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Innovative biomarkers of insulin resistance and lipid metabolism predicting diabetes

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Chapter 1

GENERAL INTRODUCTION AND AIMS OF THE THESIS

INTRODUCTION

Type 2 diabetes (T2D) is among the most prevalent chronic diseases and according to the World Health Organization (WHO) the number of adults living with diabetes is expected to increase to 693 million by 2045 [1]. T2D development is triggered by multiple factors including genetic and environmental factors [2]. However, since diabetes disease is a complex disease, not all relevant disease causes and pathophysiological changes are completely understood [3]. While evidence is mounting to support the heterogeneity of T2D, a total of five new diabetes clusters have been developed and validated recently as an alternative to the traditional classification of T2D and T1D [4]. Those five new clusters were determined using several factors which include BMI, the age at which diabetes was first diagnosed, as well homoeostasis model assessment estimates of β -cell function (HOMA2-B) and homoeostasis model assessment insulin resistance (HOMA2-IR). For example, individuals in the cluster of very insulin-resistant represent differently compared to individuals in insulin deficient cluster as such patients are at higher risk of developing chronic kidney disease and coronary events [4].

Insulin resistance and insulin secretion are the major pathophysiological mechanisms that occur before the onset of T2D [5]. Therefore, insulin resistance is an important component determining the risk of incident T2D. Usually, the early stage of this pathology could be asymptomatic, or symptoms could be so mild that they go unnoticed. Therefore, it could be remained unrecognized for many years [6]. Accordingly, the identification of individuals at high risk of developing T2D is of great importance, as early interventions might delay or even prevent the disease [7]. A number of factors contribute to the development of T2D, including metabolic factors, lifestyle and environmental factors, medical history and psychosocial factors [8]. Novel circulating biomarkers make it possible to find early biomarkers for a disease of interest, including T2D metabolomics which is a comprehensive characterization of metabolic changes connected to disease development and progression [9].

NEED FOR NEW DIABETES BIOMARKERS

Biomarkers can be strong tools in the identification and management of a disease. Plasma glucose (measured after fasting or during a glucose tolerance test) and glycated hemoglobin (HbA1C) are used as diagnostic and screening biomarkers for T2D, and a diagnosis of diabetes is based on specific cut-off values of plasma glucose and HbA1c. Novel biomarkers may shed light on pathophysiological changes contributing to diabetes development at an early stage with the advantage that such metabolic alterations could still be reversible [10]. Preclinical model research and the application of findings from animal studies to human T2D revealed that biomarkers

such as plasma metabolomic, lipidomic, and peptidomic analysis could be used to identify people who are at risk of developing T2D [11]. Therefore, there is a pressing need for biomarkers that improve T2D prediction. Health-related resources could focus on preventing disease progression in individuals at higher risk of developing T2D. This is not only important for the prevention of T2D but also for its complications, thereby lowering morbidity and mortality.

PERSONALIZED MEDICINE

Early diagnosis of T2D is critical for identifying individuals at a higher risk of the disease. In this respect, precision medicine is an emerging approach that includes evaluations, tests, decisions and treatments that are adapted to the characteristics of an individual patient. With regard to T2D, precision medicine manages a wealth of “omics” data (genomic, metabolic, proteomic, environmental, clinical, and paraclinical) to increase the number of clinically validated biomarkers to identify patients in the early stages, even before the prediabetic phase [12]. The urgent demand for novel biomarkers to reduce the incidence or even delay the onset of T2D suggests that many potential biomarkers may be helpful in the prediction and early diagnosis of T2D. However, there is an emerging need to identify novel biomarkers for specific groups of people at risk, since each biomarker has a different impact on diabetes-related pathophysiology. Also, each biomarker has its own interconnected dossier and can be interrogated for reported function(s), relevant publications and patents, relation with other biomarkers (e.g. within biomarker panels) and differential behaviour in multiple diseases [13]. We may be able to gain a better understanding of the biomarker function and its use in different populations when it comes to clinical practice.

Altogether, this means that the validation of predictive biomarkers for incident T2D needs to be examined in different subgroups of people with different clinical characteristics as indicated by their status of insulin secretion and insulin resistance, kidney or liver function, as well as body composition.

