MISSION-T2D

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

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Deliverable 3.5

Report on the integration of stress induced inflammation model to the overall workflow

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Responsible Author	Name	Pietro Lio	Partner	UniCAM
	Email	pl219@cam.ac.uk		

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Executive Summary	In this task, we describe the extension of the model first presented in D3.1 that is used in the integrated model workflow (WP6). The extension consisting in taking into account the effects of gut microbiota to inflammation and therefore to beta cell function.
Keywords	mTOR, inflammation, gut microbioma, butyrate

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1 Introduction

In this deliverable we describe the work done in updating a new ODE system model for diabetes incorporating many aspects of published models, which includes inflammation and incorporates the effects of the gut microbiota.

1.1 Postprandial inflammation, obesity

Bacterial endotoxin is a potently inflammatory antigen that is abundant in the human gut. Endotoxin circulates at low concentrations in the blood of all healthy individuals, although elevated concentrations are associated with an increased risk of endotoxemia and insulin resistance (IR). Postprandial hyperlipidemia with accumulation of remnant lipoproteins is a common metabolic disturbance associated with atherosclerosis and vascular dysfunction, particularly during chronic disease states such as obesity, the metabolic syndrome and, diabetes. Remnant lipoproteins become attached to the vascular wall, where they can penetrate intact endothelium causing foam cell formation. Postprandial remnant lipoproteins can activate circulating leukocytes, upregulate the expression of endothelial adhesion molecules, facilitate adhesion and migration of inflammatory cells into the subendothelial space, and activate the complement system. This process is sketched in figure Figure 1.

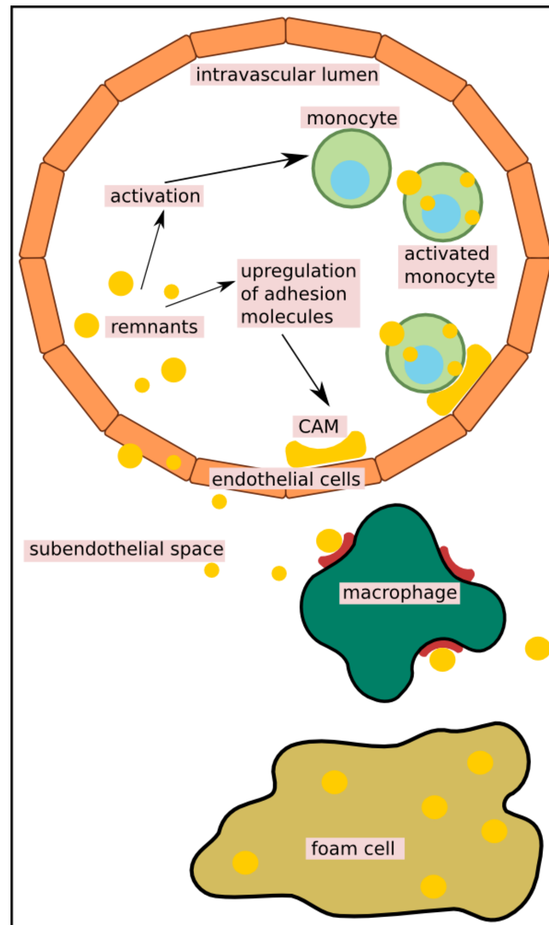

Figure 1

Recent works have shown that gut bacteria can initiate the inflammatory state of obesity and IR through the activity of lipopolysaccharide (LPS), a component of the Gram-negative bacteria cell wall that is present in large quantities in the human gut and which can trigger the inflammatory process by binding to specific complex at the surface of innate immune cells. Consequently, high concentration of LPS increases adipose tumor necrosis factor TNF-α and interleukin IL-6 concentrations and promoted IR. A high-fat, high-carbohydrate meal induced significant postprandial plasma LPS elevation, accompanied by an increased nuclear factor-κB (NF-κB), and suppressor of cytokine signaling-3 (SOCS-3), an adipokine involved in IR. In concordance with these data, some studies have shown that a high-fat meal leads to an increase in postprandial endotoxemia. Indeed, there is evidence that metabolic plasma LPS levels are modulated by food content: the higher the fat content, the higher the concentration of plasma LPS. On one hand, small amounts of LPS are absorbed from the gut in healthy individuals; on the other hand, obese subjects tend to the consumption of a

high-fat diet, and gram-negative bacteria in gut microbioma is higher in obese subjects than in lean subjects. Thus, these conditions would enhance the translocation of endogenous LPS from the gut during fat absorption, which would lead to the low-grade inflammation observed in these patients.

