Date: 12/2/2024

Effects of Glucagon-Like Peptide-1 Agonists on Metabolism and Ectopic Fat Deposition in Chronic Kidney Disease: A Pilot and Feasibility Study

T. Alp Ikizler, MD, Professor
Division of Nephrology
Vanderbilt University Medical Center
S-3223 Medical Center North

Rengin Elsurer Afsar, MD,
Professor of Nephrology at
Suleyman Demirel University, Turkey
Visiting Scholar in Vanderbilt University Medical Center
Department of Nephrology

Lale Ertuglu, MD,
Post Doc Fellow, Ikizler Lab
Vanderbilt University Medical Center,
Department of Nephrology

Jorge Gamboa, M.D., Ph.D.
Research Assistant Professor of Medicine
Vanderbilt University Medical Center,
Division of Clinical Pharmacology

Katherine N. Bachmann, M.D., MSCI
Assistant Professor of Medicine
Vanderbilt University Medical Center,
Division of Diabetes, Endocrinology, and Metabolism

1.0 Specific Aims

Specific Aim 1: To test the hypothesis that GLP-1RA decreases intermuscular fat deposition in patients with stage 3-4 CKD.

Primary Outcome: We will measure intermuscular fat deposition by magnetic resonance imaging (MRI) before and after 12 weeks of dulaglutide 1.5 mg/wk. administration in patients with stage 3-4 CKD.

Specific Aim 2: To test the safety and feasibility of 12 weeks of dulaglutide 1.5 mg/wk administration as an adjunct therapy to the standard care of patients with stage 3-4 CKD.

Safety and tolerability will be evaluated throughout the study by assessment of weight change, hypoglycemic episodes, continuous glucose monitoring (only in patients with type 2 diabetes mellitus), serious adverse events (AE), treatment-emergent AE, discontinuations attributable to AE, laboratory tests, vital signs, 12-lead electrocardiograms, and allergic/hypersensitivity reactions.

2.0 Inclusion/Exclusion Criteria

We propose to study 10 patients with stage 3-4 CKD.

Inclusion criteria:

1. Patients with stage 3-4 CKD (eGFR 15-59 ml/min/1/73 m^2)

2. Age ≥ 18 years and ≤75 years

Exclusion criteria:

1. Patients with type 1 diabetes mellitus

2. Patients with T2D who are on insulin therapy or who started a new antidiabetic medication within 1 month prior to study or who received incretin-based therapy within 3 months prior to study

3. BMI <25 kg/m^2, BMI >40 kg/m^2

4. HbA1c>8% measured within 1 month prior to study, or a history of hypoglycemic episode within 1 year prior to study, or a history of diabetic ketoacidosis

5. Uncontrolled hypertension (>200/100 mmHg) despite optimal antihypertensive therapy

6. Arrythmia, heart failure (NYHA class III-IV), valve disease or heart diseases other than coronary artery disease

7. History of major gastrointestinal surgery, inflammatory bowel disease, pancreatitis or cholelithiasis

8. Personal or family history of medullary thyroid cancer, or personal history of Multiple Endocrine Neoplasia (MEN)-2

9. Pregnancy, breast feeding or intention to become pregnant

10. Previous renal transplantation

11. Acute or chronic infectious diseases

12. Cancer or chemotherapy within 3 years prior to study

13. Treatment with systemic corticosteroids within 3 months prior to study

14. Known or suspected allergy to dulaglutide

15. Claustrophobia or other contraindications for MRI

3.0 Study Enrollment and Design

The study participants will be primarily selected from Vanderbilt's outpatient clinics and the Nashville Veterans Affairs outpatient clinics. The study visits will be conducted at the Vanderbilt University Medical Center (VUMC) Department of Nephrology Outpatient Clinic and Vanderbilt University Institute of Imaging Science (VUIIS). Institutional review board approval and written informed consent will be obtained from all study subjects. Patients meeting inclusion-exclusion criteria will be identified by physician investigators and coordinators. Patients will be first approached by clinical staff. Those who express interest will be informed about the study by the study personnel. The subjects will be selected based on the above inclusion/exclusion criteria.

