

Publishable summary

1.1. RESOLVE context and objectives

1.1.1. The concept of RESOLVE

The metabolic syndrome (MetS) is defined as a cluster of interrelated common clinical disorders, including abdominal obesity, elevated blood pressure, loss of glycemic control, high triglycerides (TG), and low HDL-C. MetS is widely used as simple clinical definition of overweight individuals at increased risk of a large number of comorbidities such as type 2 diabetes (T2DM), cardiovascular disease (CVD) and non-alcoholic fatty liver disease (NAFLD). It is expected that by 2030 33% of the total population (200 million individuals) in the 27 countries of the EU will be obese. Many of them will have one or more of the above co-morbidities. At the current prevalence at least 20 million individuals will suffer from diabetes, 60 million have NAFLD and 10 million CVD. Without successful interventions, besides the toll on lives of EU citizens, costs of treating the comorbidities will increase to more than 100 billion Euros per year beyond 2030. Clearly there is an urgent clinical as well as economic need to conquer the sequelae and comorbidities of MetS which is at the center of RESOLVE's interest.

The aim of RESOLVE directly relates to the call topic HEALTH.2012.2.1.2-2. Systems medicine: **RESOLVE will combine the outcome of basic pre-clinical and clinical research and network analysis to further develop a comprehensive computer model allowing targeting the underlying mechanism** of low HDL-C, high triglyceride and loss of glycemic control in MetS patients. This will be translated into novel avenues for development of therapeutic intervention resulting, in close collaboration with the European biotech/pharmaceutical sector, in a profound impact on European citizen' health.

1.1.2. The Scientific and Technological objectives of RESOLVE

- **Project objective I:** To build a computational model for analyzing the kinetics of plasma lipids, lipoproteins and their interactions with glucose metabolism. Objective will be targeted by WP2 through data generated and managed in all other WPs and secured via deliverables D2.10-15 and milestones M1 and M2
- **Project objective II:** To apply the iterative systems biology cycle for calibrating, validating and improving the computational model in dedicated studies **in mice**. Objective will be targeted by WP2, 3 and 4 secured via deliverables D3.18–21 and milestone M2
- **Project objective III:** To build, calibrate and validate the computational model for use **in humans**. Objective will be targeted by WP2, 5 and 6 and secured via deliverables D5.29-34 and milestone M1
- **Project objective IV:** To analyze – based on model and experimental data – which processes in the **murine** metabolic network regulate the physiological response to perturbations in lipid, lipoprotein and glucose metabolism and how these interact. Objective will be targeted by WP2, 3 and 4 and secured via deliverables D4.22-28 and milestones M2 and M3
- **Project objective V:** To analyze – based on model and experimental data – which processes in the **human** metabolic network regulate the physiological response to perturbations in lipid, lipoprotein and glucose metabolism and how these interact. Objective will be targeted by WP2, 5 and 6 and secured via deliverables D6.35-42 and milestones M1 and M4
- **Project objective VI:** To use the **human** model **to identify network-based drug targets** aimed at restoring the metabolic dyslipidemia and glycemic control in patients with MetS and associated comorbidities. Objective will be targeted by WP2, 5 and 6 and secured via deliverables D6.35-42 and milestone M5.

1.2. Summary of the work performed during Period 4 (M37-M48)

In period 4, the RESOLVE project continued according to the planning outlined in the report of period 3. The mathematical model MINGLeD (Model INtegrating Glucose and Lipid Dynamics) developed in P3 has now been further extended and can be used in a modular fashion tailored to a particular experimental set-up. The model was tested on experimental (mouse) data sets derived from UMCG and UKE. At UMCG MINGLeD was applied to analyse the extensive heterogeneity in the long-term diet response and development of MetS in APOE*3-Leiden.CETP mice. Despite the same genetic