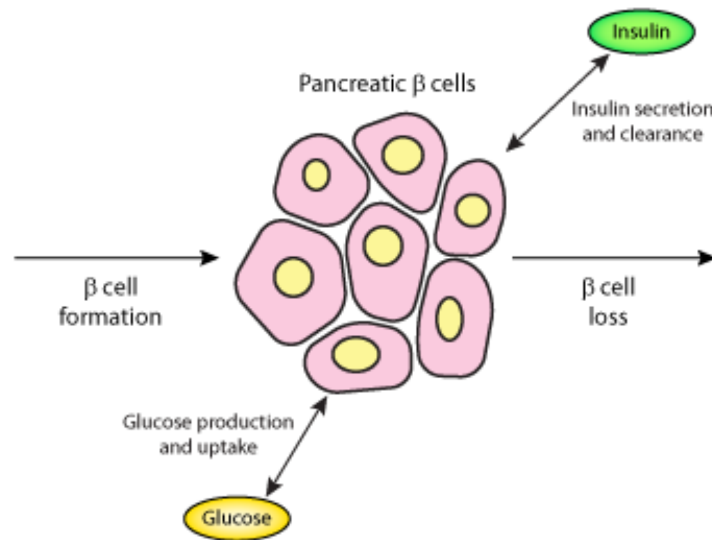

Figure 2. Relationship between beta cells and glucose/insulin

The first important step is coupling the effect of gran-negative bacteria to the postprandial inflammation; the second aspect consists in identify the role of the microbioma in increasing IR. Taken together, the above studies support the concept that gut microbioma, during food ingestion, affects plasma endotoxin levels contributing to the postprandial inflammatory state, and that low-grade endotoxemia plays a key role in the pathogenesis of obesity-associated inflammatory state.

2 The model

To address the problem, we propose to integrate the βIG model introduced by Brian Topp [1] (firstly described in deliverable D3.1 and partially adopted to be integrated to the overall MISSION-T2D workflow) with two coupled ODEs for the insulin resistance and mTOR and the equations mimicking the postprandial inflammation and the gram-negative bacteria induced endotaxemia:

$$G'(t) = R_0 - G(t)\left(E_{G0} + \frac{S_I\ I_{ns}(t)}{1+I_R(t)}\right) + g\ F(t)$$

$$I'_{ns}(t) = \frac{\sigma\ G(t)^2 \beta_f(t)}{\alpha + G[t]^2} - k_I\ I_{ns}(t)$$

$$\beta(t) = \left[r_1\ G(t) - r_2\ G(t)^2 - d_0\right]$$

$$I'_R(t) = qI_{ns}(t) + m\ M_{TOR}(t) + k_b\ B(t) - i_0\ I_R(t)$$

$$M'_{TOR}(t) = c_1\ G(t) + c_2\ \frac{I_{ns}(t)}{I_R(t)+1} - m_0\ M_{TOR}(t)$$

$$F(t) = \sum_i F_i\ \Theta(t - T\left\lfloor \tfrac{t}{T} \right\rfloor - t_i)\ \Theta(t_i + \Delta t + T\left\lfloor \tfrac{t}{T} \right\rfloor - t)$$

$$B'(t) = \left\{\frac{2}{1+\exp[B_0 - b\ F(t)]} + 1\right\} B(t)\ \log\left(\frac{B(t)}{L_B}\right)$$

The first three equations are derived from the model in [1]. The disruption of normal glucose dynamics is central to the pathogenesis of both diabetes type 1 and type 2. Despite the spatial complexity of its distribution, however, it has been demonstrated that when considering an appropriate time-scale (days to years), glucose dynamics can be accurately represented via a single-compartment model. This assumes an instantaneous glucose distribution relative to the time-scale of its measurement, implying that blood glucose levels depend only upon its production and uptake. The rates of its production and uptake depend linearly on blood glucose and insulin levels, relationships, which have been well established via the glucose clamp technique and hyperinsulemic clamp studies.

In the first equation, the parameter R_0 is the net rate of production at zero glucose, E_{G0} is the total glucose effectiveness at zero insulin, and S_I is the total insulin sensitivity.

