Project description:

<u>Title</u>:

Effects of metformin on hepatic fatty acid metabolism in patients with type 2 diabetes assessed by positron emmission tomography.

Investigator and sponsor:

Lars Christian Gormsen, consultant, PhD
Department of Nuclear Medicine & PET Center
Palle Juul-Jensens Boulevard 165
Dk-8200 Aarhus N
Tel: +45 7845 6260
Cell: +45 2281 1631
lars.christian.gormsen@clin.au.dk

Version 2.4.1 - date 4/2 2013

Regional Scientific Committee Region Midtjylland: 1-10-72-522-12
Clinical Trials Gov ID: NCT01729156
Data Protection Agency: 1-16-02-303-12

<u>Introduction and purpose:</u>

Type 2 diabetes (T2D) is characterized by insulin resistance, impaired glycemic control and elevated levels of potentially harmful circulating lipids. One of the most widely used drugs to control blood sugar levels in patients with T2D is metformin, which has well known inhibitory effects on hepatic gluconeogenesis, but also possesses poorly understood lipid lowering effects. Thus, metformin supresses circulating triglycerides to an extent that is not fully explained by decreased hepatic de novo lipogenesis. The pharmacological effects of metformin are thought to principally occur in the liver, which has been a serious obstacle to human studies. The liver is an inaccessible organ and any experimental procedures designed to uncover intracellular reactions has previously involved surgical procedures with biopsies. Advances in molecular imaging may change that. Positron emission tomography (PET) is a new and advanced technology in which tracer amounts of positron emitting isotopes are attached to the molecules under investigation, in this case fatty acids. This non-invasive technique allows quantification of the fate of fatty acids in the liver in vivo and can therefore be used to assess how large a proportion of lipids that are oxidized, reesterified or resecreted from the liver. It is therefore our aim to employ the PET scanning technique to study the effect of metformin on hepatic fatty acid handling in the liver in patients with type 2 diabetes.

Specific purposes:
- By [11C]palmitate PET scans to investigate hepatic fatty acid uptake, oxidation and resecretion as very-low-density-lipoprotein (VLDL) in a population of patients with newly diagnosed T2D. Patients will be studied in a parallel group, placebo controlled, double-blinded study before initiation of metformin therapy and after 3 months treatment. An age- and BMI matched group treated with metformin for 3 months will serve as healthy controls.
- By whole body [1-14C]VLDL-TG to quantitate hepatic production and secretion of VLDL-TG before and after metformin treatment.
- By 14CO2-breath collection to quantitate the effect of metformin on whole body oxidation of VLDL derived fatty acids
- By [3-3H]glucose infusion to quantitate the impact of metformin on whole body glucose disposal as well as endogenous glucose production and to correlate any changes with changes in lipid metabolism
- By dual X-ray absorptiometry to determine the impact of metformin treatment on body composition

It is our hypothesis that T2D hypertriglyceridemia is primarily caused by defective hepatic oxidation of fatty acids with subsequent increased resecretion as VLDL-TG. We also hypothesize that treatment for 3 months with metformin will normalize hepatic fatty acid oxidation resulting in diminished reesterification and resecretion as triglycerides.

<u>Background:</u>

The prevalence of diabetes in general and T2D in particular has been growing steadily during the past decades(1). T2D is most often associated with obesity and is characterized by insulin resistance and elevated blood glucose but also by profound dyslipidemia. This is the so-called diabetic lipid triad of elevated low-density-lipoprotein (LDL) cholesterol, decreased high-density-lipoprotein (HDL) cholesterol and hypertriglyceridemia(2). Each component of the dyslipidemic triad is associated with

increased risk of cardiovascular disease and mortality and is therefore a potential risk factor for diabetic patients.

