Study Protocol

Title: Metabolic Phenotyping During Stress Hyperglycemia in Cardiac Surgery Patients

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Title: **Metabolic Phenotyping During Stress Hyperglycemia in Cardiac Surgery Patients**

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ABSTRACT:

Research: Stress hyperglycemia is common in the perioperative period and is associated with increased risk of postoperative mortality. Counterregulatory hormones and inflammatory mediators appear to modulate the acute biological response to stress; however, the pathophysiological pathways that result in stress hyperglycemia and its link to poor clinical outcomes are not well understood. Comprehensive metabolic profiling (metabolomics) represents a novel tool to examine metabolic changes during stress conditions. The current approach to treat hyperglycemia with insulin has major limitations including high resource utilization and high risk of hypoglycemia. The overarching hypotheses of this research proposal are that: **1)** Stress-hyperglycemia is associated with changes in unknown and known metabolites (i.e. branched-chain amino acids, acylcarnitines, ceramides) and path-specific biomarkers (representing inflammation, coagulation, cell stress, and immune modulation) in the perioperative period in cardiac surgery patients, and **2)** with a low risk of iatrogenic hypoglycemia, exposure to a long acting glucagon like peptide-1 receptor agonist (GLP-1 RA) will ameliorate the hyperglycemic and inflammatory response to surgical stress. Using comprehensive metabolic profiling methods, this study will provide new insights into the mechanisms underlying the metabolic and inflammatory responses to surgical stress **(Aim 1)**. This study will also examine whether exposure to a long acting GLP-1 RA can improve glycemic control and ameliorate the inflammatory response to acute surgical stress **(Aim 2)**. These findings will provide proof-of principle to support the use of novel therapies to prevent and manage stress hyperglycemia in the inpatient setting.

SPECIFIC AIMS About 400,000 patients undergo coronary artery bypass graft surgery (CABG) yearly. Stress hyperglycemia (blood glucose [BG] >140 mg/dl) occurs in ~60-80% of non-diabetic patients during CABG and is associated with increased perioperative complications and mortality. The underlying mechanisms that lead to hyperglycemia in response to stress during cardiac surgery are not well understood.

Even though stress hyperglycemia and diabetes are primarily disorders of glucose metabolism, other dimensions including lipids (i.e. acylcarnitines, ceramides) and amino acids [i.e. branched-chain amino-acids (BCAA)] have been identified in association with hyperglycemia and diabetes through high-resolution metabolomics (HRM) techniques. Furthermore, HRM has identified BCAA, ceramides, and acylcarnitines as important markers of cardiovascular disease (CVD) risk. In acutely ill patients presenting with severe hyperglycemia we observed a positive association between BCAA and delayed resolution of hyperglycemia during intravenous insulin therapy. These relationships suggest that circulating and increased release of amino-acids and acylcarnitines during hormone-mediated catabolism during stress could mediate insulin resistance, beta-cell dysfunction, and cardiovascular (CV) risk in patients with CAD undergoing surgery. In addition, path-specific biomarkers representing inflammation [C-reactive protein (CRP)], hyper-coagulation [fibrin degradation products (FDP)], cell stress [heat shock protein-70 (HSP-70)], and immune modulation [soluble urokinase-type plasminogen activator receptor (SuPAR)] have emerged as strong predictors of death in patients with CVD. Previous studies suggest hyperglycemia can lead to activation of these pathways.

Continuous insulin infusion therapy (CII) is the treatment of choice of stress hyperglycemia. Although effective in improving BG control, CII is costly and labor intensive requiring hourly measurements of glucose levels; and carries a high risk of iatrogenic hypoglycemia (12% to 35%), which is associated with increased risk of complications and death. Incretin-based therapies have emerged as an effective alternative to insulin therapy in improving glycemic control with minimal risk of hypoglycemia and additional cardiovascular benefits. By augmenting glucose-dependent insulin secretion and inhibiting glucagon secretion, glucagon-like peptide-1 receptor agonists (GLP1-RA) target the underlying pathophysiology of stress-induced hyperglycemia and could represent a novel prevention and treatment approach with a very low risk of hypoglycemia.

This project will fill several knowledge gaps by characterizing the underlying mechanisms and metabolic signatures of stress hyperglycemia with the use of comprehensive metabolic profiling methods and provide proof-of principle with the use of novel therapies for the prevention and management of stress-hyperglycemia.

Aim 1. To examine baseline and postoperative metabolic profiles of non-diabetic CABG patients with stress hyperglycemia. With a case-control design we will conduct the following analyses:

1.1 Targeted Approach: To determine cross-sectional and longitudinal changes of BCAA, ceramides, acylcarnitines, inflammatory markers, and a biomarker panel for CV risk [CRP + FDP + HSP-70, SuPAR] and their relationship with stress hyperglycemia (BG >140mg/dl) and insulin resistance estimates. *We hypothesize that elevated ceramides, acylcarnitines, branched-chain amino acids (BCAAs) are associated with incident stress hyperglycemia, and that hyperglycemia leads to changes in path-specific biomarkers related to CV risk.*

1.2 Untargeted Analysis: Utilizing untargeted HRM analyses, we aim to identify additional candidate metabolites and metabolic pathways associated with incident stress hyperglycemia during cardiac surgery. *We hypothesize that known (candidate metabolites) and previously unrecognized metabolites and pathways are associated with hyperglycemia during surgical stress.*

1.3 Integrative analysis: Metabolome-wide association studies (xMWAS) and integrative network analyses will be conducted to find multilevel associations between metabolites, biomarkers, and stress-hyperglycemia. *We hypothesize that multiple factors associated with diabetes and cardiovascular disease are interrelated and predict hyperglycemia during stress conditions.*

Aim 2. To obtain preliminary estimates of the effect of a long-acting GLP-1 RA on the prevention of stress-hyperglycemia and modulation of metabolic stress during cardiac surgery. We propose a randomized study with assignment to dulaglutide or placebo three days prior to surgery in non-DM obese patients (BG<126 mg/dL, HbA1c <6.5%, BMI ≥25, age≥40 years) undergoing CABG surgery.

2.1 Primary endpoint: to determine if dulaglutide can prevent stress-hyperglycemia.

2.2 Secondary endpoints: to determine if dulaglutide can result in a lower mean BG during ICU and hospital stay. We will also explore the need of rescue therapy with insulin and differences in clinical outcomes.

2.3 Comparison of changes in candidate metabolites and path-specific biomarkers, as well as beta-cell function and insulin resistance estimates in response to dulaglutide or placebo.

We will test the hypothesis that exposure to a single dose of dulaglutide can ameliorate the hyperglycemic and inflammatory response to surgical stress with a low risk of iatrogenic hypoglycemia.

A) SIGNIFICANCE Coronary artery disease (CAD) remains a predominant cause of death globally[1,2] About 400,000 patients undergo coronary artery bypass graft surgery (CABG) each year in the United States[5] and more than 80% of patients with a known history of diabetes (DM) and ~60% of non-DM patients develop stress-hyperglycemia [blood glucose (BG) >140 mg/dl] after surgery.[6-9] Perioperative hyperglycemia is associated with higher rates of wound infections,[10,11] kidney injury,[8] longer hospital stay,[12,13] and mortality.[10,12,14-17] In non-DM subjects, stress-hyperglycemia is associated with up to 4-fold increase in complications and a 2-fold increase in death compared to patients with normoglycemia and to subjects with DM.[18-27]

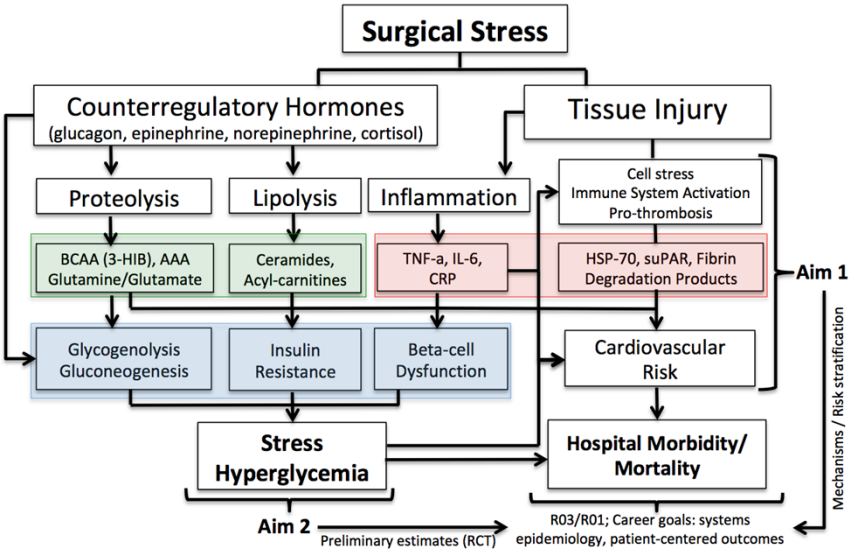

Figure 1. Study overview. Aim 1 is focused on understanding the underlying mechanisms that lead to stress hyperglycemia and increased cardiovascular risk in cardiac surgery patients (see Table 1). Green box: main groups of candidate metabolites to be assessed with metabolomics techniques. Red box: inflammatory markers and biomarker risk score components known to predict CV outcomes. Blue box: assessments of glycemia, insulin secretion (HOMA-B) and insulin resistance (HOMA-IR). Aim 2 is focused on obtaining preliminary estimates on stress hyperglycemia prevention and glycemic control (RCT) with a strategy with low risk of iatrogenic hypoglycemia.

