Clinical Study Protocol

Drug Substance	AZD7687
Study Code	D2710C00002
Edition Number	1

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

Sponsor: AstraZeneca AB, 151 85 Södertälje, Sweden

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment

Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change

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PROTOCOL SYNOPSIS

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

Principal Investigator

Study centre and number of subjects planned

This study will be conducted at 1 study centre and the planned number of overweight to obese but otherwise healthy volunteers (hereafter called subjects) to be enrolled is up to 45.

Study period		Phase of development
Estimated date of first subject enrolled	Q2 2010	Clinical Pharmacology (Phase I)
Estimated date of last subject completed	Q3 2010	

Objectives

Primary Objective

To investigate the safety and tolerability of AZD7687 following administration of multiple ascending doses.

Secondary Objectives

- To evaluate the pharmacokinetics (PK) (plasma and urine) of AZD7687 and its glucuronic acid metabolite after single and multiple doses.
- To investigate if AZD7687 inhibits DGAT1 activity in subcutaneous adipose tissue.
- To investigate if AZD7687 inhibits DGAT1 activity in the gut.

Exploratory Objectives

- To investigate the effect of AZD7687 on gastrointestinal peptides.
- To investigate if treatment with AZD7687 will decrease body weight.
- To investigate if treatment with AZD7687 will decrease waist circumference.
- To investigate if treatment with AZD7687 will affect production of chylomicrons.
- To investigate if treatment with AZD7687 has an effect on energy balance.
- To investigate if treatment with AZD7687 has an effect on insulin sensitivity.
- To investigate if treatment with AZD7687 results in a delayed gastric emptying
- To collect and store adipose tissue samples for potential future exploratory analysis of AZD7687 concentration in adipose tissue if no effect on TAG/DAG is seen, and for potential future exploratory research aimed at exploring biomarkers involved in nutrient metabolism and endocrine function.
- To collect and store blood samples for potential future exploratory research aimed at exploring factors involved in DGAT1 effects.
- To investigate the presence and/or identity of drug metabolites of AZD7687 after multiple doses of AZD7687 and, if appropriate, characterise their pharmacokinetics.
- To collect and store samples for potential future exploratory genetic research aimed at identifying/exploring genetic variations that may affect PK and PD, safety and tolerability related to AZD7687. The exploratory genetic research will not be reported in the clinical study report for this study.

Study design

This is a Phase I, randomised, single blind, placebo-controlled multiple ascending dose study in overweight to obese but otherwise healthy male subjects conducted at a single centre. The planned study design includes up to 5 dose steps (panels).

Nine subjects will participate in each panel and will receive either AZD7687 or placebo, randomised 6:3. Each subject will only be dosed in one panel.

The study will be divided into 3 visits; Visit 1 (enrolment visit), Visit 2, Part A (randomisation and single dose of AZD7687 or placebo) and Part B (7-days repeated dosing with AZD7687 or placebo) and Visit 3 (follow-up).

Target subject population

Overweight to obese but otherwise healthy male subjects aged ≥20 to ≤45 years with a body mass index (BMI) between 27 and 35 kg/m^2.

Investigational product, dosage and mode of administration

AZD7687 will be administered as an oral suspension. AZD7687 will be provided as a frozen oral suspension, 0.1 mg/mL and 5 mg/mL. Single and multiple oral doses of AZD7687 will be administered starting with 1 mg once daily.

The starting dose will be 1 mg, based on data from the single ascending dose (SAD) study. Administration of the next and subsequent doses of AZD7687 will be based on review of the available safety, PK and pharmacodynamic (PD) data from the previous doses and data from the SAD study.

Comparator, dosage and mode of administration

Oral suspension of placebo to match AZD7687 will be provided as a white oral suspension.

Duration of treatment

Each subject will receive a single dose of AZD7687 or placebo on Day 1. Repeated dosing will commence on Day 3 with AZD7687 or placebo once daily for 7 days.

Outcome variable(s):

- Safety

  Adverse events, vital signs (blood pressure and pulse), safety laboratory variables, fat content in faeces, electrocardiograms (ECGs) and physical examinations.

- Pharmacokinetics

  The following PK parameters will be determined for AZD7687 and its glucuronide metabolite .

  Following the single dose part of the study:

  Maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), area under the curve from zero to 24 hours (dosing interval for the multiple dosing) ($AUC_{(0-\tau)}$), area under the curve from zero to 48 hours($AUC_{(0-48)}$), area under the curve from zero to the time of the last quantifiable concentration ($AUC_{(0-t)}$), and area under the curve from zero to infinity (AUC), terminal elimination rate constant (λ_z), terminal half-life ($t_{1/2,\lambda z}$), oral plasma clearance (CL/F) [for AZD7687 only], amount of analyte excreted unchanged in the urine (A_e), fraction of drug excreted in the urine (fe%), renal clearance (CL_R), and metabolite ratios for AUC and C_{max}.

  Following the multiple dose part of the study:

Maximum plasma concentration at steady state ($C_{max, ss}$), time to steady state C_{max} ($t_{max,ss}$), minimum plasma concentration at steady state ($C_{min, ss}$), average plasma concentration at steady state (C_{avg}), area under the plasma concentration-time curve during a dosing interval ($AUC_{(0-\tau), ss}$), terminal elimination rate constant at steady state ($\lambda_{z, ss}$), terminal half-life at steady state ($t_{1/2\lambda z, ss}$), apparent plasma clearance at steady state (CL_{ss}/F) [for AZD7687 only], amount of analyte excreted unchanged in the urine at steady state ($A_{e,ss}$), fraction of drug excreted into the urine at steady state ($fe_{\%, ss}$) renal clearance at steady state ($CL_{R, ss}$), percent fluctuation (%Fluct), and metabolite ratios for $AUC_{(0-\tau), ss}$ and $C_{max, ss}$. In addition the accumulation ratio for C_{max} and $AUC_{(0-\tau)}$ of AZD7687 will be calculated after single and multiple dosing.

- Pharmacodynamics

  The following PD variables will be determined: TAG, DAG and TAG/DAG in adipose tissue, serum TAG_{total}, plasma $TAG_{refined,}$ and DAG, insulin and FFA.

- Exploratory variables

  Weight, waist circumference, gastric peptides (total GLP-1, GIP, and PYY3-36), ApoB48, adiponectin and leptin will be summarised as observed and change from baseline. Paracetamol plasma levels (paracetamol challenge for delayed gastric emptying) will also be summarized.

Statistical methods

No formal statistical hypothesis testing will be performed. The analyses of safety, tolerability, pharmacokinetic and pharmacodynamic data will be summarised descriptively including tables, listings and graphs, as appropriate.

Dose proportionality of AZD7687 will be analysed following the single dose and multiple dose regimen for primary pharmacokinetic parameters using the power model approach. Least square estimates and 90% confidence intervals for slope and intercept will be presented.

The time dependency of the PK will be evaluated by comparing $AUC_{(0-\tau),ss}$ (Day 9) with AUC (Day 1). Least squares geometric means will be used to estimate the ratio $AUC_{(0-\tau),ss}$ /AUC and will be presented with 90% confidence intervals based on an appropriate statistical model. Similarly the accumulation ratios $AUC_{(0-\tau),ss}$ / $AUC_{(0-\tau)}$ and $C_{max,ss}/C_{max}$ will also be estimated by calculating ratios of the geometric least square means and will be presented with confidence intervals.

For the PD variables of primary interest (the inhibitory effect on TAG/DAG in adipose tissue and the relative change in incremental serum TAG_{total} AUC), each dose will be compared with placebo using 95% confidence intervals by parametric or non-parametric methods. The results will also be presented as descriptive statistics.

For all other PD variables, the primary analysis will be descriptive, using suitable statistics and graphical presentations of mean values and individual values over time, where appropriate.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study protocol.

Abbreviation or special term	Explanation
ACTH	adreno corticotrophin hormone
AE	Adverse event (see definition in Section 6.3.1)
A_e	Amount of analyte excreted in the urine
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under plasma concentration time curve
$AUC_{(0-48)}$	Area under plasma concentration time curve from zero to 48 hours
$AUC_{(0-t)}$	Area under plasma concentration time curve from zero to last quantifiable concentration
$AUC_{(0-t),ss}$	Area under plasma concentration time curve from zero to last quantifiable concentration at steady state
$AUC_{(0-\tau)}$	Area under plasma concentration time curve during a dosing interval
$AUC_{(0-\tau),ss}$	Area under plasma concentration time curve during a dosing interval at steady state
BLQ	Below limit of quantitation
BMI	Body Mass Index
BNP	Brain natriuretic peptide
BP	Blood pressure
C_{avg}	Average plasma concentration
CFR	Code of Federal Regulations
CL/F	Oral plasma clearance
CL_{ss}/F	Oral plasma clearance at steady state
CL_R	Renal clearance
$CL_{R,\ ss}$	Renal clearance at steady state
C_{max}	Maximum plasma concentration
$C_{max,\ ss}$	Maximum plasma concentration at steady state
$C_{min,\ ss}$	Minimum plasma concentration at steady state
Co-A	Coenzyme A

Abbreviation or special term	Explanation
CPA	Clinical Pharmacology Alliance
CPD	Clinical Pharmacology, & Drug Metabolism and Pharmacokinetics
CRF	Case Report Form (electronic/paper)
CRO	Contract research organisation
DAG	Diacylglycerole
dECG	Digital ECG
DGAT	Diacylglycerol acyltranferase
DHEA-s	Dehydroepiandrosterone sulphate
EC	Ethics committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram
EClysis©	User-interactive computer-based system for analysis of digital ECGs and measurement of ECG intervals and wave amplitudes used by the AstraZeneca ECG Centre
EDC	Electronic data capture
fe%	Fraction of analyte excreted in the urine
FFA	Free fatty acid
GIP	Gastric inhibitory protein
GLP-1	Glucagon-like peptide-1
HDL-C	High density lipoprotein-C
HIV	Human Immunodeficiency Virus
hs-CRP	High sensitivity C reactive protein
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IP	Investigational Product
λ_z	Terminal elimination rate constant
LDL-C	Low density lipoprotein-C
LLOQ	Lower limit of quantification
MAD	Multiple ascending dose
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum

Abbreviation or special term	Explanation
$MRC_{max,ss}$	Metabolite to parent ratio for $C_{max,ss}$
$MRAUC_{(0-\tau),\ ss}$	Metabolite to parent ratio for $AUC_{(0-\tau),\ ss}$
MTD	Maximum tolerated dose
NQ	Not quantifiable
OAE	Other Significant Adverse Event (see definition in Section 11.1.1)
PD	Pharmacodynamics
pECG	Paper print-out ECG
PK	Pharmacokinetics
PR(PQ)	ECG interval measured from the onset of the P wave to the onset of the QRS complex.
PT	Preferred term
PYY3-36	Peptide YY 3-36
QRS	ECG interval measured from the onset of the QRS complex to the J point.
QT	ECG interval measured from the onset of the QRS complex to the end of the T wave
QTcF	QT interval corrected for heart rate using Fridericia's formula
RR	The time between corresponding points on 2 consecutive R waves on ECG
SAD	Single ascending dose
SAE	Serious adverse event (see definition in Section 6.3.2).
SD	Standard deviation
SOC	System organ class
SOP	Standard operating procedure
SRC	Safety Review Committee
ss	Steady state
SUSAR	Suspected unexpected serious adverse reaction
$t_{1/2\ \lambda z}$	Terminal half life
$t_{1/2\ \lambda z,ss}$	Terminal half life at steady state
TAG	Triacylglycerole
$TAG_{refined}$	Triacylglycerole (refined measurement)
TAG_{total}	Total triacylglycerole
t_{max}	Time of maximum plasma concentration
$t_{max,ss}$	Time of maximum plasma concentration at steady state

Abbreviation or special term	Explanation
ULN	Upper limit of normal
WBDC	Web Based Data Capture

1. INTRODUCTION

1.1 Background

Type 2 diabetes is a metabolic disease that results from a combination of insulin resistance and pancreatic insufficiency. According to the World Health Organisation, a worldwide epidemic of type 2 diabetes has been in progress since the mid-1980s. The worldwide number of diabetics was 30 million in 1985 and is predicted to increase to at least 360 million by 2030. The vast majority of this increase is due to type 2 diabetes. The epidemic of type 2 diabetes is largely driven by a markedly increased prevalence in obesity, especially in the industrialized world and in emerging industrial countries such as India and China. The worldwide number of obese and overweight adults was 0.3 billion and 1 billion respectively in 2005. Obesity is also a well-known and significant risk factor for the development of other diseases, such as cardiovascular disease, cancer, venous thromboembolism and joint and musculoskeletal disability.

Diglyceride acyltransferase-1 (DGAT1) inhibition may be an approach for improving insulin resistance and diabetes control and for weight reduction and/or weight maintenance.

DGAT1 is one of the two DGAT enzymes that catalyze the biosynthesis of triacylglyceroles (TAG) at the final step of the process, converting diglyceride and fatty acyl-coenzyme A (CoA) into TAGs. DGAT1 is therefore an important factor in lipid storage. Although most tissues generate TAGs, DGAT1 is most highly expressed in small intestine and adipose, and these tissues are therefore believed to be the key target tissues; however, DGAT1 is also present in the liver, pancreas, skeletal muscle and heart.

AZD7687 is a novel potent, selective and reversible small-molecule inhibitor of DGAT1 activity. Due to its action at two of the key target tissues for metabolic syndrome, gastrointestinal tract and adipose tissue, it is expected to deliver weight loss through reduction of food intake, increased lipid oxidation and decreased adiposity. Furthermore, published studies in mouse have demonstrated that inhibition of DGAT1 is associated with improvement in insulin sensitivity and glucose disposal and hence should deliver beneficial effects on glucose metabolism in type 2 diabetes. Animal data and hypothetical mechanisms in humans thus point towards a therapeutic potential for the DGAT1 inhibitor in treatment of type 2 diabetes and/or weight management.

Currently, there are no products of this novel mechanism available on the market and there are no clinical results with DGAT1 inhibitors available in the public domain.

Further information regarding pre-clinical data can be found in the Investigator's Brochure (IB).

The Sponsor will immediately notify the Principal Investigator if any additional safety or toxicology information becomes available during the study.

1.2 Rationale for conducting this study

This is the first study with repeated oral administration in man with AZD7687. It is designed to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of AZD7687 in overweight to obese but otherwise healthy male subjects at increasing doses when given for 7 days.

The results from this study will form the basis for the doses of AZD7687 to be used in future studies.

1.3 Benefit/risk and ethical assessment

No medical benefit is expected for the healthy subjects included in the study.

The study design (randomised and placebo-controlled) is standard for a clinical multiple ascending dose (MAD) study evaluating safety, tolerability and pharmacokinetics of an investigational product. Safety will be monitored thoroughly during the study. At least 4 dose levels in the single ascending dose study must have been evaluated before giving the first dose in this study. In the preceding single ascending dose (SAD) study, doses of the investigational product AZD7687 have been given which gave at least the C_{max} which is expected in this repeated dose study.

DGAT plays an important role in energy metabolism. The enzymatic activity of DGAT is present in all cell types because of the necessity of producing TAGs for cellular needs. The amounts of TAGs synthetised varies widely from cell to cell, with adipocytes, hepatocytes and intestinal enterocytes producing much more TAGs than other cell types. Thus, alterations of the expression and/or activity of DGAT1 not only affect intracellular TAGs levels, but can also impact on tissue/organ and systemic energy metabolism. To address potential secondary metabolic effects, markers of lipid and glucose metabolism (total cholesterol, HDL-C, LDL-C and TAGs, free fatty acid (FFA), ketones, S-glucose, insulin, lactate) will be monitored carefully as a part of safety assessments.

There is also a hypothetical risk that changes in lipid metabolism in the heart could cause pathological changes to the heart including effects on contractile function. In case of myocardial ischemia, a potential issue could be the increased demand of oxygen if the heart switches from carbohydrate to lipid oxidation. However, no changes indicating an effect on the heart have been observed in the nonclinical studies. In the absence of a more complete understanding of the exact mechanism involved, heart function will be monitored closely by 24-hour ECG monitoring and brain natriuretic peptide (BNP) will be assessed.

In the mouse and dog dose-related sebaceous gland atrophy was noted in the skin at all dose levels. This finding was considered to be minor and not adverse as it was not associated with any effects on the clinical condition of the fur or skin, nor any adverse microscopic change in the skin. In this multiple dose study, standard pharmacovigilance (physical examination and AE collection) is considered sufficient to address this preclinical observation.

Previous research on DGAT1-inhibitors with limited selectivity over the closely related enzyme acetyl-coenzyme A acetyltransferase 1 (ACAT1) indicate a potential risk of concomitant ACAT1-inhibition causing adrenal toxicity with degenerative histopathology findings in the dog. In vitro data indicate that AZD7687 is more than 400 fold selective for DGAT1 inhibition over ACAT1 inhibition. Nonetheless, adrenal function (morning and evening ACTH and cortisol, as well as dehydroepiandrosterone (DHEA-S), renin, aldosterone, S-sodium and S-potassium) will be monitored in this study.

Mild elevations in liver chemistry tests were observed at the highest dose level in the multiple dose mouse study. These findings were no longer present after a drug withdrawal period of 4 weeks, indicating reversibility. In the dog 14 day toxicity study, marked increases of liver chemistry tests were seen at the high dose level. At the mid dose level mild elevations only in alanine transaminase (ALT) and glutamate dehydrogenase (GLDH) were seen in some animals; there were no degenerative or other adverse histopathological findings in the liver at any dose level. In this study, there will be close monitoring of liver chemistry tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], bilirubin [total and conjugated], gamma glutamyl transpeptidase [GGT], albumin, and International Normalised Ratio [INR]).

A paracetamol test will be done in the study (as described in Sections 3.1 and 3.2). A single low dose of paracetamol will be administered and liver enzymes will, as previously mentioned, be closely monitored. In order to enable evaluation of paracetamol measurements in plasma, additional paracetamol will not be allowed 24 h prior to and 8 h after the test. Paracetamol will be generally allowed in the study, up to a maximum of 2 g per day.

Before proceeding to the next dose level in this study a safety review committee will evaluate safety, PK and PD data (see Section 3.1.1, Stopping criteria for dose escalation).

Dose evaluation will include safety data and available PK and PD data.

The maximum exposure achieved in this study will be no higher than a C_{max} of 70 µmol/L or AUC ($AUC_{(0\text{-}24h)}$ at steady state) of 800 µmol*h/L, or a lower exposure based on the MTD in the ongoing SAD study, or due to safety findings in this study. The provisional dose escalation is 1 mg, 2.5 mg, 5 mg, 10 mg and 20 mg once daily.

For further information regarding an overall risk benefit assessment, see the Investigator's Brochure (IB). For information regarding overdose of AZD7687 see Section 13.2, of the clinical study protocol (CSP).

2. STUDY OBJECTIVES

2.1 Primary objective

To investigate the safety and tolerability of AZD7687 following administration of multiple ascending doses.

2.2 Secondary objectives

- To evaluate the pharmacokinetics (PK) (plasma and urine) of AZD7687 and its glucuronic acid metabolite after single and multiple doses.
- To investigate if AZD7687 inhibits DGAT1 activity in subcutaneous adipose tissue.
- To investigate if AZD7687 inhibits DGAT1 activity in the gut.

2.3 Exploratory objectives

- To investigate the effect of AZD7687 on gastrointestinal peptides.
- To investigate if treatment with AZD7687 will decrease body weight.
- To investigate if treatment with AZD7687 will decrease waist circumference.
- To investigate if treatment with AZD7687 will affect production of chylomicrons.
- To investigate if treatment with AZD7687 has an effect on energy balance.
- To investigate if treatment with AZD7687 has an effect on insulin sensitivity.
- To investigate if treatment with AZD7687 results in a delayed gastric emptying.
- To collect and store adipose tissue samples for potential future exploratory analysis of AZD7687 concentration in adipose tissue if no effect on TAG/DAG is seen, and for potential future exploratory research aimed at exploring biomarkers involved in nutrient metabolism and endocrine function.
- To collect and store blood samples for potential future exploratory research aimed at exploring factors involved in DGAT1 effects.
- To investigate the presence and/or identity of drug metabolites of AZD7687 after multiple doses of AZD7687 and, if appropriate, characterise their pharmacokinetics.
- To collect and store samples for potential future exploratory genetic research aimed at identifying/exploring genetic variations that may affect PK and PD, safety and tolerability related to AZD7687. The exploratory genetic research will not be reported in the clinical study report for this study.

3. STUDY PLAN AND PROCEDURES

This Clinical Study Protocol has been subject to a peer review.

3.1 Overall study design and flow chart

This is a Phase I, randomised, single-blind, placebo-controlled MAD study in overweight to obese but otherwise healthy male subjects conducted at a single centre. Up to 45 overweight to obese but otherwise healthy male subjects aged ≥20 to ≤45 years will participate in up to 5 dose steps (panels).

Nine subjects will participate in each panel and will receive either AZD7687 or placebo, randomised 6:3. Each subject will only be dosed in one panel.

Subjects will receive a single dose of AZD7687 or placebo on Day 1 followed by once daily dosing for 7 days (commencing on Day 3). The starting dose will be 1 mg and there will be up to 4 dose escalations. Administration of the next and subsequent doses of AZD7687 will be based on review of available safety, tolerability, PK and PD data from the previous doses.

Subsequent panels will be administered a maximum predicted dose up to 5 times higher than the previous dose until either a non-tolerated dose is reached (Section 3.1.1) or the maximum allowed exposure has been achieved. The maximum exposure achieved in this study will be no higher than a C_{max} (or $C_{ss,max}$) of 70 µmol/L or AUC ($AUC_{(0-24h)}$ at steady state) of 800 µmol*h/L, or a lower exposure based on the MTD in the ongoing SAD study, or due to safety findings in this study. The provisional dose escalation is 1 mg, 2.5 mg, 5 mg, 10 mg and 20 mg once daily.

After each cohort a Safety Review Committee (SRC) (Section 3.1.3) will assess available data to make a decision on the next dose level (Section 3.1.1). The decision may be to give the intended dose, a greater or smaller dose increment than the intended dose, a repeated dose, a lower dose or to stop dosing. Dose escalation will occur following review of safety (ECG, vital signs and clinical laboratory safety tests), tolerability (adverse event profile), available PK and PD information. A minimum of 5 datasets from subjects receiving AZD7687 will be reviewed prior to dose escalation.

Following a screening period (Visit 1, within 30 days prior to Visit 2) there will be one admission period (Visit 2) from Day -3 (3 days before dosing starts) until discharge on Day 11. Visit 2 will comprise of two parts: in Part A subjects will be randomised and administered a single dose of AZD7687 or placebo and in Part B subjects will receive 7-days multiple dosing with AZD7687 or placebo. There will be a follow-up visit (Visit 3) 7 to 14 days after administration of the final dose (see Study Flow Chart Figure 1). Subjects are required to fast for 10 hours prior to Visits 1, 2 and 3.

Visit 1

Visit 1 (enrolment) will take place within 30 days before Visit 2. Informed consent will be signed before any study related procedures are initiated.

Visit 2

At Visit 2, subjects will arrive at the clinic in the morning of Day -3. An evening meal will be served in order to keep standardised conditions. Prior to intended dosing on Day 1, baseline PD measurements will be performed on Day -2 and Day -1. Weight will be recorded and waist circumference will be measured. A standardised meal (standardised mixed meal [SMM], see Appendix E) will be served, followed by repeated blood sampling to measure postprandial lipid levels and gastrointestinal peptides. Paracetamol challenge will be done. In addition, an adipose tissue biopsy will be taken.

Prior to and during the recording of the dECG subjects are not allowed to have the upper body elevated with more than 30 degrees hip flexion from the horizontal supine position.

For Part A (single dose) on Day 1 subjects will receive a single dose of AZD7687 or placebo in the morning and a full PK profile will be taken (blood and urine). The PK data will be collected up to 48-hours after administration of the single dose.

For Part B (multiple dose) repeated dosing will commence on Day 3. Subjects will receive AZD7687 or placebo once daily for 7 days. It is anticipated that this will be sufficient to achieve and maintain steady state for several days and evaluate the safety of multiple dosing adequate for the intended target. Throughout the residential period there will be safety monitoring and serial blood samples for PK and PD evaluation and PK urine collections (for details see Table 1, Table 2 and Table 3. For fasting restrictions, see Section 5.1.

All meals given at the clinic will be standardised.

Subjects will be discharged from the clinic approximately 48 hours after administration of the last dose (Day 11). Prior to discharge, the physician will ensure the necessary safety assessments have been performed.

Visit 3

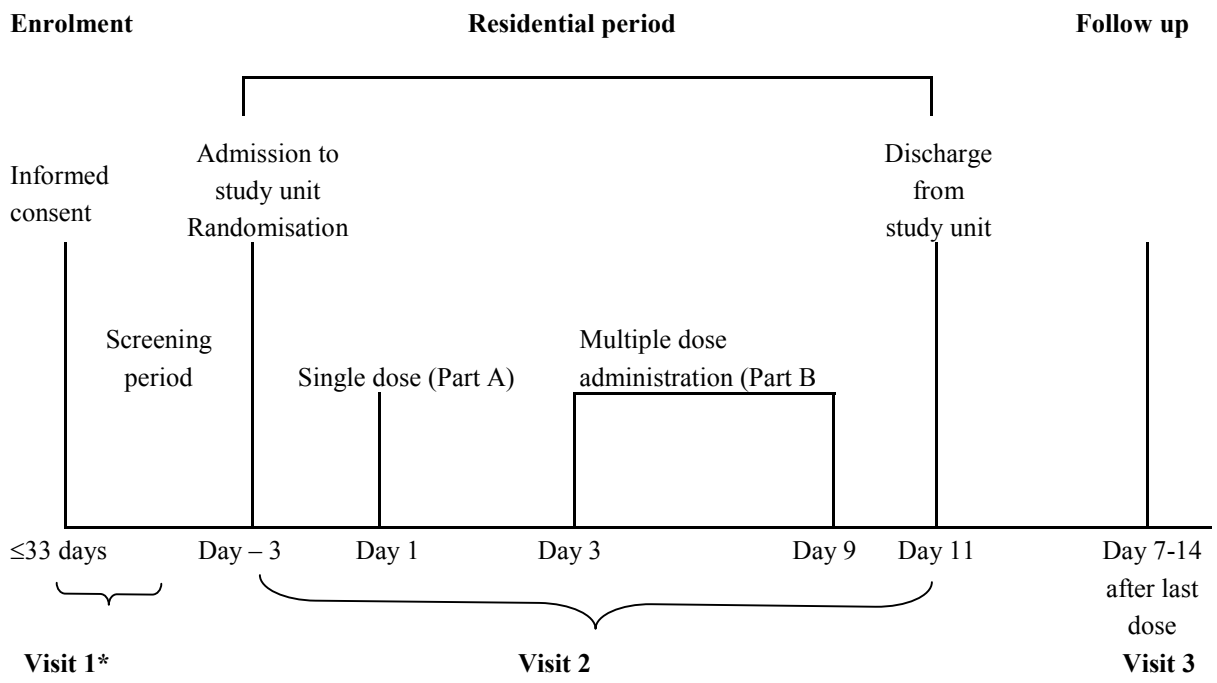
At Visit 3 (follow-up) subjects will attend the clinic after an overnight fast and will remain in the clinical unit for approximately 12 hours. Safety measurements will be performed and blood samples for PK and safety will be taken. Details of the procedures performed at the follow-up visit are provided in Table 1.

