CMA syringes, CMA60 microdialysis catheter, dressing pack and local anaesthetic (1% lidocaine, with needle and syringe), betadine solution, tegaderm dressing.

Method:

A single microdialysis catheter (CMA60, CMA Microdialysis ltd) will be inserted under local anaesthetic (1ml of 1% lignocaine) into the subcutaneous adipose tissue 5cm to one side of the umbilicus. Using the CMA107 microdialysis pump, a microdialysate solution (physiological sterile saline solution) will be introduced into the catheter (perfusion rate = 0.3μl/minute). Microdialysis will take place over the duration of the hyperinsulinaemic clamp (including the basal phase). Microdialysate fractions will be analyzed by automated analyzer (ISCUS flex) for glycerol, glucose, lactate and pyruvate. After the clamp the catheter will be removed.

APPENDIX 6

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Recommendations guiding physicians in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly
Helsinki, Finland, June 1964
and amended by the
29th World Medical Assembly, Tokyo, Japan, October 1975
35th World Medical Assembly, Venice, Italy, October 1983
41st World Medical Assembly, Hong Kong, September 1989
and the
48th General Assembly, Somerset West, Republic of South Africa, October 1996

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The Health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it

impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE (Clinical Research)

In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, reestablishing health or alleviating suffering.

The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.

The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS (Non-Clinical Biomedical Research)

In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

The subject should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

In research on man, the interest of science and society should never take precedence over considerations related to the wellbeing of the subject.