Triglycerides are transported in the circulation packaged in lipoproteins that are large particles consisting of scaffolding and surface proteins (among them ApoB's) with a core of various lipids. ApoB48 containing chylomicrons dominate in the immediate postprandial period, whereas ApoB100 containing VLDLs are secreted continuously albeit at a slower rate after feeding(3). VLDL associated TG (VLDL-TG) is formed in the liver by a series of processes involving reesterification of fatty acids released from subcutaneous and visceral adipose tissue depots. VLDLs serve as transport molecules ensuring a constant exchange of lipids between adipose tissue, muscle and the liver and also "absorb" any surplus fatty acids not oxidized by fuel consuming tissue and released due to bursts of exercise or catecholamine stimulation. In healthy humans, the total pool of circulating VLDLs is surprisingly stable as is the production, secretion and elimination of VLDL particles. In patients with diabetes, by contrast, release of fatty acids from adipose tissue depots exceeds what is oxidized in the liver and in muscle, and as a result, more fatty acids are reesterified and resecreted as VLDL-TG. This expands the VLDL-TG pool leading to diabetic hypertriglyceridemia. What causes this inbalance between released and oxidized fatty acids is still somewhat unclear. It has been argued that the major culprit is adipose tissue resistance with increased release of fatty acids, but also defects in insulin action in the liver and increased de novo lipogenesis due to elevated blood sugar has been suggested. To date, the precise pathophysiology remains obscure.

Treatment of diabetic hypertriglyceridemia is aimed at preventing formation of VLDL particles in the liver(4). Statins are a series of drugs originally designed to inhibit cholesterol production that also prevent hepatic formation of mature VLDL particles due to lack of cholesterol content. Inhibition of VLDL-TG substrate delivery to the liver can be achieved by blocking the hormone sensitive lipase with acipimox, a nicotinic acid analogue(5), or by increasing fatty acid oxidation with fibrates(6). Somewhat surprisingly, the glucose lowering biguanide, metformin, also reduces levels of circulating triglycerides. This has previously been attributed to diminished conversion of glucose to lipids via hepatic de novo lipogenis, but recent studies indicate that metformin may affect lipid metabolism more directly(7), either in the liver or in the periphery. In vitro and animal studies have thus demonstrated that metformin may increase hepatocyte fatty acid oxidation(8). Metformin has also been shown to increase endothelial lipolysis of VLDL derived TGs(9) releasing more fatty acids for oxidation in muscle. Whether the effect of metformin is primarily hepatic or peripheral or a combination of both is still as unclear as whether diabetic dyslipidemia is caused by hepatic or peripheral insulin resistance. The advent of the PET technique may very well change this.

In short, the PET scan technique involves tracing the fate of certain metabolites in vivo. This is done by incorporating a positron emitting isotope into any molecule being investigated (the radiotracer), inject it as a bolus into the patient and subsequently monitor time-activity curves in various organs. Organ time-activity curves reflect radiotracer exchange between the circulation and intracellular pools e.g. lipid droplets in the liver or VLDL-TG particles ready for secretion. Using dynamic PET scans, Iozzo et. al have validated a kinetic model that accurately describe the hepatic fate of [C11]palmitate(10, 11) but [C11]palmitate can also be used to estimate myocardial fatty acid uptake and oxidation(12). PET scans are non-invasive and are in theory only limited by the field of view of the scanner, typically ~20 centimeter. Estimating fatty acid metabolism in a single scan is thus feasible in neighboring organs like the liver, visceral fat and myocardium (see figure 1).

In addition to the fatty acid tracer, we have in our lab developed and validated a whole body VLDL-TG tracer, [1-14C]VLDL, which enables measurements of VLDL-TG production, secretion and oxidation in tissues outside the PET scanner field of view(13, 14). Combining whole body [1-14C]VLDL tracer kinetics with [C11]palmitate PET scans thus yields simultaneous measurements of hepatic and peripheral lipid metabolism (see figure 2).

It is therefore our aim to establish whether the lipid lowering effect of metformin is a result of increased hepatic fatty acid oxidation or increased oxidation of VLDL derived TGs in skeletal muscle. Insights into the mechanisms of metformin action will provide us with an opportunity to tailor lipid lowering therapy in diabetes patients.

