PROTOCOL IDENTIFICATION

Protocol Title	***Region-specific Adipose Tissues and Liver Changes Associated With Semaglutide Treatment in Chronic Kidney Disease Patients (ADIPOLIVE)***
Unique Protocol Id	**U1111-1328-1600**
Therapeutic Area	CKD, Liver, and Cardiovascular
Primary Product	Semaglutide (drug not requested)
Requested Support (i.e., type of support, dollar amount)	$125,000

INVESTIGATOR IDENTIFICATION

Department Name	Medicine-Cardiology
Institution Name	University of Alberta
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PROTOCOL SCHEDULE

Study Duration	12 months
Recruitment Rate Per Month [if applicable]	6-8
Study Start Date*	September 1, 2025
First Patient First Visit (FPFV) [if applicable]	September 15, 2025
First Patient Last Visit (FPLV) [if applicable]	September 15, 2026
Last Patient Last Visit (LPLV) [if applicable]	November 2027
Study Completion Date	November 2027
Date of Final Study Report Submission to NNCI	January 2028
Anticipated Publication Submission Date	February 2028

Version: 1.1
Date: 15 May 2025

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PROTOCOL SUMMARY

Section 1

1.1 Protocol Summary

Cardio-kidney-liver metabolic disorders (CKLMD) are highly prevalent in western societies and are associated with increased risk of cardiovascular disease. Visceral adipose tissue and accumulation of lipids in the liver are phenotypical changes often seen in patients with CKLMD.
The proposed focus of our project is to phenotype the visceral adipose tissue (AT) of patients with chronic kidney disease (CKD) and assess the prevalence and severity of metabolic associated steatotic liver disease (MASLD), as they correlate with cardiovascular disease (CVD) complications, in patients receiving and not receiving semaglutide. GLP-1RAs represent a very important advancement in the management of diabetes mellitus and the frequently associated CKD. A few investigations suggested that GLP-1RAs have a remodelling effect on AT. Ours will be the first study to link changes in organ-specific AT deposits (epicardial and perirenal) associated with GLP-1RAs therapy to stages of renal function and cardiometabolic load; we will identify different adiposopathy profiles within each CKD stage.
The proposal has two specific objectives, divided into precise tasks related to the general purpose of this project.
In the short-term, our project will contribute to increasing our knowledge of the potential mechanisms of actions of GLP-1RA (i.e modification of AT deposits) and may provide a new way to classify CKD patients as far as their CV risk is concerned.
In the medium to long term, the results derived from this project will bring physicians and healthcare staff closer to a personalized treatment approach, to reduce not only the progression of renal failure but also a set of associated cardiometabolic complications.

PROTOCOL BACKGROUND AND SPECIFIC AIMS

Section 2

2.1 Background, Purpose, and Study Aims/Objective(s)

Chronic kidney disease (CKD) is one of the most prevalent non-communicable chronic diseases, affecting more than 10% of the worldwide population, ie more than 800 million individuals (1,2). According to the WHO, CKD is among the leading causes of morbidity and mortality worldwide, and it is expected to become the 5th most common chronic disease by 2040 (3,4). The progression to end-stage kidney disease (ESKD) is associated with a substantial increase in the risk for cardiovascular disease (CVD), such as stroke, myocardial infarction and heart failure (5,6), which translates into a poor prognosis for affected patients, with annual mortality rates higher than 10% and an estimated five-year survival around 50% (7,8). Similarly, MASLD is the most frequent form of liver disease in