Following review of data from each panel of subjects, or from the SAD study, assessments may be omitted, the timing of assessments and/or blood samples and meals may be adjusted, the composition of the SMM may be replaced and the length of washout period between single and multiple dosing and dosing regimen may be adjusted (once or twice daily dosing). Fasting periods might be moved in case measurements requiring a fasting condition are moved. Additional assessments, visits or sampling times may be added if indicated by the data however the maximum blood volume taken from each subject will not exceed 600 mL. One extra adipose tissue biopsy may be added.

Following review of data from each panel of subjects or from the SAD study dosing times may be amended.

There will be a minimum 7-day interval between the last dose of one dose level and initiation of the next dose level.

Figure 1 Study Flow Chart



*Visit 1 may be conducted over one or more days during the screening period

Table 1 Study Plan

Visit number	**1** Screening Period	**2** Residential Period														**3** Follow-up
Part		**Baseline PD measurements**			**Part A**		**Part B**									
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Informed consent	X															
Demography	X															
Medical/surgical history	X															
Inclusion/exclusion criteria	X	X														
Physical examination[a]	X	X[b]													X[b]	X
Randomisation		X														
Administer study drug					X		X	X	X	X	X	X	X			
Weight	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X															
Waist circumference	X		X												X	
Vital signs[c]	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Paper ECG (pECG)	X				X	X					X	X	X	X		X
Digital ECG (dECG)[d]					X	X	X						X			
Telemetry					X	X	X	X	X	X	X	X	X	X	X	
Drugs of abuse screen	X	X														
PK blood sampling					X	X	X					X	X	X		
PK urine collection					X	X							X			
Exploratory blood sampling					X									X		
Safety laboratory tests[e]	X	X		X[f]	X	X	X	X[f]		X		X[f]	X[f]	X		X

Visit number	**1**	**2**														**3**
	Screening Period	**Residential Period**														**Follow-up**
Part		**Baseline PD measurements**			**Part A**		**Part B**									
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Standardised mixed meal			X				X					X				
Paracetamol challenge			X				X					X				
TAG_{total}, $TAG_{refined}$ and DAG blood sampling			X	X			X	X				X	X			
Insulin blood sampling			X		X	X	X			X	X					
Total GLP-1, GIP and PYY3-36 blood sampling			X				X					X				
ACTH and cortisol blood sampling				X^{g}							X^{g}			X^{g}		X^{g}
Renin, aldosterone and DHEA-S blood sampling					X						X			X		
Insulin, glucose, ketones, lactate and BNP blood sampling					X									X		
ApoB48, FFA and hsCRP blood sampling			X	X			X	X				X	X			
Leptin and adiponectin blood sampling					X	X								X		
Fat content faecal sampling		X----------X										X----------X				
Genetic blood sampling		X^{h}														
Adipose tissue biopsy				X									X			
Adverse events[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

a Including special attention to dry skin, skin inflammation (especially face) and hair loss.
b Updates to physical examination will be recorded at admission and discharge.
c Vital signs include blood pressure, pulse and body temperature.
d Detailed information regarding the timing of dECGs is provided in Table 3.
e Blood and urine sampling for haematology, clinical chemistry (including coagulation, lipid panel and liver chemistry, see Section 6.3.5) and urinalysis parameters.

f Only liver chemistry tests, See Section 6.3.5 for definition.
g It is important that the samples are taken at the same time each morning and evening, if applicable. The samples for cortisol and ACTH should be obtained at a time point where reference values for cortisol and ACTH exist, if possible.
h The genetic sampling can be done at any day during Visit 2 and 3.
i SAEs will be recorded from signing of informed consent, non-serious AEs will be recorded from randomisation .

Table 2 Time Schedule During Visit 2

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
-3	Morning	Clinical chemistry[a]/haematology Genetic sampling[d]		Urinalysis Drugs of abuse and alcohol screen	Sample D-3–D-1 start		Vital signs	Admission to the clinic
-2	00:00[e]	FFA Glucose Insulin $TAG/TAG_{ref}/DAG$ ApoB48 PYY3-36						Weight recorded. Waist circumference measured.
-2	00:30	hsCRP $TAG/TAG_{ref}/DAG$						
-2	01:00 pre-SMM	FFA GLP-1/GIP PYY3-36 ApoB48 Glucose Insulin $TAG/TAG_{ref}/DAG$						
-2	01:00	Paracetamol sample						SMM start
-2	01:15							SMM finish Paracetamol administered
-2	01:30	FFA GLP-1/GIP PYY3-36 Paracetamol sample						
-2	01:45	Paracetamol sample						
-2	02:00	FFA GLP-1/GIP Insulin PYY3-36 $TAG/TAG_{ref}/DAG$ Paracetamol sample						
-2	02:20	Paracetamol sample						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
-2	02:40	Paracetamol sample						
-2	03:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	05:00	ApoB48 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	06:00	FFA Glucose Insulin TAG/TAG_{ref}/DAG Paracetamol sample						
-2	07:00	ApoB48 GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	08:00	TAG/TAG_{ref}/DAG						
-2	09:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-1	00:00[e]	Liver chemistry ACTH/cortisol[f] ApoB48 TAG/TAG_{ref}/DAG			Sample D-3–D-1 end			Weight recorded
-1	04:00					Adipose biopsy		
-1	12:00	ACTH/cortisol[f]						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
1	Predose	PK-1 Aldosterone/DHEA-S[g] BNP Clinical chemistry[a]/haematology Insulin Ketones Lactate Leptin/adiponectin Renin[g] Exploratory blood sample		Urinalysis PK-urine predose			dECG[h] pECG Telemetry start Vital signs	Weight recorded
1	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start				Empty bladder
1	00:15						dECG start	
1	00:20	PK-2					dECG stop	
1	00:35						dECG start	
1	00:40	PK-3					dECG stop	
1	00:55						dECG start	
1	01:00	PK-4					dECG stop pECG Vital signs	
1	01:15						dECG start	
1	01:20	PK-5					dECG stop	
1	01:35						dECG start	
1	01:40	PK-6					dECG stop	
1	01:55						dECG start	
1	02:00	PK-7					dECG stop Vital signs	
1	02:55						dECG start	
1	03:00	PK-8					dECG stop Vital signs	
1	03:55						dECG start	
1	04:00	PK-9		PK-urine 0-4 h end PK urine 4-8 start			dECG stop	
1	05:00							
1	05:55						dECG start	
1	06:00	PK-10					dECG stop	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
							pECG Vital signs	
1	07:55						dECG start	
1	08:00	PK-11		PK-urine 4-8 h end PK-urine 8-12 h start			dECG stop	
1	11:55						dECG start	
1	12:00	PK-12		PK-urine 8-12 h end PK-urine 12-24 h start			dECG stop Vital signs	
1	18:00	PK-13						
1	23:55						dECG start	
1	24:00	PK-14		PK-urine 12-24 h end PK-urine 24-48 h start			dECG stop pECG Vital signs	
2	Predose	Leptin/adiponectin						
2	00:00[e]	Clinical chemistry[a]/haematology Insulin		Urinalysis				Weight recorded
2	24:00	PK-15		PK-urine 24-48 h end				
3	Predose	Clinical chemistry[a]/haematology FFA Glucose Insulin TAG/TAG_{ref}/DAG		Urinalysis			Vital signs dECG[h]	Weight recorded
3	00:00 [e]		AZD7687/placebo					
3	00:30	hsCRP TAG/TAG_{ref}/DAG						
3	01:00 pre-SMM	PK-16 ApoB48 GLP-1/GIP PYY3-36 FFA Insulin TAG/TAG_{ref}/DAG						
		ApoB48						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
3	01:00	Paracetamol sample						SMM start
3	01:15							SMM end Paracetamol administered
3	01:30	FFA GLP-1/GIP PYY3-36 Paracetamol sample						
3	01:45	Paracetamol sample						
3	02:00	FFA GLP-1/GIP Insulin PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
3	02:20	Paracetamol sample						
3	02:40	Paracetamol sample						
3	03:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
3	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
3	05:00	ApoB48 TAG/TAG_{ref}/DAG Paracetamol sample						
3	06:00	FFA Insulin Glucose TAG/TAG_{ref}/DAG Paracetamol sample						
3	07:00	ApoB48						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
		GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
3	08:00	TAG/TAG_{ref}/DAG						
3	09:00	PK-17 GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
4	Predose	ApoB48 TAG/TAG_{ref}/DAG Glucose Liver chemistry					Vital signs	Weight recorded
4	00:00		AZD7687/placebo					
5	Predose						Vital signs	Weight recorded
5	00:00[e]		AZD7687/placebo					
6	Predose	Glucose Insulin Clinical chemistry[a]/haematology					Vital signs	Weight recorded
6	00:00[e]		AZD7687/placebo					
7	Predose	ACTH/cortisol[f] Aldosterone/DHEA-S[g] Clinical chemistry[a]/haematology Insulin Renin[g]		Urinalysis			pECG Vital signs	Weight recorded
7	00:00[e]		AZD7687/placebo					
7	12:00	ACTH/cortisol[f]						
8	Predose	FFA Glucose Insulin Liver chemistry TAG/TAG_{ref}/DAG			Sample D8–D10 start		pECG Vital signs	Weight recorded

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
8	00:00[e]		AZD7687/placebo					
8	00:30	TAG/TAG_{ref}/DAG hsCRP						
8	01:00 pre-SMM	PK-18 ApoB48 GLP-1/GIP PYY3-36 FFA Glucose Insulin TAG/TAG_{ref}/DAG						
8	01:00	Paracetamol sample						SMM start
8	01:15							SMM end Paracetamol administered
8	01:30	FFA GLP-1/GIP PYY3-36 Paracetamol sample						
8	01:45	Paracetamol sample						
8	02:00	FFA GLP-1/GIP Insulin PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
8	02:20	Paracetamol sample						
8	02:40	Paracetamol sample						
8	03:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
8	04:00	FFA GLP-1/GIP Insulin Glucose						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
		PYY3036 TAG/TAG_{ref}/DAG Paracetamol sample						
8	05:00	ApoB48 TAG/TAG_{ref}/DAG Paracetamol sample						
8	06:00	FFA Insulin Glucose TAG/TAG_{ref}/DAG Paracetamol sample						
8	07:00	ApoB48 GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
8	08:00	TAG/TAG_{ref}/DAG						
8	09:00	PK-19 GLP-1/GIP PYY-3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
9	Predose	PK-20 Liver chemistry ApoB48 TAG/TAG_{ref}/DAG			Sample D8–D10 end		dECG[h] pECG Vital signs	Weight recorded
9	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start				Empty bladder
9	00:15						dECG start	
9	00:20	PK-21					dECG end	
9	00:35						dECG start	
9	00:40	PK-22					dECG end	
9	00:55						dECG start	
9	01:00	PK-23					dECG end pECG Vital signs	
9	01:15						dECG start	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
9	01:20	PK-24					dECG end	
9	01:35						dECG start	
9	01:40	PK-25					dECG end	
9	01:55						dECG start	
9	02:00	PK-26					dECG end Vital signs	
9	02:55						dECG start	
9	03:00	PK-27					dECG end Vital signs	
9	03:55						dECG start	
9	04:00	PK-28		PK-urine 0-4 h end PK-urine 4-8 h start		Adipose biopsy	dECG end	
9	05:00							
9	05:55						dECG start	
9	06:00	PK-29					dECG end pECG	
9	07:55						dECG start	
9	08:00	PK-30		PK-urine 4-8 h end PK-urine 8-12 h start			dECG end	
9	11:55						dECG start	
9	12:00	PK-31		PK-urine 8-12 h end PK-urine 12-24 h start			dECG end	
9	18:00	PK-32						
9	23:55						dECG start	
9	24:00	PK-33		PK-urine 12-24 h end			dECG end	
10	00:00[e]	ACTH/cortisol[f] Aldosterone/DHEA-S[g] BNP Clinical chemistry[a]/haematology Insulin Ketones Lactate Leptin/adiponectin Renin[g]			Collection end		pECG Vital signs	Weight recorded

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
		Glucose Exploratory blood sample						
10	12:00	ACTH/cortisol[f]						
10	24:00	PK-34						
11	00:00[e]						pECG Telemetry end Vital signs	Weight recorded Physical examination Waist circumference measured

AZD7687 will be administered at 00:00 on Day 1 and Days 3 to 9.
SMM = standardised mixed meal

[a] Clinical chemistry includes lipid panel and liver chemistry

[b] PK urine collections will be done at the intervals 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h, 24 to 48 h following the single dose on Day 1 and at 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h after the last day of multiple dosing on Day 9.

[c] Faecal collection periods are as follows: Day -3 to Day -1, Day 8 to Day 10.

[d] The genetic sampling can be done at any day during Visits 2 and 3.

[e] The time 00:00 should be the same time each morning throughout the study period.

[f] It is important that the samples are taken at the same time each morning and evening, if applicable. The samples for cortisol and ACTH should be obtained at a time point where reference values for cortisol and ACTH exist, if possible.

[g] The subject should rest in bed for 10 minutes before aldosterone and renin blood sampling.

h Predose dECG recordings on Days 1, 3 and 9 will be 10 minutes long.

Table 3 Study Plan - Time schedule for digital ECG assessments during residential period

Study Days	ECG Number	Start time hour: minute[d]	Dose	Stop time hour: minute	dECG continuous[abc]	Other
1		-01:30		-01:00		Apply the electrodes[b]
		-00:40		-00:30		Rest in bed
	1	-00:30	Predose	-00:20	10 minutes[c]	
		-00:20		-00:05		Toilet use allowed
		00:00	Administration of AZD7687/placebo			
	2	00:15		00:20	5 minutes[c]	
	3	00:35		00:40	5 minutes[c]	
	4	00:55		01:00	5 minutes[c]	
	5	01:15		01:20	5 minutes[c]	
	6	01:35		01:40	5 minutes[c]	
	7	01:55		02:00	5 minutes[c]	
	8	02:55		03:00	5 minutes[c]	
	9	03:55		04:00	5 minutes[c]	
	10	05:55		06:00	5 minutes[c]	
	11	07:55		08:00	5 minutes[c]	
	12	11:55		12:00	5 minutes[c]	
	13	23:55		24:00	5 minutes[c]	
4		-01:30		-01:00		Apply the electrodes[b]
4	14	-00:30	Predose	-00:20	10 minutes[c]	
10		-01:30		-01:00		Apply the electrodes[b]
		-00:40		-00:30		Rest in bed
	15	-00:30	Predose	-00:20	10 minutes	
		-00:20		-00:05		Toilet use allowed
		00:00	Administration of AZD7687/placebo			
	16	00:15		00:20	5 minutes[c]	
	17	00:35		00:40	5 minutes[c]	

Study Days	ECG Number	Start time hour: minute[d]	Dose	Stop time hour: minute	dECG continuous[abc]	Other
	18	00:55		01:00	5 minutes[c]	
	19	01:15		01:20	5 minutes[c]	
	20	01:35		01:40	5 minutes[c]	
	21	01:55		02:00	5 minutes[c]	
	22	02:55		03:00	5 minutes[c]	
	23	03:55		04:00	5 minutes[c]	
	24	05:55		06:00	5 minutes[c]	
	25	07:55		08:00	5 minutes[c]	
	26	11:55		12:00	5 minutes[c]	
	27	23:55		24:00	5 minutes[c]	

a The subject must be in the same supine body position (max. 30 degrees flexion in the hip) at each time point and at all visits. Subject's feet should not contact the footboard of the bed.

b Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied at least 30 minutes before first recording.

c Subject must rest in bed for at least 10 minutes prior to each ECG time point.

d Time points for dECG may be adjusted according to emerging PK data.

3.1.1 Stopping criteria for dose escalation

After each cohort a Safety Review Committee will review and assess all available data (safety, tolerability, PK and PD) to make a decision on how to proceed the dose escalation.

If any of the following criteria are met no further dose escalation should be performed unless subsequent investigations clearly indicate an alternative cause that is not related to the investigational drug. The SRC should then determine whether the dose level should be repeated, whether a lower dose should be tested or whether the study should be terminated.

- One or more subjects, who receive AZD7687 report a serious adverse event (SAE) possibly related to study medication or experience a non-tolerable adverse event (AE) possibly related to study medication.

- Two or more subjects that receive AZD7687 have QTc prolongation defined as QTcF >500 ms, or an increase of QTcF greater than 60 ms above baseline to a value greater than 480 ms on the 12-lead ECG, confirmed (persistent for greater than 5 minutes) on a repeat 12-lead ECG.

- Two or more subjects, who receive AZD7687, have signs of hepatic toxicity defined as one or more of

  - Confirmed alanine aminotransferase (ALT) or aspartate aminotransferase (AST) increase to >3 x the upper limit of normal (ULN).

  - Confirmed isolated total bilirubin increase to > 2 x ULN.

  - Confirmed ALT or AST increase to >2 x ULN concurrent with an increase in total bilirubin to >1.5 x ULN.

- Two or more subjects, who receive AZD7687, have other clinically significant changes in laboratory values or other safety parameters. When one or more subjects, who receive AZD7687, have a documented tachyarrhythmia of concern, these may be reviewed by an expert group for opinion prior to the Safety Review Committee decision and consulting externally.

- The predefined exposure limit ($C_{max}/C_{ss,max}$ and/or $AUC/AUC_{(0\text{-}24h)}$ at steady state) has been reached in 2 or more subjects, or a lower exposure based on the maximum tolerated dose in the SAD study.

- Other findings that, at the discretion of the SRC, indicate that further dose escalation should be stopped.

The above clinical criteria may also be used as a guideline for determining when dosing should be discontinued in an individual subject.

3.1.2 Subject stopping criteria

Treatment with AZD7687 for a specific subject should be discontinued if:

- Confirmed ALT or AST increase >3 x ULN.
- Confirmed isolated total bilirubin increase >2 x ULN.
- Confirmed ALT or AST increase >2 x ULN concurrent with an increase in total bilirubin >1.5 x ULN. In the event this criterion is met, AZ will be notified immediately.

3.1.3 Safety Review Committee (SRC)

After each cohort a Safety Review Committee will evaluate the safety, tolerability and pharmacokinetics of AZD7687 and decide the next dose.

The SRC will be composed of the following core individuals:

- Principal Investigator or delegate physician (Chair).
- AZ Medical Science Director / Clinical Pharmacology Physician or delegate.
- AZ CPA Physician.
- Project Manager (non-voting member).

In addition, the Principal Investigator and AZ Medical Science Director may invite other specialist individuals when appropriate or needed for off-line input or attendance to the SRC meeting (eg, AZ PK Scientist, AZ ECG Centre, AZ Patient Safety Physician, AZ Statistician.

AZ will be unblinded. Staff at the clinical unit will remain blinded until the SRC meeting.

The decision of the SRC on the next dose will be taken in consensus between the physicians. If consensus cannot be reached then the Principal Investigator, who has the ultimate responsibility for the safety of the subjects, will take the final decision on the next dose level or whether to stop the study. The Medical Science Director, who may need to consider other factors, will be involved in this decision. The decisions and decision-making of the SRC on the next dose level will be documented and provided to the Principal Investigator and Pharmacist prior to the next scheduled dosing day.

For details on stopping criteria refer to Section 3.1.1.

3.2 Rationale for study design, doses and control groups

This multiple ascending dose (MAD) study is the second study in man with AZD7687. A standard parallel-group design is used for this MAD study to evaluate the safety, tolerability,

PK and PD of an investigational product in healthy subjects. The study is randomised and single-blind to minimise bias and includes placebo to facilitate identification of effects related to administration of drug rather than the study procedures or situation.

This study will be conducted in overweight to obese but otherwise healthy male subjects to avoid interference from disease processes or other drugs. The selection criteria are defined such that subjects selected for participation in the study are known to be free from any significant illness. As most of the patients intended for treatment with AZD7687 will be overweight or obese, subjects who are overweight or obese will provide more clinically relevant pharmacokinetic and pharmacodynamic information.

The starting dose in this MAD study is 1 mg once daily based on preliminary safety, tolerability and PK data from the ongoing SAD study, D2710C00001. Subsequent doses of AZD7687 to be given will be based on review of the available safety, tolerability, PK and PD data from the previous dose(s) and data from the SAD study. The exposure at steady state ($C_{ss,max}$ and $AUC_{(0-24h)}$) for the starting dose is predicted not to exceed the C_{max} and AUC obtained from the highest dose so far evaluated and found safe in the SAD study.

The starting dose of 1 mg is predicted to result in a trough free concentration ($C_{ss,min}$) corresponding to <50% DGAT1-inhibition as defined from in vitro enzyme inhibition experiments in adipose tissue (IC50 11 nM). This level of inhibition is expected to be below levels to produce significant pharmacological effects based on preclinical in vivo studies with AZD7687. The level of enzyme inhibition needed for therapeutic effect in man is not known. However an enzyme inhibition of 50% during the entire dosing interval at steady state is anticipated to result in a relevant therapeutic effect, and this level of inhibition is predicted to be reached at a dose of approximately 5 mg or higher. The proposed starting dose (1 mg) is then 1/5th of the lowest anticipated therapeutic dose.

Safety, tolerability and PK will be evaluated after each dose step. Doses will be escalated as to achieve the maximum exposure levels defined for this study; C_{max} (or $C_{ss,max}$) of 70 µmol/L or AUC ($AUC_{(0-24h)}$ at steady state) of 800 µmol*h/L (the exposure below which no elevation in liver enzymes were noted in individual dogs after administration of AZD7687 for 14 days), or a lower exposure based on MTD in the SAD study, or until stopped due to safety findings in this study. If exposure limits have been reached or escalation has been stopped due to safety findings after the first dose step(s), the last dose step(s) will primarily be chosen as to characterize the dose/exposure and DGAT1-inhibition relationship in man, and lower or in between doses will be given for that purpose.

In the SAD study healthy volunteers treated with AZD7687 have experienced mild to moderate gastrointestinal events (nausea, vomiting and loose stools) after a high-fat SMM 4 hours postdose. The reason form this is not known but might be a result of a delayed gastric emptying. Measurements of paracetamol in plasma after oral intake of paracetamol have previously been used as a marker of gastric emptying. In this study oral paracetamol followed by measurements in plasma will therefore be given before start of treatment with AZD7687 and after 1 and 6 days of repeated treatment.

No formal power calculations have been performed. The sample size is based on the desire to obtain adequate safety, tolerability, PK and PD data to achieve the objectives of the study whilst exposing as few subjects as possible to study medication and procedures. Exposure in this initial study with AZD7687 is limited to 8 days treatment (one single dose followed by 48 h washout and 7 days of repeated once or twice daily dosing). It is anticipated that this will be sufficient to achieve and maintain steady state in exposure of AZD7687.

4. SUBJECT SELECTION CRITERIA

Investigators should keep a record ie, subject screening log, of subjects who entered pre-study screening.

Each subject must meet all of the inclusion criteria and none of the exclusion criteria at the time of randomisation for this study. Under no circumstances can there be exceptions to this rule.

4.1 Inclusion criteria

For inclusion in the study subjects should fulfil the following criteria:

1. Provision of signed and dated, written informed consent prior to any study specific procedures.

2. Healthy male subjects aged ≥20 to ≤45 years with suitable veins for cannulation or repeated venepuncture.

3. Male subjects must use condoms from the time of dosing until 4 months after the post study medical visit both to prevent pregnancy and protect partners (and the foetus, if partner is pregnant) from potential exposure to study drug. Male subjects should inform the medical staff at the Clinical Unit if their partner becomes pregnant during the study. In addition to the use of condoms, female partners of male subjects must use additional contraception from the time of dosing until 4 months after the post-study medical visit. Acceptable methods to be used by female partners include the oral contraceptive pill, hormone implants, intra-uterine devices (IUDs) or diaphragms with spermicide. Male subjects must not donate sperm for 4 months after the post-study medical visit.

4. Have a body mass index (BMI) between 27 and 35 kg/m^2.

5. Be able to comply with the requirements of the study as judged by the investigator.

For inclusion in the genetic component of the study, subjects must fulfil the following additional criterion:

6. Provision of signed, written and dated informed consent for genetic research. If a subject declines to participate in the genetic component of the study, there will be

no penalty or loss of benefit to the subject. The subject will not be excluded from other aspects of the study described in this protocol.

4.2 Exclusion criteria

Subjects must not be randomised in the study if any of the following exclusion criteria are fulfilled:

1. History of any clinically significant disease or disorder which, in the opinion of the investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.

2. History or presence of gastrointestinal, hepatic or renal disease or any other condition known to interfere with absorption, distribution, metabolism or excretion of drugs.

3. Any clinically significant illness, medical/surgical procedure or trauma within 4 weeks of the first administration of investigational product.

4. Any clinically significant abnormalities in clinical chemistry, haematology or urinalysis results as judged by the investigator.

5. Fasting serum (S)-glucose ≥7.0 mmol/L or non-fasting S-glucose ≥11.1 mmol/L at screening.

6. Any of liver chemistry tests (AST, ALT, ALP or bilirubin) above ULN.

7. Serum TAG levels >1.5 mmol/L

8. Suspicion of or known Gilbert's disease.

9. History or presence of ezcema or psoriasis.

10. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and human immunodeficiency virus (HIV).

11. After 10 minutes supine rest abnormal vital signs (diastolic blood pressure, systolic blood pressure, heart rate) as judged by the investigator.

12. Any clinically important abnormalities in rhythm, conduction or morphology of resting ECG that may interfere with the interpretation of QTc interval changes. This includes subjects with any of the following:

    - Clinically significant PR (PQ) interval prolongation.

    - Intermittent second or third degree AV block. (2nd degree AV-block, Mobitz type 1Wenchebach during sleep is not disqualifying).

- Incomplete, full or intermittent bundle branch block (QRS <120 ms with normal QRS and T wave morphology is acceptable if there is no clear evidence of left ventricular hypertrophy).

