PROJECT FINAL REPORT

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Project acronym: DEXLIFE

Project title: Mechanisms of prevention of type 2 diabetes by lifestyle intervention in subjects with pre-diabetes or at high-risk for progression.

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1.1. Executive summary

Diabetes is a global pandemic, driven by many factors associated with modern living, not least an even bigger pandemic of obesity. There is a pressing need for better methods of detection of those at high risk and more effective methods of selection for prevention and treatment.

Despite scientific advances in the understanding of the pathophysiology of type 2 diabetes, it remains a challenge to effectively identify and manage individuals in the general population who are at high risk of developing diabetes or those who already have undiagnosed diabetes. Therefore, there is a need to identify novel early markers of glucose intolerance that reflect the underlying biology and the overall physiological, metabolic, and clinical characteristics of progression towards diabetes.

The DEXLIFE project - led by John Nolan, CEO and Head of Steno Diabetes Center in Denmark – is a type 2 diabetes prevention project, which ran between 2012 and 2015 and involved a wide range of European partners, including diabetes clinics, universities, and industry. The DEXLIFE project focuses on type 2 diabetes, from its earliest stages in high risk, but otherwise healthy, individuals through to established diabetes. The current approach to diabetes risk is based on clinical assessment including risk scoring systems along with some form of glucose measurement. The current approach to the treatment of type 2 diabetes follows generalised algorithms, applicable to all patients. DEXLIFE places major focus on a deeper individual phenotype both for people with pre-diabetes and for those with diabetes. Using new methodologies in metabolomics and lipidomics, we have characterised baseline risk, progression and response to intervention in a range of well characterised European cohorts.

The key work undertaken and results achieved in DEXLIFE include the following:

- Identified novel biomarkers that anticipate and accurately forecast abnormal glucose tolerance in asymptomatic individuals. We have validated these biomarkers and investigated their mechanism of action and determined the extent to which these biomarkers are responsive to lifestyle intervention.
- Developed a personalised model for intervention using a systems biology approach. The outputs anticipated from DEXLIFE can be used for large-scale screening programmes to identify high-risk individuals and those with undiagnosed diabetes.
- Our work has resulted in nine journal publications to date, with one more article in preparation for re-submission, and approximately 11 more publications planned. Researchers also presented on DEXLIFE and the development of novel diagnostics and predicitve biomarkers at several conferences.

In DEXLIFE, we have generated new knowledge about metabolomics, lipidomics, RNA-sequencing, methylation, intervention studies, and bioinformatics. We believe that this new knowledge provides health care providers and policy makers with a sound basis on which to shape future health policy, diabetes supports and interventions. We have also opened up avenues for further research and study, particularly in translational research and further development of diagnostic instrumentation allowing analysis of blood and other samples at an acceptable cost for clinical practice.

The ultimate legacy of DEXLIFE will be diagnostic kits to measure biomarkers, personalised intervention regimes for front-line clinical environments, automation and simplification of biomarker analysis process, as well as feeding into future personalised medicine education. Our results and findings have real potential to impact on health policy in the future and our personalised lifestyle intervention has the potential to make a significant contribution to help prevent progression to diabetes in high-risk European citizen. We believe the ultimate legacy of DEXLIFE will be to help improve diabetes screening and interventions, thereby improving health outcomes while reducing the burden on the health sector and delivering related social and socioeconomic benefits.

1.2. Summary description of project context and objectives

1.2.1. Background

Large-scale diabetes prevention studies have clearly shown that type 2 diabetes can be prevented or delayed in people at high-risk by sustained changes in both diet and physical exercise. However, despite solid evidence for preventive actions, it remains a challenge to effectively identify and manage individuals in the general population who are at high risk of developing diabetes or those who already have undiagnosed diabetes. Not only are there multiple patterns of progression towards diabetes, there is also a significant variation in individuals response to interventions on exercise and diet. Thus, even if people are accurately identified as having pre-diabetes, their subsequent response to preventive intervention remains difficult to forecast and is still highly variable.

DEXLIFE is tackling these problems by identifying novel markers of glucose intolerance that reflect the underlying biology and the overall physiological, metabolic, and clinical characteristics of progression towards diabetes. These markers should ideally be sensitive to small changes in physiology and demonstrate change with deteriorating glucose tolerance as well as with improvements in glucose tolerance in response to lifestyle intervention. The identification of new biomarkers of this kind could facilitate a completely new approach to disease prevention, based on accurate prognostic phenotyping of high-risk subjects and accurate selection of these individuals for the appropriately matched interventions.

The DEXLIFE project focuses on type 2 diabetes, from its earliest stages in high risk but otherwise healthy individuals through to established diabetes. The current approach to diabetes risk is based on clinical assessment including risk scoring systems along with some form of glucose measurement. The current approach to the treatment of type 2 diabetes follows generalised algorithms, applicable to all patients.

DEXLIFE places major focus on a deeper individual phenotype both for people with pre-diabetes and for those with diabetes. Using new methodologies in metabolomics and lipidomics we have characterised baseline risk, progression, and response to intervention, in a range of well characterised European cohorts.

1.2.2. DEXLIFE aims and objectives

The DEXLIFE project was developed to identify novel diagnostic and predictive biomarkers, based on the core pathophysiology, that underlie the progression from normal glucose tolerance through to pre-diabetes followed by type 2 diabetes. In particular, the objectives were to:

- Identify a 'metabolic signature' of circulating and tissue specific biomarkers that characterise the development of abnormal blood glucose.
- Better detect the progression toward diabetes in high-risk individuals with different phenotypes.
- Evaluate the responsiveness of these biomarkers to a lifestyle intervention known to prevent type 2 diabetes.

DEXLIFE will help to inform the development of new screening tests and interventions to help prevent progression to diabetes in high-risk European citizens. DEXLIFE uniquely provides the following:

- Besides **identifying novel biomarkers that predict the development of type 2 diabetes**, the aim of the DEXLIFE project was to investigate how many of **the biomarkers that predicted progression to diabetes also track in the opposite direction when diabetes risk is reduced**. This would enable the identification of the biomarkers most strongly associated with changes in glucose tolerance. **Healthcare providers will be able to determine whether individuals will be responsive to lifestyle intervention at an early stage or whether they require other treatment options.**
- A **lifestyle intervention,** comprising changes in physical activity and diet, has been shown to significantly reduce the risk of developing diabetes. The DEXLIFE approach differed from other intervention studies in that the intervention **focused on providing participants with choices** of different types and schedules of exercise and varieties of diet control, so they could select the best option for them. For the first time it will become possible to tailor a **personalised treatment plan with the highest likelihood of efficacy for an individual patient.**

1.2.3. DEXLIFE impact

DEXLIFE will ultimately help **prevent progression to diabetes in high-risk European citizens**. This has happened, on a small scale, during the lifetime of our research project (through our lifestyle intervention) and our results and tools are now being shared for wider uptake.

The impact of DEXLIFE extends **beyond European borders**. Type 2 diabetes is now a global epidemic with the greatest rates of increase in Asia and developing countries. However, phenotypic characteristics of patients in these countries may differ from the typical pattern of patients from European countries. For example, BMI is significantly lower in Asian patients diagnosed with type 2 diabetes. Nonetheless, the underlying biochemistry and physiology are similar. What these countries most urgently require is an inexpensive, easy to administer, widely available screening test to identify high-risk individuals. The screening capabilities resulting from DEXLIFE can therefore benefit citizens that are at risk worldwide.

The project results will also enable healthcare providers, including governments, to predict the progression to diabetes and assist with prevention strategies. **Healthcare providers will be able to determine whether individuals will be responsive to lifestyle intervention at an early stage or whether they require other treatment options.** At present, diet and exercise modifications are recommended for all patients with diabetes and, if they are not responsive or compliant, a series of pharmaceutical agents are prescribed to control glucose and lipid metabolism. Our consortium has challenged the assumption that all individuals respond to lifestyle intervention, and has show that, as with pharmaceutical treatment, some are more, or less responsive. Thanks to DEXLIFE, it is now, for the first time become possible to tailor a **personalised treatment plan with the highest likelihood of efficacy for an individual patient.**

Even though this direct benefit makes the project worthwhile, the real value of DEXLIFE is tackling the four major problems that exist in the identification of high-risk individuals:

- Glucose is the only accepted biomarker for diabetes; unfortunately this metabolite changes too late in the physiological progression to disease, so that glucose-based diagnosis is often too late.
- There is a high degree of inter-individual variation in the progression to diabetes.
- There is a high degree of variability in responsiveness to various treatment modalities, including lifestyle changes.
- The majority of people with abnormal glucose tolerance and type 2 diabetes remain undiagnosed and asymptomatic for a number of years.

These problems **require tools for population-based screening** that can be easily distributed and are cost effective. In the lifetime of the project, DEXLIFE has:

- Identified novel biomarkers that anticipate and accurately forecast abnormal glucose tolerance in asymptomatic individuals.
- Validated the DEXLIFE biomarkers and investigated their mechanism of action.
- Determined the extent to which the biomarkers are responsive to lifestyle intervention.
- Developed a personalised model for intervention using a systems biology approach.

