MISSION-T2D

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

Work Package 6

Deliverable 6.3

Report on the validation of the computational model and refinement of the integrated model

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Executive Summary	In this deliverable we describe the work done in task 6.5 (PM24-PM36). In the previous deliverable D6.2 we have described the work done in integrating the various model components into a unified simulation tool. In the present document we describe the work done in setting up the parameters to obtain a dynamics that is as close as possible to either literature available data or data provided by partner TNO in WP7. This constituted the validation of the model implementation up to date. We also discuss the issue of parameter sensitivity with respect to (although limited) user-defined input.
Keywords	Diabetes, inflammation, system simulator, model validation, available data, physical exercise, oral-tolerance test, diet.

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1 Deliverable Description

This deliverable describes the work performed to set the model parameters in a way to be able to reproduce (qualitatively but also quantitatively) real data coming from cohort studies. This data has been either identified and extracted from literature or provided by partner TNO as described in the previous deliverable D7.1 of WP7.

Before describing the work done in the validation phase we report about the parameter scanning procedure (sensitivity analysis) performed to understand the dependence of important output variables such as body mass index (BMI) and fasting glucose (or glucose base level, Gbl). Mainly partner CNR (WP6), in close collaboration with the other partners responsible for the various components, has conducted the validation phase. In particular, partners CNR, UniRM and TNO have frequently interacted in this task.

2 Background

2.1 Background on the previous work as described in D6.2

In the previous deliverable D6.2 we have described the various sub-models (M1-M5) provided by the different partners of the MISSION-T2D consortium to account for inflammation, metabolism, gut, and physical exercise, their mutual relationship and the strategy for combining them altogether to make up the unified model. In the same deliverable D6.2 we have described the strategy, the problems and the solutions we have adopted in the task of integrating the different sub-models.

Finally, few details of the overall model needed attention and further development. This included a term for insulin resistance in peripheral tissues, the parameter tuning of the sub-model for alanine and triglycerides dynamic modifications during meal needs, and a modification of the physical activity module to use of the heart rate as model input which required the relationship between the heart rate and percentage of VO_2max.

3 Other (not minor) tasks related to model development

During the last project period (year 3) we have also performed a number of related activities such as speeding up the computation in order to be able to perform a large number of simulations needed for i) the sensitivity analysis, ii) the validation itself and for iii) producing the look-up table for the mobile app under development by partner MED in WP8 that will be described in forthcoming deliverables at PM38. This task,

which resulted in an improvement of the model performances close to 400%, included some architectural optimisations, clean up and careful rewriting of portions of code and identification of bottlenecks in the numerical solver execution that suggested the points of intervention. In fact, most of the speed up has been obtained by avoiding those number crunching critical sections of certain metabolic variables dynamics, which arose because of a poor choice of the parameters. In this way we reduced the running time of a typical simulation from 4 days to 32 hours and in some cases, luckily the majority, to 6 hours (and even less).

4 Considerations about parameter sensitivity

To get a large picture of the effect of some parameters on the overall dynamics of some "crucial" variables, we performed a systematic set of numerical experiments. The results are shown in Figure 1.

Each of the six panels of the figure shows the time dependent dynamics of one variable (the one in the label of each figure's panels) with respect to changes of few key input parameters or initial conditions, which will ultimately be associated to the user input, in particular the physical activity and dietary habits. The top-left panel shows changes in the body mass index (for different initial conditions of the BMI itself) as a function of a different life style corresponding to calorie intake and number *n* of physical activity sessions of moderate intensity.

Although the scope of these simulations was not to draw conclusions about general characteristics of the dynamics observed, we are nevertheless able to note interesting features that are worth to be mentioned here. The first of these regards the BMI itself which seems uncorrelated (at least within the simulated time frame of 24 weeks) to the number *n* of PA sessions, in other words, *the weight is not significantly affected by physical activity alone* and, as we will show in the following panels, *it is the dietary habits which is more important*. This is, of course, something that we already know but it is nevertheless interesting to observe how the model is in agreement with our daily life experience.

The top-centre panel shows the fasting glucose (that we call glucose base-level or Gbl) as a function of time, parameterised by *n* the number of PA sessions, under different dietary regimens. The observation to make here is that the cases of no PA (i.e., *n=0*) and to a certain extend low PA (i.e., *n=1*), are certainly correlated to a faster progress toward insulin sensitivity and ultimately T2D. The right-most panel in the first row

shows the level of inflammation under the same conditions. Again, interestingly, here we note that *the physical activity alone does not make any difference is ameliorating the inflammation arising from a poor quality nutritional style*; i.e., the rising curves identify high caloric intakes whereas the descending or platooning ones are the result of a balanced diet (the details of the diet are not reported here for brevity).

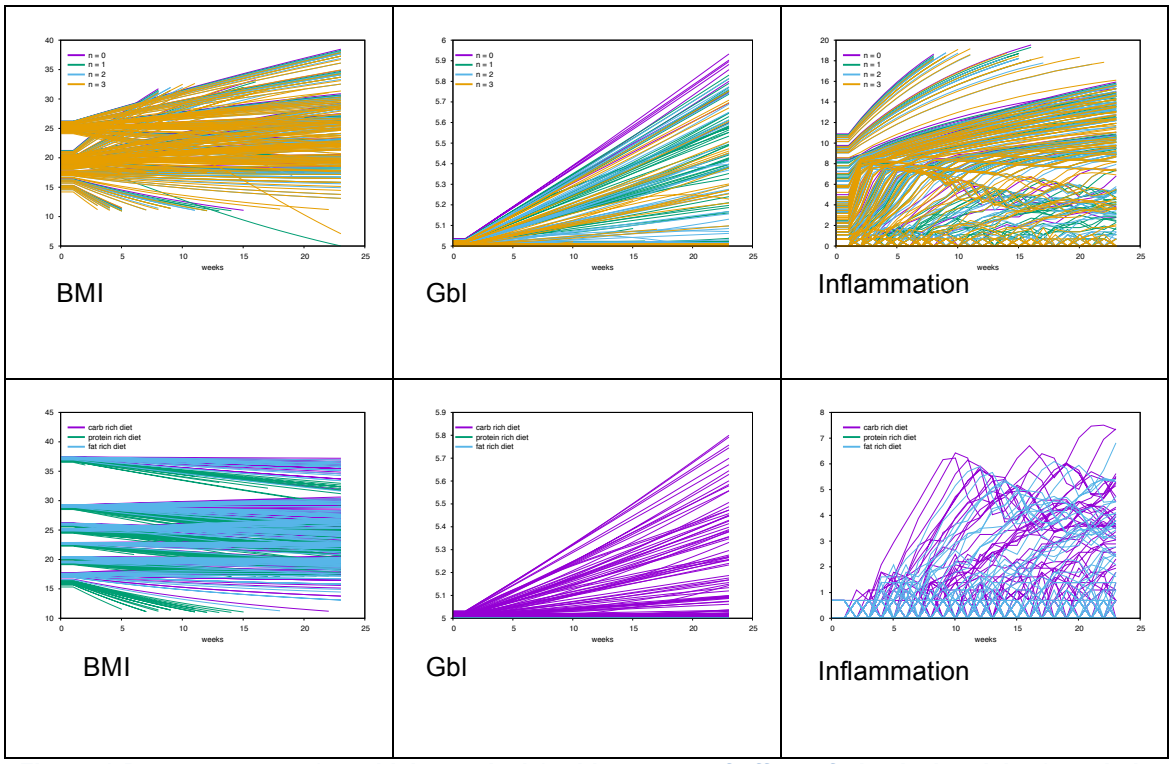


Figure 1 Parameter scan (sensitivity analysis) with respect of effect of physical activity (panels in the first row) on BMI, fasting glucose and inflammation level, and effect of various diets on the same variables, panels in the second row.

When we classify the diet in three classes, 1) those rich of carbohydrates, 2) rich in proteins and 3) rich in fat (each with the other two components set respectively to "scarce"), we obtained a clearer picture of the BMI as shown the left-bottom panel. What is evident is that a carb rich diet is the one that prevents a decrease of BMI (which in some cases even increases) notwithstanding the scarcity of the other two dietary components and the action of physical activity in reducing the weight. The glucose base level is also depending on the carbohydrate (thus glucose) intake (bottom-centre panel) whereas the level of inflammation (that is in logarithmic scale – but arbitrary units - in the plot) is equally determined by a carb rich or fat rich diet with the protein rich diet appearing as the most virtuous one.

Further parameter sensitivity tests have been performed on each model component separately as for example shown in other deliverables (D5.4) and in Figure 2 for the physical activity module. This plot shows the level of insulin for a varying intensity physical exercise of 50 minutes duration.

