Biomarkers for the Prediction and Early Diagnosis of Diabetes and Diabetes-related Cardiovascular Complications
Type 2 Diabetes Mellitus (DM2) affects more than 800,000 people in the Netherlands alone, and by the time it is diagnosed, life-threatening complications such as myocardial infarction, cerebrovascular incidents and kidney failure may have developed. It is expected that early diagnosis and treatment reduces the burden of disease, which subsequently lead to the notion that screening might be warranted.

The aim of the PREDICCt (Biomarkers for the Prediction and Early Diagnosis of Diabetes and Diabetes-related Cardiovascular Complications) consortium was to build on the latest insights into the etiology of DM2, and develop innovative biomarker based technologies to allow identification of individuals at increased risk of DM2 and DM2-related complications. To achieve that aim, the PREDICCt project has adopted two complementary strategies. By exploiting new insights into the mechanisms leading to DM2 and its complications we developed biomarkers, which were based novel technologies for visualizing or measuring the associated processes. Additionally, the PREDICCt project has studied the clinical, epidemiological, and economic aspects of improved identification of people at risk of DM2 or cardiovascular complications.

Major Findings
Markers of the etiology of DM2
• In a rodent model, high fat diets given to mothers during the pregnancy and pre-weaning stage increased the susceptibility to develop fatty liver disease and metabolic syndrome.
• As DM2 can be predicted quite well with existing biomarkers (weight, plasma markers), there is little room for innovation. Our studies show that the emphasis should be on understanding metabolic programming as a novel source of biomarkers, to complement existing genetic markers.

Markers of DM2 complications
• NASH and its morbidity should be regarded as a complication of diabetes, rather than a cause.
• Kupffer cell activation appears to be an important factor in Non-Alcoholic Steato-Hepatitis (NASH), and can be detected by plasma levels of CETP and lysosomal enzymes, including cathepsin D.
• Two devices, have been further developed and validated through testing in large cohorts. These include the AGE Reader to measure the accumulation of advanced glycated endproducts in the skin and the Glycocalyx, to measure the inner lining of the blood vessels, the glycocalyx, as a factor of vascular health.
• Unique prospective cohorts with prevalent DM2 developing various degrees of cardiovascular disease have been used to test mainly inflammatory proteins as biomarkers, and have improved our ability to predict the onset of morbidity.

Valorization potential
Short term valorization of the scientific attainments of PREDICCt into clinical and economic successes turned out unlikely. The current challenges in reducing the overall societal burden of DM2 requires lifestyle changes, and will not be resolved by more accurate risk quantification. However, we have identified the cardiovascular complications as the area were the gains should be made, and most of our progress has been in this area. Clinical and economic impact of novel biomarkers will depend strongly on the development of new, more personalized treatments to reduce cardiovascular risk in DM2 patients and the improvement of the healthcare system that provides the service.
Diabetes Mellitus Type 2

The current obesity epidemic has led to a strong increase in the prevalence of DM2.

Affected individuals suffer from disturbances in the control of both glucose and fat metabolism, which is associated with an atherogenic lipid profile in blood and with deleterious fat accumulation in various organs (e.g. liver, heart) and tissue (muscle). Inflammation in the adipose tissue and the liver plays an important causal role in this process.

As a consequence, DM2 patients are at high risk for developing cardiovascular complications, including stroke, myocardial infarction and kidney failure.

Treatment for this so-called “end-organ damage” is difficult and often leads to a permanent and significant loss of quality of life.

Therefore it is of utmost importance to prevent development of diabetes and its associated cardiovascular complications.

Clinical Need

- Establish the performance of the current prediction (risk) models for development of DM2 and DM2 related vascular complications
- Improvement of these prediction models
- Insight in causes of DM2 development (non-alcohol steatohepatitis - NASH) and DM2 related vascular complications in order to define new targets and strategies for prevention and treatment of DM2

Tools

- To measure Diabetes Mellitus Type 2 related biomarkers (risk factors).
- To combine outcome of biomarker measurements in an algorithm to predict individual risk (risk calculator).
- New targets for prevention and treatment of Diabetes Mellitus Type 2.
Public-Private Partnership

GENERATE KNOWLEDGE…

…TRANSLATE INTO APPLICATIONS

…NEW CURE/CARE SOLUTIONS

APPLICATION

SCIENCE

PATIENT

New tools for stratification of patients developing diabetes and its cardiovascular complications