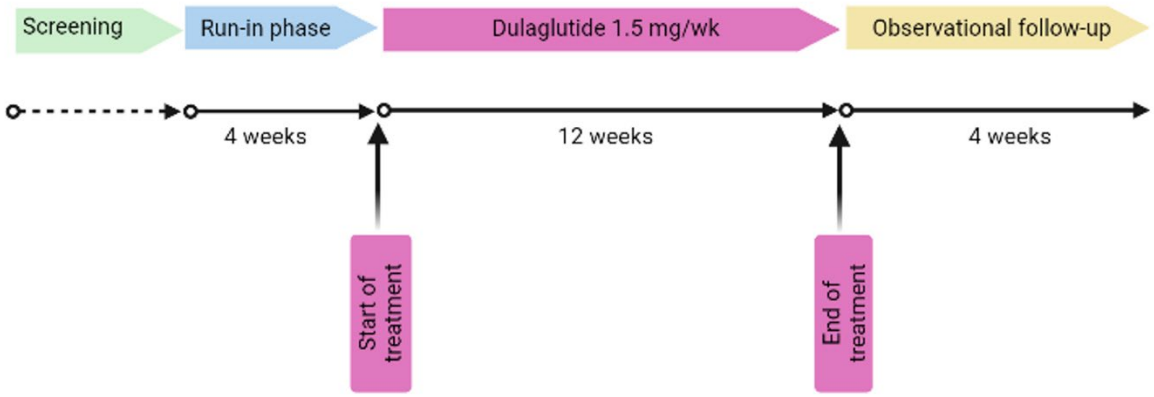

Figure 5.1

All participants will undergo a 4-week run-in phase followed by 12 weeks of treatment (Dulaglutide 1.5 mg/wk), followed by 4 weeks of follow up after discontinuing the study medication (Figure 5.1).

6.0 Study Procedures

After recruitment and review of eligibility, patients who gave informed consent will undergo the following procedures:

Screening Visit: Patients will have a screening visit 4 weeks prior to the beginning of the study intervention to confirm that they meet the inclusion-exclusion criteria. Subjects will be presented detailed information regarding the study with an opportunity to discuss the study as needed for a complete consent discussion. This visit will include a medical history extracted from the patients' medical records, demographic information, a medication history, HbA1c test (for patients with T2D), 12-lead electrocardiogram, vital signs and BMI measurement, urine, or blood pregnancy test (for females of childbearing potential) and serum creatinine test to confirm eGFR. Since the presence of any metal introduces unnecessary risk for MRI, a screening X-ray exam will be performed to confirm the absence of any metal in subjects where that is a possibility.

Visit 1 (Week -4): The purpose of this visit is to determine baseline characteristics of the patients enrolled. We will review medical records, obtain dietary and physical activity recalls

and 12-lead electrocardiogram. We will measure baseline vital signs and perform anthropometric measurements and scheduled blood and urine laboratory tests. We will calculate basal HOMA-IR, QUICKI, ADIPO-IR and LAR. We will perform MRI to assess basal visceral and ectopic fat distribution characteristics and ^{31}P MRS to assess basal skeletal muscle mitochondrial function. Baseline skeletal muscle endurance will be evaluated by six-minute walk test.

Visit 2 (Week 0): Prior to the first dose of the drug, we will review medical records, measure vital signs, perform anthropometric measurements, obtain 12-lead electrocardiogram, dietary and physical activity recalls, laboratory tests in blood and urine, six-minute walk test and perform MRI and ^{31}P MRS (Table 6.1).

The first dose of the study drug Dulaglutide (Trulicity®) will be administered while the participant is at the Nephrology Outpatient Clinic. Patients will be instructed on administration and given information about the instructions for use. Participants will administer dulaglutide on a weekly basis. Safety measures and compliance will be assessed for each patient by phone calls every two weeks.

Visit 3 (Week 12): We will perform the same procedures as week 0 at week 12 (Table 6.1). Study drug administration will be terminated with the last dose given within 7 days before Visit 3.

Visit 4 (Week 16): We will perform the same procedures as week 0 at week 16 (Table 6.1).