METHODS:

Design: The study will be a randomized, double-blinded, placebo-controlled parallel study with 24 newly diagnosed type 2 diabetes patients and 12 age, sex and BMI matched healthy controls. Patients and controls will be studied before intervention and after 3 months treatment with either metformin (2 x 1000 mg) or placebo (T2D patients). Healthy controls will be studied after 3 months metformin treatment (see figure 3).
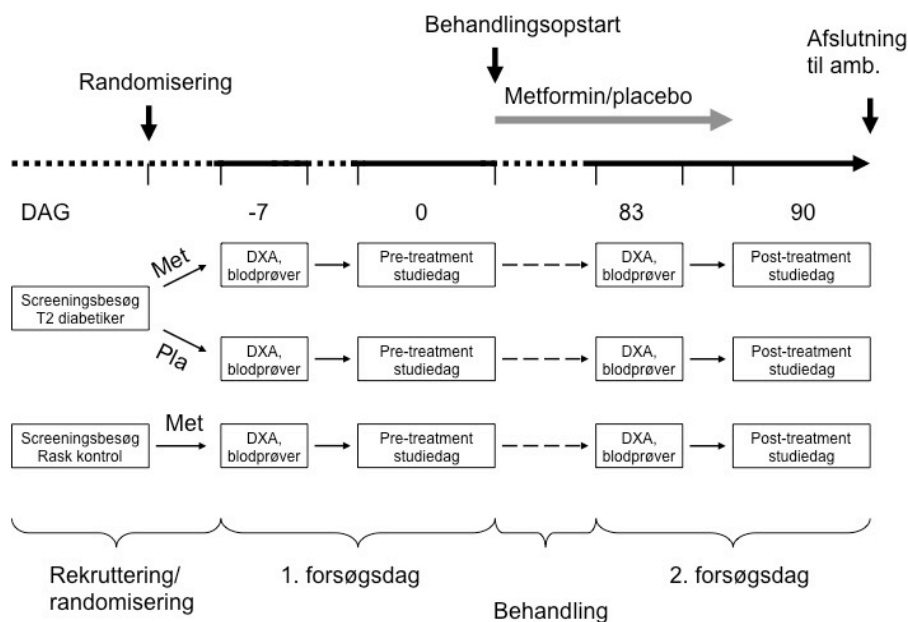
Intervention T2D patients: Treatment with metformin or metformin placebo: 500 mg twice daily for 2 to 4 weeks followed by 1000 mg daily for the remaining 8-12 weeks. Dose will be reduced in patients with moderate renal insufficiency (eGFR 50-80 ml/min. = 25-50%, eGFR 30-50 ml/min. = 50-75%). Treatment will be initiated after day 0 for a total of 90 days. In this patient cohort, the study is double-blinded and randomized.

Intervention healthy controls: Treatment with metformin or metformin placebo: 500 mg twice daily for 2 to 4 weeks followed by 1000 mg daily for the remaining 8-12 weeks. Treatment will be initiated after day 0 for a total of 90 days. In this patient cohort, the study is not blinded –subjects serve as their own controls (before and after treatment).

Study subjects: 24 newly diagnosed T2D patients >50 years will be recruited by physicians in the endocrine outpatient unit (MEA, Aarhus University Hospital). 12 healthy controls will be recruited by advertisements in local papers.

Inclusion criteria patients:
- Newly diagnosed (>3 and <12 måneder) Type 2 diabetes
- Age 50-70 year
- BMI<40

Inclusion criteria healthy controls:
- Age 50-70 year
- Sex and BMI matched with included patients

Exclusion crtiteria patients
- Metformin treatment >6 months
- Liver disease in the form of NASH (non-alkoholic steatohepatitis)
- Cancer
- Anaemia
- HbA1C>8.5 %
- Other clinically relevant abnormal biochemical parameter
- Chronic or acute pancreatitis
- Known substance or alcohol abuse
- Allergy towards metformin
- Claustrophobia
- Extreme obesity > 130 kg