The mechanisms leading to stress-hyperglycemia and the association with poor clinical outcomes are not well understood. Acute metabolic and hormonal changes associated with the response to injury can result in insulin resistance, increased hepatic glucose production, and relative insulin deficiency.[28-31] Elevated Inflammatory mediators can also contribute to hyperglycemia during injury; conversely, hyperglycemia itself can promote inflammation, oxidative stress, and a pro-thrombotic state [32-36] (Fig. 1). Metabolomics analysis of human biosamples has undergone a rapid technological evolution in recent years and represents a powerful tool to investigate the complexity of all the interactions within a complex individual. Even though hyperglycemia and diabetes are primarily considered disorders of glucose metabolism, other dimensions including lipids and amino acids have been consistently identified in association with prediabetes and diabetes through high-resolution metabolomics techniques (HRM).[37,38] Analyses of large cohorts using HRM have reported positive associations of branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs) with incident prediabetes or diabetes.[37-39] Products of fatty acid metabolism (i.e. acylcarnitines) are also associated with insulin resistance, insulin secretion defects, and incident diabetes.[40-42] These relationships suggest that circulating and increased release of amino acids and acylcarnitines during hormone mediated catabolism during stress could mediate insulin resistance and beta-cell dysfunction in surgical patients, Table 1 and Fig 1. Furthermore, HRM has identified BCAA, ceramides, and acylcarnitines as important markers of CVD risk.[43-46]

Table 1.	Candidate metabolites and known biomarkers	Rationale / Clinical relevance
Protein Metabolism	BCAA (Isoleucine, leucine, valine) Aromatic Acids (tyrosine, phenylalanine) Other: glycine, glutamine/glutamate ratio	Meta-analyses of multiple cohorts show that BCAA, AAA increase the risk of chronic hyperglycemia. Some AA are protective (glyceine, glutamine/glutamate ratio)[37,47]. BCAA lead to mTOR/S6K1 kinase pathway activation and serine phosphorylation of IRS1 leading to insulin resistance. BCAA catabolic flux can lead to glutamate transamination to alanine and increased gluconeogenesis, as well as insulin secretion defects.[48-50] BCAA are also associated with CVD.[42] The product of valine catabolism, 3-HIB has emerged as a potential mediator linking BCAA to regulation of fatty acid flux and insulin resistance (IR).[51,52] We observed higher abundance of 3-HIB in subjects with hyperglycemic crises and delayed response to insulin suggesting IR. Recently, 3-HIB was associated with incident diabetes.[53]
Lipid Metabolism	Acylcarnitines, ceramides / sphingosine, triacylglycerols (TAGs),	Elevated medium chain acylcarnitines lead to pancreatic β-cell dysfunction.[40] Ceramides, and acylcarnitines are associated with insulin resistance and incident CVD[41,44-46,54,55] Sphingosine, a ceramide derivate is elevated in prolonged ketoacidosis, and is associated with insulin resistance.[56]
Carbohydrate Metabolism	Glucose, Insulin	Hyperglycemia can lead to inflammation, oxidative stress, and a pro-thrombotic state [32-36] HOMA-IR and HOMA-B are well-described surrogates of insulin resistance and insulin secretion derived from insulin and glucose levels.

Hormonal response	Cortisol, glucagon	Counterregulatory hormones are expected to increase during cardiovascular surgery (GLUCO CABG).[3,4]
Inflammatory pathways	TNF-a, IL-6, CRP	TNF-a, IL-6 are Inflammatory markers known to cause insulin resistance (TNF-a, IL-6) and insulin secretion defects[57-62] and associated with cardiovascular risk. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans.[36] Levels of inflammatory markers are increased for days after CABG surgery.[3] CRP is an established CVD risk factor.[63]
Cell stress	Heat shock protein (HSP)	Hyperglycaemia upregulates HSP60 & HSP70.[64] HSP70 is associated with incident CVD.[2,65,66] (Quyyumi)
Immune pathways	suPAR	SuPAR is a marker of immune activation associated with all-cause death and myocardial infarction in patients with CVD.
Pro-thrombosis	Fibrin degradation products (FDP)	Hyperglycemia and diabetes are associated with a prothrombotic state. FDP associated with incident cardiovascular disease in patients with and without diabetes.[2,65,66]
BCAA: branched-chain amino acids, AAA aromatic amino acids, 3-HIB 3-Hydroxyisobutyric acid		

Recently, four biomarkers identified susceptible individuals and predicted meaningful clinical endpoints.[65-68] A simultaneous evaluation of biomarkers representing inflammation (C-reactive protein), coagulation (fibrin degradation products), cell stress (HSP-70), and immune pathways (suPAR) significantly predicts risk of death in patients with coronary artery disease (CAD).[65,66] Previous research suggests that hyperglycemia can lead to activation of these pathways[36,64,69,70], however; no longitudinal assessment of the relationship between biomarkers and stress-hyperglycemia has been conducted. It is important to characterize the molecular changes that occur during stress hyperglycemia to identify preventive strategies and maximize the potential to find cost-effective interventions. To fill this knowledge gaps, we will conduct targeted and untargeted analyses to further understand the underlying biology of metabolic changes during surgical stress.

Large number of observational and randomized control studies have shown that improvement of glycemic control reduces perioperative complications in critically and non-critical ill patients but is also associated with high risk of hypoglycemia. Glycemic control with continuous insulin infusion therapy (CII) can reduce complications and mortality,[11,71-76] particularly among non-DM subjects.[77] In the GLUCO-CABG trial[4], we reported a significant reduction in a composite of perioperative complications in non-DM patients with stress-hyperglycemia treated with intensive vs conservative glycemic control. Although effective,[71,72,76] the use of CII is costly and labor intensive, and carries a 12-35% risk of hypoglycemia.[78-82] Hypoglycemia after cardiac surgery has been associated with increased risk of complications and mortality.[83-85]

Hospital use of incretin-based therapies is a safe alternative to complex insulin regimens. To simplify the management of hyperglycemia and avoid the risk of hypoglycemia we have examined the role of DPP-4 inhibitors[86] and GLP-1 receptor analogs (RA) in the hospital setting (see preliminary results). GLP-1 is an incretin hormone secreted in the gut in response to meal ingestion, targeting multiple tissues. One of its main targets is the pancreatic β-cell, resulting in increased insulin secretion and inhibition of glucagon production.[87] In patients with and without diabetes,[88-90] native GLP-1 and GLP1-RA treatments have shown to improve glycemic control similar to insulin administration during corticosteroid-induced hyperglycemia,[91] acute cardiovascular (CV) events,[91] in the perioperative period of patients undergoing CABG surgery,[92] and mixed ICU populations.[93] In patients with CV disease, compared to insulin therapy native GLP-1 resulted in better glycemic control, fewer arrhythmias,[94] and improved cardiac function,[95,96] CV events.[97,98]

Dulaglutide, a long-acting GLP-1RA suitable for once-weekly administration,[99] have demonstrated improved glycemic control compared to other antidiabetic agents.[100-102] Phase 1 pharmacodynamics analysis in non-DM subjects showed median maximum plasma concentrations of dulaglutide between 24-48 hours after a single dose.[103] Evaluation with stepped glucose infusion 3 days after dulaglutide administration revealed a dose-dependent rise in insulin secretion and associated decline in serum glucose concentration.[103] Compared to short-acting GLP-1 RA, dulaglutide results in fewer and dose-dependent gastrointestinal side effects.[103,104] Dulaglutide has also been shown to be better tolerated compared to other long-acting GLP-1RA.[105] GLP-1 RA can be used before surgery in patients with diabetes.[106,107] The above data suggest that a single subcutaneous administration of dulaglutide can improve glucose-dependent insulin secretion and decrease glycemic excursions that may result in stress-hyperglycemia prevention with low risk of hypoglycemia.

B) INNOVATION

1) The underlying mechanisms leading to stress hyperglycemia and poor hospital outcome are not well understood. It is unknown if hyperglycemia is a mediator in the development of hospital complications, or a bystander marker only representing severity of disease. Serial assessments of relevant candidate metabolites and biomarkers before and after the acute stress episode will facilitate our understanding of potential mediators of stress hyperglycemia and the metabolic response to stress. CABG surgery represents a unique human model to study in depth these relationships.

2) Metabolomics (HRM) is an emerging scientific field that enables profiling of thousands of metabolites to provide a functional readout of cellular metabolism in plasma and other bio samples. The use of metabolomics can identify metabolites and metabolic pathways implicated in the development of stress hyperglycemia. We expect to find an association of metabolites derived from protein and fatty acid catabolism (such as BCAA, and specifically 3-HIB) with stress hyperglycemia. In addition, serial assessments of glucose levels, candidate metabolites and biomarkers as well as an integrative network analysis to find multilevel associations between them will facilitate for the first time an approximation to causality interpretations. No previous studies have applied metabolomics analysis to understand stress-hyperglycemia. Novel bioinformatics tools such as metabolome-wide association studies (xMWAS) have the capability to facilitate an integrative network analysis to find multilevel associations and identify metabolic pathways and network structures in association with stress-hyperglycemia.

3) We will examine biomarkers associated with meaningful cardiovascular disease outcomes that represent inflammation (C-reactive protein), coagulation (fibrin degradation products), cell stress (heat shock protein-70), and immune activation (suPAR). Hyperglycemia can lead to activation of these pathways. This study will for the first time examine longitudinally the relationship between clinically relevant biomarkers and stress-hyperglycemia in patients with established cardiovascular disease.

4) Current approach to manage hyperglycemia is a reactive care model. In a setting associated with high incidence of stress-hyperglycemia, we will test for the first time (Aim 2) if the use of a GLP-1 RA can prevent the onset of stress-hyperglycemia (proactive care) during the perioperative period of cardiac surgery. The use of novel agents with prolonged action is ideal for this purpose.

5) The proposed study will provide critical first steps aiming at understanding and preventing stress-hyperglycemia and will lay the groundwork to study the links between stress-hyperglycemia and hospital complications (career goal). This strategy has the potential to impact millions of patients undergoing cardiac surgery and to other scenarios associated with high risk of stress hyperglycemia and related complications.

