MISSION-T2D

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

Work Package 5

Deliverable 5.3

Report on validation and refinement of the physical activity module in the overall workflow

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Executive Summary	This document was originally supposed to include the results of Task 5.4 (Validation and refinement of the model in the overall workflow). However, as reported in the DoW, Task 5.4 will start at PM 24 and will end at PM 36. Due to this material error in indicating the expected date of delivery for the present document (PM24 in the DoW) we are unable to include the planned content of the deliverable, which should have referred to the model validation and refinement. We have however decided to use this opportunity to present an update about the activities carried out in Task 5.3 (*Integration in the overall workflow*) and a description of the activities that will be carried out in Task 5.4, showing also an important aspect concerning the integration with a USFD project that would allow the collection of experimental data to be used for the validation of the Mission-T2D physical activity model. Please note also that for its present confidential nature, this deliverable will be disseminated only within the consortium. The final version of Deliverable 5.3 will be produced at the end of Task 5.4 (PM 36) and publically disseminated as expected.
Keywords	Physical activity, mathematical model, heart rate, work rate.

Contents

1 Deliverable description

This document was supposed to include the results of Task 5.4 (Validation and refinement of the model in the overall workflow). However, as reported in the DoW, Task 5.4 will start at PM 24 and will end at PM 36. Due to this error in reporting the date of delivery for the present document (PM24 in the DoW) with respect to the actual content of the deliverable, i.e., validation and refinement, we are unable to include the planned content of the deliverable. Thus, in this document we have included an update of the results of Task 5.3 (Integration in the overall workflow). In particular, the following sections and subsections have been updated with respect to the previous deliverable: 2.3.2, 2.4 and 2.5.4.

We have also included in this report the description of the future work highlighting an important new element with respect to the original DOW. Thanks to an ongoing collaboration with the clinical group in Sheffield, it has been possible to organise, leveraging also on parallel funding that has been locally granted in the meanwhile, an ad hoc experimental campaign, which will allow to collect data for the direct validation of the WP5 model. After significant effort needed for the definition of the protocol, partner USFD has now been granted local ethical approval to start a project titled “Validation and Feasibility Study of Physical Activity Monitors in Diabetes” which includes also patients with T2D. The outcome of this project, that represents a significant added value for Mission-T2D, allowing to directly experimentally validate the relationships included in WP5 model, will be included in the final version of Deliverable 5.3, that will be produced at the end of Task 5.4 (PM36). Please note also that for its present confidential nature, this deliverable will be disseminated only within the consortium. The final version of Deliverable 5.3 will be publically disseminated as expected.

2 Deliverable results

2.1 Modelling the exercise effects on the metabolic system

As reported in Deliverable 5.1, exercise affects different variables related to the Type 2 diabetes (T2D) onset and progression. Briefly, physical exercise has been shown to boost the availability of glucose transporter-4 (GLUT-4) in muscle cells, both by translocation to the cell membrane and by increased transcription. These changes have been shown to be associated with an increase in both insulin sensitivity and

insulin-independent glucose uptake. The pathways of exercise-induced translocation and augmented transcription have not been entirely elucidated yet. Nevertheless, muscle fibres contractions have been proven to be at the basis of these changes, promoting an anti-inflammatory action. Thus, to give a comprehensive description of the effects of exercise on T2D, two complementary aspects need to be modelled: the **metabolic** and the **inflammatory** processes.

A few mathematical models of physical exercise have been proposed. Some of them deal with the description of the metabolic pathways as limited to the muscles (Korzeniewski et al. 1996; Lambeth et al. 2002). Others, i.e., the models of Breton, Derouich and Dalla Man (Breton 2008; Derouich et al. 2002; Dalla Man et al. 2009), describe the metabolic phenomena looking at the dynamics of glucose and insulin at the whole body level. More in details, Derouich et al. (Derouich et al. 2002), starting from a minimal model representation, developed a model that simulates the effect of physical activity on the dynamics of glucose and insulin. The effectiveness of this model has been confirmed and expanded by the results of Breton (Breton 2008), who proposed a parsimonious model of physical exercise. Breton's model links the change in insulin action and glucose effectiveness to the changes in heart rate (HR). In a subsequent work, Dalla Man et al. (Dalla Man et al. 2009) proposed and tested three possible extensions of the Breton's model (Breton 2008). Recently, Kim et al. (Kim et al. 2007) proposed and validated a model incorporating cellular metabolism of tissue/organs system in the whole-body responses of glucose and insulin. It is important to highlight that none of the existing mathematical models provides a description of the mechanisms regulating the inflammatory processes.

RESULT 1: FEW MATHEMATICAL MODELS OF EXERCISE HAVE BEEN PROPOSED SO FAR, AND NONE OF THEM DESCRIBES THE INFLAMMATORY PROCESS. AMONG THESE MODELS, THE KIM MODEL IS THE MOST SUITED STARTING POINT FOR THE DEVELOPMENT OF THE OVERALL MISSION-T2D MODEL.

2.2 Physical exercise representation in the Kim model

The mathematical model of the whole-body metabolism proposed and validated by Kim et al. (Kim et al. 2007) 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 balances and major cellular metabolic reactions, which lead to substrates (glucose, lactate, pyruvate, alanine, fatty acids and glycerol) “conversion” in ATP. The glucagon-insulin system is incorporated into the whole-body model to predict hormonal changes during exercise (see Figure 1).

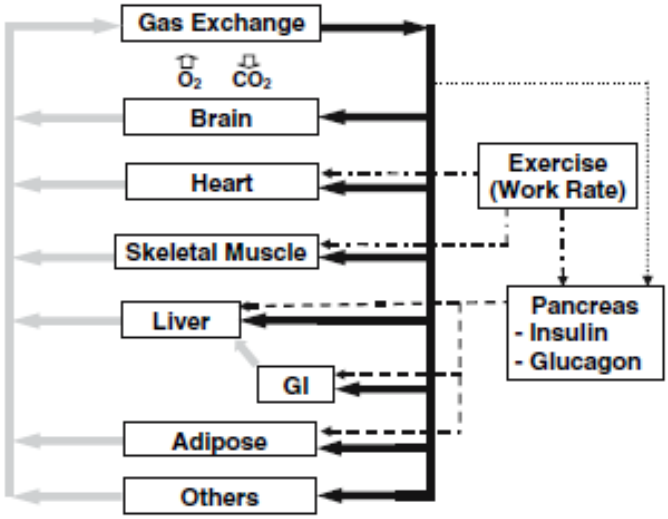

Figure -1 Whole body system diagram of the Kim model (Kim et al. 2007). Each tissue is connected via the blood supply that carries substrates to organs/tissues in arterial blood (black solid arrows). Venous blood (grey solid arrows) leaving these tissues/organs takes away by products and becomes arterial blood to re-start the circulation after releasing carbon dioxide and taking up oxygen in lungs (gas exchange).