Academic partners

Supporting Foundations

Industrial partners
Organisation and Partners

**Advisory board**
- ISAC CTMM

**CTMM**
- Workpackage leaders
  - WP1: Prof. B. Groen, Dr. T. Plosch (UMCG)
  - WP2: Prof. K. Willems van Dijk (LUMC)
  - WP3: Prof. J. Glatz (MUMC+)
  - WP4: Prof. H. Vink (MUMC+)
  - WP5: Prof. C. Schalkwijk (MUMC+)
  - WP6: Prof. J. Smit (LUMC, Radboudumc)
  - WP7: Prof. Y. v.d. Schouw, Dr. J. Beulens (UMCU)
  - WP8: Prof. P. Hilbers (TU/e)
  - WP9: Prof. E. Buskens, Dr. D. Postmus (UMCG)

**SteeringCie**
- Partner Representatives
- CTMM

**Project Team**
- Prof. M. Hofker, PI (UMCG)
- Dr. M. Schreurs (UMCG)
- Dr. C. Verger (UMCG)
- All WP leaders
- Dr. B. v.d. Heijning (Nutricia)
- Dr. E. Erdsieck-Ernste (CTMM)

**Coordination**
- Finance
- Publications

**Partners**
- Coordination
- Finance
- Publications

**Dutch Heart Found.**
- Microscan
- Dutch Diabetes Found.
- Dutch Kidney Found.

**MUMC+ FABPulous**
- Topic
- U-Protein Express
- Nutricia

**UMCG**
- DiagnOptics

**TU/e CTMM**
- LUMC Leiden University
- Medipark
- Percuros
- TNO
- MSD Spinnovation
- Da Vinci Laboratory Solutions
- Oroboros (AT)
- BG Medicine (USA)
Budget: CTMM manages the flow of funds

Funding:
- 25% Academia
- 25% Industrial
- 50% Government Subsidy

Project costs:
- Personnel
- Materials
- Use of existing equipment
- Investments
- Third parties
- Management (5%)
Distribution of the PREDICCT consortium budgets to perform the R&D activities

Budget: 18.4 M€
Start: 2008
End: 2014
Partners: 23

Academic cash costs:
- PhD: 5.000.000
- PostDoc: 2.000.000
- Supp. Staff: 1.000.000
- IT-Staff: 3.000.000
- M&S: 5.000.000
- Investments: 4.000.000

Total industrial cash costs:
- Large: 1.000.000
- SME: 2.000.000
- CTMM - Investments: 3.000.000

Academic kind costs:
- PhD: 6.000.000
- PostDoc: 4.000.000
- Supp. Staff: 2.000.000
- IT-Staff: 1.000.000
- M&S: 3.000.000
- Investments: 5.000.000

Total industrial kind costs:
- Large: 3.000.000
- SME: 6.000.000
- CTMM - Investments: 5.000.000
### Facts & Figures

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<td><strong>Budget</strong></td>
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<td><strong>Start</strong></td>
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<td><strong>Papers</strong></td>
<td>86 papers in submission - mean impact factor all published PREDICCT papers: 5.9</td>
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<tr>
<td><strong>Theses</strong></td>
<td>7 - 15 planned for 2014</td>
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<td><strong>Patent Filings</strong></td>
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<td><strong>Licenses</strong></td>
<td>1 - license agreement of MUMC+ Glycocalyx patent by GlycoCheck BV</td>
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<tr>
<td><strong>Spin-off Companies</strong></td>
<td>1 - Glycocheck BV</td>
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</table>
2010: The UKPDS risk engine overestimates chronic heart disease and cardiovascular risk. The discriminative ability of this model is moderate, irrespective of various subgroup analyses and should be updated (UMCU).

2010: A very low calorie diet (VLCD) in patients with DM2 results in remission of DM2, reduction in ectopic fat compartments and improvement in cardiac function (assessed by advanced MR phenotyping) and may serve as a reverse model for pathogenesis of DM2. This model can be used for identification of biomarkers related to pathogenesis of DM2 (LUMCG).

2011: When cardiac lipid content is increased by prolonged exercise, it does not hamper cardiac function (MUMC+).

2012: Animal experiments show that exposure to increased fat intake early in development predisposes the offspring to obesity and primes susceptibility to develop liver hypertrophy and hepatic steatosis (UMCG).

2012: TNFR1 shedding is not an essential feedback mechanism in preventing development of hepatic steatosis or insulin resistance, but that it is pivotal in attenuating the progression from ‘simple steatosis’ towards a more serious NASH-like phenotype (UMCG).

2012: Levels of Advanced Glycation Endproducts in carotid atherosclerotic plaques in individuals with and without diabetes correlate with characteristics of rupture-prone lesions (MUMC+).

2012: Basic prediction models perform well to identify people at high risk of developing diabetes. Models including novel biomarkers do show a slight improvement in risk stratification, but the results from early health economic modeling suggest that the order of magnitude of this improvement is not sufficient to make the routine assessment of these biomarkers economically viable (UMCG).

2012: Pericardial fat compartment responds differently to dietary interventions than visceral fat and is not clearly associated with the development of DM2 (LUMC).