METHODS

Anthropometric Measurements:

1. Weight
2. Height
3. Waist circumference (measured at the midpoint between the top of the iliac crest and the lower margin of the last palpable rib in the mid axillary line (inch))
4. Hip circumference (measured at the largest circumference of the buttocks (inch))
5. Waist-to-hip ratio (waist circumference (inches)/hip circumference (inches))
6. Body mass index will be calculated by the following formula:
   BMI= weight (kg)/height^2 (m^2)

Research Laboratory Measurements:

Subjects will be asked to come to the VUMC Department of Nephrology Outpatient Clinic in the morning under fasting conditions for blood and urine sampling. All blood and urine sampling will be performed at the VUMC Nephrology Outpatient Clinic. After blood sampling, samples for serum creatinine and hemoglobin will be transported at room temperature and all other samples will be transported on ice and centrifuged at 3,000 revolutions/min for 15 min before being kept frozen at −80°C. The following parameters will be assessed in blood.

1. Fasting plasma glucose (by glucose oxidase method (Glucose analyzer 2; Beckman Coulter, Brea, CA))
2. Fasting insulin (by double-antibody radioimmunoassay (MilliporeSigma))
3. Fasting free fatty acids (by Methanolic HCl from Supelco (Cat. 3-3051); Measured by GC-FID from Agilent)

4. Leptin (by MILLIPEXMAP Human Serum Adiponectin Panel A kit (MilliporeSigma))
5. Adiponectin (by MILLIPEXMAP Human Serum Adiponectin Panel A kit (MilliporeSigma))
6. Creatinine (will be studied in The Vanderbilt Clinic (TVC) Patient Lab)
7. Hemoglobin (will be studied in TVC Patient Lab)
8. Albumin (by bromcresol green technique)
9. High-sensitivity C-reactive protein (by high-sensitivity particle enhanced turbidometric Unicel Dxl Immunoassay system (Beckman Coulter))
10. Total cholesterol (enzymatically (Cliniqa, San Marcos, CA))
11. Triglycerides (enzymatically (Cliniqa, San Marcos, CA))
12. HDL-C (enzymatically (Cliniqa, San Marcos, CA)

We will assess the following parameters in spot urine samples.

1. Protein (will be studied in TVC Patient Lab)
2. Albumin (Albuwell Hu ELISA kit from Exocell)
3. Creatinine (Creatinine Companion kit from Ethos Biosciences)
4. Sodium (IL 943 flame photometer, Instrumentation Laboratory, 180 Hartwell Road, Bedford, MA)

Albumin/creatinine ratio (mg/g) and protein/creatinine ratio (mg/g) will be calculated in spot urine samples.

eGFR will be calculated by the following formula:

CKD-EPI creatinine equation: eGFR: 141 x $\min(SCr/\kappa, 1)^{\alpha}$ x $\max(SCr/k, 1)^{-1.209}$ x 0.993^{Age} [x 1.018 if female] [x1.159 if black], where SCr is serum creatinine (in mg/dl), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min is the minimum of SCr/κ or 1, and max is the maximum of SCr/κ or 1[1].

Systemic and peripheral IR will be calculated by the following formulas:

HOMA-IR= Fasting plasma glucose (mmol/L)× Fasting insulin (μU/L)/22.5

QUICKI= 1/[log (Insulin μU/mL) + log (Glucose mg/dL) [2].

Adipose tissue IR will be calculated by the following formula:

ADIPO-IR= Fasting FFA concentration (mmol/L) x fasting insulin concentration (pmol/L) [3].

Individual FFA concentrations of palmitic, oleic, linoleic, linolenic, eicosapentaenoic, docosahexaenoic, palmitoleic, palmiteladic, arachidonic, myristic, elaidic and stearic acid will be added to calculate total FFA [3].

Adipose tissue dysfunction will be calculated by the following formula:

LAR: Leptin (ng/ml)/adiponectin (mg/ml) [4].