However, discovery and validation are not sufficient to achieve clinical implementation. The process of translating biomarker discovery to application is challenging and few validated biomarkers are ultimately implemented in daily patient care. A large challenge is that there is no 'gold standard approach' that guarantees a 100% success rate in biomarker validation research, hampering biomarker implementation.

INSULIN, C-PEPTIDE, PROINSULIN

Pancreatic β -cell dysfunction plays a fundamental role in the pathogenesis of T2D. Pancreatic β -cell biomarkers are known as insulin, c-peptide, and proinsulin which represent β -cell function and could be measured by plasma [11,12]. Insulin, which is produced solely in β -cells, is a critical metabolic regulator. Insulin, a peptide composed of 51 amino acids, is synthesized as preproinsulin and processed into proinsulin. Proinsulin, a peptide composed of 81 amino acids, consists of three domains: an amino-terminal B-chain, a carboxy-terminal A-chain and a connecting peptide in the middle, known as the C-peptide (Figure 1). During passage through the endoplasmic reticulum, the precursor folds, and the prohormone convertases PC1/3 and -2 subsequently excise the C-peptide, generating the mature form of insulin and C-peptide. Subsequently, they are stored in secretory granules awaiting release on demand [14,15].

Mature insulin is secreted into plasma via intracellular vesicles. Several previous studies have used insulin concentration to infer pancreatic insulin secretion as a clinical biomarker of insulin resistance [16]. Insulin is primarily degraded in the liver with a 3-5 min half-life [17].

C-peptide, which is almost always co-secreted with insulin is not cleared by the liver during first-pass transit, despite a substantial portion of insulin being released into the portal vein from pancreatic islets [18]. Thus, we hypothesized that C-peptide would more accurately reflect β -cell function and represent a possible reliable biomarker for predicting T2D. Therefore, in **Chapter 2**, we longitudinally investigated the association of C-peptide levels with the risk of developing T2D in a population-based cohort.

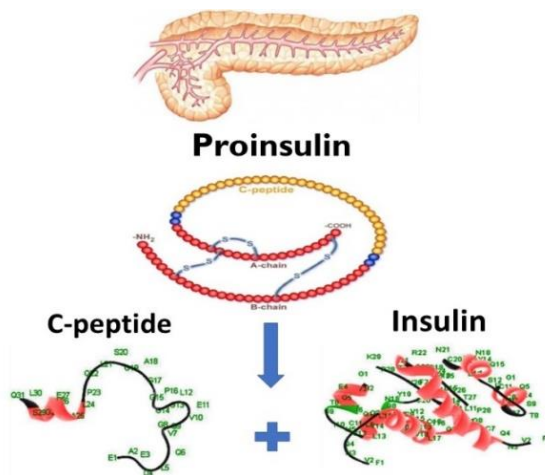


Figure 1. Release of C-peptide in biosynthesis of human insulin [19]

Moreover, C-peptide is primarily cleared by renal filtration and mostly metabolized by the kidneys. Renal extraction of C-peptide accounts for approximately 85% of the total metabolic clearance [20]. Therefore, we hypothesized that C-peptide level is a biomarker that could be affected by renal impairment. We examined the association between C-peptide with the risk of developing T2D other than in a population with normal kidney function and focused on the subgroups with kidney dysfunction.

The next important biomarker, proinsulin, has been investigated for pancreatic β -cell function. Proinsulin is cleared slower from the plasma than insulin and is suggested as an early subclinical β -cell dysfunction biomarker [21,22]. Higher levels of proinsulin has been shown to be associated with insulin resistance and T2D [23–27]. In the past, nonspecific assays showed high cross-reactivity, which could lead to incorrect conclusions regarding β -cell dysfunction and prediction of diabetes. A new, specific, and intact proinsulin ELISA (no cross-reactivity) has been developed that can be easily used in routine laboratories [28]. Therefore, in **Chapter 3**, we longitudinally investigated the association between proinsulin levels and the risk of incident T2D. Moreover, proinsulin is substantially removed by the kidneys, and proinsulin degradation can be affected by kidney dysfunction [29,30]. Accordingly, we investigated the potential effects of modification by variables related to kidney function.