Study power: We have previously studied T2D patients and found that their average VLDL-TG secretion was ~87 µmol/min. Interventional studies using metformin to treat patients with type 2 diabetes report TG concentration decreases of ~10 %(15), 10 %(16), 13 %(17), 17 %(18) and 17 %(19) and an extensive meta-analysis of clinical studies have shown an average decrease of ~10 %(20). Total TG concentration reflects TG in all lipoprotein fractions but there are strong indications that metformin primarily affects VLDL associated TG. Since the proportion of TG in VLDL particles is 60-75 % (21), VLDL-TG concentration will decrease more than 10 %, estimated 14 %. In addition, we have previously observed a 50 % greater decrease in VLDL-TG *production* than VLDL-TG *concentration* during insulin stimulation (22). In our view, metformin treatment in the dose proposed by us will therefore result in a decrease in hepatic VLDL-TG production ~20 % or 0.20 x 87 µmol/min = 17 µmol/min. Based on our previous studies in comparable patient populations(23), the SD of VLDL-TG secretion

change is 13 µmol/min. α is set at 0.05 and power β set at 0.8. We will then have to recruit ~11 participants in each group.

Scans: *PET/CT scans:* PET/CT scans will be preceded by at least 6 hours fasting. A catheter will be placed in an antecubital vein for injection of the radiotracer. First, a low dose CT scan will be performed in order to aid anatomical localization of the liver and for attenuation correction of the subsequent PET scan. Second, 380 MBq [C11]palmitate is injected as a bolus over 10 seconds. Dynamic imaging of [C11]palmitate will be done by list mode aquisition of data in 32 frames of increasing length (from 5 s a frame in the first minute to 600 seconds a frame in the end of the PET study). The PET scan will last a total of 50 minutes. Arterialized blood samples will be obtained in order to measure radioactive palmitate metabolites (TGs, phospholipids and CO_2). The input function will be corrected for radioactive metabolites.

PET data analysis: *Kinetics:* Hepatic metabolism of the radiotracer [C11]palmitate will be calculated based on a 3-compartment model developed and validated by Iozzo et. al at Turkku PET Center. In this model, the input function is based on image derived data from regions of interest (ROIs) in the aorta, portal vein and hepatic right lobe. The dual input from the hepatic artery and the portal vein will be weighted with 80 % to the portal vein and the remaining 20 % from the artery. The analysis yields the parameters hepatic fatty acid uptake (K1), hepatic fatty acid oxidation (k2), resterification (k3) and resecretion as VLDL-TG (k4).

[1-14C]VLDL-TG metabolism: Study subjects will be transferred to the Medical Research Lab immediately after the PET scan and a bolus (~10 mCi) ex-vivo labeled [1-14C]VLDL will be injected. Blood samples will be taken every five minutes for 30 minutes and every 30 minutes for the following 2,5 hours in order to estimate VLDL-TG secretion. Oxidation of VLDL-TG will be assessed by collecting breath samples every 30 minutes and analyzing them for $14CO_2$ content.

[3-3H]glucose metabolism: [3-3H]glucose will be infused continously during the basal period and subsequently for another 2 hours during a hyper-insulinemic euglycemic clamp (HE clamp). HE-clamping is utilized to estimate whole body insulin sensitivity in subjects and can also be employed to asses hepatic endogenous glucose production.

Body composition: Both patients and healthy controls will have their body composition assessed by dual X-ray absorptiometry (DXA) (Hologic). In the DXA scanner, minute amounts of X-rays are used to determine various tissue attenuation patterns that reflect body composition.

Blood samples: Serum insulin, C-peptide, cortisol and glucagone will be measured by Radio-Immuno-Assay (RIA); interleukin-6 (Luminex); retinol binding protein 4 (Elisa); plasma

glucose (YSI STAT 2300 Glucose Analyzer), glycerol and lactate (Floruometric enzymatic method), serum non-esterified FFAs (radiochemical assay), plasma acyl og desacyl ghrelin.

Patient data: Demographic data and other information regarding patients and disease history are collected before the onset of the trial. All information are recorded in the Case Report Form (CRF).

Data and permissions: All electronically collected data are archived on the PET Center server (Tiger). Manual data are kept in a locked room at the PET Center. All participants are registered under a study subject ID with initials provided by the blinding and randomization authority – The Hospital Pharmacy. Participants are aware that the local Ethical Committee and the Danish Data Agency must have access to all data. The study is approved by the Danish Medicines Agency (EUDRA-CT 2012-000808-16), The Danish Data Agency and the local Ethical Committe (1-16-02-303-12).