1.2.4. The DEXLIFE approach

DEXLIFE addresses the core physiological parameters underlying the current pandemic of type 2 diabetes, driven by modern trends in diet and physical activity. Central to DEXLIFE has been the examination of the **progression from a state of normal metabolism with normal glucose tolerance through to pre-diabetes followed by type 2 diabetes**. The range of cohorts available to DEXLIFE means that we have been able to map the natural background progression to diabetes in certain groups. While mapping and analysing **background** progression to diabetes is important, we chose in addition to analyse the responses to lifestyle (diet and exercise) **intervention**. Thus, our research sought to map as completely as possible the full range of the process of the evolution of diabetes in the population, from young adults through to middle-aged and older subjects at various stages of progression of metabolic disease. By achieving this degree of clinical 'coverage' of the phenotypes represented in the community, the project has provided its basic

science/mechanistic partners with an appropriately broad range of clinical settings from which to derive the range of cell, tissue and circulating biomarkers needed to classify and predict the relevant clinical endpoints under consideration. The overall research strategy was a top-down and iterative evaluation of combinations of established and novel biomarkers, leading to new insights into diabetes risk and progression. This included examination of clinical phenotypes, metabolome/lipidomics, protein expression, transcriptomics, epigenetics and genetics at a baseline, over the natural progression of type 2 diabetes and in response to active intervention.

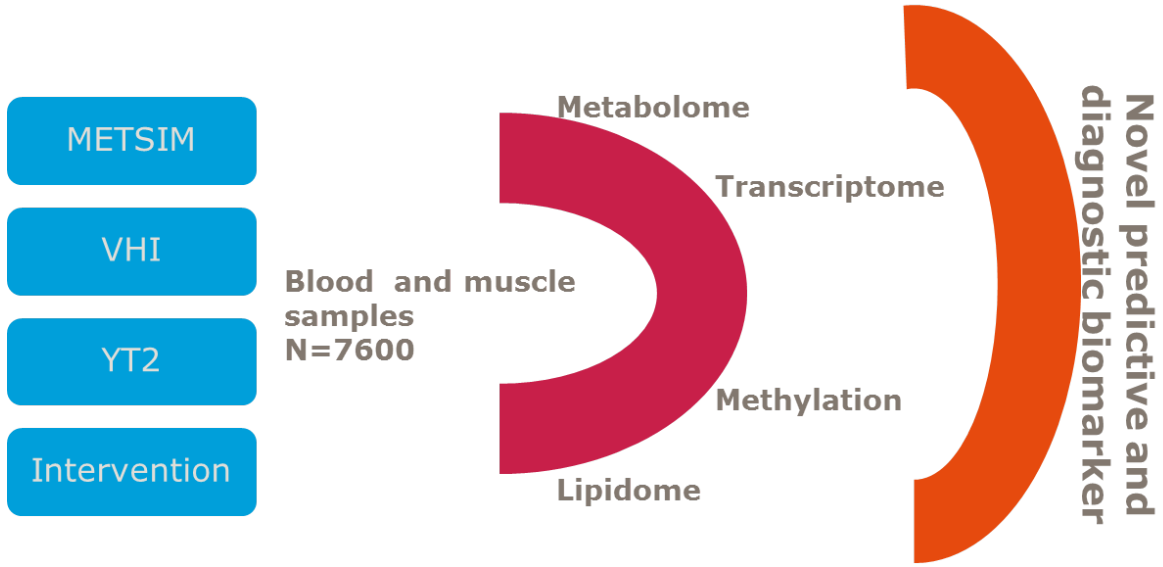

Figure 1 DEXLIFE: During the course of DEXLIFE more than 7600 blood and muscle samples have been analysed for a multitude of different metabolite levels. The comparison of the samples allowed us to develop novel predictive diagnostic biomarkers. YT2 (young type 2), OT2 (Old type 2)

The DEXLIFE project encompasses a **uniquely broad and deep research population**. In DEXLIFE, several unique and specially selected clinical cohorts provided the basis for a series of clinical, physiological and mechanistic investigations. These investigations were combined with a range of the new 'omic technologies to construct a detailed profile of the spectrum of progressors/non-progressors across multiple cohorts. In addition, an exercise and dietary intervention study was conducted, enabling us to assess the impact of personalised interventions on both biomarkers and specific functional tissue-based markers. These groups include:

- METSIM (Metabolic Syndrome in Men) cohort (a longitudinal study following 10,197 men aged 45 to 73 from the city of Kuopio in Finland);
- VHI Diabetes Mellitus and Vascular Health Initiative (DMVhi) cohort (the VHI screening study identified 700 participants out of the 30,000 VHI policy holders who participated in this first large-scale screening study);
- YT2 (135 Young Type 2 participants were recruited from the outpatients clinics at St. James' Hospital Dublin) cohort; and
- The longitudinal multicentre RISC cohort (1,500 Europeans, recruited in 2003 and followed-up in 2008 and 2013).
- The intervention study also comprised of 373 participants from Ireland who have a sedentary lifestyle and are deemed at higher risk for developing type 2 diabetes.

1.3. Description of the main S&T results/foregrounds

1.3.1. Introduction

The DEXLIFE project focuses on type 2 diabetes, from its earliest stages in high risk but otherwise healthy individuals through to established diabetes. Type 2 diabetes develops gradually over the course of many years. The prodromal stage of disease is clinically silent, and without symptoms. Previous approaches to diabetes risk are based on clinical assessment, including risk scoring systems along with some form of glucose measurement. Treatment of type 2 diabetes follows generalised algorithms which are applied to all patients.

Progression to type 2 diabetes has been the major focus of DEXLIFE. This progression is thought to result from two major pathologies: (i) gradual loss of insulin secretion along with (ii) persistent and increasing insulin resistance, with ongoing demand for compensatory increased insulin secretion. Vulnerability to diabetes is not simple and may include factors that are not obvious from a simple clinical risk assessment. There is increasing interest in mapping trajectories of progression prior to the diagnosis of diabetes in carefully phenotyped cohorts. The DEXLIFE approach is novel because it places major focus on a deeper individual phenotype both for people with pre-diabetes and for those with diabetes. Using new methodologies in metabolomics and lipidomics we have characterised baseline risk, progression, and response to intervention, in a range of well characterised European cohorts.

The scientific work of the project was divided into the following work packages:

- **WP1: Cohort Management.** The main objective of WP1 was to assemble, coordinate and manage the various large cohorts of human subjects.
- **WP2: Circulating Biomarkers and *omics.** WP2 studied changes in circulating metabolites and lipids that characterise the progression from normal glucose tolerance through the intermediate prediabetic, dysglycemic stages of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) and, finally, on to overt type 2 diabetes (T2D).
- **WP3 Cell and Tissue Analyses.** The major objective of WP3 was to study changes in the muscle transcriptome, metabolome (including lipidome), and methylome (promoter CpG methylation) as a result of lifestyle interventions (exercise or diet).
- **WP4 Intervention.** The primary purpose of WP4 was to deliver a lifestyle intervention programme that is expected to improve insulin sensitivity and decrease the risk of progression to type 2 diabetes in a high risk cohort.
- **WP5 Integration & Translation.** WP5 consisted of work on integrating data from multiple cohorts in order to obtain environmental risk factors related to different stages of type 2 diabetes. The data from multiple cohort studies (WP1, WP2, WP3) was pooled and analysed in WP5. Biomarkers indicative of higher diabetes risk and/or prediabetes were identified. These identified biomarkers were subsequently evaluated in the intervention studies in WP4.

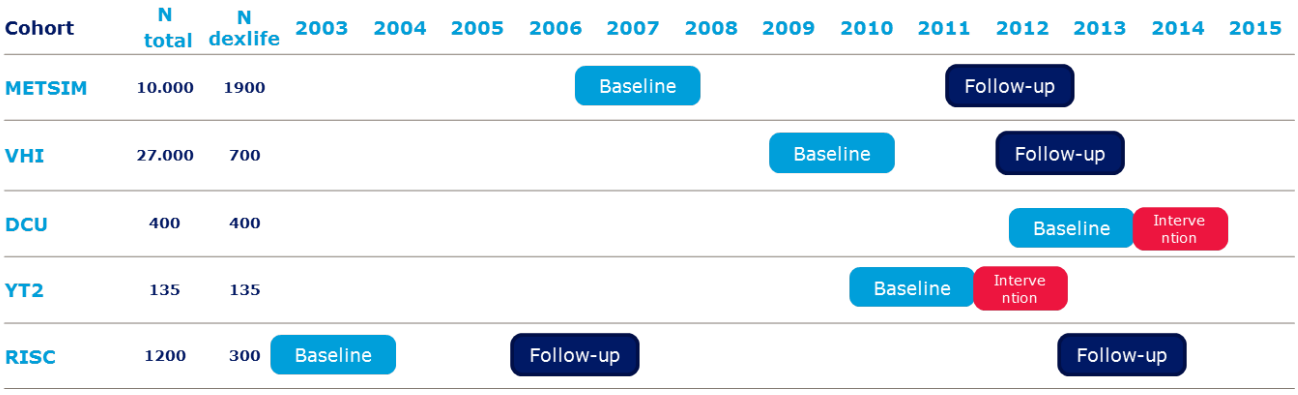

Figure 2: The DEXLIFE cohorts

The range of cohorts available to DEXLIFE (Figure 2) has made it possible to map the natural background progression to diabetes in certain groups. The methods have been described thoroughly in a recent DEXLIFE publication (Andersen et al., 2014). The main components of the project were:

- Novel circulating metabolomic and lipidomic profiles of progression to diabetes were identified in two cohorts, the METSIM study in Finland and the Vhi Healthcare cohort in Ireland.
- A small cohort of young people with type 2 diabetes, matched controls and older people with type 2 diabetes, were used to identify biomarkers of early onset diabetes.
- A prospective lifestyle intervention was implemented in a high risk group of individuals to determine if the new candidate biomarkers tracked with improved glucose tolerance.
- The new candidate biomarkers were validated using the RISC cohort of healthy European people from all over the continent and other samples from the Vhi Healthcare cohort that were not included in the original analysis.