2012: Mass spectrometry (MS), targeted multiple reaction monitoring (MRM) and isobaric tags for relative and absolute quantitation (iTRAQ) revealed potential biomarkers differentially expressed in DM2 versus controls as well as before and after a very low calorie diet. (LUMC, TU/e).

2012: Many cardiovascular risk scores are available that can be applied to patients with type 2 diabetes. A minority of these risk scores has been validated, with only a few showing a discriminative value of ≥0.80 (UMCU).

2012: In vitro evidence indicated that inhibition of the plasma membrane protein CD36 prevents fat accumulation and contractile dysfunction in cardiac muscle (MUMC+).

2013: Skin auto fluorescence and/or plasma pentosine may be a useful tool to predict and monitor the development of vascular complications because both are associated with higher pulse wave velocity in individuals with type 2 diabetes mellitus (MUMC+).

2013: Plasma CETP is predominantly derived from hepatic macrophages, and may be a plasma biomarker for the hepatic macrophage content in NASH (LUMC).

2013: Exendin-4 could be a valuable strategy to treat NASH (LUMC/TNO).

2013: large scale proteomics, lipidomics and metabolomics in extensively MR pheno-typed human intervention studies was used to find novel biomarkers and pathways involved in pathogenesis of DM2 and its complications (LUMC, LU).

2013: Multi-Criteria Decision analysis can be used for priority setting and optimized allocation in translational research (UMCG).

**Highest Impact Papers – mean 17.7**

### Scientific Value Creation - Theses

<table>
<thead>
<tr>
<th>Thesis</th>
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<tr>
<td>Joep Schmitz</td>
<td>TU/e</td>
<td>2012</td>
</tr>
<tr>
<td>Jacqueline Jonker</td>
<td>LUMC</td>
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<tr>
<td>Kim van de Ven</td>
<td>Radboudumc</td>
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<tr>
<td>Ali Abbasi</td>
<td>UMCG</td>
<td>2013</td>
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<td>Dorien Zelle</td>
<td>UMCG</td>
<td>2013</td>
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<tr>
<td>Tineke van de Weijer</td>
<td>MUMC+</td>
<td>2013</td>
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<td>Susan van Dieren</td>
<td>UMCU</td>
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<td>Mieke Louwe</td>
<td>LUMC</td>
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<td>Patricia Nunes</td>
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<td>Mirjam Lips</td>
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<td>Eline van Ewijk</td>
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<td>Wen Liang</td>
<td>TNO</td>
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<td>Gimon de Graaf</td>
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<td>Tim Hendrikx</td>
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<td>Marcelle van Eupen</td>
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<td>Ralph Widya</td>
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<td>Natanja Tjeerdema</td>
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<tr>
<td>Sam van der Tuin</td>
<td>LUMC</td>
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Mouse models inducing NASH-like phenotype
- ApoE3*Leiden.hCETP mice treated for 10 weeks with high fat diet +0.4% cholesterol or carbohydrates to induce vacuolization and hepatocellular hypertrophy (TNO)
- TNFR1-non-sheddable knock-in mice treated with high fat diet for 8 weeks to induce inflammation, apoptosis, fibrosis and hepatocellular carcinoma. Model for low-grade chronic inflammation (UMCG)
- LDLR knock-out mice treated with a high fat and cholesterol diet to induce Kupffer cell activation by oxidized lipids causing lysosomal dysfunction (MUMC+)
- CD36 knock-out mice show increased whole body insulin sensitivity. Fatty acids are directed to the liver, eliciting steatosis and steato-hepatitis (UMCG, MUMC+)

Mouse models for gestational malnutrition
- Mouse gestational protein-restriction model: application of isocaloric 8% protein diet induce hyper-methylation of specific CpG dinucleotides in the hepatic Lxra promoter and long-term sex-dependent effects in offspring (UMCG)
- Mouse gestational & lactation over-nutrition model: application of Western-style semi-synthetic diet promotes hepatic inflammation in male offspring (UMCG)

Rodent models with cardiac damage
- Male RP105 knock-out mice with permanent ligation of the left anterior descending coronary artery to induce myocardial infarction (MI) (LUMC)
- Rats fed for 3 weeks on a high fat diet induces insulin resistance and cardiac dysfunction. Model for DM2 and its cardiac complications (MUMC+)
- ATGL knock-out mouse model develops cardiac lipid accumulation and mitochondrial dysfunction (MUMC+).
- AGAT show increased whole-body and muscle insulin sensitivity with a high fed food intake (MUMC+)

Cell model
- Isolated rat cardiac myocytes cultured in high fat containing medium induce cardiac lipid accumulation (MUMC+).