Radioactivity dose:

PET scan with low dose CT to determine hepatic fatty acid metabolism ([^{11}C]palmitate):

According to the lastest ICRP 106, maximal realistic effective dose for C11-labeled tracers is 0.011 mSv/MBq. We plan to inject 380 MBq per study day and the total injected dose will then be:

0.011 mSv/MBq x 380 MBq/day x 2 days = 8,36 mSv.

Low dose CT of the abdominal region is 1 mSv.

Total PET related radiation dose is then 9,36 mSv. The [14C]VLDL tracer yields an additional 0,4 mSv per study day and the DXA scan 0,15 mSv.

Accumulated radiation dose: <u>9,91 mSV</u>.

This dose is roughly equivalent to half a diagnostic CT of the thorax, abdomen and pelvis (18 mSv). It is also euquivalent to 3 times the normal background radiation (3 mSv per year).
For persons aged 60 this corresponds to an additional risk of dying from cancer of about 0.0005 (0.05 %) which is comparable to smoking 9 packets of cigarettes or driving 6000 kilometers in a car (http://www.xrayrisk.com/ (24)).

Ethics:

The study is performed according to the principles of the Helsinki declaration II and has been approved by the local Ethical Committee, the Danish Medicines Agency and the Data

Protection Agency. All PET related procedures have been performed at the PET Center numerous times both in an experimental and a clinical setting. The Clinical Research laboratory has extensive experience in doing metabolic studies and has experienced no serious adverse study related events for the past 5 years.

Rationale for the study:

Hypertriglyceridemia is almost universally present in patients with T2D and has

been convincingly demonstrated to be a serious risk factor for the development of cardiovascular disease (CVD). Since CVD is associated with an increased morbidity and mortality, many ongoing studies are designed to foster ideas of how to lower potentially dangerous circulating lipids. In this context, it is of great interest to determine whether the lipid lowering effect of metformin is independent of its beneficial effects on glucose metabolism and whether the effect indeed hinges on improved or "normalized" hepatic fatty acid oxidation. If that is the case, future lipid lowering treatment strategies in patients with T2D should include drugs with a different mode of action, e.g. statins or liraglutide rather than fibrates. This study is therefore directly designed to modify treatment of a large group of patients and the risk of using ionizing radiation (category 2B) is in our opinion acceptable. To further minimize radiation related risks, the study is restricted to patients >50 years.

Publication:

Lars Gormsen, Søren Nielsen and other associated researchers will cooperate in gathering data, interpretating results and writing the papers. All results, both negative and positive, will be reported. The Vancouver Criteria will be adhered to.

Reference list

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes care 2004;27(5):1047-53.
2. Goldberg IJ. Diabetic Dyslipidemia: Causes and Consequences. Journal of Clinical Endocrinology & Metabolism 2001;86(3):965-71. doi: 10.1210/jc.86.3.965.
3. Beisiegel U. Lipoprotein metabolism. European heart journal 1998;19 Suppl A:A20-3.
4. Haffner SM. Management of dyslipidemia in adults with diabetes. Diabetes care 1998;21(1):160-78.
5. Taskinen MR, Nikkila EA. Effects of acipimox on serum lipids, lipoproteins and lipolytic enzymes in hypertriglyceridemia. Atherosclerosis 1988;69(2-3):249-55.

6. Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. Biochimica et biophysica acta 1996;1302(2):93-109.

7. Davidson MB, Hu T, Sain G, Hoar B, Stevenson C, Hoogwerf BJ. The relationship of glycaemic control and triglycerides in patients with diabetes mellitus: a PreCIS Database Study. Diabetes, obesity & metabolism 2009;11(2):118-22. doi: 10.1111/j.1463-1326.2008.00912.x.

8. Zhou G, Myers R, Li Y, et al. Role of AMP-activated protein kinase in mechanism of metformin action. The Journal of clinical investigation 2001;108(8):1167-74. doi: 10.1172/jci13505.