background and diet, some animals do not develop MetS (NonResponders). Fig. 1 shows the result of ADAPT simulations of 6 months on a high-fat diet with 0.25% cholesterol (HDF+C) for the NonResponders and the animals that do develop MetS (Responders). ADAPT quantifies the precision of the model predictions, plotted as shaded areas, representing the median (solid lines) $\pm$ median absolute deviation. Simulations confirm that body fat (triglycerides, TG) increases slower and less excessive in the NonResponders compared to the Responders. Intestinal TG levels are low compared to total body TG, but are predicted to be higher in the NonResponders than Responders. According to the model the effect can be best explained by a reduced fat absorption in NonResponders compared to Responders. This prediction could be validated by measuring fecal fat excretion in the mice. A linear relation between the percentage of fat absorption and increased body weight was observed explaining at least part of the heterogeneity in the experimental results. Further studies are required to elucidate the underlying molecular mechanism for the variation in lipid uptake. At UKE experiments were performed to study the effect of activation of brown adipose tissue (BAT) on whole body lipid metabolism. Animals were exposed for various time periods to cold (no cold; 4 hours, 1 day, 7 days cold) under different conditions of brown adipose tissue activation (described in D3.18). The kinetics of TRL plasma clearance were strongly dependent on the activation status of BAT, namely accelerated by cold, reaching a maximum after 1 day cold exposure. Consequently, 1 day cold exposure was used as intervention in further experiments. In addition, beta-adrenergic stimulation by CL316,243 (CL) for short-term activation of BAT was applied. Using MINGLeD in a snapshot simulation fashion, short term effects of cold activation of BAT on plasma triglycerides and cholesterol could be successfully modelled also providing predictions on the effects of organ lipids. a paradoxical increased hepatic VLDL production after BAT activation. This hypothesis was confirmed by metabolic studies in various mouse models by UKE. Further validation of predictions by the model will be carried out in P5.

UZH completed the investigation of the role of sphingolipids in development of MetS in male and female apoE3L.CETP mice. The heterogeneity in response on the HDF+C observed by UMCG was confirmed including a gender effect. However, besides elevated plasma and liver TGs, the mice did not show impaired glucose homeostasis which contrasts the observations by UMCG. Feeding the mice with a serine enriched HDF+C diet reduced 1-deoxysphingolipids but no significant increase was seen in the alanine enriched diet group. Interestingly, a significant lowering of plasma and liver TGs in animals fed with amino acid enriched HFD compared to the HFD only group was found indicating that supplementation of the diet with L-alanine or L-serine had a positive effect on steatosis and NAFLD in these mice independent of the effects on deoxysphingolipids. RYGB surgery on the APOE*3-Leiden.CETP mice has now been established by UZH and first cohorts show promising results with complete amelioration of MetS symptoms. Livers have been send to FORTH for microarray analysis and a full report on gene expression changes on both responders and non-responders will be reported in P5. FORTH performed a transcriptomic analysis in livers of apoE3L.CETP mice that were infected with AAVs expressing FOXO1 shRNAs or FOXO1 controls and identified pathways that are regulated by this factor.

The increasing flow of data generated by the beneficiaries during P4 requires adequate storage according to the FAIR principles. To accomplish this option GeneXplain has developed software consisting of a combining technologies developed earlier: SEEK database management system (<http://www.seek4science.org/>) developed by the FAIRDOM community efforts (fairdomhub.org) and geneXplain platform developed on the basis of open source community driven project BioUML (biouml.org). The executable modules encompass a scalable reactive programming container that is capable of providing a web server to present HTTP interfaces. Besides the programming interface, the server exposes an http/XML microservice as well as a first set of HTML pages. This construct forms the basis for the project-specific database as a software service. The integrated database in these two platforms consists of three main parts, 1) an annotation database, 2) an ontology database, 3) experimental data and SOPs. The first two parts are located in the geneXplain platform database module and the last part is located at the SEEK database module. the developed solution achieves the possibility to identify and easily pull together data samples across studies, which can be identified by common curation properties like studied cell type, as well as generation of custom data subsets for specific comparisons interest, possibly from large scale studies consisting of hundreds of samples. This possibility is achieved by applying so called « tags » to the data documents. « Tags » help to classify and respectively search and browse the samples according to various criteria. The microarray

