

Project title

Metabolic Signatures: Decoding incretin and glucagon pathways and their interaction with metabolic traits in cardiometabolic disease progression

**Brief project description (max 2000 characters including spaces.
Current count: 2088)**

People at high risk of type 2 diabetes are not currently being systematically identified, primarily because of variation in who progresses to diabetes. This project investigates the causal role of natural incretin and glucagon responses, aiming to identify the primary drivers of progression toward diabetes and diabetes related complications. The project will use the deep phenotyped data in the Fenland Study.

The Fenland Study is well suited as the selected database because it combines OGTT based incretin and glucagon measurements, genetics, proteomics, metabolomics, DEXA derived adiposity, liver function biomarkers, and long term cardiometabolic outcomes in a general population cohort. The study includes three phases of examination from 2005 to 2025, which allows for the assessment of individual trajectories in cardiometabolic risk. We will validate findings in the Danish ADDITION PRO study and the UK Biobank. The project is divided into three parts. First, using traditional and newer methods of causal inference, including genetically predicted and phenotypically measured traits, we aim to determine the causal relationship between incretin and glucagon responses and long term cardiometabolic outcomes and to identify mediating pathways such as liver health, inflammation and metabolite profiles. Second, we will investigate incretin and glucagon responses together with metabolic traits that contribute to heterogeneity in metabolic dysfunction risk, such as liver health and inflammation, and aim to clarify the impact of each metabolic trait on long term cardiometabolic risk. Third, we will identify and predict machine learning based metabolic clusters using clinical and omics data.

By using new epidemiological methods to characterize individual risk of progression toward cardiometabolic disease, and by clarifying the causal role of natural incretin and glucagon release, including important mediating pathways, this proposal aims to help identify individuals with the greatest potential benefit from early intensive intervention and to pinpoint targetable biomarkers.

**Lay project description (max 1000 characters including spaces.
Current count: 637)**

Type 2 diabetes develops differently from person to person, and today we cannot easily identify who is most at risk. This project uses data from the large Fenland Study to understand how two natural gut hormones, incretin and glucagon, influence the body's ability to control blood sugar. By combining hormone measurements with information on liver health, body fat, inflammation, genes and metabolism, we aim to discover why some people progress to diabetes or other cardiometabolic diseases while others do not. The project will also create simple tools that can help identify people who may benefit from early and targeted prevention.

**Project description (max 20,000 characters including spaces.
Current count: 36,000)**

Specific aim

This Inter-SUSTAIN Part 2 project aims to use the Fenland study to elucidate the role of natural incretin and glucagon responses as drivers of heterogeneity in the long-term risk of diabetes and related complications, and to map clinical and biological traits associated with these responses, with particular emphasis on liver health, adiposity, and inflammation. In doing so, we aim to identify patterns of metabolic dysfunction that may predict progression to type 2 diabetes and inform personalized treatment strategies.

The specific aims are to:

- a) Phenotypically and genotypically investigate the causal role of natural incretin and glucagon responses to an oral glucose tolerance test (OGTT) in determining the risk of regression to normoglycemia, progression to type 2 diabetes, and the development of diabetes-related complications. This will be conducted using data from the Fenland study and the Danish ADDITION-PRO cohort, linked to comprehensive outcomes from the Danish National Health Registries.
- b) Identify metabolites and biomarkers for liver health, adiposity and inflammation that mediate the association between phenotypically measured (Fenland study, ADDITION-PRO) and genetically predicted (Fenland study, ADDITION-PRO, UK Biobank) incretin and glucagon responses and cardiometabolic outcomes, with particular emphasis on direct and indirect markers of liver function.
- c) Map dimensions of metabolic traits in conjunction with incretin and glucagon responses to decipher their role in deteriorating glucose metabolism and their relationship to long-term cardiometabolic health.

- d) Predict clustered dimensions of metabolic traits using clinical markers, metabolites, and genetics.

Background

Pre-diabetes is a complex state associated with increased risk of progression to type 2 diabetes and related complications^{1–3}. However, not all people with pre-diabetes progress to diabetes; many persist in the prediabetic state or regress to normoglycemia over the course of various years². Remission to normoglycemia has been shown to reduce the risk of cardiovascular morbidity and mortality⁴, however, it remains unclear to which extent if the remission is driven by lifestyle changes or by a heterogeneous low-risk subgroup.