Exercise sends neuroendocrine signals (dash-dot arrows) to heart, skeletal muscle and pancreas. In addition, feedback signal (dotted arrow) from the arterial glucose concentration can be sent to pancreas. Finally, glucagon–insulin ratio signal (dash arrow) from pancreas is sent to liver, GI (gastrointestinal) tract and adipose tissue.

The basic hypothesis behind the model is that exercise-induced change in the hormone epinephrine (described by eq. (1)) 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 arterial epinephrine level CE(t) varies in time with a step increase in the work rate WR, which represents the modelled exercise intensity, according to the following equation:

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

where ω(WR) represents a steady state gain in response to WR, and τ_E is a time constant, which accounts for the epinephrine dynamics.

During the exercise, the whole body glucose turnover rate increases by three to four times according to the exercise intensity. According to the "Glucose Shunt" theory (Bergman et al. 1999), most of the increased glucose utilization is shunted to the essential tissue/organ during exercise (i.e., skeletal muscle), making glucose uptake by skeletal muscle consequently increase by almost ten times. Conversely, the plasma glucose utilization in other tissues is kept almost constant. On this ground, exercise is implemented in the Kim model as an increase in ATP utilization rates in both the heart and the skeletal muscles.

According to Cabrera et al. (Cabrera et al. 1999), the ATP hydrolysis rate in the skeletal muscles is a function of WR:

$$\Phi_{m,ATP\rightarrow ADP}(WR) = \Phi_{m,ATP\rightarrow ADP}(rest) + \gamma_m \cdot WR \qquad (2)$$

where γ_m is a conversion factor relating regional blood flow to oxygen uptake in skeletal muscle (Cabrera et al. 1999; Kim et al. 2007).

RESULT 2: THE INPUT OF THE KIM MODEL OF THE METABOLIC PROCESSES DURING EXERCISE IS REPRESENTED BY THE WORK RATE. THE INTEGRATION OF THE WP5 MODEL IN THE OVERALL MODEL WILL HENCE REQUIRE ITS INPUT TO BE LINKABLE WITH THE WORK RATE.

2.2.1 Evaluation of suitability of the Kim model for WP5 purposes

MISSION-T2D aims at developing and validating an integrated, multilevel patient-specific model for the simulation and prediction of the **metaboli**c and **inflammatory processes** in the onset and progression of T2D. The Kim model is a validated model of glucose homeostasis during exercise that links cellular metabolism to the whole-body responses and incorporates the effects of hormonal control on fuel metabolism of various tissues/organs. The advantage of this model is that it provides dynamic

predictions of the metabolite concentrations and of the flux rates in various tissues. However, as previously mentioned, the model does not include any description of the modification that the exercise induces on inflammatory processes, which is one of the MISSION-T2D aims. Thus, **a novel set of equations needs to be developed to link the two aspects**.

The input of the Kim model is the work rate, which is not an obvious quantity to measure or to estimate in order to describe the energy expenditure from the daily physical activity, therefore our choice for the inputs of the WP5 model is steps per day and heart rate, which account for the duration and the intensity of physical activity, respectively. Since both work rate and heart rate are indicators of exercise intensity, a correspondence between the two measurements needs to be established. It has to be noted, that, in terms of multi-scale modelling, heart rate and work rate are suitable inputs for whatever concerns a minutes/hour time scale. On the other hand, steps per day, which will not be an input to the Kim model equations, will provide the information needed to account for the inflammatory processes happening at the months-year time scale (Figure 2).

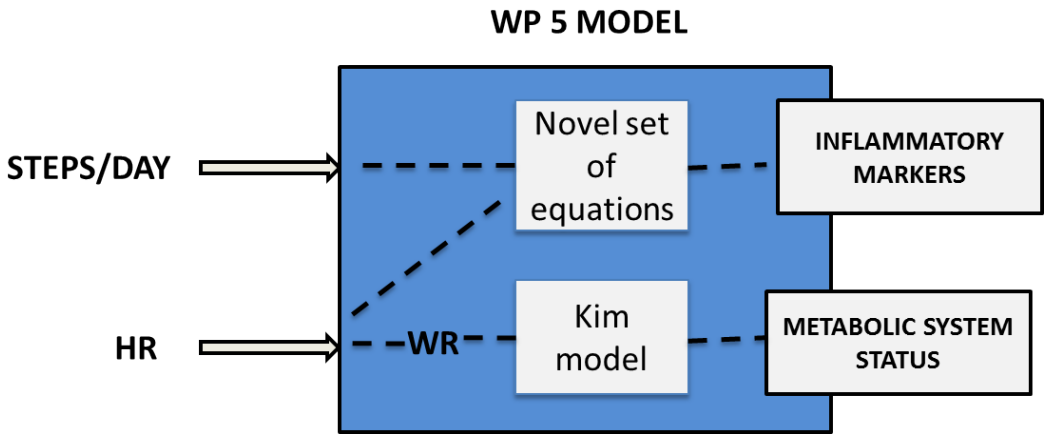

Figure -2 Integration of the input-output relationships of the WP5 model in the overall MISSION-T2D model.

A further obstacle toward the integration concerns the assumptions made in the Kim model about the exercise intensity. The modelled exercise is a moderate intensity exercise performed by a **healthy young man** at 60% of maximal oxygen uptake ($\dot{V}O_{2max}$), which corresponds to a WR = 150 watt. This WR is considered to be the one that does not exceed the lactate threshold of the subject. However, if we consider a typical subject taken from a population with T2D or at risk of developing T2D (e.g., obese, middle aged, etc.) a moderate intensity exercise performed at 60% $\dot{V}O_{2max}$

generally corresponds to WR = 55 watt (Sales et al. 2011). This value could likely exceed the lactate threshold of a specific subject. Thus, different assumption on exercise intensity should be made if an individual at risk of T2D is considered and the Kim model should be used only if the exercise considered is lower than the lactate threshold of the subject.