the world and the most frequent cause of cirrhosis and liver transplant. Diabetes mellitus (DM), particularly type 2 (T2DM), arterial hypertension and obesity are well-known risk factors for CKD and MASLD (9-11). In the recent past, visceral AT has garnered attention as the organ potentially connecting these risk factors and underlying cardiovascular risk in patients with CKD. Various methodologies have been used to explore the role of AT dysfunction as a cardiovascular risk and mortality indicator in patients diagnosed with different stages of CKD. Body composition assessment through bioimpedance revealed that the percentage of total body fat has an inverse correlation to kidney function (12) and is an independent risk factor for all-cause mortality in patients with CKD (13). Imaging methods (computed tomography, magnetic resonance and ultrasonography) were also used by diverse research groups, ours amongst others, to evaluate differences in the AT accumulated in specific body areas (region-specific AT), such as the intrabdominal, thoracic (epicardial) and around the kidneys (perirenal), in patients with CKD. Their augmented radiodensity and thickness/volume are associated with an increased cardiovascular risk and a worse prognosis in CKD (14-17). From a proteomic point of view, the cytokines secreted by AT, known as adipokines, have both anti- and pro-inflammatory both locally and systemically. An imbalance in the actions of adipokines in favor of those with a pro-inflammatory function, also associated with insulin resistance and cardiometabolic injury, has been reported in patients with CKD and MASLD (18,19).The aforementioned highlights that obesity, T2DM, CVD, CKD and MASLD are strongly linked through AT dysfunction (*adiposopathy*) and should be considered different aspects of the same spectrum of diseases newly termed adiposity-based chronic diseases (ABCD) (20,21). Accordingly, new trends in CKD management are focusing efforts on adopting an adipocentric approach, in which the identification of AT alteration serves as an earlier diagnosis of kidney dysfunction, allowing a more precise categorization of the cardiometabolic risk in patients with CKD and the implementation of personalized therapeutic plans that prioritize targeting the AT to effectively reduce the morbidity and mortality of this disease (22). Glucagon-like peptide 1 receptor agonists (GLP-1RA) are recommended for patients with T2DM and obesity, and are well suited to address this adipocentric view point in light of their ability to induce weight loss and provide cardiometabolic and renal protection beyond glycemic control (23-25).

The purpose of the study is to explore the link between adiposity and cardio-renal-liver metabolic dysfunction, within a multidisciplinary adipocentric approach (using clinical, imaging and biochemical markers of adiposopathy) across different stages of CKD in patients treated and not treated with semaglutide, both oral and injectable. This study will unveil the association of AT, renal dysfunction and MASLD and provide a view on the potential mechanism of action of GLP-1RA. This may pave the way toward new adipocentric diagnostic and therapeutic models in CKD.

PROTOCOL SIGNIFICANCE

Section 3

3.1 Protocol Significance

a) Adipose tissue as a dynamic endocrine organ

AT is a connective tissue mainly composed of adipocyte cells, which can be divided into three different types according to structure, location and functions. AT should be considered a dynamic tissue, as its cells are in a continuous state of remodeling depending on many factors, such as insulin sensitivity, energy expenditure and inflammatory status (26). Thus, white adipocytes, which contain large globules of fat (lipid droplets), are mainly energy-storing cells that give rise to the white AT (WAT), mainly located in the subcutaneous layer and around the organs (perivisceral), such as the epicardial and perirenal AT (27). On the contrary, brown adipocytes contain smaller lipid droplets and a higher number of mitochondria, that are capable of thermogenic activities via the uncoupling protein-1 (UCP-1). Brown AT (BAT) is located in small depots in the sub-clavicular and axillary areas (28). Beige adipocytes (BeAT) are white AT (WAT) adipocytes that have undergone partial “browning”. Browning is induced by exposure to cold, sympathetic stimulation and certain drugs (29). Some diseases or metabolic disorders could generate the opposite effect, known as whitening, in which BAT becomes white or more pathologic, as described further on.
AT secretes multiple adipokines involved in glucose metabolism, appetite regulation and inflammation, which exert their functions on nearby (paracrine actions) and distant organs (endocrine actions) (26). Leptin and adiponectin are the best known adipokines. Whereas adiponectin is cardioprotective and has anti-inflammatory and antioxidant properties, by inhibiting the expression of inducible nitric oxide synthase (iNOS) and stimulating cyclooxygenase-2 (COX-2) production (30,31), leptin induces cardiac cell hypertrophy, the production of reactive oxygen species (ROS) and macrophage infiltration among endothelial cells, leading to atherosclerosis (32,33). Other less commonly studied adipokines are resistin, visfatin and omentin. Resistin and visfatin behave similarly to leptin, whereas omentin has properties analogous to adiponectin (34-36).

b) The link between adiposity, cardiometabolic dysfunction and kidney disease

As previously mentioned, ABCD is a new concept used to encapsulate the diverse conditions related to adiposopathy, which includes the process of hypertrophy, inflammation and fibrosis of the AT. This process, which ultimately has a negative impact on cardiovascular health, is shared by metabolic diseases (obesity, T2DM) MASLD and CKD (20,21). The adiposopathy process initiates with the expansion of the AT (hypertrophy) via increased accumulation of lipids in the adipocytes, which in the process of gaining volume compress adjacent cells leading to reduced regional blood flow and hypoxia. The hypoxic process activates ROS production and the nuclear factor kappa B (NF-kB). This initiates a pro-inflammatory cascade, with release of interferon-gamma (IF-γ), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL)-6 and IL-1β (37). These factors activate macrophages, which shift to a pro-inflammatory M1 phenotype and infiltrate the vascular endothelium via the expression of adhesion molecules and selectins (38), leading to endothelial dysfunction and, eventually, atherosclerosis, thrombosis and cardiovascular injury. Chronic inflammation and immune cell infiltration among adipocytes eventually lead to the deposit of fibroblasts (fibrosis) (26). The hypoxia secondary to cellular hypertrophy that initiates the the phenotypical adiposopathy transformation, involves a process of transcriptomic and