Recently the idea of metabolic heterogeneity in type 2 diabetes has been extended to pre-diabetes, and traits that distinguish individuals most likely to progress to diabetes, such as insulin-resistant fatty liver and visceral adiposity-related renal dysfunction, have been identified¹. In recent years, incretins (Glucagon Like Peptide 1 [GLP 1] and Gastric Inhibitory Polypeptide [GIP]) and the hormone glucagon have gained increasing attention as targets in therapeutics for prevention of type 2 diabetes and diabetes-related complications^{5–9}. GLP-1 and GIP enhance post-meal insulin secretion, supporting efficient nutrient handling¹⁰. Glucagon serves as a counter-regulatory hormone to insulin and maintains glucose balance both during fasting and following glucose intake¹¹. However, in the context of heterogeneity in pre-diabetes, the role of natural incretin and glucagon responses for the progression to type 2 diabetes and diabetes-related complications remains unexplored. Prior research in the Danish ADDITION-PRO study showed that, individuals with pre-diabetes and type 2 diabetes exhibited up to 25% lower natural GLP-1 responses to oral glucose¹². Additionally, impaired glucagon suppression and elevated fasting glucagon levels have been observed in individuals with insulin resistance and early glucose dysregulation¹³, involving hepatic insulin resistance that impairs the turnover of branched-chain amino acids¹⁴. However, these findings have not been limited investigated in relation to prospective cardiometabolic outcomes. A limitation of the ADDITION-PRO cohort is the selection of individuals at high risk of diabetes based on diabetes-specific risk scores, which limits the generalizability of the findings to the broader populations.

The Fenland Study represents a pioneering population-based cohort that has substantially advanced the precision of identifying individuals at high risk of type 2 diabetes¹⁵ and elucidating causal pathways underlying cardiometabolic disease [16]¹⁷[18]¹⁹. Leveraging extensive phenotypic and genetic data, the study has established causal relationships between liver function markers, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), and type 2 diabetes¹⁶, as well as between Dual Energy X-ray Absorptiometry (DEXA) derived adiposity traits, fat-mass ratios, and metabolic dysfunction-associated liver disease¹⁹. Moreover, Fenland data have uncovered mechanistic links between interleukin-6-mediated inflammation and type 2 diabetes²⁰, highlighting the interplay between inflammatory and metabolic pathways. The cohort’s precise characterization of adiposity, inflammation,

and hepatic health, combined with detailed assessments of incretin, glucagon, insulin, and glucose dynamics in a general population, provides an unparalleled resource for investigating the constellation of metabolic traits and their long-term impact on cardiometabolic health. Their cohort provide an unique timing for studying constellations of metabolic traits relevant to progression to cardiometabolic disease and how the play in conjunctions to glucose and hormones.

This project is grounded in the hypothesis that dysregulated incretin secretion and inadequate glucagon suppression, together with insulin resistance, accelerate the progression of metabolic dysfunction by activating hepatic and inflammatory pathways in individuals with at higher risk of diabetes.

Work Package 1: Investigate causal relationship of neutral release of incretin and glucagon and long-term cardiometabolic disease and identify mediating pathways.

Hypothesis for Work Package 1: Natural incretin and glucagon responses play a causal role as drivers of the risk of progression from pre-diabetes to type 2 diabetes and diabetes-related complications. In this pathway, biomarker and metabolites related to liver health play a mediating role.

Natural incretin and glucagon responses in epidemiological studies have been mostly investigated cross-sectionally, with a few exceptions. The Fenland study includes ~12,000 individuals at baseline who underwent measurement of natural incretin and glucagon responses during a 2-point (0, 120 min) oral glucose tolerance test (OGTT) in 2005-2015, i.e. before the introduction of incretin based therapies. This unique timing provides an opportunity to prospectively analyze the associations between individuals's natural hormonal responses to glucose and progression to diabetes, regression from prediabetes to normoglycemia, and risk of diabetes-related complications.

Preliminary findings from ADDITION PRO in a population with high risk of diabetes showed that per SD higher glucagon (incidence rate ratio IRR: 1.38 CI: 1.15 to 1.67) and GLP-1 responses (IRR: 1.34 CI: 1.05 to 1.71) at 120 minutes during the OGTT are associated with incident heart failure over an 11-year follow-up period.