data sets from the liver biopsies from the UZA cohort have been stored in the data base. Analysis of the microarray data has generated a number of novel leads. In P3 IPL reported that apoF negatively correlated with incidence of NAFLD/NASH. Subsequent investigation revealed a role of the protein phospholipid metabolism. A module describing phospholipid metabolism in detail was incorporated in MINGLeD. TU/e has used the transcriptomics data from the UZA cohort to develop hepatic metabolic network models for each subject. Two different Genome-Scale Metabolic Models (GSMM's) were used, the Recon2 hepatocyte model and ihepatocytes232. For each subject the liver transcriptomics data has been mapped to both network models using GPR (gene – protein – reaction) information. This resulted in two metabolic network models for each patient, one with the Recon2 hepatocyte model as template and one model with ihepatocytes2322 as template. For each patient both models were solved to obtain flux distributions consistent with the gene expression data. Solutions for both models of each patient differed, because the template models (Recon2 hepatocyte model and ihepatocytes2322) are different in their reconstruction and level of detail. Only reaction sets that were predicted by both models have been defined as 'enriched sets' for each patient and have been analysed further. The enriched sets were clustered using a histology based NAFLD score. Differences in fatty acid metabolism, carnitine shuttle and inflammatory metabolism are associated with different stages of NAFLD. Data analysis revealed that the capacity of the carnitine shuttle limited transport of fatty acids into the mitochondria, leading to an impaired carnitine shuttle and thus affected fatty acid oxidation. New microarray analysis of a second set of liver biopsies of UZA cohort to increase the number of analyzed samples and hence increase the statistical power of analysis by IPL revealed that the classical pathway of bile acid synthesis, detoxification and systemic efflux are modulated in the livers of NASH patients compared to "no NASH" patients. Since literature reports from mouse studies but also bariatric surgery patients suggest an important role of bile acids in glycemic and lipid control as well we decided to extend MINGLeD^{hk}, with a gut module. This module describes the dynamics of different BA species (primary and secondary, conjugated and unconjugated) as function of time and location in the intestine (duodenum, jejunum, ileum and colon). Since the mathematical framework of MINGLeD is based on ordinary differential equations (ODE's) also the gut module was modelled with ODE's. The model results for a standard meal are shown in figure 1. The BA response dynamics in different compartments are described and predicted, including liver, gallbladder (GB), ileum, proximal colon and plasma. Through the modelling we obtained a fascinating novel insight into BA kinetics. The initial rise (within 30-60 minutes) in plasma BA's after a meal is caused by propulsion of BA's residing in the gastrointestinal (GI) tract from prior meals, which precedes gallbladder emptying induced by the new meal. In normal, healthy subjects BA's released from the gallbladder are responsible for the peak in plasma BA's observed around 90 minutes. The shoulder in the response results from BA recycling through the enterohepatic circulation. Data analysis from patients in which the gallbladder has been removed confirmed these results. It should be noted that BA's in plasma, which can also be measured, are only a minor fraction of the total BA's in other compartments.

