MISSION-T2D

Multiscale Immune System SImulator for the Onset of Type 2 Diabetes integrating genetic, metabolic and nutritional data

Work Package 6

Deliverable 6.2

Report on the integration of the overall workflow

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Executive Summary	In this deliverable we describe the work done in task 6.2 (PM13-PM24). In the previous deliverable D6.1 we have provided a brief introduction about diabetes and inflammation and details on the identification and definition of the immunological knowledge necessary to model inflammation in diabetes-related tissues such as the adipose tissue. In the present deliverable D6.2 we describe the updates on the overall model of inflammation and the other modules (metabolism, gut, physical exercise) that have been integrated to the simulation architecture.
Keywords	Diabetes, inflammation, immune cells, agent-based immune system simulator, model integration

Contents

1 Deliverable Description

This deliverable describes the work concerning the integration of the different sub-models of gut microbiota, food absorption, insulin/glucagon controller and physical activity together with the Agent-based model (ABM, or M1) of the immune system and inflammation, i.e., the integrated model backbone, into a unified simulation workflow. As the interaction includes all sub-models (M2-M5), all partners participate to this task.

The integration has been performed adopting the following two general strategies:

1) ABM model inputs are provided by using the preceding sub-model outputs in a sequential manner (pipeline);
2) 2) the sub-models have been entirely embedded into the overall ABM simulator and executed at run-time (live) at each (macro-) time step of the ABM.

The following consideration applies: whereas the former is generally preferable to speed-up execution, it is not always the a viable option since the model output sometimes cannot be computed in advance without compromising the overall model accuracy or, in general terms, without changing the “semantic” of the algorithm. The case-by-case description below will clarify this point.

2 Background

2.1 Background on the previous work as described in D6.1

In the previous deliverable D6.1 we have described the work done in identifying and defining the immunological knowledge needed to customize the general-purpose immune system simulator that we have decided to employ for the purpose of the project MISSION-T2D so to have an accurate representation of the phenomena underpinning the innate immune activation (e.g., damage signals from hypertrophic adipocytes and from a high-glucose environment) and onset of the inflammatory process (e.g., macrophage chemotaxis and migration to adipose tissues). We have described the ABM model itself and we mentioned the specific/pivotal role of T-helper lymphocytes and macrophages. To this purpose we have described the modifications

to the original model to account for a much more detailed description of the known subclasses of T-helper (Th) cells and of the macrophage (MA) as well as of all cytokines and interleukines involved in the differentiation process of these immune cells.

In the present deliverables we will briefly describe the other sub-models (extensively discussed in the relative deliverables from our MISSION-T2D partners), their mutual relationship and the strategy for combining them altogether to make up the unified model.

Before mentioning the other model we will describe the updates to the ABM simulation of inflammation implemented to detail the differentiation of T-helper lymphocytes (implemented and operative) and macrophages (in progress).

The list of abbreviations of cells and signalling molecules can be found in the appendix.

3 Description of all modelling components

3.1 General description of integration levels

Task 6.2 has been devoted to the definition of the prerequisites for the integrated model (CNR, UniBO, UniCAM, UniRM, TNO, USFD) has been defined in Task 6.2. WP6 has collaborated with the other WPs in order to define the interfaces between the ABM and the other sub-models.

A data exchange format for linking the various models developed in WP2-WP5 to the integrated modeling platform has been identified and used. The outputs/inputs from/to each level have been identified and reported in D6.1, and summarized here in Table 1. Figure 1 describes the general architectural interdependencies and relationships, and the main input/output fluxes among the M1-M5 models.

The actual strategy for model integration has been also taken into account in this task.

This present Task 6.3 has been carried out in cooperation with other WPs to define the interfaces among the agent-based (M1, WP6) and the other sub-models (M2 to M5), and to outline the actual strategy for whole-model integration. A preliminary data exchange strategy for linking the various models (WP2 to WP5) to the integrated modelling platform (WP6) has been identified and described as follows.

Model	Input / Output (→ M1 input)
M2 (gut)	Nutritional pattern Enterotype SCFAs IFNγ/STAT1 signaling, cytokine production
M3 (inflammatory pathways)	Cytokines Cytokines
M4 (metabolic)	Energy intake and expenditure Major metabolites
M5 (PA)	Physical Activity Energy expenditure Specific metabolites

Table 1: General scheme of major input (in red)/output (in green) parameters linking the various models. Model M1 will input/output from/to all other models.

The general integration scheme relies on the exchange of the identified, critical parameters from one model with the other, such as the outputs of a model are used as inputs for the following one in a hierarchical way of operation (this has been called "summarize and jump").

- M1: Partner CNR (WP6) has implemented and integrated in the ABM a gene regulatory network level of description and simulation. Such level allows to give account for the complex differentiation processes of a) the CD4+ T helper (Th) lymphocytes into the subtypes Th1, Th2, Th17, Treg, and b) the macrophages (MA) into the subtypes MA1 and MA2. Such cell differentiation processes shape the form and the range of the immune response to different antigenic challenges, and are considered a critical feature of the immune responses. The model integration performed in this task allows bridging a gap between gene level information and cell level population, and give accounts on how the model M1 is able to describe a coherent immunological behaviour when challenged with different stimuli.
- M2: Partner UNIBO (WP2) has developed a simplified gut dynamics model (M2) able to relate patient's nutritional patterns (mainly described by quantity of ingested lipids from food) and patient's enterotype as inputs to yield levels of short chain

fatty acids (SCFAs) such as butyrate and pyruvate, major metabolites in colonic lumen that regulate (colonic) inflammation via the inhibition of the IFNγ/STAT1 signalling pathways. Specific mechanistic associations relate the presence (and quantity) of such SCFAs outputting from M2 with the production of given cytokines (such as TNF-α, IL-2, IL-6 and IL-10) by lymphocytes and other cell types in model M1.