In this context, it must be recalled that, when setting a value for WR different from WR = 150 watt, particular attention needs to be given to rescaling the values of the parameters related to WR. In details, since the epinephrine secretion is a function of the exercise intensity, the parameters ω(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.

RESULT 3: THE WR ABSOLUTE VALUE, ITS TRANSLATION INTO HR, AND ITS RELATED PARAMETERS NEED TO BE TUNED ACCORDING TO THE CONSIDERED SUBJECT CHARACTERISTICS. IN ADDITION, THE MODELLED EXERCISE NEEDS TO BE A MODERATE INTENSITY EXERCISE THAT IS LOWER THAN THE LACTATE THRESHOLD OF THE SUBJECT.

2.3 Modelling the inflammatory processes

2.3.1 A mathematical model of Interleukin-6 dynamics during exercise

As a first step toward the complete description of the inflammatory processes, **we formulated and validated a new mathematical model of the Interleukin-6 (IL-6) dynamics during exercise**. Since IL-6 dynamics depends on the intensity of the exercise performed and considering that heart rate correlates well with the exercise intensity, the heart rate signal was used as input to the model.

As described in Deliverable 5.1, the 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 et al. 2005), which eventually induce a reduction of the insulin resistance (Hotamisligil et al. 1993). IL-6 has been identified as the first cytokine increasing in the circulation during exercise and stimulating the activation of an anti-inflammatory cascade (see Figure 3) given by the secretion of cytokine inhibitors (sTNF-R and IL-1ra) and of the cytokine IL-10 (Febbraio et al. 2004; Pedersen et al. 2005). Plasma IL-6 has been shown to augment

up to 100-fold depending on the exercise intensity and duration. The exercise-induced increase of plasma IL-6 is not linear over time: repeated measurements during exercise showed an increase of the IL-6 in plasma that happens in an almost exponential manner (Ostrowski et al. 1998). Furthermore, the peak level of IL-6 is reached at the end of the exercise, or shortly thereafter, and is then followed by a rapid decrease towards pre-exercise levels (Ostrowski et al. 1998).

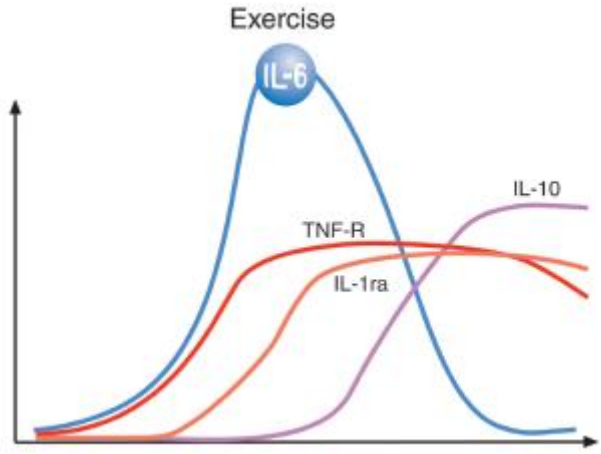

Figure -3 Cytokine responses to exercise (adapted from (Petersen et al. 2005)). IL-6 is the first cytokine increasing in the circulation during exercise. IL-6 provokes an anti-inflammatory cascade stimulating IL-1ra and IL-10 secretion.

The model that has been developed (Morettini et al. 2014 (a); Morettini et al. 2014 (b)) to integrate the above aspects is constituted by the following two non-linear differential equations:

$$\frac{dIL6(t)}{dt} = k_1 e^{\frac{Y(t)}{\tau}} - k_2 IL6(t) + \frac{Ra_{IL6}}{V} \qquad (3)$$

$$\frac{dY(t)}{dt} = -\frac{1}{T_{HR}}[(HR(t) - HR_b) - Y(t)] \qquad (4)$$

In eq. (3), IL6(t) represents the IL-6 concentration in the plasma compartment. In eq. (4), taken from Dalla Man et al. (Dalla Man et al. 2009), Y(t) is a delayed version of the suprabasal heart rate signal (HR(t)-HR_b), in which HR(t) indicates the heart rate signal, HR_b represents the basal heart rate (i.e., the heart rate value measured at rest) and T_{HR} represents a time-delay in the HR signal. The first nonlinear term on the right hand

side of eq. (3) accounts for the plasma IL-6 increase from its basal value in response to muscle contraction during exercise. This term is conceived as non-linearly dependent on a delayed version of the suprabasal heart rate signal. The second term of the same equation represents the IL-6 removal from the circulation after exercise. Finally, the third term accounts for the physiological production of IL-6, and is mainly representing the adipose tissue contribution (Mohamed-Ali et al. 1997). V is the IL-6 volume of distribution. The initial conditions are: IL6(0) = $IL6_b$ and Y(0) = 0. The value of Ra_{IL6} has been calculated by imposing the steady-state condition (time derivative equal to zero).

The model output was initially tested against the mean values of IL-6 plasma concentration taken from an experimental study that investigated the dynamics of several cytokines in ten male athletes before, during and after 2.5 hours of treadmill running at 75% of $\dot{V}O_{2max}$ (Ostrowski et al. 2000). Plasma IL-6 concentration was measured for each subject at 0, 30, 60, 90, 120, 150, 210, 270, 330, 390, 450 and 510 minutes after the beginning of the experiment (t = 0 min). The exercise started at t = 0 min and ended at t = 150 min. Heart rate values were measured during the exercise at 30, 60, 90, 120 and 150 minutes from the beginning of the experiment. For the construction of the HR(t) signal, heart rate values were interpolated between t = 0 and t = 510. Since no information was available for HRb, which corresponds to HR(0), its value was set to HR_b = 80 bpm (Sherwood et al. 2008). Similarly, it was assumed the heart rate to return to its basal value HR_b at t = 210 min.

The model consists of five independent parameters (Table 1). V and T_{HR} were assigned numerical values as obtained from reported observations (Dalla Man et al. 2009; Xu et al. 2011), while k_1, and k_2 were let free to vary and their optimal value was estimated by setting up a weighed least squares (WLS) fitting procedure implemented in Matlab (Mathworks®) based on the previously described data (Ostrowski et al. 2000). The value for τ was chosen ensuring that the model output would better match the experimental data.

