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Glucagon-like peptide-1 and interleukin-6 interaction in response to physical exercise: An in-silico model in the framework of immunometabolism

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ABSTRACT

Background and objective: Glucagon-like peptide 1 (GLP-1) is classically identified as an incretin hormone, secreted in response to nutrient ingestion and able to enhance glucose-stimulated insulin secretion. However, other stimuli, such as physical exercise, may enhance GLP-1 plasma levels, and this exercise-induced GLP-1 secretion is mediated by interleukin-6 (IL-6), a cytokine secreted by contracting skeletal muscle. The aim of the study is to propose a mathematical model of IL-6-induced GLP-1 secretion and kinetics in response to physical exercise of moderate intensity.

Methods: The model includes the GLP-1 subsystem (with two pools: gut and plasma) and the IL-6 subsystem (again with two pools: skeletal muscle and plasma); it provides a parameter of possible clinical relevance representing the sensitivity of GLP-1 to IL-6 (k_0). The model was validated on mean IL-6 and GLP-1 data derived from the scientific literature and on a total of 100 virtual subjects.

Results: Model validation provided mean residuals between 0.0051 and 0.5493 pg·mL^{-1} for IL-6 (in view of concentration values ranging from 0.8405 to 3.9718 pg·mL^{-1}) and between 0.0133 and 4.1540 pmol·L^{-1} for GLP-1 (in view of concentration values ranging from 0.9387 to 17.9714 pmol·L^{-1}); a positive significant linear correlation ($r = 0.85$, $p<0.001$) was found between k_0 and the ratio between areas under GLP-1 and IL-6 curve, over the virtual subjects.

Conclusions: The model accurately captures IL-6-induced GLP-1 kinetics in response to physical exercise.

1. Introduction

Glucagon-like peptide 1 (GLP-1) is an incretin hormone [1] secreted by the intestinal L cells in response to nutrients ingestion and, acting on the pancreatic beta cells, it potentiates glucose-stimulated insulin secretion [2]. However, it is now evident that GLP-1 is a multifaceted hormone playing a role in several metabolic processes [3], including the inhibition of glucagon secretion [4], the delaying of gastric emptying [5] and the promotion of satiety [6]. All these findings supported the development of GLP-1-based pharmacological approaches for the treatment of type 2 diabetes (T2D) and obesity [2].

Besides the classical viewpoint in which nutrients ingestion represents the main stimulus for GLP-1 secretion, evidence showed that other stimuli, such as physical exercise, may enhance GLP-1 plasma levels [7,8]. This exercise-induced GLP-1 secretion has been recently demonstrated to be mediated by interleukin-6 (IL-6) [9], thus confirming in human subjects what previously observed in mice [10]. IL-6 is a cytokine secreted by contracting skeletal muscle during physical exercise, but also by other organs (for example adipose tissue) and, as recently reviewed [11], it has various physiological and pathophysiological functions not only in the immune system but also in metabolism, not fully elucidated yet.

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On the basis of these observations regarding the role of both GLP-1 and IL-6, it is evident that the study of the link between them may impact the understanding of skeletal muscle/intestinal crosstalk, which is crucial to explain the beneficial effect of exercise on metabolic health in a new perspective defined as "exercise immunometabolism" [12].

In-silico models have always helped to gain insight into glucose homeostasis [13]; in particular, compartmental modelling has been widely used since it represents the most common pharmacokinetic modelling technique [14]. As regards GLP-1, some attempts have been made to model its kinetics [15–20] and also its insulinotropic effect [16,19,20]. However, the proposed models focused on nutrient ingestion as a stimulus for GLP-1 secretion and none on physical exercise. Thus, the aim of the study is to fill this gap, by proposing a mathematical model of IL-6-induced GLP-1 secretion and kinetics in response to physical exercise.