- Abnormal T wave morphology, particularly in the protocol defined primary lead.

13. Prolonged QTcF >450 ms or shortened QTcF <350 ms or family history of long QT syndrome.

14. Any eating disorder or actively attempting to loose weight within 3 months prior to enrolment.

15. Major change in body weight within 3 months prior to enrolment as judged by investigator.

16. History of bariatric surgery.

17. Known or suspected history of drug abuse as judged by the investigator.

18. Smoking more than 7 cigarettes per week from time of consent.

19. History of alcohol abuse or excessive intake of alcohol as judged by the investigator.

20. Positive screen for drugs of abuse at screening or on admission to the clinic.

21. Use of anabolic steroids.

22. History of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the investigator or history of hypersensitivity to drugs with a similar chemical structure or class to AZD7687.

23. Excessive intake of caffeine containing drinks eg, coffee, tea, caffeine containing energy drinks and cola (more than 5 cups of coffee or equivalent per day).

24. Use of drugs with enzyme inducing properties such as St John's Wort within 3 weeks prior to the first administration of investigational product.

25. Use of any prescribed or non-prescribed medication including antacids, analgesics (other than paracetamol/acetaminophen), herbal remedies, vitamins and minerals during the 2 weeks prior to the first administration of IP or longer if the medication has a long half-life. Occasional use of paracetamol/acetaminophen, for minor pains and headache (maximum 2 g per 24 h), and nasal anticongestants are allowed.

26. Any intake of liquorice, grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade or other products containing grapefruit or Seville oranges within 2 weeks of the first administration of investigational product.

27. Plasma donation within 1 month of screening, blood donation within 3 months prior to screening, or any blood loss >500 mL during the 3 months prior to screening.

28. Has received another new chemical entity (defined as a compound which has not been approved for marketing) or has participated in any other clinical study that included drug treatment within 3 months of the first administration of investigational product in this study. The period of exclusion begins at the time of the last visit of the prior study. Note: subjects consented and screened, but not dosed in this study or a previous Phase I study, are not excluded.

29. Previous randomisation of treatment in the present study.

30. Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff, staff and staff at the study site).

31. Judgment by the investigator that the subject should not participate in the study if they are considered unlikely to comply with study procedures, restrictions and requirements.

In addition, any of the following is considered a criterion for exclusion from the genetic research:

32. Previous bone marrow transplant.

33. Non-leukocyte depleted whole blood transfusion within 120 days of the date of the genetic sample collection.

For procedures for handling incorrectly randomised subjects see Section 5.3.

5. STUDY CONDUCT

5.1 Restrictions during the study

The following restrictions apply for the specified times during the study period:

1. Fast from at least 10 hours before Visit 1 and Visit 3. A moderate amount of water is allowed up to one hour before the visit to the clinic.

   On intensive PK assessment days fast for 10 hours overnight before the morning dose. A moderate amount of water is allowed up to one hour prior to each morning dose and may be resumed one hour after dosing. A meal can be given 5 hours after the morning dose.

On days where the standardised mixed meal is administered subjects be fasted overnight and should remain fasted during the 8 hour lipid blood sampling periods following the standardised mixed meals.

On days where adipose tissue biopsies are taken subjects should fast until the biopsy procedure is finished.

On other days during the repeated dosing phase of the study, fast for 10 hours overnight prior to the morning dose and delay breakfast until 1 hour after dosing (except for days when the standardised mixed meal is given). Water is allowed up to one hour before and from one hour after the morning dose.

2. Eat and drink only the standardised meals and drinks provided (apart from water) during the residential period in the unit.

3. Abstain from consuming any of the following:

   - Alcohol from 72 hours before admission, during the residential period and for 72 hours before the study follow-up visit.

   - Energy drinks containing taurine or glucuronolactone eg, Red Bull from 72 hours before admission, during the residential period and for 72 hours before the study follow-up visit.

   - Caffeine-containing drinks during the residential period apart from any provided as part of a standardised meal. Excessive intake of caffeine should be avoided between discharge from the unit and the study follow-up visit.

   - Poppy seeds found in speciality bread from time of consent until after the final medical examination at the study follow-up.

   - Grapefruit, grapefruit juice, Seville oranges, Seville orange marmalade or other products containing grapefruit or Seville oranges from 14 days before admission until after the final medical examination at the study follow-up.

4. Abstain from nicotine use, smoking for 42 hours before admission, during the residential period and for 24 hours before the study follow-up. Abstain from drugs of abuse from time of consent until after the final medical examination at the study follow-up.

5. Abstain from taking any medication (prescribed or over the counter products), other than paracetamol/acetaminophen and nasal anticongestants, from 2 weeks prior to the first administration of investigational product until after the final medical examination at the study follow-up. However, this should not obviate necessary medical treatment. If any medication is necessary during the residential period, it

should be prescribed by the investigator and the AstraZeneca CPA Physician should be informed.

6. On days where the paracetamol challenge is done the subjects should abstain from taking paracetamol 24 hours prior to the start of the test and throughout the paracetamol challenge blood sampling period. On all other days a maximum daily dose of paracetamol of 2 g will be allowed.

7. Subjects should refrain from strenuous physical activity, which is not within the subject's normal daily routine, from 7 days prior to admission to the unit until after the final medical examination at the study follow-up.

8. Abstain from blood or plasma donation until 3 months after the final medical examination at the study follow-up.

9. Male subjects should use a condom to prevent pregnancy and drug exposure of a partner and refrain from donating sperm or fathering a child from the first administration of investigational product until 3 months after the last administration of investigational product

5.2 Subject enrolment and randomisation

The Principal Investigator will ensure:

1. Signed informed consent is obtained from each potential subject before any study specific procedures are performed.

2. Each potential subject is assigned a unique enrolment number, beginning with ""E0001001".

3. The eligibility of each subject is determined See Sections 4.1 and 4.2

Randomisation will be performed on Visit 2.

Randomisation codes will be assigned strictly sequentially as subjects become eligible for randomisation.

If a subject withdraws his/her participation in the study, then his/her enrolment/randomisation code cannot be reused.

5.2.1 Procedures for randomisation

A randomisation scheme will be produced by AstraZeneca using the global randomisation system (GRand). Within each panel subjects will be allocated to AZD7687 or placebo in a ratio of 6:3. The randomisation will be done for each panel using consecutive randomisation codes (subject numbers).

5.3 Procedures for handling incorrectly randomised subjects

Subjects who fail to meet the inclusion/exclusion criteria should not, under any circumstances, be randomised into the study. There can be no exceptions to this rule.

Where a subject, who does not meet the selection criteria, is randomised in error and this is identified before dosing, the subject should be withdrawn from the study. A discussion should occur between the AstraZeneca CPA Physician and the investigator regarding whether a replacement may be considered.

Where a subject, who does not meet the selection criteria, is randomised in error and started on treatment, or where a subject subsequently fails to meet the study criteria post initiation, a discussion should occur between the AstraZeneca CPA Physician and the investigator regarding whether to continue or discontinue the subject from treatment. If treatment is discontinued the subject should be advised to continue assessments to ensure their safety. In situations where an agreement cannot be reached, the subject should have their study treatment discontinued.

The AstraZeneca CPA Physician is to ensure all decisions are appropriately documented.

5.4 Blinding and procedures for unblinding the study

5.4.1 Methods for ensuring blinding at the clinical unit

At each dose level, both the active and the placebo dose will be of the same volume to ensure blinding. In addition the suspension will be administered from a coloured syringe to mask any difference in the colour of the placebo and active suspension,

Staff at the clinical unit will remain blinded with regard to treatment (AZD7687 or placebo) at each dose level until the SRC meeting. The following personnel will have access to the randomisation list:

- The AstraZeneca personnel carrying out the labelling and packaging of study drug.
- The pharmacy personnel preparing study drug at the site.
- The personnel analysing the PK samples and the personnel preparing the SRC data for PK, PD and PK/PD comparison.

The randomisation list should be kept in a secure location until the end of the study. Paper copies of the allocation will be produced and checked by internal quality control procedures. Copies will be given to the un-blinded Pharmacist(s) for dispensing of study medication. One paper copy will be placed in a sealed envelope and retained in a secure restricted access area within the Data Management Department of

5.4.2 Methods for unblinding the study

Individual treatment codes, indicating the treatment randomisation for each randomised subject, will be available to the investigators or pharmacists at the study centre.

Individual sealed subject codes (1 for each subject) with instructions for code breaking will be provided to the Principal Investigator.

The treatment code should not be broken except in medical emergencies when the appropriate management of the subject requires knowledge of the treatment randomisation. The investigator documents and reports the action to AstraZeneca.

After each dose group the SRC will determine the dose for the next cohort. All members of the SRC will have access to unblinded data.

5.5 Treatments

5.5.1 Identity of investigational product(s)

Table 4 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
AZD7687[a]	0.1 mg/mL oral suspension	AstraZeneca
	5 mg/mL oral suspension	AstraZeneca
Placebo[a]	0 mg/mL oral suspension	AstraZeneca

a Details of the batch numbers will be included in the trial master file.

AZD7687 and matched placebo oral suspensions are white to off-white suspensions filled into bottles. The suspensions will be stored in a freezer (below -15 °C).

The IP will be supplied as study specific labelled bulk containers for each treatment.

The responsible staff will dispense the IP into subject specific labelled containers, according to the randomisation scheme and handling instructions, provided by AstraZeneca.

AstraZeneca will provide the site with instructions for preparing and handling of the study drugs.

Formulation number and batch number will be presented in the clinical study report.

5.5.2 Doses and treatment regimens

Each subject will receive a single oral dose of AZD7687 or placebo on Day 1. The dose will be administered after an overnight fast of at least 10 hours as 240 mL suspension and water in total and according to the Handling Instruction.

After dosing, subjects will remain on their bed or sitting for as long as necessary for the required study procedures.

AZD7687 or placebo will be given as 240 mL suspension and water in total, according to the Handling Instruction, once daily for 7 days.

The first cohort will receive 1 mg AZD7687 or placebo. The actual dose for subsequent cohorts will be determined after review of data from the previous dose. The provisional doses for panels 2 to 5 are 2.5 mg, 5 mg, 10 mg and 20 mg.

5.5.3 Labelling

Labels will be prepared in accordance with Good Manufacturing Practice and local regulatory guidelines. The labels will fulfil Good Manufacturing Practice Annex 13 requirements for labelling.

Each bottle of IP will have an investigational-use label permanently affixed to the outside, stating the study drug is for clinical trial use.

5.5.4 Storage

All study drugs should be kept in a secure place under appropriate storage conditions. The IP label on the bottle and the Investigator Brochure specifies the appropriate storage conditions. The storage location will be locked and only accessible to authorised personnel.

5.6 Concomitant and post-study treatment(s)

Apart from paracetamol/acetaminophen (up to 2 g per day including when the paracetamol challenge is administered) and nasal anticongestants no concomitant medication or therapy will be allowed. The subjects should be instructed that no other medication is allowed including herbal remedies, vitamin supplements and over-the-counter products without the consent of the investigator.

Medication, which is considered necessary for the subject's safety and well-being, may be given at the discretion of the investigator during the residential period. When any medication is required, it should be prescribed by the investigator who should inform the AstraZeneca CPA Physician. Following consultation with the CPA Physician, the investigator should determine whether or not the subject should continue in the study.

5.7 Treatment compliance

The administration of all medication (including investigational product) should be recorded in the appropriate sections of the Case Report Forms.

Treatment compliance will be assured by supervised administration of the investigational product by the investigator or delegate. The dose, dates and times of administration of the investigational product will be recorded and checked by the monitor at monitoring visits.

5.7.1 Accountability

It is the investigator's responsibility to establish a system for handling study treatments, including investigational medicinal products, to ensure that:

1. Deliveries of such products from AstraZeneca are correctly received by a responsible person (eg, pharmacist).

2. Such deliveries are recorded.

3. Study treatments are handled and stored safely and properly.

4. The study drug provided for this study will be used only as directed in the study protocol.

5. The study personnel will account for all drugs received at the site, dispensed for the subject and returned to the pharmacy. Any discrepancies should be documented, investigated and appropriately resolved.

6. At the end of the study, site personnel will account for all unused drugs and for appropriate destruction. Certificates of delivery and destruction should be signed.

5.8 Discontinuation of investigational product and withdrawal from study

Subjects may be discontinued from investigational product in the following situations:

- Subject decision. The subject is at any time free to withdraw his/her participation in the study, without prejudice.
- Adverse events.
- Subject stopping criteria have been reached (see Section 3.1.2).
- Severe non-compliance to the study protocol as judged by the investigator and/or AstraZeneca.
- Randomisation in error (see Section 5.3).

Subjects who discontinue investigational product will undergo the assessments and procedures scheduled for the follow-up visit.

Subjects who discontinue treatment with the IP due to adverse events after the start of dosing will not be replaced. Subjects who withdraw for any reason before the first dose or for reasons other than adverse events after the start of dosing may be replaced.

5.8.1 Procedures for withdrawal of a subject from the study

Subjects are at any time free to withdraw from the study (study treatment and assessments), without prejudice (withdrawal of consent). Such subjects will always be asked about the reason(s) and the presence of any adverse events. If possible, subjects who withdraw from the study after the start of dosing and before completion should be seen by an investigator and undergo the assessments and procedures scheduled for the follow-up visit. Adverse events should be followed up (see Sections 6.3.3 and 6.3.4).

6. COLLECTION OF STUDY VARIABLES

The study assessments are described in the sections below and the timing of these assessments are detailed in the Study Plans (see Table 1, Table 2 and Table 3).

It is important that PK sampling occurs as close as possible to scheduled time. In order to achieve this, other assessments scheduled at the same time may be initiated prior to the time point. The sequence at a particular time point is:

1. dECG.
2. Vital signs.
3. Pharmacokinetic blood sample.
4. Safety laboratory sampling.
5. Serum TAG_{total} blood sample.
6. Plasma $TAG_{refined}$ and DAG blood sample.
7. ApoB48.
8. PK urine sample.

Pre-dose assessments may be performed up to 60 minutes prior to dosing.

6.1 Recording of data

For this study, subject data will be collected by EDC. Where electronic data capture is not possible, the source data will be captured on paper.

The Investigator will ensure that data are recorded on the EDC as specified in the study protocol. He/she ensures the accuracy, completeness, and timeliness of the data recorded, for data queries and all required reports according to any instructions provided.

6.1.1 Electronic data capture

Data are collected electronically for each subject by an EDC data management and workflow system. Data are directly captured at the bedside where the data are collected electronically from instrumentation, or data are entered through touch-pad entry screens by the site personnel at the bedside. Investigators and study personnel will be responsible for the data capture and will respond to queries within the EDC data management system. For subjects who discontinue or terminate from the study, the site personnel will complete a termination screen that clearly documents the reason for termination on the end of study screens.

EDC collected data is real-time data collection and reflects the latest observations on the subjects participating in the study. Correction of any data errors and other such changes are

made by changing or updating the data in the system, which also requires the entry of the users name and a password for each change to be captured in the electronic audit trail.

Clinical data (including AEs and concomitant medications) will be entered into a 21 Code of Federal Regulations Part 11-compliant data management system provided by . The data system includes password protection and internal quality checks, such as automatic verification range checks, to identify data that appear to be out of the specified ranges. Programmed edit specifications identify discrepancies in the data, which may be addressed by the site.

When data have been entered reviewed, edited and source data verification performed by the AstraZeneca representative, the data will be frozen to prevent further editing.

6.2 Enrolment and screening procedures

At enrolment (Visit 1), each potential subject will provide informed consent prior to starting any study specific procedures.

Demographic data and other characteristics will be recorded and will include date of birth, race and alcohol consumption.

Each subject will undergo screening during the 30 days prior to admission to confirm eligibility. This will consist of:

1. A standard medical, medication and surgical history with review of the inclusion and exclusion criteria with the subject.
2. A complete physical examination.
3. Height, weight and calculation of BMI.
4. Vital signs – resting supine blood pressure, pulse and body temperature.
5. Recording a resting 12-lead paper ECG.
6. Blood samples for routine clinical chemistry, haematology and screening for hepatitis B surface antigen, antibodies to hepatitis C virus and antibodies to HIV.
7. A urine sample for routine urinalysis, drugs of abuse and alcohol screen.
8. Waist circumference.

After admission and before randomisation the investigator should reassess each subject to reconfirm eligibility.

6.2.1 Follow-up procedures

A post-study medical examination will be performed 7 to 14 days after the last dose. This will be similar to the one performed at screening and will include a complete physical examination, measurement of weight, vital signs, recording a 12-lead paper ECG, a blood sample for clinical chemistry (including lipid panel and clinical chemistry) and haematology, a urine sample for urinalysis, blood sampling for PK analysis, blood sampling for adreno corticotrophin hormone (ACTH) and cortisol and assessment of any adverse events or required medication.

6.3 Safety

The Principal Investigator is responsible for ensuring that all staff involved in the study is familiar with the content of this section.

6.3.1 Definition of adverse events

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, laboratory findings, electrocardiogram). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used generally to include any AE whether serious or non-serious.

6.3.2 Definitions of serious adverse event

A serious adverse event is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:

- Results in death.
- Is immediately life threatening.
- Requires in-patient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability or incapacity.
- Is a congenital abnormality or birth defect.
- Is an important medical event that may jeopardise the subject or may require medical intervention to prevent one of the outcomes listed above.

For further guidance on the definition of a SAE, see Appendix B of this Clinical Study Protocol. For definition of other significant adverse events (OAE) see Section 11.1.1.

6.3.3 Recording of adverse events

Time period for collection of adverse events

Adverse Events will be collected from randomisation throughout the treatment period and including the follow-up period.

SAEs will be recorded from the signing of the informed consent form.

Follow-up of unresolved adverse events

Any AEs that are unresolved at the subject's last visit in the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF. AstraZeneca retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Variables

The following variables will be collected for each AE:

- AE diagnosis/description;
- the date and time when the AE started and stopped;
- intensity;
- whether the AE is serious or not;
- investigator causality rating against the investigational product (yes or no);
- action taken with regard to investigational product;
- AE caused subject's withdrawal from study (yes or no);
- outcome.

Additional variables will be collected for all SAEs including treatment given for the event.

The following intensity ratings will be used:

1. mild (awareness of sign or symptom, but easily tolerated).
2. moderate (discomfort sufficient to cause interference with normal activities).
3. severe (incapacitating, with inability to perform normal activities).

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.3.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for

several hours may be considered severe nausea, but not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be an SAE.

Causality collection

The investigator will assess causal relationship between investigational product and each adverse event, and answer "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?"

For SAEs causal relationship will also be assessed for other medication and study procedures. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as "yes".

A guide to the interpretation of the causality question is found in Appendix B of this Clinical Study Protocol.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel: "Have you had any health problems since you were last asked?", or revealed by observation will be collected and recorded in the CRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from protocol mandated laboratory tests, vital signs, ECGs and other safety assessments will be summarised in the clinical study report. Deterioration as compared to baseline in protocol-mandated laboratory values, vital signs, ECGs and other safety assessments should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory value or vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result or vital sign will be considered as additional information. Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in non-mandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

6.3.4 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the CRF.

For studies in countries implementing the EU Clinical Trials Directive, informing Ethics Committees and Regulatory Authorities will be performed by AstraZeneca. If any SAE occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives within one day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The designated AstraZeneca representative works with the investigator to ensure that all the necessary information is provided to the AstraZeneca patient safety data entry site **within one calendar day** of initial receipt for fatal and life threatening events and **within five calendar days** of initial receipt for all other SAEs.

For fatal or life-threatening adverse events where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day ie, immediately but **no later than the end of the next business day** of when he or she becomes aware of it.

The reference document for definition of expectedness/listedness is the Investigator's Brochure for the AstraZeneca drug.

6.3.5 Laboratory safety assessment

Blood and urine samples for determination of clinical chemistry, haematology, and urinalysis will be taken at the times indicated in the Study Plans (see Table 1 and Table 3). The date and time of collection of all laboratory tests will be recorded in the appropriate CRF.

The following laboratory variables will be measured:

Clinical Chemistry

Serum (S)-Albumin

S-GGT

S-Calcium, total

S-Creatinine

S-Glucose

S-Potassium

S-Sodium

S-Creatine kinase

S-Insulin

Lipid Panel

S-TAG

S-HDL-C

S-LDL-C

S-Cholesterol, total

Liver Chemistry Tests

S-Bilirubin, total

S-Bilirubin, conjugated

S-ALP

S-AST

S-ALT

Coagulation

P-Prothrombin time

P-INR

Haematology

Blood (B)-Haemoglobin

B-Leukocyte

B-Absolute leukocyte differential count

B-Platelet count

Urinalysis

Urine (U)-Glucose

U-Haemoglobin

U-Protein

U-Ketones

Project Specific Clinical Chemistry

S-ACTH

S-Cortisol

S-DHEA-S

S-FFA

S-Ketones

S-Lactate

S-hs-CRP

S-Renin

S-Aldosterone

S-BNP

Additionally, at screening all subjects will be tested for HIV, hepatitis B surface antigen and antibodies to hepatitis C. Urine will be tested for the following drugs of abuse at screening and admission: amphetamines, barbiturates, tricyclic antidepressants, cocaine, methadone, morphine, phencyclidine, tetrahydrocannabinol and opiates. An alcohol screen will be

performed at screening and admission. If a subject tests positive to any of these screening tests he will be excluded from the study.

Further analysis of the faecal samples may be performed to determine the lipid classes that are increased (ie, analyses of TAG and FFA in faeces).

Safety laboratory values outside the reference limit suspected to be of any clinical significance will be repeated. Subjects in whom suspected clinical significance is confirmed will either not be included or if already randomised will be followed until normalisation or for as long as the investigator considers necessary. Additional laboratory variables may be performed for safety reasons if judged appropriate by the investigator.

In the event a subject has any of the following: AST, ALT, bilirubin, ALP >1.5 x ULN there will be intensified liver monitoring for the subject with all liver chemistry tests taken every day until the value(s) begin to improve. Thereafter liver chemistry tests will be performed at an interval decided to be appropriate by the investigator. The subject must be followed until the liver chemistry tests have returned to baseline or until a firm explanation (diagnosis) to the elevated liver chemistry tests has been established.

The samples for Hepatitis B, Hepatitis C and HIV, clinical chemistry, lipid panel, liver chemistry, coagulation, haematology and urinalysis will be analysed using routine methods at

.

Cortisol, ACTH and DHEA-S will be analysed using routine methods at

.

Lactate will be analysed by

Aldosterone, FFA, ketones, rennin and BNP will be analysed using routine methods at

.

The amount of paracetamol in plasma will be analysed by .

For blood volume see Section 7.1.

6.3.6 Physical examination

The timing of individual examinations are indicated in the Study Plan (Table 1). A complete physical examination will be performed at screening and follow-up and include an assessment of the following: general appearance, skin, head and neck, lymph nodes, thyroid, abdomen, musculo-skeletal, cardiovascular, respiratory and neurological systems. On admission prior to dosing and at discharge from the unit only a brief physical examination is required.

At all occasions when physical examination is performed special attention will be given to, dry skin, skin inflammation (especially face) and hair loss.

Height will be measured in centimetres and weight in kilograms (to one decimal place). Details of weight measurement are given in Section 6.3.7. Measurements should be taken without shoes and the same scale used for all measurements. BMI will be calculated from the height and weight.

6.3.7 ECG

For timing of assessments refer to the Study Plans (Table 1, Table 2 and Table 3).

6.3.7.1 Resting 12-lead ECG

A 12-lead ECG will be obtained after the subject has been resting in the supine position for at least 10 minutes. All ECGs will be documented by recording date, time of collection, heart rate, PR, RR, QRS, QT, QTcB and QTcF intervals from the 12-lead ECG.

If indicated, additional ECG assessments can be made at the discretion of the Investigator. These assessments should be entered as an unscheduled assessment. The investigator will judge the overall interpretation as normal or abnormal. If abnormal, it will be decided as to whether or not the abnormality is clinically significant and the reason for the abnormality will be recorded. All ECG readings will be stored as source documents both digitally and as a paper printout.

6.3.7.2 Electronic capture of 12-lead continuous digital ECG (dECG)

The AZ ECG Centre is performing the ECG analysis in the study, using the EClysis© system, version 2.7 or higher.

At protocol indicated time points, 12-lead continuous digital ECGs (dECG) will be recorded over at least 5 minutes (Table 1, Table 2 and Table 3) with the Schiller Cardiovit CS-200 recorder (Schiller AG, Baar, Switzerland) and transmitted to the AZ dECG central repository, according to AstraZeneca standard procedures for settings, recording, and transmission of dECGs.

The same recorder will be used for each subject at each time point, if possible. Date and time settings must be checked at the start of each study day and aligned with an official timekeeper for all machines used in the study.

Skin preparation must be thorough and electrode positions must be according to standard 12-lead ECG placement. Electrode positions for dECG take precedence over telemetry electrodes. Electrode positions will be marked with an indelible pen at the start of the study days to ensure exact reposition. Permanent electrodes will be applied at least 30 minutes before first study recording and left in place for the duration of each relevant study day.

Subjects will rest in a supine position for at least 10 minutes before the start of every recording. The subject should be in the same supine body position (maximum 30 degrees flexion in hip and feet not in contact with the footboard) at each recording time point during the study.

In this study lead V2 will be analysed and reported as primary. Lead V5 will be analysed, for all visits, as backup for the individual where analysis in lead V2 is not deemed possible for predose, or for significant parts of whole visits or whole visits. The analysis is performed blinded to treatment.

The meta data for all dECG files will be checked, and if needed, corrected by the responsible personnel at the study site before the files are electronically transferred to the AZ central dECG files repository.

From the AZ dECG central repository, the dECG files will be imported into the EClysis© system. As standard, 10-second ECGs are extracted by the EClysis system twice per minute from the continuous recording and automatically analyzed.

To provide a decision basis for dose escalation, the ECG Scientific Advisor will perform a preliminary analysis including at least also the first 24 hours of dECG recordings Day 10, in, in lead V2, with main focus on QT changes, wave morphology changes, and dysrrythmia. The ECG Centre Cardiologist will review the data, perform an evaluation and interpretation of findings, and will provide a safety report.

The ECG Scientific Advisor will ensure that all protocol-defined dECGs have been imported into EClysis© databases and will perform all required manual corrections to the ECG annotations provided by EClysis©. Finally, an external cardiologist will review the data and perform all necessary adjustments before locking the EClysis© database into a read-only state.

From the locked database the numerical values for ECG intervals and amplitudes will be then exported in secure, checksum-protected files, made accessible on the AZ dECG Central repository to accredited data management specialists for conversion into SAS files.