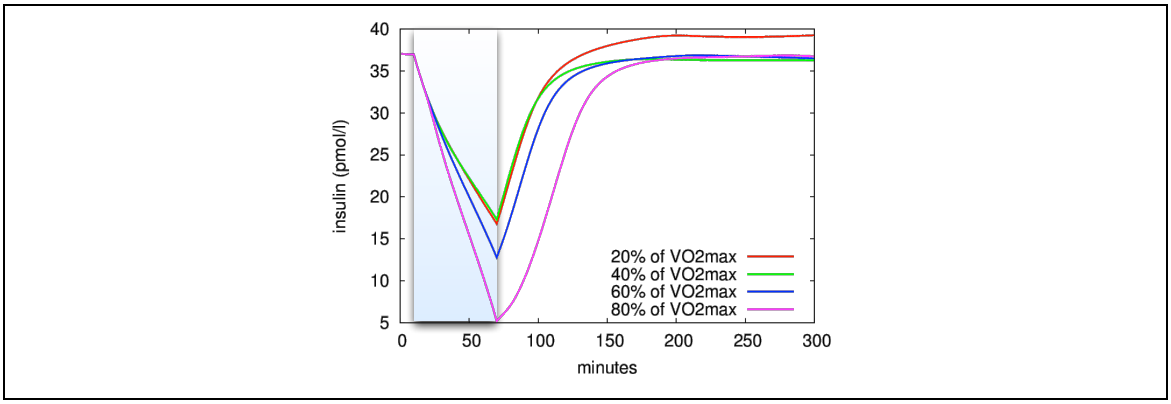


Figure 2 Insulin as a function of the intensity of the physical activity session starting at minute 10 and ending at minute 60.

5 Model validation

The next three sections describe the work done for what concerns the validation of the model. We have identified suitable data from literature from cohort studies pertaining either the effect of physical activity or the calorie intake (OGTT=oral glucose tolerance tests, OLTT=oral lipid tolerance tests) on anthropometric, metabolic and hormonal variables. The other source of data was internal to the project, namely, one of the partners, TNO, provided cohort studies as described in deliverable D7.1 of WP7.

All data sets are briefly summarised here and in the following Table 1, Table 2 and Table 3.

Before describing the data and corresponding simulations, it is worth to make a premise. This is about the *variability* observed in the data. To exemplify this issue we plot in Figure 3 the glucose measured by different studies (Nauck et al., Campioni et al., Muscelli et al., Bernsmeier et al., and Kardinaal et al.) as the average of variable size cohorts with uneven gender balance and quite heterogeneous anthropometric parameters. Even if the standard deviation is not displayed, the variability is quite evident.

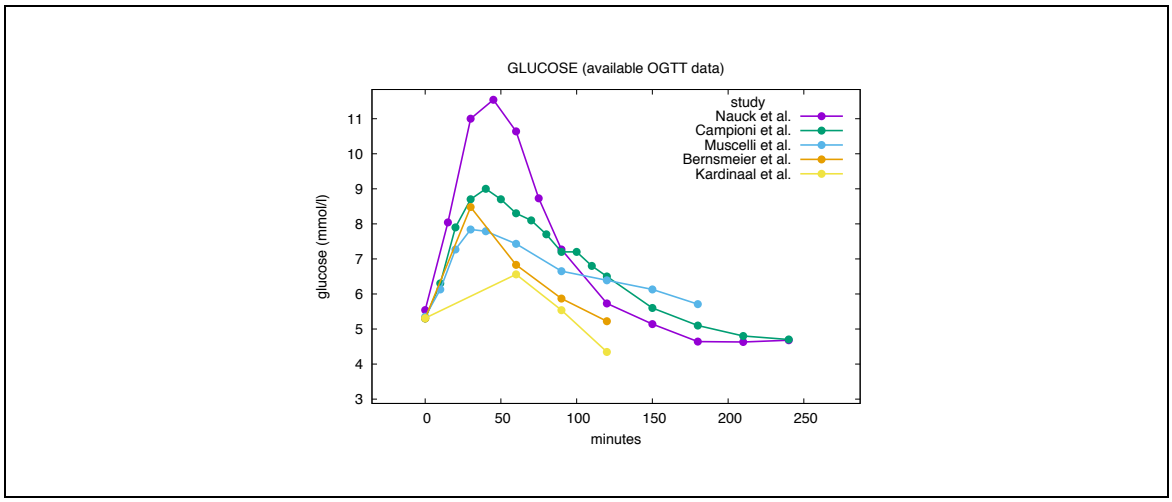


Figure 3 The high variability of available literature data in the glucose measured in quite heterogeneous cohorts.

This fact exemplifies the situation we faced in trying to validate the MISSION-T2D model in the fact that, as it will be show later in this document, it was extremely difficult, and sometimes impossible, to match the observables in a *statistically* meaningful way (we mean "statistically meaningful" in a quite broad or weak sense as to be within one standard deviation or standard error of the mean, SEM, from the average value). The heterogeneity of the various studies, and within each study, of the individuals constituting the cohort, is probably the main reason for the discrepancies among real and in-silico data. Expecting to match such heterogeneity is probably asking too much to the model. A possible partial resolution could consist in stratifying the cohort data in a more detailed manner (we do have stratified the data but avoiding to create countless cases) and compare that with tailored simulations; this could in some (not all) cases reduce the error observed but would inevitably require 1) more data in order to have statistically relevant abundances in each class, and 2) more time and effort; therefore we resorted to a coarser comparison leaving the "finer" validation for a more detailed study to be done at a later stage.

5.1 The effect of physical activity

The first set of data regards the effect of physical activity on the model dynamics.

For the validation of physical activity effects on whole body metabolism we referred to four different studies, namely:

1. Hirsch (Hirsch, 1991),
2. Bergman (Bergman, 1999),

3. Wahren (Wahren, 1975),
4. Bloom (Bloom, 1976).

In all of these studies different exercise protocols, in terms of duration and intensity, are taken into account. Moreover, the considered subjects have different physical fitness status (e.g., trained, T, or untrained, UT).

The first three studies are used by Kim and colleagues (Kim, 2007) for the validation of the original model, although they consider only data regarding a specific exercise (60%VO_2max), only one kind of subject (trained) and do not include the recovery period after exercise.

Characteristics of each of the four studies considered are reported in the following Table 1.

Table 1 Data sets for Physical Activity

Study	Aim	Input parameters								Observables	
		Sex (m/f)	Age (yrs)	Weight (kg)	Height (m)	BMI (kg/m^2)	PA duration (min)	PA intensity (%VO_2max)	VO_2max (ml/kg/min)	Hormones	Metabolites
Hirsch (1991)	Response to an exercise session	13/0	25 ± 3	75.4 ± 9.4	1.75	24.65	60	60	44	Insulin, glucagon, epinephrine	Glucose, FFA
Bergman (1999)	Response to an exercise session (UT)	7/0	22.1 ± 1.3	75.6 ± 6.2	1.8	23.3	120 90	40 60	38.55		Lactate
	Response to an exercise session (T)	7/0	25.1 ± 1.8	73.7 ± 3.5	1.8	22.7	120 90	40 60	58		
Wahren (1975)	Response to an exercise session	8/0	27.25 ± 5.34	74.75 ± 9.36	1.83 ± 0.05	22.3 ± 1.8	40	60	46.4	Glucagon	Glucose, lactate, pyruvate, glycerol, FFA
Bloom (1976)	Response to an exercise session (UT)	6/0	26 ± 0.8	74 ± 4	1.84 ± 0.04	21.85	8 x 4	30, 45, 60, 75	35	Glucagon, epinephrine	Glucose, lactate, pyruvate, glycerol, alanine, FFA
	Response to an exercise session (T)	6/0	29 ± 1	69 ± 4	1.75 ± 0.03	22.53	8 x 4	30, 45, 60, 75	50		

UT = untrained
T = trained

Hirsch and coworkers in (Hirsch, 1991) analyzed the decrements of glucagon and increments of insulin in the prevention of hypoglycemia during moderate exercise (60%VO_2max for 60 minutes). Thirteen untrained (UT) young men after an overnight fast performed a physical exercise on a cycle ergometer. Observations were continued for 120 minutes. Insulin, glucagon, epinephrine and FFA were measured. Results of the simulation superimposed to experimental data are reported in Figure 4 and show a decent agreement with the experimental data.