2. Methods

2.1. Model formulation

2.1.1. Model equations

The proposed model includes the GLP-1 subsystem, composed of two pools (gut and plasma) and the IL-6 subsystem, composed as well of two pools (skeletal muscle and again plasma). IL-6 skeletal muscle secretion is supposed to be driven by changes in oxygen uptake. In plasma, IL-6 increases for the contributions from skeletal muscle and adipose tissue, whereas it is cleared by the liver. IL-6 in the skeletal muscle is supposed to exert a derivative control on GLP-1 secretion from the gut. GLP-1 in plasma accounts for secretion from the gut and partially from other organs. GLP-1 also undergoes degradation in the gut and clearance from plasma. The compartmental description of the model is shown in Fig. 1.

The model is based on the hypothesis that during an exercise session GLP-1 is secreted in a IL-6-dependent manner [9]. To model IL-6 secretion and kinetics during physical exercise, we exploited our previously proposed model [21] in which IL-6 secretion is supposed to be dependent on the characteristics of the exercise bout, as duration and relative intensity expressed as a percentage of individual's maximal oxygen uptake and indicated as $\%VO_{2max}$, with values ranging from 0 to 100%. In the model, the variations of the percentage of supra-basal oxygen uptake ($PVO_{2max}(t)$) that occur during the exercise are described according to the following equation:

$$\frac{dPVO_{2max}(t)}{dt} = -0.8 \cdot PVO_{2max}(t) + 0.8 \cdot u(t) \tag{1}$$

in which $PVO_{2max}(0) = 0$ and $u(t)$ is the system input described as follows:

$$u(t) = \begin{cases} 0 & 0 \le t < t_0 \\ T_v & t_0 \le t \le t_f \\ 0 & t > t_f \end{cases} \tag{2}$$

where t_0 and t_f (both expressed in min) represent the initial and final time point of the exercise bout, respectively.

The value of T_v (target value of intensity for the specific exercise bout) is set considering the difference between relative exercise intensity (expressed in terms of $\%VO_{2max}$) and basal oxygen uptake equal to 8%, thus T_v assumes values from 0% to 92%. Once T_v has been set in relation to exercise intensity, coefficients in (1) are fixed to 0.8 min^{-1} to have $PVO_{2max}(t)$, which starts from 0%, reaching the value assumed for T_v in 5-6 minutes [21–23].

IL-6 secretion and kinetics are described by the following equations, accounting for IL-6 in the skeletal muscle ($IL6_m(t)$, $pg \cdot mL^{-1}$) and in the plasma ($IL6_p(t)$, $pg \cdot mL^{-1}$):

$$\frac{dIL6_m(t)}{dt} = SR_{ex} \cdot PVO_{2max}(t) - k_m \cdot IL6_m(t) \tag{3}$$

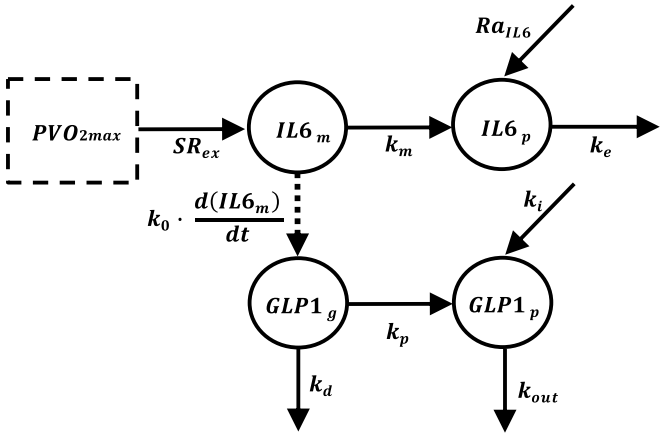

Fig. 1. Compartmental description of the model representing glucagon-like peptide-1 (GLP-1) secretion and kinetics induced by interleukin-6 (IL-6) in response to physical exercise. $GLP1_g(t)$: GLP-1 gut compartment; $GLP1_p(t)$: GLP-1 plasma compartment; $IL6_m(t)$: IL-6 skeletal muscle compartment; $IL6_p(t)$: IL-6 plasma compartment; SR_{ex}: IL-6 skeletal muscle secretion rate; $PVO_{2max}(t)$: oxygen uptake; k_m and Ra_{IL6}: skeletal muscle and adipose tissue contribution to plasma IL-6, respectively; k_e: IL-6 clearance from plasma; k_0: control on GLP-1 secretion from the gut; k_p and k_i: gut compartment and other organs contribution to plasma GLP-1; k_d and k_{out}: GLP-1 degradation in the gut and clearance from plasma, respectively.