Genetic variants, clinical biomarkers and metabolites serve as valuable indicators of individual predisposition for specific phenotypes (genes) and mediating pathways (metabolites and clinical biomarkers) that link phenotypes to disease outcomes. Genetic variants can strengthen causal inference by serving as instrumental variables in Mendelian randomization studies²¹. Identification of metabolic pathways that mediate the effect of genetically determined phenotypes on the incidence of diseases such as type 2 diabetes helps us understand the causal biological mechanisms linking traits to disease. This understanding in turn supports the development of potential new targeted interventions based on metabolic pathways.

Evaluating the risk associated with incretin and glucagon responses to a glucose load in isolation may be limited without considering concurrent insulin and glucose regulation. To address

this in WP1, principal component analysis (PCA) and unfold manifold approximation and projection (UMAP) will be applied to extract the strongest sources of variance in the concurrent OGTT response across all 4 hormones and glucose.

Research design and methods

To quantify the causal relationship between incretins, glucagon and cardiometabolic disease, we will first apply traditional epidemiological time-to-event analysis methods (Poisson and Cox regression) within the Fenland Study, using adjustments informed by pathways outlined in directed acyclic graphs (DAGs). In addition, we aim to characterize trajectories of cardiometabolic profiles including glucose, lipids, kidney function, adiposity markers from DEXA, liver markers and inflammatory biomarkers using linear mixed effects models. To strengthen causal inference and account for unmeasured confounding, we will conduct Mendelian randomization using pathway specific polygenic risk scores (PRS) as instruments for incretins and glucagon [daveysmith2014], leveraging data from the Fenland Study, ADDITION-PRO and the UK Biobank [mrcepidemiologyunit].

To sequence mediating pathways and identify key metabolites linking incretin and glucagon responses to cardiometabolic disease, we will apply a structural causal algorithm (NetCoupler)²² to identify sequential causal pathways involving metabolites. Based on the identified metabolites, structured causal mediation analyses²³ will be conducted to assess and quantify the direct and indirect effects of individual metabolites seen as most likely to be causal mediators in NetCoupler on cardiometabolic outcomes.

Work Package 2: To map dimensions of metabolic traits in conjunction with incretin and glucagon responses, in order to decipher their role in the context of deteriorated glucose metabolism

Hypothesis for Work Package 2: natural incretin and glucagon responses may exert protective, compensatory effects under conditions of obesity, hyperglycemia, insulin-resistance, and loss of beta-cell function. We further hypothesize that liver health and low-grade inflammation may act as an effect modifier in the associations between incretin-glucagon responses and metabolic outcomes.

Obesity and insulin resistance have long been regarded as central components in the development of type 2 diabetes. Recent clustering analyses have identified liver fat as a main determinant heterogeneity among people with pre-diabetes with regard to their risk of progression to type 2 diabetes¹. Reduced liver function interacts with insulin resistance, glucose and glucagon responses, and is accompanied by low grade inflammation and morphological changes to the liver, particularly MAFLD (Metabolically Associated Fatty Liver Disease)^{11,24,25}. The biological actions of GLP-1 and GIP include reducing hepatic inflammation²⁶. Liver function therefore appears to be a central pathway modulating diabetes risk and interacting with