1.3.2. Personalised medicine

Personalised medicine emerged as a medical model using characterisation of individuals' phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, to determine the predisposition to disease, and deliver timely and targeted prevention. Essentially, personalised medicine relies on using biological markers to stratify patients into specific sub-groups, which may respond best to specific interventions and therapies. Integrating the biological and lifestyle components offers the best opportunity to stratify subgroups for personalised prevention or treatment programmes. This approach promises to reduce the burden of disease, by targeting prevention and treatment more effectively.

In DEXLIFE, we have taken a unique approach by stratifying both the biological markers and the response to lifestyle intervention.

- Biological markers: The success of personalised medicine requires the development of accurate and reliable diagnostics and on the identification of predictive biomarkers. For the identification of suitable biomarkers, research on early disease processes is important in order to identify which factors to study as potential predictors of disease and of treatment response.
- Lifestyle intervention: Stratified phenotypes will be strengthened if they include responses to lifestyle intervention and are not solely based on biological progression. Individual responses to dietary and exercise interventions are varied and can have a major impact on biological biomarkers.

When effective therapy can be selected for a given patient based on individualised profile, the treatment can be more effective and/or adverse effects can be avoided. This will in turn reduce healthcare costs by minimising costs associated with ineffective treatment and avoidable adverse events. The goal of this approach is also to focus healthcare resources on prevention, moving from illness to wellness, and from treating disease to maintaining health.

1.3.3. Metabolomics of diabetes and pre-diabetes

Metabolites are small, non-protein molecules having molecular weights of less than 1500 Daltons. Several thousand metabolites can be detected in human plasma, although considerably fewer are measured in a typical experiment. Circulating metabolites may result from a number of sources, including endogenous production, diet, gut microbes, drugs, and personal care products, and their levels can be affected by ethnicity, age, BMI, and sex. Metabolite levels can change rapidly along with changes in flux through metabolic pathways (e.g. after a meal) and can reflect the current metabolic status of an individual.

Discovery metabolomics and lipidomics were carried out using the DMVhi, YT2, and METSIM cohorts – as a result, 23 candidate metabolite biomarkers were identified. In order to validate the 23 metabolites that were shortlisted, a new METSIM subcohort was used. 1,293 samples from non-diabetic or newly diagnosed diabetic cases from METSIM baseline study (with gene expression data available) were selected for the Metabolon discovery platform (all of these Metabolon naïve). Models to identify impaired glucose tolerance (IGT) using fasting plasma glucose (FPG) and our candidate metabolites were generated - the Quantose IGT model. This model has been found to be sensitive and specific, and may serve as a convenient alternative to the oral glucose tolerance test (OGTT) to identify subjects at high-risk state for developing type 2 diabetes. A second, representative subset of METSIM samples, not used for discovery, and samples from the RISC cohort, are currently used to validate the candidate biomarkers.

Metabolite	Classification
α-Hydroxybutyric acid (AHB)	Methionine/Threonine metabolism
β-Hydroxybutyric acid (BHB)	Ketone body
2-Aminoadipic acid (2AAA)	Lysine metabolism
3-Hydroxyisobutyric acid (3HIB)	Valine metabolism
3-Methyl-2-oxobutyric acid (3MOB)	Valine metabolism
3-Methyl-2-oxopentanoic acid (3MOP)	Isoleucine metabolism
4-Methyl-2-oxopentanoic acid (4MOP)	Leucine metabolism
α-Ketobutyric acid (AKB)	Methionine/Threonine metabolism
α-Ketoglutaric acid (AKG)	Krebs cycle intermediate
Creatine	Creatine metabolism
Glycine	Amino acid
Hydroxyisovaleroyl carnitine	Leucine metabolism
Isoleucine	Branched-chain amino acid
Leucine	Branched-chain amino acid
lysoPC(18:2(9Z,12Z)); also known as LGPC	Lysophosphatidylcholine
Oleic acid	Fatty acid
Phenylalanine	Aromatic amino acid
Serine	Amino acid
Trigonelline (N'-methylnicotinate)	Nicotinate metabolism;
Tyrosine	Aromatic amino acid
Valine	Branched-chain amino acid
Vitamin B5 (pantothenic acid)	Vitamin; precursor to CoA
X-12063	Unknown

Table 1 Quantitative Assay List – Panel of 23 Metabolites identified and validated by the DEXLIFE project.

The panel of quantitative assays was used to validate the metabolites in 1,620 diabetes-free subjects from the RISC cohort and the Vhi Healthcare cohort not used in the original discovery phase. A set of 23 metabolites that predict progression to type 2 diabetes have been identified and validated by the DEXLIFE project. In most cases, the metabolite changes were present in pre-diabetes, indicating they could be used as early indicators of diabetes risk.

Steno scientists will continue research in biomarkers that predict the development of diabetes. The research will be extended to biomarkers that can predict complications in both T1D and T2D patients. The POPS study (currently ongoing at Steno) will examine the hypothesis that: a patient profile including psychosocial/demographic data and physical tests, complimented by metabolomics, lipidomics, and proteomics data on biological samples may be used to obtain a more effective treatment recommendation. The results will be directly translated into better personalised treatment and clinical care.

1.3.4. Personalised lifestyle interventions

The DEXLIFE lifestyle intervention was a 12-week, partially supervised, exercise training programme accompanied with dietary advice, to improve insulin sensitivity and assist with body fat reduction. Lifestyle interventions, comprising changes in physical activity and diet, have been shown to significantly reduce the incidence of diabetes.

The aim of our programme was to decrease the risk of progressing to diabetes by improving fitness levels, changing dietary intake and decreasing body fat levels. In so doing we hypothesised that blood glucose levels would decrease and that some of the biomarkers that predict progression to diabetes would move in the expected direction of change. We wanted to see how many of the biomarkers that predicted progression to diabetes also track in the opposite direction when diabetes risk is reduced. This would help us identify the biomarkers most strongly associated with changes in glucose tolerance.

Participants were recruited from the Vhi Healthcare cohort and the general public, who meet one of the following criteria: (i) impaired fasting glucose or impaired glucose tolerance; (ii) normal glucose tolerance but a score > 12 on the FINDRISC questionnaire (suggesting a 1 in 6 chance of developing type 2 diabetes in the next 10 yrs.). Participants who provided consent were invited to attend for medical screening and baseline measures of aerobic capacity with ECG monitoring, an oral glucose tolerance test, heart rate variability and body composition (DEXA and abdominal ultrasound). They completed a series of questionnaires to assess quality of life, physical activity, health & lifestyle. A sub-group of the control (n=40) and intervention (n=100) participants had a skeletal muscle biopsy from the vastus lateralis. We focused on two key components to modify lifestyle and achieve our aim:

- Exercise: We set a target of 4-hrs of exercise per week and encouraged participants to join group classes that were specially designed in the University (DCU) sports complex. We also developed a special web diary that allowed us track all of the exercise sessions, even for those that could not come to the sports complex.
- Diet: Our aim was to develop a self-selected healthy eating food plan based on participant's (estimated 3-day) food diaries. The dietician assisted in identifying food choices that were unhealthy, offering alternative healthy options and advice on reducing intake by 600 kcal per day. Each participant was contacted every four weeks to ensure that they were following their dietary plan.

The intervention focused on providing choices for participants so they could select the best option for them.

- There were over 50 exercise classes available each week, at all times of the day, and to suit all activities. The group classes were specially designed for chronic disease management and based on a circuit of exercise stations that worked the upper and lower body. Other options included yoga, pilates and spinning classes or participants could exercise themselves using the gym equipment.
- The dietary intervention was minimally invasive and focused on offering alternatives to unhealthy food choices that were healthier and had a lower calorie content.

- The online diary was used to record exercise sessions and to monitor body weight. This was regularly monitored and participants were contacted if they had not entered a recent exercise session or body weight had not changed.

What did we measure before and after the intervention?

- **Body size and shape**: We measured changes in body weight, shape and composition. We measured the circumference of the waist and hip using standard protocols. Using a DEXA scanner, we were able to detect changes in the amount of fat and muscle in the body, and we also measured the thickness of fat in the abdomen using ultrasound.
- **Physical fitness**: Participants walked on a treadmill until they could no longer keep going, using a standard exercise stress test protocol (Figure 3). They had ECG, blood pressure and oxygen consumption measured during the test. A higher amount of oxygen consumed is associated with better aerobic fitness. Prior to the test they also had heart rate variability measured using the Vagus system.
- **Glucose tolerance**: After an overnight fast, participants were given a standard 75g oral glucose tolerance test. Blood samples were taken before glucose ingestion and at 30-, 60-, 90-, 120- and 180-mins post.
- **Questionnaires**: Each participant completed a series of questionnaires to assess quality of life and several indicators of health and wellbeing.

Significant differences were found between the intervention and control groups. Overall, participants in the intervention group decreased their body mass index, waist circumference, and body fat, as well as increased their aerobic fitness. Blood glucose levels were significantly lower in the intervention group and health related quality of life and dietary intake improved significantly. It was also concluded that individuals who exercised in the gym did better than individuals who mainly exercised at home.

