Protocol synopsis
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The effect of Liraglutide treatment on postprandial chylomicron and VLDL kinetics, liver fat and *de novo* lipogenesis.

A single-center randomized controlled study.

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EudraCT: 2013-005075-40

Principal investigators: Niina Matikainen[1,2,3,4], Jan Borén[5]
Other Investigators: Stephano Romeo[5], Sanni Söderlund[1,2,3], Martin Adiels[5],Kirsi Pietiläinen[2,3], Leonie-Helen Bogl [2,3], and Marja-Riitta Taskinen[1,2,3]

[1] Heart and Lung Centre, Cardiovascular Research Unit, Helsinki University Central Hospital; [2]Research Programs' Unit, Diabetes & Obesity, University of Helsinki; [3]Clinical Research Institute, Huch ltd. Biomedicum Helsinki, Finland; [4]Department of endocrinology, Helsinki University Central Hospital, [5]Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

Background

The substantial residual risk for cardiovascular disease (CVD) risk that persists in patients with optimal low-density lipoprotein (LDL) cholesterol associates with the atherogenic dyslipidemia and postprandial lipemia, is a significant but clinically neglected CVD risk factor in particular in Type 2 diabetes (T2DM) patients [1]. The well recognized dyslipidemia in people with T2DM consists of high fasting and non-fasting plasma triglycerides (TG), low high-density lipoprotein (HDL) -cholesterol and preponderance of small dense LDL particles nominated as the atherogenic lipid triad. Recent studies consistently show that non-fasting triglyceride values are better associated with CVD risk and death than fasting triglyceride values. In addition, non-fasting triglycerides also predicted stroke risk [2]. Indeed, humans are mostly in a postprandial rather than fasting state and therefore non-fasting TG values reflect more accurately the continuous exposure of arterial wall to triglyceride rich lipoproteins (TRLs) and more importantly, to substantial cholesterol load that these particles deliver. In circulation TRLs undergo lipoprotein lipase (LPL)-induced lipolysis and remodeling with the formation of a spectrum of heterogeneous particles referred as TRL remnant particles with a progressive reduction of particle size that allows particles to penetrate the arterial wall. Although LDL cholesterol is recognized as the most important lipid factor in early stages of atherogenesis, postprandial TRLs may be relevant to later atherometabolic processes. TRL remnants contribute significantly to intimal cholesterol deposition as remnants contain more cholesterol per particle than LDL.

Postprandial lipemia is highly prevalent even in T2DM patients with normal fasting TG concentrations. Intestinal overproduction of chylomicrons (CM s) and the structural protein apolipoprotein (apo)-B48 has been identified as an integral feature of postprandial lipemia in T2DM and insulin resistance [3,4]. Each CM particle contains one apo-B48 molecule that remains the integral part of CM remnant particles during the lipolysis of the particles by LPL. Consequently apo-B48 can be utilized in vivo to track the secretion and metabolic fate of intestinally derived CM particles [5]. CMs contribute significantly but not exclusively to the elevation of postprandial triglyceride levels. Hepatic very low-density lipoprotein (VLDL) particles are quantitatively the major component leading to increased number of remnant particles.

We recently reported that elevation of plasma TG in abdominally obese dyslipidemic subjects at high CVD risk is due to dual metabolic defects; 1. overproduction of $VLDL_1$ particles linked to increased liver fat and subcutaneous abdominal fat, and 2. impaired catabolic rate of $VLDL_1$ particles associated with increases of

apo-CIII [6]. Our recent data suggest that the capacity of the lipolytic pathway is a determinant of both fasting and non-fasting triglyceride levels [7]. *Therefore it is clinically important to elucidate the mechanism for delayed postprandial lipemia and the interactions between dysglycemia and dyslipidemia in T2DM subjects.*

Incretins are hormones released from the gut that respond to the ingested nutrients and potentiate glucose-induced insulin secretion from the pancreatic β-cells. The most important incretins are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), both of which are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4) [8-10]. Incretins are important in the regulation of postprandial glucose levels [11-13]. Importantly the incretin effect is strongly reduced or lost in T2 DM patients. Recently new approaches that targets incretin hormones including the GLP-1 receptor (GLP-1R) agonists and DPP4 inhibitors have been developed with a mechanism of action distinct from existing glucose lowering agents [10,14]. Incretin based therapies with GLP-1 agonists are accepted as an ideal add on therapy to intensify glucose control in combination therapy as they offer benefits like weight loss and no hypoglycemia [15].