The errors associated with the IL-6 measurements were assumed to be normally distributed random variables, with a zero mean and a constant coefficient of variation (CV) equal to 6.9% (Ostrowski et al. 1998). The precision of the parameter estimates was expressed as CV(pi)%= SDpi/pi•100, where pi is the ith component of the model parameters vector and SDpi is the associated standard deviation (computed as the square root of the diagonal terms of the inverse of the Fisher information matrix).

The estimates (with CV% in round brackets) of the free model parameters are reported in Table 1 (asterisk marked parameters).

Parameter	Value (CV %)	Units
$k_1(*)$	0.006 (4)	$pg \cdot ml^{-1} \cdot min^{-1}$
τ	20	beats per min
$k_2(*)$	0.004 (4)	min^{-1}
V	8250	ml
T_{HR}	5	min

Table -1 Values of independent IL-6 model parameters.

In Figure 4, the model predicted profile (solid line) of IL-6 plasma concentration in response to exercise is compared to the experimental data from Ostrowski et al. (Ostrowski et al. 1998).

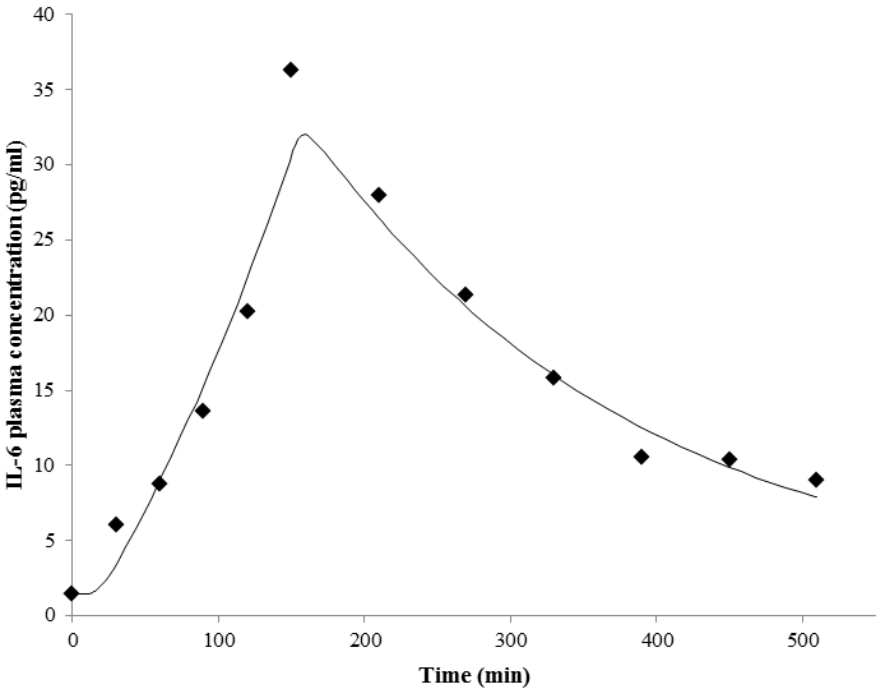

Figure -4 The profile of the mean value of plasma IL-6 measured in 10 healthy human males during and after 2.5 hour running (black diamonds) is compared with the corresponding IL-6 profile estimated by the proposed model (solid line).

The model was also used to simulate the IL-6 response to a different exercise protocol. It was hypothesized that a virtual subject ran for 60 minutes at 65% of $\dot{V}O_{2max}$, which corresponds to 80% of his maximal heart rate (HRmax). The exercise starts at t = 15 min and ends at t = 75 min and is supposed to account for the square-wave variation in

suprabasal heart rate reported in Figure 5 (Panel A). Simulated data were compared with the experimental data from a very similar exercise protocol (Scott et al. 2011) (Figure 5, Panel B). Notably, the model reproduced with reasonable verisimilitude the findings obtained from Scott et al. (Scott et al. 2011), despite the fact that a generic impulse was used as input, since exact experimental data were not available. The main difference between the predicted and measured values was recorded right at the end of the exercise session, i.e. when the step-change assumption is less realistic.

The good approximation of experimental data shows that the proposed model is a suitable tool to reproduce IL-6 time course during exercise. This model constitutes the first step in describing the complete immune system response during exercise. This description will include modelling the anti-inflammatory cascade constituted by sTNF-R, IL1-ra and IL-10. As previously mentioned, this anti-inflammatory cascade, initiated by muscle contraction, might represent a “defence” mechanism against pro-inflammatory actions caused by TNF-α secreted by “inflamed” adipose tissue.