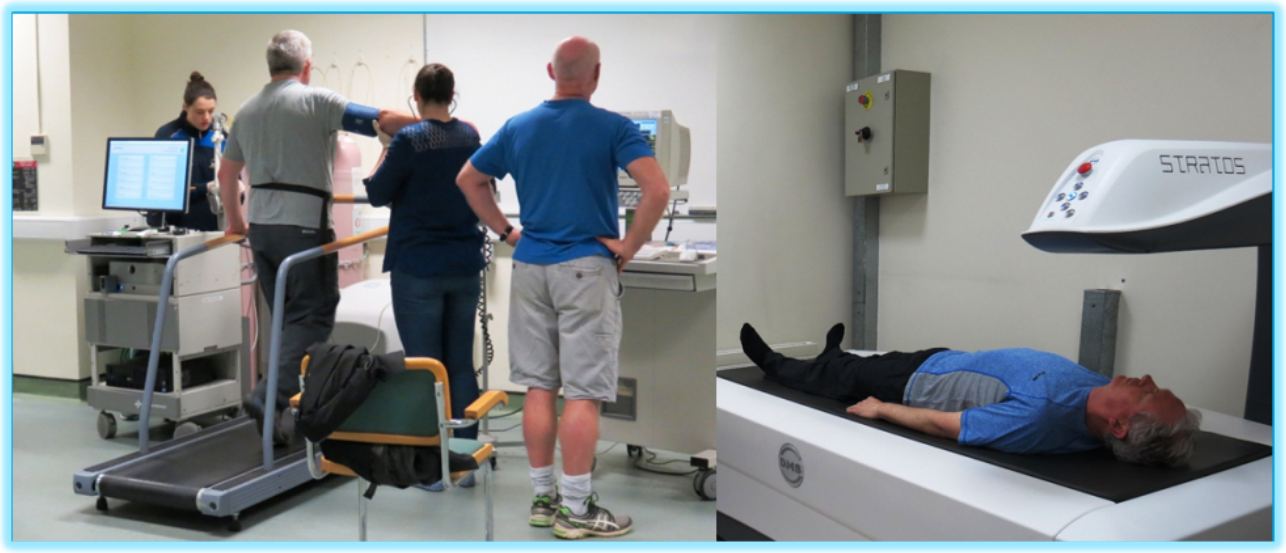

Figure 3. Testing the physical fitness of participants in the lifestyle intervention.

Main Results:

Physical characteristics: After completing the 12-week programme the intervention, but not control, group had a significant reduction in body weight, BMI, waist circumference, subcutaneous fat thickness, and whole body fat percentage. There was no difference in the visceral fat thickness in either the control of intervention groups. The intervention group also significantly improved their aerobic fitness while the control group did not change from baseline.

Glucose tolerance: Fasting blood glucose and 2-hr blood glucose, following the OGTT, were significantly lower in the intervention group following the intervention programme. There were no differences in the control group.

Quality of life: The overall quality of life score significantly improved in the intervention group following the 12-week intervention. The significant improvements in physical functioning, general health and vitality seem to contribute most to the change in quality of life. The control group had no change in quality of life or any of the individual components.

1.3.5. Identification of metabolites that track with the diet and exercise intervention

Our DEXLIFE prospective lifestyle intervention helped to determine if the new candidate biomarkers tracked with improved glucose tolerance. Analysis of intervention study samples showed that ten of the 23 metabolites changed significantly with the intervention. The 23 metabolite panel was measured pre- and post-intervention for a sub-group of subjects that completed the 12-week intervention. The new candidate biomarkers were validated using the RISC cohort of healthy European people from all over the continent and other samples from the Vhi Healthcare cohort that were not included in the original analysis.

We identified two potential biomarkers for tracking diet and exercise intervention effectiveness. The best overall biomarker to track responses in the diet and exercise intervention is tyrosine, which is already measured in standard amino acid clinical panels. Changes in tyrosine correlated best with change in FPG while changes in an unidentified metabolite correlated best with change in body weight. Metabolon is currently working on elucidating the structure of the unidentified metabolite.

δ-Metabolite	δ-FPG	δ-2hPG	δ-Weight
Tyrosine	0.34	0	0.4
Alpha-ketoglutaric acid	0.22	0.13	0.1
3-Methyl-2-oxobutyric acid	-0.03	0.16	-0.02
Phenylalanine	0.17	-0.01	0.26
2-Aminoadipic acid	0.29	0.03	0.14
Leucine	0.23	0.05	0.27
Isoleucine	0.25	0.08	0.15
alpha-ketobutyric acid	0.07	0.21	-0.2

Table 2 Pearson Correlations for Change in Metabolite Concentration Versus Change in Glucose

The above metabolite changes reflect a single time point (12 weeks) after initiation of a diet and exercise lifestyle modification. More research is needed to understand metabolite changes in both shorter and longer timeframes for this intervention. In addition, metabolite changes with exercise only and diet only interventions need to be investigated as well as responses to drug therapy (e.g. metformin).

The DEXLIFE programme has greatly expanded our knowledge of metabolite biomarkers associated with the pathophysiology of prediabetes and type 2 diabetes. Future work in this area should include their incorporation into new tests for the prediction of incident diabetes and for use in monitoring intervention studies for the prevention or delay of incident diabetes.

1.3.6. Identification of methylomic and transcriptomic alterations in muscle samples

A list of possible altered gene pathways has been generated based on a comparison of typical type 2 diabetic patients and BMI- and age-matched non-diabetic subjects. Although methylome has been shown to be directly altered by diets, such as those deficient in folate or methionine, epigenetic alterations of key metabolic genes that may determine the molecular basis of diabetes development have not been characterised in detail yet.

Analysis of the genome-wide methylation showed that the intervention program (diet and exercise) significantly alters the CpG methylation profiles at specific regulatory sequences. Controls do not differ in their genome-wide methylation profiles. However, we could identify 158 CpGs that change after the intervention in all individuals. Most importantly, 61 of these CpGs were located into the regulatory genes and were selected as the panel to cross with the transcriptome – derived candidates. Gene ontology analyses reveal an enrichment of genes associated with regulatory functions (such as transcription factors), Apk signalling pathway and immune defense (CD1, CD22, IL1RE).

Ultimately, we have identified novel differentially expressed gene pathways in skeletal muscle in high risk individuals subjected to a lifestyle intervention programme (WP4). We report gene pathways involved in the oxidative phosphorylation, cellular respiration all enclosed in mitochondrial function as well as skeletal muscle remodelling. RNA sequencing of the skeletal muscle samples gave us a higher overview of the gene expression in muscle before and after lifestyle intervention programme. Moreover, RNA seq information allows us to analyse not only the gene expression per se but also of the isoforms associated a particular gene. The successful sequencing of RNA allowed us to validate the benefit in quality of life linked to the lifestyle intervention. Methylation studies in the same cohort, provided a completely new information of the regulation of mitochondrial function and skeletal muscle remodelling gene expression. All these data led to the identification of specific pathways that can be investigated in the future in a more specific programme where exercise and diet can be analysed separately. The DEXLIFE project has been useful as a platform for the generation of very important data base in the physiology of skeletal muscle in the way of type 2 diabetes development.

1.3.7. Identification and validation of lipid molecular networks associated with progression of T2D

Lipids are a diverse group of metabolites that have many key biological functions, acting as structural components of cell membranes, energy storage sources and intermediates in signaling pathways. Due to their importance lipids are under tight homeostatic control and exhibit spatial and dynamic complexity at multiple levels. It is thus not surprising that altered lipid metabolism plays important roles in the pathogenesis of most of the common diseases including in diabetes. Lipidomics emerged as a discipline closely related to metabolomics, which is dedicated to global study of lipidomes, including pathways and networks of lipids in biological systems. Advanced analytical techniques, particularly mass spectrometry, have played the crucial role in the recent progress of lipidomics.

Lipidomic analysis identified several co-regulated clusters of lipids associated with progression of type 2 diabetes. Using network analysis and sub-sequent stepwise regression analysis, a subset of individual lipids was identified as a biomarker candidate panel to predict progression of type 2 diabetes. These lipids included lysophosphatidylcholine (lysoPC(18:2)), one ether phosphatidylcholine and three triacylglycerols. In an independent study setting, these lipids were then confirmed as predictive markers of type 2 diabetes. It has also been shown this lipid biomarker panel is an independent predictor of type 2 diabetes which alone outperforms the FINDRISK score, as well as improves the prediction if combined with glucose and FINDRISK.

A targeted assay was also developed which is applicable in clinical setting. The panel was selected based on literature survey and the available results in DEXLIFE. Furthermore, metabolites associated with diabetes complications, not studies in DEXLIFE, were also considered for the inclusion in the assay. Specifically, the panel of metabolic biomarkers (50 compounds) for prediction/diagnosis of (pre)diabetes and its co-morbidities and complications, and for follow-up of interventions includes the following:

- Markers of prediabetes
- Markers of status of glycemic control
- Markers of fatty liver
- Predictive markers for complications

The method developed is based on ultra-high performance liquid chromatography combined with triple quadruple mass spectrometry (UHPLC-QqQMS). During the method development, the conditions for sample preparation and conditions for the UHPLC-QqQMS have been optimised both with standard samples and with serum and plasma samples.

Specifically, in the sample preparation, the conditions for protein precipitation were optimised; conditions for the derivatisation of amino acids have similarly been optimised. The main goal was to perform the protein precipitation in aqueous conditions, using acidic conditions, and to modify the derivatisation reagents to be better compatible with the MS. The method that is generally used for the derivatisation can cause ion suppression of MS, leading to less robust quantitation. In the UHPLC separation, several different columns have been tested in order to find optimal conditions and good separation for also very polar species, as well as to obtain separation of specific isobaric compounds, such as leucine and isoleucine.

The ongoing and future studies will establish if the lipid-based risk score can identify specific responders or non-responders to specific interventions aimed at diabetes prevention.