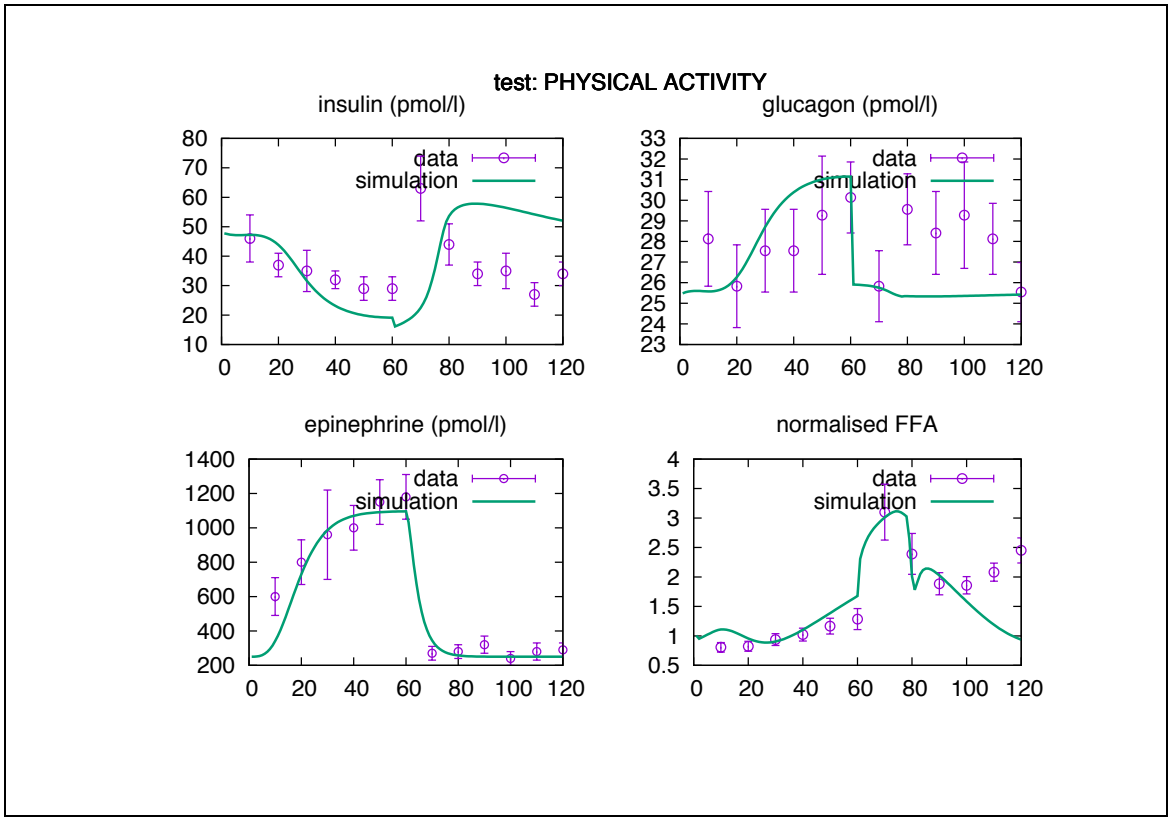


Figure 4 Model predictions vs. experimental data from the study by Hirsch et al. of insulin (left-upper panel), glucagon (right-upper panel), epinephrine (left-lower panel) and FFA (right-lower panel). Data are mean ± SEM. FFA has been normalised with respect to basal value.

Bergman et al. in (Bergman, 1999) show the lactate concentrations in 7 strongly trained (T) and 7 untrained (UT) healthy men while they were exercising on a cycle ergometer over a wide range of relative exercise intensities after an overnight fast. Trained (40 and 60% VO_2max, with VO_2max = 50 ml/kg/min) and (40 and 60% VO_2max, with VO_2max = 38.55 ml/kg/min) male with age between 19 and 32 years were analyzed. Trained subjects were licensed category 2 or 3 racers in the United States Cycling Federation (USCF). Exercise was performed at 40% VO_2max for 120

minutes and at 60% for 90 minutes. The results of the corresponding simulations are reported in Figure 5.

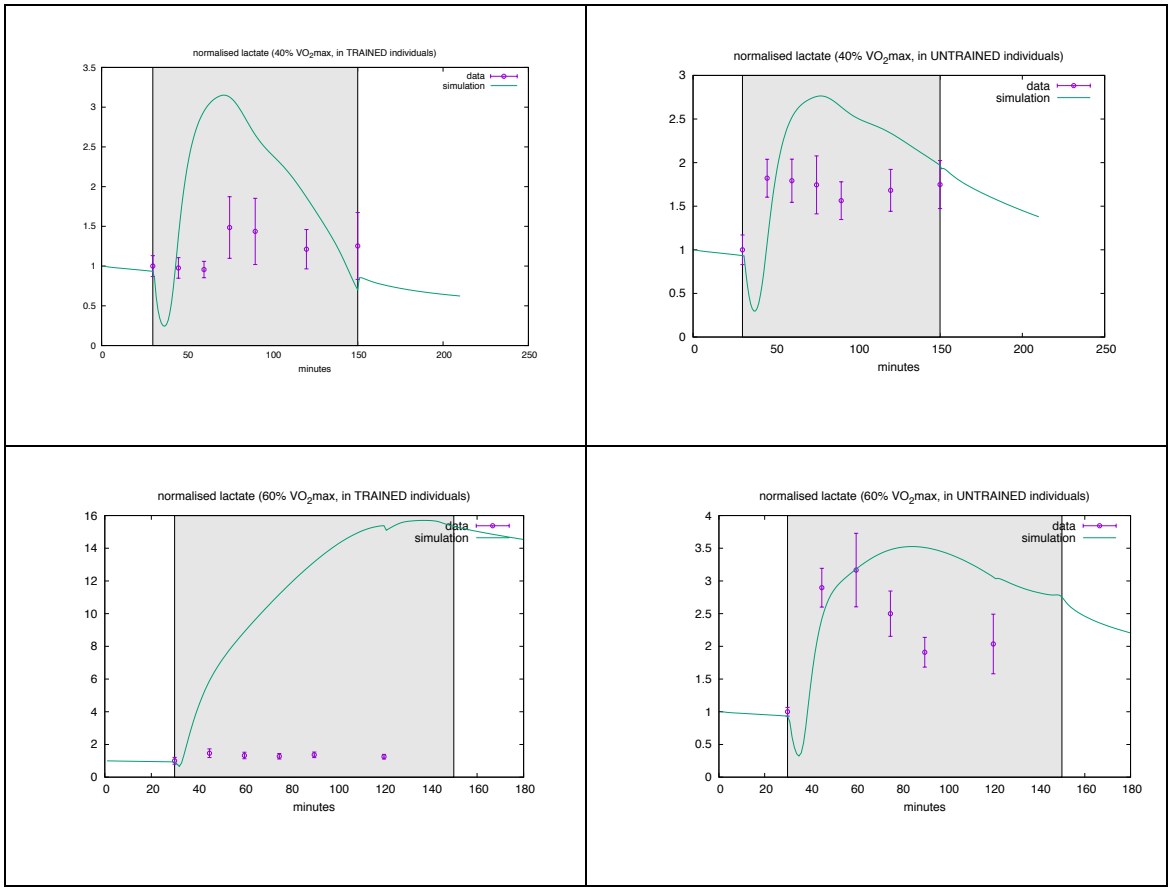


Figure 5 Model predictions of the lactate concentration in response to 120 min of exercise at 40% VO_2max in a trained (upper left panel) and an untrained individual (upper right panel). Simulations are superimposed to experimental data from the study by Bergman et al. Data are mean ± SEM. Lactate concentration has been normalised with respect to its basal value. Lower left and right panels show the same values for an exercise at 60% VO_2max in trained (left panel) and an untrained individual (right panel).

Despite a slight overestimation, model predictions of lactate concentration during 40%VO_2max exercise show a decent agreement with experimental data in both trained and untrained subjects (Figure 5, top left and right panels).

With regards to the results of the performance at 60%VO_2max, the simulation shows a good agreement with data for untrained subjects (Figure 5, bottom right), whereas there is a clear overestimation for the trained ones (Figure 5, bottom left panel). In this case, it should be pointed out, however, that the athletes, and the phenomena associated to the high level of physically activity that they might reach, are certainly of

less interest for the scope of MISSION-T2D. These subjects (category 2 or 3 racers in the USCF) in fact, are certainly much less prone to develop T2D with respect to non-physically active subjects.

In the study by Wahren (Wahren, 1975) arterial concentrations of substrates (glucose, lactate, pyruvate, glycerol and FFA) and glucagon were measured in eight healthy male volunteers at rest and during 40 minutes of exercise performed at 60% VO_2max (VO_2max = 46.4) on a cycle ergometer. Simulations and data are plotted in Figure 6.

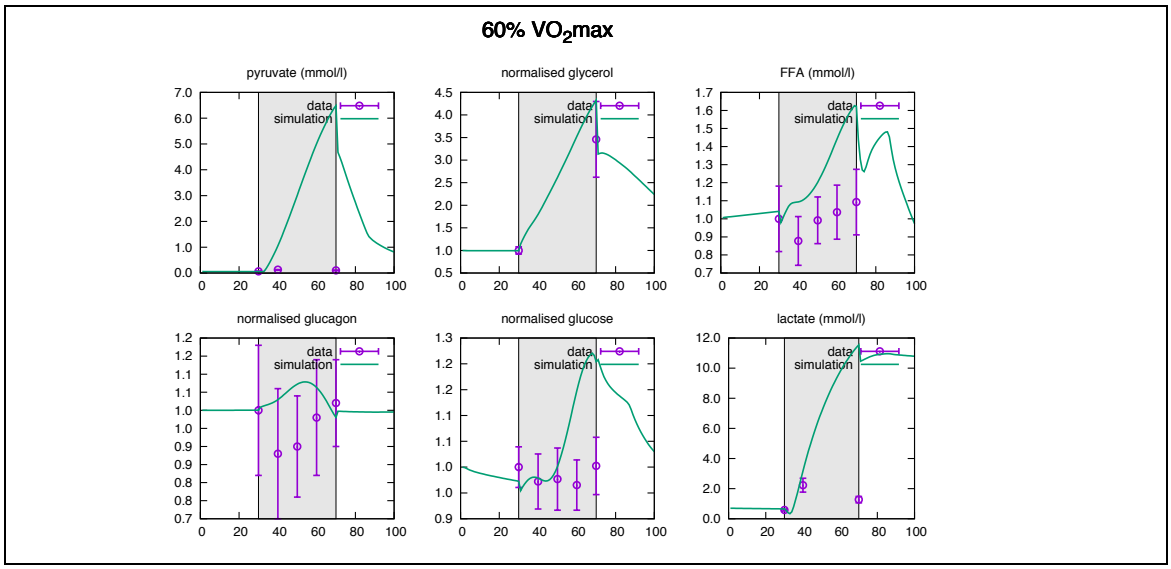


Figure 6 Model predictions of pyruvate, glycerol, FFA, glucagon, glucose and lactate vs. experimental data from the study by Wahren et al. Data are mean ± SEM. In some plots normalization with respect to basal value is shown

Figure 6 shows that there is a good agreement between data and simulation results for glycerol and glucagon; FFA and glucose concentrations are slightly overestimated in the final part of the physical activity. Pyruvate and lactate are overestimated; this might be due to an incorrect estimation of the maximum rate coefficient in the model that needs to be adjusted.