proteomic conversion (reprogramming) of the AT, by which the secretion of anti-inflammatory adipokines, such as adiponectin and omentin, is reduced, together with an upregulation of the pro-inflammatory leptin, visfatin and resistin, further contributing to the dysfunctional AT state (15,39). AT reprogramming also affects the brown and beige adipocytes, that lose their thermogenic properties through autophagic degradation of mitochondria and reduced expression of UCP-1, to end up exhibiting characteristics typical of white adipocytes in a whitening process (40,41).

In patients with CKD, the adiposopathy process is both induced and aggravated by a chronic low-grade systemic inflammation secondary to the progressive accumulation of uremic toxins in the circulation derived from kidney dysfunction (42). This pro-inflammatory state is supported by the early elevation of biomarkers of inflammation and kidney damage, such as the soluble urokinase-type plasminogen activator receptor (suPAR) and/or kidney injury molecule 1 (KIM-1) in initial CKD stages, even before the estimated glomerular filtration rate (eGFR) decreases or other classical risk markers, such as microalbuminuria, emerge (43,44). Hence, adipokines in patients with CKD seem to behave slightly differently from patients with obesity, T2DM and other related conditions. Unlike what happens in these three situations (15), adiponectin levels in patients with CKD are increased up to three-fold in relation to physiological conditions, and their concentrations correlate with the progression of kidney dysfunction, so that patients with ESKD are found to have the highest levels of adiponectin (45). Along with high serum levels of adiponectin, in patients with CKD there is an increased production of adiponectin by the AT and its receptor, marking a state of adiponectin resistance and altered adiponectin signaling induced by uremia (19,46). What is still unclear is the role of adiponectin as a cardiovascular risk marker in patients with CKD, as certain studies revealed that lower levels of this adipokine are predictive of CVD in renal populations (47,48), whereas others found that cardiovascular mortality increases with the rise in adiponectin concentrations (49). As expected, given their pro-inflammatory status, patients with CKD show elevated leptin levels, with up to a four-fold increase in ESKD. The reasons for hyperleptinemia in kidney failure are multifactorial, including reduced renal elimination and increased production of leptin by the adipocytes (15,50), as demonstrated by an upregulated expression of the leptin gene in the subcutaneous AT from patients with CKD (18). Only rare studies focused on the role of less common adipokines in CKD: visfatin and resistin levels increase with declining renal function and they appear to actas independent CVD risk markers (51,52). Simultaneously, whereas significantly lower concentrations of omentin concentrations have been reported in CKD, particularly in the subgroup of patients with T2DM (53).

One hallmark feature of adiposopathy is the reprogramming and increase in size of certain region-specific AT. Perivisceral AT plays a pivotal role in ABCD as it releases adipokines and cytokines that not only contribute to the systemic pro-inflammatory and oxidative stress processes but may also influence the function of the organs surrounded by this tissue (54). Epicardial AT (EAT) is located below the visceral pericardium (epicardium), in direct contact with the coronary arteries and the myocardium. Patients with CKD show increased EAT thickness (ultrasonography evaluation) and volume (measured through computed tomography or magnetic resonance) (55). This increase in size serves as an