1.3.8. 10 year follow up on the RISC cohort

The DEXLIFE project has provided the resource and structure to conduct detailed clinical phenotyping of a subset of the RISC cohort of healthy subjects. The project has resulted in the 10yr follow-up of 235 subjects in the RISC cohort (via UNEW), who have previously undergone baseline (0yr) and 3yr follow-ups. Such detailed longitudinal data provide unique and valuable opportunities to investigate the behaviour and robustness of novel biomarkers in an otherwise healthy population. A key output will be the assessment of metabolomic biomarkers as predictors of glucose tolerance.

DEXLIFE has provided the impetus and catalyst to attract additional funding to the RISC cohort to conduct 10yr follow-up in a further 400 subjects. Importantly, the groundwork to prepare the protocols/ethics approvals and sampling schedules will be transferable from DEXLIFE to the extended work. This provides added value to the DEXLIFE cohort and will increase the cohort to over 600 subjects with 0, 3 and 10yr follow-up data.

1.3.9. Quantose Impaired glucose tolerance (IGT) test

An IGT test, Quantose IGT, which can identify IGT subjects in a mixed IGT/NGT population was developed, in part, using data from the 23 metabolite panel in the Vhi cohort.

The 23 metabolite data was used to develop a novel IGT test by identifying metabolites whose plasma levels correlated with the 2 hour plasma glucose (2hPG) from the OGTT and those which had predictive value in identifying subjects with IGT. In general the best metabolites were those previously identified as being biomarkers of insulin resistance as measured by the clamp and this may be a reflection of the peripheral insulin resistance associated with IGT. The variables with the highest correlation with 2hPG are fasting plasma glucose, AHB, LPGC, and oleic acid. These four metabolites had higher correlations than did anthropometric and metabolic parameters such as insulin, age, BMI, and HOMA-IR.

A number of models were generated and one, in particular, including FPG, AHB, LGPC, oleic acid, serine, 4MOP, β-hydroxybutyric acid, and pantothenic acid had AUCs of 0.82 and 0.83 in RISC and Vhi respectively. This IGT test is called Quantose IGT and the score is called Q^{IGT}. This test estimates the probability that a subject is IGT. The higher the score, the more likely the subject is IGT. The test has a sensitivity/specificity of 78%/72% and may serve as a convenient alternative to the OGTT to identify subjects at risk of being IGT. The test is currently available at Metabolon, Inc. (Durham, NC USA, metabolon.com) and is expected to be commercialised soon in Europe. Metabolon's clinical diagnostics team is working to create metabolomics-driven tests that give healthcare providers actionable information to aid in patient management.

Quantose IGT is designed to easily identify IGT using a single, fasted blood draw. This simple test is a convenient surrogate for the oral glucose tolerance test (OGTT), the currently accepted clinical practice for measuring IGT. Although the OGTT can effectively measure IGT, patients must fast prior to having the test, and after establishing a fasting glucose level by means of a blood draw, the patient must then drink a glucose-rich beverage and undergo multiple blood draws over the course of about two hours. Because it is time consuming and can cause patients to feel nauseated, sweaty, or lightheaded, the OGTT is unpopular with primary care physicians and patients. DEXLIFE results will also inform Metabolon's Quantose diabetes product line and are expected to result in new diagnostic and monitoring products. Another area of focus could be to identify the best biomarkers for monitoring intervention response; our results so far suggest that the best metabolites for diagnosis may not be the best for monitoring therapy response or disease progression.

1.3.10. Network of Excellence and Future Collaboration

Beyond the commercial and future research opportunities described above, the DEXLIFE project has resulted in the creation of a new network of excellence in the area of diabetes and personalised medicine. Continued collaboration within and beyond the current partner consortium will help to meet the rapidly growing screening and intervention needs Europe and worldwide. The DEXLIFE mailing list will facilitate future collaboration. A follow on meeting has also been scheduled after completion of the project to facilitate discussion of collaboration on publications, future research, and exploitation directions.

To date, the DEXLIFE work has resulted in nine publications (discussed in some depth in Section 1.4.2 below). Furthermore, 11 publications are planned within the next year. Partners will continue the analysis of the results. A meeting is scheduled for 20 September 2016 at Steno, where partners will gather to further

discuss our continued publication strategy and strategies for novel research projects and applications. Steno will host this meeting.

After the end of the DEXLIFE project there is excellent potential for further research and translation. DEXLIFE researchers will keep searching for new funding opportunities for research in the clinical implementation of measuring biomarkers as routine clinical care.

The DEXLIFE intervention has collected a large amount of phenotypic and biological data on individuals at high risk of developing diabetes. A major resource has been created by DEXLIFE and it will be used to continue the collaboration between DEXLIFE partners by facilitating new research questions and applications for funding.

1.3.11. Conclusion

DEXLIFE has harnessed and built upon a wide range of expertise (from 'omics to lifestyle intervention) and technologies (bioinformatics and profiling) over a wide range of national contexts. As a result, DEXLIFE results will have an impact on in a wide range of scientific disciplines:

- **Metabolomics:** Non-targeted metabolite profiling of fasted EDTA plasma samples provided semi-quantitative measurements of hundreds of small molecules (molecular weight less than ~1500 Daltons). This data were used to identify candidate biomarkers ("discovery") associated with dysglycemia and type 2 diabetes and quantitative assays were developed for the most promising candidates for validation studies in additional samples not used in the discovery phase.
- **Lipidomics:** Global lipidomic profiling of plasma (i.e. molecular lipids such as phosphatodylcholines, sphingomyelins, triacylglycerols) was used in DEXLIFE cohorts in order to identify specific lipids associated with progression of type 2 diabetes, both in discovery and validation studies. Targeted quantitative platform was also established for use in the clinic, based on the project findings. DEXLIFE project provides evidence that profiling of molecular lipids is a powerful tool for identifying and utilising type 2 diabetes biomarkers in clinical setting.
- **RNA-sequencing:** RNA sequencing (RNA-Seq) provided a highly sensitive and accurate measurement of expression across the transcriptome in muscle samples from high-risk type 2 diabetes cohort. RNA-seq was used to provide visibility to previously undetected changes occurring in type 2 diabetes. RNA-seq allowed us to detect transcript isoforms, single nucleotide variants, allele-specific gene expression and other features without the limitation of prior knowledge before DEXLIFE project.
- **Methylation:** Illumina 450K methylation arrays provided a quantitative interrogation of the methylation sites across the genome at single-nucleotide resolution in muscle samples from a high- risk type 2 diabetes cohort. Methylation arrays were also used to explore the effect of lifestyle intervention programs. Statistical tools were used to select a list of candidate genes with differential methylation to be validated in specific validation models by bisulfite sequencing.
- **Intervention studies:** It is clear from the DEXLIFE intervention that offering choice to participants when undertaking lifestyle programmes has a major influence on their adherence, quality of life, and responsiveness. It is not necessary to differentiate between the effects of diet and physical activity when assessing the optimal intervention for reducing the risk of diabetes. DEXLIFE has identified metabolites that are significantly different even with varying contribution from diet and physical activity.
- **Bioinformatics:** Large amounts of data generated in DEXLIFE, particularly in the domains of lipidomics/metabolomics, demanded utilisation of advanced bioinformatics methods to facilitate metabolite identification, pathway analysis, and ultimately identification of key molecular features (metabolites/lipids, genes) associated with insulin resistance and type 2 diabetes, which were subsequently also validated. Multiple approaches were used, together demonstrating that bioinformatics is an essential and powerful tool for the discovery of clinical biomarkers of type 2 diabetes.

1.4. Potential impact, main dissemination activities and exploitation of results

1.4.1. The DEXLIFE impact and future use

1.4.1.1. Introduction

The DEXLIFE project has the potential to impact significantly on future health policy and future research agendas in relation to diabetes screening and interventions. The DEXLIFE screening programme has the potential for use in a variety of settings and with different populations, to deliver better personalised intervention programmes, and result in better long term health and better quality of life for people within at risk groups, thereby also reducing associated healthcare costs. Some of the key lessons we have learnt and their potential implications for the future are set out below.

There is potential for the impact of the DEXLIFE intervention to be felt on a number of different levels:

- *General practice and specialist clinical practice:* DEXLIFE provides an approach with the potential to improve screening, capacity for personalised intervention, patient health, and quality of life.
- *At the community and patients' level*: Our results show health benefits for people who participate in the personalised intervention. DEXLIFE has the potential to offer improvements in health and quality of life for patients who are at high-risk.
- *Research and development level:* The DEXLIFE intervention has collected a large amount of phenotypic and biological data on individuals at high risk of developing diabetes. A major resource has been created by DEXLIFE and it will be used to continue the collaboration between DEXLIFE partners by facilitating new research questions and applications for funding.

1.4.1.2. Lessons learnt - Diagnostic kits to measure biomarkers

A **screening panel** of 23 metabolite biomarkers linked to present and future dysglycemia and type 2 diabetes. The panel was developed using discovery findings from the DEXLIFE project, internal Metabolon data, and the literature. These biomarkers have been run in samples from the RISC, DMVhi, and METSIM cohorts, and the DCU diet and exercise intervention study cohorts, and have **consistently shown value in discriminating normal subjects from dysglycemic and diabetic subjects**. In particular, selective metabolite biomarkers of isolated impaired glucose tolerance (IIGT) have been identified (alpha-hydroxybutyric acid (AHB), linoleoylglycerophosphocholine (LGPC), oleic acid) as well as selective metabolite biomarkers of impaired fasting glucose (3-methyl-2-oxobutyric acid, 4-methyl-2-oxopentanoic acid). We hope to make this panel available to researchers interested in prediabetes and diabetes.