The following variables will be reported by the ECG centre: RR, PR, QRS and QT intervals from the primary lead of the digital 12-lead ECG. Derived parameters will be calculated by the study statistician.

6.3.7.3 Real time display (telemetry)

For timing of telemetry assessments refer to Table 1 and Table 2.

A 6-lead real-time telemetry ECG will be displayed starting at predose on Day 1 and continuing while the subjects are in the clinical unit until Day 12.

6.3.8 Vital signs

6.3.8.1 Pulse and blood pressure

For timings of the assessment of BP and pulse refer to Table 1 and Table 2.

Supine blood pressure and pulse rate will be measured using a semi-automatic blood pressure recording device with appropriate cuff size after 10 minutes rest on a bed.

6.3.8.2 Body temperature

Body temperature will be measured in degrees Celsius using an automated thermometer at the times indicated in the Study Plans (Table 1 and Table 2).

6.4 Pharmacokinetics

6.4.1 Collection of pharmacokinetic samples

Venous blood samples (approximately 2.0 mL) for the determination of concentrations of AZD7687 in plasma will be taken at the times presented in the Study Plans (Table 1 and Table 2).

Urine samples (approximately 10 mL) for determination of concentrations of AZD7687 in urine will be taken from the total urine sample provided during each collection period presented in the Study Plans (Table 1 and Table 2).

The weight of each urine collection will be recorded.

The date and time of collection of each sample will be recorded on the EDC system.

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

The timing and number of PK samples may change based on emerging data, however the total blood volume collected will not exceed the maximum limits for this study.

For blood volume see Section 7.1.

6.4.2 Determination of drug concentration in pharmacokinetic samples

Samples for determination of AZD7687 concentration in the plasma and urine, will be analysed by on behalf of AstraZeneca, using an appropriate validated bioanalytical method. Full details of the analytical method used will be described in a separate bioanalytical report.

All samples still within the known stability of the analytes of interest at time of receipt by the bioanalytical laboratory will be analysed.

For each placebo treatment period and subject, only one plasma sample around the expected time of maximum plasma concentration will be analysed to confirm that these subjects have not been given any study drug.

Additional analyses may be conducted on the biological samples to further investigate the presence and/or identity of drug metabolites. Any results from such analyses may be reported separately from the clinical study report.

6.5 Pharmacodynamics

6.5.1 Collection of pharmacodynamic samples

6.5.1.1 Adipose tissue biopsies

Samples for biopsies of adipose tissue (for determination of TAG and DAG in adipose tissue) will be taken at presented in the Study Plans (Table 1 and Table 2). The biopsies will be obtained on the lateral part of the abdomen; with skin penetration approximately at a point located 1/3 from the iliac crest in direction towards the umbilicus. The goal is to obtain 1 to 3 g of adipose tissue in each biopsy.

The adipose tissue samples may be analysed for AZD7687 concentrations if deemed appropriate.

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

6.5.1.2 Assessment of postprandial lipaemia

Samples for the determination of serum total triacylglycerole (TAG_{total}) and samples for the refined plasma triacylglycerole and diacylglycerole ($TAG_{refined}$ and DAG) will be taken at time points specified in the study plans (Table 1 and Table 2).

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

For blood volume see Section 7.1.

6.5.1.3 Insulin and FFA

Samples for the determination of plasma insulin and FFA will be taken at time points specified in the study plans (Table 1 and Table 2).

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

For blood volume see Section 7.1.

6.6 Pharmacogenetics

See Appendix D of this Clinical Study Protocol.

For blood volume see Section 7.1.

6.7 Collection of samples for biomarker research

Samples for determination of exploratory biomarkers involved in nutrient metabolism, metabolic diseases, and/or pharmacokinetics of AZD7687 will be taken at time points specified in the study plans (Table 1 and Table 2). The exploratory biomarker variables will include gastric peptides (GLP-1, GIP, and PYY3-36), ApoB48, leptin and adiponectin.

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

GIP, total GLP-1 and PYY-36 will be analysed using routine methods at

.

Apo B48 will be measured by

TAG (total and refined) and DAG will be measured at a laboratory sub-contracted by

For blood volume see Section 7.1.

Additional exploratory blood sampling will take place on Day 1 and Day 10. Blood samples obtained will be divided into serum and plasma samples (1:1). The volume of blood drawn will be decided in respect to the maximum blood volume that can be drawn from each subject. The timing of these samples may be changed based on the results from the ongoing SAD study and this MAD study.

Body Weight

Body weight will be measured at time points specified in the study plans (Table 1 and Table 2). Body weight for each subject will be measured daily on the same calibrated scale every morning in only underwear or scrubs, before breakfast, after lavatory visit. The weight should be measured with one decimal.

Waist circumference

Waist circumference will be measured at time points specified in the study plans (Table 1 and Table 2). Waist circumference will be measured at the part of the trunk midway between the most caudal part of the lateral costal arch and the iliac crest in the morning before breakfast, after lavatory visit with the person standing with feet about 25 to 30 cm (10 to 12 in) apart. The measurer should stand beside the individual and fit the tape snugly, without compressing any underlying soft tissues. The Gulick II Tape will ensure accurate measurements by applying a constant tension. At the calibration point one coloured bead and the edge of the silver disk is seen. For further information, please see the instruction manual to Gulick II. The tape should be hold in parallel to the floor. The circumference should be measured to the nearest 0.5 cm, at the end of a normal expiration.

Paracetamol Challenge

A paracetamol challenge will be performed on all days when the SMM is administered (see Table 1 and Table 2). Directly after the SMM is finished (at protocol 01:15) 500 mg of paracetamol elixir will be administered. Blood sampling will be performed to determine the paracetamol levels in plasma.

Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

For blood volume see Section 7.1.

7. BIOLOGICAL SAMPLING PROCEDURES

7.1 Volume of blood

The total volume of blood that will be drawn from each subject in this study is as follows:

Table 5 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)[a]	No. of samples	Total volume (mL)
Safety	Clinical chemistry (including lipid panel and liver chemistry)	5.0	5	25.0
	Liver chemistry	3.5	3	10.5
	Haematology	2.0	5	10.0
	Coagulation	1.8	1	1.8
	Serology	3.5	1	3.5
ACTH, Cortisol		3.5	6	21.0
Aldosterone, DHEA-S		3.5	3	10.5
Renin		2.0	3	6.0
FFA, hsCRP		3.5	2	7.0
FFA		2.0	18	36.0
Insulin		3.5	16	56.0
BNP		2.0	2	4.0
Ketones		4.0	2	8.0
Lactate		2.0	2	4.0
Pharmacokinetics		2	34	68
TAG_{total},		3.5	28	98
$TAG_{Refined}$ and DAG		3.5	28	98
PYY3-36		2.0	17	34
GLP-1, GIP		2.0	19	38
Apo B48		3.5	2	7.0
Adiponectin, leptin		3.5	4	14.0
Paracetamol challenge		0.5	36	18.0
Pharmacogenetics		10	1	10
Total				588.3

a Where an indwelling cannula is used an additional 1 mL of blood will be drawn and discarded.

The number of samples taken, as well as the volume required for each analysis, may be changed during the study as new data on AZD7687 become available. However, the maximum volume to be drawn from each subject will not exceed 600 mL.

Faecal samples will be collected at the times indicated in Table 1 and Table 2 for the determination of fat content.

Urinalysis parameters will be measured from a 20 mL fresh urine sample at the times indicated in Table 1 and Table 2.

7.2 Handling, storage and destruction of biological samples

The samples will be used or disposed of after analyses or retained for further use as described here.

7.2.1 Pharmacokinetic and/or pharmacodynamic samples

Samples will be disposed of after the clinical study report has been finalised, unless retained for future analyses, see below.

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples and reported in the bioanalytical report. Key samples for validation of long term stability in incurred samples may be retained at UK on behalf of Clinical Pharmacology & DMPK, Astra Zeneca R&D Mölndal, Sweden, for a maximum of 1 year following the finalisation of the clinical study report (CSR). The results from the validation will not be reported in the CSR but separately in the bioanalytical method validation report.

Key samples (blood and urine) from the analysis of AZD7687 will be shipped to AstraZeneca R&D Mölndal, on behalf of Clinical Pharmacology & DMPK, AstraZeneca R&D Mölndal, Sweden for investigation of the presence and/or identity of drug metabolites. These samples will be stored for a maximum of 3 years. The results from the investigation will not be reported in the Clinical Study Report but separately in a bioanalytical/metabolism report.

7.2.2 Pharmacogenetic samples

See Appendix D of this Clinical Study Protocol.

7.3 Labelling and shipment of biohazard samples

The Principal Investigator ensures that samples are labelled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B regulations (materials containing or suspected to contain infectious substances that do not meet Category A criteria), see Appendix C of this Clinical Study Protocol 'IATA 6.2 Guidance Document'.

Any samples identified as Infectious Category A materials are not shipped and no further samples will be taken from the subject unless agreed with AstraZeneca and appropriate labelling, shipment and containment provisions are approved.

7.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

The Principal Investigator keeps full traceability of collected biological samples from the subjects while in storage at the centre until shipment or disposal (where appropriate) and keeps documentation of receipt of arrival.

The sample receiver keeps full traceability of the samples while in storage and during use until used, disposed of or until further shipment and keeps documentation of receipt of arrival.

AstraZeneca keeps oversight of the entire life cycle through internal procedures, monitoring of study sites and auditing of external laboratory providers.

Samples retained for further use are registered in the AstraZeneca bio bank system during the entire life cycle.

7.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed, if not already analysed and the action documented. If samples are already analysed, AstraZeneca is not obliged to destroy the results of this research.

As collection of donated biological samples is an optional part of the study then the subject may continue in the study.

The Principal Investigator:

- Ensures subject's withdrawal of informed consent to the use of donated samples is notified immediately to AstraZeneca
- Ensures that biological samples from that subject, if stored at the study site, are immediately identified, disposed of/destroyed and the action documented.
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed, the action documented and the signed document returned to the study site.
- Ensures that the subject and AstraZeneca are informed about the sample disposal

AstraZeneca ensures the central laboratory (ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed, the action documented and the signed document returned to the study site.

8. ETHICAL AND REGULATORY REQUIREMENTS

8.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH) Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

8.2 Subject data protection

The Informed Consent Form will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

See Appendix D of this Clinical Study Protocol for additional precautions for genetic data.

8.3 Ethics and regulatory review

The Ethics Committee should approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The investigator will ensure the distribution of these documents to the applicable Ethics Committee, and to the study site staff.

The opinion of the Ethics Committee should be given in writing. The investigator should submit the written approval to AstraZeneca before enrolment of any subject into the study.

The Ethics Committee should approve all advertising used to recruit subjects for the study.

AstraZeneca should approve any modifications to the Informed Consent Form that are needed to meet local requirements.

If required by local regulations, the protocol should be re-approved by the Ethics Committee annually.

Before enrolment of any subject into the study, the final study protocol, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

AstraZeneca will handle the distribution of any of these documents to the national regulatory authorities.

AstraZeneca will provide the Regulatory Authority, Ethics Committee and Principal Investigator with safety updates / reports according to local requirements, including SUSARs (Suspected Unexpected Serious Adverse Reactions), where relevant.

8.4 Informed consent

Any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation should be described in the informed consent form that is approved by an Ethics Committee.

The Principal Investigator will:

- Ensure that each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study.
- Ensure that each subject is notified that they are free to withdraw from the study at any time.
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided.
- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study.
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File.
- Ensure a copy of the signed Informed Consent Form(s) is/are given to the subject.

8.5 Changes to the protocol and Informed Consent Form

Study procedures will not be changed without the mutual agreement of the investigator and AstraZeneca.

If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment and where required in a new version of the study protocol (Revised Protocol).

The amendment should be approved by the Ethics Committee and if applicable, also the national regulatory authority, before implementation. Local requirements should be followed for revised protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the protocol to the Principal Investigator. For distribution to the Ethics Committee see Section 8.3.

If a protocol amendment requires a change to the Informed Consent Form, AstraZeneca and the Ethics Committee should approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by the Ethics Committee.

8.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an ethics committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonisation (ICH), and any applicable regulatory requirements. The investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the centre relating to the study.

9. STUDY MANAGEMENT

9.1 Pre-study activities

Before the first subject is entered into the study, it is necessary for a representative of AstraZeneca to:

- Determine the adequacy of the facilities
- Determine availability of subjects appropriate for the study
- Discuss with the investigators (and other personnel involved with the study) their responsibilities with regard to protocol adherence, and the responsibilities of AstraZeneca or its representatives. This will be documented in a Clinical Study Agreement between AstraZeneca and the investigator.

9.2 Training of study site personnel

Before the first subject is entered into the study, an AstraZeneca representative will review and discuss the requirements of the Clinical Study Protocol and related documents with the investigational staff and also train them in any study specific procedures and system(s) utilised, and review training of the staff for the EDC system to be used in the study.

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The Principal Investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

9.3 Monitoring of the study

During the study, an AstraZeneca representative will have regular contacts with the study site, including visits to:

- Provide information and support to the investigators

- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the CRFs, that biological samples are handled in accordance with the Laboratory Manual and that investigational product accountability checks are being performed
- Perform source data verification (a comparison of the data in the CRFs with the subject's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating subjects. This will require direct access to all original records for each subject (eg, clinic charts)
- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the subject.

The AstraZeneca representative will be available between visits if the investigators or other staff at the centre needs information and advice about the study conduct.

9.3.1 Source data

Refer to the Clinical Study Agreement for location of source data.

9.4 Study agreements

The Principal Investigator should comply with all the terms, conditions, and obligations of the Clinical Study Agreement or equivalent for this study. In the event of any inconsistency between this Clinical Study Protocol and the Clinical Study Agreement, the Clinical Study Protocol shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, the terms of the Clinical study Agreement shall prevail.

Agreements between AstraZeneca and the Principal Investigator should be in place before any study-related procedures can take place, or subjects are enrolled.

9.4.1 Archiving of study documents

The investigator follows the principles outlined in the Clinical Study Agreement.

9.5 Study timetable and end of study

The end of the study is defined as "the last visit of the last subject undergoing the study".

The study is expected to start in Quarter 2, 2010 and to end by Quarter 3, 2010.

The study may be terminated if the study procedures are not being performed according to Good Clinical Practice, or if recruitment is slow. AstraZeneca may also terminate the entire study prematurely if concerns for safety arise within this study or in any other study with AZD7687.

10. DATA MANAGEMENT BY

A 21 Code of Federal Regulations (CFR) part 11 compliant EDC system will be used for this study. Electronic CRFs will be produced by for each subject. The majority of study data collected will be either directly entered by clinical research staff or directly captured from devices onto the electronic CRF. Data will be available for AstraZeneca review via pre-defined reports extracted from the database at agreed intervals. The CRFs must be kept in order and up-to-date so that they reflect the latest observations on the enrolled subjects.

When direct data entry onto the electronic CRF is inappropriate or impractical data will be collected on paper source documents and subsequently transcribed, where necessary, onto the electronic CRFs by the clinical research staff of Quintiles Ltd. All source documents will be retained by Photocopies of completed source documents will be provided only if essential (ie, for regulatory purposes) at the request of the AstraZeneca.

Laboratory data are managed within the laboratory information management system (LIMS) and only the date and time of sampling are recorded in the electronic CRF. Data that is not directly captured eg, safety laboratory results and AE coding, are managed externally from the main study database. These data will be merged with the data from the main study database in post-production. Datasets supplied to the Sponsor will contain all study data.

The informed consent will be kept with a copy of the completed source documents in the appropriate file folder provided, or a note to indicate where the records can be located. All records should be kept in conformance to applicable national laws and regulations.

All electronic CRF entries, corrections, and alterations must be made by the Investigator or other, authorized, study-site personnel and only by individuals who have received training on the electronic data capture system. Site staff may be allowed access to the system only after training is completed. Training must be documented and a log of all electronic data capture users and their rights within the system be maintained.

The Data Management Plan will describe the methods used to collect, check and process clinical data in detail. It will also clarify the roles and responsibilities for the different functions and personnel involved in the data management process.

Validity and consistency of data will be checked by employing pre-programmed data validation rules that will be applied to the data extracted from the EDC system during the course of the study. The data management team will raise queries in the EDC system to resolve discrepancies. The Investigator must verify that all data entries in the electronic CRFs are accurate and correct. After completion of the study and when all collected data is validated, the database will be locked, pursuant to the prior approval by AstraZeneca. Final data will be extracted from the EDC system and delivered to AstraZeneca in the form of SAS® datasets in accordance with defined project standards. A PDF copy of the electronic CRF will be produced for each study subject and included in the final delivery.

The EDC system will keep track of all data entry, alterations and query resolution in an audit trail. The audit trail will form an integral part of the database and will be archived alongside with the Dictionary coding. Medical coding is done using the most current version of MedDRA and AstraZeneca Drug Dictionary.

Management of external data

Data Management determines the format of the data to be received from external vendors and coordinates the flow of data to an external environment or clinical database. External data reconciliation will be done with the clinical database as applicable.

SAE/AE Reconciliation

Serious Adverse Event Reconciliation Reports are produced and reconciled with the Patient Safety database and/or the Investigational Site.

Data verification and validation

The study data verification will be carried out by or delegate and will involve comparison of the database against source documents (ie, ECG print-outs, laboratory results and other health records at the study site). Questions and corrections will be noted and verified by the investigator.

See Appendix D of this Clinical Study Protocol for handling of genetic data.

11. EVALUATION AND CALCULATION OF VARIABLES

11.1 Calculation or derivation of safety variable(s)

11.1.1 Other significant adverse events

During the evaluation of the AE data, an AstraZeneca medically qualified expert will review the list of AEs that were not reported as SAEs or AEs leading to discontinuation of investigational product and withdrawal from the study. Based on the expert's judgement, significant adverse events of particular clinical importance may, after consultation with the Global Safety Physician, be considered OAEs and reported as such in the Clinical Study Report. A similar review of other data from laboratory tests, vital signs, ECGs and other safety assessments will be performed for identification of OAEs.

Examples of these are marked haematological and other laboratory abnormalities, and certain events that lead to intervention (other than those already classified as serious), dose reduction or significant additional treatment.

11.2 Calculation or derivation of pharmacokinetic variables

The PK analyses of the plasma and urine concentration data for AZD7687 will be performed at . The actual sampling times will be used in the PK parameter calculations. Pharmacokinetic parameters will be derived

using standard non-compartmental methods using WinNonlin v5.2 or higher and/or SAS® version 9.1 or higher.

For single dose administration, predose plasma concentrations that are missing or below lower limit of quantification (LLOQ) will be assigned a value of zero for the calculation of PK parameters. Any other concentrations less than LLOQ will be assigned a value of zero if they precede quantifiable samples in the initial portion of the profile. If values <LLOQ occur at the end of the collection interval (after the last quantifiable concentration), these will be treated as missing data. Handling of anomalous concentration values, concentrations <LLOQ occurring between quantifiable data points, or consecutive values <LLOQ followed by quantifiable concentrations in the terminal portion of the concentration curve, will be treated as described in global standard operating procedures (SOPs).

Where possible, the following PK parameters will be determined for AZD7687 using the plasma or the urine concentration data.

Following the single dose part of the study: Day 1

C_{max}	Maximum concentration in the plasma (µmol/L), obtained directly from the observed concentration versus time data
t_{max}	Time of maximum plasma concentration (h), obtained directly from the observed concentration versus time data
$AUC_{(0-\tau)}$	Area under the concentration-time curve in plasma from zero (predose) to 24 hours (µmol*h/L) (dosing interval for the multiple ascending doses).
$AUC_{(0-48)}$	Area under the concentration-time curve in plasma from zero (predose) to 48 hours (µmol*h/L)
$AUC_{(0-t)}$	Area under the concentration-time curve in plasma from zero (predose) to time of last quantifiable concentration (µmol*h/L), calculated by linear up/ log down trapezoidal summation. A minimum of 4 quantifiable post-dose concentration values will be required for AUC calculation.
AUC	Area under the concentration-time curve in the plasma from zero (predose) extrapolated to infinite time (µmol*h/L), calculated by linear up/ log down trapezoidal summation and extrapolated to infinity by addition of the last quantifiable concentration divided by the elimination rate constant: $AUC_{(0-t)} + C_{last}/\lambda_z$. If the extrapolated area ($C_{last}/\lambda z$) is greater than 20% of AUC, then AUC will be set to missing.
λ_z	Apparent terminal rate constant (1/h), determined by linear regression of the terminal points of the log-linear concentration-time curve. Visual assessment will be used to identify the terminal linear phase of

	the concentration-time profile. A minimum of 3 data points and a regression coefficient (Rsq) value of >0.8 will be used as the criteria for reliable estimation of λ_z.
$t_{1/2,\lambda z}$	Apparent terminal half-life (h), determined as ln2/λz
CL/F	Apparent oral plasma clearance (L/h), calculated as dose divided by AUC (calculated for AZD7687 only)
A_e	Amount of analyte excreted in the urine (µmol)
fe (%)	Fraction of drug excreted in the urine calculated as A_e/Dose *100 (calculated for AZD7687 only)
CL_R	Renal clearance (L/h) estimated by dividing $A_{e(0-48)}$ by $AUC_{(0-48)}$
MR C_{max}	Metabolite/parent ratio for C_{max}
MR AUC	Metabolite/parent ratio for AUC

Following the multiple dose part of the study: Day 9

$C_{max,ss}$	Maximum concentration in the plasma(µmol/L) at steady state, obtained directly from the observed concentration versus time data
$C_{min,ss}$	Minimum concentration in the plasma (µmol/L) at steady state, obtained directly from the observed concentration versus time data
$C_{avg,ss}$	Average concentration (µmol/L) at steady state, calculated as $AUC_{(0-\tau),ss}$ divided by the dosing interval
$t_{max,ss}$	Time of maximum plasma concentration at steady state (h), obtained directly from the observed concentration versus time data
$AUC_{(0-\tau),ss}$	Area under the concentration-time curve in plasma area under the plasma concentration-time curve during a dosing interval at steady state, (µmol*h/L) calculated by linear up/ log down trapezoidal summation.
$\lambda_{z,ss}$	Apparent terminal rate constant (1/h), determined by linear regression of the terminal points of the log-linear concentration-time curve. Visual assessment will be used to identify the terminal linear phase of the concentration-time profile. A minimum of 3 data points and a regression coefficient (Rsq) value of >0.8 will be used as the criteria for reliable estimation of λz.
$t_{1/2,\lambda z,ss}$	Apparent terminal half-life (h), determined as ln2/λz,ss

CL_{ss}/F	Apparent oral plasma clearance (L/h), at steady state (calculated for AZD7687 only)
$A_{e(0-\tau)ss}$	Amount of analyte excreted in the urine during a dosing interval at steady state (µmol)
$f_{e,\ ss}$ (%)	Fraction of drug excreted in the urine at steady state calculated as $A_{e(0-\tau)\ ss}$ /Dose *100
$CL_{R,ss}$	Renal clearance (L/h) estimated by dividing $A_{e(0-\tau),ss}$ by $AUC_{(0-\tau),ss}$
RC_{max}	Accumulation ratio for C_{max} calculated as $C_{max,\ ss}$ (estimated after multiple dose)/C_{max} (estimated after single dose)
$RAUC_{(0-\tau)}$	Accumulation ratio for $AUC_{(0-\tau)}$ calculated as $AUC_{(0-\tau),ss}$ (estimated after multiple dose)/ $AUC_{(0-\tau)}$ (estimated after single dose)
%Fluct	Percent fluctuation calculated as $[(C_{max,ss} - C_{min,ss})/ C_{avg}] * 100$
$MRC_{max,ss}$	Metabolite /parent ratio for $C_{max,ss}$
$MRAUC_{(0-\tau),\ ss}$	Metabolite /parent ratio for $AUC_{(0-\tau),\ ss}$

The following PK parameters will be calculated for diagnostic purposes and listed, but will not be summarised:

- The t_lower and t_upper of the log-linear regression to determine $t_{½\lambda z}$.
- Number of data points ($t_{½}$, N) included in the log-linear regression analysis.
- Goodness of fit statistic for calculation of λ_z [Regression coefficient: Rsq].
- Percentage of AUC obtained by extrapolation (%AUCex).Paracetamol Challenge

11.3 Calculation or derivation of pharmacodynamic variable(s)

Two PD variables are of special interest: (1) the inhibitory effect on the TAG/DAG ratio in adipose tissue (biopsies) and (2) the inhibitory effect on incremental serum TAG_{total} AUC (blood samples).

1. The inhibitory effect on the TAG/DAG ratio in adipose tissue compared to baseline is defined as:

$$\frac{(TAG/DAG_{\ day\text{-}1} - TAG/DAG_{\ day9})}{(TAG/DAG_{\ day\text{-}1} - TAG/DAG_{\ max\ inhibition\ day\text{-}1})}$$

TAG/DAG $_{\text{max inhibition}}$ is defined as the ratio observed after direct incubation of the biopsy with AZD7687 on the respective day.

ie, for each individual the inhibitory effect is related to the maximal inhibitory effect of AZD7687 at baseline. (Note: the TAG/DAG $_{\text{max inhibition day9}}$ is not used in the analysis but will be compared descriptively with TAG/DAG $_{\text{max inhibition day-1}}$ as a confirmation of the expected stability of the value at max inhibition).

2. The incremental serum TAG $_{\text{total}}$ AUC is defined as the difference between the area under the TAG versus time curve from 0 to 8 hours and the area of the rectangle with height = pre-meal TAG value and width = 8 hours, where the pre-meal value is the average of the last two pre-meal measurements.

The effect on incremental serum TAG_{total} AUC is measured by the relative change from Day -1 to Day 3 and Day 8.

Change from baseline will also be calculated for insulin and FFA.

11.4 Calculation or derivation of exploratory variables

11.4.1 Weight and waist circumference

The change from baseline in body weight will be calculated for study days Day 2 to Day 11 and also follow up where the baseline for weight will be an average of three measurements done on Day -2, Day -1 and Day 1 predose.

The change from baseline in waist circumference will be calculated for Study Day 11 where baseline is defined as the Day -2 value.

11.4.2 Gastrointestinal peptides (total GLP-1, GIP, PYY3-36)

The change from baseline in total GLP-1, GIP, and PYY3-36 will be calculated for Day 3 and Day 8, with the time matched Day-2 data as the baseline.

11.4.3 ApoB48 and hsCRP

The change from baseline in ApoB48 and hsCRP will be calculated for Day 3, Day4, Day 8, and Day 9 with average of Day -1 and Day -2 value as baseline.

11.4.4 Leptin and adiponectin

The change from baseline in leptin and adiponectin will be calculated for Day 2 and Day 10 with Day 1 predose value as baseline.