independent predictor for myocardial perfusion defects (56), as well as an independent predictor for mortality in patients on dialysis (57). These findings were later corroborated by other authors (16), who highlighted that not only an augmented size but also a higher EAT radiodensity are predictors of mortality in CKD populations (58). Perirenal AT (PAT) is in the retroperitoneal space surrounding the kidneys (54); therefore, its accumulation may mechanically compress the kidney with renal and/or cardiovascular consequences (59). Nonetheless, the consideration of its role as an endocrine organ influencing kidney function is relatively new. PAT thickness has been reported to have a negative correlation with eGFR, and to be associated with renal functional decline and cardiometabolic risk factors (14,60). MASLD (61) is the ultimate liver manifestation of cardiometabolic disorders, and potentially closely associated with CKD. Intrahepatic fat may act as a mediator in the pro-inflammatory signaling pathway activation that occurs in patients with CKD, rendering it a potential independent predictor of kidney damage (62,63). Non-perivisceral AT, such as the subcutaneous AT (SAT) and the preperitoneal AT (pPAT), have also been associated with CKD. SAT is the fat accumulated subcutaneously in the anterior abdominal wall. Its volume is positively correlated with leptin levels and insulin resistance measures in non-dialysis populations (64). An increase in its radiodensity is also considered an independent predictor of kidney damage progression (65). A less explored abdominal depot in CKD populations is the pPAT, which is AT located between the liver surface and the linea alba situated between the two recti abdominal muscles. Classically associated with arterial stiffness (vascular aging) and cardiometabolic risk markers, such as hypertension, T2DM and high triglyceride levels (66,67), it appears that certain non-coding microRNA (miRNAs 17-5p and 130b) secreted by the pPAT are predictors of survival and cardiovascular events in candidates for dialysis (68).

As described, region-specific AT seems to be a risk factor for CVD and kidney disease progression in CKD. However, no study to date has simultaneously explored region-specific Ats, such as EAT and PAT, MASLD and adiposopathy biomarkers in the CKD population to generate knowledge of how they vary across different stages of the disease and to distinguish those region-specific AT with a strongest association with renal function. Also, no studies have observed these adiposopathy markers during treatment with GLP-1RA.

c) GLP-1 receptor agonists: first steps towards an adipocentric therapeutic approach

The new antidiabetic GLP-1RAs, together with the sodium-glucose cotransporter inhibitors (SGLT-2i), have made a revolution in the comprehensive management of diabetic patients. Besides their glucose-lowering effects, both medications have weight-loss effects beneficial in obesity, they slow diabetic kidney disease progression, and exert anti-inflammatory actions that would alleviate the pro-inflammatory state associated with ABCD (69). However, the beneficial mechanisms of both drugs beyond their glycemic control are complex and not completely understood; in particular, their effects on AT remodeling and adiposopathy are still under evaluation. With the proposal we present here, we intend to take a step forward into the global assessment of these antidiabetic medication effects on adiposopathy by evaluating the changes triggered by GLP-1RA on

different AT depots from a clinical, biochemical and imaging perspective.

GLP-1RA stimulates the receptor for glucagon-like peptide-1 (GLP-1), an incretin-like hormone released in the large intestine that reduces serum glucose concentrations by stimulating the glucose-dependent release of insulin, inhibiting the hypersecretion of glucagon (except in hypoglycemic periods) and promoting satiety (70). There are different GLP-1RAs released to the market, such as exenatide, liraglutide, dulaglutide, and semaglutide; the latter has the longest duration of action allowing once weekly dosing (71). GLP-1RA reduced the incidence of CVD and cardiovascular death in patients with T2DM compared with placebo (72). In the randomized placebo-controlled FLOW trial (NCT03819153), that addressed the effects of semaglutide on CKD populations, this GLP-1RA significantly decreased the incidence of major kidney events, also reducing the progression of kidney dysfunction and the risk of death (73). The cardiorenal protective effects from GLP-1RA derive not only from glycemic and weight control, but also from direct involvement in the regulation of the apoptotic process of myocardial cells (74), and from inhibition of the sodium-hydrogen antiporter 3 (NH3) in the renal proximal tubule, increasing natriuresis and reducing pressure on the glomerulus (75). In animals, the observed morphological changes generated by GLP-1RA could be underlined by potential actions on AT remodeling, as these drugs upregulated the expression of AT-browning related genes in perivisceral WAT from murine models (76), although the transcriptomic effects from GLP-1RA on the adiposopathy process are still unknown. At the same time, semaglutide has been shown to reduce liver inflammation, fibrosis and lipid accumulation in patients to MASH (77). Whether the beneficial effects on AT, liver and kidney proceed together has not been studies yet.