- M3: Partner UniCAM (WP3) developed a model for mTOR signaling in immune cells, pivotal in eliciting an inflammatory process, that has strong influence on the efficiency of pancreatic beta-cells for the production of insulin (firstly introduced in D3.1 and then further developed deliverables D3.5). This model (M3) has been embedded in the agent-based model M1 to drive the inflammation process from metabolic deregulation. Relevant parameters such as cytokines (e.g., TNF-α) and, possibly, genes involved in the major intra- and intercellular processes described in model M1 have been identified and accounted for the link between the two models. Binary states ("on/off") or continuous quantitative parameters, when available and relevant, related to the involved players will be taken into account.
- M4: Partner TNO (WP4) adapted, extended and further developed a model (M4) for metabolism (described in deliverable D4.1). Dynamic mass balances and major cellular metabolic reactions describe seven tissue compartments. A number of parameters (identified in Table 1) are used as inputs for the agent-based model M1 to get an integrated description of inflammation and metabolism. The metabolic model M4 has been also linked to the model M5 developed by partner USFD/UniRM (WP5) regarding the influence of physical activity on specific metabolites. Feedback loops M1→M4 are taken into account.
- M5: Partners USFD and UniRM (WP5) have identified a model (M5) to introduce the physical activity (PA) in the project's global architecture. Preliminary work in this respect has been described in D5.1. This model impinges upon few variables of the metabolic model (M4). In particular the parameter "work rate" which stands for a measure of the intensity of the physical activity, influences the dynamics of the metabolic model M4. This parameter in M5 is related to the heart rate, either measured by means of suitable equipment, or calculated on the basis of patters of physical activity as declared by the user. Moreover, M5 also add a relationship between PA and the level of the inflammatory cytokine IL-6. This realizes another (important) link between M1 and M5.

The integrated prototype of the models (M1+M2+M3+M4+M5) has been implemented in WP6 by partner CNR, and it results in a model that describes the inflammation in obese individuals (but also in healthy individuals) developing over time as a function of nutritional habits, lifestyle habits, and other personal parameters like age, gender, body mass index, etc.

In what follows we describe in more detail the integration issues of the various sub-models into the unified MISSION-T2D architecture.

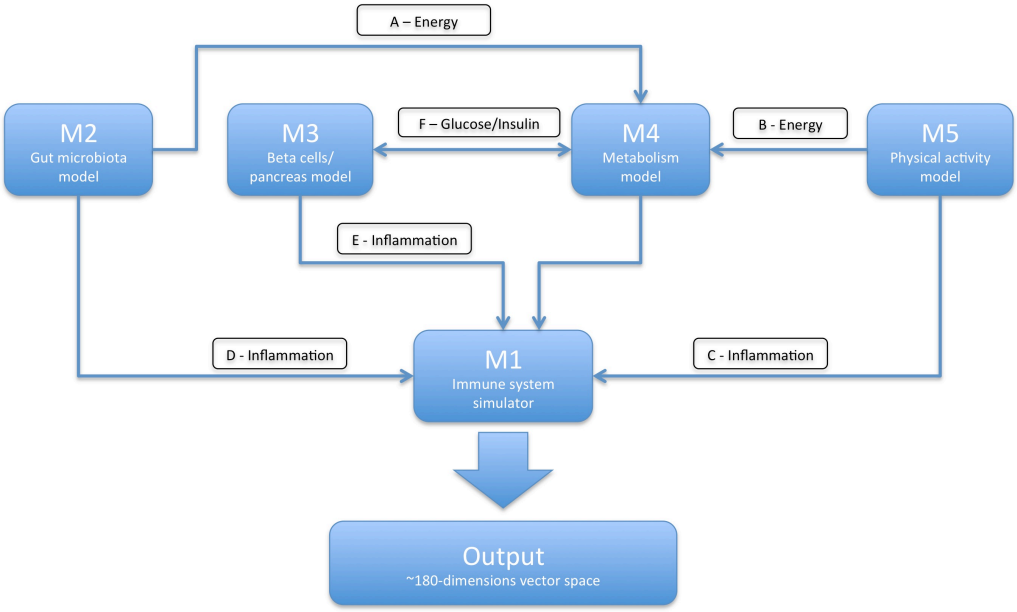

Fig 1: schematic representation of the models' interdependences and relations. M1 serves as the "hub" of the other models in that it uses all inputs and produces the output. Sub-models feed M1 either pre-computing variables/parameters values or running in parallel with it and passing the values computed at predefined time steps (as in the summarize-and-jump strategy).

3.2 Integration gene regulation-inflammation

Here we describe the integration of the Th1/Th2/Th17/Treg lymphocytes differentiation model (already implemented) and the macrophage MA1/2 switch (work in progress).

3.2.1 Th differentiation

This integrative step expands the previous version of the ABM to include not only Th1 and Th2 but also Th17 and Treg fates in the differentiation model. The model has been described in detail in Tieri et al., and such enhancement has been carried out on the ABM as described in D6.1 “Customization of immune simulator”.

Figure 2 schematizes the gene regulatory network originally from Martinez-Sosa et al. The network has been organised according to the different functional compartments of the cell. The differentiation fate of a simulated CD4+ T cell depends on the input stimuli sensed by its membrane receptors, in particular, by the TCR (T-cell receptor, able to bind antigens presented by MHC-II complex on APCs) and by various cytokine receptors (i.e., receptors for IL-6, IL-23, IL-10, TGF-b, IL-2, IL-12, IL-18, IL-4, IFN-β and IFN-γ). Upon the activation of these network nodes, the activation level of key transcription factors and genes ultimately leading to the production of Th cell subset hallmark cytokines (i.e., IL-10, IL-17, IL-6, IFN-g and IL-4) is computed. Every node can assume a binary deactivated (0) or activated (1) state, and is linked to a set of experimentally associated nodes, which can contribute to activate (continuous black line) or to inhibit (dotted red line), as reported in fig. 1. The global network state is synchronously updated according to a common rule used for updating single nodes. A node is activated (i.e., turning its state to 1 at time step t + 1) if and only if at time t there is at least one node in the set of its activators are turned on (state 1) and all nodes in its inhibitory set are turned off (state 0); otherwise, its state is set to 0 (deactivated). This is a typically used simplification of a more complex realistic situation but represent the only viable modelling choice in absence of knowledge about the activation of each single gene. The Boolean updating function is formally defined as follows:

$$x_{i,t+1} = \left(\vee_{j \in A_i} x_{j,t} \right) \wedge \left(\neg \wedge_{j \in I_i} x_{j,t} \right)$$

where we use the notation $x_{i,t}$ to define the state of node i at time t, Ai is the set of nodes activating node i, and Ii is the set of nodes inhibiting node i. For simplification, all cells release the same amount (indicated by ω) of cytokines. This implies that all cytokines have the same efficacy in exercising their action. At each macro time step every Th0 cell ‘senses’ the concentration of the input cytokines in the same lattice point and sets the activation level of the corresponding input nodes. The activation is modelled as a stochastic event with probability p given by a sigmoid function depending on the concentration c of the input cytokine:

$$p = c2/(\omega 2 + c2).$$

As shown in the Fig. 2 the network is made of 40 nodes and 67 edges. In order to distin- guish the input nodes from the internal ones (that have a feedback effect) we have added four nodes from the original network, namely, 'IFN-γ input', 'IL-4 input', 'IL-6 input' and 'IL-10 input'. We then performed the dynamical simulation according to the rule reported above, until a fixed point is reached (this requires not more that 20 iterations). Four different fixed points are reported in Martinez-Sosa et al., identifying the four Th subtypes: Th1, Th2 Th17 and Treg (Th0 nodes remain Th0 if no input is activated). These are characterized by the activation of a set of signature genes as follows:

1. Th1: IFN-γ, IFN- γR, SOCS1, TBET
2. Th2: GATA3, IL-10, IL-10R, IL-4, IL-4R, STAT3, STAT6
3. Th17: IL-17, IL-6, IL-6R, JAK3, ROR−γt, STAT3
4. Treg: FOXP3

According to the final network configuration, and after the discrete dynamics is applied, the transition to the new Th phenotype is operated. The phenotype differences are mirrored by a different pattern of secreted cytokines, which greatly influences the overall immune response dynamics.

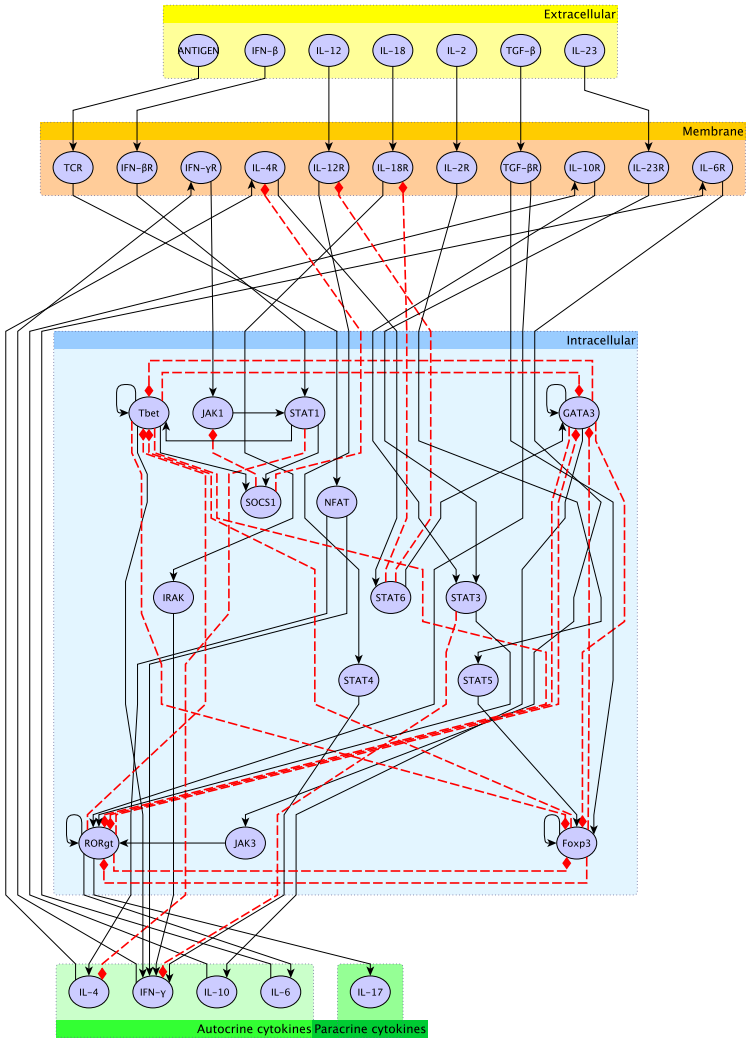

Fig. 2: Scheme of the gene regulatory network of Th differentiation adapted from Martinez-Sosa et al. Black lines: activation; red lines: repression/deactivation. Depending on the input stimuli that engage cell surface receptors (namely, antigen presenting MHC-peptide complex on TCR, IL-6, IL-23, IL-10, TGF-b, IL-2, IL-12, IL-18, IL-4, IFN-b and IFN-g), the network computes the activation level of key transcription factors for each cell fate (Th1, Th2, Th17 and Treg) and the expression level of genes resulting in the production of signature cytokines such as IL-10, IL-17, IL-6, IFN-g and IL-4.

3.2.2 Differentiation of the Macrophage into type 1 and 2

We here describe a perspective work not previously planned in the DoW that takes into account the macrophage MA1/MA2 differentiation, a critical immune regulation process.

In line with the Th differentiation, a phenotypic switch of macrophages (MA) towards a

pro-inflammatory (MA1) as opposed to anti-inflammatory (MA2) profile is observed in the dynamics of the MA population (Martinez et al.).

In a similar manner to the Th differentiation, we are therefore detailing the agent-based rule for macrophage differentiation by means of a Boolean gene-regulatory network (see Fig. 3). Also in this case the differentiation fate of the cell is determined by the cytokine background, specifically IFN-gamma and IL-4 besides the presence on the antigen's surface, of lipopolysaccharide molecules. This is a work in progress and constitutes the aim of an on-going collaboration with the American University of Sharjah (UAE) and Virginia Bioinformatics Institute (USA).

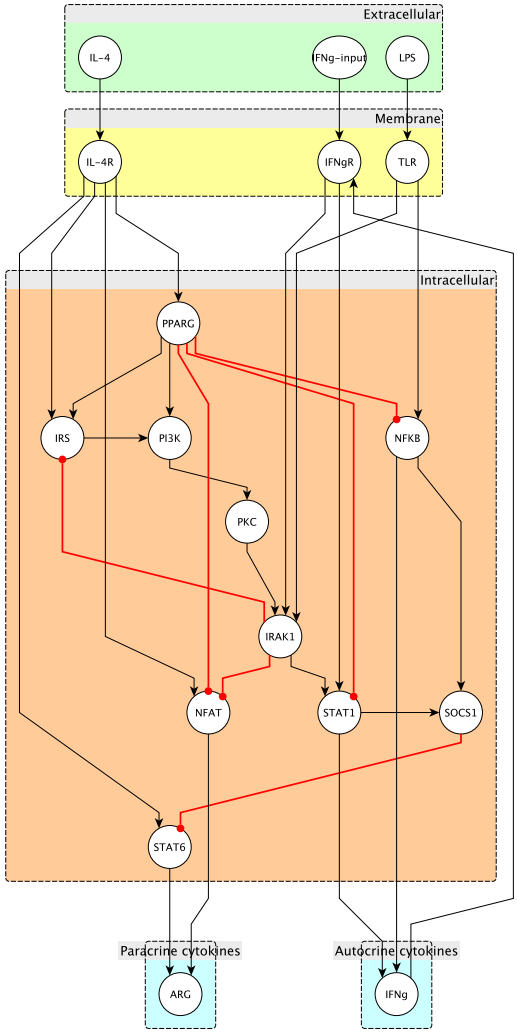

Fig. 3: Scheme of the gene regulatory network of macrophage MA1/MA2 differentiation. Four cellular compartments are taken into account. Black lines: activation; red lines: repression/deactivation.