6) Next steps (R03/R01 applications): Results from Aim 1: once the most relevant metabolites are identified I plan to conduct primed continued tracer infusions to better understand if concentration changes are related to faster, slower production or both. I will conduct metabolic flux analysis in vivo to further examine the dynamic flow of specific metabolites through metabolic pathways during stress conditions. To understand what tissues are responsible for these changes, I will collaborate with scientists such as Dr. Kibbey to design additional studies. I also plan to integrate multi-omics approaches (metabolomics along with genomics/epigenomics) and reproduce findings in independent cohorts. Results from Aim 2 will provide preliminary estimates on hyperglycemia prevention, insights into risk stratification, and characterization of subjects most likely to respond to GLP-1RA to design additional RCTs.

7) In summary: this project will under the guidance of a mentoring/advisory team: **a)** leverage the state-of-the-art innovative technology available at Emory by applying new high-resolution metabolic profiling capabilities developed by co-mentor Dr. Jones and collaborators Dr. Yu, Dr. Uppal and Dr. Ziegler; **b)** integrate data with clinically meaningful biomarkers; **c)** help understand the underlying biology of stress hyperglycemia (under the guidance of Dr. Kibbey and Dr. Alvarez); and **e)** test potential solutions along with experts in inpatient diabetes (Dr. Umpierrez and Dr. Inzucchi).

C) PRELIMINARY DATA Our research team has extensive research experience in inpatient management of hyperglycemia and has published several randomized controlled trials in ICU and non-ICU settings.[24,27,86,108-114]

C.1. Impact of perioperative stress-hyperglycemia: In a retrospective analysis of glycemic control and hospital outcomes in 1971 patients with confirmed preoperative normoglycemia, we observed an increase in complications and mortality in patients developing stress-hyperglycemia.[115] Compared to patients with normoglycemia (<140 mg/dl), patients with stress-hyperglycemia had a longer length of hospital stay (LOS) and higher rates of complications and mortality (all, p<0.001). After adjusting for age, gender, BMI, race, and Charlson comorbidity score, compared to patients with normoglycemia, those with postoperative BG 140-180 mg/dl had higher odds for both complications [odds ratio (OR) 1.7 (1.2 – 2.4)] and mortality [OR 1.7 (0.4-7.1)]. The OR

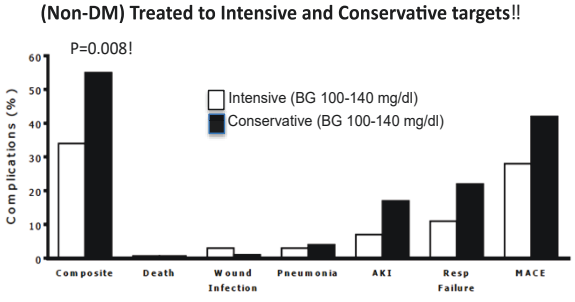

Figure 2. Non-DM patients randomized to the intensive group had a lower composite of complications compared to subjects in the conservative target.[4]

for complications and mortality in patients with BG >180 mg/dl were 3.5 (2.2-5.4) and 6.6 (2.1-20.3), respectively.

C.2. *GLUCO-CABG Trial:*[24] in this RCT we aimed to determine whether intensive BG control (target: 100-140 mg/dl) could reduce perioperative complications compared to conservative BG control (141-180 mg/dl) in hyperglycemic patients undergoing CABG. A total of 302 patients were randomized to intensive (n=151) or conservative (n=151) glucose control. The mean ICU daily BG was 132±14 mg/dl (IQR 124-139) in the intensive and 154±20 mg/dl (IQR 142-164) in the conservative group (p<0.001). Overall, we observed a similar number of patients in the intensive and conservative groups experiencing ≥ 1 complications (42% vs. 52%, p=0.08). We observed no differences in the composite or individual complications in patients with DM; however, the composite of complications was lower in non-DM patients with stress-hyperglycemia assigned to the intensive group, p=0.008, (Fig.2). Since all patients enrolled GLUCO-CABG patients had stress-hyperglycemia, we recently *analyzed phenotypic and biological characteristics of non-diabetic individuals at risk for perioperative stress hyperglycemia during CABG.* The two most important features in patients who developed >2 episodes of BG >140 mg/dL were older age (67.0 ± 9.2 vs 59.1± 9.4, p=0.004) and higher BMI (28.9 ± 5.8 vs 26.1 ± 4.3, p=0.06). All six patients older than 50 years and BMI>30kg/m^2 developed hyperglycemia. These characteristics were confirmed in an additional cohort (i.e. age >45 years). Older age and obesity are well-established risk factors for insulin resistance and DM.

C.3. Inflammatory markers during CABG: Circulating levels of cortisol, inflammatory and oxidative stress markers increase for several days in the perioperative period (Fig. 3). No difference was observed between conservative and intensive insulin therapy groups in the perioperative period of patients undergoing CABG surgery. Changes in these markers did not explain the benefits associated with tighter glycemic control with insulin therapy.[3]

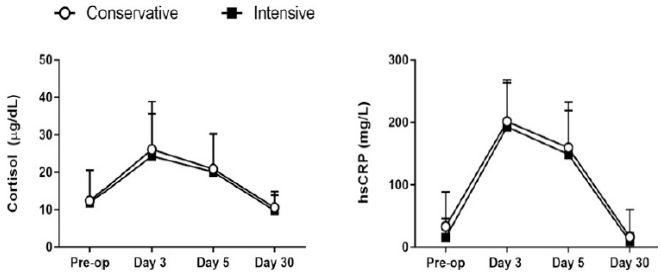

Figure 3. Cortisol and inflammatory markers during CABG surgery (Source: GLUCO-CABG trial)[3,4]

C.4. *GLP-1 RA safety and non-inferiority to insulin therapy.* In a recent RCT, we observed that a similar proportion of subjects reached goal (70-180mg/dL) with exenatide alone (61%) compared to basal bolus (63%) in the hospital. Exenatide (twice a day) was well tolerated, with higher, but not significant incidence of gastrointestinal side effects. Longer acting (once a week) GLP-1 RAs have been shown to be non-inferior or superior to insulin therapy and have a lower incidence of gastrointestinal side effects compared to exenatide or other short acting GLP-1 RAs.[116-119] Recently dulaglutide was shown to have lower incidence of side effects compared to another long acting GLP-1 RA, semaglutide. Native GLP-1 continuous infusion (very short half-life) was well tolerated in patients undergoing CABG.[120] GLP-1 RA may be used before surgery in patients with DM.[106,107]

C.5. *Biomarkers and CV Death: Dr.* Quyyumi et al. have demonstrated that a biomarker risk score (BRS) comprising suPAR (a biomarker of immune system activation and of inflammation) in addition to CRP (inflammation), FDP (coagulation), and HSP-70 (cell stress) significantly predicts all major outcomes in patients with coronary disease independently of traditional risk factors (Fig. 4). Correlations between biomarkers are modest suggesting that they act independently involving separate biological pathways.[30] This findings have been recently reproduced in CABG patients (BARI-2 trial).[2]

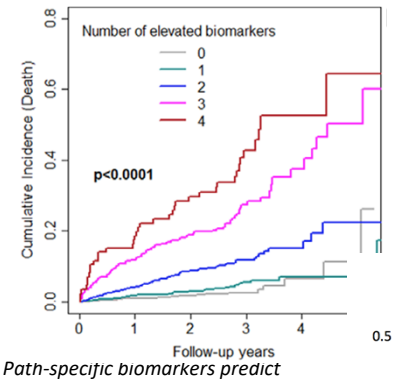

Figure 4. Path-specific biomarkers predict Mortality in patients with CAD. [30]

C.6. M*etabolomics in patients with hyperglycemic crisis:* Following a metabolomics analysis flow developed in the lab of Dr. Jones (Fig. 7) we examined plasma samples from 29 adults with diabetic ketoacidosis (DKA). Out of 4,144 metabolites, we found 216 significant baseline features that differed between patients with severe and non-severe DKA; 87 features were down-regulated and 129 were up-regulated in severe DKA. A total of 373 metabolites differed according to duration of continuous insulin infusion therapy.

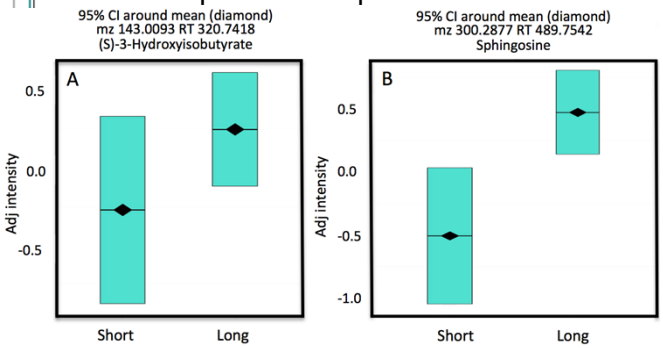

Figure 5. 3-Hydroxyisobutyrate (Panel A) a product of BCAA and sphingosine (Panel B), a product of lipid metabolism was associated with prolonged resolution of hyperglycemic crises in patients with diabetes, suggesting prolonged resistance to insulin (Pasquel et al. preliminary data)

C.7. Protein and lipid catabolism: We observed that

3-Hydroxyisobutyrate (3-HIB), and sphingosine were associated with prolonged resolution of ketoacidosis suggesting resistance to insulin therapy (Fig. 5) BCAA are implicated in the development of insulin resistance based on epidemiological and experimental data.[49] 3-HIB a catabolic intermediate of the BCAA valine is secreted from muscle cells, activates endothelial fatty acid transport, stimulates muscle fatty acid uptake in vivo and promotes lipid accumulation in muscle, leading to insulin resistance.[52] Recently, 3-Hydroxyisobutyrate has been associated with insulin resistance in humans and has also been identified as a predictor of incident type 2 diabetes.[52,53,121] In addition, sphingosine, a sphingolipid derived from ceramide was also associated with prolonged resolution of ketoacidosis. Ceramides and acylcarnitines are associated with insulin resistance and incident CVD. [41,44-46,54,55] These initial metabolomics analyses represent an innovative approach in diabetes related research. To better understand mechanisms leading to stress-hyperglycemia in hospitalized patients (career goal) we will apply these technologies in a setting were a baseline and longitudinal assessment is more feasible (elective CABG surgery) and very clinically relevant.