PROTOCOL RESEARCH STRATEGY: STUDY DESIGN AND STUDY POPULATION

Section 4

a. Endpoints

a) Primary and Secondary Endpoints

The primary endpoint of this proposal is to establish a connection between kidney disease progression and cardiometabolic changes, focusing on region-specific adipose tissues such as perirenal, epicardial and hepatic steatosis in patients with or without semaglutide treatment. Additionally, adiposopathy biomarker comparisons between different CKD severity stages will be investigated. Given the current indication of GLP-1RA, the sample will comprise patients with T2DM and CKD (diabetic kidney disease or DKD). Thus, this endpoint can be split into two secondary endpoints, also identified as Work Packages (WP) 1 & 2, each one divided into different tasks:

WP1. To distinguish cardiometabolic profiles within each CKD mild to moderate severity stage (1 to 4). Patients will already be classified into their corresponding stages based on their eGFR and albuminuria. Patients on semaglutide or standard of care (SoC) regimen, within each CKD stage, will be categorized based on clinical, imaging and biochemical markers of adiposopathy. These markers will be associated with variables of both renal function and cardiometabolic risk. Creatinine, urea, cystatin C, intact parathyroid hormone (iPTH), vitamin D, calcium/phosphate, and uric acid will be used as markers of renal function in serum; in urine, microalbuminuria, albumin/creatinine ratio, and microalbuminuria/creatinine ratio will be measured. Serum total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, apolipoprotein A, triglycerides, glycated hemoglobin (HbA1C) and N-terminal pro b-type natriuretic peptide (NT-proBNP) will be considered cardiometabolic risk markers. Hepatic steatosis will be assessed using various non-invasive serum biomarkers. These include the Fatty Liver Index (FLI), NAFLD Liver Fat Score (NAFLD-LFS), Hepatic Steatosis Index (HSI), FIB-4 score and the TyG index (triglyceride × glucose).

Task 1a. To connect clinical markers of adiposopathy with renal/hepatic/cardiometabolic function. The clinical markers of adiposopathy will be based on anthropometric measures such as weight and body mass index, among others.

Task 1b. To establish associations between biochemical (proteomics) biomarkers of adiposopathy/inflammation and renal/hepatic/cardiometabolic function. The biochemical markers will include serum and urinary profile of kidney function (creatinine, eGFR, urea, microalbuminuria, protein-creatinine ratio, KIM1, the protein Klotho); adipokines (leptin, adiponectin, visfatin, omentin, resistin). Serum inflammatory markers, such as TNF-α, INF-γ, interleukins (IL-6, IL-8 and IL-10) and suPAR, will also be measured. Additionally, the SteatoTest and Fibromax panel will be used for a more comprehensive assessment.

- **Task 1c.** To establish a link between imaging (MRI) markers of adiposopathy via

assessment of region-specific adipose tissue and renal/hepatic/cardiometabolic function. Three region-specific AT will be assessed (perirenal, epicardial and steatotic liver), measuring their total volume, fatty acid composition (78) and vascularization (79).

WP2. To establish a link between the clinical improvement observed in patients receiving GLP-1RA (Semaglutide) and the changes in adiposopathy markers. Patients receiving semaglutide or SoC will be invited to participate.

Task 2a. To compare the changes in clinical (anthropometric) markers of adiposopathy with GLP-1RA or SoC and correlated with the improvement observed in renal, hepatic and cardiometabolic variables. Comparisons will be made at one point in time, in which the total body composition measures mentioned above will also be correlated with the renal, hepatic and cardiometabolic risk markers.

Task 2b. To make comparisons in biochemical markers of adiposopathy/inflammation throughout GLP-1RA or SoC treatment and establish correlations with the changes in renal function and cardiometabolic variables. The biochemical markers measured in urine and serum will be compared to correlate these differences with the ones in the cardiometabolic, hepatic, and renal profile of the patient.

Task 2c. To compare the imaging aspect of each region-specific AT in GLP-1RA or SoC regimens and how each one associates with kidney and liver function and cardiometabolic markers.

b. Study Type

We propose cross sectional, real-life, single-center, and observational study. We will enroll patients with diabetic kidney disease at different stages of CKD receiving the GLP-1RA Semaglutide or SoC regimen, depending on their medical indication and cardiometabolic and hepatic steatosis stage.

c. Study Population

Patients will be recruited from the Divisions of Family Medicine, Cardiology, Nephrology, Internal Medicine and Endocrinology at the University of Alberta by PR (PI), and associates, according to the following selection criteria:

Inclusion Criteria

- Patients ≥ 18 years of age.
- Patients diagnosed with T2DM (>18 months) and CKD in stages G1, G2, G3a, G3b and G4; the CKD staging will be established according to the eGFR as per the KDIGO guidelines (G1: eGFR ≥90 ml/min/1.73m^2; G2: 60-89 ml/min/1.73m^2; G3a: 45-59 ml/min/1.73m^2; G3b: 30-44 ml/ min/1.73m^2; G4: 15-29 ml/ min/1.73m^2).
- Patients with T2DM and CKD, with or without semaglutide treatment.
- Patients who voluntarily agree to participate and sign informed consent.