Cohorts & Biobanks
- Seven small human DM2 cohorts (n=14-80) with (dietary) intervention, i.e. very low caloric diet, fasting and bariatric surgery. Extensive phenotyping by MR.
- NEO (Netherlands Epidemiology of Obesity) Cohort: Observational study with individuals aged 45 to 65 years with a BMI of ≥27 kg/m² (n=6000).
- Acipimox trial (n=20): DM2 patients in a randomized cross-over design with either placebo or Acipimox, thereby lowering plasma fatty acid levels.

Data-driven Methods
- A systems biology model for the analysis of skeletal muscle physiology adaptations that underlie changes in mitochondrial capacity that occur in DM2 (TU/e)

Molecular Diagnostics & Imaging
- Miniaturized sampling methods coupled to a nano-scale LC-MS platform to measure intermediates in plasma and tissue by metabolomics technologies (LUMC)
- Platform for metabolomic profiling >90 biogenic amines and acetyl carnitines (LC-MS/MS), >300 lipids (LC-MS), >100 oxy-lipids (LC-MS), and >60 central metabolites (GC-MS). (LU)
- Histological scoring system for analysis of non-alcoholic fatty liver disease (NAFLD/NASH) in rodents (TNO)
- Validated multi-array technique for known pathophysiological mechanisms in relation to CVD (MUMC+)
- Protocol 3 Tesla MRI: pulse wave velocity as measure for aorta stiffness (LUMC)
- ELISA assays: CD36, FABP 4/5, H-FABP, CRP, SAA, sICAM-1, sVCAM-1, MGO-derived THP (MUMC+)
- NMR analysis of tissue, plasma and urine of rodents (Spinnovation)
Clinical and Economic Value Creation of the PREDICCT consortium

New ‘products’ for clinical care
Main Product Pipelines

**Technology Development**

- **Discovery or Selection promising markers**
  - Methylation screen ongoing
  - Partially financed: ZONMW-Top, STW

**Clinical & Experimental Verification**

- **Clinical Validation Cohort 1**
  - Cathepsins CETP, Lysosomal markers
  - Morbid Obesity cohort
  - NEO cohort
  - Not financed

- **Clinical Validation Cohort 2**
  - Mitochondrial function
  - Human intervention studies
  - Not financed

**RISK for Development of DM2**

- **Programming**
  - Methylation screen ongoing

- **NASH -ELISA**
  - Cathepsins CETP, Lysosomal markers
  - Morbid Obesity cohort
  - NEO cohort
  - Not financed

- **Oxygraph®**
  - Mitochondrial function
  - Human intervention studies
  - Not financed

- **GlycoCheck®**
  - Glycocalyx
  - Various
  - Helius cohort
  - Not financed

- **AGE Reader®**
  - Skin AGE
  - Various
  - Maastricht Study
  - Not financed

**DM2 risk score**

- Performance $C_{stat} = 0.8-0.9$

**RISK DM2-related Vascular Complications**

- **Lipid Metabolomics**
  - EPIC cohort
  - ZODIAC-CVR
  - Not financed

- **Vascular markers**
  - FABPs, CD36, others
  - Not financed

- **Multiplex ELISAs**
  - 7 vascular pathways
  - “outside PREDICCT”
  - EPIC-NL cohort
  - SMART cohort
  - Not financed

- **Complement Factors**
  - 7 selected factors
  - CODAM cohort
  - Not financed

**DM2-CVD risk score**

- Performance $C_{stat} = 0.6-0.7$
  - Adapted
  - Not financed

**DM2-Renal risk score**

- Performance $C_{stat} = 0.7-0.8$
  - Adapted
  - Not financed

**Risk calculators**

- Performed within PREDICCT
Very early DM2 risk: metabolic programming and genetic imprinting markers

Dietary conditions during the pre- and early postnatal period are crucial for defining the set-points for the energy balance, which determines the glucose and lipid homeostasis. Dysregulation of these set-points is known to be associated with obesity and metabolic syndrome.

The aim of the PREDICCT consortium was to define this metabolic programming at the molecular level and to investigate the mechanism(s) in order to identify suitable imprinting biomarkers such as DNA methylation markers, histone modifications and others.

![Figure: Impression of DNA methylation](By Christoph Bock (Max Planck Institute for Informatics) (Own work) [CC-BY-SA-3.0 http://creativecommons.org/licenses/by-sa/3.0], via Wikimedia Commons)

Device
Too early in pipeline development

Leading Partner
UMCG

Current Diagnostics
Not available

Main results in CTMM

Development of gestational imprinting models
During the first years of PREDICCT various gestational animal models have been developed and validated in order to investigate the molecular basis of metabolic programming. One of the key findings is that maternal dietary fat intake during early development programmed susceptibility to liver disease in male offspring, mediated by disturbances in lipid metabolism and inflammatory response. Long-lasting epigenetic changes may underlie this dysregulation

Discovery imprinting markers
Livers of the above mentioned model have been used to perform a large screen to find genetic methylation markers.