$$\frac{dIL6_p(t)}{dt} = k_m \cdot IL6_m(t) - k_e \cdot IL6_p(t) + \frac{Ra_{IL6}}{V} \tag{4}$$

in which $IL6_m(0)=0$ and $IL6_p(0)=IL6_b$. IL-6 is produced in the skeletal muscle depending on PVO_{2max} through the muscular secretion rate (SR_{ex}, $pg \cdot mL^{-1} \cdot min^{-1}$) and reaches the plasma with fractional rate k_m (min^{-1}). IL-6 changes in plasma with respect to its basal value ($IL6_b$) are due not only to skeletal muscle (during exercise) but also to other body sources, especially the adipose tissue. Such extra-muscle contribution (which is not null even at rest) is accounted for by Ra_{IL6} ($pg \cdot min^{-1}$), normalized by IL-6 distribution volume (V) assumed equal to 14 L [24]; Ra_{IL6} can be determined by imposing the steady-state condition for (4):

$$Ra_{IL6} = k_e \cdot IL6_b \cdot V. \tag{5}$$

IL-6 is then cleared by the hepato-splanchnic viscera with fractional rate k_e (min^{-1}). Further details on the IL-6 model can be found in the original study [21]. GLP-1 secretion and kinetics are modelled according to the following differential equations, accounting for GLP-1 in the gut ($GLP1_g(t)$, $pmol \cdot L^{-1}$) and in the plasma ($GLP1_p(t)$, $pmol \cdot L^{-1}$):

$$\frac{dGLP1_g(t)}{dt} = k_0 \cdot \frac{dIL6_m(t)}{dt} - (k_p + k_d) \cdot GLP1_g(t) \tag{6}$$

$$\frac{dGLP1_p(t)}{dt} = k_p \cdot GLP1_g(t) - k_{out} \cdot GLP1_p(t) + k_i \tag{7}$$

in which $GLP1_g(0)=0$ and $GLP1_p(0)=GLP1_b$. The first term on the right-hand side of (6) identifies the control exerted (through k_0, $10^{-3} \cdot pmol/pg$) by IL-6 in the skeletal muscle on the intestinal production of GLP-1; the second term accounts, through k_p and k_d (both expressed in min^{-1}), for the portion of GLP-1 leaving the gut partly directed to blood and partly degraded by dipeptidyl peptidase-4 (DPP-4) protein [3], respectively.

Equation (7) expresses changes in plasma-circulating GLP-1, starting from the basal value $GLP1_b$. The first term of the right-hand side of (7) accounts for exercise-stimulated GLP-1 secretion; the second term accounts for degradation operated by DPP-4 and the kidneys (through the fractional rate k_{out}, expressed in min^{-1}) [3], whereas the third term (k_i, expressed in $pmol \cdot L^{-1} \cdot min^{-1}$) accounts for GLP-1 production in non-stimulated conditions [3]. By imposing the steady-state condition for (7), the following expression for k_i is found:

$$k_i = k_{out} \cdot GLP1_b. \tag{8}$$

The fractional rate k_{out} is fixed to 0.1 min^{-1}, as reported in [25] and in other models [19,20]. It is deemed that the output rate (k_{out}) of GLP-1 from the plasma is equal to the output rate of GLP-1 from the gut, thus yielding:

$$k_{out} = k_p + k_d. \tag{9}$$

Moreover, according to Müller et al. [3], a large portion of intestinal GLP-1 is degraded in the distal gut because of the elevated expression of DPP-4, and only the 10-15% reaches the systemic circulation. Thus, the following relationships hold:

$$k_p = 0.15 \cdot k_{out} \tag{10}$$

$$k_d = 0.85 \cdot k_{out}. \tag{11}$$

2.1.2. Structural identifiability analysis

The model parameters, k_{out}, k_p, k_d and V as well as the initial conditions for (4) and (7) ($IL6_b$ and $GLP1_b$, respectively) are fixed, whereas SR_{ex}, k_m, k_e and k_0 are estimated; Ra_{IL6} and k_i are computed imposing steady-state conditions. Structural (*a priori*) identifiability was tested by using *GenSSI* (Generating Series for testing Structural Identifiability) 2.0, under MATLAB® (The MathWorks, Natick, MA, USA) [26].

