

FINAL REPORT

**EFFECT OF LIRAGLUTIDE ON EPICARDIAL FAT
IN SUBJECTS WITH TYPE 2 DIABETES**

INVESTIGATOR-INITIATED STUDY

NCT 2014740

University of Miami Clinical Trial # 20120811

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SUMMARY

Background Epicardial adipose tissue (EAT) is a peculiar visceral fat depot with anatomical and functional contiguity to the myocardium and coronary arteries. EAT is a highly inflammatory tissue enriched with genes involved in endothelial function, coagulation, immune signalling, lipid metabolism, potassium transport and apoptosis. EAT is associated with coronary artery disease, diabetes and obesity. EAT is easily measurable and an emerging therapeutic target that responds and modifies to medications targeting the adipose tissue. Liraglutide, an analogue of glucagon-like peptide-1 (GLP-1), is indicated for the treatment of type 2 diabetes mellitus. Liraglutide has recently shown to reduce cardiovascular risk. Nevertheless, whether liraglutide could reduce EAT is unknown.

Aim The primary objective of this interventional study was to evaluate whether liraglutide can cause a significant and rapid reduction of EAT, organ-specific visceral fat and cardiovascular risk factor itself, in overweight/obese type 2 diabetic patients.

Methods To test this hypothesis we performed a 6-month randomized, open-label, controlled study in 100 type 2 diabetic subjects with body mass index (BMI) $\geq 27 \text{ kg/m}^2$ and HemoglobinA1c (HbA1c) $\leq 8\%$ on metformin monotherapy. Individuals were randomized in 2 groups to receive additional liraglutide up to 1.8 mg sc once daily ($n=55$) or to remain on metformin up to 1000 mg twice daily ($n=45$). Ultrasound-measured EAT thickness was measured at baseline, 3 and 6-month follow ups.

Results In the liraglutide group EAT decreased from 9.6 ± 2 to 6.8 ± 1.5 and 6.2 ± 1.5 mm ($p < 0.001$), accounting for a -29% and -36% of reduction at 3 and 6 months, respectively, whereas there was no EAT reduction in the metformin group; BMI and HbA1c improved only in the liraglutide group after 6 months.

Conclusions. For the first time, we found that liraglutide added to metformin caused an unprecedented, close to 40%, reduction of EAT. EAT reduction was not only quantitatively important, but also very rapid, as the earliest reduction, of almost 30%, occurred within 3 months. We suggest that the recently reported (LEADER study) beneficial cardiovascular effects associated with liraglutide treatment may be EAT-mediated.

PROTOCOL

STUDY RATIONALE

This study was designed to reflect a real-life and common clinical scenario of type 2 diabetic patients presenting with fairly good glucose control, but at mild-moderate cardiovascular risk. We intentionally chose HbA1c $\leq 8\%$ to rule out the confounding effect of a poor diabetes control on study outcomes. Also, as prior treatment with metformin was a criteria for entry into the study, we could evaluate the independent effect of liraglutide on EAT. Epicardial fat has recently shown to be a modifiable and measureable cardiovascular risk factor, and therefore it could well serve as primary outcome of the study. In addition, EAT is known to be higher in subjects with diabetes, due to its strong correlation with visceral adiposity and insulin resistance.

METHODS

Study design

This was a randomized, open-label controlled 6-month study in 100 overweight/obese (body mass index (BMI) $\geq 27 \text{ kg/m}^2$) type 2 diabetic subjects on metformin monotherapy. Patients were screened among those routinely referred to the Division of Endocrinology, Diabetes and Metabolism out-patient clinic at the University of Miami. Approximately 500 patients were screened between January 2014 and January 2016. All the patients provided written informed consent before participation. The study was approved by the University of Miami Institutional Review Board (# 20120811) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Study Criteria and Treatment Arms