Differently from the work in [1], the first equation has been modified by including the extra term g F(t) which mimics the increase of glucose in the blood at rate g after the food ingestion F(t). Similar assumptions can be made when considering blood insulin dynamics, leading to a single-compartment equation dependent on the rates of its secretion and clearance. Given the slow time-scale of the model we assume a clearance rate proportional to its concentration, representing its continuous uptake via

the kidneys, liver and peripheral insulin receptors. The secretion function of insulin, by contrast, has been demonstrated to be a sigmoidal function of glucose concentration. While Topp et al. also assumed it to be proportional to β-cell mass, one of the improvements made by Nelson [2] was to point out that its secretion is in fact only proportional to the number of functioning β-cells (see Figure 2). The parameter k is the insulin clearance constant, σ is the maximal rate at which each of the functional β-cells secretes insulin, $\frac{G2}{\alpha + G2}$ is a Hill function describing a sigmoid ranging from 0 to 1, reaching half its maximum at $G = \alpha^{1/2}$. In the model of Brian Topp [1] and Patrick Nelson [2], hence assume that the rate of β-cell formation is equal to their rate of replication. Their replication rate, however, is nonlinearly dependent on plasma glucose concentrations. At normal levels, as the glucose concentration increases, so does the β-cell replication rate, yet above a certain threshold (> 400mg/ml) the replication rate decreases.

In the third equation, the behaviours β-cells are modelled via a simple second-degree polynomial, using three different rate constants: r_1, r_2 and d_0. Similarly, apoptotic death of β-cells have also been shown to vary non-linearly with glucose levels, although remain consistently low above glucose concentrations of around 110mg/ml.

The forth equation describes the dynamics of the insulin resistance in function of the insulin in the blood and the overproduction of mTOR. The fifth equation describes the mTOR inflammatory marker overproduction as a function of glucose, insulin and insulin resistance. The sixth equation takes into account the ingestion of food. At each meal i, which occurs at time t_i and ends after a time Δt, the quantity of food ingested is F_i. The parameter T refers to the periodicity of the process, which in this case is fixed at 24 hours. In order to consider all the meals in the period T describes, on the right hand side, we sum over the index i. A product of step functions gives the dynamics of a meal and the periodicity of the process is guaranteed by the term $T\left\lfloor\frac{t}{T}\right\rfloor$ which appears with opposite sign in respect to the time t. The last equation describes the population of gram-negative bacteria producing inflammation, which goes as a Gompertz function with a rate modulated by the quantity of food. The limiting value of the bacteria population is given by the capacitive parameter L_B. The parameter F_0 represents an average quantity of food ingested per meal and is chosen bigger than 1. If the food ingested passes the threshold given by the average quantity F_0, the exponential

function in the denominator of the modulating rate decreases, and the rate of growth of bacteria increases causing further inflammation. Primary results show that the system behaves properly when parameters are chosen in the rage representing healthy subjects and obese subjects.

3 Inflammation and T2DM

Considering a steady influx and efflux of macrophages through a small, fixed volume of the pancreas, we can assume, for the development of type two diabetes, that some harmful influence has disrupted normal metabolic or immunologic function and liberated some β-cell antigenic proteins. The antigenic proteins will be engulfed and processed by the macrophages, before being presented via their MHC class II molecules. As this occurs, the macrophages are said to become "activated". The total population of macrophages within the considered volume is hence a sum of both the inactive and active macrophages.

Aside from the difficulty in quantifying the rates of change in β-cell formation through neogenesis and trans-differentiation, a growing body of experimental evidence suggests that they make negligible contributions (other than during development). In the model of Topp et al. [1], the loss of β-cells is due only to apoptotic death, however, it is not the only source of functional loss. The loss of ability to produce insulin is taken into account in [2], and is assumed to be due to Th1 and cell infiltration. This is consistent with the experimental findings, as mentioned in next section, where it was shown that as the ratio of Th17/Treg cells increases in pro-inflammatory adipose tissue, this shift stems from an increase in Th1 cells, which the Treg cells fail to regulate. An additional consideration made by [2], which is also incorporated in the present model, is the possibility that the non-functional β-cells may only be temporarily dysfunctional and may regain their ability to secrete insulin after some time, t.