LIPIDS AND LIPOPROTEINS

T2D is known to be accompanied by lipoprotein abnormalities, including elevations in triglyceride-rich apolipoprotein B (apoB)-containing lipoproteins, very low-density lipoproteins (VLDL) and smaller sized low-density lipoproteins (LDL), and low levels of high density lipoproteins (HDL) [31,32]. These abnormalities have also been associated with insulin resistance and development of T2D [33–35]. Moreover, VLDL, LDL, and HDL particles are heterogeneous and vary considerably in composition, size, and function, which may lead to differential associations with incident T2D [36–39]. This may be due to accumulation of cholesterol in the pancreatic β cells by VLDL and LDL particles, and deficient HDL function leading to pancreatic steatosis, and β cell dysfunction [40–46].

In this thesis, we make use of a newly developed nuclear magnetic resonance (NMR)-derived algorithm called LP4, which provides information regarding the concentration of lipoprotein particles categorized into different particles and subspecies according to their size [47–51]. This includes five triglyceride rich lipoprotein (TRL) particles (very large, large, medium, small and very small), three LDL particles (large, medium and small) and seven HDL subspecies (H1P to H7P), categorized in three HDL particles (small, medium, and large). They were quantified using the conventional

deconvolution method and the amplitudes of their spectroscopically distinct lipid methyl group NMR signals [52]. Total TRLP is calculated as the sum of the concentrations of very large, large, medium, small, and very small TRLP. Total LDLP is calculated as the sum of the concentrations of large, medium, and small LDLP. Total HDL particles is calculated by the sums of the concentrations of small, medium, and large HDL particles. Mean TRL,LDL, and HDL sizes were calculated using the weighted averages derived from the sum of the diameters of each subfraction.

In **Chapter 4**, we investigated the associations of TRL and LDL particle and subfraction concentrations using this newly developed NMR algorithm with incident T2D in the general population. In addition, considering the effect of statins on glucose tolerance and insulin secretion, we aimed to investigate these associations in different subgroups of individuals of statin users and nonusers [53–55]. Furthermore, the association could also be affected by alcohol consumption which we aimed to investigate in individuals who consumed high levels of alcohol [56,57].

In **Chapter 5**, we explored the associations of HDL particles (large, medium, and small HDL particles), seven HDL subspecies and HDL size with incident T2D in the general population. In addition, as a result of the composition and functional alterations in HDL composition in the context of obesity, insulin resistance, and sex [58–60], we aimed to examine the impact of obesity, insulin resistance, and sex on the association between these HDL parameters and newly developed T2D.

Finally, we corroborated recent findings from the Brazilian Longitudinal Study of Adult Health cohort by Carvalho et al. regarding the association between TRL size and T2D incidence [61]. That study demonstrated that incorporating TRL particle diameter into a risk prediction model improved the accuracy of T2D risk prediction [61]. In **Chapter 6**, we evaluated the improvement in incident T2D prediction when lipoprotein diameter markers (such as LDL and HDL size) were included in the risk prediction models, in addition to TRL size alone.

POSTTRANSPLANTATION DIABETES IN KIDNEY TRANSPLANT RECIPIENTS

Kidney transplantation is well-established preferred treatment for most patients with end-stage kidney disease, which improves their quality of life and survival compared to dialysis treatment [62,63]. However, kidney transplant recipients (KTRs) are susceptible to chronic transplant-associated comorbidities [64]. Posttransplantation diabetes mellitus (PTDM) is one of the main metabolic complications after kidney transplantation, which is estimated to affect from 7% to 39% at one year after transplantation and from 10% to 30% at 3 years post-transplantation. PTDM may be

associated with adverse effects on both short- and long-term outcomes in KTRs, including infections, graft failure, cardiovascular disease and survival [65–68]. Accordingly, early identification of KTRs at higher risk of developing PTDM allows for early intervention, preventive measures, and optimal therapeutic approaches in KTRs.