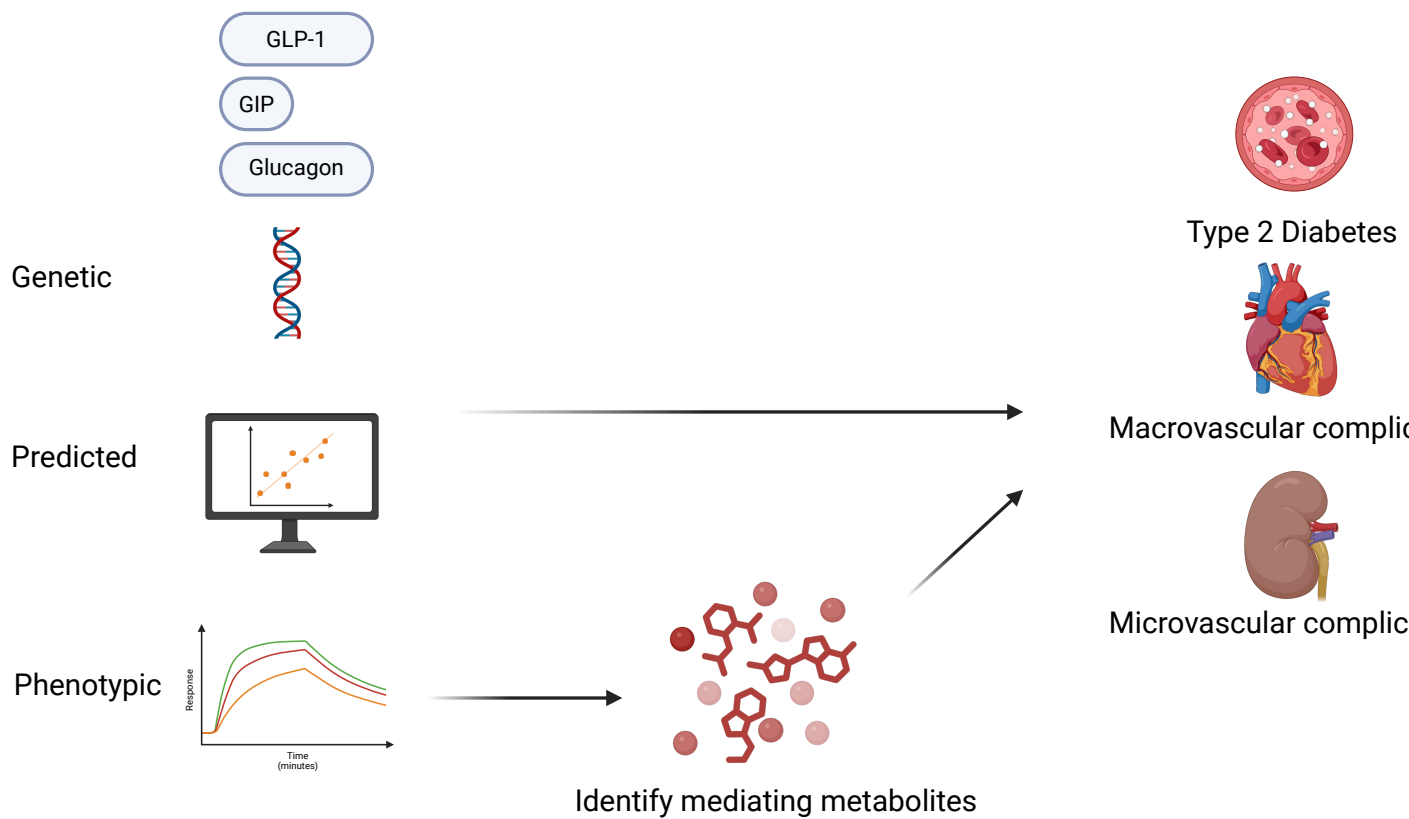


Figure 1: **Figure 1: Structured causal analysis framework of incretin and glucagon responses and their association with the risk of progression to cardiometabolic disease**

or mediating the impact of natural incretin and glucagon responses. The Fenland study includes baseline measurements of γ -glutamyl transferase and alanine transaminase, and 11,559 participants had DEXA²⁷ performed allowing to estimate regional fat mass.

[Deep learning processing of ultrasound images] In conjunction with liver function biomarkers, this will enable the calculation of a general indication of liver health.

We will further integrate dimensions of metabolically relevant low-grade inflammation by integrating markers of Interleukin 6 (IL-6), adiponectin, and C-reactive protein²⁸ in our multidimensional characterization of liver health. In addition, fasting branched-chain amino acids (BCAA) and lipid-related metabolites during the OGTT²⁹ were obtained in Fenland study. These data allow us to characterize aspects of metabolic function, such as liver fat accumulation³⁰ and tissue-specific insulin resistance^{31,32}.

Based on phenotypes, metabolites, and genotypes, we want to identify metabolic traits that, in conjunction with incretin and glucagon responses, contribute to cardiometabolic risk. Machine learning-based dimensionality reduction techniques help identify and characterize distinct constellations of metabolic traits across individuals. From phenotypic clusters, we want to map dimensions of metabolic function that either provide compensatory protection against or contribute to cardiometabolic disease. Then, we will add dimensions of metabolite-based profiles to the existing clusters to enhance their characterization.