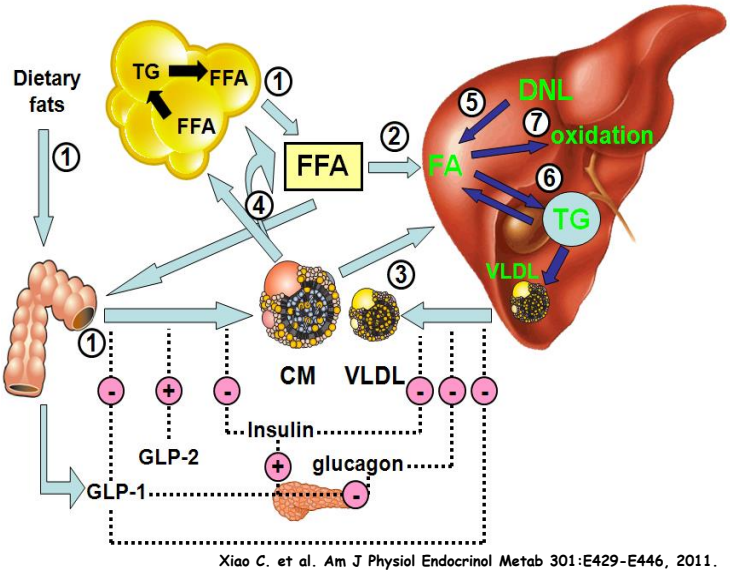


Figure 1.*Coordinated TRL metabolism during the fasting-to-fed transition*. *The intestine actively produces chylomicrons and secrets gut hormones, including GLP-1 and GLP-2. A shift of TRL metabolism from fasting to fed state involves 1) dietary fat absorption and chylomicron secretion, 2) decreased adipose tissue lipolysis and free fatty acid (FFA) flux to liver and intestine, with net FA uptake by adipose tissue, 3) increased chylomicron remnant uptake by the liver, 4) fatty acid "spillover" (i.e., FA released by intravascular lipolysis of TRL not taken up and stored in adipose tissue) and utilization for VLDL and possibly chylomicron synthesis, 5) increased hepatic de novo lipogenesis (DNL), 6) increased liver lipid droplet storage, and 7) relative reduction in fatty acid (FA) oxidation vs. esterification of FA in liver. Altered hormonal secretion plays an important role in coordinating this transition, including glucose and GLP-1-stimulated insulin secretion and suppressed glucagon secretion from the pancreas. GLP-1 suppresses, GLP-2 stimulates, and insulin suppresses intestinal chylomicron secretion, whereas insulin and glucagon suppress hepatic VLDL secretion. The effect of GLP-1 on hepatic VLDL secretion is currently poorly characterized in humans.*

Emerging data indicate that incretins modulate not only glucose metabolism but also lipid metabolism via GLP-1 receptor activation. GLP-1 plays an important role in the assembly and secretion of chylomicrons (Figure 1) [16]. Pharmacological augmentation of GLP-1 receptor signaling by GLP-1 agonists or DPP-4 inhibition reduces intestinal lipoprotein secretion in experimental studies, suggesting that DPP-4 inhibitors may ameliorate dyslipidemia and thus improve cardiovascular risk profile in patients with T2DM. [17].

Recently we reported that alogliptin (DPP-4 inhibitor) therapy over 16 weeks produced significant reductions in postprandial TG and TRL lipoproteins as well as fasting levels of TG, apo-B48 and RLP cholesterol indicating that burden of remnant particles is ameliorated in a randomized double-blind placebo controlled study [18]. Our data are in line with previous studies showing that vildagliptin, sitagliptin as well as exenatide, a GLP-1 agonist, reduces postprandial lipemia in type 2 diabetic patients [19-21]. Recently exenatide was shown acutely to suppress intestinal lipoprotein production [22]. Likewise Liraglutide was reported to reduce postprandial responses of TG and apoB48 after a fat rich meal [23]. However long-term studies are needed to elucidate the lipid effects of incretin-based therapies. Thus, the available data suggest that the incretin-based therapies have a class effect on intestinal lipid metabolism but there may be some difference in the efficacy to modulate lipid metabolism.

Importantly, GLP-1 receptor expression was recently demonstrated in human hepatocytes and GLP-1 treatment reduced VLDL production in mice fed a high-fat diet [24]. We reported that alogliptin not only reduced postprandial $VLDL_1$ apo-B48 and TG incremental area under curve (iAUC) but also $VLDL_1$ apo-B100 iAUC. Recently GLP-1 receptor agonists have been reported to decrease hepatic de novo lipogenesis (DNL) linked with reduced VLDL production and hepatic steatosis in two different animal models [25,26].