Six well-trained male cyclists (aged 22-27 years) and six male untrained volunteers (aged 25-33 years) were studied by Bloom and colleagues in (Bloom, 1975) during and immediately after four successive 7 min periods of exercise at 30, 45, 60 and 75 % of their maximal work capacity (VO_2max = 35 ml/kg/min for untrained subjects and VO_2max = 50 for cyclists). Blood samples were taken at rest, at the end of each exercise period and 5 min following the end of exercise, for estimation of metabolites in

blood (pyruvate, alanine, glycerol, FFA), plasma insulin, glucagon, glucose and epinephrine. The physical activity was performed on a cycle ergometer. In Figure 7 we compare simulations and experimental data.

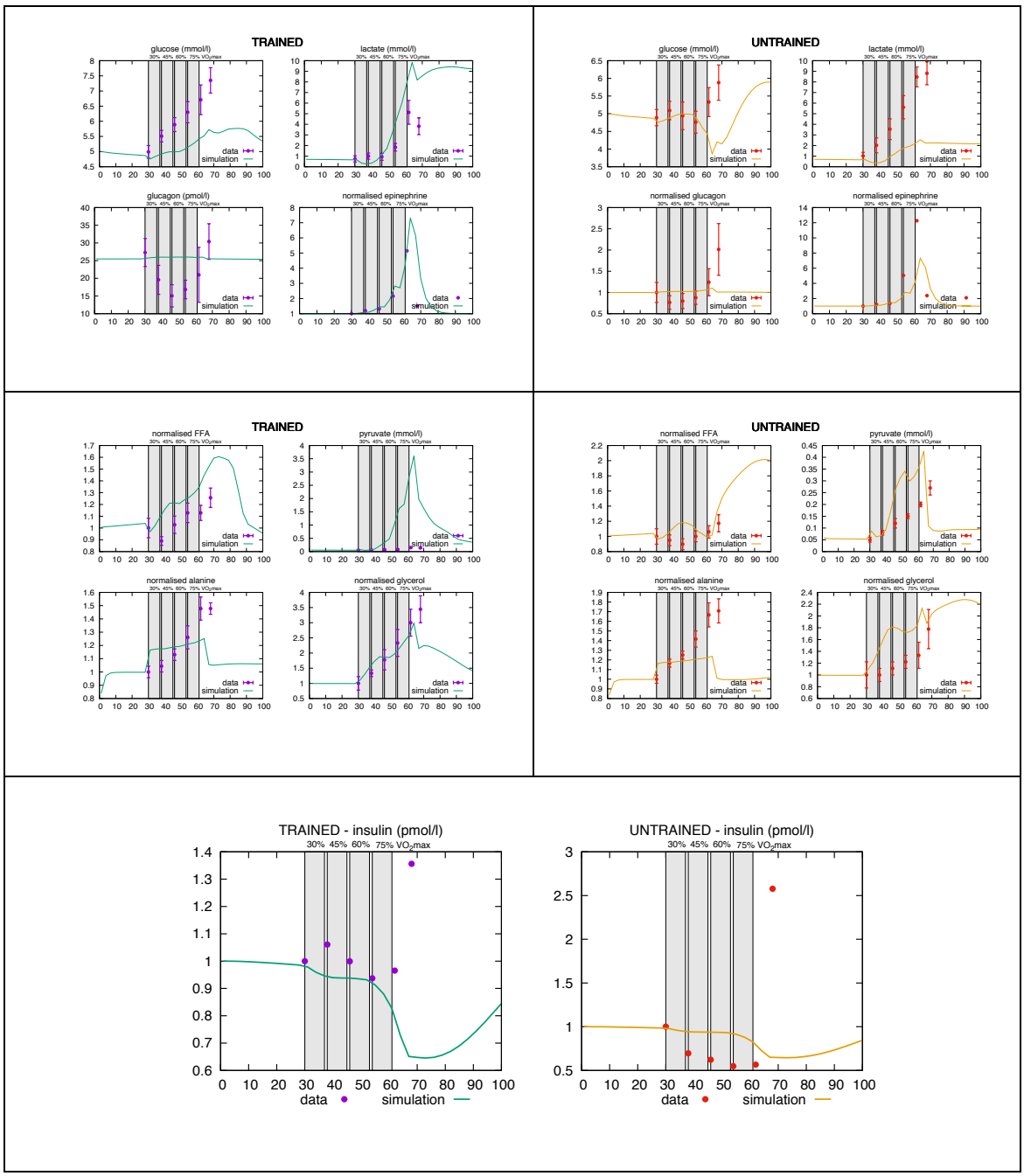


Figure 7 Comparison of simulation with data coming from the study of Bloom et al. about the effect of physical activity on different metabolites in trained and untrained individuals. Data points indicate averages and are shown with error bars for standard deviations, whereas simulations are continuous lines. The grey boxes reveal the periods with different activity intensity, 30, 45, 60 and 75% of VO_2max.

FFA, glycerol, insulin and epinephrine are well estimated in both cases; alanine too,

even if a little bit underestimated. Pyruvate and glucagon are good for untrained, whereas overestimated for trained. Lactate is underestimated for untrained. Glucose is slightly underestimated for trained and does not match the final part of the exercise for untrained.

5.2 Oral challenge tests

The second set of data deals with the validation of the model of meal ingestion and oral absorption of glucose, alanine and triglycerides after the intake of carbohydrates, proteins and fat. This model was implemented by introducing the Dalla Man formulation of rate of appearance (Dalla Man, 2006) based on gastric empting description reported by Elashoff (Elashoff, 1982) for glucose intake. The same formulation was adopted to represent the rate of appearance of alanine and triglycerides. The implementation of alanine and triglycerides dynamic modifications during meal needed the estimation of proper parameters, provided by TNO.

For the validation of meal ingestion on whole body metabolism we referred to five different studies, namely:

1. the study by Campioni (Campioni, 2007),
2. the study by Muscelli (Muscelli, 2006),
3. the study by Nauck (Nauck, 2004),
4. the study by Kardinaal (Kardinaal, 2015),
5. the study by Bernsmeier (Bernsmeier, 2014).

In all these studies the volunteers, after an overnight fast, received an OGTT (oral glucose tolerance test) of 75g, composed by 417 mmol of glucose, for the total of 286Kcal. In the study by Kardinaal and colleagues, even the response to an OLTT challenge test was analyzed. Such a challenge test is composed by 99mmol of glucose, 112mmol of alanine and 90mmol of triglycerides, for the total of 816Kcal. In all these studies healthy subjects and patients with diseases related to diabetes or metabolic syndrome. For what concerns MISSION-T2D purposes, only data regarding healthy subjects were taken into account.

Table 2 Data sets for Oral Challenge Tests (OGTT and OLTT)

Study	Aim	Input parameters									Observables		
		Sex (m/f)	Age (yrs)	Weight (kg)	Height (m)	BMI (kg/m^2)	Glucose (mmol)	Alanine (mmol)	Triglycerides (mmol)	Kcal	Hormones	Metabolites	Inflammatory markers
Campioni (2007)	Response to OGTT	5/5	28.8 ± 1.8	72	1.7	24.8 ± 0.7	417	0	0	286	Insulin	Glucose	
Muscelli (2006)	Response to OGTT	4/7	44	75	1.7	25.8	417	0	0	286	Insulin	Glucose, FFA	
Nauck (2004)	Response to OGTT	6/4	45 ± 13	75	1.7	26.1 ± 4.2	417	0	0	286	Insulin	Glucose	
Kardinaal (2015)	Response to OGTT	10/0	42.5	78.4 ± 8	1.8	24.1 ± 1.8	417	0	0	286	Insulin	Glucose	
	Response to OLTT	10/0	42.5	78.4 ± 8	1.8	24.1 ± 1.8	99	112	90	816	Insulin, glucagon	Glucose, FFA, TG	IFNg, IL-1b, IL-6, IL-10, IL-12, IL-18, Lep, TNFa, lymphocytes, monocytes
Bernsmeier (2014)	Response to OGTT	50	35.9 ± 1.9	71 ± 1.1	1.77	22.7 ± 0.2	417	0	0	286	Insulin, glucagon	Glucose	