11.4.5 Paracetamol challenge

The analyses of plasma concentration of paracetamol will be performed at . The actual sampling times will be used in the parameter calculations. Pharmacokinteic parameters will be derived using standard non-compartmental methods using WinNonlin v5.2 or higher and/or SAS® version 9.1 or higher.

The following parameters will be calculated for paracetamol:

C_{max}	Maximum concentration in the plasma (µmol/L), obtained directly from the observed concentration versus time data
t_{max}	Time of maximum plasma concentration (h), obtained directly from the observed concentration versus time data
AUC(0-t)	Area under the concentration-time curve in plasma from zero (predose) to time of last quantifiable concentration (µmol*h/L), calculated by linear up/ log down trapezoidal summation. A minimum of 4 quantifiable post-dose concentration values will be required for AUC calculation.

11.5 Calculation or derivation of pharmacogenetic variables

See Appendix D of this Clinical Study Protocol.

12. STATISTICAL METHODS AND SAMPLE SIZE DETERMINATION

12.1 Description of analysis sets

12.1.1 General principles

The analysis of data will be based on different subsets according to the purpose of analysis, ie, for safety, PK and PD, respectively. The decision regarding validity of data for each of the analysis sets will be based on a blind review of data.

The as-treated principle will be applied to all evaluations; ie, subjects who received another treatment than the one assigned in the randomisation list will be analysed as belonging to the actual treatment group and not that assigned by randomisation.

12.1.2 Analysis of Safety population

All subjects who received at least one dose of randomised investigational product, AZD7687 or placebo and for whom any post-dose data are available will be included in the safety population.

12.1.3 PK analysis set

The PK analysis set will include all subjects who received at least 1 dose of AZD7687 and have at least 1 measured AZD7687 plasma or urine concentration at a scheduled PK time point postdose. The PK analysis set should include all evaluable PK data appropriate for the evaluation of interest (with no major protocol deviations or violations thought to significantly affect the PK of the drug) from all subjects who received AZD7687. A strategy for dealing with data affected by protocol violations and deviations will be agreed

by the study team physician, pharmacokineticist and statistician prior to clean file and code break.

Subjects that receive placebo will not be part of the PK analysis set.

12.1.4 PD analysis set

The PD analysis set will include all subjects in the safety analysis set and who have evaluable postdose PD data appropriate for the evaluation of interest (with no major protocol deviations or violations thought to significantly affect the PD of the drug) from all subjects who received AZD7687 or placebo.

12.2 Methods of statistical analyses

12.2.1 General principles

Given the exploratory nature, no formal statistical hypothesis testing will be performed in this study.

Data will be presented by actual dose (not by cohort), and subjects receiving placebo will be pooled across dosing cohorts for the purposes of summarising the safety and pharmacodynamic results.

Since no planned formal testing will be performed in this study, and the confidence intervals that will be calculated are only for descriptive purposes, no corrections for multiplicity will be used.

Missing data will be result in a reduced sample size for that parameter. Since the statistical analyses will be predominantly presentations in tables and individual data listings, no action will be taken to handle missing data.

A subject who withdraws prior to the last planned observation in a study period will be included in the analyses up to the time of discontinuation.

12.2.2 Subject characteristics

Continuous variables will be summarised using descriptive statistics (n, mean, SD, min, median, max) by treatment group. Categorical variables will be summarised in frequency tables (frequency and proportion) by treatment group.

12.2.3 Safety and tolerability

Continuous variables will be summarised using descriptive statistics (n, mean, SD, min, median, max) by treatment group. Categorical variables will be summarised in frequency tables (frequency and proportion) by treatment group. Graphical presentations will be used as appropriate. Examples may include line graphs showing individual or mean development over time, and shift plots showing pre-treatment values on horizontal axis and post-treatment values on vertical axis.

All SAEs will be collected for each subject from the time when informed consent is obtained (Visit 1) until the follow-up visit. All non-serious AEs will be collected from randomisation. AEs that occur before dosing will be reported separately.

Adverse events will be summarised by PT (Preferred term) and SOC (System organ class) using Medical Dictionary for Regulatory Activities (MedDRA) vocabulary. Furthermore, listings of serious adverse events and adverse events that led to withdrawal will be made and the number of subjects who had any adverse events, serious adverse events, adverse events that led to withdrawal, and adverse events with severe intensity will be summarised.

ECG parameters will be summarised for the absolute value at each scheduled assessment, together with the corresponding changes from the pre-dose value. The QT correction factor will be based on the Fridericia's formula. Further categorical summaries of absolute QT and QTcF values (> 450 ms, > 480 ms, > 500 ms) and change from pre-dose values in QT and QTcF values (> 30 ms, > 60 ms) may also be produced.

12.2.4 Pharmacokinetics

The PK tables and data listings and individual PK figures as well as the statistical analysis of the PK variables will be the responsibility of the study biostatistician at (using SAS® version 9.1 or higher and, where appropriate, additional validated software).

A listing of PK blood sample collection times by individual, as well as derived sampling time deviations will be provided. A subject listing of all plasma AZD7687 concentration-time data for each dose will be presented. Individual urine sample collection times, AZD7687 urine concentrations and amounts will be listed by dose.

PK parameters for plasma and urine will be summarised by appropriate descriptive statistics such as n, mean, SD, geometric mean, coefficient of variation (CV%), geometric CV (%GCV), minimum, median and maximum. Graphical presentations will be used when appropriate.

The geometric mean is calculated as the exponential of the arithmetic mean calculated from data on a log scale. The %GCV is calculated as $100 \cdot \sqrt{\left(\exp\left(s^2\right)-1\right)}$ where s is the standard deviation of the data on a log scale.

LLOQ values of plasma concentrations will be handled as follows:

- If, at a given time point, 50% or less of the plasma concentrations are <LLOQ, the mean, geometric mean, SD, and CV% will be calculated by substituting the lower limit of quantification (LLOQ) for values which are <LLOQ.
- If more than 50%, but not all, of the concentrations are <LLOQ, the mean, geometric mean, SD, and CV% will be reported as not calculable (NC).

- If all the concentrations are <LLOQ, the geometric mean and mean will be reported as <LLOQ, and the SD and CV% as NC

global SOPs will be followed for the rounding conventions.

Dose proportionality will be analysed for AZD7687 of AUC, $AUC_{(0-t)}$ and C_{max} as the dependent variable and the logarithm of the dose as the independent variable. The intercept α and the slope β (in [AUC, $AUC_{(0-t)}$ or C_{max}]=α*dose$^{\beta}$) together with confidence intervals (2-sided 90%) will be estimated and presented for AUC, $AUC_{(0-t)}$ and C_{max}.

Similarly, dose proportionality will be analysed following multiple dose regimen using the power model approach with the logarithm of $AUC_{(0-\tau),ss}$, and $C_{max,ss}$ as the dependent variable and the logarithm of the dose as the independent variable. The intercept α and the slope β (in [$AUC_{(0-\tau),ss}$, and $C_{max,\ ss}$]=α*dose$^{\beta}$) together with confidence intervals (2-sided 90%) will be estimated and presented for $AUC_{(0-\tau)}$and $C_{max,ss}$

The time dependency of the PK will be evaluated by comparing $AUC_{(0-\tau),ss}$ (Day 9) with AUC (Day 1). For each dose level, a linear mixed-effect analysis of variance (ANOVA) model using the logarithm of AUC_{τ} (AUC) as the response variable and day as a fixed effect will be used. Transformed back from the logarithm scale, true geometric means will be estimated and the ratios of true geometric means together with confidence intervals (2-sided 90%) $AUC_{(0-\tau),ss}$ /AUC will be estimated and presented. From this model, the ratios of accumulation $AUC_{(0-\tau),ss}$ / $AUC_{(0-\tau),}$ and $C_{max,ss}/C_{max}$ will also be estimated by calculating ratios of the geometric least square means and will be presented with confidence intervals. Since these analyses are of an exploratory nature, no formal conclusions will be drawn.

Steady state condition will be assessed graphically.

12.2.5 Pharmacodynamics

The PD tables and data listings as well as the statistical analysis of PD variables will be the responsibility of the study biostatistician at (using SAS® version 9.1 or higher and, where appropriate, additional validated software).

All PD endpoints will be summarized by treatment. Placebo subjects from all dose groups will be combined into a single treatment.

A listing of PD blood sample collection times by individual, as well as derived sampling time deviations will be provided. The serum TAG_{total}, plasma $TAG_{refined,}$ and DAG concentrations as well as $TAG_{refined}$/DAG ratios will be summarized using descriptive statistics by dose. The derived variable, incremental serum TAG_{total} AUC, will be listed and summarized using appropriate descriptive statistics.

In addition the TAG and DAG concentrations in the adipose tissue and TAG/DAG ratio in the adipose tissue (including measurements after direct incubation) will be listed and summarized by dose.

For the PD variables of primary interest (the inhibitory effect on TAG/DAG in adipose tissue and the relative change in incremental serum TAG_{total} AUC), each dose will be compared with placebo using 95% confidence intervals by parametric or non-parametric methods. The analysis will be on the original scale or after a suitable transformation, depending on the distribution of the actual data. The results will also be presented as descriptive statistics.

For all other PD variables, the primary analysis will be descriptive, using suitable statistics and graphical presentations of mean values and individual values over time, where appropriate.

12.2.6 Pharmacokinetic - pharmacodynamic correlations

Scatterplot to evaluate the correlation between the inhibitory effect on TAG/DAG in adipose tissue (as described in Section 11.3) and AZD7687 plasma concentration at time of biopsy Day 9.

Scatterplot to evaluate the correlation between the percentage change from baseline in incremental serum TAG_{total} AUC (as described in Section 11.3) and AZD7687 plasma concentration pre-meal.

12.2.7 Exploratory biomarkers

12.2.7.1 Weight and waist circumference

Individual observed and change from baseline weight will be listed and summarised by study day and dose. Change from baseline weights versus study day will be graphically displayed by dose. Individual observed and change from baseline waist circumference will be listed and summarised by dose. Placebo subjects will be combined into a single group for this analysis.

12.2.7.2 Gastrointestinal peptides (total GLP-1, GIP, PYY3-36)

A listing of exploratory blood sample collection times by individual, as well as derived sampling time deviations will be provided. The exploratory biomarker concentrations and their change from baseline values (PYY 3-36, GIP and total GLP-1) will be listed and summarised by study day and dose wherever applicable. Graphical presentations will be made where appropriate.

12.2.7.3 ApoB48

A listing of exploratory blood sample collection times by individual, as well as derived sampling time deviations will be provided. The ApoB48 concentrations and its change form baseline results will be listed and summarised by study day and dose wherever applicable. Graphical presentations will be made where appropriate.

12.2.7.4 Leptin and adiponectin

A listing of exploratory blood sample collection times by individual, as well as derived sampling time deviations will be provided. The exploratory biomarker concentrations (leptin

and adiponectin) and their change form baseline results will be listed and summarised by study day and dose wherever applicable.

The data from all the exploratory biomarkers may be presented in the CSR or in a separate report.

12.2.7.5 Weight and waist circumference

A listing of paracetamol blood sampling times by individual will be provided. Subject listings of all plasma paracetamol concentration time data for each dose and sampling occasion will be presented. Plasma concentration versus time data will be presented graphically. Descriptive statistics will be presented for C_{max}, t_{max} and $AUC_{(0\text{-}t)}$.

12.3 Determination of sample size

Due to the exploratory nature of the study the sample size is not based on formal statistical considerations. The sample size is based on experience from previous similar Phase I studies with other compounds.

13. IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

13.1 Medical emergencies and AstraZeneca contacts

The Principal Investigator is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. **A medical emergency usually constitutes an SAE and is to be reported as such, see Section 6.3.4**

In the case of a medical emergency the investigator may contact the CPA Physician. If the CPA Physician is not available, the CPA Programme at AstraZeneca Research and Development may be contacted.

Name	Role in the study	Address & telephone numbers
	AZ CPA Physician	
	AZ CPA Programme Director	

Name	Role in the study	Address & telephone numbers
	Principal Investigator	
	Project Manager	

13.2 Overdose

There are no human data on overdosing since only single doses of AZD7687 have been given to humans previously. There is no known antidote to AZD7687.

In the event of an overdose the subject should be monitored closely and treated symptomatically.

13.3 Pregnancy

All pregnancies in the partners of subjects in this study and their subsequent outcome (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should be reported to AstraZeneca using the appropriate forms.

13.3.1 Maternal exposure

Women of childbearing potential are not allowed to be included in this study.

13.3.2 Paternal exposure

Pregnancy of a subject's partner is not considered to be an adverse event. However, any conception occurring from the date of dosing until three months after dosing should be reported to AstraZeneca and followed up for its outcome.

14. LIST OF REFERENCES (NOT APPLICABLE)

Clinical Study Protocol Appendix A

Drug Substance	AZD7687
Study Code	D2710C00002
Edition Number	1

Protocol

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

This Clinical Study Protocol has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol.

AstraZeneca Research and Developmer
site representative

This document contains confidential information, which should not be copied, referred to, released or published without written approval from AstraZeneca. Investigators are cautioned that the information in this protocol may be subject to change and revision.

ASTRAZENECA SIGNATURE(S)

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

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AstraZeneca Research and Development site representativ

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SIGNATURE OF PRINCIPAL INVESTIGATOR

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

---

I agree to the terms of this study protocol. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations and I ensure that all relevant site staff follows the instructions given in the latest version of the Laboratory Manual for Investigators.

Signature:

This document contains confidential information, which should not be copied, referred to, released or published without written approval from AstraZeneca. Investigators are cautioned that the information in this protocol may be subject to change and revision.

Clinical Study Protocol Appendix B

Drug Substance	AZD7687
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Appendix B
Additional Safety Information

FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

Life threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (eg, hepatitis that resolved without hepatic failure).

Hospitalisation

Outpatient treatment in an emergency room is not in itself a serious AE, although the reasons for it may be (eg, bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in situations where important medical events may not be immediately life threatening or result in death, hospitalisation, disability or incapacity but may jeopardize the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious.

Simply stopping the suspect drug does not mean that it is an important medical event; medical judgement must be used.

Examples of such events are:

- Angioedema not severe enough to require intubation but requiring iv hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (eg, neutropenia or anaemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse.

A GUIDE TO INTERPRETING THE CAUSALITY QUESTION

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the drug.

- Time Course. Exposure to suspect drug. Has the subject actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of the suspect drug?

- Consistency with known drug profile. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?

- Dechallenge experience. Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?

- No alternative cause. The AE cannot be reasonably explained by another aetiology such as the underlying disease, other drugs, other host or environmental factors.

- Rechallenge experience. Did the AE reoccur if the suspected drug was reintroduced after having been stopped? AstraZeneca would not normally recommend or support a rechallenge.

- Laboratory tests. A specific laboratory investigation (if performed) has confirmed the relationship?

A "reasonable possibility" could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a "reasonable possibility" of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?

- Is there a known mechanism?

Ambiguous cases should be considered as being a "reasonable possibility" of a causal relationship unless further evidence becomes available to refute this. Causal relationship in cases where the disease under study has deteriorated due to lack of effect should be classified as no reasonable possibility.

Clinical Study Protocol Appendix C

Drug Substance	AZD7687
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Appendix C
International Airline Transportation Association (IATA) 6.2 Guidance Document

LABELLING AND SHIPMENT OF BIOHAZARD SAMPLES

International Airline Transportation Association (IATA) classifies biohazardous agents into 3 categories **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm).** For transport purposes the classification of infectious substances according to risk groups was removed from the Dangerous Goods Regulations (DGR) in the 46th edition (2005). Infectious substances are now classified either as Category A, Category B or Exempt. There is no direct relationship between Risk Groups and categories A and B.

Category A Infectious Substances are infectious substances in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Category A pathogens are eg, Ebola, Lassa fever virus:

- are to be packed and shipped in accordance with IATA Instruction 602.

Category B Infectious Substances are infectious Substances that do not meet the criteria for inclusion in Category A. Category B pathogens are eg, Hepatitis A, B, C, D, and E viruses, Human immunodeficiency virus (HIV) types 1 and 2. They are assigned the following UN number and proper shipping name:

- UN 3373 – Biological Substance, Category B
- are to be packed in accordance with UN3373 and IATA 650

Exempt - all other materials with minimal risk of containing pathogens

- Clinical trial samples will fall into Category B or exempt under IATA regulations
- Clinical trial samples will routinely be packed and transported at ambient temperature in IATA 650 compliant packaging **(http://www.iata.org/whatwedo/cargo/dangerous_goods/infectious_substances.htm)**
- **Biological samples transported in dry ice require additional dangerous goods specification for the dry-ice content**
- IATA compliant courier and packaging materials should be used for packing and transportation and packing should be done by an IATA certified person, as applicable

- Samples routinely transported by road or rail are subject to local regulations which require that they are also packed and transported in a safe and appropriate way to contain any risk of infection or contamination by using approved couriers and packaging / containment materials at all times. The IATA 650 biological sample containment standards are encouraged wherever possible when road or rail transport is used.

AstraZeneca

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Appendix D
Pharmacogenetics Research

TABLE OF CONTENTS PAGE

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or special term	Explanation
CSR	Clinical Study Report
DNA	Deoxyribonucleic acid
LIMS	Laboratory information management system
PGx	Pharmacogenetics

1. BACKGROUND AND RATIONALE

AstraZeneca intends to perform genetic research in the AZD7687 clinical development programme to explore how genetic variations may affect the clinical parameters associated with AZD7687. Collection of DNA samples from populations with well described clinical characteristics may lead to improvements in the design and interpretation of clinical trials and, possibly, to genetically guided treatment strategies.

It is emphasised that AstraZeneca will only look for markers within genes relevant to the mode of action of, and response to AZD7687 under study within the current Clinical Study Protocol. No other research will be performed on the samples.

2. GENETIC RESEARCH OBJECTIVES

The objective of this research is to collect and store DNA for future exploratory research into genes/genetic variation that may influence response (ie, distribution, safety, tolerability and efficacy) to AZD7687.

3. GENETIC RESEARCH PLAN AND PROCEDURES

3.1 Selection of genetic research population

3.1.1 Study selection record

All subjects will be asked to participate in this genetic research. Participation is voluntary and if a subject declines to participate there will be no penalty or loss of benefit. The subject will not be excluded from any aspect of the main study.

3.1.2 Inclusion criteria

For inclusion in this genetic research, subjects must fulfil all of the inclusion criteria described in the main body of the Clinical Study Protocol **and**:

- Provide informed consent for the genetic sampling and analyses.

3.1.3 Exclusion criteria

Exclusion from this genetic research may be for any of the exclusion criteria specified in the main study or any of the following:

- Previous allogeneic bone marrow transplant
- Non-leukocyte depleted whole blood transfusion in 120 days of genetic sample collection

3.1.4 Discontinuation of subjects from this genetic research

Specific reasons for discontinuing a subject from this genetic research are:

Withdrawal of consent for genetic research: Subjects may withdraw from this genetic research at any time, independent of any decision concerning participation in other aspects of the main study. Voluntary discontinuation will not prejudice further treatment. Procedures for discontinuation are outlined in Section 7.5 of the main Clinical Study Protocol.

3.2 Collection of samples for genetic research

The blood sample for genetic research will be obtained from the subjects at Visit 2 after randomisation. Although genotype is a stable parameter, early sample collection is preferred to avoid introducing bias through excluding patients who may withdraw due to an adverse event (AE), such patients would be important to include in any genetic analysis. If for any reason the sample is not drawn at Visit 2, it may be taken at any visit until the last study visit. Only one sample should be collected per subject for genetics during the study. Samples will be collected, labelled, stored and shipped as detailed in the Laboratory Manual.

For blood volume, see Section 7.1 of the Clinical Study Protocol.

3.3 Coding and storage of DNA samples

The processes adopted for the coding and storage of samples for genetic analysis are important to maintain subject confidentiality. Samples will be stored for a maximum of 25 years, from the date of last subject last visit, after which they will be destroyed. DNA is a finite resource that is used up during analyses. Samples will be stored and used until no further analyses are possible or the maximum storage time has been reached.

For all samples irrespective of the type of coding used the DNA will be extracted from the blood sample. The DNA sample will be assigned a unique number replacing the information on the sample tube. Thereafter, the DNA sample will be identifiable by the unique DNA number only. The DNA number is used to identify the sample and corresponding data at the AstraZeneca genetics laboratories, or at the designated contract laboratory. No personal details identifying the individual will be available to any person (AstraZeneca employee or contract laboratory staff working with the DNA.)

The samples and data for genetic analysis in this study will be single coded. The link between the subject enrolment/randomisation code and the DNA number will be maintained and stored in a secure environment, with restricted access WITHIN the Clinical Genotyping Group Laboratory Information Management System (LIMS) at AstraZeneca. The link will be used to identify the relevant DNA samples for analysis, facilitate correlation of genotypic results with clinical data, allow regulatory audit and to trace samples for destruction in the case of withdrawal of consent.

4. ETHICAL AND REGULATORY REQUIREMENTS

The principles for ethical and regulatory requirements for the study, including this genetics research component, are outlined in Section 8 of the main Clinical Study Protocol.

4.1 Informed consent

The genetic component of this study is optional and the subject may participate in other components of the main study without participating in the genetic component. To participate in the genetic component of the study the subject must sign and date both the consent form for the main study and the genetic component of the study. Copies of both signed and dated consent forms must be given to the subject and the original filed at the study centre. The principal investigator(s) is responsible for ensuring that consent is given freely and that the subject understands that they may freely discontinue from the genetic aspect of the study at any time.

4.2 Subject data protection

AstraZeneca will not provide individual genotype results to subjects, any insurance company, any employer, their family members, general physician or any other third party, unless required to do so by law.

Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a subject. For example, in the case of a medical emergency, an AstraZeneca Physician or an investigator might know a subject's identity and also have access to his or her genetic data. Also Regulatory authorities may require access to the relevant files, though the subject's medical information and the genetic files would remain physically separate.

5. DATA MANAGEMENT

Any genotype data generated in this study will be stored in the AstraZeneca genotyping LIMS database, or other appropriate secure system within AstraZeneca and/or third party contracted to work with AstraZeneca to analyze the samples.

The results from this genetic research may be reported in the CSR for the main study, or in a separate report as appropriate.

Some or all of the clinical datasets from the main study may be merged with the genetic data in a suitable secure environment separate from the clinical database.

6. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The number of subjects that will agree to participate in the genetic research is unknown. It is therefore not possible to establish whether sufficient data will be collected to allow a formal statistical evaluation or whether only descriptive statistics will be generated. A statistical analysis plan will be prepared where appropriate.

7. LIST OF REFERENCES (NOT APPLICABLE)

Clinical Study Protocol Appendix E

Drug Substance	AZD7687
Study Code	D2710C00002
Edition Number	1

Appendix E
Standardised mixed meal

1. PROTOCOL FOR THE SERVING AND CONSUMPTION OF FOOD AND DRINK

The composition of the standardised mixed meal (SMM) may be changed in the case of averse events based on SAD and MAD results.

The standardised mixed meal (SMM) should be prepared according to the method of Karpe (Karpe. Effects of diet on postprandial lipaemia: a suggestion for methodological standardization. Nutr Metab Cardiovasc Dis 1997, 7;44-55). It consists of pasta, boiled chicken breast, green peas and mayonnaise, and should be served with a liquid containing up to 17 g of carbohydrates. The energy content of the SMM is 1000 kcal for an individual of less than 85 kg and 1200 kcal for a person with a body weight of more than 85 kg. Fat, CHO and protein contributes to 60%, 27% and 13% of the total energy content, respectively. The nutrient composition of the 1000 kcal and 1200 kcal meals was 68 and 82 g of fat, 66 and 79 g of CHO and 33 and 37 g of protein respectively. The meal should be consumed within 15 minutes

1.1 Serving Pasta Salad

Utensils needed when serving:

Disposable plate, disposable fork, disposable knife, disposable teaspoon, serviette, disposable glass, rubber scraper and plastic jug.

1. Put all the ingredients (except for the mayonnaise) that are needed for the specified energy level on a disposable plate. If the patient prefers, mix the ingredients together on the plate. The meal is served cold but can be heated (not the mayonnaise). The food can be seasoned with herbs without salt, (e.g. tarragon, basil, dried pizza herbs).

2. Tell the patient that **the mayonnaise is not to be mixed with the pasta salad**, but must be added as the salad is eaten, as the **mayonnaise is the ingredient which must always be eaten**. Point out the importance of using the rubber scraper to get all the mayonnaise out of the jar.

3. Drink: 17 g carbohydrates are allowed with the meal. This is equivalent to **150 to 200 mL frozen or pasteurised ready-to-drink orange juice or apple juice or just under 150 mL fruit drink**. In addition, the patient may consume energy-free drinks such as water or drinks sweetened with aspartame or xylitol. A total of no more than 600 mL drink is allowed. NB: Do not forget to note down the type and amount of energy free drinks consumed. The patient is to consume the same amount of drink at the second meal as at the first.

4. Tell the patient that the meal must be **finished within 15 minutes**. Even if the patient cannot manage to eat all the pasta salad, **it is still necessary that all the mayonnaise is consumed**. Any food that has not been eaten should be weighed

directly, the ingredients in the pasta salad and the mayonnaise being weighed separately. The weights should be entered in the protocol in whole grams. NB: Always use the same scale when weighing any leftover food.

1.2 Preparation of Pasta Salad

1. Calculate the amount of ingredients needed for the study subjects in question, and add **1 extra portion** of each ingredient to cover waste. See separate recipe.

2. Order the ingredients from your kitchen or purchase them in a shop.

3. Defrost the amount of chicken that will be needed for the coming week's study subjects, plus a little extra. Cut away any excess fat or tendons.

4. Weigh the exact amount of chicken for the energy levels which have been determined for each subject. **Make sure that you label the pans in which the chicken is to be prepared if the study subjects have different energy levels.**

5. Cook the chicken fillets either in the oven or in a shallow cooking pot on the stove.

   - **In the oven**: put the chicken fillets in a baking dish. Season lightly with salt and pepper. Cover and bake (200 degrees) for **approx. 15 min** or until you get a clear juice when pricking meat for doneness.

   - **On the stove**: put the chicken fillets in a shallow pan. Season lightly with salt and pepper. Add enough water to just cover the chicken. Bring to the boil, lower the heat, cover and let simmer until the chicken feels done when tested; **approx. 5 min**. Prick the skin to see that the juice runs clear.

6. Let the chicken cool. Dice each portion and store the portions individually. **Label the containers with the energy level contained in each portion and the weight after cooking** (in case leftover chicken must be weighed at the laboratory after the meal).