Furthermore, we showed that vildagliptin reduced remnant-like lipoprotein particles with a concomitant increase in LDL size [27]. These findings suggest an improvement in the metabolism of the TRLs and decrease in atherogenic modification of LDL particles. Today, it is not known if incretin-based therapies improve reduce production rate of large VLDL particles or their clearance in humans.

As abnormal concentrations of blood lipids can results from changes in the production, conversion or clearance of lipoprotein particle, static measurements of lipid levels do not reveal the underlying mechanism of these lipid disorders. Therefore it is necessary to confirm the effects of GLP-1 agonists on the kinetics of TG, apo-B48 and apo-B100 in the postprandial state using stable isotopes concomitantly with quantification of liver fat to demonstrate how GLP-1 system modulates lipoprotein metabolism in T2DM. Kinetic studies in humans provide an important and unique tool to quantitatively elucidate the dynamics of lipid metabolism providing estimates of lipoprotein secretion and clearance underlining the pathophysiology of lipid disorders.

We have established and validated novel gas cromatography/mass spectrometry (GC/MS) methods to analyze the enrichment of combined load of stable isotopes; $^{2}H_5$ glycerol, to track enrichments into VLDL-TG, $^{2}H_3$leucine incorporated to apo-B48 and apo-B100, $^{13}C_4$palmitate a to track the contribution of dietary lipids to VLDL triglyceride secretion and $^{2}H_2O$ heavy water to quantitate DNL in the VLDL particles released by liver (Table 1, Figure 2).

Table 1. **Stable isotopes**
$^{2}H_5$-Glycerol Injected tracer that is esterified to triglycerides in the liver but not the intestine as it lacks glycerol kinase activity. It is thus only incorporated into VLDL–triglycerides.
$^{2}H_3$-Leucine Injected tracer that is incorporated into apoB100 and apoB48. Chylomicrons secreted from the enterocytes contain apoB48, while VLDL secreted from the liver contain apoB100.
$^{13}C_4$-Palmitate The dietary tracer [1,2,3,4-^{13}C]-palmitate will be served in the meal to track the contribution of dietary lipids to VLDL–triglyceride secretion.
$^{2}H_2O$ (heavy water) Given orally. Incorporated into all fatty acids. Used to quantify the amount of fatty acids derived from liver *de novo* lipogenesis into $VLDL_1$ and $VLDL_2$.

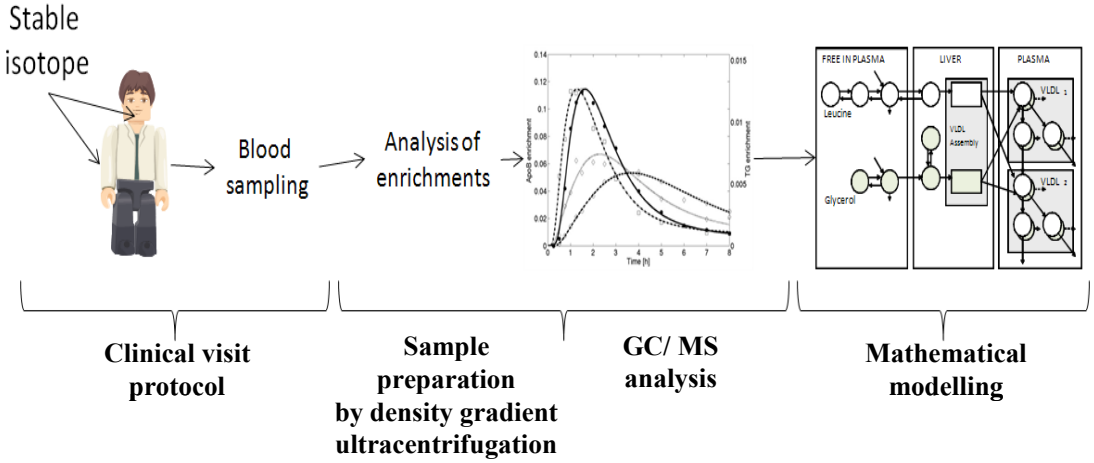


Figure 2.*Outline of a kinetic study* *that includes three major steps:* ***(i)*** *clinical visit protocol,* ***(ii)*** *sample preparation/processing and enrichment analysis by GC/MS, and* ***(iii)*** *mathematical modeling. Subjects ingest* ***heavy water*** *and* ***$^{13}C_4$-Palmitate*** *and receive infusions of* ***$^{2}H_5$-Glycerol*** *and* ***$^{2}H_3$-Leucine (Table 1)****.After GC/MS-detection of stable isotope enrichments in isolated lipoprotein fractions it is possible to mathematically follow the metabolism of both dietary and endogenous derived triglycerides, apoB-100 and apoB-48 and calculate the amount of de novo –lipogenesis in TRL fractions.*