Magnetic Resonance Imaging and Intermuscular Fat (IMAT) Measurements

On the same day as the VUMC Nephrology Outpatient Clinic Visits, patients will undergo MRI to measure VAT and IMAT. Intermuscular fat will be calculated using consecutive cross-sectional images of the mid-thigh region between the patella and ischial spine. The analysis will be performed in all the quadriceps muscle heads using a custom-written Matlab (Mathwworks, Natick, MA) program, as previously described [5, 6]. IMAT will be defined as the fat beneath the deep fascia of the muscle. IMAT infiltration will be quantified as the ratio between IMAT and muscle volumes [6].

^{31}P Magnetic Resonance Spectroscopy

We will measure *in vivo* knee extensor's mitochondrial function using ^{31}P-MRS to obtain the phosphocreatine recovery time constant, a measure of mitochondrial function, as we described previously [6]. During the 2 days prior to testing, we will ask that subjects 1) to perform no moderate or heavy intensity exercise within 1 day of testing; 2) not to consume alcohol within the 1 day prior to testing; 3) not to consume caffeine or use tobacco within 6 hours prior to testing; and 4) not to eat a meal 3-6 hours before testing. Mitochondrial function will be evaluated using the non-invasive technique ^{31}P-MRS, which provides the quantification and kinetics of phosphocreatine (PCr), other phosphate-energy carrier molecules, and inorganic phosphate in limb muscles. Each subject will lie prone for approximately 30 minutes with a coil positioned over the belly of the vastus lateralis muscle. The position of the coil will be confirmed using scout localizer images and a reference positioned within the center of the coil (Figure 6.1.A).

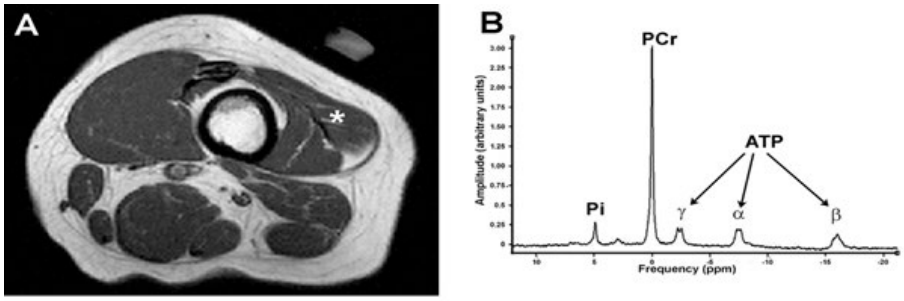

Figure 6.1

After basal measurements, subjects will be asked to perform cycles of knee extension and rest using a research exercise device. Measurements will be recorded during the exercise protocol (approximately 90 seconds) and for additional 5 minutes (recovery period). We will repeat the exercise and recovery cycle at least two times. Spectra will be used to evaluate the kinetics of PCr. The primary endpoint will be the half-time of PCr recovery (time to achieve half of basal concentration during the recovery period). A spectra obtained by the investigators is shown in Figure 6.1.B. This method is reliable and reproducible and has been considered the "gold standard" to measure mitochondrial function [7, 8].

Six-Minute Walk Test

On the same day of VUMC Nephrology Outpatient Clinic Visits, we will measure physical performance by the six-minute walk test. This test will be performed as previously

described [9, 10]. Briefly, patients will be instructed to on a 30-meter corridor at a self-determined pace for a total of 6 minutes. Patients will be allowed to rest briefly by leaning against the wall or sitting during the test, or to stop prematurely if they are unable to complete the test. There will be no practice test, warm up period, or incentive provided for performance. The distance in meters will be recorded. Patients will not be encouraged to perform the test, but they will be informed about the time remaining. The detailed time schedule of the study procedures is shown in Table 6.1.

Study Drug Administration

Dulaglutide (Trulicity®) will be supplied as a 1.5 mg/0.5 mL single dose pen-injector for subcutaneous administration. Drug will be dispensed by the study personnel. Drug will be provided to the participant as labelled by the participant's name at one dispence/monthly. A sharps container and injection cleaning supplies will be provided to the participant. Each participant will be instructed how to self-administer the study drug. Patients will be asked to administer dulaglutide subcutaneously once weekly, on the same day of the week, independent of meals, at any time of the day (but always at the same time of the day in subsequent administrations) in abdomen, thigh or upper arm and will be advised to rotate the injection site. In T2D patients who are using concomitant insulin secretagogues (i.e., sulfonylurea), the dose of the insulin secretagogues will be reduced (i.e., by one-half) to reduce the risk of hypoglycemia.