3.3 Integration gut-inflammation M1-M2

Basing mainly on the work of Wu et al. and the review of Scott et al., Partner UniBO has gathered a list of important gut microbiota (GM) bacteria and assessed how different diet component affect their abundance in the gut has been defined in WP2, as reported in D2.4.

Starting from the gained knowledge, a table summarizing bacterial species, nutritional patterns and pro- or anti-inflammatory trend has been produced in order to implement the gut-inflammation integrated modelling layer. In table X, symbol "+" means a positive correlation (presence of the bacterial strain) between the bacteria and the corresponding nutritional pattern, while "-" stands for negative correlation. If no correlation has been observed in these works, no symbol is included. The main products of the considered bacteria have been report in the following tables, with particular attention to the short-chain fatty acids butyrate and propionate, equipotent and directly linked to inflammation modulation (Tedelind et al). Such table shows a diet or nutritional, through its effects on GM, affects both the host metabolism and the immune system, contributing to the metabolic flexibility switch impairment that is observed in Insulin Resistant and Type 2 Diabetes subjects. In particular, in the presence of butyrate, TNF, IL-6 and IL-1β levels decrease dose-dependently. With 10 mM butyrate, TNF, IL-6 and IL-1β levels reach control values, while 2 mM butyrate strongly inhibits LPS induced TNF production by PBMC in healthy and non-healthy (Chron's disease) subjects (Segain et al.).

In conclusion the knowledge distilled in this table allows inputting TNF, IL-6 and IL-1β parameters in M1 on the basis of individual's enterotype and nutritional pattern, carrying out the integration level M1-M2.

Anti- (green) or pro- (red) inflammatory action of bacteria specie in presence of the corresponding nutritional pattern

Species of bacteria	PUFA	SATURATED FATTY ACIDS	CLA FATTY ACIDS	TRANS FATTY ACIDS	VEGETABLE PROTEINS	ANIMAL PROTEIN	TOTAL PROTEINS	PREBIOTICS	SUGARS	TOTAL CARBOHYDRATES	FIBER	PRODUCTS	Butyrate production	Propionate production	OTHER INFO
Bacteria.**Actinobacteria**											-				
Bacteria.Actinobacteria.Actinobacteria.Actinomycetales.Actinomyces				+							-				pathogenic bacteria
Bacteria.Actinobacteria.Actinobacteria.Actinobacteridae.Bifidobacteriales.Bifidobacteriaceae.Bifidobacterium					+	-		+				acetate, butyrate, ammonia, molecular hydrogen	Yes		control intestinal pH (decrease that amount of ammonia in the colon), regulate the intestinal microbial homeostasis, inhibit pathogens and harmful bacteria that colonize and/or infect the gut mucosa, modulate the local and systemic immune responses,
Bacteria.Actinobacteria.Actinobacteria.Actinobacteridae.Bifidobacteriales.Bifidobacteriaceae.Bifidobacterium.Bifidobacterium adolescentis						-						SCFAs (propionate, butyrate, and acetate), lactate, vitamins, cyanocobalamin, nicotine, thiamin, folic acid, pyridoxine	Yes	Yes	control intestinal pH (decrease that amount of ammonia in the colon), regulate the intestinal microbial homeostasis, inhibit pathogens and harmful bacteria that colonize and/or infect the gut mucosa, modulate the local and systemic immune responses,
Bacteria.Actinobacteria.Actinobacteria.Coriobacteriales							+								
Bacteria.Actinobacteria.Actinobacteria.Coriobacteriales.Coriobacteriaceae							+								enriched in obese
Bacteria.**Bacteroidetes**		+		+											gram negative, decrease in obesity
Bacteria.Bacteroidetes.Bacteroidia		+	+	+											
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales		+	+	+											
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Bacteroidaceae	-	+	+	+		+	+		-	-					
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Bacteroidaceae.Bacteroides	-	+	+	+		+	+	-	-	-		acetic acid, iso valeric acid, and succinic acid			Gram-negative, pathogenic bacteira
Bacteria.Bacteroidetes.Bacteroidetes.Bacteroidales.Bacteroidaceae.Bacteroides thetaiotamicron											+	acetic acid, iso valeric acid, and succinic acid			Gram-negative, pathogenic bacteira
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Prevotellaceae	+	-				-	-				-	SCFAs (Acetate, propionate), succinate		Yes	
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Prevotellaceae.Paraprevotella		-					-		+			succinate, acetate			
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Prevotellaceae.Prevotella	+						-	-				SCFAs			
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Porphyromonadaceae		+	+	+	-				-		-				
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Porphyromonadaceae.Barnesiella									-			succinate, acetate			
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Porphyromonadaceae.Butyricimonas												butyrate, isobutyrate, succinate, acetate, propionate	Yes	Yes	
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Porphyromonadaceae.Odoribacter		+					+				-	Succinate, acetate, iso-valeric acid			
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Porphyromonadaceae.Parabacteroides		+	+		-					-	-	acetic acid,succinic acids,isovaleric acid, propionic acid, isobutyrate,formic acid, lactic acid, catalase, Mannose, raffinose, L-Rhamnose, Trehalose	Yes		
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Porphyromonadaceae.Parabacteroides diastonis												catalase			
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Rikenellaceae											-				
Bacteria.Bacteroidetes.Bacteroidia.Bacteroidales.Rikenellaceae.Alistipes											-	succinate, acetate, mannose, raffinose, propionic acid			
Bacteria.**Firmicutes**			-	-											increase with obesity
Bacteria.Firmicutes.Bacilli									+	+					
Bacteria.Firmicutes.Bacilli.Lactobacillales									+	+					
Bacteria.Firmicutes.Bacilli.Lactobacillales.Lactobacillaceae															
Bacteria.Firmicutes.Bacilli.Lactobacillales.Lactobacillaceae.Lactobacillus					+	-		+				lactate			help maintain the pH level
Bacteria.Firmicutes.Bacilli.Lactobacillales.Lactobacillaceae.Lactobacillus.Lactobacillus ruminis											+	lactate			help maintain the pH level
Bacteria.Firmicutes.Bacilli.Lactobacillales.Streptococcaceae									+	+					
Bacteria.Firmicutes.Bacilli.Lactobacillales.Streptococcaceae.Streptococcus									+	+		lactate			pathogenic bacteria
Bacteria.Firmicutes.Clostridia			-	-		+									
Bacteria.Firmicutes.Clostridia.Clostridiales			-	-											
Bacteria.Firmicutes.Clostridia.Clostridiales.Acidaminococcaceae.Phascolarctobacterium				-							+	propionate		Yes	gram negative, succinate consumer
Bacteria.Firmicutes.Clostridia.Clostridiales.Clostridiaceae.Anaerotruncus					-				+						
Bacteria.Firmicutes.Clostridia.Clostridiales.Clostridiaceae.Anaerotruncus.Faecalibacterium prausnitzii								+			+	formate, D-lactate, butyrate			
Bacteria.Firmicutes.Clostridia.Clostridiales.Clostridiaceae.Clostridium												butyrate	Yes		
Bacteria.Firmicutes.Clostridia.Clostridiales.Clostridiaceae.Clostridium.Clostridium histolyticum								-				organic solvents such as butanol			