In the development of the model we have utilized the data from 5 subjects who have had a kinetic study (1) in the fasting state and (2) repeated together with an oral fat load test. We have established for the first time that our protocol allows us to follow the production, transfer and direct catabolism of hepatic ($VLDL_1$ and $VLDL_2$) and intestinal (chylomicrons) lipoprotein particles and triglycerides in vivo, and to elucidate how DNL and dietary lipids contribute to postprandial TRLs. We also have demonstrated that our new non-steady state model allows the mathematical modeling of the data.

RESEARCH HYPOTHESIS

The aim of this study is to elucidate the mechanisms underlying the positive effect of incretin therapy on lipoprotein metabolism in type 2 diabetic subjects and for the first time establish the potential effect on liver DNL. Our study design allows differentiating the impact of weight loss dependent and independent effects of liraglutide on lipoprotein and carbohydrate metabolism.

We hypothesize that:

1. Reversal of the incretin defect by liraglutide will result in reduced production and/or improved clearance rate of chylomicrons and remnants
2. GLP-1 receptor activation in the liver will be associated with decreased hepatic DNL, consequently, reduced production of large $VLDL_1$ particles and decrease in liver fat content.
3. Positive effects of liraglutide onTRL kinetics and liver fat are further potentiated with weight loss.

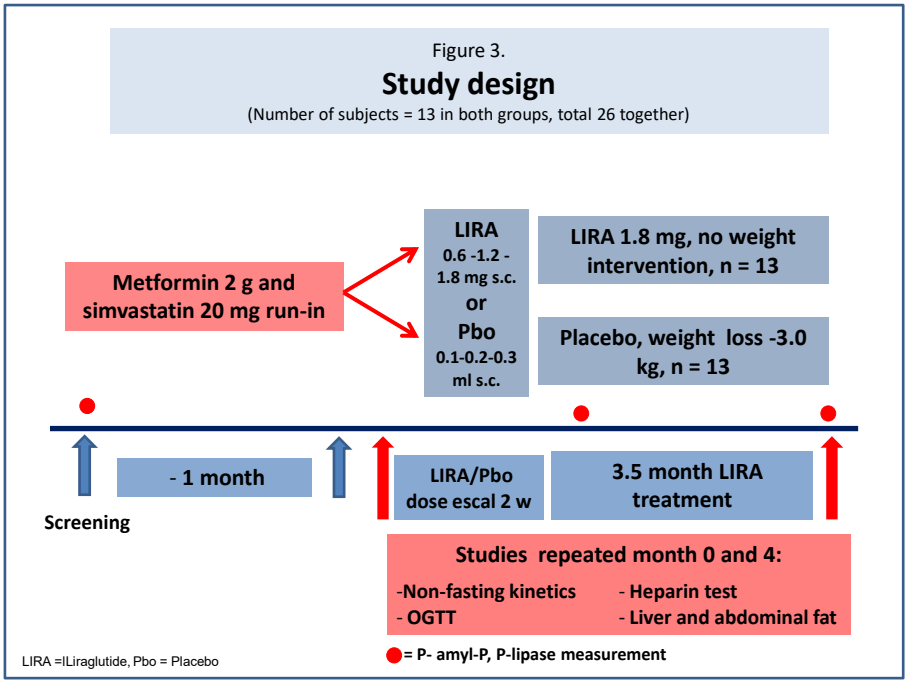
TREATMENT

The study will be single blinded into two groups matched for age, sex and BMI in order to achieve similar weight loss in liraglutide and placebo arms. In addition to a fixed dose of metformin (2 g/day) and simvastatin (20 mg/day) treatments, patients will be assigned to liraglutide therapy once daily and randomized to liraglutide group with uncontrolled diet or to placebo group with weight loss of 3.0 kg supervised by the study dietician for 4 months (Figure 3). Previous studies in type 2 diabetes show that the average weight loss with liragutide is 3-3.5 kg and stable weight is achieved within 3-3.5 months (LEAD1-6). The study cohort will comprise male and female (non-fertile or of child-bearing potential using a medically approved birth control method) with T2DM eligible for treatment with metformin or on stable dose of metformin.