Similar to the pathogenesis of T2D, potential mechanism of transplant-associated hyperglycemia (TAH) and PTDM development could be explained as a result of insulin resistance or impaired insulin secretion by the pancreatic β -cell [64,69]. Moreover, specific transplant determinants including, pretransplantation insulin resistance in the final stage of kidney failure, obesity, less physical activity, inflammatory activity of viral infections, particularly cytomegalovirus (CMV) and hepatitis C virus (HCV), and chronic exposure to calcineurin inhibitors and corticosteroids aggravate insulin resistance and TAH in KTRs [70–74]. Apart from the occurrence of hyperglycemia immediately after transplantation, which is typically related to surgical stress and high glucocorticoid dosing, incident long-term PTDM could be of great importance in renal transplant healthcare in stable KTRs. This necessitates the identification of new biomarkers and factors that can predict PTDM in KTRs in epidemiological or clinical studies. Indirect insulin resistance indices including HOMA-IR, visceral adiposity index (VAI), lipid accumulation product (LAP), or triglycerides-glucose (TyG) index, are accepted for epidemiological or clinical studies in the general population because of their simplicity [72,75,76]. However, it is unknown to which extent those indices could be useful for determining insulin resistance and PTDM development in KTRs. Therefore, in **Chapter 7**, we aimed to prospectively investigate the association between indirect insulin resistance indices and incident PTDM.

Moreover, HDL itself may play a role in preservation of insulin secretion and prolong β -cell survival. HDL remodeling is altered in insulin-resistant states leading to less large and more small HDL particles [51,77]. NMR measured HDL particles and HDL subspecies as novel lipoprotein biomarkers have not been investigated before in KTRs [50]. In **Chapter 8**, we aimed to determine the association between HDL particle characteristics and the risk of developing PTDM in KTRs.

Another important factor that is known to be associated with hyperglycemia is medication. Statins and diuretic use have been associated with development of T2D [78–80]. Diuretic-induced hyperglycemia and glucose intolerance have been mainly attributed to the impairment of insulin secretion, secondary to potassium loss following diuretic treatment [81,82]. These medications are frequently prescribed in KTRs. The association between statin use and increased risk of PTDM in KTRs has been investigated recently [83]. In **Chapter 9**, we aimed to investigate the association between diuretic use and increased risk of PTDM.

POPULATION INVESTIGATED IN THIS THESIS

Lower risk population for renal dysfunction

The first part of the thesis (**Chapters 2-6**) concerns a general population-based cohort, at low risk for renal dysfunction. The study was performed within the frame of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study, an observational, general population-based, longitudinal cohort study which investigated vascular and renal damage among inhabitants of the city of Groningen, The Netherlands (Figure 2). Briefly, all residents of Groningen aged 28 to 75 years, were invited to participate in this study from 1997 to 1998. Pregnant women and participants with type 1 diabetes and T2D using insulin were excluded. After further exclusion of individuals who were unable or unwilling to participate in the study, a total of 6000 individuals with a urinary albumin concentration of 10 mg/L or greater and a randomly chosen control group of 2592 individuals with a urinary albumin concentration of less than 10 mg/L completed the screening protocol and constituted the PREVEND cohort (n = 8592). A second screening was performed from 2001 to 2003 with 6894 participants, which was the baseline of our studies.

Higher risk population for renal dysfunction

The second part of the thesis (**Chapters 7-9**) concerns a KTR cohort, a population at high risk for recurrent renal dysfunction. The study described **Chapter 7** was performed within the frame of the prospective cohort study in KTRs who survived with a functioning allograft beyond the first year after transplantation between August 2001 and July 2003. Patients with known systemic illnesses, such as congestive heart failure, cancer other than cured skin cancer, endocrine disorders other than diabetes, or overt generalized infections were excluded. A total of 606 from an eligible 847 RTR (72% consent rate) signed written informed consent.

The **Chapter 8 and 9** were conducted within the TransplantLines Food and Nutrition Biobank and Cohort Study. All adult KTR (age ≥ 18 years) ≥ 1 year after transplantation were approached for participation during outpatient clinic visits at the University Medical Centre Groningen (UMCG), Groningen, the Netherlands between 2008 and 2011. Included KTRs had no history of substance abuse or alcohol addiction. Patients with an estimated life expectancy of less than one year, particularly those with severe congestive heart failure (New York Heart Association class III and class IV) and those diagnosed and treated with cancers other than non-melanoma skin cancer, were not included in this cohort study. Of 817 initially invited KTRs, 707 signed a written informed consent form to participate in the study.

HYPOTHESIS AND AIM OF THIS THESIS

The general hypotheses of this thesis are: a) that biomarkers related to insulin secretion, in particular C-peptide and proinsulin and b) that biomarkers related to lipoprotein metabolism, in particular triglyceride-rich lipoproteins, LDL and HDL subfractions are specifically associated with new onset T2D.