2.2. Model implementation

The model was implemented in MATLAB® environment using *Simulink*. Free model parameters (i.e., SR_{ex}, k_m, k_e and k_0) were estimated by solving a weighted non-linear least square problem through the *lsqnonlin* function, in which the weighted residual sum of square function (WRSS) has the following expression:

$$WRSS = \sum_{i=1}^{n} \left(\frac{GLP1(t_i) - GLP1_{exp}(t_i)}{\Gamma_{GLP1,i}} \right)^2 + \sum_{k=1}^{n} \left(\frac{IL6(t_i) - IL6_{exp}(t_i)}{\Gamma_{IL6,i}} \right)^2, \tag{12}$$

where $GLP1(t_i)$ and $IL6(t_i)$ are the model predicted GLP-1 and IL-6 time course during physical exercise at i-th time point; $GLP1_{exp}(t_i)$ and $IL6_{exp}(t_i)$ represent the corresponding measured experimental data. The weights $\Gamma_{GLP1,i}$ and $\Gamma_{IL6,i}$ are assumed equal to 9.1% and 4.8% of $GLP1_{exp}(t_i)$ and $IL6_{exp}(t_i)$, respectively, under the assumption of normal distribution with zero mean for the measurement errors [27,28]. The Levenberg-Marquardt algorithm has been used by the *lsqnonlin* function and the following lower and upper bounds have been applied to the parameters: (0; 1) for k_m and k_e; (0; ∞) for SR_{ex} and k_0. Function and step-size tolerances have been set to 10^{-20} and 10^{-7}, respectively. All the non-specified options have been retained at the default value set by MATLAB®.

The precision of all parameter estimates was expressed as percent coefficient of variation: $CV(p_i)\% = SDp_i/p_i \cdot 100$, where p_i represents the i-th free model parameter and SDp_i is the standard deviation of p_i, which is computed as the square root of the diagonal terms of the inverse of the Fisher information matrix [27].

The Simulink model representation and the pseudocode for the parameter estimation procedure are provided in Appendices A and B, respectively.

2.3. Model validation

2.3.1. Reported mean experimental data

Mean experimental data reported by Islam et al. [28] were considered to initially validate the model. In such study, active young males were asked to perform an exercise bout consisting of: i) 5 min of warm-up; ii) 30 min of continuous running at 65% VO_{2max}, indicated as Moderate-Intensity Continuous Training (MICT); iii) 5 min cool-down. In the 30-min interval before the beginning of the exercise bout, participants were given a standardized test meal (Chocolate Chip Clif Bar, 7 kcal/kg; 68% carbohydrates, 17% fat, 15% protein). IL-6 and GLP-1 concentrations were measured by venous blood sampling before the exercise, immediately post-exercise, 30-min post-exercise and 90-min post-exercise. Commercially available enzyme-linked immunosorbent assay kits were used to determine plasma concentrations of active GLP-1 (EMD Millipore) and IL-6 (R&D Systems, Minneapolis, MN), with intra-assay coefficients of variation of 9.1 and 4.8%, respectively.

Table 1
Model parameters values for validation on mean data from Islam et al. [28].

Parameter	Value (CV%)	Units	Reference
SR_{ex}	0.0015 (61%)	pg·mL^{-1}·min^{-1}	estimated
k_m	0.0119 (51%)	min^{-1}	estimated
k_e	0.0277 (50%)	min^{-1}	estimated
k_0	42.0010 (71%)	10^{-3}·pmol/pg	estimated
k_{out}	0.1	min^{-1}	fixed [19,20,25]
k_p	0.015	min^{-1}	fixed [3]
k_d	0.085	min^{-1}	fixed [3]
V	14	L	fixed [24]
k_i	0.795	pmol·L^{-1}·min^{-1}	steady-state for (7)
Ra_{IL6}	0.5468	pg·min^{-1}	steady-state for (4)

2.3.2. Virtual population generation

A virtual population composed of 100 subjects was created using a Monte Carlo approach [29,30]. Each virtual subject was conceived as a single realization of a pair of IL-6 and GLP-1 curves in response to the exercise bout, in which IL-6 and GLP-1 concentrations at each time sample were randomly and independently generated, based on normal distributions with mean and standard deviation (SD) derived for each time point and for each of the two substrates (IL-6 and GLP-1) by the study described in Section 2.3.1 [28], and considering spanning in the mean ± 3·SD interval.