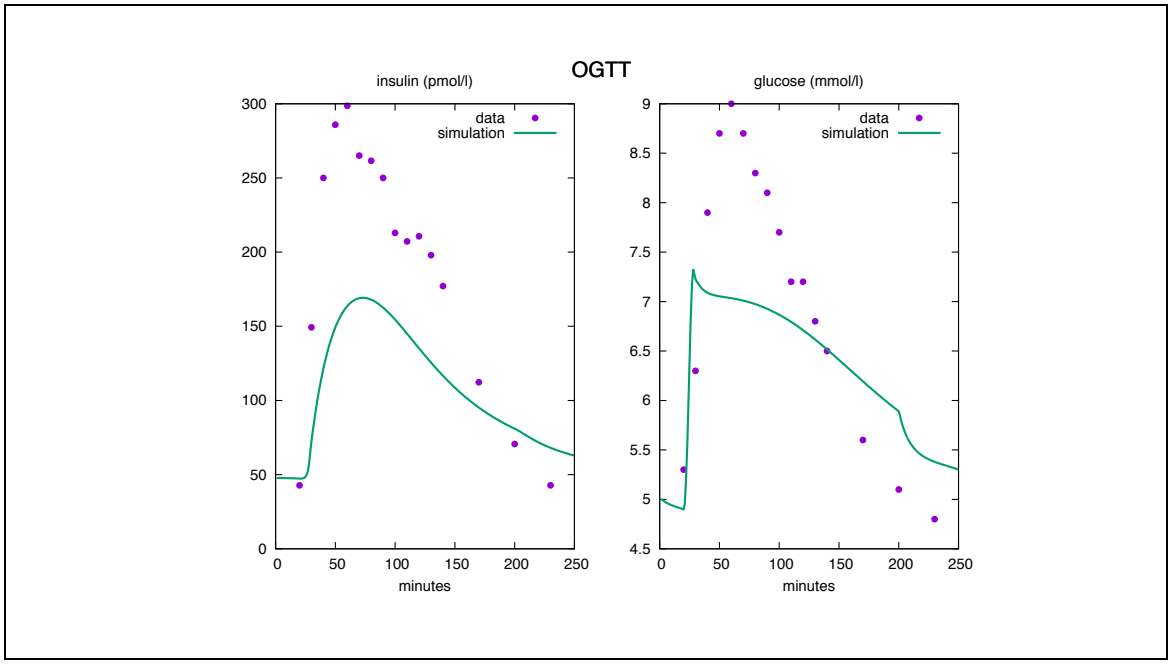


Figure 8 Plasma insulin (left panel) and glucose (right panel) concentration during an OGTT. Data from (Campioni et al. 2007).

In the study conducted by Campioni and coworkers (Campioni, 2007), 5 male and 5 female non-obese volunteers received an OGTT and blood samples were collected until 240 minutes after the test. In Figure 8 the simulated and the experimental measures are shown. For this set of data, the simulated dynamics for both the insulin and the glucose concentration are slightly underestimated.

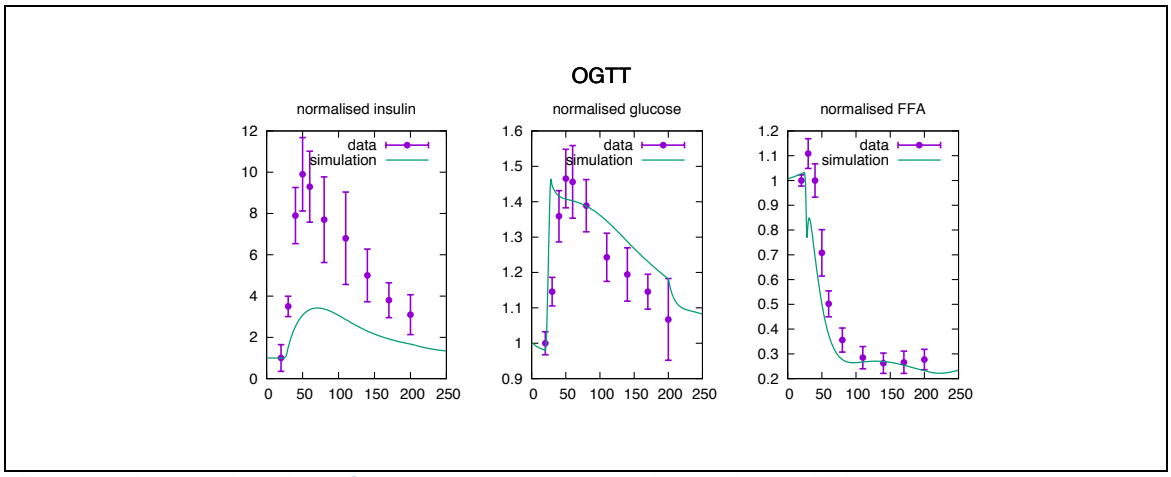


Figure 9 Plasma insulin (left panel), glucose (central panel) and FFA concentration in the 10 subjects during an OGTT. Data are normalized with respect to the initial value and experimental measures are expressed as mean +- SEM. Time is plotted on the x-axis, expressed in minutes. Data from (Muscelli, 2006).

In the study conducted by Muscelli et al. (Muscelli, 2006) 11 healthy volunteers (4 men and 7 women) received an oral glucose tolerance test after an overnight fast. Blood samples for analysis were collected until 180 minutes and glucose, insulin and FFA were observed as reported in Figure 9.

The comparison between the model predictions and the experimental data shows a good agreement for glucose and FFA. The insulin dynamics, as in the previous validation of the dataset provided by Campioni's study (Figure 8), results to be underestimated.

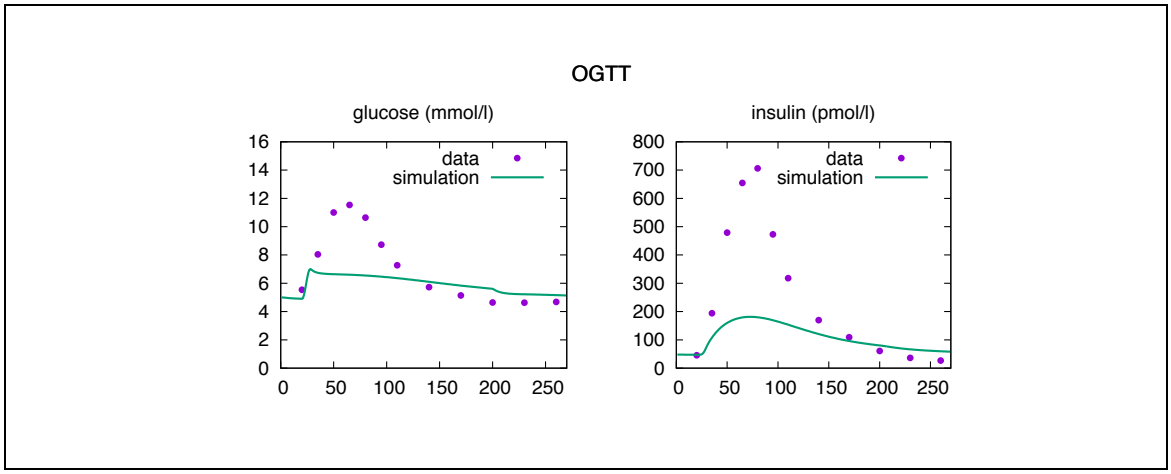


Figure 10 Glucose (left panel) and insulin (right panel). Experimental data are expressed as mean +- SEM. Time is plotted on the x-axis, expressed in minutes. Data from Nauk et al. (Nauk, 2004).

Data from 10 healthy controls (6 male, 4 female, 45 +- 13 years, 26.1 +- 4.2 kg/m2) were taken into account for validation from Nauck et al. (Nauck, 2004). The tests were performed in the morning after an overnight fast and blood was drawn over 240 min to measure glucose and insulin concentrations. Comparison between the model predictions and experimental data are reported in Figure 10. It shows a slightly underestimation of simulated data with respect to the experimental ones.

Kardinaal and colleagues in (Kardinaal, 2015) studied metabolism homeostasis in different condition after three challenge tests: high-fat high-calorie (HFHC) diet, OGTT and OLTT. For MISSION-T2D purposes, we took into account the data regarding the responses to the tests of 10 healthy men.

These subjects consumed an HFHC diet for 4 weeks, consisting of a fixed amount of commercially available foods high in fat, sugar, or both. The nutritional composition of HFHC diet resulted in a daily surplus of 1300Kcal (138g carbohydrates, 31g protein, 63g fat). Moreover, the volunteers received an OGTT after an overnight fast before the HFHC diet and responses to high fat challenges (OLTT) were measured at the beginning and at the end of the overfeeding period.

In this paragraph we report the comparison between the simulation and experimental data for OGTT and OLTT tests.

- The OGTT test was performed in the morning after an overnight fast and blood was drawn over 120 min to measure glucose and insulin concentrations.
- The OLTT test was performed in the morning after an overnight fast and blood was drawn over 480 min to measure glucose, insulin, glucagon, FFA, triglycerides and inflammatory markers (IFNg, IL-1b, IL-6, IL-10, IL-12, IL-18, Lep, TNFa, lymphocytes, monocytes) concentrations.

In Figure 11, Figure 12 and Figure 13, the results of the simulation superimposed to experimental data are reported.