The aims of this thesis are, therefore, to investigate the longitudinal associations of innovative insulin resistance and lipid biomarkers predicting incident diabetes in the general population and KTRs.

The first part of this thesis focuses on the general population. In **Chapters 2 and 3**, we investigated the relation between fasting C-peptide and proinsulin levels with incident T2D. We aimed to evaluate the predictive value of these insulin resistance biomarkers for the risk of incident T2D, added to a base model of clinical predictors. Moreover, we examined potential effect modification by variables related to kidney function. In **Chapters 4,5 and 6**, we investigated the association between newly developed NMR-measured lipid particles and incident T2D. We also questioned whether the later association was different in different groups of the general population based on their gender, BMI, insulin resistance status and statin use.

The second part of this thesis concerns the kidney transplant population. In such patients, it was unknown whether indirect insulin resistance indices are valid for predicting PTDM. This was investigated in **Chapter 7**. In **Chapter 8**, we investigated the association between newly developed NMR-measured HDL subclasses and subspecies with incident PTDM. In **Chapter 9**, we aimed to investigate the association between diuretic use and increased risk for PTDM.

Finally, in **Chapter 10**, the results of the thesis are discussed and summarized.

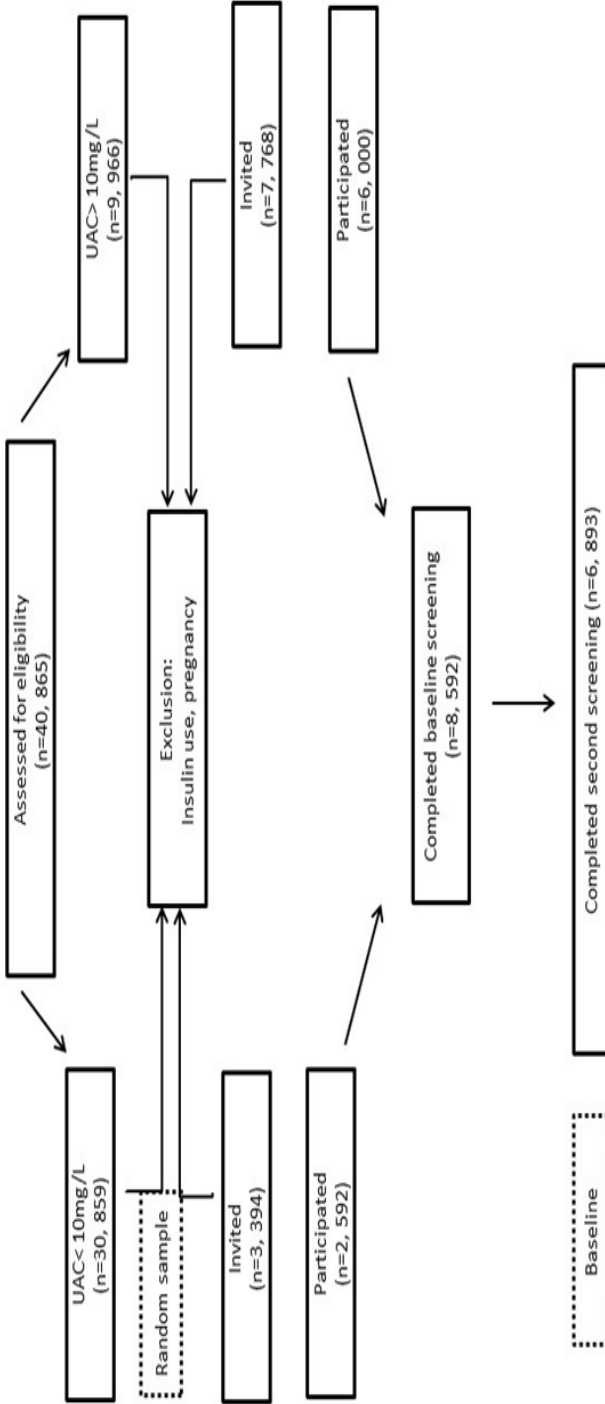


Figure 2. Outline the PREVENT study

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PART ONE

**STUDIES IN THE
GENERAL
POPULATION**