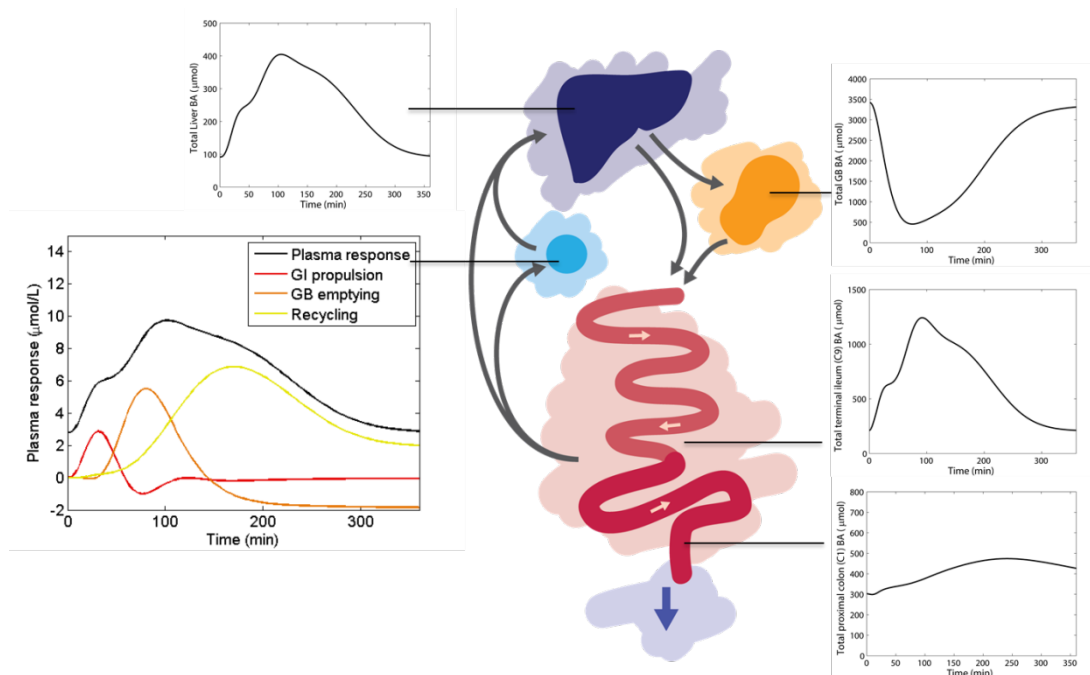


Figure 1: Simulation results of the postprandial response to a standard meal. The BA response dynamics in different compartments are described and predicted, including liver, gallbladder (GB), ileum, proximal colon and plasma. It is shown how the plasma response (that can also be measured) is decomposed into three subsequent postprandial contributions: GI (gastrointestinal) propulsion, GB emptying and recycling of BA's.

Analysis of BA data derived from the bariatric surgery intervention study in WP6 will be carried out in P5. To be able to adequately model the effects of bariatric surgery on glucose metabolism during a hyperinsulinemic euglycemic clamp (HIEC) short-term effects (2 weeks) of bariatric surgery on glucose and lipid metabolism were analysed (AMC; WP5). Glucose metabolism and total triglyceride hydrolysis in the basal state and during a HIEC using stable isotopes were studied in 18 (pre-menopausal) women before and after RYGB. Endogenous Glucose Production (EGP), peripheral glucose uptake (rate of disappearance [Rd]), hepatic and peripheral insulin sensitivity and triglyceride hydrolysis were calculated using the steady-state information of the data. A dynamic model composed of differential equations was developed to describe the (unpublished) raw data during the clamp experiments, including the isotopic tracers. The ADAPT method has been applied to compute metabolic dynamics in each of the 18 obese subjects before and after RYGB. The model captures the glucose dynamics accurately. It should be noted that the glucose levels are frequently sampled (many more time points available than for the other concentrations) and measured accurately (small error bars). The model captured the glucose dynamics accurately and hence is ready for application to the extended data sets being produced in WP6. In P4 UGOT has continued developing and validation of the human model describing postprandial lipid handling. The new version of the model is able to describe both VLDL- and chylomicron kinetics whereas the previous version only described chylomicron kinetics. Importantly, in addition, the effect of apoC-III on LPL activity is incorporated into the model. ApoC-III is considered to play an important role in the TG-HDL axis. To study the interaction between carbohydrate and lipid metabolism an intervention study with Fructose has been carried out. The clinical part of this intervention study (75 g fructose per day for 3 months) was finalized 2015 (Clinical Trials NCT01445739). The UH and UGOT team finalized quantitative analyses of liver, visceral and subcutaneous fat contents and multiple cardiometabolic risk factors before and after fructose feeding in the combined group of 71 subjects. The data are now available for computational analysis.