A **simple test for impaired glucose tolerance** using metabolites from the panel of 23 metabolites along with plasma glucose which requires a single, fasted blood draw. This test, called **Quantose IGT,** allows for the identification of subjects with, or at risk of, IGT without performing an oral glucose tolerance test (OGTT). Metabolon have begun **commercialisation** of the test in the United States, through True Health Diagnostics in Frisco, Texas. This test is to be used in subjects thought to be at risk of having IGT.

1.4.1.3. Lessons learnt - Personalised intervention regimens for front-line clinical environments

Steno is currently working on **including lipid testing as routine analysis for newly diagnosed type 2 diabetes patients referred to the Steno Clinic.** In the short term, the following study is planned: "A Prospective Observational investigation of metabolomic, lipidomic, and proteomic Profiles in blood from type 2 diabetes patients at Steno Diabetes Center" (POPS).

With POPS, Steno wants to test the hypothesis that a patient profile including psychosocial/demographic data and physical tests, complemented by an array of biological tests from the omics-arena (metabolomics, lipidomics, and proteomics) may be used to obtain a more effective treatment recommendation. The concept is referred to as profile-omics. Previous studies at Steno suggest that substantial weight loss achieved by non-surgical means can be sustained in the long term, and that there is a significant effect on the ability to perform physical activity. As proof of concept, they recently worked out an index based on profile-omics which is informative specifically of the health value of losing weight. They identified a

metabolomic risk profile which seems particularly responsive to weight loss. It is desirable to compliment these studies by applying the profile-omics approach to short and long term data, particularly studying the blood proteome by a completely new technology. A profile-omics informed recommendation on choice of treatment will improve precision and will move evidence-based medicine closer to personalised medicine.

- The primary purpose of POPS is to measure the metabolomic, lipidomic, and proteomic blood profiles of type 2 diabetes patients before and after the 12 months course program in the type 2 diabetes clinic at Steno Diabetes Center.
- The secondary purpose of POPS is to add the omics-data generated to other clinically available pre- and post-treatment data in order to work out a predictive tool for which patients benefit from what treatments based on the complete pre-treatment profiles.

After evaluation of the POPS study, the goal is to implement routine testing of DEXLIFE identified metabolites plus POPS identified peptides on all patients at Steno Diabetes Center.

1.4.1.4. Lessons learnt - Automation and simplification of the biomarker analysis process

DEXLIFE identified and validated a panel of biomarkers predictive of progression of type 2 diabetes, which are also sensitive to patients' responses to preventive therapy. Based on these findings, clinical assays were developed which can be implemented in a typical clinical chemistry laboratory and thus can be D6.2 Exploitation & Up-Scaling Plan applied by GPs in the clinic. The assay outputs allow simple read-outs such as added/decreased risk of progression to type 2 diabetes, and can thus be used to assess the efficacy of particular therapy.

1.4.1.5. Education - Diabetes modules for personalised medicine

During the course of DEXLIFE Steno has established a formal affiliation with Copenhagen University and John Nolan has been appointed professor at Copenhagen University. In the future, Steno will take part in the education of medical students, especially in the field of personalised medicine.

1.4.1.6. Preventing diabetes - using DEXLIFE personalised medicine to manage patients diagnosed with diabetes

Preventive action at an early stage is one of the most important steps that can be taken to reduce diabetes prevalence. Pre-diabetes, in particular, is an important clinical state in which type 2 diabetes can be prevented. This is an important and growing issue as the global obesity and diabetes pandemic continue to accelerate. The current results and the emerging new biomarkers discovered by the DEXLIFE project provide a **completely new tool to determine vulnerability to progression to type 2 diabetes**. Following simple risk based screening with a risk-score, the use of a novel, simple blood test for a targeted set of metabolomic and lipidomic markers will deliver a better method to define in simple terms 'high risk' or 'low risk' for progression to diabetes. The new knowledge being gained by DEXLIFE in relation to biomarkers that track with lifestyle intervention response will provide the clinician with an additional tool to assess and monitor response to a chosen approach with exercise and diet. Vulnerability to diabetes is complex and multi-factorial. The new DEXLIFE biomarkers will provide the clinician with a relatively simple tool to more accurately define biological vulnerability in the early stages of diabetes progression.

1.4.1.7. Managing diabetes - using DEXLIFE personalised medicine to manage patients diagnosed with diabetes

The application of metabolic and lipid biomarkers in diabetes treatment will need further study. Taking the approach from DEXLIFE, the next step is to test the responses of the leading biomarkers in patients, following the introduction of various targeted therapies. The current approach to the medical treatment of type 2 diabetes consists of a wide menu of options after metformin, increasing from dual to triple therapy and up to combined oral and injected therapy.

What DEXLIFE makes available now is the capability to inform these therapeutic choices, at each step, with new phenotypic information (regarding metabolites and lipids), and to test patient responses to treatment early in the course of care. This will help to define individual response or non-response to treatment (including early response to diet and exercise).

These new capabilities will support a new era of more personalised treatment of type 2 diabetes, based on the individual metabolic phenotype. Such a scenario is possible and follows a natural extension of the DEXLIFE project. It will however require further study in settings where the new biomarkers are available in the clinic for all patients at baseline and during carefully selected time points during treatment. The POPS study will be initiated at Steno in 2016, and will provide the basis for personalised treatment of diabetes at Steno.

1.4.1.8. Conclusion

In DEXLIFE we have generated new knowledge about metabolomics, lipidomics, RNA-sequencing, methylation, intervention studies, and bioinformatics. We believe that this new knowledge provides health care providers and policy makers with a sound basis on which to shape future health policy, diabetes supports and interventions. We have also opened up avenues for further research and study, particularly in translational research and further development of diagnostic instrumentation allowing analysis of blood and other samples at an acceptable cost for clinical practice.

The ultimate legacy of DEXLIFE will be diagnostic kits to measure biomarkers, personalised intervention regimes for front-line clinical environments, automation and simplification of the biomarker analysis process, as well as feeding into future personalised medicine education; each of which will also help to reduce the burden on the health sector and deliver related social and socioeconomic benefits.

1.4.2. DEXLIFE dissemination

Our target audience included academics and researchers, health professionals, voluntary organisations, policymakers, patients and the general public. Our aim has been to raise awareness about diabetes screening and intervention, and the findings from our research and the DEXLIFE intervention. Below we describe the dissemination activities undertaken including online, peer reviewed publications, conference presentations and posters, project materials, events and exhibitions, DEXLIFE on Euronews, and the DEXLIFE final conference.

1.4.2.1. Website and online

The project website is at http://dexlife.eu/ and was established in July 2012. The website includes the following sections:

- Homepage (including a brief summary of the project and contact details, this includes news and updates regarding recent developments and events).
- Results (setting out more detail about the project's aims and objectives).
- Partners List (details of each beneficiary, including the leads from each organisation).
- Management (provides information on the coordinator and the external advisory committee)
- Press/Media (provides project information and a project press release)
- Publications (lists the project publications)
- Diabetes Risk Score (gives access to the 'Diabetes Risk Score' quiz)

The website has represented an important communication tool for DEXLIFE and has been regularly updated throughout the project.