In summary, our preliminary studies indicate that stress-hyperglycemia is common and associated with increased rate of complications. Plasma BCAA, ceramides and acylcarnitines provide an intermediate link between metabolic stress, overnutrition, and mechanisms underlying insulin resistance and cardiovascular disease (CVD). Metabolic changes during hyperglycemia are complex and further explorations before and after stress in patients with and without perioperative stress-hyperglycemia are needed. Previous studies suggest that protein catabolism related products are associated with increased risk of pre-diabetes and diabetes. Our results suggest that 3-Hydroxyisobutyrate (3-HIB) a product of valine catabolism, and sphingosine are associated with prolonged response to insulin in acutely ill patients. A simultaneous and longitudinal assessment of biomarkers and relevant metabolites before and after surgery is essential to understand these complex relationships.

D) APPROACH

General overview: The proposed research will test in a nested case- control study associations of individual metabolites and broader metabolomics profiles as well as counter-regulatory hormones and biomarkers with stress-hyperglycemia (Aim 1). For this analysis patients will be stratified according to the outcome of stress-hyperglycemia (cases) and compared to controls (no hyperglycemia). For Aim 2 a randomized clinical trial will be conducted to examine the effects of exposure to dulaglutide on the prevention of stress-hyperglycemia and the metabolic inflammatory response in the perioperative period, (Fig. 6).

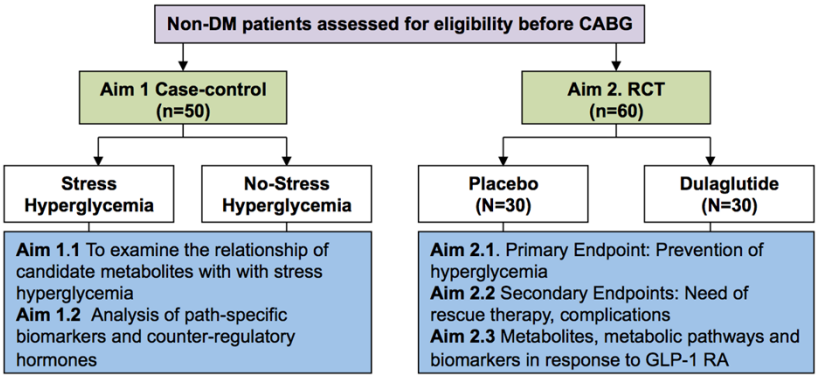

Figure 6. General overview. Aim 1 will be tested with a case-control study design. In Aim 2 we will conduct a clinical trial.

Specific Aims

D1. Case – control study

Aim 1. To examine baseline and postoperative metabolic profiles of non-diabetic CABG patients with stress hyperglycemia.

Rationale: the underlying mechanisms leading to stress-hyperglycemia and its association with poor outcomes in cardiac surgery patients are not well understood. We aim to study metabolic signatures associated with hyperglycemia and path-specific biomarkers. We will test the temporal relationships between candidate metabolites/pathways (Table 1) and specific biomarkers with stress-hyperglycemia.

Study design: In a nested case-control study (N=50) we will compare the characteristics of patients developing postoperative stress-hyperglycemia (cases) compared to patients that do not develop stress-hyperglycemia (age-matched controls). A proportion of patients will be derived from the placebo arm of the randomized trial. This design will allow efficient recruitment from our cohort of CABG patients to allow substantial savings in cost

and time. *Case definition:* stress-hyperglycemia is defined as a BG >140mg/dl on arrival to the ICU. Please see additional details in Human Subjects section.

Study Population: we will include patients with ages between 18-80 years without a history of diabetes [BG ≤125 mg/dl and HbA1c <6.5%] undergoing CABG surgery. Patients will be recruited from three academic hospitals, including Emory University Hospital, Emory Hospital Midtown (EUHM), and Grady M Hospital in Atlanta, GA. Dr. Halkos (Chief of Division and Director of the CTS Center for Clinical Research) is a key collaborator and will facilitate recruitment and perform CABG surgeries.

Aim 1.1 Targeted Approach: We will determine cross-sectional and longitudinal changes of ceramides, acylcarnitines, BCAA, and a biomarker panel for CVD outcomes and their relationship with stress hyperglycemia (BG >140mg/dl on ICU arrival). Path-specific biomarkers include: TNF-α and C-reactive protein (inflammation), fibrin degradation products (pro-thrombosis), heat shock protein-70 (cell stress), and suPAR (immune modulation and inflammation). Counterregulatory hormones include: cortisol, glucagon. Glucose, insulin and indexes of insulin sensitivity/insulin resistance: will be derived from glucose, insulin and c-peptide values. **Methods:** Plasma samples will be stored at − 80 °C until analysis. Serum CRP and FDP including fragments D and E, D -dimer, and additional intermediate cleavage products will be determined (FirstMark, San Diego, CA). Serum HSP-70 will be measured with a sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Plasma suPAR levels will be measured using commercially available kits (suPARnostic kit; Virogates, Copenhagen, Denmark). Minimum detectable CRP, FDP, HSP-70, and suPAR have been reported at 0.1 mg/L, 0.06 μg/mL, 0.313 ng/ml, and 0.1 ng/ml, as described previously.[65-68] Liquid chromatography tandem mass spectrometry techniques will be used to quantitatively profile ceramides, acylcarnitines, branched chain amino acids (leucine, isoleucine, valine), and aromatic amino acids (tyrosine and phenylalanine) in plasma samples along with standard reference samples. Glucose levels will be analyzed by the hexokinase method (Beckman-Coulter, Los Angeles, CA). Counterregulatory hormones, insulin, C-peptide levels by two-site sequential chemiluminescent immunometric assays. Surrogates of beta cell function (HOMA-B) and insulin resistance (HOMA-IR) will be derived from fasting glucose, c-peptide and insulin. Analysis: Student's t test and $\chi 2$ tests will be used when appropriate. Mann–Whitney U or Kruskal–Wallis nonparametric tests will be used for non-normally distributed variables. Analysis will account for age-matching. The relationship between biomarkers and stress-hyperglycemia and surrogates of insulin sensitivity and beta cell function will be determined using the Cox proportional hazards regression in unadjusted models and in models adjusted by gender, and smoking status to evaluate the impact of each 1 U increase in a biomarker risk score based on previously defined cut points for CRP, FDP, HSP-70, and suPAR (3 mg/L, 1.0 μg/mL,0.313 ng/ml, and 3.5 ng/ml, respectively) as previously described.[65,66,68]

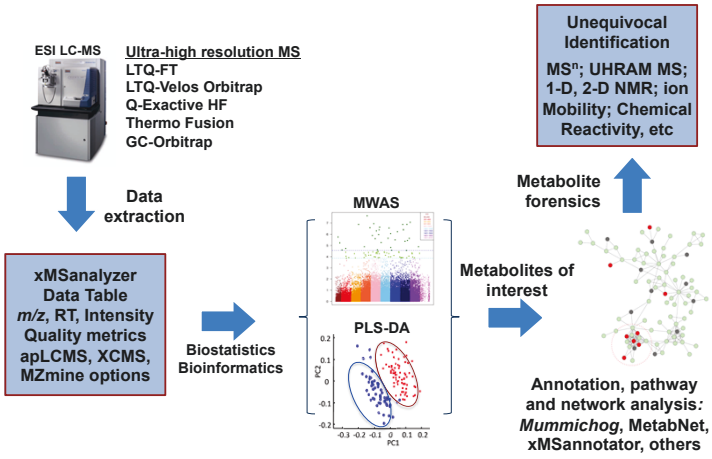

Figure 7. Workflow for high-resolution metabolomics. Samples are processed and analyzed in triplicate using liquid chromatography coupled to ultra-high resolution mass spectrometry. Data extraction and quality assessment is automated with stringent quality control requirements. Data are analyzed with well-established univariate and multivariate methods, with false discovery rate adjustment as appropriate. Selected features are tested for pathway enrichment, and suspect metabolites are confirmed by coelution and MS/MS with authentic standards. Reference standardization is used for quantification of metabolites of interest. (Uppal, Jones et al)