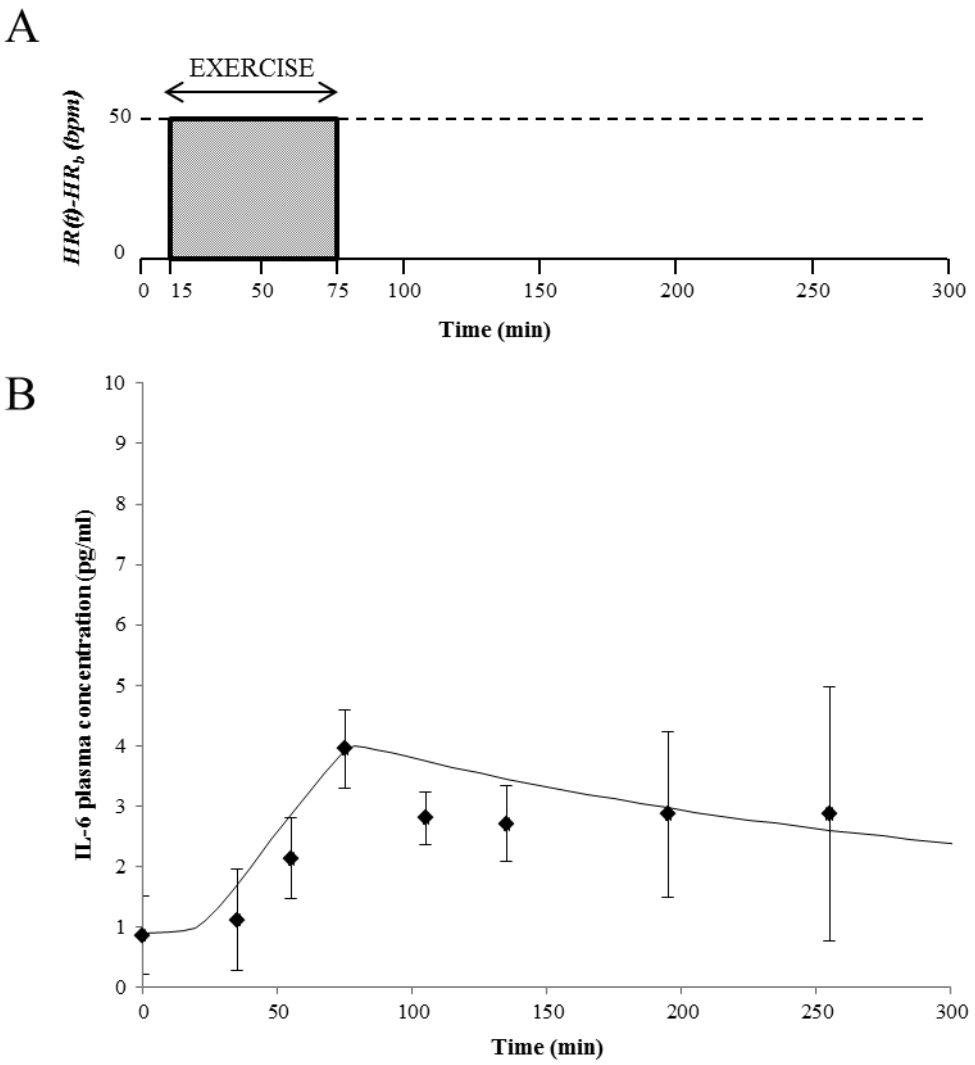

Figure -5 The square-wave variation in suprabasal heart rate hypothesized during a 60-minute running exercise at 65% VO2max (Panel A) originates the IL-6 model prediction (solid line, Panel B). The model prediction is superimposed to reported experimental data (closed diamonds, Panel B) from a similar exercise protocol (Scott et al. 2011). Values for reported experimental data are mean ± SD.

RESULT 4: AS A FIRST STEP TOWARD THE COMPLETE DESCRIPTION OF THE INFLAMMATORY PROCESSES TO BE INTEGRATED IN THE KIM MODEL, A NEW MATHEMATICAL MODEL OF THE INTERLEUKIN-6 (IL-6) DYNAMICS DURING EXERCISE HAS BEEN FORMULATED AND VALIDATED. THE HEART RATE SIGNAL (HR) WAS USED AS INPUT TO THE MODEL.

2.3.2 Updates on the definition of the relationship between steps per day and Interleukin-6

According to the MISSION-T2D DoW, a systematic review of the literature (Morettini et al. (c)) has been performed in WP5 in order to systematize the knowledge about the

relationship between the walking activity and inflammatory status, by looking at the most classic pro-inflammatory markers, namely Interleukin-6 (IL-6), C-Reactive Protein (CRP) and Tumour Necrosis Factor-α (TNF-α). From the literature review, it can be concluded that moderate-intensity walking interventions lasting several weeks can reduce low-grade inflammation. However, the different methods of assessment of physical activity in free-living conditions represent one of the major limitations to the comparison of studies results.

For this reason, as anticipated in Deliverable 5.2, partner USFD has started in January 2015 an experimental study aiming at exploring the existence of a correlation between the number of steps walked per day, measured by an objective method of assessment of physical activity (activity monitor) and the concentration of inflammatory markers (in particular IL-6, which is prominently involved in mediating the acute-phase response). In order to determine this correlation, results of Yates et al. study (Yates at al. 2010) reported in Figure 6 and previously in Deliverable 5.1 has been considered as a starting point. The adjusted regression model (for age, ethnicity, sex, BMI and medication status) extrapolated from Yates data suggested that a difference in change in ambulatory activity of around 2,500 steps/day, equivalent to 25 minutes of moderate-intensity walking activity per day (American Diabetes Association 2013), was needed to induce each 0.5 pg/ml difference in IL-6 concentration. The value determined by this relationship will be the value of IL6b for the model previously described (Morettini et al. 2014 (a); Morettini et al. 2014 (b)). The USFD study will provide the data for validating the relationship proposed by Yates (Yates et al. 2010). Details of the study protocol are reported in section “Future works”.

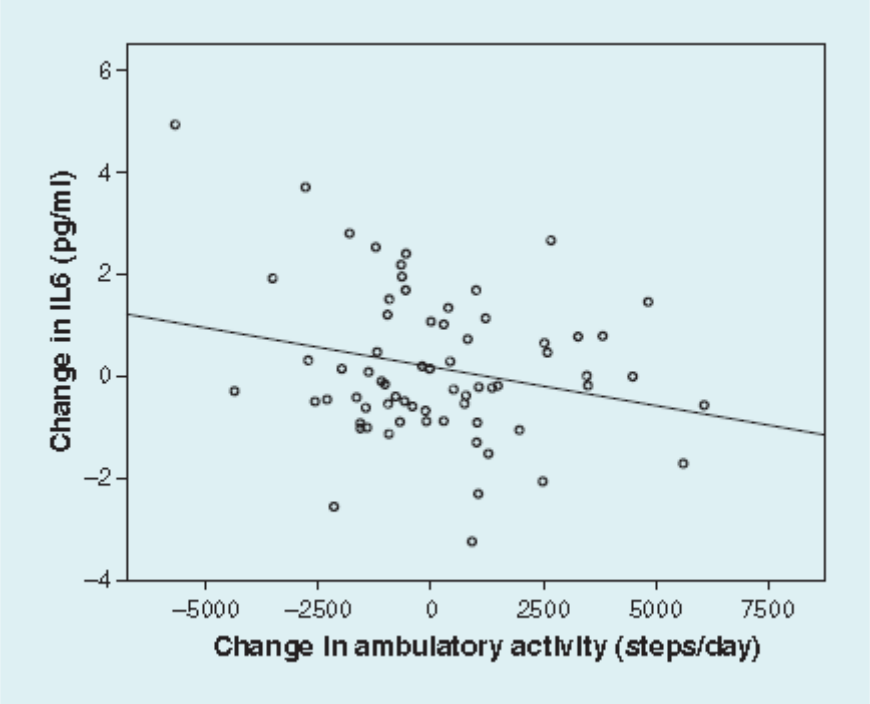

Figure -6 Relationship between change in IL-6 and change in ambulatory activity (adapted from Yates et al. 2010).