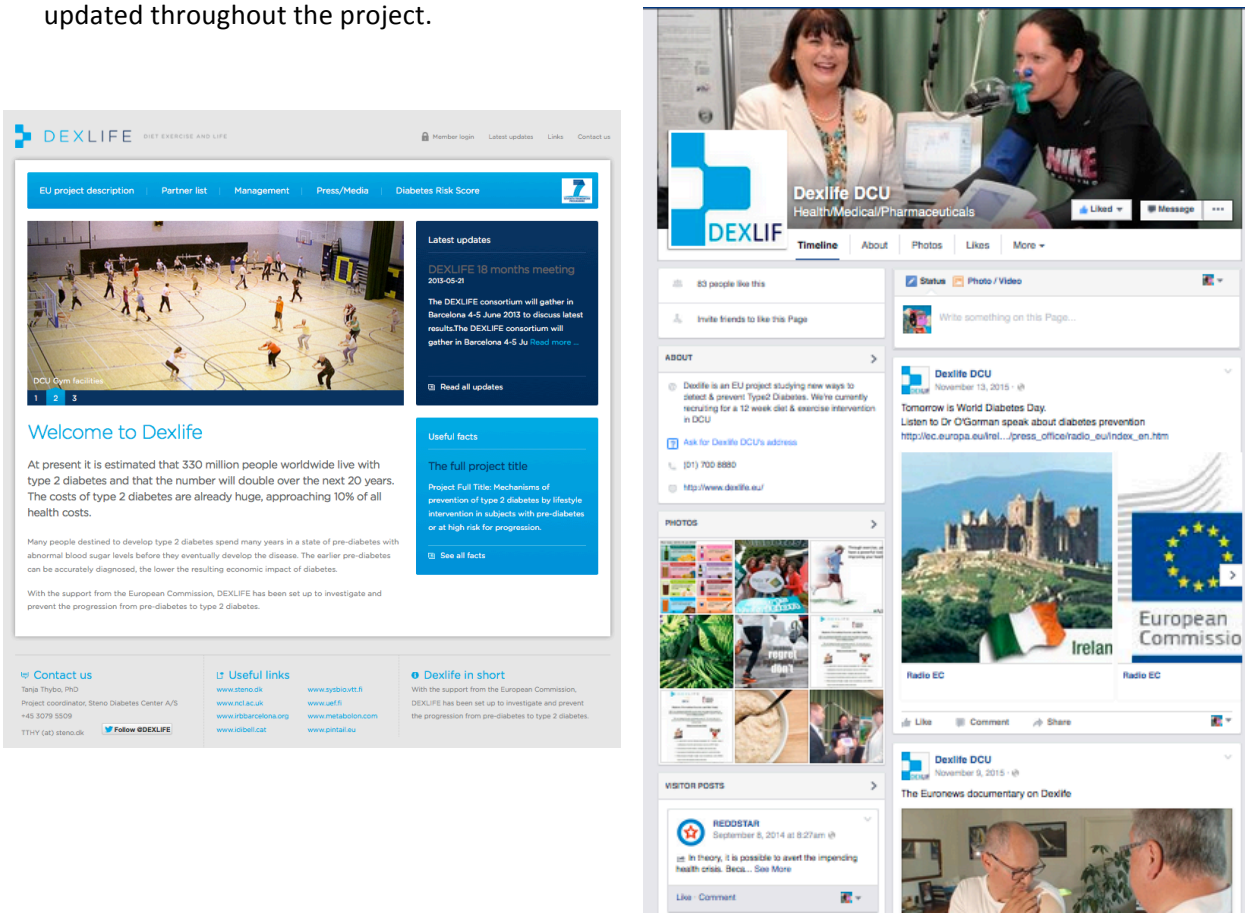

Figure 4 DEXLIFE website and facebook pages

The @dexlife and @dexlifeDCU twitter pages and the project facebook page (illustrated in Figure 4 above) have provided an online forum with which to engage with people directly as well as to share useful results from related studies and articles. Participants engaged in the DEXLIFE programme have been able to communicate with the team through facebook. Some participants tagged their DEXLIFE exercise related activities – on instagram.com and flickr.com (social photograph sharing communities), the #dexlife hash tag was used to tag photos taken as part of the intervention regime whereas on twitter #dexlife has been linked to #nikeplus running results.

1.4.2.2. Project Materials

The project materials issued over the course of the project included the following:

- DEXLIFE flyer and leaflets (distributed at the "Research in Action" exhibition, 9th July - 20th July 2012, Ireland; European Diabetes Leadership Forum 25th – 26th July 2012, Copenhagen; Open Doors Brussels, 17th May 2014.)
- DEXLIFE Handbook (draft presented at the DEXLIFE conference 20th May 2015; update planned for early 2016)

The **project leaflets** (Figure 5) set out the project's aims and objectives. They also summarised the risks of diabetes and provide tips on preventing people from developing type 2 diabetes.

The **DEXLIFE handbook** (Figure 6) provides clinical guidelines and "recipes" which can be used by a clinician or general practitioner to identify the optimal intervention regimen for an individual. It presents biomarkers and personalised medicine for predicting diabetes and preparing individualised treatment regimes. The purpose of the handbook is to aid translation of the DEXLIFE project results to the clinical environment.

An important output of our integrational analysis (across the different 'omic analyses that took place in DEXLIFE) is the identification of our biomarkers, which indicate higher diabetes risk or pre-diabetes. Also, it has been possible to identify biomarkers that track with a lifestyle intervention study. As these biomarker sets are simple (consisting of few and/or easily measured biomarkers) this will represent an important tool for large-population screening programmes.

The draft version of this handbook was presented at the DEXLIFE conference 20th May 2015. The conference audience was 100 clinicians working with diabetes. The clinicians represented the entire world and all in attendance received a copy of the draft version of the handbook. An updated (with new graphic design) version was shared online on 2nd June 2015.

Figure 5 DEXLIFE flyers

Figure 6 DEXLIFE handbook screen shots. The full version can be found online: http://dexlife.eu/eu-project-description.html

1.4.2.3. Events and exhibitions

The DEXLIFE team organised a series of regional and international events in order to discuss project results, implementation of interventions and potential impact.

- DEXLIFE participated in the European Commission's "**Research in Action**" **exhibition**, 9th July - 20th July 2012, in 'The European Commission Representation' in Ireland, European Union House, 18 Dawson Street, Dublin 2. DEXLIFE had an interactive exhibition stand for the duration of the event providing information about the DEXLIFE project to the general public, in addition to information regarding diabetes prevention. Members of the public in attendance, over the two weeks, were able to assess their own risk of developing type 2 diabetes, using the FINDRISC questionnaire based assessment. DCU provided the equipment necessary to complete the questionnaire, so that people were able to weigh themselves, measure their height, calculate BMI, and measure their waist circumference.
- The DEXLIFE team in the School of Health and Human Performance at DCU ran several public type 2 **Diabetes Risk Screening Events.** These occurred on the 25-26th July 2012 at DCU 13th August 2012 in Phibsboro, Dublin, 20th and 23rd August 2012 in Ballymun, Dublin, and the 31st August 2012 in Clontarf, Dublin. Twitter and Facebook were used to publicise these events. At these events, the public were invited to complete the FINDISC assessment, were provide with information about their risk for developing diabetes and had the opportunity to volunteer for the 12-week diet and exercise intervention.
- DEXLIFE participated in the EU Commission Stand at the **Annual BT Young Scientist and Technology Exhibition** in Dublin in January 2013, showcasing the work done by researchers in the DCU DEXLIFE project. This exhibition showcases the work of primary and secondary school children in Ireland and allows them the opportunity of engaging with scientists.
- DEXLIFE participated in the **European Diabetes Leadership Forum***,* hosted by Organisation for Economic Co-operation and Development (OECD) and the Danish Diabetes Association in Copenhagen on 25th -26th April 2012.
- Steno hosted an **exhibition of research at Steno Diabetes Center** on 22nd February 2013. At this event, a presentation was made to the Danish Health Minister Astrid Kragh. This included an overview of the DEXLIFE project.
- DEXLIFE participated in **Open Doors Brussels** 17th May 2014. Eoin Durkan from DCU and Tanja Thybo from Steno participated in this event with an exhibition stand where the message was delivered that that early discovery of diabetes makes it possible to avoid or reverse the disease. There was a movie on type 2 diabetes and flyers were handed out. Furthermore +500 people assessed their FINDRISC score on paper. Eoin and Tanja assisted in weighing and measuring waist circumference. After completion of the FINDRISC score, advice was given according to results and a pedometer handed out.
- An exhibition was held at **Steno to open the new metabolomics lab** on 5th November 2014. Matej Orešič and Tuulia Hyötyläinen, formerly at VTT, moved to Steno Diabetes Center in April 2014, where they since established a new systems medicine research group and a metabolomic laboratory.

1.4.2.4. DEXLIFE on Euronews

Euronews produced a 10 min TV documentary on latest European research on type 2 diabetes early detection and diagnosis, including the DEXLIFE project (http://www.euronews.com/2015/11/09/confronting-the-epidemy-that-is-diabetes/). It was aired from 9th November 2015 for a whole week (22 times) on the programme Futuris. Futuris is programme that features European science, research and innovation. The documentary was broadcast to 430 million households in 130 countries in EuroNews´ thirteen broadcasting languages (English, German, Spanish, French, Greek, Italian, Russian, Portuguese, Arabic, Turkish, Persian, Ukrainian, and Hungarian).

Figure 7 The movie was announced on LinkedIn and Twitter and is also available at dexlife.eu.

Figure 8 John Nolan was interviewed for the Euronews documentary.

1.4.2.5. DEXLIFE Conference

The DEXLIFE conference, hosted by Steno, was held on 20 May 2015 in conjunction with the Steno Frontiers on personal medicine in diabetes care. During that conference, the first day was dedicated to DEXLIFE. The following DEXLIFE partners gave talks on DEXLIFE results:

- The DEXLIFE study: Overview and main results. John Nolan, Steno.
- Lifestyle intervention in pre-diabetes. Donal O'Gorman, DCU.
- Metabolite markers of glucose tolerance and insulin resistance. Jeff Cobb, Metabolon.
- Use of metabolomics in prevention of type 2 diabetes. Matej Oresic, Steno.

120 international senior diabetologists attended the conference in order to be updated on frontline diabetes research and clinical diabetology.

1.4.2.6. Publications

Publishing in peer-reviewed journals has been a key aim of our dissemination strategy in order to reach academics, researchers, and medical professionals. In total there are now nine project publications registered on the EU portal. These publications are listed in part 2 Template A1 of this final report and include the following:

- G. S. Andersen, T. Thybo, H. Cederberg, M. Orešič, M. Esteller, A. Zorzano, B. Carr, M. Walker, J. Cobb, C. Clissmann, D. J. O'Gorman, J. J. Nolan; on behalf of the DEXLIFE Consortium. The DEXLIFE study methods: Identifying novel candidate biomarkers that predict progression to type 2 diabetes in high risk individuals. Diabetes Res. Clin. Pract. 106, 383-389 (2014).
- O' Donoghue G, Cunningham C, Murphy F, Woods C, Aagaard-Hansen J.; Assessment and management of risk factors for the prevention of lifestyle-related disease: a cross-sectional survey of current activities, barriers and perceived training needs of primary care physiotherapists in the Republic of Ireland. *Physiotherapy. 2014 Jun;100(2):116-22*.
- J. Cobb et al. A Novel Test for IGT Utilizing Metabolite Markers of Glucose Tolerance. Journal of Diabetes Science and Technology 2015; 9(1):69-76.
- T. Hyötyläinen and M. Orešič. Systems biology strategies to study lipidomes in health and disease. Prog. Lipid Res. 55, 43-60 (2014).
- Hyötyläinen T, Orešič M. Optimizing the lipidomics workflow for clinical studies-practical considerations. Anal Bioanal Chem. 2015 Apr 9. PubMed PMID: 25855150.
- Margaret Sinnott , Brendan T. Kinsley , Abaigeal D. Jackson , Cathal Walsh , Tony O'Grady , John J. Nolan , Peter Gaffney , Gerard Boran , Cecily Kelleher, Bernadette Carr: Fasting Plasma Glucose as Initial Screening for Diabetes and Prediabetes in Irish Adults: The Diabetes Mellitus and Vascular Health Initiative (DMVhi). PLoS OneVol. 10/Issue 4
- Jørgenrud B, Jäntti S, Mattila I, Pöhö P, Rønningen KS, Yki-Järvinen H, Orešič M, Hyötyläinen T. The influence of sample collection methodology and sample preprocessing on the blood metabolic profile. Bioanalysis. 2015 May;7(8):991-1006. doi: 10.4155/bio.15.16.
- Hyötyläinen T, Orešič M: Analytical Lipidomics in Metabolic and Clinical Research: Trends in Endocrinology and Metabolism S1043-2760(15)00158-7.
- Grainne M. O'Donoghue , Aileen Kennedy , Gregers Stig Andersen , Eoin Durkan , Tanja Thybo , Margaret Sinnott , John J. Nolan , Donal J. O'Gorman: An evaluation of the DEXLIFE 'self-selected' lifestyle intervention aimed at improving insulin sensitivity in people at risk of developing type 2 diabetes: study protocol for a randomised controlled trial. Trials. Vol. 16, 529-534.