1.2 Untargeted Analysis: Utilizing untargeted LCMS-derived high-resolution metabolomics (HRM) analysis we aim to identify additional candidate metabolites and new metabolic pathways associated with incident stress hyperglycemia during cardiac surgery. **Methods:** HRM analysis is a major component of my career development plan. Data analysis will be conducted under the supervision of Dr. Jones. Data Interpretation will be conducted under the guidance of Dr. Richard Kibbey (expert in metabolism). I will also have local support from Dr. Alvarez for interpretation of metabolomics results, as well as statistical support from Dr. Tianwei Yu. Dr. Uppal will provide guidance for hands-on use of metabolomics tools. Co-mentors and collaborators are experts in the data management requirements of the LCMS-derived high-resolution metabolomics (HRM) analysis proposed in this project. Serial samples will be collected: at baseline (up to seven days before surgery), and intra-op at 15 minutes (after cardiac bypass has started for on pump surgeries) or 2hrs after sternum incision (for off pump surgeries), 24hrs, 72-96hrs and 30 days after surgery. Plasma samples will be stored at −80°C until analysis. The methods use UPLC with ultra-high resolution mass spectrometry with a dual chromatography, dual ionization approach with each sample being analyzed with three technical replicates.[122-124] With advanced data extraction provided by xMSanalyzer and apLCMS, I will be able to also measure a spectrum of high and low abundance metabolites. We will utilize a well-established computational metabolomics workflow with rigorous standard operating procedures outlined in Fig. 7.[124] Data Selection and Annotation (Biostatistics and Bioinformatics Tools): HRM data will be processed using apLCMS with xMSanalyzer [125,126]. Feature annotation will be performed using xMSannotator.[127] xMSannotator provides confidence scores for more than 2,000 metabolites, with more than 300 metabolites having confirmed identities and including most major pathways. The quality of data will be judged based on established rigorous criteria (e.g. coefficient of variation within replicatesand QC samples, the percentage of missing values, the percentage of data exceeding detection limits of the machinery, batch-effects, and the percentage of outliers, etc.). Principal Component Analysis (PCA) will be used for batch-effect evaluation and ComBat[122] will be used for batch- effect correction if necessary. Identifying expressed metabolites across different time points: we will use univariate and multivariate statistical methods, such as linear mixed effects models with post-hoc Tukey tests, LIMMA, and multilevel sparse Partial Least Squares Discriminant Analysis (msPLSDA).[128,129] Multiple hypotheses testing correction will be performed using the Benjamini-Hochberg method.[130] Identifying putative biomarkers: we will use a consensus feature selection approach such that metabolic signatures identified by both univariate and multivariate approaches will be used for downstream analyses. K-fold and leave-one-out cross-validation using Support Vector Machine (SVM) will be performed to evaluate predictive accuracy of putative biomarkers. Two-way hierarchical clustering analysis will be performed to identify clusters of correlated metabolic features and to evaluate the ability of the discriminatory features to separate samples based on stress-hyperglycemia. Pathway enrichment analysis: Pathway analysis will be performed with pathway enrichment software for untargeted metabolomics data (Mummichog and MetabNet)[131,132] Where appropriate, additional chemical verification will be performed for significant metabolites of interest according to retention time and characteristic ion dissociation (MS/MS) patterns relative to authentic standards. Metabolome-wide association study (MWAS) of specific metabolites will be conducted (MetabNet) to facilitate detection of their related metabolic pathways and network structures in association with glucose levels (continuous) and stress-hyperglycemia as a discrete dichotomous variable.

1.3 Integrative Analysis: A metabolome-wide association study (MWAS) of specific metabolites will be conducted (using MetabNet) to facilitate detection of their related metabolic pathways and network structures in association with stress-hyperglycemia. In addition, we will use xMWAS[133] for integrative network analysis to find multilevel associations between glucose levels, metabolites and biomarkers.

Expected outcomes of Aim 1: During our analysis of candidate metabolites and discovery phase in Aim 1 we expect to find an association between candidate metabolites (i.e. BCAA, ceramides, acylcarnitines) and pathways associated with stress-hyperglycemia. We also expect to find an association between pathway-

specific biomarkers and stress-hyperglycemia with a higher degree of association according to the number of biomarkers based on pre-specified cut points (biomarker risk score).[66] We expect to find multilevel associations between glucose, biomarkers and candidate metabolites. Serial measurements including measurements before the acute stress episode will facilitate causal interpretations.

Potential Pitfalls, and Alternative Considerations: It is possible that some comparisons do not yield false discovery rate (FDR)-significant metabolites. In this scenario, and to provide a balance between type I and type II statistical error, metabolites with raw $p<0.05$ will be used for subsequent analyses, with statistical tests for pathway enrichment. Pathway enrichment analysis is less susceptible to type II error.[134] We hypothesize that stress-hyperglycemia leads to elevation of path-specific biomarkers, however it is possible that pre-surgical elevation of biomarkers predicts the development of stress-hyperglycemia. Elucidating this scenario is important as identifying susceptible subjects before surgery (baseline elevation of biomarkers) will facilitate the design of cohort studies as well as recruitment of at-risk patients in future trials.

D2. Randomized Clinical Trial

Aim 2. To obtain preliminary estimates of the effect of a long-acting GLP-1 RA on the prevention of stress-hyperglycemia and modulation of metabolic stress during cardiac surgery.

Rationale: At least half of non-DM patients undergoing cardiac surgery develop stress hyperglycemia,[8,9,72,135] shown to be an independent risk factor of morbidity and mortality.[10,12,14-16] To maximize exposure to subjects at high risk of stress hyperglycemia, we will enroll obese individuals older than 50 years of age. Most patients with stress-hyperglycemia require insulin infusion in the ICU and about half continue to receive subcutaneous insulin after transition to regular wards.[24,136] GLP1 treatment has been shown to improve glycemic control similar to insulin administration in critically ill patients,[88-90] CABG surgery,[92] and in mixed ICU populations.[93] Dulaglutide is effective in improving glycemic control in patients with DM,[100-102] with lower rate of side effects compared to other long acting GLP-1-RA.[105] Phase 1 pharmacodynamics analysis in non-DM participants demonstrated median maximum plasma concentrations of dulaglutide between 24-48 hours after single-dose administration[103] with a dose-dependent rise in insulin secretion and progressive reduction in glucose concentration.[103] The rapid onset and sustained action, safety and efficacy with low-rate of hypoglycemia,[102,137] indicate that dulaglutide is an ideal agent for the prevention of stress-hyperglycemia.

Study Design: This randomized, placebo controlled study will include 60 obese subjects with ages between 40-80 years of age without a history of diabetes [BG ≤125 mg/dl and HbA1c <6.5%] undergoing CABG surgery. Due to the study design (i.e. need of surgical care), there will be no run-in period. Women in childbearing potential will have urine β-HCG measured before participating in the study.

Study population:

Inclusion criteria: Males or females between the ages of 40 and 80 years and BMI ≥25kg/m^2 undergoing elective CABG surgery. No previous history of diabetes or hyperglycemia.

Exclusion criteria: a) Hyperglycemia (BG>125 mg/dl or HbA1c > 6.5%) or previous treatment with antidiabetic agents; b) impaired renal function (GFR < 30 ml/min) or clinically significant hepatic failure; c) subjects with gastrointestinal obstruction expected to require gastrointestinal suction; d) patients with clinically relevant pancreatic or gallbladder disease; e) treatment with oral or injectable corticosteroid; f) mental condition rendering the subject unable to understand the possible consequences of the study; g) pregnancy or breast-feeding at time of enrollment. Additional considerations are outlined in the Human Subjects section.

Aim 2.1 Prevention of hyperglycemia (primary endpoint):

The primary endpoint of this exploratory study is to compare the frequency of stress-hyperglycemia defined as any post-CABG hospital-obtained BG > 140 mg/dl between patients randomized to dulaglutide or placebo.

Aim 2.2 Secondary Endpoints: To determine the difference between groups in mean BG level in the ICU. Additional endpoints include differences between treatment groups on: 1) the number of patients with stress-hyperglycemia requiring rescue therapy with SC insulin after discontinuation of CII; 2) need for CII treatment of hyperglycemia in the ICU; 3) mean ICU and floor BG concentration; 4) mean insulin dose during ICU (insulin infusion per hour (unit/hour) and per day); 5) duration of CII (hours); 6) days of SC insulin after discontinuation of CII; 7) amount of SC insulin in ICU and non-ICU stay; 8) hyperglycemic events (BG ≥ 200 mg/dl) in ICU and non-ICU; 9) hypoglycemic events in ICU and non-ICU (<70, < 54, and <40 mg/dl); 10) composite of mortality and complications including sternal wound infection, bacteremia, pneumonia], acute kidney injury, and acute myocardial infarction; 11) gastrointestinal adverse events (nausea, vomiting, ileus, pancreatitis); 12) ICU and hospital LOS, and ICU readmissions; 13) cerebrovascular events; 14) ICU and hospital mortality.

Methods for Aims 2.1 and 2.2:

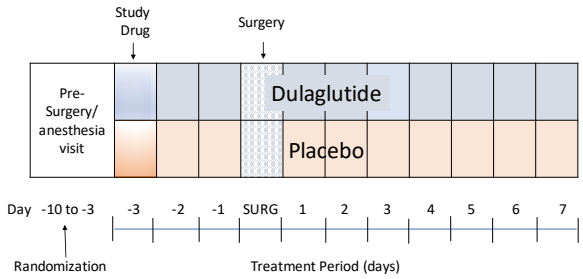

Figure 8. Treatment groups in Aim 2. Patients will be assigned to receive dulaglutide or placebo 1-3 days before surgery.

Treatment Groups: Patients will be randomized at pre-surgery/anesthesia visit. Patients without a history of DM will receive a single injection of dulaglutide/placebo 1-3 days prior to surgery (Fig. 8).

Inform consent and randomization: Patients will be consented during the preoperative evaluation on admission at least 24-72 hours prior to scheduled surgery. The investigators or study coordinators will review and explain the contents of the informed consent document to the eligible patient. Additional details are outlined in the Human Subjects section.

Study Sites: This pilot study will be conducted at Emory University Hospital, Emory Midtown Hospital, and Grady Memorial Hospital, Atlanta.

ICU Stay: Study patients who develop stress-hyperglycemia (BG >140 mg/dl) in the ICU will be started on intravenous CII adjusted to maintain a BG target between 100-140 mg/dl following a standard hospital protocol.

Transition from ICU to Regular Floor: Patients with fasting and/or premeal BG >140 mg/dl will receive coverage with sliding scale insulin (supplements), those with 2 consecutive BG >180 mg/dl, or average daily BG >180 mg/dl will be started on rescue therapy with SC basal insulin (glargine or levemir) once daily.[111]

Rescue Therapy with Basal Insulin in Non-ICU Settings[111]:

- BG between 140-200 mg/dl= start basal insulin at 0.1 units per kg weight per day.
- BG between 201-400 mg/dl= start basal insulin at 0.2 units per kg weight per day.
- Basal insulin will be given once daily, at the same time of the day.