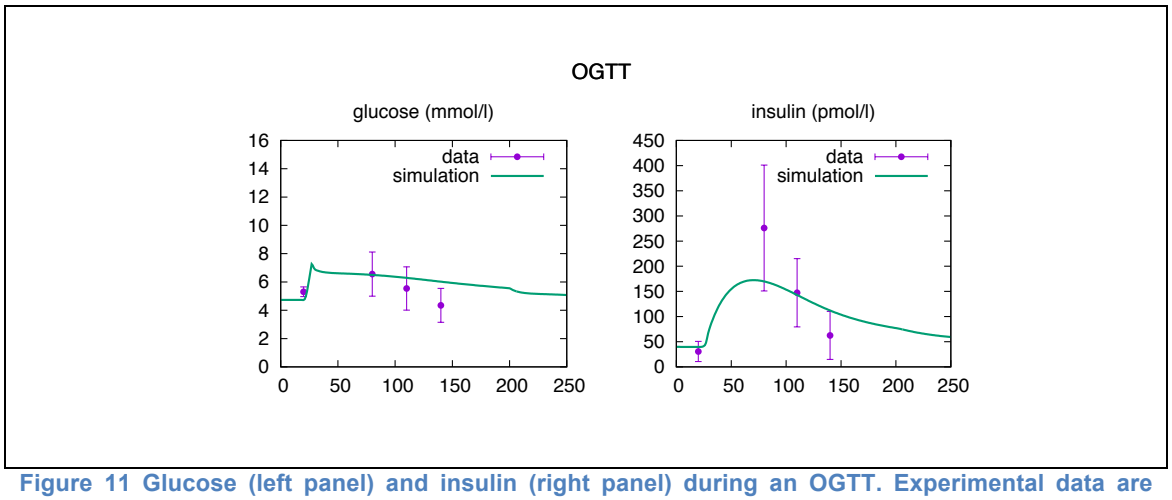


Figure 11 Glucose (left panel) and insulin (right panel) during an OGTT. Experimental data are expressed as mean +- SEM. Time is plotted on the x-axis, expressed in minutes. Data from Kardinaal et al. provided by partner TNO (Kardinaal, 2015)

As shown in Figure 11 and Figure 12, model predictions reflect a good correspondence with experimental measures for respectively OGTT and OLTT tests.

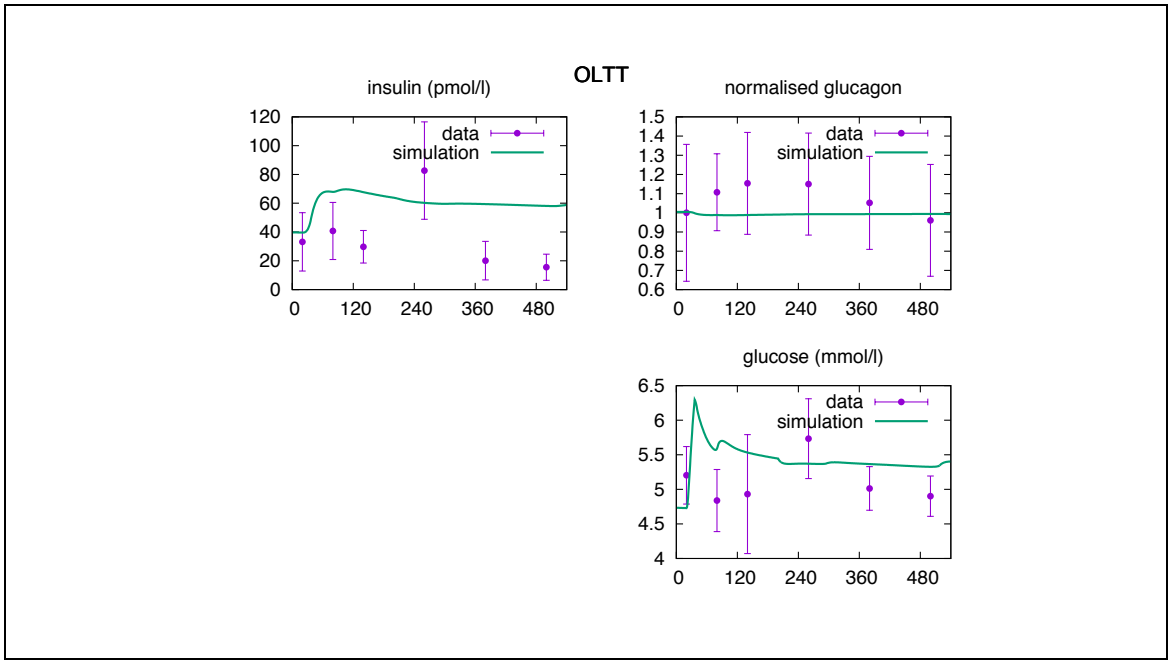
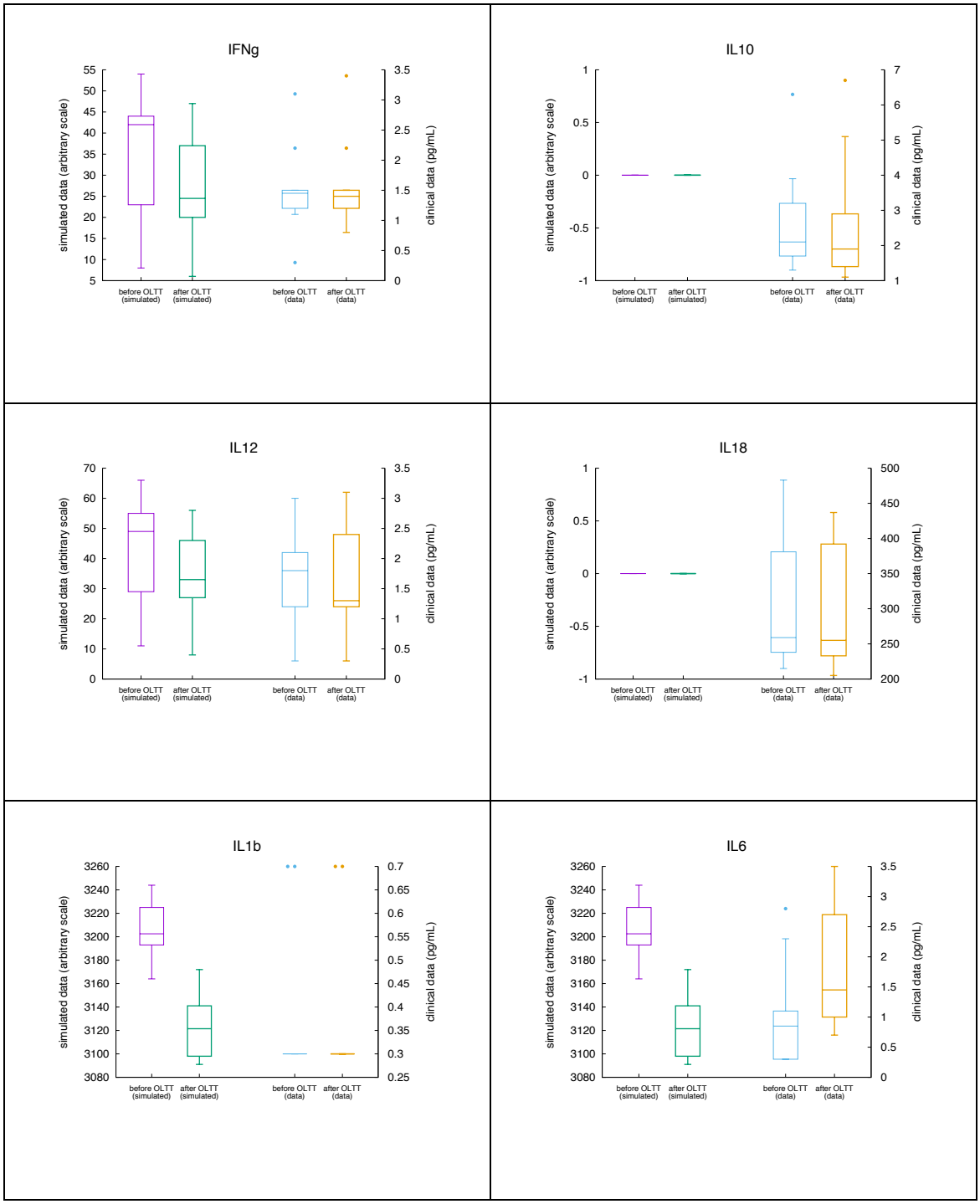


Figure 12 Insulin (left upper panel), glucagon (right upper panel) and glucose during an OLTT. Data on the glucagon concentrations are normalized with respect to the initial value. Experimental data are expressed as mean +- SEM. Time is plotted on the x-axis, expressed in minutes. Data from Kardinaal et al. (Kardinaal, 2015) provided by partner TNO.

Figure 13 shows comparison between inflammatory markers before and after an oral-lipid tolerance test (OLTT). The various markers, IFNg, IL-10, IL-12, IL-18, IL-1b, IL-6,

leptine and TNFa together with percentage of monocytes and lymphocytes are plotted together with corresponding simulated data.