Further research leading to the discovery of novel markers to identify an adverse metabolic programming state is now funded by NWO-TOP and STW.

Future Outlook:
This research line has become a rapidly maturing field of research, and it is very likely that within 5 – 10 years such markers will be in place to carry out presymptomatic testing

Expected Impact of the product
Metabolic programming and imprinting markers can be detected in the peripheral blood. As these markers occur even before the onset of obesity, it is likely to be the earliest test that can be used in combination with genetic information to offer lifestyle advice.

Although the tests are not there yet, once feasible, it will be an affordable test with high impact on the quality of life. This test can be used in combination with personal monitoring systems (e.g. “Fitbit”), that have become popular very recently.

Key References: 3, 4, 27.
DM2 risk: non-alcoholic steato-hepatitis markers

Non-alcoholic steato-hepatitis (NASH) is a fatty inflamed liver strongly associated with metabolic syndrome and DM2. Progression of NASH leads to serious morbidity including liver failure and cancer. However, NASH is rarely used in diagnosis, prognosis, therapy selection or therapy evaluation. This is due to the invasive nature and risk associated with taking needle biopsies, which is currently the only available method for assessing the amount of lipid and inflammation in the liver. Thus, there is an urgent and immediate need for reliable and less-invasive biomarkers of NASH.

Assay: ELISA on plasma or serum for cholesterol-ester transfer-protein (CETP) mass. ELISA on plasma or serum for Cathepsin D.

Figure: Macrophage expressing CETP: Nuclei are stained blue (Dapi), Macrophages are stained red (F4/80), CETP is stained green.

Leading Partners: LUMC, UMCG, MUMC

Current Diagnostics: percutaneous needle biopsy of the liver and histological analysis

Main results in CTMM
Discovery NASH markers using mouse models
Plasma CETP is a marker for activity of liver macrophages. This discovery was made by studying the APOE3Leiden-CETP mouse. This model showed that diet induced hepatic inflammation was accompanied by an increase in Kupffer cell expression of CETP. This finding could be confirmed in studies with obese NASH patients. Similarly, the cholesterol fed LDLR knock-out mouse proved to be an excellent model for NASH, and increased our insight into its etiology. It was discovered that modified lipoproteins lead to lysosomal dysfunction, inflammation and the secretion of lysosomal proteins. As a result Cathepsin D is released in the blood as a marker of both Lysosomal dysfunction and of NASH.

Experimental & Clinical Verification
Verification was performed by epidemiological correlations and measurements in liver biopsies from severely obese patients. Also other cohorts where liver biopsies are available have been tested and are currently being analyzed.

Future Outlook:
Commercial ELISAs for plasma CETP are available. This finding adds a novel application for these tests. Cathepsin D and possibly other Lysosomal markers can be also immediately tested on clinical cohorts.

Expected impact of the product
Low-risk assessment of an important aspect of liver inflammation will add relevant information to diagnosis of suspected NASH patients. Moreover, these tests makes it feasible to evaluate the success of the selected therapy to reduce NASH. In principle ELISAs can be adjusted to be run on clinical analyzers and will therefore be relatively easy to implement.

Broader use of this test can address which obese subjects are still relatively healthy and which ones are at increased risk of developing severe metabolic and cardiovascular morbidity.

Key References: 7, 53
Main results in CTMM
Protocols were developed for the simultaneous measurement of mitochondrial membrane potential using tetraphenylphosphonium ions (TPP⁺) as reporter cation and mitochondrial respiration. Results included a set of spreadsheet templates for the calculation of mitochondrial membrane potential from the measured data, taking into account unspecific binding, substance specific effects, mitochondrial volume, and amount of mitochondria and TPP⁺ in the chamber (initially and after dilution effects). The measurement of TPP⁺ concentration was extended beyond isolated mitochondria to permeabilized fibers and homogenates allowing qualitative and semi-quantitative assessment of the mitochondrial membrane potential in these samples. The calculation of absolute values of the mitochondrial membrane potential is currently restricted to isolated mitochondria due to missing reference data for unspecific binding.

Expected Impact of the product
The simultaneous measurement of mitochondrial respiration and membrane potential provides most accurate measurement of mitochondrial uncoupling. The latter process is involved in the generation of mitochondrial ROS species. Mitochondrial ROS play an important role in the aging process, as well as the glucotoxic effects on endothelial and vascular function and is thereby an important link between hyperglycemia and cardiovascular disease. Further research should be directed to targets that can affect mitochondrial uncoupling and thereby lower ROS-induced oxidative damage.

Device
OROBOROS Oxygraph-2k, the modular system for high-resolution respirometry (HRR) in mitochondrial physiology and pathology, equipped with the O2k-ISE (Ion selective electrode) module for measuring TPP⁺ (tetraphenylphosphonium ions) concentrations.