As well as the articles that have already been published, the team is working on a number of additional papers, many of which are already at an advanced stage.

1.4.2.7. Presentations

Since the beginning of the project, the DEXLIFE team have recorded 55 dissemination activities (as per the EU portal and included in part 2 template A2 of this final report), including presentations at numerous key conferences and events. Some notable presentations and posters are set out below:

- Prof María Berdasco (IDIBELL) attended the EASD Berlin, on 4th October 2012 and presented on 'Epigenetic modifications in complex diseases'.
- Steno presented a poster at the World Congress of the Prevention of Diabetes and its complications, on 13th November 2012 in Madrid on 'Mechanisms of prevention of type 2 diabetes by lifestyle intervention in subjects with pre-diabetes'.
- VTT gave a presentation at EASD Barcelona on 13th September 2013 on 'Serum lipidomic profiling identifies biomarkers associated with progression to type 2 diabetes in the METSIM study'. Jeff Cobb (Metabolon) presented a poster at the same event, titled 'Metabolomic Profiling Identifies Biomarkers Associated with Dysglycemia and Incident Type 2 Diabetes in the METSIM Study'.
- Jeff Cobb (Metabolon) presented two poster at the American Diabetes Association's meeting on 13th June 2013 in San Francisco; 'Metabolomic Characterization of Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT)' and 'Novel Metabolite Models that Distinguish Impaired Glucose Tolerance (IGT) from Normal Glucose Tolerance (NGT)'.
- Project coordinators Steno presented the DEXLIFE project at a meeting in The Shanghai Institutes for Biological Sciences on 10th January 2014.
- Aileen Kennedy represented DEXLIFE at the EASD Vienna on 15th September 2014, where she presented a poster on 'The Identification of Novel Metabolites that Track with Improvements in Glycaemia Following a 12-Week Lifestyle Intervention in High Risk Individuals'. Jeff Cobb (Metabolon) also gave an oral presentation at this meeting on, 'Novel Metabolite Models that Distinguish Impaired Glucose Tolerance (IGT) From Normal Glucose Tolerance (NGT)'.
- Jeff Cobb (Metabolon) gave an oral to the American Association of Clinical Endocrinologists annual meeting in Nashville, US on 13th May 2015, on 'Selective Biomarkers of Isolated IFG (iIFG) and Isolated IGT (iIGT)'.
- Jeff Cobb (Metabolon) presented a poster at the American Diabetes Association annual meeting in Boston, US on 5th June 2015, 2015 entitled, 'AHB and LGPC are Selective Biomarkers of Impaired Glucose Tolerance (IGT)'.
- DEXLIFE were represented at the EASD 2015 meeting in Stockholm. Oral presentations included (Steno) 'Serum lipidome as an independent predictor of progression to type 2 diabetes: the METSIM study' and (Metabolon) 'Selective metabolite biomarkers of isolated IFG (iIFG) and isolated IGT (iIGT)'.
- Steno presented a poster at the First Annual Danish Bioinformatics Conference, 27th August 2015, in Odense, Denmark. The poster was titled, 'Lipidome-Based Early Predictors of Type 2 Diabetes'.
- Steno gave two oral presentations at the 8th World Congress on Prevention of Diabetes and Its Complications, Cartagena, Colombia (15th – 17th October 2015) including: 'The DEXLIFE project' and 'Prediction of type 2 diabetes using molecular profiling (opening plenary lecture)'.
- UNEW represented DEXLIFE at the DICP Symposium (XXXIX) on functional metabolomicson the 9th November 2015 in Dalian, China. They gave a talk on 'Serum lipidome as an independent predictor of progression to type 2 diabetes'.
- DCU presented a poster at the Danish Diabetes Academy & Cambridge Metabolic Network Workshop 10th December 2015 titled 'The identification of novel metabolites that track with improvements in glycaemia following a 12-week lifestyle intervention in high risk individuals'.

- Kristine Færch (Steno) will an oral presentation at ACSM's 63rd Annual Meeting, 7th World Congress on Exercise is Medicine in Boston, Massachusetts on 1st June 2016. Her talk is entitled, 'Heterogeneity in fitness response to a lifestyle intervention: The DEXLIFE intervention study'.

1.4.2.8. Other

The DEXLIFE team has also engaged with other stakeholders and initiatives over the course of the project.

Steno (Tanja Thybo) explored the possible collaboration with "SIBS-Novo Nordisk Translational Research Centre for Pre-Diabetes". The Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy Sciences (CAS) and Novo Nordisk have decided to collaborate in certain respects in the area of pre-diabetes research and development, and established in 2008 a joint research centre for pre-diabetes named "SIBS-Novo Nordisk Translational Research Centre for Pre-Diabetes".

Their annual review was held at the key lab of system biology, SIBS, Shanghai on January 10, 2014, and Tanja Thybo was invited to present DEXLIFE. Several of the PDC results are particularly exciting, as the DEXLIFE consortium has obtained similar results in Caucasian cohorts. Different scenarios for collaboration were explored including a H2020 application.

Each project partner has their own relationship with regional and national stakeholders, in particular diabetes associations, and continues to engage with them locally (e.g. local meetings, diabetes guidebooks etc.).

1.4.2.9. Conclusion

DEXLIFE partners have achieved several significant publications, with several more in preparation. Researchers also presented on DEXLIFE and the development of novel diagnostics and predictive biomarkers conferences.

Throughout the DEXLIFE project, we have grown our presence online through the project website and Twitter account. We have issued project resources and our project handbook (uploading them to our website and distributing them in hard copy). The DEXLIFE conference took place on 20th May 2015 in Copenhagen and provided a valuable opportunity for the team to engage with external experts, gather valuable feedback about the project, and consider next steps for the DEXLIFE outputs.

We believe that our work on dissemination (within WP6) has laid a solid foundation for screening tests and interventions to help prevent progression to diabetes in high-risk European citizens. Our dissemination strategy has spread the DEXLIFE message about the future of personalised lifestyle interventions and the importance of focusing on the individual when characterising baseline risk and subsequent interventions. The work undertaken will continue to bear fruit beyond the life of the project.

We believe that our results and findings have real potential to impact on health policy in the future and that our personalised lifestyle intervention has the potential to make a significant contribution to self-management support to help prevent progression to diabetes in high-risk European citizen.

1.5. Project website and contact details

Contact Details

Project Coordinator: Professor John Nolan, jjnl@steno.dk
Project Scientific Coordinator: Tanja Thybo, tthy@steno.dk
For further information, please see our project website: http://dexlife.eu/ (Figure 9).

Project partners are listed in Figure 10.

Figure 9 DEXLIFE website

steno diabetes center	**Steno Diabetes Center, Denmark** Steno Diabetes Center will lead and coordinate the DEXLIFE project www.steno.dk
Centre for Preventive Medicine	**Centre for preventive Medicine, Dublin City University, Ireland** The University is responsible for the lifestyle interventions to identify novel biomarkers www.preventivemedicine.ie
Vhi HEALTHCARE	**Voluntary Health Insurance Board, Ireland** VHI will manage a large population group of members previously examined for diabetes www.vhi.ie
IRB BARCELONA	**Fundacio privada institut de recerca biomedica IRB, Spain** The institute will perform cell and tissue analysis for novel biomarkers www.irbbarcelona.org/index.php/en/research/programmes/molecular-medicine/molecular-pathology-and-therapy-in-heterogenic-and-polygenic-diseases
IDIBELL	**Fundació Institut d'Investigació Biomèdica de Bellvitge, Spain** The institute will perform cell and tissue analysis for epigenetics studies www.idibell.cat
UNIVERSITY OF EASTERN FINLAND	**University of Eastern Finland** The university will identify new type 2 diabetes cases from a long-term study in a Finnish population group previously examined for diabetes www.uef.fi/coe/etusivu
METABOLON	**Metabolon, US** The institute will perform metabolomics analysis for identifying and validating biomarkers www.metabolon.com
Newcastle University	**University of Newcastle Upon Tyne, UK** The university will re-study participants from an ongoing large European research study who have been previously examined for diabetes www.ncl.ac.uk/crf
	**Pintail Ltd, Ireland** The company will be responsible for administration of the project together with Steno Diabetes Center www.pintail.eu

Figure 10 DEXLIFE partner list