To support phenotypic clustering, genetic data will be incorporated through partitioned polygenic risk scores (PRS) for type 2 diabetes^[33]³⁴. These scores will be added with underlying metabolic traits such as glucagon and incretin, inflammation, beta-cell function, obesity, liver fat, and insulin resistance. This approach enables us to decipher phenotypes driven by genetic risk scores that contribute to cardiometabolic disease.

Research design and methods

We use data from previous work in The Fenland study and ADDITION-PRO, including genotypes, metabolomic profiles, and markers of metabolism and inflammation^{12,13,28,29,35–37}.

Combined with liver function biomarkers, this approach will enable us to generate a general, multidimensional indication of liver health.

We will apply PCA and UMAP to integrate variables and identify a restricted number of metabolic dimensions that reflect the interplay between incretin and glucagon responses along with concurrent glucose-insulin dynamics during the OGTT³⁸. We will further add dimensions of obesity, insulin resistance, inflammation, liver health markers, subsequently examine these dimensions in relation to diabetes risk³⁹. Finally, metabolite-based profiles and partitioned PRS will be analyzed separately in relation to cardiometabolic outcomes and subsequently incorporated to enrich the dimensional metabolic profiling.

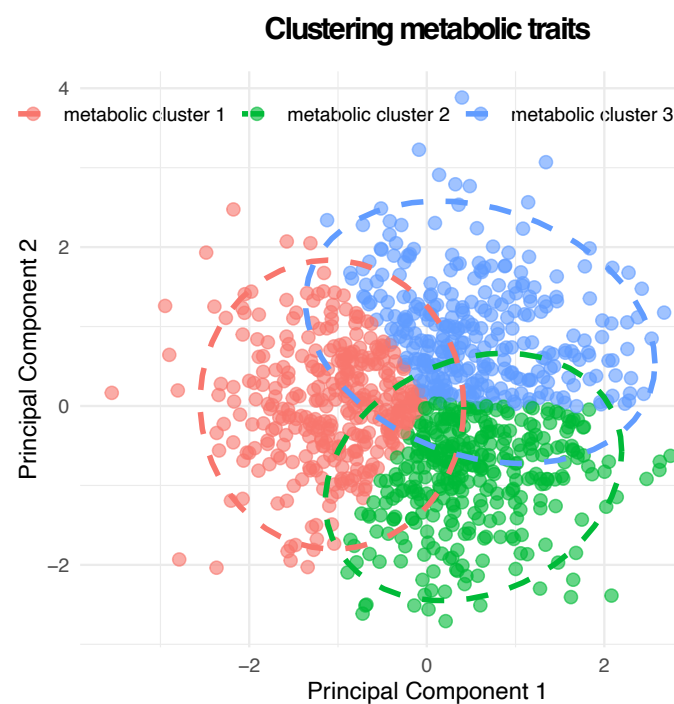
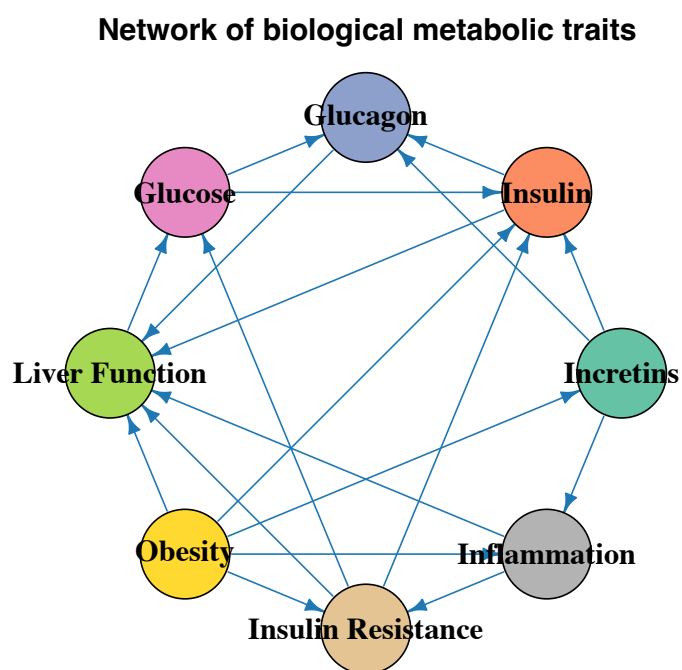


Figure 2: Figure 2: Illustration of dimensionality reduction of metabolic traits into metabolic clusters

Work Package 3: To predict incretin and glucagon responses in conjunction with metabolic traits based clinical and biological traits

Hypothesis for Work Package 3: population heterogeneity of incretin and glucagon responses in conjunction with metabolic traits can be predicted to a clinically useful degree based on clinical, genetic and metabolic biomarkers, and these predictions can stratify individuals by their risk of progression to type 2 diabetes and related complications.