Leading Company
OROBOROS INSTRUMENTS Corp.

Current Diagnostics
High resolution respirometry.
Cardiovascular complications result from insufficient vascular protection against risk factors such as cholesterol, smoking, hypertension and diabetes. Vascular protection is mediated by the endothelial glycocalyx, a “Teflon” like gel-coating on the luminal surface of blood vessels.

Device
In the PREDICT consortium, we developed a clinical Glycocalyx Measurement System that allows quality controlled acquisition of video microscopic recordings of sublingual micro vessels and automated analysis of glycocalyx dimension in over 3000 vessels per patient within 5 minutes.

Leading Company
Spin-off MUMC+: Glycocheck BV

Current Diagnostics
GycoCheck RI (for Research Institutes) and GlycoCheck VC (Vascular Care for professionals)

Progress obtained in translational pipeline

Main results in CTMM
Development of Demonstrator In order to allow translation of the Glycocalyx Measurement System to a clinical setting, real time processing is required. Together with the two industrial partners (TOPIC, Microscan) new hardware and software are being developed to improve the glycocalyx measurements (camera and interface) for “real time” analysis directly after the measurement.

Experimental Verification To establish importance and impact of the glycocalyx in DM2 in various experimental set-ups

Clinical Validation Good reproducibility and sensitivity to detect glycocalyx loss in T2D patients compared to age-matched healthy controls. In 2012-2014 glycocalyx data will be collected in a large cohort study (Helius, n=6000, ethnic groups) to test the prognostic value of with respect to cardiovascular risk and the onset of diabetes/insulin resistance

Future Outlook:
Further development towards handheld device at reduced price to allow individual consumers and/or healthcare professionals to identify personal vascular vulnerability and monitor efficacy of personal treatment.

Expected Impact of the product
Identification of individuals with increased vascular vulnerability enables early personalized treatment.

Further development towards lower priced handheld device for consumer market will provide much greater access for individual households and will enable individuals / healthcare practioners to monitor effect of changes in lifestyle and diet on improvement of vascular health and thereby prevention of vascular complications as well as monitoring of effect of treatment on reduction of vascular vulnerability.

Key References: 1, 13, 14, 59, 75, 84.
## Main results in CTMM

1. **Comparative AGE Reader measurements between both generations AGE Reader** were available in 160 persons (algorithm development) and 477 persons (algorithm validation) (healthy controls and patients). The results show that measurement results with the AGE Reader mu show the same or smaller differences from those with the AGE Reader SU. It was concluded that the AGE Reader mu and SU provide the same results.

2. **In the first dataset of the Maastricht study (n=740)** we found that skin autofluorescence, as measured with the AGE reader, is associated with higher pulse wave velocity and with 24-hours ambulatory systolic blood pressure and pulse pressure. These associations were more pronounced in individuals with type 2 diabetes. These data support the hypothesis that AGEs are involved in the development of hypertension and arterial stiffness.

*Total number of AGE Reader measurements: 4000*

## Future Outlook:

More clinical trials and a cost-effectiveness analysis will be performed as part of the Maastricht Study in collaboration with the UMCG and Diagnoptics.

### Expected Impact of the product

Performing AGE Reader measurements on diabetes patients will lead to an early diagnosis of diabetic complications. Early detection of diabetic complications allows early intervention. It is already well known that interventions in early stages of the development of complications are highly effective. This can significantly improve the quality of life of diabetes patients and reduce the healthcare costs associated with diabetic complications.

### Number of Patients

$\geq 650,000$

### Yearly Costs

$\geq 1000$ M€
As a first application FABPulous has developed a rapid CE-marked test for FABP3 (heart-type FABP) to aid in the diagnosis of acute myocardial infarction (MI) in first line medical care. The test integrates FABPulous innovative plasma separation technology with proven high-quality lateral flow immunoassay detection, allowing for a time to result of 2–5 min. The simple and robust work flow allows for testing at the doctor’s office and at visits to the patients home as well.

Device for rapid FABP3 screening in plasma

**Leading Company**
FABPulous BV

**Current Diagnostics**
High-sensitive immunochemical assay

**Expected Impact of the product**

The low cost of the product (point-of-care test) allows a widely accessible use in primary care and thus aid in the assessment of the risk of DM2 patients for developing cardiovascular complications.

Early recognition of these complications will allow for a more early treatment and thus will add to the quality of life of these DM2 patients.

**Number of Patients** > 650.000

**Yearly Costs** >1000 M€

**Main results in CTMM**

In the PREDICCT program it was investigated whether FABP3 is also predictive for cardiovascular complications in DM2 patients.

For this purpose an FABP3 ELISA 96 wells quantitative assay for large scale samples screening was used.