The set of equations describing how the immune response influence the activity of functioning β-cells is:

$$\beta'_f(t) = \left[r_1\, G(t) - r_2\, G(t)^2 - d_0\right] \beta_f(t) - \frac{\delta_1 T(t)\beta_f(t)}{\tau + T(t)} - c_2\, M_{AC}(t)\, \beta_f(t) + \epsilon\beta_{nf}(t)$$

$$\beta'_{nf}(t) = \frac{\delta_1\, T(t)\beta_f(t)}{\tau + T(t)} + c_2\, M_{AC}(t)\beta_f(t) - \epsilon\, \beta_{nf}(t) - d_1\left[\gamma + c_3\, M_{AC}(t)\right]\beta_{nf}(t)$$

$$M'(t) = a - g\, A(t)M(t) - c_1\, M(t) + (b + k)M_{AC}(t)$$

$$M'_{AC}(t) = g\, A(t)\, M(t) - k\, M_{AC}(t)$$

$$A'(t) = n\left\{d_0\, \beta_f(t) + d_1\left[\gamma + c_3\, M_{AC}(t)\right]\beta_{nf}(t)\right\} - A(t)\delta_2$$

$$T'(t) = h\frac{CD44}{CD44_0}T(t)\, M_{AC}(t) - d_2\, T(t)$$

The first two equations take into account the dynamics of functioning and non functioning β-cells, respectively. These two equations are used in place of the equation for all the β-cells used in [1] and adopted in the previous section. By contrast to the equation for $\beta'_f(t)$ in [2], however, we shall consider the effects of the cytokines released by the activated macrophages. These will increase both the rates at which β-cells lose their functionality as well as the rate at which non-functioning β-cells are destroyed. The parameter d_0 is the β-cell death rate at zero glucose, while r_1 and r_2 are β-cell replication rate constants. The process of non-functional β-cells regaining their functionality is described with a simple linear function and ε is the rate at which the process takes effect. The parameter δ_1 is a constant representing the maximal rate at which Th17 cells may cause functional β-cells may lose their functionality, τ represents the half-saturation rate for this process and c_2 corresponds to the cytokine-induced loss of functionality. Similarly, in the second equation for the non-functional β-cell population, the parameter d_1 marks the elevated rate at which the non-functioning β-cells undergo apoptosis, γ represents the rate at which cytotoxic CD8+ T-cells may directly destroy them and c_3 characterises the rate of cytokine-induced destruction. The third and fourth equations describes the macrophages entering the pancreas and their activation/deactivation process as function of antigenic proteins. The parameters a and c_1 denote the steady influx and efflux rates of macrophages, respectively, g is their rate of activation, as stimulated by the presence of the β-cell antigenic proteins, A. Once they become activated they then release pro-inflammatory cytokines, which in turn recruit more macrophages at a rate denoted by b. The parameter k is then the rate at which the macrophages may become deactivated.

The fifth equation mimics the dynamics of β-cell antigenic proteins triggering the immune response attack. The deaths of β-cells leads to the release of β-cell antigenic proteins, therefore the quantity of antigens released is given by the functioning β-cells undergo apoptosis and by non-functioning β-cells, either by apoptosis or necrosis. The parameter n determines how many antigenic proteins in average are released by each β-cells. The parameter δ_2 is the rate of depletion of antigenic proteins. The sixth equation consider the concentration of pro-inflammatory pathogenic Th17 cells, T. Th17-lymphocytes naturally circulate around the body and are stimulated to proliferate when they recognise their complementary MHC-peptide complexes, which are present upon activated macrophages. We therefore assume that their rate of formation within a small, fixed volume of the pancreas is proportional to both their concentration within the tissue and the concentration of activated macrophages. Here, the parameter h represents the Th17 cell proliferation rate when it recognises an MHC-peptide complex on a macrophage. d_2 is the removal rate of T-lymphocytes, which will occur at a constant rate in the absence of a stimulation.

Taking the equations for the dynamics of the glucose and insulin driven by food ingestion and β cells and perturbed by inflammation and bacteria in the gut together with the inflammatory response, the full system of ODEs is:

$$I'_{ns}(t) = \frac{\sigma\ G(t)^2 \beta_f(t)}{\alpha + G[t]^2} - k_I\ I_{ns}(t)$$

$$G'(t) = R_0 - G(t)\left(E_{G0} + \frac{S_I\ I_{ns}(t)}{1+I_R(t)}\right)$$

$$\beta'_f(t) = \left[r_1\ G(t) - r_2\ G(t)^2 - d_0\right]\beta_f(t) - \frac{\delta_1 T(t)\beta_f(t)}{\tau+T(t)} - c_2\ M_{AC}(t)\ \beta_f(t) + \epsilon\beta_{nf}(t)$$

$$\beta'_{nf}(t) = \frac{\delta_1\ T(t)\beta_f(t)}{\tau+T(t)} + c_2\ M_{AC}(t)\beta_f(t) - \epsilon\ \beta_{nf}(t) - d_1\left[\gamma + c_3\ M_{AC}(t)\right]\beta_{nf}(t)$$