Exclusion Criteria

- Patients <18 years of age.
- Pregnant, breastfeeding, or an intention of becoming pregnant or not using adequate contraceptive measures (including country-specific adequate measures, if any)
- Patients diagnosed with T2DM and CKD in stage G5 or stage G4 requiring dialysis as per KDIGO guidelines.
- Previous participation in this trial (screened or randomized)
- Patients diagnosed with neuropsychiatric diseases that prevent them from understanding the benefits/risks associated with the project or voluntarily choosing to participate.
- Known or suspected allergy to trial medication(s), excipients, or related products
- Contraindications to study medication(s), worded specifically as stated in the Product Monograph
- Refusal to participate or consent revocation.

d. Withdrawal Criteria

- The subject may withdraw at will at any time.
- Consent revocation
- Patient dies

e. Diversity and Inclusion

Effects of gender inclusion in the

content of the proposal

- ***Research focus*****:** The prevalence of T2DM is slightly higher in men than in women. However, the latter have a higher risk of suffering cardiovascular events. Nonetheless, the effect of GLP-1RA, in terms of glycaemic control and weight loss, is greater in women (80). Therefore, the impact of the results derived from this project in terms of improving the metabolic profile and adiposopathy and prevention strategies will benefit both sexes and genders.
- ***Literature review*****:** the literature review carried out on the scientific framework of this project includes, for the most part, clinical and preclinical studies with participants of both sexes. Some of them also investigate differences in the levels of adipokines linked to gender (81), and differences in the effects of GLP-1RA related to sex (80).

- ***Research hypothesis*:** Our research hypothesis, based on the ability of Semaglutide to modify adiposopathy markers and the metabolic profile of patients with T2DM and CKD, is aimed at both sexes, being aware that the effects of these drugs may vary in men and women. Hormonal variations during the female life stage (menstrual and postmenopausal) can influence the metabolic effects derived from Semaglutide. Once the global results of the study population have been obtained, a stratified analysis will be carried out concerning sex, which will allow us to observe differences in the results between men and women.
- ***Research methods*:** the recruitment of patients will ensure that similar populations of men and women are obtained, so that solid results differentiated in their magnitude can be obtained for both sexes if required. The analytical extractions will have a volume proportional to the patient's weight. The participant's hormonal stage will be considered in the interpretation of the values of certain parameters, such as adipokines, parathyroid hormone, and inflammation parameters.
- ***Ethical issues*:** the particular implications for certain life stages of women have been considered, such as pregnancy, which has been excluded from the study due to the possible risks of GLP-1RA on the mother and foetus.
- ***Dissemination of results*:** the dissemination of results will be performed in a truthful manner, reporting all differences in the results between men and women, providing evidence-based explanations related to biological (sex), cultural variations and social (gender) status that can justify the observed differences.

PROTOCOL RESEARCH STRATEGY: METHODS

Section 5

5.1 Study Procedures/Assessments

Collection of the following data by research assistants and PI will occur at the time of MRI imaging appointment or within 10 days of the imaging visit:

• Clinical data collection from participants and institutional clinical databases, including age, sex, cause and duration of CKD

• Mean blood pressure value after three consecutive blood pressure measurements.

• Fasting blood sample collection will be performed by the nursing staff at the hospital hospital to measure serum levels of routine renal biomarkers (creatinine, urea, iPTH, vitamin D, calcium/phosphate, cystatin C, renin and uric acid) and cardiometabolic markers, such as serum total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, apolipoprotein A, triglycerides, glycated hemoglobin (HbA1C), Pro-adrenomedullin and N-terminal pro b-type natriuretic peptide (NT-proBNP).

• An additional blood volume (~10mL) will be taken from the same extraction to be transported, upon centrifugation, to the laboratories at the University of Alberta to be frozen at -80ºC.