9. Ohira M, Miyashita Y, Murano T, Watanabe F, Shirai K. Metformin promotes induction of lipoprotein lipase in skeletal muscle through activation of adenosine monophosphate-activated protein kinase. Metabolism: clinical and experimental 2009;58(10):1408-14. doi: 10.1016/j.metabol.2009.04.024.

10. Iozzo P, Bucci M, Roivainen A, et al. Fatty acid metabolism in the liver, measured by positron emission tomography, is increased in obese individuals. Gastroenterology 2010;139(3):846-56, 56 e1-6. doi: 10.1053/j.gastro.2010.05.039.

11. Guiducci L, Jarvisalo M, Kiss J, et al. [11C]palmitate kinetics across the splanchnic bed in arterial, portal and hepatic venous plasma during fasting and euglycemic hyperinsulinemia. Nuclear medicine and biology 2006;33(4):521-8. doi: 10.1016/j.nucmedbio.2006.02.003.

12. Tamaki N, Fujibayashi Y, Magata Y, Yonekura Y, Konishi J. Radionuclide assessment of myocardial fatty acid metabolism by PET and SPECT. Journal of nuclear cardiology : official publication of the American Society of Nuclear Cardiology 1995;2(3):256-66.

13. Gormsen LC, Jensen MD, Nielsen S. Measuring VLDL-triglyceride turnover in humans using ex vivo-prepared VLDL tracer. Journal of lipid research 2006;47(1):99-106. doi: 10.1194/jlr.M500205-JLR200.

14. Sorensen LP, Gormsen LC, Nielsen S. VLDL-TG kinetics: a dual isotope study for quantifying VLDL-TG pool size, production rates, and fractional oxidation in humans. American journal of physiology Endocrinology and metabolism 2009;297(6):E1324-30. doi: 10.1152/ajpendo.00366.2009.

15. Carlsen SM, Rossvoll O, Bjerve KS, Folling I. Metformin improves blood lipid pattern in nondiabetic patients with coronary heart disease. Journal of internal medicine 1996;239(3):227-33.

16. Chu NV, Kong AP, Kim DD, et al. Differential effects of metformin and troglitazone on cardiovascular risk factors in patients with type 2 diabetes. Diabetes care 2002;25(3):542-9.

17. Lawrence JM, Reid J, Taylor GJ, Stirling C, Reckless JP. Favorable effects of pioglitazone and metformin compared with gliclazide on lipoprotein subfractions in overweight patients with early type 2 diabetes. Diabetes care 2004;27(1):41-6.

18. DeFronzo RA, Goodman AM. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. The New England journal of medicine 1995;333(9):541-9. doi: 10.1056/nejm199508313330902.

19. Eriksson A, Attvall S, Bonnier M, Eriksson JW, Rosander B, Karlsson FA. Short-term effects of metformin in type 2 diabetes. Diabetes, obesity & metabolism 2007;9(4):483-9. doi: 10.1111/j.1463-1326.2006.00624.x.

20.   Wulffele MG, Kooy A, de Zeeuw D, Stehouwer CD, Gansevoort RT. The effect of metformin on blood pressure, plasma cholesterol and triglycerides in type 2 diabetes mellitus: a systematic review. Journal of internal medicine 2004;256(1):1-14. doi: 10.1111/j.1365-2796.2004.01328.x.

21.   Mittendorfer B, Patterson BW, Klein S. Effect of sex and obesity on basal VLDL-triacylglycerol kinetics. The American journal of clinical nutrition 2003;77(3):573-9.

22.   Sorensen LP, Sondergaard E, Nellemann B, Christiansen JS, Gormsen LC, Nielsen S. Increased VLDL-triglyceride secretion precedes impaired control of endogenous glucose production in obese, normoglycemic men. Diabetes 2011;60(9):2257-64. doi: 10.2337/db11-0040.

23.   Sondergaard E, Nellemann B, Sorensen LP, et al. Similar VLDL-TG storage in visceral and subcutaneous fat in obese and lean women. Diabetes 2011;60(11):2787-91. doi: 10.2337/db11-0604.

24.   Cohen BL. Catalog of risks extended and updated. Health Phys 1991;61(3):317-35.