Patients were enrolled on the basis of the following inclusion criteria: type 2 diabetes, HbA1c $\leq 8\%$ measured at least 1 month prior to the study, BMI $\geq 27 \text{ kg/m}^2$, prior treatment with metformin only, age ≥ 18 and ≤ 65 years old. Exclusion criteria were the following: type 1 diabetes, current use of insulin, other GLP-1 receptor agonists, dipeptidyl peptidase 4 (DPP-4) inhibitors, pramlintide, sulfonylureas, thiazolidinediones, history of diabetic ketoacidosis, history of diabetic macro or micro vascular complications, known contra-indications to liraglutide, such as previous history of pancreatitis or medullary thyroid carcinoma, personal or family history of multiple endocrine neoplasia type 2, renal or liver or heart failure, acute or chronic infective diseases, cancer or chemotherapy, current use of systemic corticosteroids or in the 3 months prior this study, known or suspected allergy to liraglutide, excipients, or related products, pregnant, breast-feeding or the intention of becoming pregnant. Eligible individuals were randomly assigned to receive additional liraglutide, or to remain on metformin. The liraglutide-group was started on liraglutide (Victoza®) up to 1.8 mg s.c. once daily. Liraglutide was administered with a starting dose of 0.6 mg (for a least one week) and subsequent increments to 1.2 mg (after a least one week) and to 1.8 mg (after at least a week on 1.2 mg). Liraglutide-group subjects had to achieve the final dose of 1.8 mg by at least three weeks from the starting dose. Subjects who were not able to tolerate the dose of 1.8 mg, were advised to lower the dose to 1.2 mg. Patients received full training and titration

instructions by a registered nurse. Metformin regimen was continued. Metformin-group was treated with metformin monotherapy for the duration of the study at the dosage from 500 mg twice daily to a maximum of 1000 mg twice daily. Both groups received lifestyle and diabetes education, as part of the standard care. Patients in both groups were advised to continue with their usual dietary habit and physical activity. Once patient eligibility and written consent were obtained, the study participants were scheduled for the baseline clinic visit. Each patient underwent a transthoracic echocardiographic study, full physical examination and a fasting blood drawn for measurement of blood glucose and haemoglobinA1c (HbA1c) and other parameters. Study patients were then scheduled for follow-up visits at 3 and 6 months and the same visit procedures were repeated at each follow-up visit.

Study procedures

Echocardiographic Epicardial Fat Thickness. EAT thickness was measured according to the method first described and validated by Iacobellis et al. Briefly, EAT was identified as the echo-free space between the outer wall of the myocardium and the visceral layer of pericardium. EAT thickness was measured perpendicularly on the free wall of the right ventricle at end-systole in three cardiac cycles. Maximum EAT thickness was measured at the point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aortic annulus, used as the anatomical landmark for this view. The average value of three cardiac cycles was calculated and used for analysis. Echocardiograms were done by an experienced operator who was blinded to the patients' data. Intra-observer reproducibility of echocardiographic measurement of EAT was assessed by the intra-class correlation coefficient.

Physical Examination All anthropometric measures were obtained by the study nurse at each visit. Height (in cm) and weight (in kg) were measured, and BMI was automatically calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured in the seated position, using an automated cuff and digital readout, and entered by the study nurse into the electronic database. Resting heart rate was also measured

Blood Measurements Fasting blood samples for fasting blood glucose (FBG) and HbA1c were obtained from all individuals according to the study design timetable. Blood parameters were measured according to standard and previously described procedures. Fasting comprehensive metabolic panel and lipid profile were also measured at each visit as standard diabetes care.

Follow up of glucose profile Study patients were advised to monitor their capillary glucose twice daily, fasting in the morning and post-prandially. Glucose profile was tracked weekly by the research coordinator and/or principal investigator who were in contact with study patients throughout MyUHealth e-mail system or by telephone.

STATISTICAL ANALYSIS

Primary outcome of the study was EAT changes on liraglutide combined to metformin vs metformin monotherapy; secondary outcomes were determinants of HbA1c changes during treatment. Assuming a difference of 1 ± 0.5 mm in EAT to be clinically significant and using a significance level of $p<0.05$ and 90% power, the current study sample size was sufficient to detect statistical significant differences in EAT between liraglutide and metformin group. Continuous variables were considered as age-adjusted and sex-adjusted means with their standard deviations (SDs). Patients were randomized consecutively using a computer generated randomization table. Two-sample t test with 95 % CI for difference was performed to evaluate differences between the two groups at baseline. Two-way ANOVA was used to calculate changes (Δ) in EAT and other study variables between baseline, 3 and 6 months in both groups. Relations between study variables were calculated using simple linear regression analysis. Two-tailed $p < 0.05$ indicates statistical significance. Statistical analysis was performed using Minitab 17 Software.