Figure 3.

Study design

(Number of subjects = 13 in both groups, total 26 together)



INCLUSION CRITERIA

Obese subjects with T2D and a large waist (waist > 88 cm in women and > 92 cm in men, BMI 27–40 kg/m2, triglycerides between 1.0 – 4.0 mmol/L and LDL < 4.5 mmol/l) treated with a lifestyle or metformin (any dose) (HbA1c 6.5-9%) and aged 30 – 75 years will be included. Subjects may or may not use statins. ***Only subjects who sign an informed consent are included to the study.***

EXCLUSION CRITERIA

- Type 1 diabetes
- Apo E2/2 phenotype
- ALT/AST > 3x ULN
- GFR < 60 ml/min, clinically significant TSH outside normal range
- Lipid-lowering drugs other than statins within 6 months
- Treatment with pioglitazone, insulin, sulphonylureas, gliptines, glinides, SGLT-2 inhibitors or thiazide diuretics (at a dose of > 25 mg / day) within 6 months
- Blood pressure > 160 mmHg systolic and/or > 105 diastolic
- History of pancreatitis or stomach / other major bleeding, thyroid neoplasia, persistent hypothyroidism or persistent hyperthyroidism
- Any medical condition that puts the patient in the risk of dehydration
- Concurrent medical condition that may interfere with the interpretation of efficacy and safety data during the study.
- Females of childbearing potential who are not using adequate contraceptive methods
- Subjects who have experienced side-effects previously from GLP-1 agonists
- Non-compliance or withdrawal of consent
- ***Any information or clinical event decribed in liraglutide SPC that is a contraindication for the use of liraglutide (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001026/WC500050017.pdf)***

WHITHDRAWAL FROM THE STUDY

- Withdrawal of consent
- Clinical information/condition/adverse event that potentially endangers study subject/study results judged by the PI of the study
- ***any information or clinical event decribed in liraglutide SPC that is a contraindication for the use of liraglutide (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/001026/WC500050017.pdf)***

Restrictions during the study:

- No aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) within 1 week before heparin tests (LPL / HL) and biopsies
- No alcohol or strenuous exercise within 72 hours of each special test
- Serum amylase and lipase is followed at screening, week 8 and end of liraglutide treatment period or at clinical suspicion of pancreatitis

STUDY VISITS

All subjects will undergo a screening visit. Before screening, the subjects may visit the study center for screening laboratory tests. After giving extensive oral and written information, a written informed consent form will be obtained from the subjects at the first visit before screening procedures. Subjects will be informed on all adverse events related to all the study procedures they will be submitted to both orally and in writing.

Subjects eligible for the study will enter the run-in phase and treated with Metformin 2 g/day and Simvastatin 20 mg/day for 4 weeks. In the treatment period, subjects are randomized to Liragutide or placebo.To keep subjects blinded, Liraglutide and placebo will be visually identical and be packed. Liraglutide and placebo will be provided by Novo Nordisk A/S. Investigators are not blinded in order to advice subjects

in placebo group to lose weight 3 kg during the study. Dose will be escalated to Liraglutide 0.6 mg / placebo 0.1 ml for one week, Liraglutide 1.2 mg/ placebo 0.2 ml one week and thereafter Liraglutide 1.8 mg / placebo 0.3 ml s.c. daily injections.

Thereafter the baseline visits will include an OGTT, heparin test, non-fasting kinetics and abdominal MRI investigation days. The investigational product (Liraglutide or placebo) will be given to the subjects together with detailed instructions about the usage and storage ***(Shelf life 30 months. After first use: 1 month. Storage in a refrigerator (2°C - 8°C). Do not freeze. Store away from the freezer compartment.)*** ~~(in +2 -+8 degrees, opened product in +2 -+29 degrees)~~ of the product during these visits. After the study period of total 4 moths these investigations are repeated. In addition to the screening visit(s), the subjects will undergo 8 investigational visits. The subjects meets dietician after randomization. The follow-up about weight, diet and possible adverse events takes place with weekly phone calls/e-mail contacts by the study nurse/dietician/doctor. Subjects fill in dietary records for 3 days before and during the investigational drug. At week 8 after initiation of the treatment period a blood sample for safety is draw, weight is recorded and the patient meets dietician/study nurse if necessary. In case of unsuccessful weight loss, weight loss > 1.5 kg/month or adverse events, an additional visit is arranged. The duration of the study is 1-1,5 month run in and 4 to 4,5 month investigational phase i.e. 5 to 6 months. The subjects will be followed by a phone call/e-mail/visit 2 weeks after the end of the treatment period.