7. In the same way measure the appropriate amount of pasta for each subject. **Label the pots if the portions to be boiled at the same time contain different energy levels**. Boil the pasta for the time specified on the package and then rinse in cold running water. Weigh the pasta and divide it into the number of portions which were boiled and into different energy levels, when appropriate. **Package the portions individually and label them in the same way as the chicken**.

8. The ingredients for the mayonnaise should be at **room temperature**. Calculate the amount of mayonnaise needed for all the study subjects (see separate recipe) and **one extra portion** (to cover waste in the jar, etc.). The recipe can be easily doubled (the mayonnaise can be kept in the refrigerator for 3 to 4 weeks). Place the bowl of the food processor on the scale and set to zero. **Weigh out the egg yolk, vinegar and mustard** separately according to the required weights. **NB: <u>Do not forget to</u>**

set the scale to zero after each ingredient is weighed. Place **a jug with a spout** on the scale and set to zero. Weigh the **oil according the calculated weight plus a few extra grams, which must be recorded**. This is done so that the exact amount of oil is added to the mayonnaise. The easiest way to do this is to put the jug back on the scale now and then to see how much oil has been added. Thus, the extra amount of oil will be left in the jug when the mayonnaise is ready. Turn on the food processor at the highest speed and add the oil, first as a fine stream, and then increasing gradually.

9. Weigh out the different sized portions and package them individually. **Make sure to mark the different energy levels they represent and the weight of each portion**. (Please note the weight of the container which the mayonnaise is served in to make it easier for the lab to weigh any leftovers).

10. Calculate the amount of peas needed for the coming week's study subjects. **Label the pots or containers if different sizes portions are to be cooked**. Boil the peas according to the directions, either in a pot or in a microwave oven and then rinse them in cold running water. Next, weight the peas and divide them into the number of portions prepared, and, if applicable, according to different energy levels. **Package the portions individually and label the energy levels and the weight after cooking.**

11. Re: **drink to be served with the pasta salad**: the drink **is to be delivered in a ready- to-drink form** at the same time as the pasta salad and is to be **labelled with weight only** (the same volume is to be used for all energy levels).

12. At meal time, the ingredients for the **pasta salad** are to be delivered separately, but they **can be mixed on the plate before eating**, if desired. **However, the mayonnaise, which is always served in its container, is not to be mixed with the pasta salad.**

1.2.1 Preparation Time

These times are calculated on 2 study patients/occasion and 2 occasions/week

Chicken: removing skin, trimming, weighing and placing in labelled pan (s) **approximately 15 min.**

Cooking time: **max 15 min** regardless of cooking method

Dicing cooled chicken and packaging: **approximately 30 min**

Pasta: weighing and any labelling of pots **approximately 15 min.**

Cooking time: **approximately 15 min** including rinsing.

Weighing out the pasta portions and packaging: **approximately 15 min.**

Peas: weighing and any labelling of pots **approximately 15 min.**

Cooking time: **approximately 15 min** including rinsing and packaging portions.

Mayonnaise: weighing out all ingredients **approximately 30 min.**

Preparation time: **approximately 30 min** including weighing out and packaging portions.

At serving time: laying disposable utensils, putting pasta salad in serving dishes and mixing the drink **approximately 15 min/2 study patients**

TOTAL PREPARATION TIME: approximately 3.5 hours

1.2.2 Recipes

Meal Recipe

	1000 kcal	**1200 kcal**
Chicken fillet; without skin	85 g	102 g
Pasta Penne	88	106
Peas, frozen	35	42
Yolk	7	8.4
Wine-vinegar, white	3	4
Mustard, sweat	1	1
Rape-oil	64	76.8

Mayonnaise Recipe (300 g)

Yolk	26 g
Wine-vinegar, white	13 g
Mustard, sweet	5 g
Rape-oil	225 g

1 portion = 75 g (1000 kcal energy level). At 1200 kcal energy level 1.2 x 75 g = 90 g.

Please observe that when cooking the last portion will be a “waste-portion” due to waste in container and tools.

Clinical Study Protocol Amendment	
Amendment Number	1
Drug Substance	AZD7687
Study Code	D2710C00002
Date	
Protocol	

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

This submission /document contains trade secrets and confidential commercial information, disclosure of which is prohibited without providing advance notice to AstraZeneca and opportunity to object.

Sponsor:

AstraZeneca AB, 151 85 Södertälje, Sweden.

Centres affected by the Amendment:

Quintiles Drug Research Unit

The protocol for the study is to be amended as follows:

Section of protocol affected:

List of Appendices

Previous text:

LIST OF APPENDICES

Appendix A Signatures

Appendix B Additional Safety Information

Appendix C IATA 6.2 Guidance document

Appendix D Pharmacogenetic Research

Appendix E Standardised Mixed Meal

Revised text:

Appendix A Signatures

Appendix B Additional Safety Information

Appendix C IATA 6.2 Guidance document

Appendix D Pharmacogenetic Research

Section of protocol affected:

Section 3.1 Overall study design and flow chart

Previous text:

Visit 2

At Visit 2, subjects will arrive at the clinic in the morning of Day -3. An evening meal will be served in order to keep standardised conditions. Prior to intended dosing on Day 1, baseline PD measurements will be performed on Day -2 and Day -1. Weight will be recorded and waist circumference will be measured. A standardised meal (standardised mixed meal [SMM], see Appendix E) will be served, followed by repeated blood sampling to measure postprandial lipid levels and gastrointestinal peptides. Paracetamol challenge will be done. In addition, an adipose tissue biopsy will be taken.

Revised text:

At Visit 2, subjects will arrive at the clinic in the morning of Day -3. An evening meal will be served in order to keep standardised conditions. Prior to intended dosing on Day 1, baseline PD measurements will be performed on Day -2 and Day -1. Weight will be recorded and waist circumference will be measured. A standardised meal (standardised mixed meal [SMM]) containing between 30 and 60 % fat (the exact composition could vary between the cohorts) will be served, followed by repeated blood sampling to measure postprandial lipid levels and gastrointestinal peptides. Paracetamol challenge will be done. In addition, an adipose tissue biopsy will be taken.

Section of protocol affected:

Section 3.1 Overall study design and flow chart

Following review of data from each panel of subjects, or from the SAD study, assessments may be omitted, the timing of assessments and/or blood samples and meals may be adjusted, the composition of the SMM may be replaced and the length of washout period between single and multiple dosing and dosing regimen may be adjusted (once or twice daily dosing). Fasting periods might be moved in case measurements requiring a fasting condition are moved. Additional assessments, visits or sampling times may be added if indicated by the data however the maximum blood volume taken from each subject will not exceed 600 mL. One extra adipose tissue biopsy may be added.

Revised text:

Following review of data from each panel of subjects, or from the SAD study, assessments may be omitted, the timing of assessments and/or blood samples and meals may be adjusted, the composition of the SMM (containing between 30 and 60 % fat) may be replaced and the length of washout period between single and multiple dosing and dosing regimen may be adjusted (once or twice daily dosing). Fasting periods might be moved in case measurements requiring a fasting condition are moved. Additional assessments, visits or sampling times may be added if indicated

by the data however the maximum blood volume taken from each subject will not exceed 700 mL. One extra adipose tissue biopsy may be added.

Section of protocol affected:

Section 3.1, Table 1, Table 2 and Table 3.

Previous text:

Table 1 Study Plan

Visit number	**1**	**2**														**3**
	Screening Period	**Residential Period**														**Follow-up**
Part		**Baseline PD measurements**			**Part A**		**Part B**									
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Informed consent	X															
Demography	X															
Medical/surgical history	X															
Inclusion/exclusion criteria	X	X														
Physical examination[a]	X	X[b]													X[b]	X
Randomisation		X														
Administer study drug					X		X	X	X	X	X	X	X			
Weight	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X															
Waist circumference	X		X												X	
Vital signs[c]	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Paper ECG (pECG)	X				X	X					X	X	X	X		X
Digital ECG (dECG)[d]					X	X	X						X			
Telemetry					X	X	X	X	X	X	X	X	X	X	X	
Drugs of abuse screen	X	X														
PK blood sampling					X	X	X					X	X	X		
PK urine collection					X	X							X			
Exploratory blood sampling					X									X		

Visit number	**1**	**2**													**3**
	Screening Period	**Residential Period**													**Follow-up**
Part		**Baseline PD measurements**			**Part A**		**Part B**								
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Safety laboratory tests[e]	X	X		X[f]	X	X	X	X[f]		X		X[f]	X[f]	X		X
Standardised mixed meal			X				X					X				
Paracetamol challenge			X				X					X				
TAG_{total}, $TAG_{refined}$ and DAG blood sampling			X	X			X	X				X	X			
Insulin blood sampling			X		X	X	X			X	X					
Total GLP-1, GIP and PYY3-36 blood sampling			X				X					X				
ACTH and cortisol blood sampling				X[g]							X[g]			X[g]		X[g]
Renin, aldosterone and DHEA-S blood sampling					X						X			X		
Insulin, glucose, ketones, lactate and BNP blood sampling					X									X		
ApoB48, FFA and hsCRP blood sampling			X	X			X	X				X	X			
Leptin and adiponectin blood sampling					X	X								X		
Fat content faecal sampling		X----------X										X----------X				
Genetic blood sampling		X[h]														
Adipose tissue biopsy				X									X			
Adverse events[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

a Including special attention to dry skin, skin inflammation (especially face) and hair loss.
b Updates to physical examination will be recorded at admission and discharge.
c Vital signs include blood pressure, pulse and body temperature.
d Detailed information regarding the timing of dECGs is provided in Table 3.

e Blood and urine sampling for haematology, clinical chemistry (including coagulation, lipid panel and liver chemistry, see Section 6.3.5) and urinalysis parameters.
f Only liver chemistry tests, See Section 6.3.5 for definition.
g It is important that the samples are taken at the same time each morning and evening, if applicable. The samples for cortisol and ACTH should be obtained at a time point where reference values for cortisol and ACTH exist, if possible.
h The genetic sampling can be done at any day during Visit 2 and 3.
i SAEs will be recorded from signing of informed consent, non-serious AEs will be recorded from randomisation .

Table 2 Time Schedule During Visit 2

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
-3	Morning	Clinical chemistry[a]/haematology Genetic sampling[d]		Urinalysis Drugs of abuse and alcohol screen	Sample D-3–D-1 start		Vital signs	Admission to the clinic
-2	00:00[e]	FFA Glucose Insulin TAG/TAG_{ref}/DAG ApoB48 PYY3-36						Weight recorded. Waist circumference measured.
-2	00:30	hsCRP TAG/TAG_{ref}/DAG						
-2	01:00 pre-SMM	FFA GLP-1/GIP PYY3-36 ApoB48 Glucose Insulin TAG/TAG_{ref}/DAG						
-2	01:00	Paracetamol sample						SMM start
-2	01:15							SMM finish Paracetamol administered
-2	01:30	FFA GLP-1/GIP PYY3-36 Paracetamol sample						
-2	01:45	Paracetamol sample						
-2	02:00	FFA GLP-1/GIP Insulin PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	02:20	Paracetamol sample						
-2	02:40	Paracetamol sample						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
-2	03:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	05:00	ApoB48 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	06:00	FFA Glucose Insulin TAG/TAG_{ref}/DAG Paracetamol sample						
-2	07:00	ApoB48 GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	08:00	TAG/TAG_{ref}/DAG						
-2	09:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-1	00:00[e]	Liver chemistry ACTH/cortisol[f] ApoB48 TAG/TAG_{ref}/DAG			Sample D-3–D-1 end			Weight recorded
-1	04:00					Adipose biopsy		
-1	12:00	ACTH/cortisol[f]						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
1	Predose	PK-1 Aldosterone/DHEA-S[g] BNP Clinical chemistry[a]/haematology Insulin Ketones Lactate Leptin/adiponectin Renin[g] Exploratory blood sample		Urinalysis PK-urine predose			dECG[h] pECG Telemetry start Vital signs	Weight recorded
1	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start				Empty bladder
1	00:15						dECG start	
1	00:20	PK-2					dECG stop	
1	00:35						dECG start	
1	00:40	PK-3					dECG stop	
1	00:55						dECG start	
1	01:00	PK-4					dECG stop pECG Vital signs	
1	01:15						dECG start	
1	01:20	PK-5					dECG stop	
1	01:35						dECG start	
1	01:40	PK-6					dECG stop	
1	01:55						dECG start	
1	02:00	PK-7					dECG stop Vital signs	
1	02:55						dECG start	
1	03:00	PK-8					dECG stop Vital signs	
1	03:55						dECG start	
1	04:00	PK-9		PK-urine 0-4 h end PK urine 4-8 start			dECG stop	
1	05:00							
1	05:55						dECG start	
1	06:00	PK-10					dECG stop pECG	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
							Vital signs	
1	07:55						dECG start	
1	08:00	PK-11		PK-urine 4-8 h end PK-urine 8-12 h start			dECG stop	
1	11:55						dECG start	
1	12:00	PK-12		PK-urine 8-12 h end PK-urine 12-24 h start			dECG stop Vital signs	
1	18:00	PK-13						
1	23:55						dECG start	
1	24:00	PK-14		PK-urine 12-24 h end PK-urine 24-48 h start			dECG stop pECG Vital signs	
2	Predose	Leptin/adiponectin						
2	00:00[e]	Clinical chemistry[a]/haematology Insulin		Urinalysis				Weight recorded
2	24:00	PK-15		PK-urine 24-48 h end				
3	Predose	Clinical chemistry[a]/haematology FFA Glucose Insulin TAG/TAG_{ref}/DAG		Urinalysis			Vital signs dECG[h]	Weight recorded
3	00:00[e]		AZD7687/placebo					
3	00:30	hsCRP TAG/TAG_{ref}/DAG						
3	01:00 pre-SMM	PK-16 ApoB48 GLP-1/GIP PYY3-36 FFA Insulin TAG/TAG_{ref}/DAG						
		ApoB48						
3	01:00	Paracetamol sample						SMM start
3	01:15							SMM end

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
								Paracetamol administered
3	01:30	FFA GLP-1/GIP PYY3-36 Paracetamol sample						
3	01:45	Paracetamol sample						
3	02:00	FFA GLP-1/GIP Insulin PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
3	02:20	Paracetamol sample						
3	02:40	Paracetamol sample						
3	03:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
3	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
3	05:00	ApoB48 TAG/TAG_{ref}/DAG Paracetamol sample						
3	06:00	FFA Insulin Glucose TAG/TAG_{ref}/DAG Paracetamol sample						
3	07:00	ApoB48 GLP-1/GIP PYY3-36						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
		TAG/TAG_{ref}/DAG Paracetamol sample						
3	08:00	TAG/TAG_{ref}/DAG						
3	09:00	PK-17 GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
4	Predose	ApoB48 TAG/TAG_{ref}/DAG Glucose Liver chemistry					Vital signs	Weight recorded
4	00:00		AZD7687/placebo					
5	Predose						Vital signs	Weight recorded
5	00:00[e]		AZD7687/placebo					
6	Predose	Glucose Insulin Clinical chemistry[a]/haematology					Vital signs	Weight recorded
6	00:00[e]		AZD7687/placebo					
7	Predose	ACTH/cortisol[f] Aldosterone/DHEA-S[g] Clinical chemistry[a]/haematology Insulin Renin[g]		Urinalysis			pECG Vital signs	Weight recorded
7	00:00[e]		AZD7687/placebo					
7	12:00	ACTH/cortisol[f]						
8	Predose	FFA Glucose Insulin Liver chemistry TAG/TAG_{ref}/DAG			Sample D8–D10 start		pECG Vital signs	Weight recorded
8	00:00[e]		AZD7687/placebo					
8	00:30	TAG/TAG_{ref}/DAG hsCRP						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
8	01:00 pre-SMM	PK-18 ApoB48 GLP-1/GIP PYY3-36 FFA Glucose Insulin TAG/TAG_{ref}/DAG						
8	01:00	Paracetamol sample						SMM start
8	01:15							SMM end Paracetamol administered
8	01:30	FFA GLP-1/GIP PYY3-36 Paracetamol sample						
8	01:45	Paracetamol sample						
8	02:00	FFA GLP-1/GIP Insulin PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
8	02:20	Paracetamol sample						
8	02:40	Paracetamol sample						
8	03:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
8	04:00	FFA GLP-1/GIP Insulin Glucose PYY3036 TAG/TAG_{ref}/DAG Paracetamol sample						
8	05:00	ApoB48						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
		TAG/TAG_{ref}/DAG Paracetamol sample						
8	06:00	FFA Insulin Glucose TAG/TAG_{ref}/DAG Paracetamol sample						
8	07:00	ApoB48 GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
8	08:00	TAG/TAG_{ref}/DAG						
8	09:00	PK-19 GLP-1/GIP PYY-3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
9	Predose	PK-20 Liver chemistry ApoB48 TAG/TAG_{ref}/DAG			Sample D8–D10 end		dECG[h] pECG Vital signs	Weight recorded
9	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start				Empty bladder
9	00:15						dECG start	
9	00:20	PK-21					dECG end	
9	00:35						dECG start	
9	00:40	PK-22					dECG end	
9	00:55						dECG start	
9	01:00	PK-23					dECG end pECG Vital signs	
9	01:15						dECG start	
9	01:20	PK-24					dECG end	
9	01:35						dECG start	
9	01:40	PK-25					dECG end	
9	01:55						dECG start	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
9	02:00	PK-26					dECG end Vital signs	
9	02:55						dECG start	
9	03:00	PK-27					dECG end Vital signs	
9	03:55						dECG start	
9	04:00	PK-28		PK-urine 0-4 h end PK-urine 4-8 h start		Adipose biopsy	dECG end	
9	05:00							
9	05:55						dECG start	
9	06:00	PK-29					dECG end pECG	
9	07:55						dECG start	
9	08:00	PK-30		PK-urine 4-8 h end PK-urine 8-12 h start			dECG end	
9	11:55						dECG start	
9	12:00	PK-31		PK-urine 8-12 h end PK-urine 12-24 h start			dECG end	
9	18:00	PK-32						
9	23:55						dECG start	
9	24:00	PK-33		PK-urine 12-24 h end			dECG end	
10	00:00[e]	ACTH/cortisol[f] Aldosterone/DHEA-S[g] BNP Clinical chemistry[a]/haematology Insulin Ketones Lactate Leptin/adiponectin Renin[g] Glucose Exploratory blood sample			Collection end		pECG Vital signs	Weight recorded
10	12:00	ACTH/cortisol[f]						
10	24:00	PK-34						
11	00:00[e]						pECG	Weight recorded

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
							Telemetry end Vital signs	Physical examination Waist circumference measured

AZD7687 will be administered at 00:00 on Day 1 and Days 3 to 9.
SMM = standardised mixed meal

[a] Clinical chemistry includes lipid panel and liver chemistry

[b] PK urine collections will be done at the intervals 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h, 24 to 48 h following the single dose on Day 1 and at 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h after the last day of multiple dosing on Day 9.

[c] Faecal collection periods are as follows: Day -3 to Day -1, Day 8 to Day 10.

[d] The genetic sampling can be done at any day during Visits 2 and 3.

[e] The time 00:00 should be the same time each morning throughout the study period.

[f] It is important that the samples are taken at the same time each morning and evening, if applicable. The samples for cortisol and ACTH should be obtained at a time point where reference values for cortisol and ACTH exist, if possible.

[g] The subject should rest in bed for 10 minutes before aldosterone and renin blood sampling.

h Predose dECG recordings on Days 1, 3 and 9 will be 10 minutes long.

Table 3 Study Plan - Time schedule for digital ECG assessments during residential period

Study Days	ECG Number	Start time hour: minute[d]	Dose	Stop time hour: minute	dECG continuous[abc]	Other
1		-01:30		-01:00		Apply the electrodes[b]
		-00:40		-00:30		Rest in bed
	1	-00:30	Predose	-00:20	10 minutes[c]	
		-00:20		-00:05		Toilet use allowed
		00:00	Administration of AZD7687/placebo			
	2	00:15		00:20	5 minutes[c]	
	3	00:35		00:40	5 minutes[c]	
	4	00:55		01:00	5 minutes[c]	
	5	01:15		01:20	5 minutes[c]	
	6	01:35		01:40	5 minutes[c]	
	7	01:55		02:00	5 minutes[c]	
	8	02:55		03:00	5 minutes[c]	
	9	03:55		04:00	5 minutes[c]	
	10	05:55		06:00	5 minutes[c]	
	11	07:55		08:00	5 minutes[c]	
	12	11:55		12:00	5 minutes[c]	
	13	23:55		24:00	5 minutes[c]	
4		-01:30		-01:00		Apply the electrodes[b]
4	14	-00:30	Predose	-00:20	10 minutes[c]	
10		-01:30		-01:00		Apply the electrodes[b]
		-00:40		-00:30		Rest in bed
	15	-00:30	Predose	-00:20	10 minutes	
		-00:20		-00:05		Toilet use allowed
		00:00	Administration of AZD7687/placebo			
	16	00:15		00:20	5 minutes[c]	
	17	00:35		00:40	5 minutes[c]	

Study Days	ECG Number	Start time hour: minute[d]	Dose	Stop time hour: minute	dECG continuous[abc]	Other
	18	00:55		01:00	5 minutes[c]	
	19	01:15		01:20	5 minutes[c]	
	20	01:35		01:40	5 minutes[c]	
	21	01:55		02:00	5 minutes[c]	
	22	02:55		03:00	5 minutes[c]	
	23	03:55		04:00	5 minutes[c]	
	24	05:55		06:00	5 minutes[c]	
	25	07:55		08:00	5 minutes[c]	
	26	11:55		12:00	5 minutes[c]	
	27	23:55		24:00	5 minutes[c]	

a The subject must be in the same supine body position (max. 30 degrees flexion in the hip) at each time point and at all visits. Subject's feet should not contact the footboard of the bed.
b Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied at least 30 minutes before first recording.
c Subject must rest in bed for at least 10 minutes prior to each ECG time point.
d Time points for dECG may be adjusted according to emerging PK data.

Revised text:

Table 1 Study Plan

Visit number	**1**	**2**														**3**
	Screening Period	**Residential Period**														**Follow-up**
Part		**Baseline PD measurements**			**Part A**		**Part B**									
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Informed consent	X															
Demography	X															
Medical/surgical history	X															
Inclusion/exclusion criteria	X	X														
Physical examination[a]	X	X[b]													X[b]	X
Serology	X															
Randomisation		X														
Administer study drug					X		X	X	X	X	X	X	X			
Weight	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X															
Waist circumference	X		X												X	
Vital signs[c]	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Paper ECG (pECG)	X				X	X					X	X	X	X		X
Digital ECG (dECG)[d]					X	X	X						X			
Telemetry					X	X	X	X	X	X	X	X	X	X	X	
Drugs of abuse screen	X	X														
PK blood sampling					X	X	X					X	X	X		
PK urine collection					X	X							X			

Visit number	**1**	**2**														**3**
	Screening Period	**Residential Period**														**Follow-up**
Part		**Baseline PD measurements**			**Part A**		**Part B**									
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Exploratory blood sampling					X									X		
Safety laboratory tests[e]	X[k]	X[k, f]		X[f,j]		X[k, f]		X[f]		X		X[f]		X		X[k]
Standardised mixed meal			X				X					X				
Paracetamol challenge			X				X					X				
TAG_{total}, $TAG_{refined}$ and DAG blood sampling			X				X					X				
Insulin blood sampling			X		X		X				X	X				
Glucose blood sampling			X				X	X				X				
Total GLP-1, GIP and PYY3-36 blood sampling			X				X					X				
ACTH and cortisol blood sampling				X[g]							X[g]			X[g]		X[g]
Renin, aldosterone and DHEA-S blood sampling					X						X			X		
, ketones, lactate and BNP blood sampling					X									X		
ApoB48 blood sampling			X	X			X	X				X	X			
FFA blood sampling			X				X					X				
hsCRP blood sampling					X							X				
Leptin and adiponectin blood sampling					X	X								X		
Fat content faecal sampling		X----------X										X----------X				
Genetic blood sampling		X[h]														
Adipose tissue biopsy				X									X			

Visit number	**1** Screening Period	**2** Residential Period														**3** Follow-up
Part		**Baseline PD measurements**			**Part A**		**Part B**									
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Adverse events[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

a Including special attention to dry skin, skin inflammation (especially face) and hair loss.
b Updates to physical examination will be recorded at admission and discharge.
c Vital signs include blood pressure, pulse and body temperature.
d Detailed information regarding the timing of dECGs is provided in Table 3.
e Blood and urine sampling for haematology, clinical chemistry (including coagulation, lipid panel and liver chemistry, see Section 6.3.5) and urinalysis parameters.
f Liver chemistry tests only, see Section 6.3.5 for definition.
g It is important that the samples are taken at the same time each morning and evening, if applicable. The samples for cortisol and ACTH should be obtained at a time point where reference values for cortisol and ACTH exist, if possible.
h The genetic sampling can be done at any day during Visit 2 and 3.
i SAEs will be recorded from signing of informed consent, non-serious AEs will be recorded from randomisation .
j Coagulation tests only, see Section 6.3.5 for definition.
k Clinical chemistry and haematology only, see Section 6.3.5 for definition.