In the EPIC-NL cohort it was found that plasma FABP3 adds significantly for assessing the risk for DM2-related cardiovascular complications.

Currently FABP3 ELISA measures are performed in cardiovascular case versus controls of the Dutch SMART cohort.

**Future Outlook:**
Together with a reader (to be developed) the device will allow the rapid assessment of plasma FABP3 in a point-of-care setting (doctor’s office) thus providing added value to risk assessment in DM2 patients.
Basic prognostic functions for the development of vascular and renal complications in DM2 patients have been constructed, based on current knowledge. Next, the added value of risk factors that have emerged more recently have been incorporated.

The added prognostic value of the biomarkers selected by PREDICTt have then been tested in large, relevant, and to a certain extent unique, populations of people at low or high risk for DM2 and vascular complications (EPIC-NL, SMART). This has resulted in improved prognostic functions for prediction of vascular and renal complications among patients with type 2 diabetes.

**Main results in CTMM**

Prognostic functions for DM2 perform very well to identify individuals at high risk of DM2. Because this provides very little room for improvement, the consortium did not invest in biomarkers for DM2. Prognostic functions for vascular complications of DM2 were only moderately able to identify those at high risk of these complications.

PREDICTt identified 13 biomarkers, from 31 tested markers, that were able to improve prediction of vascular complication by up to 15-20%.

In an explorative metabolomic approach, 13 promising metabolites were identified that improved prediction of vascular complications in DM2 by 5-10%.

Replication of the results for 13 biomarkers and 13 metabolites is currently ongoing.

**Future Outlook:**

The most promising biomarkers and metabolites can be combined in one chip to test in patients in order to improve their risk prediction.

**Expected Impact of the product**

Adding 6 biomarkers to the prognostic function for vascular complication in DM2 resulted in a 6.6% reduction in the amount of patients receiving intensive treatment while not affecting the overall burden of vascular complications in the study population.

Whether the savings associated with providing a less expensive treatment to 6.6% of the target population are sufficient to offset an increase in initial screening cost as a result of adding 6 biomarkers is still an open question.

Key References: 40, 69
Basic prediction models for chronic kidney disease (CKD) have been developed in the general population, but only a few of these prediction models include DM2 as a predictor and are, therefore, applicable to patients with DM2. Despite the high risk of CKD in DM2, the prediction of development of CKD has been less studied in populations of subjects with DM2.

We externally validated the performance of seven models predicting incident (micro)albuminuria and CKD that are applicable to subjects with DM2 in two independent Dutch cohorts (i.e. a general population and a cohort consisting of subjects with DM2) with over 10 years of follow-up.

At this moment, only one study (ADVANCE) specifically developed prediction models for the 5-year risk of renal complications in DM2. However, since development of diabetic nephropathy progresses over a long period of time (i.e. 10 to 20 years), 5-year is relatively short compared to disease progression. Therefore, we developed basic prognostic functions for the 10-year risk of early- (i.e. [micro]albuminuria) and late-stage (i.e. 50% increase in serum creatinine [SCr]) renal complications in patients with DM2.

“Device” Scoring algorithms for development of early- and late-stage renal complications of DM2

**Leading Partner** UMCG

**Current Risk Scores** ADVANCE

**Main results in CTMM**

External validation of the existing renal risk scores that included DM2 as a risk factor showed a moderate discrimination (i.e. the ability of the model to distinguish between patients with and without the outcome) and a poor calibration (i.e. the agreement of predicted probabilities with observed risks) in subjects with DM2. Calibration of the models improved after recalibration.

Furthermore, we developed two basic prognostic functions for the 10-year risk prediction of early- and late-stage renal complications of DM2. Age, gender, systolic blood pressure, HbA1c, ACR, smoking, and macrovascular complications were predictors for early-stage renal complications. The model predicting late-stage renal complications included the predictors age, BMI, systolic blood pressure, ACR, and macrovascular complications.

**Future Outlook:**

The additional prognostic value of novel biomarkers for risk prediction of early- and late-stage renal complications in DM2.

**Expected Impact of the product**

The developed risk prediction models are applicable to patients with type 2 diabetes treated in primary care.

The use of the developed renal risk scores can help in the early identification of patients with type 2 diabetes at risk for nephropathy and may allow optimization of preventive measures to reduce the incidence of diabetic nephropathy and its complications.

**Key References:** 38, in submission

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**Product**

**Partnership**

**Patient**

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**DM2 & vascular complications: Renal RISK calculator**

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**Expected Impact of the product**

- Number of Patients: > 650,000
- Yearly Costs: > 1000 M€
**Key findings**

- It is difficult to improve current methods of DM2 prediction with the use of novel biomarkers, because we already can predict it very well, based on traditional markers (weight, plasma markers).