Insulin adjustment and supplemental (correction) insulin: we will use a standard insulin treatment protocol.[4,86]

Investigational drugs:

- Dulaglutide injection: 0.75 mg/0.5 mL solution in a single-dose pen will be provided to patients
- Saline injection/0.5 mL pre-drawn solution, a tuberculin syringe will be provided to patients
- A research pharmacist at the site-specific Clinical Research Network Unit will provide dulaglutide and saline. Patients will be instructed on self-injection technique.

Withdrawal Criteria (please see additional details in Human Subjects and DSMP sections):

- The subject may withdraw at any time during the study by primary care provider or research team.
- The subject may be withdrawn at the investigator's discretion due to a safety concern or for contravention to the inclusion and/or exclusion criteria.
- Subject diagnosed with acute pancreatitis by clinical and/or radiographic criteria.

Analysis: The primary endpoint of Aim 2 is the frequency of stress-hyperglycemia (BG >140 mg/dl) requiring insulin therapy during the postoperative period (Aim 2.1). We will use two-sided Chi-square test or Fisher's exact test for the primary outcome. In Aim 2.2 we will compare the difference in mean BG upon ICU arrival and the frequency of rescue therapy with SC insulin after transition to regular floor. We will also explore differences in complications between groups. Secondary outcomes of interest can be a binary outcome (e.g. whether CII is needed in ICU), or a count outcome (e.g. number of perioperative complications), or a continuous outcome (e.g. mean ICU BG concentration). We will adopt the same strategy proposed for the primary outcome to analyze any binary secondary outcomes. For secondary outcomes measured as counts, we plan to use nonparametric tests such as Krustal-Wallis tests. Univariate Poisson regression (or Negative Binomial regression) will be performed to estimate the marginal treatment effect. In addition, we will also conduct multivariate Poisson regression (or Negative Binomial regression) to assess the outcome differences between the two study groups with potential confounders considered. For secondary endpoints that produce continuous outcomes, we will use two-sample t-tests or nonparametric Wilcoxon tests to compare study groups. Transformations will be applied if normality violation is detected.

Aim 2.3. To compare changes in metabolites and biomarkers in response to dulaglutide or placebo (measured in Aims 1.1 and 1.2), as well as beta-cell function and insulin resistance estimates.

Methods: Biomarkers and assessment of candidate metabolites will follow the same procedures as specified in Aim 1. Surrogates of beta cell function and insulin resistance will be derived from glucose, insulin, and c-peptide measurements acquired during the perioperative period.

Analytic Techniques: Glucose levels will be analyzed by the hexokinase method (Beckman-Coulter, Los Angeles, CA). Insulin and C-peptide levels will be measured by two-site sequential chemiluminescent immuno-metric assays as previously described. Surrogates of beta cell function (HOMA-B) and insulin resistance (HOMA-IR) will be derived from fasting glucose, c-peptide and insulin. Only glucose and c-peptide will be measured serially after surgery (given high likelihood of exposure to exogenous insulin).
Analysis: We will integrate analyses from Aims 1 and Aims 2 to evaluate the role of dulaglutide on metabolites, metabolic pathways and biomarkers. We will use univariate and multivariate statistical methods, such as linear mixed effects models with post-hoc Tukey tests, LIMMA, and multilevel sparse Partial Least Squares Discriminant Analysis (msPLSDA), as described in Aim 1.

Expected outcomes in Aim 2: In Aim 2.1 we expect to observe a decrease in the number of patients with stress-hyperglycemia with low risk of hypoglycemia with dulaglutide therapy. We expect to find a least an 18 mg/dl (1 mmol/l) mean difference in BG between groups during ICU stay. In Aim 2.2 we don't expect to find differences in hospital outcomes. In Aim 2.3 We expect to observe differential changes in up or down regulation of metabolic pathways in response to dulaglutide compared to placebo.

Potential Pitfalls, and Possible Solutions, Alternative Consideration: Recruitment and retention of patients are important challenges in RCTs; however, Emory University and affiliated hospitals serve as cardiovascular referral center to a large population in the city of Atlanta, with over 1,000 major cardiac surgeries performed yearly. In our previous trial we recruited 340 patients over 3 years, therefore we expect to have enough time during the award period to complete enrollment (please see research timeline). It is possible that dulaglutide does not have an effect on the prevention of hyperglycemia. This information will help us understand the contribution of the incretin pathway to glucose metabolism during surgical stress.

E. Risks to Human Subjects

E.1.1 Human Subjects Involvement, Characteristics, and Design

- **Description and justification for proposed involvement of human subjects:**

  Extensive data from observational and prospective randomized controlled trials (RCT) in hospitalized patients have reported a strong association between stress hyperglycemia and poor clinical outcome, such as increased mortality, morbidity, hospital length of stay (LOS), infections and overall complications. This relationship is well established, and a dose response is consistently observed. This association is more robust among non-diabetic subjects compared to patients with diabetes or normoglycemia. Millions of cardiac surgeries are conducted globally and there is an urgent need to understand the mechanisms that lead to hyperglycemia during stress conditions and strategies to improve meaningful clinical outcomes. Cardiac surgery, in particular coronary artery bypass graft (CABG), is a unique human model for the study of metabolic changes during surgical stress. In our own study we have shown a reduction in a composite score of complications in subjects without diabetes undergoing CABG surgery randomized to a more intensive glycemic target. Our current approach to treat hyperglycemia, however, is reactive, it demands high resource utilization (staff needs to monitor glucose levels hourly and adjust intravenous insulin doses frequently), and if not done carefully is associated with a very high risk of iatrogenic hypoglycemia (up to 35%), which also carries a high risk of complications. This novel, exploratory study will characterize metabolic signatures during stress hyperglycemia, integrate findings with clinically meaningful biomarkers, and provide preliminary estimates of the effect of a long acting GLP-1 receptor agonist on the prevention of stress hyperglycemia.

- **Study Population Characteristics:** Clinically stable adults scheduled to undergo coronary artery bypass graft surgery (CABG) will be studied. We expect that a total of 90 subjects will complete this project. A proportion of patients will be derived from the placebo arm of the randomized trial. This design will allow efficient recruitment from our cohort of CABG patients to allow substantial savings in cost and time. For Aim 1 a total of 50 patients will be included. In Aim 2 patients will be randomly assigned to dulaglutide (N=30) or placebo (N=30) one to three days before surgery. For both aims subjects must meet the inclusion and exclusion criteria listed below:

Inclusion criteria: 1) Males or females between the ages of 40 and 80 years and BMI ≥25kg/m^2 undergoing elective CABG surgery; no previous history of diabetes or hyperglycemia.

Exclusion criteria: 1) Hyperglycemia (BG>125 mg/dl or HbA1c > 6.5%) or previous treatment with antidiabetic agents; 2) Severely impaired renal function (GFR < 30 ml/min) or clinically significant hepatic failure; 3) Subjects with gastrointestinal obstruction expected to require gastrointestinal suction; 4) patients with clinically relevant pancreatic (including history of pancreatitis) or gallbladder disease; 5) treatment with oral or injectable corticosteroid; 6) mental condition rendering the subject unable to understand the possible consequences of the study; 7) Pregnant female subjects or breast feeding at time of enrollment.

- **Sampling Plan:** Potential study subjects will be identified and pre-screened among patients scheduled for CABG at Emory University affiliated hospitals. Enrollment will be coordinated with cardiovascular surgeons. Dr. Halkos, Scientific Director, of the Cardiothoracic Surgery Center for Clinical Research (CCR) will facilitate enrollment in the study. A study coordinator will conduct informed consent in eligible subjects prior to any study procedures. Screening and recruitment reports will be generated monthly that include actual and expected recruitment statistics. Strategies to address potential problems with recruitment will include biweekly communications between Dr. Pasquel, Dr. Umpierrez, and Dr. Halkos. After informed consent, baseline and post CABG blood sampling will be performed. Samples will be stored at Clinical Research Network (CRN) sites of the NIH-funded Georgia Clinical and Translational Science Alliance (CTSA).

- **Special Populations:** This study does not involve special vulnerable populations. It will, however, include elderly subjects with ages between 65 and 80 years of age, who are more likely to have advanced cardiovascular disease and require CABG surgery. Older patients are also more likely to develop hyperglycemia during surgery.

- **Group Assignment / Case Definition:** Clinically stable adults scheduled to undergo coronary artery bypass graft surgery (CABG) will be studied. For logistic purposes recruitment will favor assignment to the clinical trial in the beginning of the award period (Aim 2, N=60). We expect a total of 30 subjects will be randomized to each group (dulaglutide or placebo). Thirty patients assigned to placebo in the clinical trial will contribute to the cohort of patients to be included in the nested case-control study. Cases and controls will be categorized prospectively on arrival to the surgical intensive care unit (SICU) (Cases are defined as BG >140mg/dL on ICU arrival). To further avoid selection bias, 25 age-matched controls will be selected for comparison. Once the number of cases is completed, additional potential controls will be selected (+/- 3 years) based on the characteristics of the population of cases. If additional patients develop stress hyperglycemia no further samples will be collected. This design will allow for efficient recruitment from our cohort of CABG patients to allow substantial savings in cost and time.

- **Research Sites:** All human subjects' research will be performed on the campus of Emory University, Atlanta, GA, and at Emory Affiliated Hospitals. Sites include Clinical Research Units (CTSA) where samples will be stored. Data collected are kept confidential and protected as per HIPAA regulations. Data collected at each facility will be shared only with the study team.