STUDY PROTOCOL: We will combine the novel kinetic protocol developed by us with several advanced analyses to elucidate how incretins modulate postprandial lipidemia and hepatic DNL in T2D subjects. To specifically elucidate the impact of a GLP-1 agonist on postprandial lipid metabolism, we will perform kinetic studies on all study subjects before and after the 4-months intervention with the GLP-1 agonist.

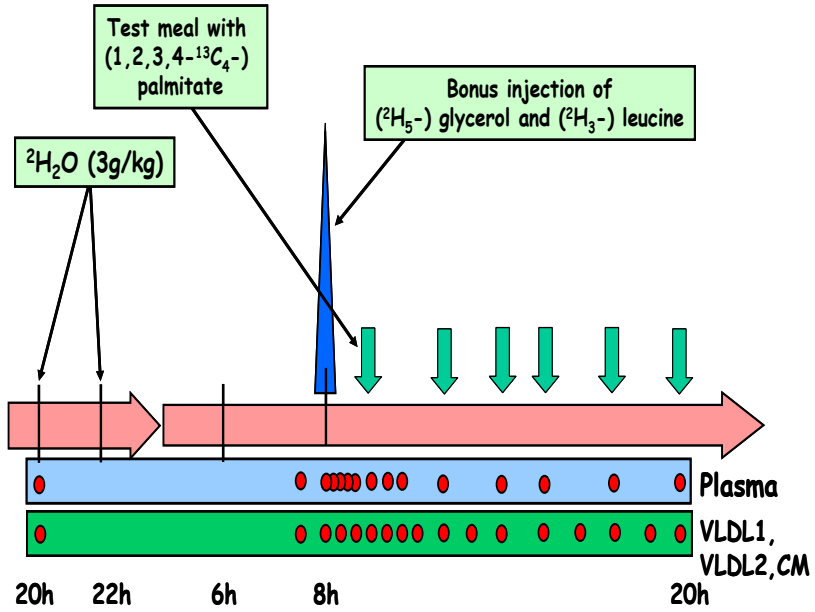


Figure 4. ***Detailed schedule for lipoprotein kinetic assessment****. Red dots represent plasma samples for kinetic data (glycerol, leucine and palmitate enrichment in TRL fractions) and green arrows illustrate time-points for plasma incretin (GLP-1, GIP), glucose and insulin sampling.*

1.Kinetic study with stable isotopes combined with an oral fat load. This study is based on the novel protocol developed by us (Figure 4). A detailed description of analysis is given in **Appendix A**.

*Tracer labelling.*On the evening of the first visit, ^2H_2O (2 g/kg) will be given between 18:00 and 22:00 to assess the contribution of de novo lipogenesis to fatty acids in VLDL (Figure 3).At 8:00 the following morning, the subjects will receive a bolus injection of $^2H_5$-glycerol (500 mg)to assess the VLDL–triglyceride kinetics, and a bolus injection of $^2H_3$-leucine (7 mg/kg) to assess VLDL–apoB100 and chylomicron–apoB48 kinetics. The fat rich mixed meal (57 g fat, 63 g carbohydrates and 40 g protein, total 934 kcal) with $^{13}C_4$-palmitate tracers (1-2 g) is served 2 h after the infusions begins.

Sampling and sample preparation. Blood samples will be taken at frequent time points as indicated by the red dots (Figure 4) and lipoprotein fractions (chylomicrons, $VLDL_1$ and $VLDL_2$) will be separated by density gradient ultracentrifugation (DGU). Concentrations of lipids and apolipoproteins (apo-B100, apo-B48) will be measured in whole plasma and in all fractions at each time point.

Quantification of lipoprotein fluxes. Enrichments of isotopes in lipids (TG, FAs) and in apo-B100 and apo-B48 are determined by GC/MS [28] and/or by proteomics [29] at each time point (red dots). Hepatic DNL is calculated from enrichment of deuterated water ingested during the kinetic study at specified time points (0, 4 and 8 hrs.) [30]. We will use a novel multicompartmental model to describe the fluxes of materials combining apoB100 and apoB48 kinetics [7, 28, 29].