Given the fact that it is impractical to assess a full incretin and glucagon response in clinical practice, WP3 aims to establish a practical set of biomarkers that jointly have the capacity to characterize incretin and glucagon responses. Analyses in the Fenland study will be carried out across three levels: (1) traditional clinical characteristics, (2) metabolomics profiles, and (3) genomic data. The utility of these predicted traits will be investigated in relation to cardiometabolic outcomes in large population-based cohorts that include the same biomarkers but have not measured incretin and glucagon responses, such as the UK Biobank, as well as using available markers in Danish registries.

[Add in conjunction with CM traits]

Research design and methods

To predict incretin and glucagon responses based on clinical and metabolite data, we will apply a two-layered modeling approach in the Fenland study. Clinical and metabolic profiles will be used separately and in combination to predict responses. For variable selection in both layers, we will apply two strategies: restricting variance and using linear LASSO regression to shrink and select the most predictive variables. To support this, we will employ machine learning models to capture undefined interactions and identify important predictors based on variable importance⁴⁰. Our approach follows established principles in machine learning and statistical modeling, adhering to standardized practices for prediction, reporting, and validation^{41–44}.

As part of the collaboration with the MRC Epidemiology Unit and Novo Nordisk Foundation Center for Basic Metabolic Research, current efforts are focused on developing PRS for GLP-1, GIP, and glucagon. These PRSs will be incorporated into the aforementioned predictive layers as composite predictors of incretin and glucagon responses. PRS for other relevant traits such as obesity, insulin resistance, beta-cell function, liver function and low-grade inflammation will also be considered where relevant.

Research design and methods

Fenland study

ADDITION-PRO Study

Baseline data collection period	2005-2015 (Phase 1)	2009–2011
Follow-up	2014-2020 (Phase 2) 2023-2025 (Phase 3)	Danish National Registries (up to 2024)
Included participants	UK general population aged 30-55 years	Individuals at high risk of diabetes
Number of participants	12,435	2,082
Measure from OGTT:	GLP-1, GIP, glucagon, glucose and insulin (t= 0, 120 min)	GLP-1, GIP, glucagon, glucose and insulin (t= 0, 30, 120 min) ^{12,13,36}
Other relevant metabolic measures	Adiposity from DEXA scan: <ul style="list-style-type: none"> • Visceral and subcutaneous fat • Bone density Liver function: <ul style="list-style-type: none"> • Ultrasound liver images • GGT, ALT • glucagon – alanine index • Alkaline Phosphatase Inflammation: <ul style="list-style-type: none"> • Interleukin 6 (IL-6), high sensitivity C-reactive protein, adiponectin 	Adiposity: <ul style="list-style-type: none"> • Visceral and subcutaneous fat from ultrasonography • adiponectin Liver function: <ul style="list-style-type: none"> • Ultrasound liver images (still B-mode images with liver protocol) • GGT, ALT • glucagon – alanine index Inflammation: <ul style="list-style-type: none"> • soluble CD163, , and high sensitivity C-reactive protein
Assessment method of metabolites	LC electrospray ionization and flow-injection analysis tandem MS (ref), targeted metabolomics	Proton nuclear magnetic resonance spectroscopy, targeted metabolomics ³⁵
Sample tissue	Fasting plasma blood samples	Both fasting and during the OGTT ^{29,35}
Number of metabolites	175 (acylcarnitines, amines, sphingolipids and phospholipids)	231 lipid-related and 3 BCAA (fasting isoleucine, leucine and valine levels)
Genotyping	Yes	Yes
Follow-up period	10-15 years	13-years