- Novel biomarkers for identifying individuals at increased risk of DM2 add little to the success of subsequent (preventive) treatment.

- Risk stratification for, and early detection of cardiovascular complications in DM2 patients is only useful when this can be used to treat patients differently according to risk. Currently the biggest need are the novel treatments. The novel biomarkers should facilitate the development of novel therapies and motivate lifestyle changes in high risk subjects.

- The impact of investments in translational research can be increased by using all available evidence and insights to set research priorities before research is initiated. This can be done using Multi Criteria Decision Analysis and will lead to more justifiable and replicable investment decisions.

**Screening & Prevention**

The current options to identify patients at risk of DM2 (risk questionnaires) perform good, are easy to administer, and are very inexpensive. As a result, there is no market for biomarkers in this area of application.

There are numerous aspects that contribute to the success of a screening program for patients at risk for DM2 and the early detection of DM2. These are, amongst others, the accuracy of a risk prediction test, the cost of that test, the response of patients to the screening and the effects of the treatment provided to the patients identified in the screening. We found that currently, the response of patients to the test and consequent treatment is a far greater problem than the accuracy of tests.

**Early Diagnosis**

Due to increased attention for DM2, many patients are now detected quite early in the course of their disease. As a result, structured screening would only bring forward the diagnosis of DM2 by about three years. It has been found that this short period of earlier detection has little impact on the burden of disease, i.e. mortality and incidence of cardiovascular complications. Early diagnosis of cardiovascular complications may bring benefits to those patients. Evidence on that is currently still lacking.

**Patient Stratification**

The current preventive treatments for DM2 patients with a high risk for cardiovascular complications, i.e., statins and blood pressure lowering drugs are cheap and have positive effects in a very broad group of patients. As a result, there is very limited economic value in biomarker tests that can stratify DM2 patients on cardiovascular risk. Biomarker tests for the quantification of (specific) cardiovascular risk are only useful when new treatments are developed that are more expensive, or only have an effect in a subgroup of DM2 patients.

**Key References:** 51, in submission
Stepwise screening for prediabetes

Screening for prediabetes has found its way into several guidelines. However, because of low patient response rates and limited effects of earlier treatment of screen-detected DM2, it remains uncertain whether the favorable effects in those who enroll in a lifestyle intervention to prevent or delay the onset of DM2 are sufficient to offset the total cost of screening in the population. To address this issue, we conducted a cost-effectiveness analysis based on combining a model of the screening program with a disease progression model of DM2.

Cost-effectiveness analysis

The screening identified 36.5% of the prediabetes patients and 39.6% of the DM2 patients at an average cost of €588 per case detected. Downstream costs and effects of subsequent interventions on identified patients compared to no interventions were estimated at €956 and 0.0325 quality adjusted life year (QALY), respectively. Combined with the initial screening costs, this resulted in an incremental cost-effectiveness ratio of €47,503 per QALY.

Main findings

Secondary prevention has a very low probability of being the most attractive alternative (p<0.05 for all scenarios considered). The optimality of the remaining strategies is however strongly dependent on the decision makers' preferences: should decision makers favor the maximization of commercial headroom, primary prevention is the best alternative, whereas tertiary prevention of microvascular and macrovascular complications is optimal in case a safer strategy with fewer obstacles, but less gain, would be preferred.

Implications

The primary obstacle for cost-effective screening is the current treatment practice for screen-detected DM2. This obstacle therefore first needs to be overcome before attempts to improve the yield and cost of screening become economically viable. Further research into the development of a cost effective treatment protocol is thus urgently needed, including evidence on the effects of lifestyle interventions in screen-detected DM2 patients, which are currently rarely included in intervention trials.
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List of Publications


List of Publications


52. Koonen DP, Jensen MK, Handberg A. Soluble CD36-a marker of the (pathophysiological) role of CD36 in the metabolic syndrome? Arch Physiol Biochem. 2011 May;117(2):57-63


<table>
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Other Sources

B. Nationaal Kompas Volksgezondheid. 2007 Rijksinstituut voor Volksgezondheid en Milieu
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AGE</td>
<td>Advanced Glycation Endprduct</td>
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<tr>
<td>CE-marking</td>
<td>Conformité Européenne</td>
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<td>CETP</td>
<td>Cholesterol-Ester Transfer-Protein</td>
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<td>CHD</td>
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<td>CVA</td>
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<td>DM2</td>
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<td>ELISA</td>
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<td>PREDICCT</td>
<td>Prevention and Early Detection of Cardiovascular Complications in DM2</td>
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<td>TNFRns</td>
<td>Tumor Necrosis Factor Receptor non-sheddable</td>
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<td>UKPDS</td>
<td>United Kingdom Prospective Diabetes Study</td>
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<tr>
<td>VLCD</td>
<td>Very Low Caloric Diet</td>
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