• Morning urinary sample collection to measure standard urinary markers of kidney function, such as the albumin/creatinine ratio, microalbuminuria and the

microalbuminuria/creatinine ratio. An additional urine volume is stored at -80ºC in the University of Alberta laboratories.The samples from serum/urine stored at University of Alberta will be further processed and prepared under the Co-PI's lead. Aliquots from the serum samples will be separated for proteomic analyses through proximity extension assay via the Olink® Target 96 (metabolic & cardiovascular panels). This technique will allow us to simultaneously obtain a variety of measures, adipokines (adiponectin, leptin, visfatin, resistin, omentin), fatty acid-binding protein and inflammatory markers (IL-6, IL-8, IL-10, IFN-γ, TNF-α) amongst others. In addition, the Olink® proteomic technique allows us to customize a selection of protein biomarkers (Flexi panels) spanning a wide range of target areas to evaluate metabolic connections between adiposopathy-related proteins and kidney dysfunction.

The remaining serum aliquots and urine samples will be processed and analyzed at University of Alberta to determine the concentrations of Klotho, suPAR and KIM-1 (urine) through enzyme-linked immunosorbent assay (ELISA), with the use of commercially available kits.

Imaging methods:

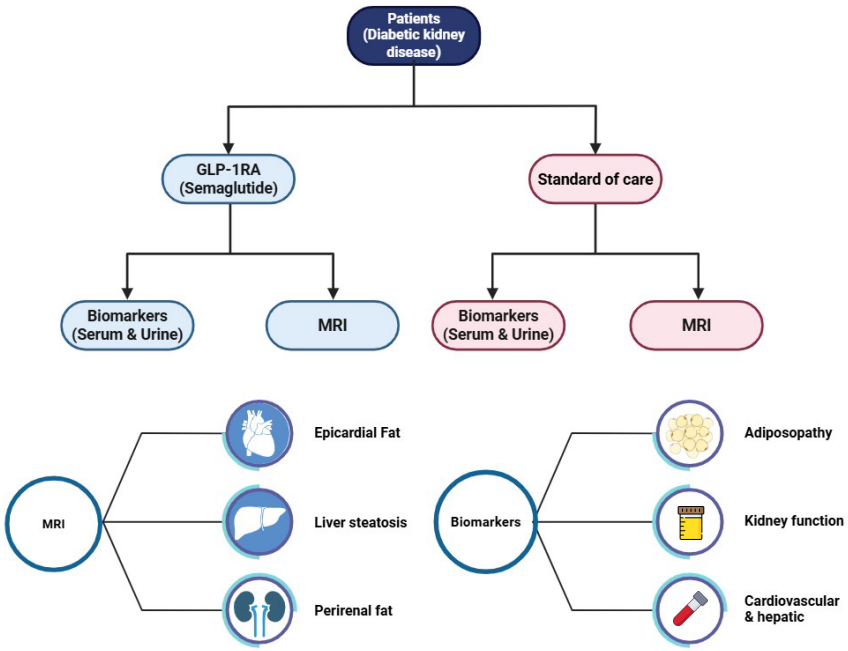
- Patients will be referred to the University of Alberta imaging centre for the MRI scan. Image interpretation will be conducted by experienced radiologists in the field.
- T1 imaging of the epicardial and PAT and proton density fat fraction (PDFF) imaging of the liver will be performed on all patients. Considering that a decrease in the oxygen supply may be related to adipose tissue hypoxia observed in CKD and dysfunctional fat, BOLD imaging will also be attempted (this technique is used to measure oxygen saturation in the myocardium and has never been attempted on visceral fat before).
- MRI images will be obtained using a 1.5 Tesla unit (Magnetom Avanto; Siemens Medical Solutions, Erlangen, Germany) and a 16-channel body coil with the patient in the supine position without sedation. Imaging will be performed in a T2-weighted turbo axial and coronal plane (T2W TSE) without fat saturation or apnoea, and a volumetric interpolated breath-hold examination (VIBE) with T1 fat-saturated gradient echo sequence.
- All images will be processed with OsiriX MD v.10.0.2 software (UCLA, Pixmeo, Bernex, Switzerland) on a MacOS-X radiology workstation (Apple Inc., Cupertino, USA). Slicing will be performed on the T2W images to remove artifacts. Measurements in the axial plane will be performed in Dixon Fat (DF) and Dixon Water (DW) sequences from an appropriate single slice passing through the level of the renal hilum to quantify the PAT.
- The estimated total radiation dose for MRI is zero and no intravenous or oral contrast will be administered to patients. All the MRI images resulting from the study will be stored in standard DICOM format for medical imaging information for further processing and evaluation.