E.1.2. Study Endpoints and Sources of Materials:

- In the case control study (Aim 1) we will compare metabolic and hormonal profiles between subjects that develop stress hyperglycemia compared to controls. In an open-label format (Aim 2) we will assess whether exposure to dulaglutide is associated with a reduction in the frequency of stress hyperglycemia compared to placebo (primary endpoint). Prospectively, we will assess the association of candidate metabolites and metabolic pathways with the onset of stress hyperglycemia. Potential mechanisms underlying these associations will be explored by examining whether the associations of exposure to dulaglutide and prevention/improvement of glycemic control can be explained by changes in metabolic pathways (and key metabolites within these pathways) measured in plasma that occur between 3 days prior to surgery (baseline) and during the perioperative period. Addressing these research questions

will be possible using innovative ultra-high resolution mass spectrometry (HRMS) with liquid chromatography (LC) and gas chromatography (GC) and advanced data extraction algorithms. LC-HRMS will also measure >2000 metabolomic intermediates in plasma at the time of surgery and at follow-up, providing coverage of >85% of human metabolic pathways. ***The overall hypotheses*** *are that candidate metabolites such as: ceramides, acylcarnitines, and branched-chain amino acids (BCAAs) are associated with incident stress hyperglycemia, and that hyperglycemia leads to changes in path-specific biomarkers related to CV risk. In addition, exposure to an agent associated with low risk of hypoglycemia can positively affect the trajectory of metabolic changes during acute surgical stress.*

Baseline measurements: Samples will be collected up to seven days prior to scheduled CABG surgery. Blood samples will be processed by the CRN Laboratory and stored at −80°C until ready for batch analysis. Fasting plasma metabolomics analysis will be performed using high-resolution metabolomics (HRM) methods established in Co-mentor Dean Jones' laboratory. Samples for measurement of biomarkers, glucose, cortisol, glucagon, insulin and c-peptide will also be collected. Indexes of insulin sensitivity/insulin resistance: will be derived from glucose, insulin and c-peptide values. Indexes of insulin secretion: pancreatic β-cell insulin secretion capacity will be assessed with HOMA-B.

Sources of materials: Research material will include medical history, demographic information, and complications obtained both from subjects, medical record, and study blood samples.

Data collected: Data to be collected is detailed in **Table 1** below, along with the source of the data. Baseline and daily information will be entered by Dr. Pasquel or a study coordinator into data collection paper forms and into an electronic database (REDCap) provided by the Emory Research Information Technology Department.

Table. Data to be collected and source

Data Item	Source
-Vitals (e.g. blood pressure, pulse, temperature)	Medical Exam
-Height, weight, BMI	Manual stadiometer and digital scale
-Demographic Information (e.g. age, race)	Self-report and medical records
-Medical history (e.g., comorbidities, surgeries)	Self-report and medical records
-Social history (e.g., education, smoking, alcohol use)	Self-report and medical records
-Indexes of insulin secretion, resistance and sensitivity	serial blood draws
-Plasma metabolomics, biomarkers, hormones	Blood draw
-Dates of hospitalization and operation	Medical records
-Daily laboratory results (e.g. blood glucose, creatinine, eGRF)	Medical records
-Treatment (IV or SC insulin dosage, use of corticosteroids)	Medical records
-Complications and adverse events (defined below)	Self-report and medical records
-Length of ICU and hospital stay	Medical records

Data Collection on Complications

- Surgical wound infection (superficial and deep sternal wound infection).
- Pneumonia (CDC criteria).
- Acute kidney injury defined as an increment in serum creatinine ≥ 0.3 mg/dL from baseline or ≥1.5 times baseline creatinine (KDIGO)
- Acute myocardial infarction (AMI): (1) typical increase and gradual decrease (troponin) or (2) more rapid increase and decrease (creatine kinase MB) of biochemical markers of myocardial necrosis with at least one of the following: (a) ischemic symptoms, (b) development of pathologic Q waves on the electrocardiogram, (c) electrocardiographic changes indicative of ischemia (ST-segment elevation or depression), or (d) coronary artery intervention (e.g., coronary angioplasty).

- **Clinical Management Guidelines:** The primary care team will provide care regarding the use of vasopressors, ventilator support, sedation, antibiotics, and treatment of co-morbid conditions.

  a. Assessment and monitoring of BG concentration. Information on BG measurements both at bedside by glucose meter and by hospital laboratory will be collected. BG will be measured every 1-2 hour during continuous insulin infusion (CII) in the ICU, and before meals and at bedtime after transition to regular wards. Hypoglycemia is the main adverse event and safety concern in the study. The number of mild (≤70 mg/dL) and severe hypoglycemia (≤ 40 mg/dL) and clinical consequences (neurological and cardiovascular) will be compared between groups.

  b. Assessment and monitoring of hospital mortality. Investigators will follow study subjects daily and the date and presumed cause of death will be recorded. Information on the attending physician's summary of events surrounding subject's demise will also be documented.

  c. Assessment and monitoring of wound infections. The investigators will review each subject's records daily from Monday to Friday regarding potential new infections. Data from the weekends will be collected the following Monday. Definitions: Deep Sternal Wound Infection (DSWI): chest wound infection involving the sternum or mediastinal tissues, including mediastinitis; Superficial Sternal Wound Infection (SSWI) (not involving the sternal bone or wires): chest wound infections involving the skin or subcutaneous tissues, or both.

  d. Assessment and monitoring of nosocomial infections. The following daily information will be collected for nosocomial infection surveillance: 1) temperature curve; 2) white blood cell counts; 3) daily progress notes; 4) daily clinical microbiology laboratory data; 5) all relevant radiographic reports; 6) orders for antimicrobial agents; 7) communication, as needed, with primary physicians and site infectious disease consultants; 8) use of the CDC guidelines for diagnosis of specific nosocomial infections.

  e. Assessment and monitoring of days on mechanical ventilation. The need for mechanical ventilation will be monitored daily. The day the subject is weaned from the ventilator will be recorded.

  f. Assessment and monitoring of length of SICU and hospital stay. The morning location of the study subject (in either the SICU or surgical ward) and date of hospital discharge will be recorded daily.

  g. Assessment and monitoring of renal function. Acute kidney injury - new-onset abnormal renal function is defined as an increment of creatinine level above 50% from baseline.

  h. Cerebrovascular accident (CVA). Defined as central neurologic deficit persisting more than 72 hours (permanent stroke), transient ischemic attack, deficit resolving within 24 hours, or deficit lasting more than 24 hours but less than 72 hours (reversible ischemic neurologic deficit).

- Data collection and data entry. Study members will enter baseline and daily data for this study into data collection paper forms and into an electronic database provided by the Emory Research Information Technology Department. Baseline data will include demographics/history form (subject gender, date of birth, ethnicity, dates of hospitalization and operation, history of diabetes, and comorbid conditions, body weight, BMI, smoking status, type of surgery, APACHE score. Daily information will be collected on treatment (insulin IV or SC and dosage, antibiotics, use of corticosteroids), nutrition support, BG and laboratory values, hospital complications and adverse events, and length of ICU and hospital stay.

E.1.3. Potential Risks

- **Potential Risks/Discomforts:**

  a. Venipuncture: There is some minor discomfort and risk of mild bruising during venipuncture. Standard sterile techniques will be used during phlebotomy; thus, infection is unlikely. Disposable pre-sterilized needles and syringes will be used for all blood drawing in this study; needles and syringes will not be reused. Discomforts associated with venipuncture are rapidly reversible. Approximately 40 mL of blood will be obtained for all the blood measurements proposed.

b. Hypoglycemia: In our previous ICU studies, we observed that <10% of cardiac surgery subjects during CII and 20-30% during SC insulin experienced hypoglycemia. No episodes of severe hypoglycemia were observed during CII in our study. The use of insulin in combination with GLP1-RA may increase the risk of hypoglycemia. The number of mild (≤70 mg/dL) and severe hypoglycemia (≤ 40 mg/dL) and clinical consequences (neurological and cardiovascular) will be compared between groups. Treatment of hypoglycemia: hypoglycemia, defined as a BG <70 mg/dL will be treated by a standard hypoglycemia protocol available at each hospital.
c. Hyperglycemia: it is very likely that a high proportion of patients randomized to placebo develop hyperglycemia. All subjects with hyperglycemia (in both groups) will be treated by standard protocols in the ICU units at each hospital. We expect that ~50-60% of subjects randomized to placebo will experience one or more episodes of hyperglycemia. The frequency of severe hyperglycemia will be analyzed statistically.
d. Gastrointestinal adverse effects: include nausea and vomiting, which are more common in patients treated with dulaglutide compared to placebo. The frequency of nausea and vomiting is reported in up to 10% of patients receiving dulaglutide in combination to oral agents compared to 3.5% in patients receiving placebo plus oral agents. If a participant developed nausea during hospitalization the research team will perform a phone call visit on post-discharge day 2 to ensure safety. There have been few reported events of acute pancreatitis with the use of GLP1-RA (typically reported among patients at higher risk of pancreatitis). Subjects should be informed of the characteristic symptoms of acute pancreatitis: persistent, severe abdominal pain. If pancreatitis is suspected, potentially suspect medicinal products should be discontinued. Patient will receive a single 0.75mg dose of dulaglutide. If the investigator suspects acute pancreatitis, all suspected drugs should be discontinued until confirmatory test have been conducted and appropriate treatment should be initiated. Subjects diagnosed with acute pancreatitis (as a minimum 2 of 3: characteristic abdominal pain, amylase and/or lipase >3xUNR or characteristic findings on CT scan/ MRI) should be withdrawn from the study.

- **Alternative treatments:** The alternative is for a participant to not participate in the trial.