Dietary recall

The participants will be asked to list the food and beverages you consume for 2 days (one weekday and one weekend day) and bring it back at your next visit (dietary recall). We will ask the patient to complete a diet recall form. The form is to ease patients to remember their diet and to increase the accuracy of diet recalls. Patients will be given the forms prior to their study visits. They will document everything they eat and drink on a weekday and on a weekend day.

Activity Monitor (Physical Activity Recall)

The physical activity monitor is about the size of a watch. It is worn on your non-dominate wrist (for example your left wrist if you are right-handed). Participants will wear this monitor for 7 days. They will wear it 24 hours per day, including when they sleep, shower, or do any other activities. We will also provide a physical activity log to document physical activity during the day.

Table 6.1. Schedule of the Procedures

		Week -4	**Week 0**	**Weeks 0-12**	**Weeks 2, 4, 6, 8, 10**	**Week 12**	**Week 16**
	Screening	**Visit ≠ 1**	**Visit ≠ 2**			**Visit ≠ 3**	**Visit ≠ 4**
Medical History	√	√	√			√	√
HbA1c	√						
Vital signs	√	√	√			√	√
Electrocardiogram	√	√	√			√	√
BMI	√	√	√			√	√
Pregnancy test (If needed)	√	√	√			√	√
Serum creatinine	√	√	√			√	√
Screening X-ray (If needed)	√						
Dulaglutide 1.5 mg/wk			√	√			
Continuous glucose monitoring (only in T2D patients)				√			
Phone calls					√		
Dietary recalls		√	√			√	√
Physical activity monitor		√	√			√	√
Anthropometric measurements		√	√			√	√
Six-minute walk test		√	√			√	√
Research Labs		√	√			√	√
Urine sampling		√	√			√	√
Systemic and peripheral IR calculation							
HOMA-IR		√	√			√	√
QUICKI		√	√			√	√
Adipose tissue IR calculation							
ADIPO-IR		√	√			√	√
Calculation of LAR		√	√			√	√
Magnetic resonance imaging		√	√			√	√
^{31}P magnetic resonance spectroscopy		√	√			√	√

REFERENCES

1 https://kdigo.org/wp-content/uploads/2017/02/KDIGO_2012_CKD_GL.pdf

2 Singh B, Saxena A. Surrogate markers of insulin resistance: A review. World J Diabetes 2010; 1 (2): 36-47.

3 Søndergaard E, Espinosa De Ycaza AE, Morgan-Bathke M et al. How to Measure Adipose Tissue Insulin Sensitivity. J Clin Endocrinol Metab 2017; 102 (4): 1193-1199.

4 Frühbeck G, Catalán V, Rodríguez A et al. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. Adipocyte 2018; 7 (1): 57-62.

5 Kumar D, Karampinos DC, MacLeod TD et al. Quadriceps intramuscular fat fraction rather than muscle size is associated with knee osteoarthritis. Osteoarthritis Cartilage 2014; 22 (2): 226-234.

6 Gamboa JL, Roshanravan B, Towse T et al. Skeletal Muscle Mitochondrial Dysfunction Is Present in Patients with CKD before Initiation of Maintenance Hemodialysis. Clin J Am Soc Nephrol 2020; 15 (7): 926-936.

7 McCully KK, Turner TN, Langley J et al. The reproducibility of measurements of intramuscular magnesium concentrations and muscle oxidative capacity using 31P MRS. Dyn Med 2009; 8: 5.

8 Layec G, Bringard A, Le Fur Y et al. Reproducibility assessment of metabolic variables characterizing muscle energetics in vivo: A 31P-MRS study. Magn Reson Med 2009; 62 (4): 840-854.

9 ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002; 166 (1): 111-117.

10 Martinson M, Ikizler TA, Morrell G et al. Associations of body size and body composition with functional ability and quality of life in hemodialysis patients. Clin J Am Soc Nephrol 2014; 9 (6): 1082-1090.