2. Determination of liver, subcutaneous and intra-abdominal fat. Using standardized protocols, we will perform a proton magnetic resonance spectroscopy (MRS) to determine liver fat content and magnetic resonance imaging to determine subcutaneous abdominal and intra-abdominal fat. [31]

3. Lipolytic enzymes, advanced lipid analysis, fat biopsies and genetic studies. We will analyze mass and enzyme activity of LPL and hepatic lipase after an injection of 75 IU/kg heparin. RLP-TG and RLP-cholesterol will be measured at 0, 4, and 8 hrs. A fasting sample is obtained for LDL and HDL size determination.

4. Oral glucose tolerance test. An oral glucose tolerance test will be performed as indicated after overnight fast at screening and the end of the 4 months intervention with the GLP-1 agonist. Glucose and insulin will be measured at 0,5,10,30,60,90,120,180 and 240 minutes and modeling of β-cell function is performed [32]. Samples of markers of energy balance (FFA, β-OHB) will be taken at three time-points (0, 4, and 8hrs) after the oral fat load.

OPTIONAL ANALYSES FOR THE FUTURE STUDIES

1. Advanced lipid analysis, fat biopsies and genetic studies

We will also utilize this protocol to collect material for optional analyses in the future not covered by this budget. We will perform lipidomics profiling of plasma and isolated lipoproteins by GC/MS technology to map lipids and proinflammatory lipid intermediates (with collaborators of principal instigators Matikainen and Borén). We will obtain needle biopsies of subcutaneous abdominal fat after fasting to measure mRNA levels of proinflammatory cytokines and biomarkers and to analyze lipids in adipose tissue. We will also take a blood sample for DNA extraction and identify common single nucleotide polymorphisms in genes that have been verified to play a role in lipid metabolism, insulin resistance, inflammation or oxidative stress

2.Analysis of biomarkers during the oral fat load; apo-CIII, apo-A5, uric acid, inflammatory biomarkers (hsCRP, TNF-α, IL-6, SAA, IL-8, PON, MPO, MCP-1) and PAI-1 at three time-points (0, 4, and 8hrs) after the oral fat load. Spare samples of plasma/serum for future angiopoetin-like protein 4 and other lipid/metabolic parameters is stored at -80 degrees at all time-points during fat rich meal test and OGTT.

3. Analysis of gut microbiome profile and urine microvesicles. Because obesity and diabetes may induce low-grade inflammation and insulin resistance due to changes in gut microbial community, the subjects will also provide a stool sample (using specific tubes and instructions) for analysis of gut microbiota profile before and after liraglutide treatment. A urinary sample for selective purification of nucleic acids and proteins that are potential markers of metabolic state and chronic diseases.

STATISTICAL METHODS AND POWER

The study continues until 13 subjects have completed in both arms. Subjects completing the protocol are included to the statistical analyses. Paired t-test (within group) and ancova (treatment effect) will be used. The number of subjects needed is based on previous studies and are in accordance with a recently published article showing that a 25% difference in VLDL kinetics can be detected with 9 subjects per each group [33].

PRIMARY OBJECTIVE

1) To examine the effect of 16 weeks of treatment with liraglutide on triglycerides (TG) and apoB48 production rates (PR) and/or fractional catabolic rates (FRC) in the lipoprotein fraction chylomicrons (Sf>400) in patients with T2DM treated with metformin. This will be fulfilled by kinetic modeling during a standardized fat-rich mixed-meal test combined with stable isotope administration.

2) To evaluate the for the first time in humans the effect of 16 weeks of treatment with liraglutide compared to baseline on hepatic DNL and liver fat content in patients with T2DM treated with metformin, assessed during a standardized fat-rich mixed meal test.

SECONDARY OBJECTIVES

To evaluate the effect of 16 weeks of treatment with liraglutide compared to baseline in patients with T2DM treated with metformin, assessed during a standardized fat-rich mixed-meal test combined with stable isotope administration on:

- production rates (PR) of TG, apo-B48 and apo-B100 in the lipoprotein fractions $VLDL_1$ (Sf 60–400) and $VLDL_2$ (Sf 20–60)
- fractional catabolic rates (FCRs) of TG, ap-B48 and apo-B100 in the lipoprotein fraction $VLDL_1$ (Sf 60–400) and $VLDL_2$ (sf 20–60)
- fasting and postprandial serum total TG, total cholesterol, and total apo-B, apo-B48, RLP-chol and RLP-TG, apo-CIII and apo-A5 as well as fasting LDL and HDL size. RLP-chol and RLP-TG are surrogate markers of remnants. Apo-CIII and apo-A5 are key regulators of the lipolytic cascade.
- TRL clearance assessed as effect on post-heparin lipoprotein lipase (LPL) and hepatic lipase (HL) activities. LPL and HL are the key enzymes regulating lipolysis of TRLs and remnant removal by the liver.
- fasting and postprandial glucose, insulin and C-peptide, assessed also during an OGTT.
- fasting visceral andsubcutaneous fat depots assessed by MRS in both fasting and in postprandial period.