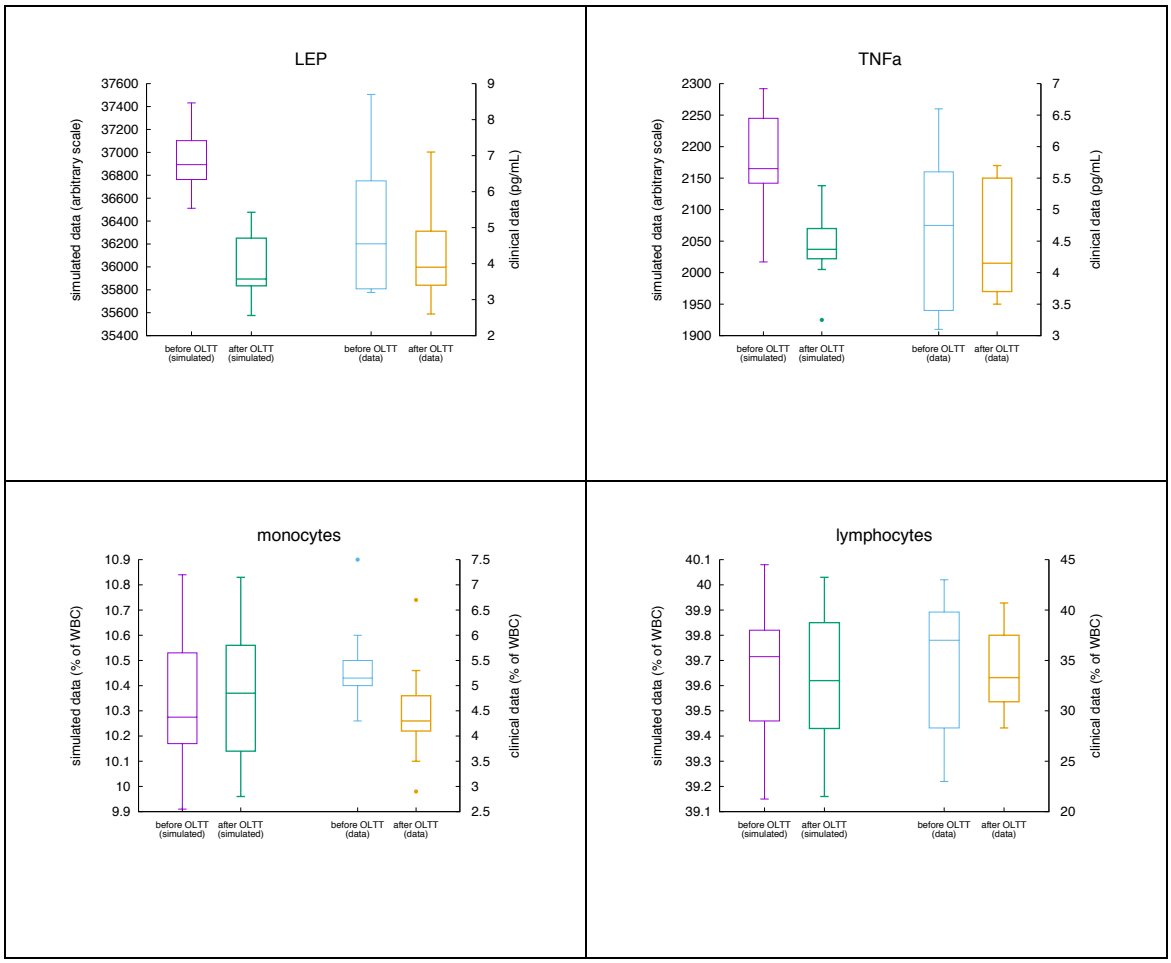


Figure 13 Inflammatory markers measured before and after an OLTT and subsequent to 4 weeks of a high-fat high-calorie (HFHC) diet. Clinical data is plotted with whisker bars on the right of each panel whereas the corresponding values coming from equivalent set-up condition simulations are plotted on whisker at the left.

This study, as reported in the article (Kardinaal, 2015) demonstrate that the 4 weeks hyper caloric intervention induced a series of anthropometric and metabolic changes related to processes of adipose tissue mass and function, metabolic flexibility, vascular health, ad glucose metabolism in healthy subjects in the fasting state, but major biomarkers of metabolic health (glucose, TGs, and CRP) were unchanged (as discussed below in Figure 16). Moreover, no significant differences were seen for most markers related to systemic stress (Kardinaal, 2015). Simulation data agree on these facts as related or equivalent variables' values were practically unchanged after OLTT but showed a slight increase during the HFHC diet. This is coherent with the picture that healthy subjects develop inflammatory stress only on a long period, something that cannot be evidenced in only 4 weeks interval.

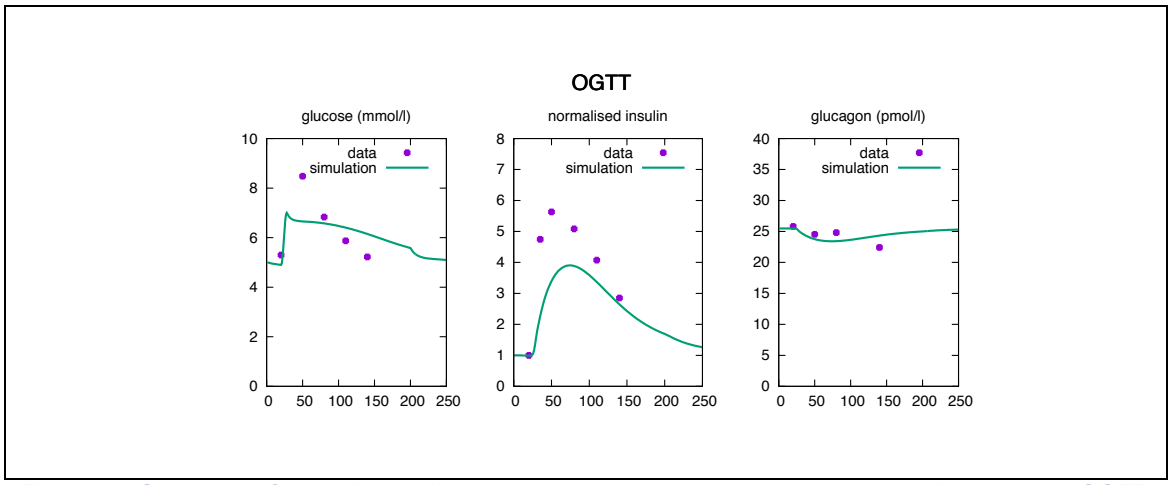


Figure 14 Glucose (left panel), insulin (central panel) and glucagon (right panel) during an OGTT. Data on the insulin concentrations are normalized with respect to the initial value. Time is plotted on the x-axis, expressed in minutes. Data from Bernsmeier et al. (Bernsmeier, 2014).

The study conducted by Bernsmeier et al. (Bernsmeier, 2014) includes 50 healthy subjects. Standardized oral glucose tolerance test was performed. Glucose, insulin, glucagon, plasma levels were measured sequentially for 120 minutes after glucose administration. Comparison between the simulation and experimental data are reported in Figure 14. As evident from the figure, simulated data reflect a good correspondence with experimental measures.

5.3 Lifestyle: diet and physical activity together

The third set of data deals with the validation of the model describing the fat mass decrease or increase in case of prolonged calorie restriction or augmentation, leading to weight loss or gain. Changes in body weight are due to variations in fat mass (FM), depending on energy balance. Weight loss and gain are modeled on the basis of Westerterp regression equations reported in (Westerterp, 1995). Energy balance is estimated taking into consideration the energy intake from diet and the energy expenditure. Energy expenditure is the resulting of resting energy expenditure (REE), thermic effect of food (TEF) and physical activity energy expenditure (AEE). REE is computed on the basis of Mifflin's estimation as in (Mifflin 1990).

TEF (also called DIT, namely diet-induced thermogenesis) is the amount of energy expenditure that occurs after eating due to the cost of digesting and processing food

and represents about 10% of the energy intake.

For the validation of this part of the model we referred to two different studies, namely:

1. the study by Heilbronn (Heilbronn, 2009),
2. the study by Kardinaal (Kardinaal, 2015).

The first study examine the effects of 6 months of calorie restriction, with or without physical exercise in overweight humans with BMI (body mass index) ranging from 25 to 30kg/m^2.

Healthy, sedentary men and women (n=46) were randomized to one of three groups with different diet protocols for 6 months:

- CR= -25% calorie restriction of baseline energy requirements (1900Kcal);
- CREX= -12.5% diet restriction of baseline energy requirements +12.5% increase in energy expenditure by structured exercise. CREX participants increased energy expenditure by 12.5% above resting by undergoing structured exercise (walking, running, cycling) five days per week. The duration of the exercise session was about 45 minutes, at the 69%VO2max (VO2max = 35 ml/kg/min).
- LCD (i.e., very low calorie diet) (890Kcal/day) until 15% reduction in body weight followed by weight maintenance diets. LCD diet was modeled by means of three meals, each composed by 226mmol of glucose, 353mmol of alanine and 5mmol of triglycerides, for the total of 890Kcal/day. Generally, target weight was achieved by week 10.

Table 3 Data sets for lifestyle (diet and diet + physical activity)

Study	Aim	Input parameters										Observables		
		Sex (m/f)	Age (yrs)	Weight (kg)	Height (m)	BMI (kg/m^2)	Diet duration (wks)	PA duration (time/wks – min)	PA intensity (%VO_2max)	Kcal /day	VO_2max (ml/kg/min)	Hormones	Metabolites	Anthropometric measures
Heilbronn (2009)	Diet + physical activity (weight loss)	20/26	37 ± 2	81.8	1.78	27.5						Insulin	Glucose	Fat mass, body weight, BMI
	CR						24			-25%				
	CREX						24	5-45	69	-12.5%	35			
	LCD						10			890				
Kardinaal (2015)	Diet (weight gain)	10/0	42.5	78.4 ± 8		24.1 ± 1.8	4			+1300				Fat mass, body weight, BMI

In Figure 15 the simulations and the experimental measures are shown for weight change, insulin and glucose blood concentrations.