Table 2 Time Schedule During Visit 2

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
-3	Morning	Clinical chemistry[a]/haematology Genetic sampling[d]		Urinalysis Drugs of abuse and alcohol screen	Sample D-3–D-1 start		Vital signs	Admission to the clinic
-2	00:00[e]	FFA Glucose Insulin $TAG/TAG_{ref}/DAG$ ApoB48 PYY3-36						Weight recorded. Waist circumference measured.
-2	00:30	$TAG/TAG_{ref}/DAG$						
-2	01:00 pre-SMM	FFA GLP-1/GIP PYY3-36 ApoB48 Glucose Insulin $TAG/TAG_{ref}/DAG$						
-2	01:00	Paracetamol sample						SMM start
-2	01:15							SMM finish Paracetamol administered
-2	01:30	FFA GLP-1/GIP PYY3-36 Paracetamol sample						
-2	01:45	Paracetamol sample						
-2	02:00	FFA GLP-1/GIP Insulin PYY3-36 $TAG/TAG_{ref}/DAG$ Paracetamol sample						
-2	02:20	Paracetamol sample						
-2	02:40	Paracetamol sample						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
-2	03:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	05:00	ApoB48 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	06:00	FFA Glucose Insulin TAG/TAG_{ref}/DAG Paracetamol sample						
-2	07:00	ApoB48 GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-2	08:00	TAG/TAG_{ref}/DAG						
-2	09:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
-1	00:00[e]	Liver chemistry ACTH/cortisol[f] ApoB48 Coagulation			Sample D-3–D-1 end			Weight recorded
-1	04:00					Adipose biopsy		
-1	12:00	ACTH/cortisol[f]						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
1	Predose	PK-1 Aldosterone/DHEA-S[g] BNP Insulin Ketones Lactate Leptin/adiponectin Renin[g] Exploratory blood sample hsCRP		Urinalysis PK-urine predose			dECG[h] pECG Telemetry start Vital signs	Weight recorded
1	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start				Empty bladder
1	00:15						dECG start	
1	00:20	PK-2					dECG stop	
1	00:35						dECG start	
1	00:40	PK-3					dECG stop	
1	00:55						dECG start	
1	01:00	PK-4					dECG stop pECG Vital signs	
1	01:15						dECG start	
1	01:20						dECG stop	
1	01:30	PK-5						
1	01:35						dECG start	
1	01:40						dECG stop	
1	01:55						dECG start	
1	02:00	PK-6					dECG stop Vital signs	
1	02:55						dECG start	
1	03:00						dECG stop Vital signs	
1	03:55						dECG start	
1	04:00	PK-7		PK-urine 0-4 h end PK urine 4-8 start			dECG stop	
1	05:00							
1	05:55						dECG start	
1	06:00						dECG stop	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
							pECG Vital signs	
1	07:55						dECG start	
1	08:00	PK-8		PK-urine 4-8 h end PK-urine 8-12 h start			dECG stop	
1	11:55						dECG start	
1	12:00	PK-9		PK-urine 8-12 h end PK-urine 12-24 h start			dECG stop Vital signs	
1	18:00							
1	23:55						dECG start	
1	24:00	PK-10		PK-urine 12-24 h end PK-urine 24-48 h start			dECG stop pECG Vital signs	
2	Predose	Leptin/adiponectin						
2	00:00[e]	Clinical chemistry[a]/haematology Insulin		Urinalysis				Weight recorded
2	24:00	PK-11		PK-urine 24-48 h end				
3	Predose	FFA Glucose Insulin $TAG/TAG_{ref}/DAG$		Urinalysis			Vital signs dECG[h]	Weight recorded
3	00:00[e]		AZD7687/placebo					
3	00:30	$TAG/TAG_{ref}/DAG$						
3	01:00 pre-SMM	PK-12 ApoB48 GLP-1/GIP PYY3-36 FFA Insulin $TAG/TAG_{ref}/DAG$						
								SMM start
3	01:00	Paracetamol sample						
3	01:15							SMM end Paracetamol administered

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
3	01:30	FFA GLP-1/GIP PYY3-36 Paracetamol sample						
3	01:45	Paracetamol sample						
3	02:00	FFA GLP-1/GIP Insulin PYY3-36 $TAG/TAG_{ref}/DAG$ Paracetamol sample						
3	02:20	Paracetamol sample						
3	02:40	Paracetamol sample						
3	03:00	GLP-1/GIP PYY3-36 $TAG/TAG_{ref}/DAG$ Paracetamol sample						
3	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 $TAG/TAG_{ref}/DAG$ Paracetamol sample						
3	05:00	ApoB48 $TAG/TAG_{ref}/DAG$ Paracetamol sample						
3	06:00	FFA Insulin Glucose $TAG/TAG_{ref}/DAG$ Paracetamol sample						
3	07:00	ApoB48 GLP-1/GIP PYY3-36 $TAG/TAG_{ref}/DAG$						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
		Paracetamol sample						
3	08:00	TAG/TAG_{ref}/DAG						
3	09:00	PK-13 GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
4	Predose	ApoB48 Glucose Liver chemistry					Vital signs	Weight recorded
4	00:00		AZD7687/placebo					
5	Predose						Vital signs	Weight recorded
5	00:00[e]		AZD7687/placebo					
6	Predose	Glucose Insulin Clinical chemistry[a]/haematology					Vital signs	Weight recorded
		Coagulation						
6	00:00[e]		AZD7687/placebo					
7	Predose	ACTH/cortisol[f] Aldosterone/DHEA-S[g] Insulin Renin[g]		Urinalysis			pECG Vital signs	Weight recorded
7	00:00[e]		AZD7687/placebo					
7	12:00	ACTH/cortisol[f]						
8	Predose	FFA Glucose Insulin Liver chemistry			Sample D8–D10 start		pECG Vital signs	Weight recorded
8	00:00[e]	PK-14	AZD7687/placebo					
8	00:30	TAG/TAG_{ref}/DAG hsCRP						
8	01:00 pre-SMM	ApoB48 GLP-1/GIP						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
		PYY3-36 FFA Glucose Insulin TAG/TAG_{ref}/DAG PK-15						
8	01:00	Paracetamol sample						SMM start
8	01:15							SMM end Paracetamol administered
8	01:30	FFA GLP-1/GIP PYY3-36 Paracetamol sample						
8	01:45	Paracetamol sample						
8	02:00	FFA GLP-1/GIP Insulin PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
8	02:20	Paracetamol sample						
8	02:40	Paracetamol sample						
8	03:00	GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
8	04:00	FFA GLP-1/GIP Insulin Glucose PYY3036 TAG/TAG_{ref}/DAG Paracetamol sample						
8	05:00	ApoB48 TAG/TAG_{ref}/DAG Paracetamol sample						

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
8	06:00	FFA Insulin Glucose TAG/TAG_{ref}/DAG Paracetamol sample						
8	07:00	ApoB48 GLP-1/GIP PYY3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
8	08:00	TAG/TAG_{ref}/DAG						
8	09:00	PK-16 GLP-1/GIP PYY-3-36 TAG/TAG_{ref}/DAG Paracetamol sample						
9	Predose	PK-17 ApoB48			Sample D8–D10 end		dECG[h] pECG Vital signs	Weight recorded
9	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start				Empty bladder
9	00:15						dECG start	
9	00:20	PK-18					dECG end	
9	00:35						dECG start	
9	00:40	PK-19					dECG end	
9	00:55						dECG start	
9	01:00	PK-20					dECG end pECG Vital signs	
9	01:15						dECG start	
9	01:20						dECG end	
9	01:30	PK-21						
9	01:35						dECG start	
9	01:40						dECG end	
9	01:55						dECG start	
9	02:00	PK-22					dECG end Vital signs	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
9	02:55						dECG start	
9	03:00						dECG end Vital signs	
9	03:55						dECG start	
9	04:00	PK-23		PK-urine 0-4 h end PK-urine 4-8 h start		Adipose biopsy	dECG end	
9	05:00							
9	05:55						dECG start	
9	06:00						dECG end pECG	
9	07:55						dECG start	
9	08:00	PK-24		PK-urine 4-8 h end PK-urine 8-12 h start			dECG end	
9	11:55						dECG start	
9	12:00	PK-25		PK-urine 8-12 h end PK-urine 12-24 h start			dECG end	
9	18:00							
9	23:55						dECG start	
9	24:00	PK-26		PK-urine 12-24 h end			dECG end	
10	00:00[e]	ACTH/cortisol[f] Aldosterone/DHEA-S[g] BNP Clinical chemistry[a]/haematology Coagulation Insulin Ketones Lactate Leptin/adiponectin Renin[g] Glucose Exploratory blood sample			Collection end		pECG Vital signs	Weight recorded
10	12:00	ACTH/cortisol[f]						
10	24:00	PK-27						
11	00:00[e]						pECG	Weight recorded

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Tissue Sampling	Clinical Assessments	Other
							Telemetry end Vital signs	Physical examination Waist circumference measured

AZD7687 will be administered at 00:00 on Day 1 and Days 3 to 9.
SMM = standardised mixed meal

[a] Clinical chemistry includes lipid panel and liver chemistry

[b] PK urine collections will be done predose and at the intervals 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h, 24 to 48 h following the single dose on Day 1 and at 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h after the last day of multiple dosing on Day 9.

[c] Faecal collection periods are as follows: Day -3 to Day -1, Day 8 to Day 10.

[d] The genetic sampling can be done at any day during Visits 2 and 3.

[e] The time 00:00 should be the same time each morning throughout the study period.

[f] It is important that the samples are taken at the same time each morning and evening, if applicable. The samples for cortisol and ACTH should be obtained at a time point where reference values for cortisol and ACTH exist, if possible.

[g] The subject should rest in bed for 10 minutes before aldosterone and renin blood sampling.

h Predose dECG recordings on Days 1, 3 and 9 will be 10 minutes long.

Table 3 Study Plan - Time schedule for digital ECG assessments during residential period

Study Days	ECG Number	Start time hour: minute[d]	Dose	Stop time hour: minute	dECG continuous[abc]	Other
1		-01:30		-01:00		Apply the electrodes[b]
		-00:40		-00:30		Rest in bed
	1	-00:30	Predose	-00:20	10 minutes[c]	
		-00:20		-00:05		Toilet use allowed
		00:00	Administration of AZD7687/placebo			
	2	00:15		00:20	5 minutes[c]	
	3	00:35		00:40	5 minutes[c]	
	4	00:55		01:00	5 minutes[c]	
	5	01:15		01:20	5 minutes[c]	
	6	01:35		01:40	5 minutes[c]	
	7	01:55		02:00	5 minutes[c]	
	8	02:55		03:00	5 minutes[c]	
	9	03:55		04:00	5 minutes[c]	
	10	05:55		06:00	5 minutes[c]	
	11	07:55		08:00	5 minutes[c]	
	12	11:55		12:00	5 minutes[c]	
	13	23:55		24:00	5 minutes[c]	
3		-01:30		-01:00		Apply the electrodes[b]
3	14	-00:30	Predose	-00:20	10 minutes[c]	
9		-01:30		-01:00		Apply the electrodes[b]
		-00:40		-00:30		Rest in bed
	15	-00:30	Predose	-00:20	10 minutes	
		-00:20		-00:05		Toilet use allowed
		00:00	Administration of AZD7687/placebo			
	16	00:15		00:20	5 minutes[c]	
	17	00:35		00:40	5 minutes[c]	

Study Days	ECG Number	Start time hour: minute[d]	Dose	Stop time hour: minute	dECG continuous[abc]	Other
	18	00:55		01:00	5 minutes[c]	
	19	01:15		01:20	5 minutes[c]	
	20	01:35		01:40	5 minutes[c]	
	21	01:55		02:00	5 minutes[c]	
	22	02:55		03:00	5 minutes[c]	
	23	03:55		04:00	5 minutes[c]	
	24	05:55		06:00	5 minutes[c]	
	25	07:55		08:00	5 minutes[c]	
	26	11:55		12:00	5 minutes[c]	
	27	23:55		24:00	5 minutes[c]	

a The subject must be in the same supine body position (max. 30 degrees flexion in the hip) at each time point and at all visits. Subject's feet should not contact the footboard of the bed.
b Skin must be cleaned, and electrode positions marked with an indelible pen. Electrodes should be applied at least 30 minutes before first recording.
c Subject must rest in bed for at least 10 minutes prior to each ECG time point.
d Time points for dECG may be adjusted according to emerging PK data.

Section of protocol affected:

Section 4.2 Exclusion criteria

Previous text:

7. Serum TAG levels >1.5 mmol/L

Revised text:

7. Serum TAG levels >1.7 mmol/L.

Section of protocol affected:

Section 7.1, Table 5.

Previous text:

Table 5 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)[a]	No. of samples	Total volume (mL)
Safety	Clinical chemistry (including lipid panel and liver chemistry)	5.0	5	25.0
	Liver chemistry	3.5	3	10.5
	Haematology	2.0	5	10.0
	Coagulation	1.8	1	1.8
	Serology	3.5	1	3.5
ACTH, Cortisol		3.5	6	21.0
Aldosterone, DHEA-S		3.5	3	10.5
Renin		2.0	3	6.0
FFA, hsCRP		3.5	2	7.0
FFA		2.0	18	36.0
Insulin		3.5	16	56.0
BNP		2.0	2	4.0
Ketones		4.0	2	8.0
Lactate		2.0	2	4.0
Pharmacokinetics		2	34	68
TAG_{total},		3.5	28	98
$TAG_{Refined}$ and DAG		3.5	28	98
PYY3-36		2.0	17	34
GLP-1, GIP		2.0	19	38
Apo B48		3.5	2	7.0
Adiponectin, leptin		3.5	4	14.0
Paracetamol challenge		0.5	36	18.0
Pharmacogenetics		10	1	10
Total				588.3

a Where an indwelling cannula is used an additional 1 mL of blood will be drawn and discarded.

The number of samples taken, as well as the volume required for each analysis, may be changed during the study as new data on AZD7687 become available. However, the maximum volume to be drawn from each subject will not exceed 600 mL.

Revised text:

Table 5 Volume of blood to be drawn from each subject

Assessment		Sample volume (mL)[a]	No. of samples	Total volume (mL)
Safety	Clinical chemistry (including lipid panel and liver chemistry)	3.5	6	21.0
	Liver chemistry	3.5	3	10.5
	Haematology	2.0	6	12.0
	Coagulation	1.8	3	5.4
	Serology	3.5	1	3.5
ACTH		2	7	14.0
Cortisol		2.5	7	17.5
Aldosterone, DHEA-S		3.5	3	10.5
Renin		2.0	3	6.0
hsCRP		2.5	2	5.0
FFA		2.5	18	45.0
Insulin		2.5	20	50.0
BNP		2.0	2	4.0
Ketones		5.0	2	10.0
Lactate		2.0	2	4.0
Glucose		2.0	14	28.0
Pharmacokinetics		2	27	54.0
TAG_{total},		2.5	32	80.0
$TAG_{Refined}$ and DAG		2	32	64.0
PYY3-36		5.0	22	110.0
GLP-1, GIP		3.0	21	63.0
Apo B48		2.0	13	26.0
Adiponectin, leptin		3.5	3	10.5
Paracetamol challenge		0.6	36	21.6
Pharmacogenetics		4.0	1	4.0
Exploratory blood sample		4.0	2	8
Total				687.5

a Where an indwelling cannula is used an additional 1 mL of blood will be drawn and discarded.

The number of samples taken, as well as the volume required for each analysis, may be changed during the study as new data on AZD7687 become available. However, the maximum volume to be drawn from each subject will not exceed 700 mL.

Previous text:

11.3 Calculation or derivation of pharmacodynamic variable(s)

Two PD variables are of special interest: (1) the inhibitory effect on the TAG/DAG ratio in adipose tissue (biopsies) and (2) the inhibitory effect on incremental serum TAG_{total} AUC (blood samples).

1. The inhibitory effect on the TAG/DAG ratio in adipose tissue compared to baseline is defined as:

$$\frac{(TAG/DAG_{day\text{-}1} - TAG/DAG_{day9})}{(TAG/DAG_{day\text{-}1} - TAG/DAG_{max\ inhibition\ day\text{-}1})}$$

   $TAG/DAG_{max\ inhibition}$ is defined as the ratio observed after direct incubation of the biopsy with AZD7687 on the respective day.

   ie, for each individual the inhibitory effect is related to the maximal inhibitory effect of AZD7687 at baseline. (Note: the $TAG/DAG_{max\ inhibition\ day9}$ is not used in the analysis but will be compared descriptively with $TAG/DAG_{max\ inhibition\ day\text{-}1}$ as a confirmation of the expected stability of the value at max inhibition).

2. The incremental serum TAG_{total} AUC is defined as the difference between the area under the TAG versus time curve from 0 to 8 hours and the area of the rectangle with height = pre-meal TAG value and width = 8 hours, where the pre-meal value is the average of the last two pre-meal measurements.

The effect on incremental serum TAG_{total} AUC is measured by the relative change from Day -1 to Day 3 and Day 8.

Change from baseline will also be calculated for insulin and FFA.

Revised text:

11.3 Calculation or derivation of pharmacodynamic variable(s)

Two PD variables are of special interest: (1) the inhibitory effect on the TAG/DAG ratio in adipose tissue (biopsies) and (2) the inhibitory effect on incremental serum TAG_{total} AUC (blood samples).

1 The inhibitory effect on the TAG/DAG ratio in adipose tissue compared to baseline is defined as:

$$\frac{(TAG/DAG_{day\text{-}1} - TAG/DAG_{day9})}{(TAG/DAG_{day\text{-}1} - TAG/DAG_{max\ inhibition\ day\text{-}1})}$$

$TAG/DAG_{max\ inhibition}$ is defined as the ratio observed after direct incubation of the biopsy with AZD7687 on the respective day.

ie, for each individual the inhibitory effect is related to the maximal inhibitory effect of AZD7687 at baseline. (Note: the $TAG/DAG_{max\ inhibition\ day9}$ is not used in the analysis but will be compared descriptively with $TAG/DAG_{max\ inhibition\ day\text{-}1}$ as a confirmation of the expected stability of the value at max inhibition).

2 The incremental serum TAG_{total} AUC is defined as the difference between the area under the TAG versus time curve from 0 to 8 hours and the area of the rectangle with height = pre-meal TAG value and width = 8 hours, where the pre-meal value is the average of the last two pre-meal measurements.

The effect on incremental serum TAG_{total} AUC is measured by the relative change from Day -2 to Day 3 and Day 8.

Change from baseline will also be calculated for insulin and FFA.

Reason for Amendment:

To clarify the blood sample collection during the study, modify the exclusion criterion for TAG levels (the higher fasting TAG levels are expected to result in greater postprandial TAG excursion), correct minor errors in the original protocol and to remove Appendix E.

Persons who initiated the Amendment:

AstraZeneca

Clinical Study Protocol Amendment No 1	
Appendix A	
Drug Substance	AZD7687
Study Code	D2710C00002
Edition Number	1
Date	
Protocol	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

This amendment has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this amendment.

AstraZeneca Research and Developmen
site representative

This document contains confidential information, which should not be copied, referred to, released or published without written approval from AstraZeneca. Investigators are cautioned that the information in this protocol may be subject to change and revision.

ASTRAZENECA SIGNATURE(S)

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

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I agree to the terms of this amendment.

AstraZeneca Research and Development site representativ

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SIGNATURE OF PRINCIPAL INVESTIGATOR

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

I agree to the terms of this amendment.

Signature:

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Clinical Study Protocol Amendment

Amendment Number	2
Drug Substance	AZD7687
Study Code	D2710C00002
Protocol Dated	

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

This submission /document contains trade secrets and confidential commercial information, disclosure of which is prohibited without providing advance notice to AstraZeneca and opportunity to object.

Sponsor:

AstraZeneca AB, 151 85 Södertälje, Sweden.

Centres affected by the Amendment:

The protocol for the study is to be amended as follows:

Section of protocol affected:

Synopsis, Study centre and number of subjects planned

Section 3.1, Overall study design and flow chart

Previous text:

Up to 45 overweight to obese but otherwise healthy male subjects aged ≥20 to ≤45 years will participate in up to 5 dose steps (panels).

Revised text:

Up to 90 overweight to obese but otherwise healthy male subjects aged ≥20 to ≤45 years will participate in up to 10 dose steps (panels).

Section of protocol affected:

Synopsis, Study centre and number of subjects planned

Previous text:

Study period		Phase of development
Estimated date of first subject enrolled	Q2 2010	Clinical Pharmacology (Phase I)
Estimated date of last subject completed	Q3 2010	

Revised text:

Study period		Phase of development
Estimated date of first subject enrolled	Q2 2010	Clinical Pharmacology (Phase I)
Estimated date of last subject completed	Q1 2011	

Reason for Amendment:

The rationale for 5additional dose panels (dose panels 6 to 10) is to define a dose range with little or no gastrointestinal intolerability concerns. In the five dose panels so far investigated, gastrointestinal symptoms (nausea, vomiting, abdominal discomfort and diarrhoea) were pronounced at the 20 mg daily dose, present albeit tolerated at the 5 mg once daily dose and absent at the 1 mg once daily dose. In the proposed new five panels, low doses, up to a total daily dose of 5 mg will initially be investigated. In the event that the gastrointestinal intolerability symptoms previously observed are ameliorated (eg, due to dose splitting or intake with food) daily doses above 5 mg may be explored. The provisional first two doses given will be 2.5 mg once daily and 2.5 mg twice daily (at 12-hourly intervals), respectively. Subsequent doses of AZD7687 will be based on review of available safety, tolerability, PK and PD data from the previous dose panels.

Dose panels in which doses/exposures are lower than those already explored and tolerated, may be run in parallel or sequence without a Safety Review Committee (SRC) meeting between cohorts.

Section of protocol affected:

Section 3.1, Overall study design and flow chart

Previous text:

In addition, an adipose tissue biopsy will be taken.

Revised text:

In addition, an adipose tissue biopsy will be taken (dose panels 1 to 5 only).

Section of protocol affected:

Section 3.1, Overall study design and flow chart

Previous text:

One extra adipose tissue biopsy may be added.

Revised text:

One extra adipose tissue biopsy may be added (dose panels 1 to 5 only).

Section of protocol affected:

Section 5.1, Restrictions during the study

Previous text:

On days where adipose tissue biopsies are taken subjects should fast until the biopsy procedure is finished.

Revised text:

On days where adipose tissue biopsies are taken subjects should fast until the biopsy procedure is finished (dose panels 1 to 5 only).

Section of protocol affected:

Section 6.5.1.1, Adipose tissue biopsy

Previous text:

Samples for biopsies of adipose tissue (for determination of TAG and DAG in adipose tissue) will be taken at presented in the Study Plans (Table 1 and Table 2).

Revised text:

Samples for biopsies of adipose tissue (for determination of TAG and DAG in adipose tissue) will be taken as presented in the Study Plans (Table 1 and Table 2) (dose panels 1 to 5 only).

Reason for Amendment:

Since evaluated dose panels (1 to 5) have not given evidence for an inhibitory effect of AZD7687 on DGAT1 in the adipose tissue, it is considered unlikely that the new proposed doses (panels 6 to 10) will provide any new useful information in this regard. Adipose tissue biopsies will therefore not be done in the additional dose panels (6 to 10).

Section of protocol affected:

Table 1, Study plan

Table 2, Time scheduling during Visit 2

Previous text:

A paracetamol challenge will be performed on all days when the SMM is administered (see Table 1 and Table 2). Directly after the SMM is finished (at protocol 01:15) 500 mg of paracetamol elixir will be administered. Blood sampling will be performed to determine the paracetamol levels in plasma.

Revised text:

A paracetamol challenge will be performed on Day -2, Day 3 and Day 8 (see Table 1 and Table 2). On Day -2 and Day 8, directly after the SMM is finished (at protocol 01:15) 500 mg of paracetamol elixir will be administered. On Day 3 the paracetamol elixir will be administered at the same time (ie, protocol time 01:15. Blood sampling will be performed to determine the paracetamol levels in plasma.

Reason for Amendment:

Data from the SAD study and the so far completed cohorts in the MAD study have already shown the magnitude of the inhibitory effect of AZD7687 on DGAT1 in the gut following single dose administration. It is considered unlikely that further analyses on Day 3 at lower exposure levels (dose panels 6 to 10) will add any new useful information. The SMM on Day 3 has therefore been removed. As a result, the text regarding the paracetamol challenge has been updated.

Section of protocol affected:

Section 3.1, Overall study design and flow chart

Previous text:

All meals given at the clinic will be standardised.

Revised text:

All meals given at the clinic, except the SMM, will be balanced and the fat content should be limited to 20 to 30%.

Reason for Amendment:

The meals provided in the clinic should be of the type recommended for type II diabetic patients (containing 20 to 30% fat).

Section of protocol affected:

Table 1, Study plan

Table 2, Time scheduling during Visit 2

Previous text:

[c] Faecal collection periods are as follows: Day -3 to Day -1, Day 8 to Day 10.

Revised text:

[c] Faecal collection periods are as follows: Day -3 to Day -1, Day 3 to Day 5 and Day 8 to Day 10. Each sampling period will be for 72 hours.

Section of protocol affected:

Section 7.1, Volume of blood

Previous text:

Faecal samples will be collected at the times indicated in Table 1 and Table 2 for the determination of fat content.

Revised text:

Faecal samples will be collected at the times indicated in Table 1 and Table 2 for the determination of the following:

- Total fat;
- Electrolytes (sodium, potassium and chloride)
- pH.

The total weight of faeces during each sampling interval will be recorded.

Section of protocol affected:

Synopsis, Exploratory objectives

Section 2.3, Exploratory objectives

Previous text:

Not applicable

Revised text:

- To collect and store faecal samples for potential future exploratory research aimed at exploring factors involved in DGAT1 effects.

Reason for Amendment:

The additional exploratory objective, analyses and sampling period have been added to aid in the evaluation of mechanisms related to gastro-intestinal adverse events.

Section of protocol affected:

Section 6.7, Collection of samples for biomarker research

Previous text:

Additional exploratory blood sampling will take place on Day 1 and Day 10.

Revised text:

Additional exploratory blood sampling will take place on Day 1 and Day 10 for dose panels 1 to 5 and pre-dose each morning on Day 1 to Day 10 for dose panels 6 to 10.

Reason for Amendment:

To evaluate mechanisms related to gastro-intestinal adverse events.

Section of protocol affected:

Section 6.5.1.2, Assessment of postprandial lipaemia

Previous text:

Samples for the determination of serum total triacylglycerole (TAG_{total}) and samples for the refined plasma triacylglycerole and diacylglycerole ($TAG_{refined}$ and DAG [dose panels 1 to 5 only]) will be taken at time points specified in the study plans (Table 1 and Table 2).

Revised text:

Samples for the determination of serum total triacylglycerole (TAG_{total}) and samples for the refined plasma triacylglycerole and diacylglycerole ($TAG_{refined}$ and DAG [sample taken for dose panels 1 to 5 only]) will be taken at time points specified in the study plans (Table 1 and Table 2).

Reason for Amendment:

The blood sample for $TAG_{refined}$ and DAG has been removed since evaluated dose panels (1 to 5) have not given evidence for a change in the TAG/DAG ratio, it is considered unlikely that additional dose panels will affect these results.

Section of protocol affected:

Section 6.3.5, Laboratory safety assessment

Previous text:

Project Specific Clinical Chemistry

S-ACTH

S-Cortisol

S-DHEA-S

S-FFA

S-Ketones

S-Lactate

S-hs-CRP

S-Renin

S-Aldosterone

S-BNP

Revised text:

Project Specific Clinical Chemistry

S-ACTH

S-Cortisol

S-DHEA-S

S-FFA

S-Ketones

S-Lactate

S-hs-CRP

S-Renin

S-Aldosterone

S-BNP

S-CCK

Reason for Amendment:

A new analysis, P-CCK has been added to gain more insight into the mechanism underlying the gastro-intestinal adverse events observed.

Section of protocol affected:

Synopsis, Duration of treatment

Previous text:

Repeated dosing will commence on Day 3 with AZD7687 or placebo once daily for 7 days.

Revised text:

Repeated dosing will commence on Day 3 with AZD7687 or placebo once or twice daily for 7 days. For twice daily dosing, doses will be separated by 12 hours.