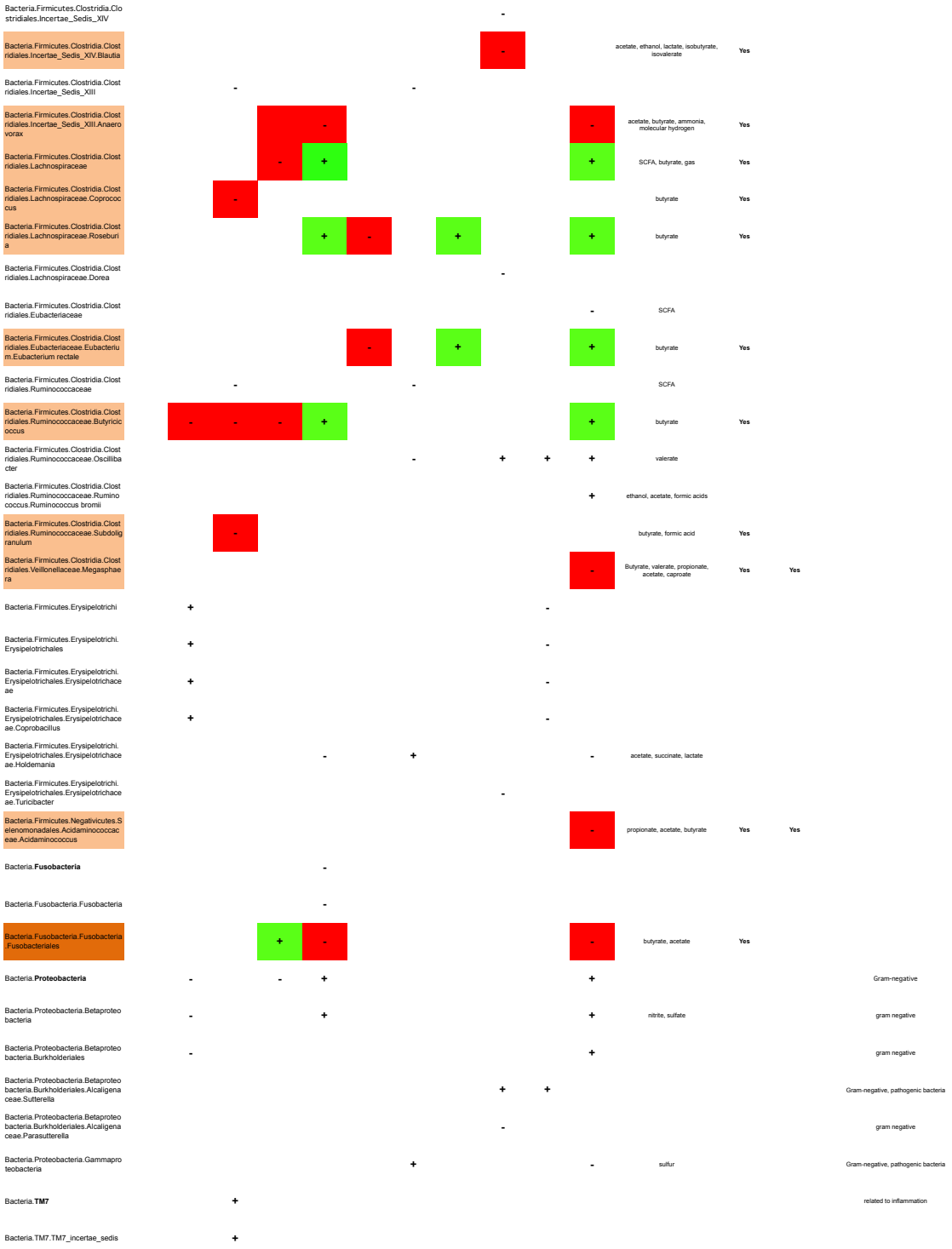

Table 2: lookup table integrating enterotype, nutritional pattern and pro- or anti-inflammatory settings in terms of production of SCFAs and related inflammatory modulation activity (TNF, IL- and IL-1b production).

3.4 Integration beta cells-metabolism-inflammation M1-M3-M4

The combined model implemented in WP3 and described in D3.1 and further in D3.5 is used to take into account functional and dysfunctional beta-cells rates of change and related insulin production; such rates are influenced by glucose dynamics, activated macrophages and T cells (a schematic representation is provided in Fig. 4). When some of the parameters considered in M3 are already implemented by M1 in native mode, then the parameters from M1 are considered, and those from M3 disregarded (namely macrophages and T cells). The main terms are Glucose concentration (G), Insulin concentration (I), Functioning beta-cells (betaf), Dysfunctional beta-cells (betanf), number of macrophages (per unity of volume, M), number of Activated Macrophages (AM), amount of beta-cells antigenic proteins (A), T cells (T), Insulin resistance (IR), expression level for mTOR (MTOR).

The model M3 provides quantitative parameterization of the T2D critical compartment of insulin-producing beta-cells and its interrelationships with inflammation and metabolism.