RESULT 5: THE DATA PROVIDED BY YATES ET AL. (2010) WILL BE USED AS A STARTING POINT FOR THE DEFINITION OF THE RELATIONSHIP BETWEEN STEPS PER DAY AND IL-6: A CHANGE IN AMBULATORY ACTIVITY OF AROUND 2,500 STEPS/DAY, EQUIVALENT TO 25 MINUTES OF MODERATE-INTENSITY WALKING ACTIVITY PER DAY, IS NEEDED TO INDUCE 0.5 PG/ML DIFFERENCE IN IL-6 CONCENTRATION. THE USFD STUDY WILL PROVIDE DATA TO VALIDATE THIS RELATIONSHIP.

2.4 Determination of the conversion factor between heart rate (HR) and work rate (WR): updating the results of Deliverable 5.2

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. In Deliverable 5.2 we stated that we need to include a conversion factor between heart rate which is the input of WP5 model and the WR.

Actually WR=125 watt used in the Kim model was obtained considering Cabrera's formula (Cabrera et al. 1999) and applying this formula to an exercise intensity equal to 60% VO_{2max}. For this reason, we changed our view and decided to consider as Kim model input the percentage of VO_{2max}. The use of $\%VO_{2max}$ instead of WR has several

advantages:

1. %VO2max represents a relative intensity whereas WR indicate an absolute intensity;
2. % VO2max can be easily convert to HR;
3. % VO2max do not require to know what exercise is performed.

In order to use the heart rate as input for MISSION-T2D model, we need to implement a relationship between HR and percentage of VO2max.

RESULT 6: WR IN THE KIM MODEL WAS OBTAINED CONSIDERED CABRERA'S FORMULA AND APPLYING THIS FORMULA TO AN EXERCISE INTENSITY OF 60% VO2max. IN ORDER TO HAVE HR AS INPUT WE HAVE IMPLEMENTED THE RELATIONSHIP BETWEEN PERCENTAGE OF VO2max AND HR.

2.5 Modelling the epinephrine dynamics as a function of WR

As previously mentioned, one of the key point to be addressed for the integration is represented by assigning a value to the parameters dependent on WR. In particular, looking at the eq. (1), which describes the epinephrine dynamics, the steady-state gain ω(WR) and the time constant τ_E have been conceived by Kim as fixed parameters which have been estimated by optimal least-squares fitting to epinephrine concentration data from a 60-min exercise test corresponding to WR = 150 Watt. Thus, in order to have suitable values for these parameters, a best-fit on epinephrine concentrations from exercise test at that specific work rate should be performed whenever the values of WR are changed. In order to do so, epinephrine experimental data for different value of the WR need to be available. This information is not promptly available in the literature. However, a possible solution to be explored to overcome this problem could be the substitution of eq. (1) with another equation, or set of equations, in which the dependence on WR is explicit. To this aim, a validated compartment model of epinephrine secretion and elimination basing its input on the physical exercise has been considered (Kildegaard et al. 2007) and adapted to our purposes. The original formulation of the model structure is shown in Figure 7.

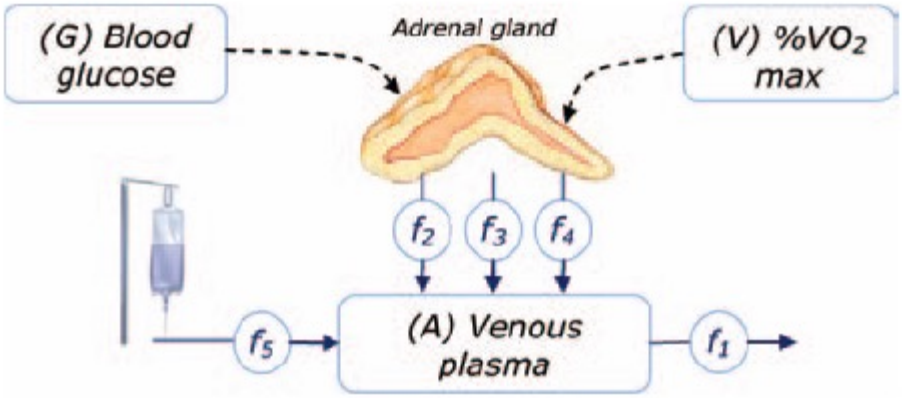

Figure -7 A compartment model structure of epinephrine secretion and elimination (adapted from Kildegaard et al. 2007).

The model consists of an epinephrine compartment (venous plasma), which has a basal secretion (f3), an epinephrine contribution (f2) depending on the blood glucose level, a contribution from the level of exercise (f4), an optional infused epinephrine (f5), and a description of epinephrine utilization (f1). The resulting differential equation describing the change in epinephrine (E, amount of epinephrine) in this system can be written as follows:

$$\frac{dE}{dt} = \left(f_2(C_{Glucose}) + f_3 + f_4\left(\%V\dot{O}_2max\right)\right) * BW + f_5(t) - f_1(E) \qquad (6)$$

It has to be noted that, for the purpose of our integration:

- no epinephrine infusion is considered: $f_5(t)=0$;
- epinephrine concentration must be used instead of epinephrine amount:
  - $C_E = E/V_d$ where C_E is epinephrine concentration and V_d is the distribution volume (which could be reasonably set to V_d = 15 L as reported by Dejgaard et al. (Dejgaard et al. 1989) for Type 1 diabetic patients);
- arterial concentration of epinephrine must be used instead of venous concentration.

On this basis, eq. (6) can be reformulated as:

$$\frac{dC_E}{dt} = \frac{1}{V_d}\left(f_2(C_{Glucose}) + f_3 + f_4\left(\%V\dot{O}_2max\right) * BW\right) - f_1(C_E) \qquad (7)$$

A detailed description of all the functions that have been modified to be adapted to the MISSION-T2D context is reported below.