$$M'(t) = a - g\ A(t)M(t) - c_1\ M(t) + (b+k)M_{AC}(t)$$

$$M'_{AC}(t) = g\ A(t)\ M(t) - k\ M_{AC}(t)$$

$$A'(t) = n\left\{d_0\ \beta_f(t) + d_1\left[\gamma + c_3\ M_{AC}(t)\right]\beta_{nf}(t)\right\} - A(t)\delta_2$$

$$T'(t) = h\frac{CD44}{CD44_0}T(t)\ M_{AC}(t) - d_2\ T(t)$$

$$I'_R(t) = qI_{ns}(t) + m\ M_{TOR}(t) + k_b\ B(t) - i_0\ I_R(t)$$

$$M'_{TOR}(t) = c_1\ G(t) + c_2\ \frac{I_{ns}(t)}{I_R(t)+1} - m_0\ M_{TOR}(t)$$

$$F(t) = \sum_i F_i\ \Theta\left(t - T\left\lfloor\frac{t}{T}\right\rfloor - t_i\right)\ \Theta\left(t_i + \Delta t + T\left\lfloor\frac{t}{T}\right\rfloor - t\right)$$

$$B'(t) = \left\{\frac{2}{1+\exp[B_0 - b\ F(t)]} + 1\right\}B(t)\ \log\left(\frac{B(t)}{L_B}\right)$$

4 Gut microbioma: the butyrate

Chronic low-grade inflammation is associated with an increased risk of insulin resistance and diabetes type 2. For this reason strategies to suppress low-grade inflammation as preventive measure for these chronic diseases are relevant to investigate, and consequently, food and food-derived substances receive increasing attention as potential factors that can modulate cells or cell functions that play a role in immunological processes. In recent studies addressing the problem of food-derived inflammation, results indicated that in healthy young subjects, an evening meal rich in non-digestible carbohydrate prevented the glucose-induced postprandial rise in plasma IL-6 and TNF-α concentrations. One of the proposed factors that could explain these effects is the increase in short-chain fatty acids (SCFA) acetate, propionate and

butyrate which are formed in the gastrointestinal tract of mammals as a result of anaerobic bacterial fermentation of undigested dietary components, and is avidly absorbed by the colonic epithelium. SCFA are rapidly absorbed from the colonic lumen and partly metabolised by colonic epithelial cells. SCFA, and especially butyrate, have for long been in the centre of interest for modulating inflammatory responses in the colonic epithelial cells and results of these studies indicate beneficial effects. Therefore, it seems worthwhile to explore whether SCFA could also affect systemic inflammation. This is especially interesting because recent studies suggest that obesity-induced inflammation is partly antigen dependent. Information about, for instance, the capacity of SCFA to reduce activation of T-cells by monocyte-presented antigens is thus highly relevant in this context. Postprandial, an inflammatory response to high-fat meals and rapidly digestible carbohydrates has been found in plasma even in normal-weight subjects, which was aggravated in obese persons. Repeated exposure to postprandial inflammatory responses is suggested to contribute to systemic inflammation and type 2 diabetes. Recent data illustrate that SCFA can even influence chemotaxis of immune cells, but its effect depends on the type and concentration of the SCFA as well as on the type of species and immune cell type. Butyrate seems to be the most potent inhibiting factor for chemotactic effects on human monocytes. Probably more effects are present but remain to be identified.

Hypertrophied adipocytes secrete abnormally high amounts of chemoattractants, which causes accumulation of macrophages in adipose tissue. These adipose tissue macrophages are considered the major source of pro-inflammatory cytokines. The pro-inflammatory T-cell populations (cytotoxic T-cells and Th17 cells) were increased in human obese adipose tissue, while anti-inflammatory T-cell populations (Treg) were decreased. In humans, a relative lack of Th17 cells in obese visceral tissue has been proposed to lead to an unfavourable Th17/Treg balance which has been shown to positively correlate with plasma IL-6 and TNF-α concentrations. Therefore, the failure of Th17 cells to counterbalance the effect of anti-inflammatory Treg cells might contribute to inflammation. Butyrate was recently demonstrated to reduce LPS migration and decreases the expression of the adhesion molecules ICAM which indicates that butyrate might prevent antigen-induced T-cell activation by preventing cell adhesion which is essential for antigen presenting cell (APC)-induced activation. By preventing chemotaxis and cell adhesion, SCFA might prevent infiltration of immune cells in peripheral tissues and can have a protective effect against systemic inflammation. As

previously said, TGF-β2 is a transcriptional target of CD44 signalling, and hence, butyrate inhibition of CD44 variant expression causes a reduction of TGF-β.