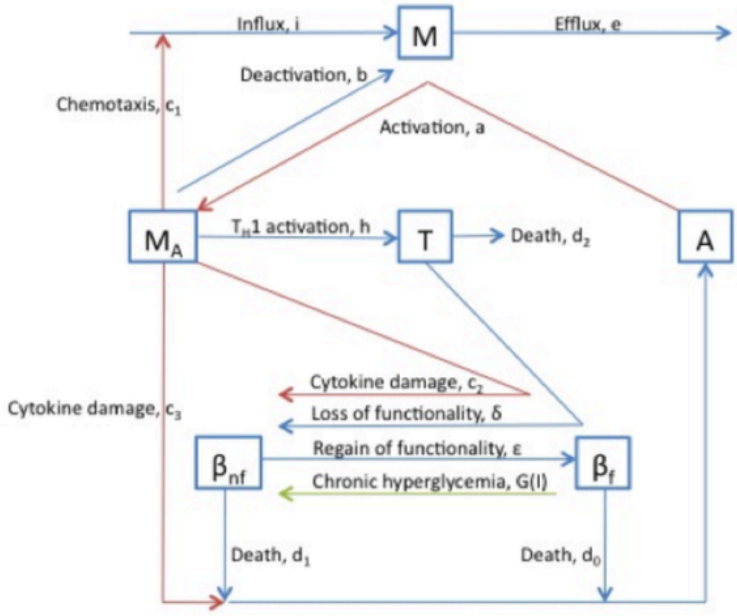

Figure 4. A schematic representation of the M3 model where the red lines represent one species causing a rate of change in another (i.e., an indirect effect, such as via the action of cytokines), the blue lines represent a rate of change of one species causing a rate of change in another (i.e. a direct effect) and the green line represents the influence of blood glucose and insulin dynamics.

3.5 Integration global metabolism-inflammation M1-M3-M4-M5

As foreseen, the integrated multiscale model will be obtained by developing and integrating models at two different aggregation levels and time scales. The short time scale, low aggregation level model (Dynamic E-MF model, see D4.3) corresponds to the challenge test time scale, i.e., minutes-to-days, and contains detail on metabolic pathways, inflammatory processes and their interactions. The long time scale, high aggregation level (MF-HOMA) model covers the week-to-months timescale and contains little mechanistic details.

The integrated model constructed in WP6 by partner CNR integrates the immune system simulator from CNR, the metabolic model contributed by TNO (see D4.1), and the models of microbiota function (WP2), beta cell function (WP3) and physical activity (WP5), contributed by the other partners.

In practice the simulator will cover a time frame of hours to years[1]. This raised the question of how to integrate the MF-HOMA model that originated at TNO and that was built inside the Marvel software tool (hereinafter Marvel, see Deliverable 4.2). Marvel is best suited to create a forecast for a much longer time frame, i.e., months to as much as 6 years. It was jointly decided to keep both models, Dyna and use the MF-HOMA model exclusively for the longer term forecast simulations.

To connect the models at the different time scales with each other, the simulator integrating the mechanisms of inflammation, metabolism, as well as the other compartments can be used to run simulations for different diabetes subgroups to arrive at personalised parameter values (strengths and speed) for the interactions between high-level variables in the Marvel model, since these are expected to be different for different subgroups.

3.5.1 The original metabolic model from Kim et al.

The original formulation of the compartmental model from Kim et al. predicts fuel homeostasis during exercise by using the hormonal control to regulate cellular metabolic processes. The whole-body model is composed of seven tissue compartments: brain, heart, liver, gastrointestinal tract, skeletal muscle, adipose tissue and “other tissues”. Each tissue compartment is then described by dynamic mass

1 In practice we are limiting the time-span of the simulations to one year for computational reasons: one year = 6 days of CPU time on a fast-processor computer. We are currently working to considerably reducing the computational requirements.

balances and major cellular metabolic reactions, which lead to substrates (glucose, lactate, pyruvate, alanine, free fatty acids and glycerol) “conversion” in ATP.

List of metabolites

1. GLC: glucose
2. PYR: pyruvate
3. LAC: lactate
4. ALA: alanine
5. GLR: glycerol
6. FFA: free fatty acids
7. TG: triglycerides
8. O2: oxygen
9. CO2: carbon dioxide
10. G6P: glucose 6-phosphate
11. GLY: glycogen
12. GAP: glyceraldehyde-3-phosphate
13. GRP: glycerol-3-phosphate
14. AcoA: acetyl coenzyme A
15. CoA: coenzyme A
16. NAD+: aldehyde dehydrogenase
17. NADH: nicotinamide adenine dinucleotide
18. ATP: adenosine triphosphate
19. ADP: adenosine diphosphate
20. PI: phosphate
21. PCR: phosphocreatine
22. CR: creatine

List of reactions

1. Glycolysis I
2. Glycolysis II
3. Glycolysis III
4. Gluconeogenesis I
5. Gluconeogenesis II
6. Gluconeogenesis III
7. Glycogenesis
8. Glycogenolysis
9. Pyruvate Reduction
10. Lactate Oxidation
11. Glycerol Phosphorylation
12. GAP Reduction
13. Glycerol 3-P Oxidation
14. Alanine Formation
15. Alanine Utilization
16. Pyruvate Oxidation
17. Fatty Acid Oxidation
18. Fatty Acid Synthesis
19. Lipolysis
20. Triglyceride Synthesis
21. TCA Cycle
22. Oxidative Phosphorylation
23. Phosphocreatine Breakdown
24. Phosphocreatine Synthesis
25. ATP Hydrolysis

Fig 4: List of metabolites and reactions taken into account in the metabolism model from Kim et al., and implemented in the M4 submodel.

The basic hypothesis behind the model is that exercise-induced change in the hormone epinephrine, affects the pancreatic secretion of glucagon and insulin. As a consequence, any change in the glucagon-to-insulin ratio can modulate in a coordinated way the metabolic flux rates of different tissues in order to prevent hypoglycaemia.

The blood epinephrine level concentration $C_E(t)$ varies in time according to the following equation 1:

$$C_E(t) = C_E(0) + \omega(WR) \cdot (1.0 - \exp\frac{1}{\tau_E})$$

$C_E(t)$ changes with a step increase in the work rate *WR,* which represents the modelled exercise intensity.

The input of the Kim model is work rate: moderate (125 W power output at 60% of peak oxygen consumption [VO2max]) exercise for 60 min was implemented.

Arterial epinephrine level was given as an input function, which directly affects heart and skeletal muscle metabolism and indirectly other tissues via a integral rein glucagon–insulin controller inspired by Saunders et al..