SAFETY REPORTING

Any adverse events will be reported to Finnish Medicines Agency (FIMEA) according to local procedures. All adverse events will be collected from the first study-related activity (from the signing of the informed consent) and in all following contacts with the study-subject through-out the project. This includes events from the first trial related activity after the subject has signed the informed consent, and until the post treatment follow-up period. The investigators will copy Novo Nordisk Finland Safety department when expediting to FIMEA any serious adverse reactions (SAR) or suspected unexpected serious adverse reactions (SUSAR) which occurred during the use of liraglutide in the study (**appendix B**). ***In addition, all pregnancies in trial subjects occurring during use of a Novo Nordisk Product will be reported to Novo Nordisk.*** The submission to Novo Nordisk Finland will be made within 15 days from the investigator's first knowledge about a valid case.

BUDGET

The study protocol utilizes state-of the art methods to measure the dynamics of postprandial lipemia and extensive and complex assessments (including magnetic resonance imaging/spectroscopy, biochemical and molecular analyses) all of which need to be performed by skilled personnel. We have calculated an overall budget of approximately 574 000€. The budget does not include study medication (liraglutide). The study is financed by an independent grant from Novo Nordisk Ag and by other research grants.

GENERAL CONSIDERATIONS

The clinical part of the study will be conducted in the research facilities of Research Programs' Unit, Diabetes & Obesity, University of Helsinki; Clinical Research Institute, Huch ltd. Biomedicum Helsinki, Finland. Laboratory work and analysis will be made in Helsinki and Gothenburg centers. The study protocol will be subjected to the consideration of the Medical Ethical committee of the Helsinki University Central Hospital and permission from the Finnish Medicines Agency (Fimea) will be obtained. The study will be conducted according to GCP and GLP standards.The subjects taking part to the study are insured by a patient insurance taken out by the Helsinki University Central Hospital.

Research responsibilities: Niina Matikainen, MD, PhD, Principal Investigator, responsible for clinical part including kinetic studies, associated laboratory work, biomarker analyses, data-analyses and report, manuscript preparation, main Clinical Investigator, subject recruitment , adipose tissue biopsies, **Kirsi Pietiläinen, MD, PhD** adipose tissue biopsies and analyses, dietary counseling, data analyses, manuscript preparation; **Marja-Riitta Taskinen, MD, PhD**, responsible for clinical part including kinetic studies, associated laboratory work, biomarker analyses, data-analyses and report, manuscript preparation; **Sanni Söderlund, MD, PhD**, Clinical Investigator, screening and recruitment of T2D subjects, kinetic studies, data analysis, manuscript preparation; **Leonie Boegl, MS** dietary counseling and analyses of dietary records**; Niina Lundblom, MD, PhD** and **Antti Hakkarainen, MD** imaging and data analysis.

Team at University of Gothenburg Sweden: **Prof. Jan Borén, MD, PhD**, Principal Investigator, Project leader for kinetic analyses, responsible for all GC/MS work and method validation, data analyses and manuscript preparation; **Martin Adiels, PhD**, responsible for multicompartmental modelling and statistics; **Marcus Ståhlman**, **PhD**, responsible for lipidomics platform and analysis; **Prof. Fredrik Bäckhed, PhD**, responsible for studies on microbiota

TIME-TABLE AND PUBLICATION PLAN

The ethical permission is obtained in January 2014. After permission, the screening will start in February 2014 and recruitment will continue for a year. First subjects enter the run-in period in March-April 2014 and the intervention phase in May 2014. The clinical studies are expected to be completed in June 2015. The laboratory and mathematical analysis will be performed in 6 months and the preparation of the manuscript(s) will take additional 3-6 months and expected to be submitted to international high-quality medical journal(s) in Jan-Jun 2016. ***All publications will be sent for a courtesy review by Novo Nordisk at least 3 weeks prior to submission.*** Conference abstracts may be submitted before the completion of the whole study. The publication policy including the authors appearing in the publication(s) is decided by the principal investigators.The investigational data is collected and filed in Research Programs' Unit, Diabetes & Obesity, University of Helsinki; Clinical Research Institute, Huch ltd. Biomedicum Helsinki, Finland according to GCP and ethical legislations.

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