University of Miami - Medical Informed Consent Form
EPROST # 20120811

Consent (Permission) to Participate in a Clinical Research Study

Title of Study:
EFFECT OF LIRAGLUTIDE ON EPICARDIAL FAT
IN SUBJECTS WITH TYPE 2 DIABETES

Principal Investigator: Dr Gianluca Iacobellis MD PhD

Department: Medicine, Division of Endocrinology, Diabetes and Metabolism

Phone Number: 305 243 3203

Email Address: giacobellis@med.miami.edu

Study Contact Name: Dr Gianluca Iacobellis MD PhD

Study Contact Telephone Number: 305 243 3203

Study Contact Email: giacobellis@med.miami.edu

READ THE FOLLOWING CAREFULLY

This consent form contains important information, so that you can decide if you wish to take part in this study. If you have any questions that remain unanswered, please ask the study doctor or one of his/her research study personnel before signing this form.

You are being asked to volunteer to participate in a research study. Before you give your consent (permission) for you to be part of this study, please read the following and ask as many questions as necessary to be sure that you understand what your participation will involve.

PURPOSE

The purpose of this research study is to learn about the effect of Liraglutide, (Victoza®), on the fat of the heart. Excessive amount of the fat around the heart is common in people with type 2 diabetes and can be associated with a poor sugar control. Victoza® is an injectable prescription medicine that can improve blood sugar control in adults with type 2 diabetes.

Revised 12/10/2010

UNIVERSITY OF MIAMI HEALTH SYSTEM
Miami, FL 33136 (305) 243-4000

CLINICAL RESEARCH CONSENT FORM



Form
D4000009E

NAME:

MRN:

AGE: _____ **DOB:**
_____/_____/_____

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NUMBER OF STUDY PARTICIPANTS

If you decide to be in this study, you will be one of approximately 100 people in this research study.

DURATION OF STUDY

The study consists of three visits. Each visit will take around 1 to 1 and 1/2 hours. Your total involvement in the study will be about 6 months.

PROCEDURES

During the first visit you will be assigned, by chance, to one of two study groups (Victoza + Metformin or Metformin only). In each visit (Visits 1-3) you will undergo the same research procedures: basic body measurements as your weight, height, waist and hip circumference; have approximately one tsp of 5 ml blood drawn; and an ultrasound of the heart.

The heart ultrasound (called echocardiogram) is a simple test that measures the morphology of the heart and that can identify the amount of the cardiac fat (called epicardial fat). The procedure is performed by placing a probe on the outside of the chest wall with a gel-like substance to transmit harmless waves into the body. The gel allows the ultrasound beams to “see” your heart. You will be asked to remove your clothing from the waist up and women will be given a gown to wear during the procedure. If you need help, the investigator will assist you in getting onto the exam table, where you will be asked to lie on your left side. The test may take 15 to 30 minutes.

We will also collect information from your medical records for research purposes.

RISKS AND DISCOMFORTS

Medication: Victoza and Metformin may cause mild and usually well-tolerable gastro-intestinal side effects. Victoza may increase the risk of thyroid C-cell tumor, a rare form of thyroid tumor, in mice. No evidences of increased risk of cancer in humans have been reported.

Blood Draw The risks of blood drawing include: fainting, the occurrence of temporary discomfort and/or bruise at the site of puncture; rarely, infection or the formation of a small clot or swelling to the vein and surrounding area may occur.

Echocardiogram

An echocardiogram is painless and safe. It does not use radiation and so carries none of the associated risks.

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There is a low chance for slight discomfort—like pulling off a Band-Aid—when the electrodes are removed from your skin.

Breach of Confidentiality (Data Collection)

There is a risk of breach of confidentiality with your medical record information; however every precaution will be taken to protect the records.

BENEFITS

This research may improve your diabetes control and may also benefit society by gaining new knowledge on the drugs available to treat diabetes.

ALTERNATIVES

You have the alternative not to participate in this study. You can decide to stop participating in this study at any time. Not participating in this study will not affect your medical care. You will receive standard care such as diabetes education, life style and dietary counseling, assessment and monitoring of glucose control, screening and prevention of diabetes complications, regardless of your participation in this study.

COSTS

There is no cost for you in participating in this research study. You are not responsible for costs of the ultrasounds, physical examination, blood draws performed. You will be **not** responsible for the cost of the drugs that will be given (neither Metformin or Victoza or both).

INCENTIVES/PAYMENTS TO PARTICIPANTS

You will not be paid for taking part in this study.