3.5.2 Summary of the original Kim model assumptions

- 7 compartments, 22 metabolites involved in 25 reactions to model the fuel homeostasis of the whole body.
- Exercise (60 min at 60% of peak oxygen consumption VO2max) is modeled as a step change in WR (125 W power).
- The blood epinephrine level concentration CE(t) varies in time according to the step change increase in WR previously described.
- The arterial glucose homeostasis is manteined at 5 mmol/l by glucagon-insulin controller inspired by Sauder's controller [2].

3.5.3 Modifications to the Kim model for MISSION-T2D purposes

Since the epinephrine secretion is a function of the exercise intensity expressed as WR, the parameters $\omega(WR)$ and τ_E in eq. (1) governing the epinephrine concentration must be adjusted in order to give a realistic description of the epinephrine dynamics. However, the value assigned by Kim for these parameters is fixed and the authors provide no mathematical relationship dependent on WR value.

Thus, in order to overcome this problem, we substituted eq. (1) with another set of equations, in which the dependence on WR is explicit. To this aim, a validated model of **epinephrine** secretion and elimination basing its input on the physical exercise has been considered (Kildegaard et al.) and adapted to our purposes.

The input of the Kim model is the WR, which is not an obvious quantity to measure or to estimate in order to describe the energy expenditure from the daily physical activity. For this reason we adopted as input the percentage of **VO2max** using the mathematical relationship linking the percentage of VO2max to WR reported by Cabrera et al.

The Kim model describes the fuel homeostasis during physical activity, but doesn't take into account the glucose intake by **meal**. To model this aspect we introduced the Dalla

Man formulation of glucose rate of appearance (Dalla Man et al.) based on gastric empting description reported by Elashoff et al. The same formulation was adopted to represent the rate of appearance of alanine and triglycerides. Such modifications were included in the gastrointestinal compartment.

The original Kim model does not include any description of the modification that exercise induces on inflammatory processes, which is one of the MISSION-T2D aims. Thus, a novel set of equation has to be proposed to link the two aspects.

As widely recognized, physical exercise can contrast and delay the evolution of T2D in light of the fact that contraction of skeletal muscles during exercise activates a series of modifications of the inflammatory pathway (Petersen AM et al), which eventually induce a reduction of the insulin resistance (Hotamisligil et al.). **IL-6** (interleukin-6) has been identified as the first cytokine increasing in the circulation during exercise and stimulating the activation of an anti-inflammatory cascade given by the secretion of cytokine inhibitors (sTNF-R and IL-1ra) and of the cytokine IL-10 (Febbraio et al., Pedersen BK et al.). As a consequence, a new model of IL-6 beneficial effects on inflammatory process has been developed (Morettini et al. (a) and Morettini et al. (b)).

Summary of modifications to the original Kim model:

- Model of **epinephrine** secretion and elimination basing its input on the physical exercise.
- Introduction of the percentage of **VO2max** as input for describing the intensity of the physical activity (replacing the fixed value of 125 W).
- Implementation of a model of oral absorption of **glucose**, **alanine** and **triglycerides**.
- Implementation of physical exercise effects on **IL-6** modifications stimulating the activation of anti-inflammatory pathways.

3.5.4 Further developments to be implemented

T2D is associated with a diminished glucose transporter expression. In addition, chronic hyperglycaemia often leads to insulin resistance in the peripheral tissues and has also been implicated in the reduction of beta cell replication rates. Transition from insulin resistance to diabetes is subsequently caused partial loss of beta-cell function. It is well known too that the development of insulin resistance can be strongly influenced

by the immune system. New model of diabetes starts from integrating different existing models combining **beta-cells**, **macrophages**, and **Th lymphocytes** mass dynamics with glucose and insulin dynamics.

The implementation of **alanine** and **triglycerides** dynamic modifications during meal needs the setting of proper parameters, still not known in literature.

In order to use the **heart rate** (HR) as input for MISSION-T2D model, we need to implement a relationship between HR and percentage of VO2max. It is wide recongnized that oxygen compsuntion and HR during physical exercise are linearly related.

4 Model integration, prototype and end-user interface (mobile app)

4.1 Difficulties/challenges encountered in the integration

The model of Kim et al. has been coded in ANSI/C language for portability and for easiness of integration in the main simulation code. During the porting a number of issues appeared as to the use of ODEPACK (including the LSODA routine) numerical integrator that has been originally developed in FORTRAN (by the Lawrence Livermore National Laboratory, USA). Of the few C-porting of this library we have chosen the SUNDIALS (SUite of Nonlinear and DIfferential/ALgebraic equation Solvers, developed by the same lab) consisting of the following five solvers (https://computation.llnl.gov/casc/sundials/main.html):

- CVODE: solves initial value problems for ordinary differential equation (ODE) systems;
- CVODES: solves ODE systems and includes sensitivity analysis capabilities (forward and adjoint);
- IDA: solves initial value problems for differential-algebraic equation (DAE) systems;
- IDAS: solves DAE systems and includes sensitivity analysis capabilities (forward and adjoint);
- KINSOL: solves nonlinear algebraic systems.

The solver fitting our purpose is the CVODES. Unfortunately it has shown a performance that is one order of magnitude slower compared to the original Fortran

solver. We have spent some time trying to understand the reason for this drawback at times considering using the original Fortran library in our code eventually sticking to the C-version for portability and the usability reasons. At the time of writing of this document we are considering to use another implementation of the same CVODE library that runs on GPU to greatly speedup the computation. However due to more pressing tasks, this job has been downgraded in the priority list.

The computational time required to run a single simulation corresponding to a query for forecast issued by the mobile app user is “to date” very demanding notwithstanding our effort to speed-up computation. Even if the six-days time required to run a real-life 1 year time span will be reduced by an order of magnitude, it is unrealistic to hope for a real-time execution of the model on a limited capacity hardware as a mobile device. This problem has already been foreseen at the beginning of the project and a solution to this problem has been long identified (pre-computing a look-up table) as discussed in deliverable D8.4.

4.2 Integration with the end-user interface

The mobile application is specified to be the tool to be provided to end users to self-monitor health and lifestyle aspects, and to be employed to receive a dynamic estimate on the risk of developing Type 2 Diabetes based on such aspects (Fig 5).
As specified in deliverable D8.4, the solution to bypass the computational capacity deficit in current day smartphones and tablets and to workaround the extensive simulation time even on much faster and more capable platforms, is to run and record an extensive amount of simulations on appropriate hardware, like university computing centers or cloud computing facilities, in order to create a vast number of results for a wide spectrum of physiological parameters.
The resulting data are then broken down into one or multiple lookup tables, which summarize results for specific configurations (or set of configurations clumped together) and subsequently enabling estimations to be made on mobile devices.