5.2 Assessments for Safety

The proposal has two clear, specific objectives, divided into precise activities (tasks) related to the general purpose of this project. The work plan is accordingly divided into two

closely related but independent WPs, so that the execution of a WP is not dependent on results originating from the other WP, which increases the success of the project. Most of the material and technological resources are already available. The research and working team are composed of competent professionals with whom the PI and Co-PI, particularly the PI, have previously worked as per their joint publications. Continuous monitoring of the research progress will be carried out. There are always several people involved in each task, with more than one as task leader, ensuring there is always one person available to take over the work in case of maternity or sick leave. To ensure homogeneous patient recruitment and sample processing, every study member has received proper previous training in the selection criteria, study protocol and sample identification for further anonymization. A route of action has been laid out (verbally and in written form with leaflets) for the laboratory staff to homogenize the sample processing, identification and storage process.

5.3 Study Flowchart



PROTOCOL RESEARCH STRATEGY: STATISTICAL CONSIDERATIONS

Section 6

6.1 Sample Size Calculation

For the sample size calculation, we assumed a 25% difference in region-specific AT (epicardial, hepatic, and perirenal) in patients in treatment with Semaglutide or SoC to be clinically relevant. In a two-sided paired-sample T-test, a minimum sample of 48

participants achieved 80% power to detect this difference, with a significance level (alpha) of 0.05. Considering an estimated 10% dropout rate, 62 participants would be needed. The sample size was calculated with the Power Module for JASP software version 0.19.0.0 (The JASP© Team, Amsterdam, The Netherlands).

6.2 Statistical Methods

Descriptive measures will be analyzed using mean (standard deviation) or median (standard error), based on their parametric/non-parametric distribution. Analysis of variance (ANOVA) tests and/or their non-parametric alternative will be used for comparisons, as well as linear regression measures for variable association studies. The results will be stratified according to sex and CKD staging, given that the effects observed may be greatly influenced by the severity of renal damage. Statistical significance will be set for an α-value <0.05. The SPSS for Windows software version 29 (SPSS Inc, Chicago, IL, USA) will be used for statistical analysis.

PROTOCOL ETHICS

Section 7

7.1 Data Handling and Recordkeeping

All data is processed anonymously and stored in a central database. All personal data is handled in accordance with appropriate national regulations.

7.2 Ethics

The inclusion of patients and procedures will be carried out in accordance with the 2013 revision of the Declaration of Helsinki, after signing and accepting the informed consent by the patient.

The SoC and or Semaglutide treatments will be prescribed by the physician in charge in accordance with current guidelines and following strict dosage escalation as per Canadian medication agency, hence the observational nature of the study.

Analytical/urinary extractions and other procedures are scheduled at one specific time point given that we are recruiting a heterogeneous sample in terms of CKD staging; however, they will try to coincide in time with the routine extractions prescribed by the physician. Imaging studies will use a non-invasive procedure (MRI) which implies no radiation to patients.

PROTOCOL ADVERSE EVENTS

Section 8

8.1 Adverse Event Reporting

The investigator will inform the subjects and the reviewing accredited medical research ethics committee (MREC) if anything occurs, based on which it appears that the disadvantages of participation may be significantly greater than were foreseen in the research proposal. The study will be suspended pending further review by the accredited MREC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

8.2 **Adverse Event Definitions**

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product or intervention. Adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.3 **Adverse Event Follow-Up**

All AEs will be followed up until they have abated or until a stable situation has been reached. Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till the end of the study, as defined in the protocol.

Pregnancy

Women of childbearing potential will be asked to perform a pregnancy test prior to performing the MRI. This test will be provided to each patient.

PROTOCOL PREMATURE STUDY TERMINATION

Section 9

9.1 Early Study Termination

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

PROTOCOL PUBLICATION PLAN

Section 10

10.1 Publication Plan

After the first year of the study, we plan to present our preliminary results in regional and national scientific meetings and congresses. The group will also aim to present

their preliminary results at the annual World Congress of Nephrology of the International Society of Nephrology, Canadian Congress of Cardiology, and American Heart Congress, among others. Additionally, we anticipate participation in the European Renal Association (ERA) and its weekly bulletin (ERA Renal Week), opening new avenues for international collaborations with other research institutions.
The preliminary results obtained at the end of the first year will be submitted as manuscripts to Q1-Q2 ranked open-access journals in the area. The submission of manuscripts to D1/Q1-ranked open-access journals, such as Nephrology Dialysis & Transplantation and Clinical Kidney Journal, is anticipated between the 2nd and 3rd year of the project.

PROTOCOL REFERENCES

Section 11

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