Outcome	Metabolic-related outcomes:	Metabolic-related outcomes:
	<ul style="list-style-type: none"> • Progression to type 2 diabetes • Regression to normoglycemia • Glucose, lipids and kidney function, inflammation, adiposity trajectories 	<ul style="list-style-type: none"> • Progression to type 2 diabetes • Regression to normoglycemia • Glucose, lipids and kidney function trajectories
	Macrovascular complications:	Macrovascular complications:
	<ul style="list-style-type: none"> • Ischemic-related cardiovascular disease • Heart failure 	<ul style="list-style-type: none"> • Ischemic-related cardiovascular disease • Heart failure
	Microvascular complications:	Microvascular complications:
	<ul style="list-style-type: none"> • Chronic kidney disease 	<ul style="list-style-type: none"> • Chronic kidney disease

To extend findings and predicted characterizations in larger population-based cohorts, will use ADDITION-PRO Study³⁶ and UK Biobank⁴⁵ and as validation cohorts.

Collaboration

The synergy between this project and the MRC Epidemiology Unit lies in the integration of deep-phenotyped metabolic data with large-scale population health surveillance. The MRC Epidemiology Unit provides an environment for precision epidemiology. By combining the Unit’s global leadership in studying the genetic and environmental determinants of obesity and type 2 diabetes with the projects focus on natural incretin and glucagon responses, we create a powerful framework to move from observational data to causal understanding. This collaboration allows for the cross-pollination of Danish clinical depth and the UK’s extensive population-based datasets, ensuring that findings regarding metabolic drivers are both biologically robust and representative of the broader population.

The MRC Epidemiology Unit, through the mentorship of experts in the Fenland Study including Professor Nick Wareham and Professor Simon Griffin, will serve as the primary international host and scientific advisor. Their role will encompass three key areas:

1. Data Provision and Oversight: Granting access to the Fenland Study ($n = 12,435$) and providing training to ensure a comprehensive understanding of the cohort and its data.

2. Methodological Mentorship: Delivering guidance in causal inference and Mendelian Randomization, with a focus on using genetic instruments to determine the causal role of incretin and glucagon in cardiometabolic outcomes.

3. Validation Expertise: Supporting the harmonization of data between the Fenland Study, ADDITION-PRO, and UK Biobank to validate identified metabolic profiles and mediating pathways, such as liver health and inflammation, across diverse geographical and demographic contexts.

The synergy with the MRC Epidemiology Unit will be an ongoing institutional bridge with visit from PI, post doctoral stay and PhD visits. Our research is strategically aligned with the Unit's mission to improve population health through better risk stratification. We will maintain regular scientific exchange through joint virtual meetings to align the analytically protocols used in the Fenland Study. This collaboration serves as a pilot for long-term data integration between Danish and UK cohorts, establishing a permanent channel for investigating the heterogeneity of type 2 diabetes and refining precision prevention strategies across borders.

Significance of the project

The Inter-SUSTAIN project supports the development of a precision approach to pre-diabetes by identifying a set of easily obtainable biomarkers that optimally distinguish individuals with a high probability of stable pre-diabetes or remission from those at greatest risk of progressing to diabetes. This work aligns with the objectives of the Novo Nordisk Foundation-funded Steno National grant, specifically [Work Package 3 \(Heterogeneity\) in the new DP-Next](#), which aims to develop new strategies for diabetes prevention⁴⁶. The project will be in close connection with the DP-Next team and it is expected that the findings from the Fenland study and ADDITION-PRO will inform the design and structure of the new DP-Next cohort.

[Add causality aspects]

The Fenland Study offers a unique opportunity through its long-term follow-up data to advance cardiometabolic prevention efforts. It enables the identification of clinical and biological metabolic traits that characterize individuals at high risk of developing type 2 diabetes and related complications, including macrovascular and microvascular conditions, as well as increased mortality. Furthermore, these traits can be linked to longitudinal changes in cardiometabolic and inflammatory profiles, allowing for the identification of individuals with accelerated progression toward cardiometabolic complications. These insights support the timely identification of high-risk individuals who may benefit from early interventions, such as intensive lifestyle modifications or targeted pharmacological treatments.