E.1.4 Adequacy of Protection Against Risks

a. ***Recruitment and Informed Consent***

- Recruitment and Informed consent: Potential study subjects will be identified and pre-screened among patients undergoing CABG surgery at Emory affiliated hospitals.
- Dr. Pasquel or the study coordinator will conduct informed consent in eligible subjects prior to any study procedures. Screening and recruitment reports will be generated monthly that include actual and expected recruitment statistics.
- Description of informed consent process**:** A written and signed informed consent will be obtained in person in a private area. After identification of a person as potentially eligible for the study, approved study personnel will explain the study in detail in the consent form as well as verbally. Potential participants will be informed of the purpose of the study, the study protocol, and the routine and potential risks associated with the study procedures. All risks, costs, and benefits will be discussed. Participants will be assured of their right to withdraw at any time without prejudice to their care. They will be assured of confidentiality in maintaining records and reporting of results. A separate consent will also be obtained for permission to store biological samples for future studies. Any additional information regarding questions or concerns from potential or confirmed study participants will be provided. Participants will have ample time to call the study team with questions/concerns and make an informed decision about their participation in the study.

  An Institutional Review Board (IRB) approved study designee will provide verbal and written consent. The study is discussed thoroughly with each potential participant, and he/she will be encouraged to ask questions regarding the study and her participation. If the potential participant decides to enroll, he/she will sign the written informed consent, as will the study team member conducting informed consent. A copy of the signed consent form will be provided to enrolled study participants. The

consent process will be documented using a template provided by the Emory IRB in the "Consent Clinical Trial Tools" section of the Emory IRB website.

Subjects must be able to read, write and understand the consent documents. Study personnel will subjectively assess the capacity to give informed consent through the use of questions to ensure understanding and comprehension of the study. Only adults will participate in this study.

- **Waivers:** We will request a partial HIPAA waiver to be able to access medical records for screening.

b. ***Protections Against Risk***

- **Protections against risks:** Adverse events are expected to be uncommon and not pose more than minimal risk to the study participants.

    - Subject identification and screening for eligibility: Our strict inclusion and exclusion criteria for entry (outlined above) will help to minimize potential risks. All subjects will be monitored closely by frequent clinical assessments and review of safety laboratory tests by experienced clinicians. In addition, we will carefully monitor capillary BG at the bedside using the hospital certified meter, b) only experienced nurses/or phlebotomist will draw blood samples, c) patients will be closely monitored in the ICU, d) no patients with history of significant pancreatic, renal or hepatic failure will be recruited in this study.
    - Blood sampling: Experienced research nurses will use aseptic techniques for all study blood draws. Participants will be asked to stay hydrated with water the day before the study visit. Universal precautions will be employed in all instances involving human specimens and all blood processed under at least BSL2 conditions. Discomforts associated with venipuncture are rapidly reversible. During surgery trained personnel will collect the blood samples.
    - Hyperglycemia: All patients that develop hyperglycemia will be treated by standard insulin protocols in the ICU and non-ICU units available at each hospital.
    - Hypoglycemia: In our previous ICU studies, we observed that <10% of cardiac surgery subjects during CII and 20-30% during SC insulin experienced hypoglycemia. No single episode of severe hypoglycemia was observed during CII in our study in CABG patients. The use of insulin in combination with GLP1-RA may increase the risk of hypoglycemia. The number of mild (≤70 mg/dL) and severe hypoglycemia (≤ 40 mg/dL) and clinical consequences (neurological and cardiovascular) will be compared between groups. Any episode of hypoglycemia (BG <70 mg/dL) will be treated by a standard hypoglycemia protocol available at each hospital.
    - Privacy/Confidentiality: The subjects' extracted medical records and data from the study will be initially stored on paper prior to transfer to electronic format. The encrypted electronic record will remain on the principal investigator's password protected computer in his locked office. Confidentiality will be assured by the use of subject codes rather than personal identifiers. The master list connecting the subject codes to identifying information will be secured in the Emory's computerized database. All paper subject records will be kept in locked file cabinets in the PI's research office and will be accessible only to the PI and the investigative team. Only institutional review board (IRB)-approved study personnel will have access to individually identifiable information about human subjects. This may include the study PI, co-investigators, research coordinators, and other approved study personnel. All information and materials will be obtained for research purposes only and the data will be kept in strict confidence for use in this proposed research only.
    - Other: Since the proposed tests are not inherently hazardous, hazard is likely to occur only as the result of impaired participant confidence or sudden unwillingness to complete a test. To avoid this possibility, study personnel will thoroughly explain all tests to potential participants prior to them signing the consent form. Potential participants will have the opportunity to see all test equipment and facilities before giving consent or undergoing testing. Every effort will be taken to prevent injury or distress that may result from this

study. Dr. Pasquel, or a study physician will be on call for support. A qualified physician will have primary responsibility for inpatient care.

- Data and Safety Monitoring: A senior faculty Safety Officer, with expertise in randomized controlled clinical trials will be designated as the Safety Officer for this study. He/she will review semi-annual reports created by Dr. Pasquel and make recommendations regarding the continuation of study. Dr. Pasquel will review this protocol on a continuing basis for subject safety and include the results of the review in semi-annual reports to the Safety Officer and annual progress reports submitted to the IRB. A full Data and Safety Monitoring Plan (DSMP) is provided in a separate document.

- **Medical intervention:** If a participant suffers from distress during a procedure, the procedure will be discontinued. The participant will then be managed in the Emory affiliated Hospital surgical suite or in the CRN unit, which, if applicable or warranted, have immediate access to EKG monitoring, cardiac resuscitation equipment and are located in close proximity to Emergency Departments.

- Every effort will be taken to prevent injury or distress that may result from this study. A study physician will be on call for support. A qualified physician will have primary responsibility for inpatient care.

E.1.5 Potential Benefits of the Proposed Research to Human Subjects and Others

- **Potential Benefits:** There may be no specific health benefit to patients' participation in the study. However, participants will receive information on their blood glucose levels during the hospital stay, which they may use to inform their own health decisions/activities.

- **Risk/Benefit Assessment:** The risks incurred are minimal relative to the potential benefit of gaining information about individuals' health. Our group has extensive experience in glycemic control in the hospital and we will follow closely all patients for timely and appropriate management of hyperglycemia and hypoglycemia.

E.1.6. Importance of the Knowledge to be Gained

- **Importance of Knowledge:** The long-term goal of this study is to identify underlying mechanisms that lead to hyperglycemia and their association with established biomarkers known to predict risk of death in patients with coronary disease. This study has the potential to identify clinically meaningful metabolites to guide the development of important biomarkers (to be assessed in independent cohort studies) that can help to stratify patients at risk for hospital complications. The findings that arise from this research will also inform scientists in developing new technologies and designing future therapies to better understand and treat metabolic derangement during acute stress. We also aim to prevent hyperglycemia with an agent associated with lower risk of iatrogenic hypoglycemia. If our hypothesis is correct, such information could guide future clinical trials aimed at preventing hyperglycemia with the goal of impacting meaningful endpoints (cost, hospital complications, and mortality).

- **Risk/Importance Assessment:** The anticipated benefits of the proposed research study outweigh the potential risks to participating subjects. The potential for serious adverse effects is small and the potential benefit to society is high enough for the studies to proceed.

E.1.7 Data and Safety Monitoring Plan (DSMP): Please see DSMP provided as a separate document.

E.1.8 ClinicalTrials.gov Requirement: This clinical trial will be registered on ClinicalTrials.gov. Final results will be published on this site.

E.*1.9 Statistical Considerations*

Sample Size Calculation and Power Analysis: with a Wilcoxon test, for an effect size >1 at alpha=0.05 and power=0.80, the proposed sample size of N=50 for this experiment will be more than adequate for the main objective of identifying differentially expressed metabolites and pathways in Aim 1, as shown in previous studies.[134,138] For Aim 2 we assumed N=60. No preliminary data on prevention of hyperglycemia is available. Assuming an effect size of ~0.50 on hyperglycemia prevention with X^2 goodness of fit for contingency tables, power 80%, alpha=0.05, a total of 52 patients would be needed. In on our previous RCT (GLUCO-CABG[4]) non-DM patients in the conservative group had a mean BG 143mg/dl vs. 127mg/dl in the intensive group. This difference was associated with a lower composite score of complications in the intensive group. It is reasonable to assume the standard deviation of the mean BG is bounded around 40 mg/dl. With two-sample t tests or Wilcoxon tests, alpha=0.05, 25 subjects for each arm would be needed to ensure 80% power to reject the hypothesis that the mean BG in patients treated with dulaglutide is not 16 mg/dl lower compared to placebo. Accounting for ~15% attrition rate this leads to a final total sample size estimate of 60 patients for both considerations. This study is not powered to assess differences in hospital complications between groups.

Research Timeline	Yr1	Yr2	Yr3	Yr4	Yr5
Aim 1. Case-Control					
Study initiation (IRB)	x				
Recruitment / sample collection	xx	xx	xxx	xx	
Lab assays / data processing				xxx	xxx
Analysis / manuscript preparation				xxx	xxx
Aim 2. Clinical Trial					
Study initiation (IRB)	x				
Randomization / sample collection	xx	xxx	xx	xx	
Lab assays / data processing				xxx	xxx
Analysis / manuscript preparation				xxx	xxx

E.1.10 Confidentiality

- Access: Only institutional review board (IRB)-approved study personnel will have access to individually identifiable information about human subjects. This may include the Emory study Investigators who are directly involved with human subjects (Francisco J. Pasquel, Guillermo E. Umpierrez, Michael Halkos), and other approved study personnel (e.g. research coordinators).

- Protection and Management of data**:** All information and materials will be obtained for research purposes only and the data will be kept in strict confidence for use in this proposed research only. Confidentiality will be assured by the use of subject codes rather than personal identifiers on questionnaires, blood collection and storage tubes. All subject paper records will be kept in locked file cabinets and will be accessible only to the investigative team. The master list connecting the subject codes to identifying information (name, birth date, phone number, address, clinic visit dates, study dates) will be secured in a computer database (REDCap). All data maintained in computerized databases will be accessible only with a login and protected password. After the study is completed, all data will be kept according to NIH and FDA regulations in a locked file.

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