The figure 5 evidences the kind of input/outputs taken/given by the MISSION-T2D integrated model. The input includes a parameterised version of the physical activity pattern of the user, e.g., total number of hours of PA per week and the average intensity or a more detailed weekly schedule or - if a measurement device is available -

the weekly pattern of activity as measured which is then assumed to repeat unchanged for each week. Another input is the nutritional habit of the user on a daily or weekly basis. The cumulative daily meal will be broken down in the macronutrients, meaning, proteins, fat, fibres and carbohydrates. A finer grain classification of nutrients accounts for the level of butyrate and propionate directly influencing the tendency of the immune system to drift toward the inflammatory state.

Other general parameters characterising the user include the age, gender, weight, etc.

All these constitute the boundary condition of the model execution, which will then forecast the meta-flammation state at the “forecasting horizon time T”. The simulation runs and returns the detailed dynamics of dozens of variables. These are ultimately used to calculate a unique value identifying the risk of T2D at time T (as indicated in the figure). The “risk of T2D” will therefore be a complex function accounting for the level of insulin resistance (e.g., efficiency of beta-cells) and the level of inflammatory cytokines and pro-inflammatory cell counts.

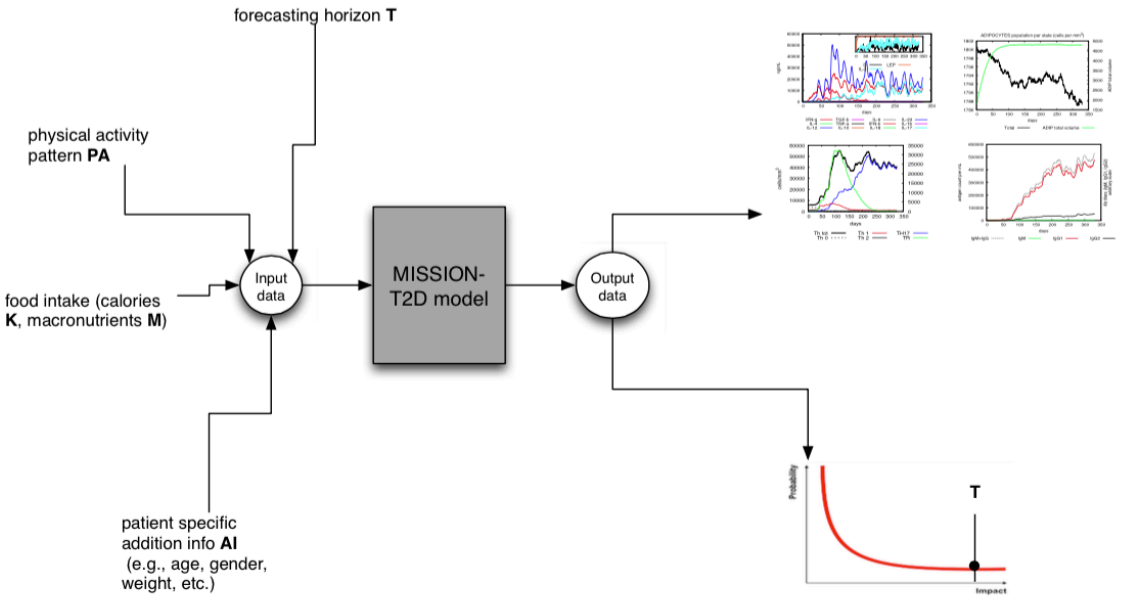

Fig 5 schematic integrated description of the user input data space, simulation engine and resulting forecasts that constitute the project pipeline for the T2D risk estimation. The user will be able to specify and continuously update temporal horizon of her/his forecast (T) as well as patient-specific data (physical activity, PA; food intake, K and M; body parameters such as age, gender, weight, enterotype, etc). The simulations, analysis and related forecasts based on these dynamic datasets will estimate the risk of the individual to develop T2D.

The final integrated simulation engine (WP6) will compute the required extensive scan of the parameter space and clusterize the results on the basis of carefully chosen identifiable outputs. This task is planned to take place within the time limit of milestone MS7. The resulting 'initial conditions/forecasted values' space will then constitute the lookup tables that will eventually be imported into the mobile app and consulted upon the user request.

5 Deliverable conclusions / next step

At the time of writing of this manuscript the unified model runs smoothly. We are performing a number of activities to:

- speedup computation;
- architectural optimisation;
- perform sensitivity analysis;
- estimate unknown integration-related parameters;
- other minor tasks.

After this phase is concluded we will start bulk execution of the simulator towards the validation and later to construct the lookup table for the mobile app. To this purpose we are evaluating the possibility to use the Amazon Cloud to perform such large-scale runs. A rough initial estimation for the cost of running a single instance of the simulation should cost no more than 40 USD at a very conservative "worst case" estimate. The calculation has been made by taking Linux virtual machine with 4 CPU cores, 15 GB of RAM and 2x40GB of SSD storage, assuming a runtime of 144 hours (i.e., 6 days). This kind of machine "costs" 0.28 USD per hour, resulting in the above mentioned 40 USD for one run.

However, if we get anywhere near the mentioned performance improvement of one magnitude, the costs may drop well below 5 USD per simulation, which, given even a tight budget, may lead to a significant number of simulations. Other configurations are available, like an 8 CPU Core, 30 GB RAM, 2 x 80 GB SSD system for twice the hourly costs. Another option includes the Amazon Elastic Block Storage into account, something that may reduce the hourly costs per instance further.

6 Appendix: Partial list of abbreviations used

AT	Adipose Tissue
APC	Antigen-Presenting Cell (immunocompetent cell type)
CD	Cluster of Differentiation (surface markers of lymphocytes)
CTL	Cytotoxic T lymphocyte (immunocompetent cell type)
DC	Dendritic Cell (immunocompetent cell type)
HSP	Heath shock protein (signalling molecule)
IL	Interleukin (signalling molecule)
IFN-γ	Interferon gamma (signalling molecule)
LPS	Lipopolysaccharide (component of pathogen cell walls, signal of danger)
MCP-1	Monocyte Chemotactic Protein-1 (signalling molecule)
MHC	Major Histocompatibility Complex (surface protein of immunocompetent cell types)
MIP-1α	Macrophage Inflammatory Protein-1 alpha (signalling molecule)
T2D	Type 2 Diabetes
TNF-α	Tumor Necrosis Factor alpha (signalling molecule)

Table 2 List of abbreviation.

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