2.5.1 Basal whole body release of epinephrine: f_3

As reported by Kildegaard et al. (Kildegaard et al. 2007), the basal release of epinephrine to plasma is regarded as constant at rest above the hypoglycaemia threshold, f_3 = b. The value of this release for adult subjects has been reported as 187.24 ng/min (1.02 nmol/min) in a study by Esler et al. (Esler et al. 1991). The expression of b as a function of BW can be obtained by a normalization of this value: b'=b/BW. For an adult subject having a BW = 70 kg, this leads to b' = 0.015 nmol/(min·kg).

2.5.2 Epinephrine elimination: f_1

As reported by Kildegaard et al. (Kildegaard et al. 2007), a first-order elimination is assumed. Clearance of epinephrine from plasma is implemented in the model according to the following equation:

$$f_1(C_E) = k \cdot C_E \qquad (8),$$

where the elimination constant k is calculated at steady-state condition:

$$k = \frac{b}{V_d \cdot C_E(0)} \qquad (9)$$

Assuming $C_E(0)$ equal to 0.25 nmol/L (Kim et al. 2007), k = 0.3 min^{-1}.

2.5.3 Epinephrine secretion dependent on blood glucose: f_2

Several studies have shown how the secretion of counter-regulating hormones increases as blood glucose decreases below a threshold of approximately 3.5 mmol/L (hypoglycaemia). In the Kildegaard model, it was hypothesized that epinephrine secretion has a maximum limit. A dose–response shaped relation between blood glucose and epinephrine secretion was therefore used (eq (10)):

$$f_2(\text{Cglucose}) = \frac{c_1}{1+e^{c_2\cdot(\text{Cglucose}-c_3)}} \quad (10)$$

where c_1 is the maximum epinephrine release, c_3 is the blood glucose level corresponding to half of maximum epinephrine release, and c_2 is the change in epinephrine release at c_3. The values provided by Kildegaard et al. are: c_1 = 0.36 nmol/(kg*min), c_2 = (nmol of epinephrine/kg/min/mmol of glucose/L) and c_3 = 2.94 mmol/L, respectively. Adrenaline secretion as a function of blood glucose is reported in Figure 8.

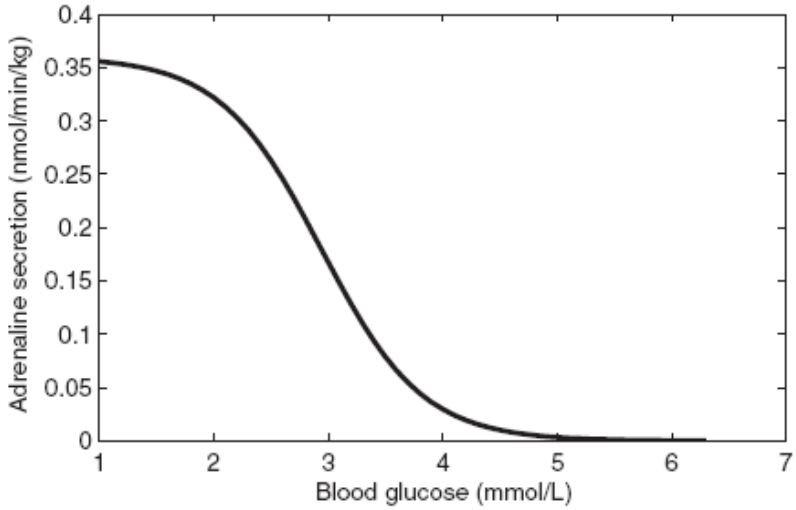

Figure -8 Adrenaline (epinephrine) secretion as a function of blood glucose (adapted from Kildegaard et al. 2007).

2.5.4 Epinephrine secretion induced by physical exercise: updating the results of Deliverable 5.2

Epinephrine secretion is increased by exercise, the intensity of which is commonly measured as a percentage of the maximal oxygen uptake (% $\dot{V}O_{2max}$). The epinephrine secretion function f4(V) proposed by Kildegaard describes the epinephrine secretion per distribution volume and uses V (% $\dot{V}O_{2max}$) as input parameter. After the initiation of an exercise, the actual level of V continues to increase until when it reaches the target level T_V , typically after 4–5 min, and remains constant hereafter (Ahlborg et al. 1974; Wharen et al. 1971). A differential equation describing this pattern has been proposed by Lenart et al. (Lenart et al. 2002), which can be used to calculate V (eq. (11)):

$$\frac{dV}{dt} = \frac{5}{3} \cdot V - \frac{5}{3} \cdot T\_V \quad (11)$$

Low exercise intensity induces low epinephrine secretion, but an upper limit for epinephrine secretion should be included at high exercise intensities in order to make the model correctly resemble the exercise physiology. The following dose–response relationship between exercise intensity and epinephrine secretion can be used to this purpose:

$$f_4(V) = \frac{d_1}{1+e^{d_2 \cdot (d_3 - V)}} \quad (12),$$

where d_1 is the maximum epinephrine secretion due to the exercise, d_3 is the level of exercise where the exercise-induced secretion is half of the maximum, and d2 is the slope of the secretion curve at d_3. The values of the parameters are d_1 = 0.47 nmol/min/kg, d_2 = 0.1, and d_3 = 82.1% VO2 (Kildegaard et al. 2007). The resulting function is shown in Figure 9.

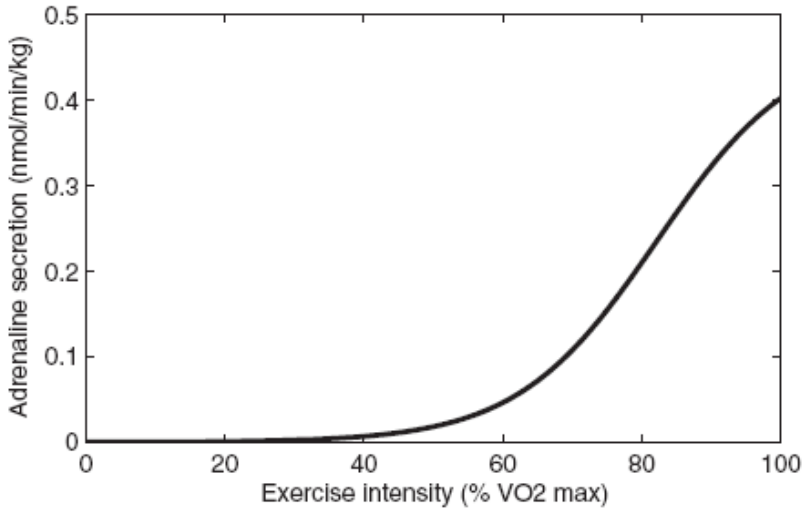

Figure -9 Adrenaline (epinephrine) secretion as a function of exercise intensity (adapted from Kildegaard et al. 2007).