COMPENSATION FOR STUDY-RELATED INJURY

You may be exposed to risk of injury from participation in this study. If injury occurs, treatment will in most cases be available. If you have insurance, your insurance company may or may not pay for these costs. If you do not have insurance, or if your insurance company refuses to pay, you will be expected to pay. Funds to compensate for pain, expenses, lost wages and other damages caused by injury are not available.

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VOLUNTARY PARTICIPATION / WITHDRAWAL FROM STUDY

You participation in this study is voluntary. You may refuse to participate, or withdraw from the study at any time, without penalty or loss of benefits to which you are otherwise entitled. This will not affect the medical care you receive from the study doctor or UM/Jackson Memorial Hospital. You must tell the study doctor if you wish to stop taking part in the study. Your participation in this study may be discontinued, without your consent, at any time by the study doctor, if he/she believes that participation in the study is no longer in your best interest. The Institutional Review Board (IRB), regulatory authorities, or the sponsor may also discontinue your participation in the study.

Dr Gianluca Iacobellis
Division of Endocrinology, Diabetes and Metabolism
1400 NW 10th Ave
Miami, FL, 33136

If you cancel your permission after you have started in the study, the study staff and the Study doctor will stop collecting your health information. Although they will stop collecting new information about you they may need to use the information they have already collected to evaluate the study results. If you start the study and then you cancel your permission, you will not be able to continue to participate in the study.

CONFIDENTIALITY

By signing this consent, you authorize the Investigator(s) and his/her/their staff to access your medical records and associated information as may be necessary for purposes of this study. Your records and results will not be identified as pertaining to you in any publication without your expressed permission. The Investigator and his/her collaborators, staff will consider your records confidential to the extent permitted by law. The Food and Drug Administration (FDA) and Department of Health and Human Services (DHHS) may review these research records. Your health care providers, including authorized University or Hospital staff not involved in the study. Your records may also be reviewed for audit purposes by authorized University of Miami employees or other agents who will be bound by the same provisions of confidentiality.

INCLUSION OF CERTAIN STUDY TEST AND PROCEDURE RESULTS IN YOUR MEDICAL RECORD

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If you are or have been a patient at a University of Miami facility, then you will have a University of Miami medical record. The University of Miami has implemented an electronic medical record system known as UChart, which will improve access to information important to your medical care.

Since this study is related to your medical care, the University of Miami electronic system will show that you are a research participant and the consent form you sign will be included in your electronic medical record. In order to provide as complete a record as possible, some of your study-related research information may also be placed in your University of Miami medical record. This information will be available to University of Miami health-care providers and other authorized University of Miami staff who may not be engaged in the research study but who are involved in the provision of your medical care. The confidentiality of the results and other documents in the University of Miami medical record will be governed by laws, such as HIPAA, that concern medical records.

It is suggested that you tell any non University of Miami provider that you are participating in University of Miami research and that this information may be made available at your request.

The patients that opt to take part in the study will have their name recorded on the consent form. All data for participants will be identified only by a number that will correspond to a list of participants. The list of participants that the identification numbers correspond to will be stored in locked filing cabinets. The list will only be accessed by the principal investigator to confirm the correct identification number is reported by the patient in all visits.

The study site personnel may use your information to notify you of appointments, send you appointment reminders, or schedule additional appointments.

WHOM TO CONTACT

If at any time you have any questions about the study, you may contact **Dr Gianluca Iacobellis** 305- 243-3203

In case of study-related injury, please contact **Dr Gianluca Iacobellis** 305- 243-3203

If you have any questions relating to your rights as a research subject, please contact **the University of Miami's HUMAN SUBJECTS RESEARCH OFFICE (HSRO)**, at **305-243-3195**.

AGREEMENT OF DECISION TO PARTICIPATE

You will receive a copy of this signed informed consent form.

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I have read this consent, which is printed in English (a language which I read and understand). This study has been explained to my satisfaction and all of my questions relating to the study procedures, risks and discomforts, and side effects have been answered. If I have any further questions regarding this study, or in the event of a study-related injury, I should contact the appropriate person named above. Based on this information, I voluntarily agree to give permission (consent) for me to take part in this study.

Signature of Participant

Date

Printed Name of Participant

Signature of Person Obtaining Consent

Date

Printed Name of Person Obtaining Consent

Revised 12/10/2010

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