1.3. Project outcomes and expected results

1.3.1. Project Outcomes

Development of the computational models in WP2 has progressed at a rapid pace in P4 and the models are now in the stage of iterative improvement. To better delineate model and type of analysis the computational model is now called MINGLeD and at the moment two types of analysis are distinguished; *Snapshot simulations* and *ADAPT*. In snapshot simulations experimental data of studies with different length is used to calibrate the model. The different longitudinal time points in the studies are simulated separately, yielding a collection of 'snapshot' models ADAPT integrates the time-course dynamics and the model predicts concentrations and fluxes at unobserved time points and provides information about control points in the system. MINGLeD and both types of analysis have been tested and developed with data sets derived from mouse studies. Important novel insights on relations between glycemic control and lipid metabolism as well as factors underlying heterogeneity in experimental data have been derived. These concepts can now be tested in the data sets that are being finalized in WP6. During the development of RESOLVE an important role for bile acids in control of energy metabolism surfaced. An intervention study in the E3L.CETP mouse model revealed that indeed activation of the bile acid receptor FXR ameliorates the MetS symptoms. This important result will be validated during P5. To be able to describe bile acid homeostasis in humans in detail MINGLeD has been extended with a gut module which was calibrated with existing data and is now waiting for the human data sets of WP6. A short term dynamic model composed of differential equations has been developed to describe the (unpublished) raw data during the euglycemic hyperinsulinemic clamp experiments with bariatric surgery patients before and after surgery. Similarly a dynamic model has been developed that describes both chylomicron and VLDL metabolism in the (human) postprandial condition. Since ADAPT has been modified for application to such data sets all methodology is in place to analyse the data produced in the human intervention studies in WP6.

1.3.2. Expected results

RESOLVE outputs	Short-Term Innovations	Long-term Innovations
Identification and quantification of the metabolic and regulatory network responsible for MetS	➤ Identification of diagnostic & therapeutic targets for MetS ➤ Fundamental insight into the relation between HDL-C, triglyceride and glucose metabolism ➤ Unravel mechanisms by which the diet, physical activity, and therapeutic interventions and bariatric surgery are impacting on MetS ➤ Systems diagnostics: tailored set of DNA, mRNA, protein, metabolite and lifestyle markers which together characterize the patient-specific MetS profile ➤ Personalized treatment of MetS due to insight into different routes leading to MetS	➤ Early identification of symptoms of MetS as undesired side/effect of novel candidate drugs ➤ Reduction of Phase I failure rates due to the availability of reliable, appropriate and predictive models and sets of biomarkers
Interactive computational model which integrates quantitative knowledge on the MetS network	➤ First calibrated computational that integrates quantitative and kinetic MetS data (pre-clinical & clinical) and is unique in that a) both short-term effects of interventions as well as long term disease progression can be described, b) whole-body and tissue-specific data are integrated, and c) lipoprotein and carbohydrate metabolism are combined. ➤ Well-annotated, structured and accessible computational model serves as an important research tool for modellers and experimentalists for future studies expanding the network or further zooming into molecular details	➤ (World-wide) use of RESOLVE's computational model by pharmaceutical companies for accelerating drugs development against MetS and co-morbidities and for predicting risk of MetS as side effect of novel candidate drugs ➤ Integration of MetS network with other disease networks to target co-morbidities in the context of underlying MetS ➤ Significant reduction in the number of animals used in the drugs development process (pre-clinical phase) ➤ Significant reduction in the

RESOLVE outputs	Short-Term Innovations	Long-term Innovations
	➤ Human and mouse models as experimentally-rooted variants of the same underlying framework allows maximal use of mouse data for human analysis, while at the same time acknowledging which mouse-human differences have functional consequences. ➤ Predictive model monitoring tools of MetS	number of human samples used in the drugs development process (clinical phase)
Strictly annotated and standardized multi -omics datasets of whole-body and tissue-specific metabolic response to MetS inducing/inhibiting interventions	➤ Impact of short-term (patho)physiological stimuli on lipid- and carbohydrate-related components of MetS in a humanized mouse model unravelled ➤ Detailed comparison of differences and similarities in quantitative network dynamics between (humanized) mouse and humans	➤ Use of data for future studies into expanded network and co-morbidities
New knowledge on pathophysiological transitions during development of specific co-morbidities	➤ Fundamental insight into the mechanism underlying pathophysiological transition into: • insulin resistance • fatty liver, and • atherosclerosis (the latter humanized mouse, but the former two in mouse and man) ➤ Clarification of whether HDL and/or triglyceride-rich lipoproteins are suitable targets to prevent co-morbidities of MetS	➤ Novel biomarkers for improving prognostics of co-morbidities ➤ Novel targets to prevent co-morbidities
Establish correlation between metabolic parameters and transcriptomic data sets	➤ Understand the development of metabolic disorders in overweight patients	➤ Prevent metabolic disorders in overweight patients

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