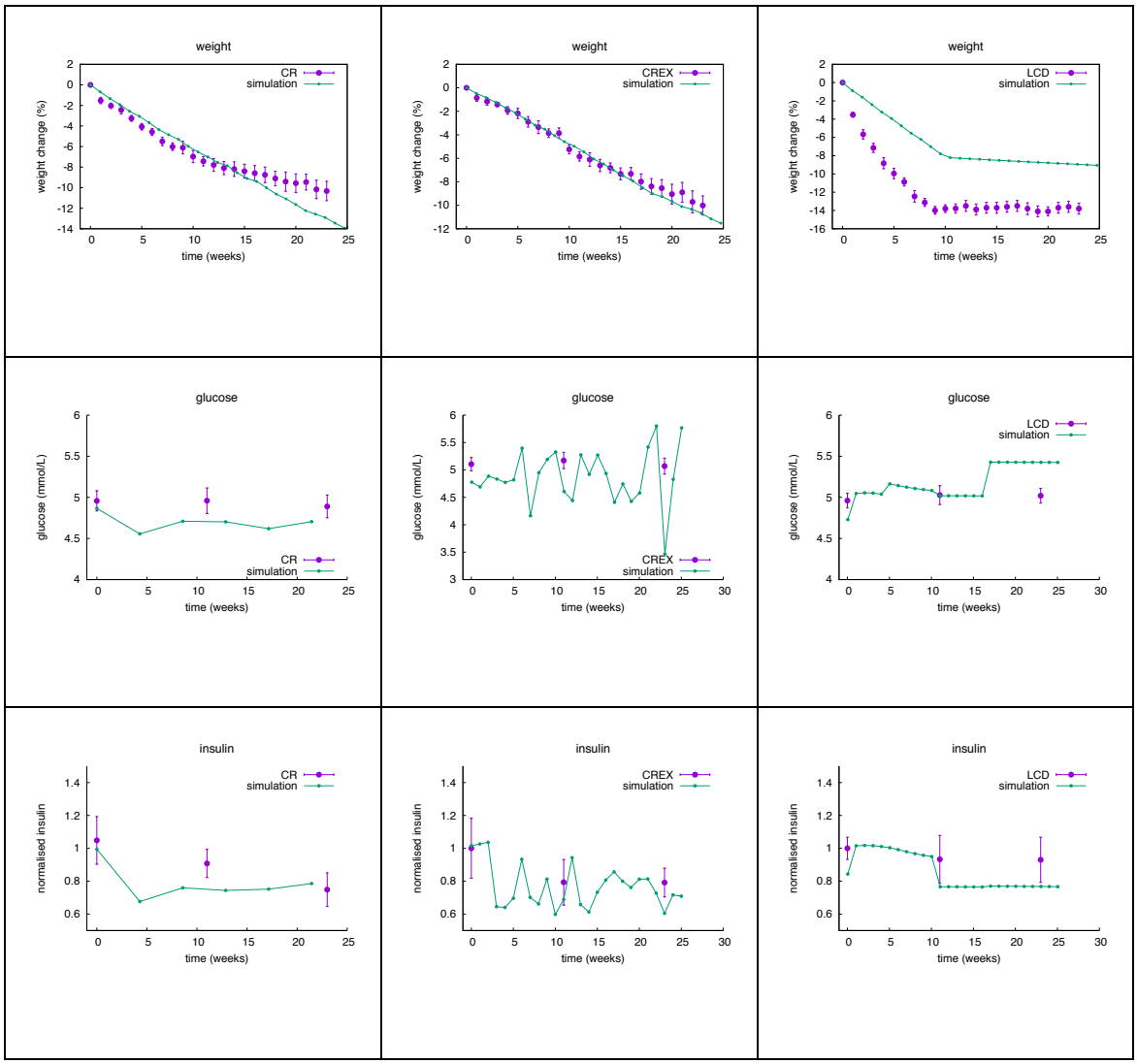


Figure 15 Diet - weight loss. Upper panels show the percentage weight loss by groups: CR (left upper panel), CREX (central upper panel), LCD (right upper panel). Central panels show the glucose concentrations for the three groups: CR (left central panel), CREX (central panel), LCD (right central panel). Lower panels show the insulin concentrations for the three groups: CR (left lower panel), CREX (central lower panel), LCD (right lower panel). Insulin data and glucose (CR) are normalised with respect to the initial value. Experimental measures are expressed as mean +- SEM. Data from Helbronn et al. (Heilbron, 2009).

A perfect match between model predictions and experimental data was observed for weight change in CR and CREX, whereas the weight change is underestimated for the LCD protocol. Probably the very low calorie diet is not properly modeled by Westerterp

regression equations (Westerterp, 1995) since physiological mechanisms other than those related to a 'normal' diet are involved in this kind of 'extreme' slimming. However, for what concerns MISSION-T2D purposes, we can assess that the LCD case is not very relevant. A good agreement between measured data and model simulations can be seen as regards glucose (panels in the central row) and insulin blood concentrations (bottom panels) for all the experimental protocols CR, CREX and LCD.

Kardinaal and colleagues in (Kardinaal, 2015) studied metabolism homeostasis in different condition after a 4 weeks high-fat high-calorie (HFHC) diet intervention. For MISSION-T2D purposes, we took into account the data regarding the responses to the tests of 10 healthy men. These subjects consumed an HFHC diet for 4 weeks, consisting of a fixed amount of commercially available foods high in fat, sugar, or both. The nutritional composition of HFHC diet resulted in a daily surplus of 1300Kcal (138g carbohydrates, 31g protein, 63g fat).

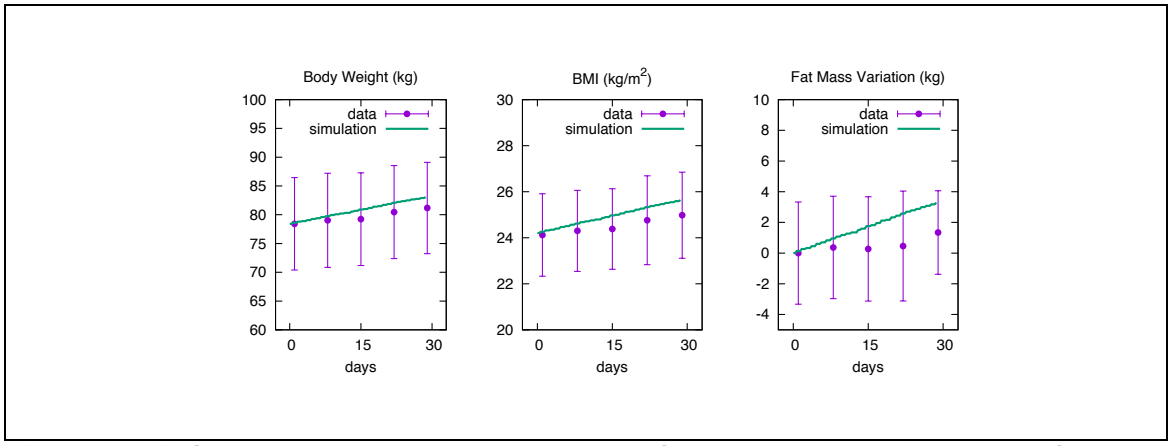


Figure 16 HFHC diet, i.e., weight gain. Body weight (left panel), BMI (central panel) and fat mass (right panel) variation with respect to the initial value. Data from Kardinaal et al. (Kardinaal, 2015).

Here we report the comparison between the simulated and the experimental data regarding body weight, BMI and FM changes after the 4 weeks HFHC diet. Results are plotted in Figure 16. A good concordance between model predictions and measured values can be seen for all the observed variables. Namely, as reported in (Kardinaal, 2015) and already mentioned above, the study demonstrates that the 4 weeks hyper caloric intervention induced a series of anthropometric and metabolic changes related to processes of adipose tissue mass and function in healthy subjects (Figure 16).

6 Deliverable conclusions

We have compared the behaviour of the MISSION-T2D model with experimental data over a wide range of conditions and found that altogether it performs reasonably well. Some aspects could be improved by carefully "tune" certain parameters, and this is an activity that is planned for the close future.

7 Appendix: List of abbreviations used

Table 4 List of abbreviations.

PA	Physical Activity
OGTT	Oral Glucose Tolerance Test
OLTT	Oral Glucose Tolerance Test
BMI	Body Mass Index
SEM	Standard Error of the Mean
STD	Standard Deviation
UT	Untrained individual
T	Trained individual
CR	Calorie Restriction
CREX	Calorie Restriction through Structured Exercise
LCD	Low Calorie Diet
USCF	United States Cycling Federation
TEF	Thermic Effect of Food
AEE	Activity Energy Expenditure
FM	Fat Mass
REE	Resting Energy Expenditure
IFNg	Interferon-gamma
IL-1b, IL-6, IL-10, IL-12, IL-18	Interleukins, signalling molecules
Lep, LEP	Leptine
TNFa	Tumor Necrosis Factor alpha

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