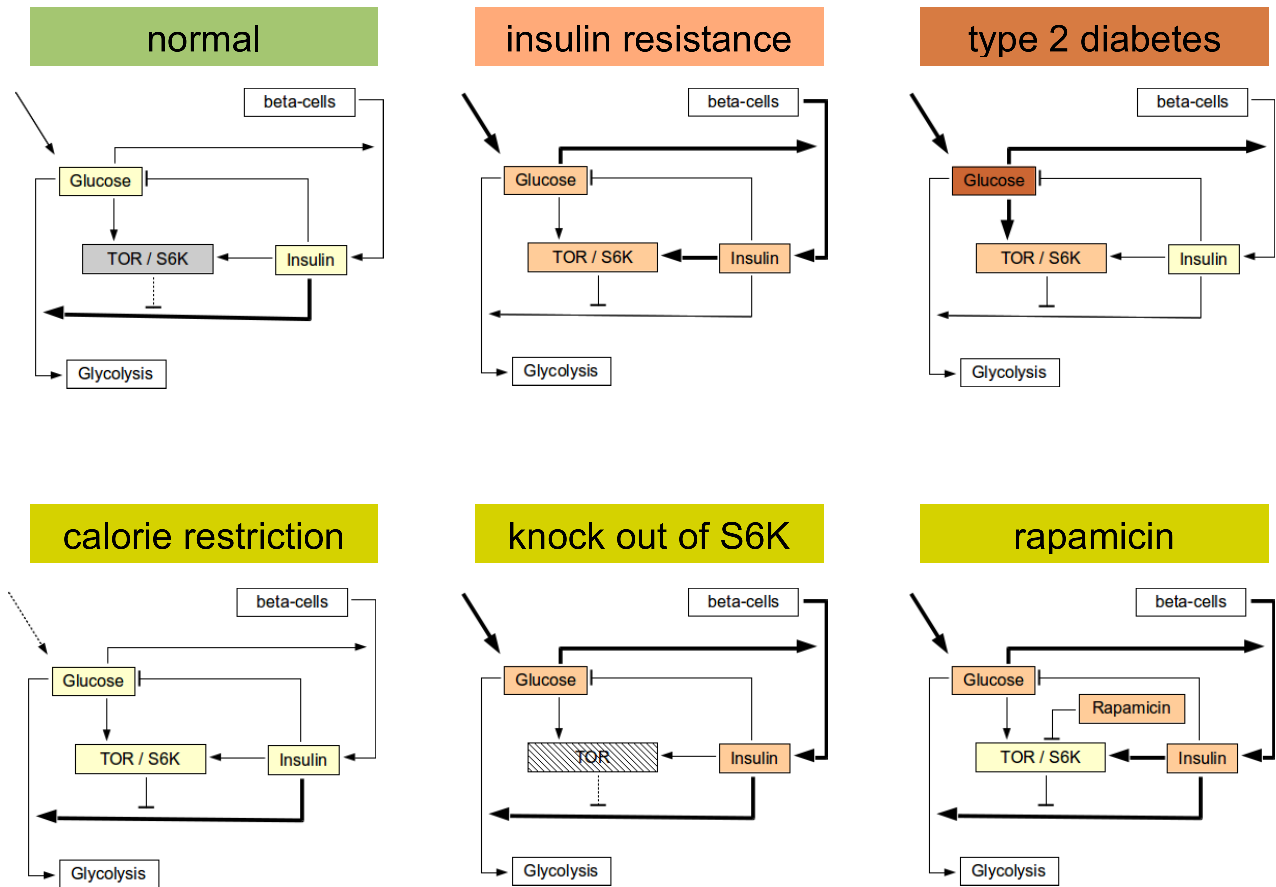

Figure 3 mTOR and CD44

The differentiation factors of Th17 cells requires that TGF-β, an anti-inflammatory cytokine, works with IL-6, a pro-inflammatory factor, to induce this highly pro-inflammatory subset of T cells. Consequently, on one side, butyrate induces a reduction Th17 and on the other side, it promotes extrathymic differentiation of Treg cells. The two phenomena induce a change in the ratio Th17/Treg, which can re-establish the immune homeostasis.

5 Conclusion

Modelling the effects of the butyrate produced in the gut as anti-inflammatory and reducing the development of IR is a fundamental step to further investigate the relation

between inflammation and T2DM. The project is moving in this direction.

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