Section of protocol affected:

Section 3.1, Overall study design and flow chart

Previous text:

Subjects will receive a single dose of AZD7687 or placebo on Day 1 followed by once daily dosing for 7 days (commencing on Day 3).

Revised text:

Subjects will receive a single dose of AZD7687 or placebo on Day 1 followed by once **or twice** daily dosing for 7 days (commencing on Day 3). For twice daily dosing, doses will be separated by 12 hours.

Section of protocol affected:

Section 3.1, Overall study design and flow chart

Previous text:

Subjects will receive AZD7687 or placebo once daily for 7 days.

Revised text:

Subjects will receive AZD7687 or placebo once or twice daily for 7 days.

Section of protocol affected:

Section 5.5.2, Doses and treatment regimens

Previous text:

AZD7687 or placebo will be given as 240 mL suspension and water in total, according to the Handling Instruction, once daily for 7 days.

Revised text:

AZD7687 or placebo will be given as 240 mL suspension and water in total, according to the Handling Instruction, once or twice daily for 7 days. For twice daily dosing, doses will be separated by 12 hours.

Reason for Amendment:

Dose splitting (twice daily) would give rise to lower peak plasma concentrations of the IMP and at the lower doses proposed may provide sufficient exposure over time while reducing the risk of intolerability symptoms. Therefore twice daily dosing will be explored in addition to once daily.

Section of protocol affected:

Section 5.1, Restrictions during the study

Previous text:

Not applicable.

Revised text:

If twice daily dosing occurs during the multiple dosing part subjects will be fasted for 10 hours before the morning dose. Subjects will then be fasted for 2 hours before the evening dose and for 1 hour afterwards. Standardized lunch, dinner and snack will be served at scheduled Unit times. A moderate amount (≤240 mL) of water is allowed up to one hour prior to dosing and may be resumed one hour after dosing.

Reason for Amendment:

As it is proposed to include twice daily dosing, the restrictions regarding fasting and meals have been updated in the event of twice daily dosing.

Section of protocol affected:

Table 1, Study plan

Previous text:

Not applicable

Revised text:

Study Plan for dose panels 6 to 10

Visit number	**1 Screening Period**	**2 Residential Period**														**3 Follow-up**
Part		**Baseline PD measurements**			**Part A**		**Part B**									
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Informed consent	X															
Demography	X															
Medical/surgical history	X															
Inclusion/exclusion criteria	X	X														
Physical examination[a]	X	X[b]													X[b]	X
Serology	X															
Randomisation		X														
Administer study drug[l]					X		X	X	X	X	X	X	X			
Weight	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X															
Waist circumference	X		X												X	
Vital signs[c]	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Paper ECG (pECG)	X				X	X					X	X	X	X		X
Digital ECG (dECG)[d]					X	X	X						X			
Telemetry					X	X	X	X	X	X	X	X	X	X	X	

Visit number	**1** Screening Period	**2** Residential Period														**3** Follow-up
Part		**Baseline PD measurements**			**Part A**		**Part B**									
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Drugs of abuse screen	X	X														
PK blood sampling					X	X						X	X	X		
PK urine collection					X	X							X			
Exploratory blood sampling					X	X	X	X	X	X	X	X	X	X		
Safety laboratory tests[e]	X[k]	X[k, f]		X[f,j]		X[k, f]		X[f]		X		X[f]		X		X[k]
Standardised mixed meal			X									X				
Paracetamol challenge			X				X					X				
TAG_{total} sampling			X									X				
Insulin blood sampling			X		X		X				X	X				
Glucose blood sampling			X				X	X				X				
Total GLP-1, GIP and PYY3-36 blood sampling			X									X				
CCK blood sampling			X									X				
ACTH and cortisol blood sampling				X[g]							X[g]			X[g]		X[g]
Renin, aldosterone and DHEA-S blood sampling					X						X			X		
Ketones, lactate and BNP blood sampling					X									X		
ApoB48 blood sampling			X	X			X	X				X	X			
FFA blood sampling			X				X					X				
hsCRP blood sampling					X							X				
Leptin and adiponectin blood sampling					X	X								X		
Faecal sampling		X----------X					X----------X					X----------X				

Visit number	**1**	**2**													**3**
	Screening Period	**Residential Period**													**Follow-up**
Part		**Baseline PD measurements**			**Part A**		**Part B**								
Activity \Day	**-33 to -3**	**-3**	**-2**	**-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**7-14 days after last dose**
Genetic blood sampling		X[h]														
Adverse events[i]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

a Including special attention to dry skin, skin inflammation (especially face) and hair loss.
b Updates to physical examination will be recorded at admission and discharge.
c Vital signs include blood pressure, pulse and body temperature.
d Detailed information regarding the timing of dECGs is provided in Table 3.
e Blood and urine sampling for haematology, clinical chemistry (including coagulation, lipid panel and liver chemistry, see Section 6.3.5) and urinalysis parameters.
f Liver chemistry tests only, see Section 6.3.5 for definition.
g It is important that the samples are taken at the same time each morning and evening, if applicable. The samples for cortisol and ACTH should be obtained at a time point where reference values for cortisol and ACTH exist, if possible.
h The genetic sampling can be done at any day during Visit 2 and 3.
i SAEs will be recorded from signing of informed consent, non-serious AEs will be recorded from randomisation .
j Coagulation tests only, see Section 6.3.5 for definition.
k Clinical chemistry and haematology only, see Section 6.3.5 for definition.
l AZD7687 may be administered once or twice daily. Twice daily administrations will occur 12 hours apart.

Section of the protocol affected

Table 2, Time schedule during visit 2

Previous text:

Not applicable

Revised text

Time Schedule During Visit 2 – Dose panels 6 to 10 single dose

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
-3	Morning	Clinical chemistry[a]/haematology Genetic sampling[d]		Urinalysis Drugs of abuse and alcohol screen	Sample D-3–D-1 start	Vital signs	Admission to the clinic
-2	00:00[e]	FFA Glucose Insulin TAG ApoB48 PYY3-36 CCK					Weight recorded. Waist circumference measured.
-2	00:30	TAG					
-2	01:00 pre-SMM	FFA GLP-1/GIP PYY3-36 CCK ApoB48 Glucose Insulin TAG					
-2	01:00	Paracetamol sample					SMM start
-2	01:15						SMM finish Paracetamol administered
-2	01:30	FFA GLP-1/GIP PYY3-36 CCK Paracetamol sample					
-2	01:45	Paracetamol sample					

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
-2	02:00	FFA GLP-1/GIP Insulin PYY3-36 CCK TAG Paracetamol sample					
-2	02:20	Paracetamol sample					
-2	02:40	Paracetamol sample					
-2	03:00	GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					
-2	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 CCK TAG Paracetamol sample					
-2	05:00	ApoB48 TAG Paracetamol sample					
-2	06:00	FFA Glucose Insulin TAG Paracetamol sample					
-2	07:00	ApoB48 GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
-2	08:00	TAG					
-2	09:00	GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					
-1	00:00[e]	Liver chemistry ACTH/cortisol[f] ApoB48 Coagulation					Weight recorded
-1	12:00	ACTH/cortisol[f]					
1	Predose	PK-1 Aldosterone/DHEA-S[g] BNP Insulin Ketones Lactate Leptin/adiponectin Renin[g] Exploratory blood sample hsCRP		Urinalysis PK-urine predose	Sample D-3–D-1 end	dECG[h] pECG Telemetry start Vital signs	Weight recorded
1	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start			Empty bladder
1	00:15					dECG start	
1	00:20	PK-2				dECG stop	
1	00:35					dECG start	
1	00:40	PK-3				dECG stop	
1	00:55					dECG start	
1	01:00	PK-4				dECG stop pECG Vital signs	
1	01:15					dECG start	
1	01:20					dECG stop	
1	01:30	PK-5					
1	01:35					dECG start	
1	01:40					dECG stop	
1	01:55					dECG start	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
1	02:00	PK-6				dECG stop Vital signs	
1	02:55					dECG start	
1	03:00					dECG stop Vital signs	
1	03:55					dECG start	
1	04:00	PK-7		PK-urine 0-4 h end PK urine 4-8 start		dECG stop	
1	05:55					dECG start	
1	06:00					dECG stop pECG Vital signs	
1	07:55					dECG start	
1	08:00	PK-8		PK-urine 4-8 h end PK-urine 8-12 h start		dECG stop	
1	11:55					dECG start	
1	12:00	PK-9		PK-urine 8-12 h end PK-urine 12-24 h start		dECG stop Vital signs	
1	23:55					dECG start	
1	24:00	PK-10		PK-urine 12-24 h end PK-urine 24-48 h start		dECG stop pECG Vital signs	
2	Predose	Leptin/adiponectin Exploratory blood sample					
2	00:00[e]	Clinical chemistry[a]/haematology Insulin		Urinalysis			Weight recorded
2	24:00	PK-11		PK-urine 24-48 h end			

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
3	Morning				Sample D3–D5 start		
3	Predose	Exploratory blood sample Glucose Insulin		Urinalysis		Vital signs dECG[h]	Weight recorded
3	00:00[e]		AZD7687/placebo				
3	01:00	Insulin					
3	01:00	Paracetamol sample					
3	01:15						Paracetamol administered
3	01:30	Paracetamol sample					
3	01:45	Paracetamol sample					
3	02:00	Insulin Paracetamol sample					
3	02:20	Paracetamol sample					
3	02:40	Paracetamol sample					
3	03:00	Paracetamol sample					
3	04:00	Insulin Glucose Paracetamol sample					
3	05:00	Paracetamol sample					
3	06:00	Insulin Glucose Paracetamol sample					
3	07:00	Paracetamol sample					
4	Predose	ApoB48 Glucose Liver chemistry				Vital signs	Weight recorded
		Exploratory blood sample					
4	00:00		AZD7687/placebo				
5	Predose	Exploratory blood sample				Vital signs	Weight recorded
5	00:00[e]		AZD7687/placebo				

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
6	Predose	Glucose Insulin Clinical chemistry[a]/haematology Coagulation Exploratory blood sample			Sample D3–D5 end	Vital signs	Weight recorded
6	00:00[e]		AZD7687/placebo				
7	Predose	ACTH/cortisol[f] Aldosterone/DHEA-S[g] Insulin Renin[g] Exploratory blood sample		Urinalysis		pECG Vital signs	Weight recorded
7	00:00[e]		AZD7687/placebo				
7	12:00	ACTH/cortisol[f]					
8	Predose	FFA Glucose Insulin Liver chemistry Exploratory blood sample			Sample D8–D10 start	pECG Vital signs	Weight recorded
8	00:00[e]	PK-12	AZD7687/placebo				
8	00:30	TAG hsCRP					
8	01:00 pre-SMM	ApoB48 GLP-1/GIP PYY3-36 CCK FFA Glucose Insulin TAG PK-13					
8	01:00	Paracetamol sample					SMM start
8	01:15						SMM end Paracetamol administered

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
8	01:30	FFA GLP-1/GIP PYY3-36 CCK Paracetamol sample					
8	01:45	Paracetamol sample					
8	02:00	FFA GLP-1/GIP Insulin PYY3-36 CCK TAG Paracetamol sample					
8	02:20	Paracetamol sample					
8	02:40	Paracetamol sample					
8	03:00	GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					
8	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 CCK TAG Paracetamol sample					
8	05:00	ApoB48 TAG Paracetamol sample					
8	06:00	FFA Insulin Glucose TAG Paracetamol sample					

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
8	07:00	ApoB48 GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					
8	08:00	TAG					
8	09:00	PK-14 GLP-1/GIP PYY-3-36 TAG Paracetamol sample					
9	Predose	PK-15 ApoB48 Exploratory blood sample				dECG[h] pECG Vital signs	Weight recorded
9	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start			Empty bladder
9	00:15					dECG start	
9	00:20	PK-16				dECG end	
9	00:35					dECG start	
9	00:40	PK-17				dECG end	
9	00:55					dECG start	
9	01:00	PK-18				dECG end pECG Vital signs	
9	01:15					dECG start	
9	01:20					dECG end	
9	01:30	PK-19					
9	01:35					dECG start	
9	01:40					dECG end	
9	01:55					dECG start	
9	02:00	PK-20				dECG end Vital signs	
9	02:55					dECG start	
9	03:00					dECG end Vital signs	
9	03:55					dECG start	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
9	04:00	PK-21		PK-urine 0-4 h end PK-urine 4-8 h start		dECG end	
9	05:55					dECG start	
9	06:00					dECG end pECG	
9	07:55					dECG start	
9	08:00	PK-22		PK-urine 4-8 h end PK-urine 8-12 h start		dECG end	
9	11:55					dECG start	
9	12:00	PK-23		PK-urine 8-12 h end PK-urine 12-24 h start		dECG end	
9	23:55					dECG start	
9	24:00	PK-24		PK-urine 12-24 h end		dECG end	
10	00:00[e]	ACTH/cortisol[f] Aldosterone/DHEA-S[g] BNP Clinical chemistry[a]/haematology Coagulation Insulin Ketones Lactate Leptin/adiponectin Renin[g] Glucose Exploratory blood sample				pECG Vital signs	Weight recorded
10	12:00	ACTH/cortisol[f]					
10	24:00	PK-25					
11	Predose				Sample D8–D10 end		
11	00:00[e]					pECG Telemetry end Vital signs	Weight recorded Physical examination Waist circumference measured

AZD7687 will be administered at 00:00 on Day 1 and Days 3 to 9.
SMM = standardised mixed meal
[a] Clinical chemistry includes lipid panel and liver chemistry

[b] PK urine collections will be done predose and at the intervals 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h, 24 to 48 h following the single dose on Day 1 and at 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h after the last day of multiple dosing on Day 9.
[c] Faecal collection periods are as follows: Day -3 to Day -1, Day 8 to Day 10. Each collection period is 72 h.
[d] The genetic sampling can be done at any day during Visits 2 and 3.
[e] The time 00:00 should be the same time each morning throughout the study period.
[f] It is important that the samples are taken at the same time each morning and evening, if applicable. The samples for cortisol and ACTH should be obtained at a time point where reference values for cortisol and ACTH exist, if possible.
[g] The subject should rest in bed for 10 minutes before aldosterone and renin blood sampling.
h Predose dECG recordings on Days 1, 3 and 9 will be 10 minutes long.

Time Schedule During Visit 2 – Dose panels 6 to 10 twice daily dosing

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
-3	Morning	Clinical chemistry[a]/haematology Genetic sampling[d]		Urinalysis Drugs of abuse and alcohol screen	Sample D-3–D-1 start	Vital signs	Admission to the clinic
-2	00:00[e]	FFA Glucose Insulin TAG ApoB48 PYY3-36 CCK					Weight recorded. Waist circumference measured.
-2	00:30	TAG					
-2	01:00 pre-SMM	FFA GLP-1/GIP PYY3-36 CCK ApoB48 Glucose Insulin TAG					
-2	01:00	Paracetamol sample					SMM start
-2	01:15						SMM finish Paracetamol administered

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
-2	01:30	FFA GLP-1/GIP PYY3-36 CCK Paracetamol sample					
-2	01:45	Paracetamol sample					
-2	02:00	FFA GLP-1/GIP Insulin PYY3-36 CCK TAG Paracetamol sample					
-2	02:20	Paracetamol sample					
-2	02:40	Paracetamol sample					
-2	03:00	GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					
-2	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 CCK TAG Paracetamol sample					
-2	05:00	ApoB48 TAG Paracetamol sample					
-2	06:00	FFA Glucose Insulin TAG Paracetamol sample					

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
-2	07:00	ApoB48 GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					
-2	08:00	TAG					
-2	09:00	GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					
-1	00:00[e]	Liver chemistry ACTH/cortisol[f] ApoB48 Coagulation					Weight recorded
-1	12:00	ACTH/cortisol[f]					
1	Predose	PK-1 Aldosterone/DHEA-S[g] BNP Insulin Ketones Lactate Leptin/adiponectin Renin[g] Exploratory blood sample hsCRP		Urinalysis PK-urine predose	Sample D-3–D-1 end	dECG[h] pECG Telemetry start Vital signs	Weight recorded
1	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start			Empty bladder
1	00:15					dECG start	
1	00:20	PK-2				dECG stop	
1	00:35					dECG start	
1	00:40	PK-3				dECG stop	
1	00:55					dECG start	
1	01:00	PK-4				dECG stop pECG Vital signs	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
1	01:15					dECG start	
1	01:20					dECG stop	
1	01:30	PK-5					
1	01:35					dECG start	
1	01:40					dECG stop	
1	01:55					dECG start	
1	02:00	PK-6				dECG stop Vital signs	
1	02:55					dECG start	
1	03:00					dECG stop Vital signs	
1	03:55					dECG start	
1	04:00	PK-7		PK-urine 0-4 h end PK urine 4-8 start		dECG stop	
1	05:55					dECG start	
1	06:00					dECG stop pECG Vital signs	
1	07:55					dECG start	
1	08:00	PK-8		PK-urine 4-8 h end PK-urine 8-12 h start		dECG stop	
1	11:55					dECG start	
1	12:00	PK-9		PK-urine 8-12 h end PK-urine 12-24 h start		dECG stop Vital signs	
1	23:55					dECG start	
1	24:00	PK-10		PK-urine 12-24 h end PK-urine 24-48 h start		dECG stop pECG Vital signs	
2	Predose	Leptin/adiponectin					
		Exploratory blood sample					
2	00:00[e]	Clinical chemistry[a]/haematology Insulin		Urinalysis			Weight recorded
2	24:00	PK-11		PK-urine 24-48 h end			
3	Morning				Sample D3–D5 start		
3	Predose	Exploratory blood sample				Vital signs	Weight recorded

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
		Glucose Insulin		Urinalysis		dECG[h]	
3	00:00[e]		AZD7687/placebo				
3	01:00	 Insulin					
3	01:00	Paracetamol sample					
3	01:15						Paracetamol administered
3	01:30	Paracetamol sample					
3	01:45	Paracetamol sample					
3	02:00	Insulin Paracetamol sample					
3	02:20	Paracetamol sample					
3	02:40	Paracetamol sample					
3	03:00	Paracetamol sample					
3	04:00	Insulin Glucose Paracetamol sample					
3	05:00	Paracetamol sample					
3	06:00	Insulin Glucose Paracetamol sample					
3	07:00	Paracetamol sample					
3	12:00		AZD7687/placebo				
4	Predose	ApoB48 Glucose Liver chemistry Exploratory blood sample				Vital signs	Weight recorded
4	00:00		AZD7687/placebo				
4	12:00		AZD7687/placebo				
5	Predose	Exploratory blood sample				Vital signs	Weight recorded
5	00:00[e]		AZD7687/placebo				
5	12:00		AZD7687/placebo				

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
6	Predose	Glucose Insulin Clinical chemistry[a]/haematology Coagulation Exploratory blood sample			Sample D3–D5 end	Vital signs	Weight recorded
6	00:00[e]		AZD7687/placebo				
6	12:00		AZD7687/placebo				
7	Predose	ACTH/cortisol[f] Aldosterone/DHEA-S[g] Insulin Renin[g] Exploratory blood sample		Urinalysis		pECG Vital signs	Weight recorded
7	00:00[e]		AZD7687/placebo				
7	12:00	ACTH/cortisol[f]	AZD7687/placebo				
8	Predose	FFA Glucose Insulin Liver chemistry Exploratory blood sample			Sample D8–D10 start	pECG Vital signs	Weight recorded
8	00:00[e]	PK-12	AZD7687/placebo				
8	00:30	TAG hsCRP					
8	01:00 pre-SMM	ApoB48 GLP-1/GIP PYY3-36 CCK FFA Glucose Insulin TAG PK-13					
8	01:00	Paracetamol sample					SMM start
8	01:15						SMM end Paracetamol administered

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
8	01:30	FFA GLP-1/GIP PYY3-36 CCK Paracetamol sample					
8	01:45	Paracetamol sample					
8	02:00	FFA GLP-1/GIP Insulin PYY3-36 CCK TAG Paracetamol sample					
8	02:20	Paracetamol sample					
8	02:40	Paracetamol sample					
8	03:00	GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					
8	04:00	FFA GLP-1/GIP Insulin Glucose PYY3-36 CCK TAG Paracetamol sample					
8	05:00	ApoB48 TAG Paracetamol sample					
8	06:00	FFA Insulin Glucose TAG Paracetamol sample					

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
8	07:00	ApoB48 GLP-1/GIP PYY3-36 CCK TAG Paracetamol sample					
8	08:00	TAG					
8	09:00	PK-14 GLP-1/GIP PYY-3-36 TAG Paracetamol sample					
8	12:00	PK-15(predose)	AZD7687/placebo				
9	Predose	PK-16 ApoB48 Exploratory blood sample				dECG[h] pECG Vital signs	Weight recorded
9	00:00[e]		AZD7687/placebo	PK-urine 0-4 h start			Empty bladder
9	00:15					dECG start	
9	00:20	PK-17				dECG end	
9	00:35					dECG start	
9	00:40	PK-18				dECG end	
9	00:55					dECG start	
9	01:00	PK-19				dECG end pECG Vital signs	
9	01:15					dECG start	
9	01:20					dECG end	
9	01:30	PK-20					
9	01:35					dECG start	
9	01:40					dECG end	
9	01:55					dECG start	
9	02:00	PK-21				dECG end Vital signs	
9	02:55					dECG start	
9	03:00					dECG end Vital signs	

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
9	03:55					dECG start	
9	04:00	PK-22		PK-urine 0-4 h end PK-urine 4-8 h start		dECG end	
9	05:00						
9	05:55					dECG start	
9	06:00					dECG end pECG	
9	07:55					dECG start	
9	08:00	PK-23		PK-urine 4-8 h end PK-urine 8-12 h start		dECG end	
9	11:55					dECG start	
9	Pre-dose 12:00	PK-24		PK-urine 8-12 h end		dECG end	
9	12:00		AZD7687/placebo	PK-urine 12-24 h start			
9	12:20	PK-25					
9	12:40	PK-26					
9	13:00	PK-27					
9	13:30	PK-28					
9	14:00	PK-29					
9	16:00	PK-30					
9	23:55					dECG start	
9	24:00	PK-31		PK-urine 12-24 h end		dECG end	
10	00:00[e]	ACTH/cortisol[f] Aldosterone/DHEA-S[g] BNP Clinical chemistry[a]/haematology Coagulation Insulin Ketones Lactate Leptin/adiponectin Renin[g] Glucose Exploratory blood sample				pECG Vital signs	Weight recorded
10	12:00	ACTH/cortisol[f]					

Study Day	Protocol time (hhmm)	Blood Sampling	Drug Administration	Urine Sampling[b]	Faecal Sampling[c]	Clinical Assessments	Other
10	24:00	PK-32					
11	Predose				Sample D8–D10 end		
11	00:00[e]					pECG Telemetry end Vital signs	Weight recorded Physical examination Waist circumference measured

AZD7687 will be administered at 00:00 on Day 1 and at 0:00 and 12:00 on Days 3 to 9.
SMM = standardised mixed meal

[a] Clinical chemistry includes lipid panel and liver chemistry

[b] PK urine collections will be done predose and at the intervals 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h, 24 to 48 h following the single dose on Day 1 and at 0 to 4 h, 4 to 8 h, 8 to 12 h, 12 to 24 h after the last day of multiple dosing on Day 9.

[c] Faecal collection periods are as follows: Day -3 to Day -1, Day 8 to Day 10. Each collection period is 72 h.

[d] The genetic sampling can be done at any day during Visits 2 and 3.

[e] The time 00:00 should be the same time each morning throughout the study period.

[f] It is important that the samples are taken at the same time each morning and evening, if applicable. The samples for cortisol and ACTH should be obtained at a time point where reference values for cortisol and ACTH exist, if possible.

[g] The subject should rest in bed for 10 minutes before aldosterone and renin blood sampling.

h Predose dECG recordings on Days 1, 3 and 9 will be 10 minutes long.

Section of protocol affected:

Section 7.1, Table 5

Previous text:

Not applicable

Revised text:

Blood volumes for dose panels 6-10

Assessment		Sample volume (mL)[a]	No. of samples	Total volume (mL)
Safety	Clinical chemistry (including lipid panel and liver chemistry)	3.5	6	21.0
	Liver chemistry	3.5	3	10.5
	Haematology	2.0	6	12.0
	Coagulation	1.8	3	5.4
	Serology	3.5	1	3.5
ACTH		2	7	14.0
Cortisol		2.5	7	17.5
Aldosterone, DHEA-S		3.5	3	10.5
Renin		2.0	3	6.0
hsCRP		2.5	2	5.0
FFA		2.5	12	30.0
Insulin		2.5	20	50.0
BNP		2.0	2	4.0
Ketones		5.0	2	10.0
Lactate		2.0	2	4.0
Glucose		2.0	14	28.0
Pharmacokinetics		2	25 (32[a])	50.0 (64.0 [a])
TAG_{total},		2.5	21	52.5
PYY3-36		5.0	15	75.0
GLP-1, GIP		3.0	14	42.0
Apo B48		2.0	9	18.0
CCK		4.0	15	60.0
Adiponectin, leptin		3.5	3	10.5
Paracetamol challenge		0.6	36	21.6

Assessment	Sample volume (mL)[a]	No. of samples	Total volume (mL)
Pharmacogenetics	4.0	1	4.0
Exploratory blood sample	4.0	10	40
Total			605.0 (619[a])

a blood volumes for subjects receiving twice daily dosing

Reason for Amendment:

Additional study schedules have been included for dose panels 6 to 10 to reflect the changes detailed in this amendment regarding sampling and analyses. In addition as twice daily dosing is proposed, an additional schedule has been included detailing administration of AZD7687/placebo and PK sampling for twice daily dosing.

Persons who initiated the Amendment:

Clinical Study Protocol Amendment No 2
Appendix A

Drug Substance	AZD7687
Study Code	D2710C00002
Edition Number	1
Protocol Dated	

Appendix A
Signatures

ASTRAZENECA SIGNATURE(S)

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese but otherwise Healthy Male Subjects

This amendment to the CSP has been subjected to an internal AstraZeneca peer review.

I agree to the terms of this study protocol/amendment.

AstraZeneca Research and Develop
site representative

This document contains confidential information, which should not be copied, referred to, released or published without written approval from AstraZeneca. Investigators are cautioned that the information in this protocol may be subject to change and revision.

ASTRAZENECA SIGNATURE(S)

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese b

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SIGNATURE OF PRINCIPAL INVESTIGATOR

A Phase I, Single Centre, Single-Blind, Randomised, Placebo-controlled, Parallel-group Study to Assess the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Oral AZD7687 after Administration of Multiple Ascending Doses in Overweight to Obese b

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I agree to the terms of this amendment.

Signature:

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