Our work will deepen understanding of heterogeneity in the risk of type 2 diabetes and provide clinical and genetic tools to enable further cohorts and registries to investigate variability in response to interventions. Such interventions could be pharmacological treatments in

populations who progress to type 2 diabetes or have obesity (e.g., time to metformin failure or effectiveness of GLP-1 analogues and SGLT2 inhibitors) as well as dietary constellation and habitual physical activity strategies in individuals with pre-diabetes. Ultimately, this aims to contribute to precision prevention approaches by enhancing tailored decision-making that improves outcomes and reduces the burden of cardiometabolic disease.

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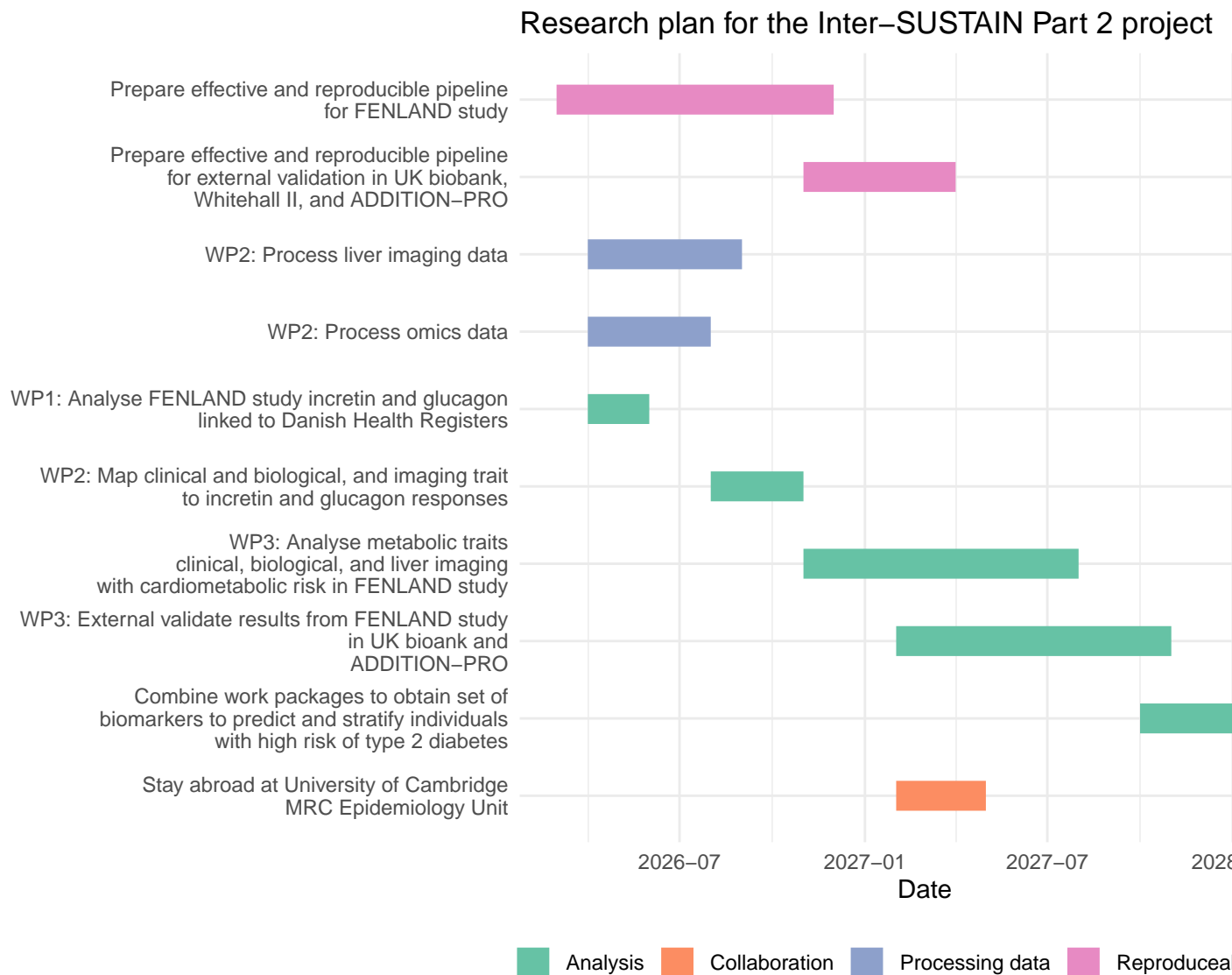


Figure 3: **Figure 3: Gantt chart**

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