We integrated the Kildegaard model with an additional differential equation in order to model the return of the variable V to its baseline values. This modification was introduced for a better representation of what happens at the end of an exercise

session.

3 Deliverable conclusions

The Kim model provides a representation of the metabolic processes during exercise. The input of the Kim model is represented by the work rate. The integration of WP5 in the overall model has been pursued by linking its input (heart rate, HR) with the work rate (WR). It has been shown that the WR (and consequently its translation into HR) has to be tuned according to the modelled subject characteristics. The proposed exercise model has to be used only for a moderate intensity exercise that is lower than the lactate threshold of the subject.

The integration of the anti-inflammatory effect of the exercise into the Kim model has been pursued by developing a new model of the IL-6 kinetics. Finally, a model of the epinephrine dynamics as a function of WR has been defined to make the Kim model adjustable to different exercise contexts.

3.1 Future works

Future work, which will be detailed in the final version of Deliverable 5.3, will include:

1. the mathematical description of the dynamics of other cytokines involved in the immune system response to exercise;
2. the validation of the model using the protocol described in the next subsection.

3.1.1 Protocol details

Sponsor Details: Sheffield Teaching Hospitals NHS Foundation Trust

Project Title: Validation and Feasibility Study of Physical Activity Monitors in Diabetes

STH Ref Number: STH18049

Protocol Version: 5.0 18/7/13

STH Directorate Affiliation: Academic Department of Diabetes and Endocrinology

DETAILS OF PROPOSAL

Specific Aims

1) Assay Validation: To determine the precision and accuracy of activity monitors in patients with diabetes. This will be performed in two distinct settings: initial laboratory validation (physical performance tests and six-minute walking test under strict experimental conditions) followed by field observational studies (where laboratory findings will be tested in the real-world).

2) Clinical Validation:

a) To establish the mutual relationships existing between PA and insulin sensitivity, endothelial function and inflammation

b) To examine clinical utility of quantifying PA in T1DM patients to inform insulin dose adjustments to prevent hypoglycaemia

3) Feasibility Assessments

a) To examine the facilitators and barriers which govern patients willingness patients to use PA monitors

b) To establish the potential number of eligible patients and determine the follow-up and response rates to the intervention

c) To examine the characteristics of the outcome measures for sample size estimations in future studies.

Subjects: 40 subjects (20 T1DM and 20 T2DM, aged 18-80) will be recruited in total. Subjects in each diabetes cohort be divided into two subgroups based on PA levels graded using validated questionnaires, functional status and exercise capacity: Group 1: sedentary or inactive, low functional status and exercise capacity; Group 2: very active or active, high functional status and exercise capacity. Exclusion criteria: chronic illness other than diabetes (e.g. COPD, CCF), unstable angina, recent myocardial infarction (within 3 months), severe ischaemic heart disease (unstable angina or exertion angina), chronic painful condition or physical disability restricting PA or mobility, uncontrolled diabetes with HbA1c>11%, alcohol consumption >3units/day for men and >2units/day for women and current smokers, patients with T2DM on insulin therapy. Subjects will be allowed to withdraw at any time during the study period. This will not affect their subsequent care.

Study Visits: There will be two study visits altogether.

Visit 1 (Screening)

1) Resting ECG and conventional cardiac autonomic function tests (O'Brien et al. 1986).

2) Functional status and the functional exercise capacity of the patients (short battery of physical performance tests (PPT) and six minutes walking test (6MWT)). During the 6MWT, patients will be equipped with the PA monitor while performing the 6MWT in order to test the reliability of the device. In addition to the activity monitors, two wireless sensors (OPAL, ADPM Inc., Portland, OR, USA) will be positioned on the left and right shanks, just above the ankle, by means of an elastic strap. Data from these sensors will be used as a gold standard for step detection.

3) Clinical laboratory tests: full blood count, hematocrit, plasma viscosity, erythrocyte sedimentation rate, hemoglobin A1C, serum concentration of blood urea and electrolytes (U&E), cholesterol profile and random urine for microalbumin and creatinine ratio. Two-field retinal photography will be used to detect the presence and grade the severity of diabetic retinopathy (obtained from annual retinal screening database).

4) Study questionnaires:

1. International Physical Activity Questionnaires (IPAQ) (Craig et al. 2003)

2. SF-36 (Brazier et al. 1992)

3. EQ-5D (Kind et al. 1998)

5) At the end of Visit 1 (two hours), patients will be provided with an activity monitor and instructed how to use it for two weeks. They will be asked to wear the instrument every day for fourteen consecutive days but not to wear it whilst bathing or swimming. Each morning, a text message will be sent to each subject to remind him/her to wear the monitor. They will be asked to maintain daily activity, blood glucose and carbohydrate portions (T1DM patients only) diary. Patients will be instructed not to alter their normal weekly routine.

6) Subjects with T1DM will also be provided with a blinded continuous glucose monitor (CGM).

Visit 2

1) A blood sample (fasting in patients with T2DM) will then be taken from each subject in order to measure insulin resistance (T2DM only) fasting insulin and homeostasis model assessment), hs-CRP, TNF-α, IL-6, fibrinogen, PAI-1 levels.

2) Diaries, PA and continuous blood glucose monitors will be collected and information downloaded, stored and analysed after study completion. Feedback from patients on use of PA monitors will be documented.

4 Appendix: List of abbreviations used

T2D	Type 2 Diabetes
TNF-α	Tumour Necrosis Factor α
GLUT-4	Glucose transporter-4
HR	Heart rate
WR	Work rate
ATP	Adenosine triphosphate
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-15	Interleukin-15
IL-1 ra	Interleukin 1 receptor antagonist
sTNF-R	Soluble TNF-α-receptors
IL-1 β	Interleukin-1 β
CRP	C-Reactive Protein
SD	Standard Deviation
$\dot{V}O_2$	Oxygen consumption
BW	Body weight

Table-2 List of abbreviations.

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