2.4. Calculations

The mean of residuals (in absolute value) on IL-6 and GLP-1 for each virtual subject was computed. Moreover, over the 100 virtual subjects, linear regression analysis was performed between k_0 and the ratio between the suprabasal area under the curve of plasma GLP-1 (AUC_{GLP-1}) to that of IL-6 (AUC_{IL-6}). In addition, Pearson correlation coefficient (r) was computed. In the case of skewed distributions, tests were applied to the log_e-transformed values. Data are reported as mean ± SD or, in case of skewed distributions, as median [25^{th} percentile; 75^{th} percentile].

2.5. Sensitivity analysis

Sensitivity analysis was performed to determine how changes in k_0, which is the model parameter of major interest, affect relevant model outputs while keeping fixed all the other model parameters. Considering parameter estimation on reported mean experimental data (Section 2.3.1), sensitivity to changes in k_0 was evaluated by assuming +10%, +25% +50% and -10%, -25% and -50% variations with respect to the estimated value.

3. Results

Analysis of identifiability proved that the model is *a priori* identifiable (globally). Model validation on mean experimental data reported by Islam et al. [28] provided the parameter estimates (with related CV%) reported in Table 1 together with the values for fixed model parameters. In Fig. 2, we reported the fit and the plot of residuals,

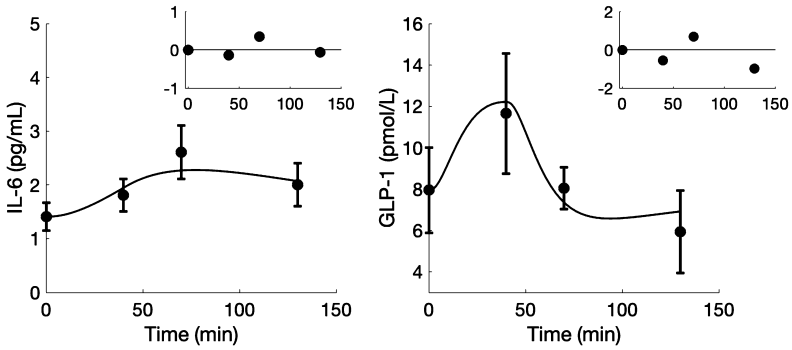

Fig. 2. Fit results for model validation over the mean experimental data by Islam et al. [28]. In detail: mean experimental data (closed circles) with related standard deviations and model output (continuous line) for IL-6 (left panel) and for GLP-1 (right panel). Related residuals are reported as insets.

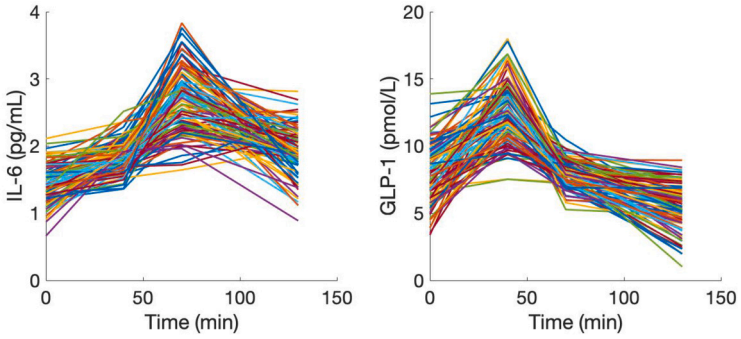

Fig. 3. Curves in the 100 virtual subjects (spaghetti plot). In detail: IL-6 (left panel) and GLP-1 (right panel).

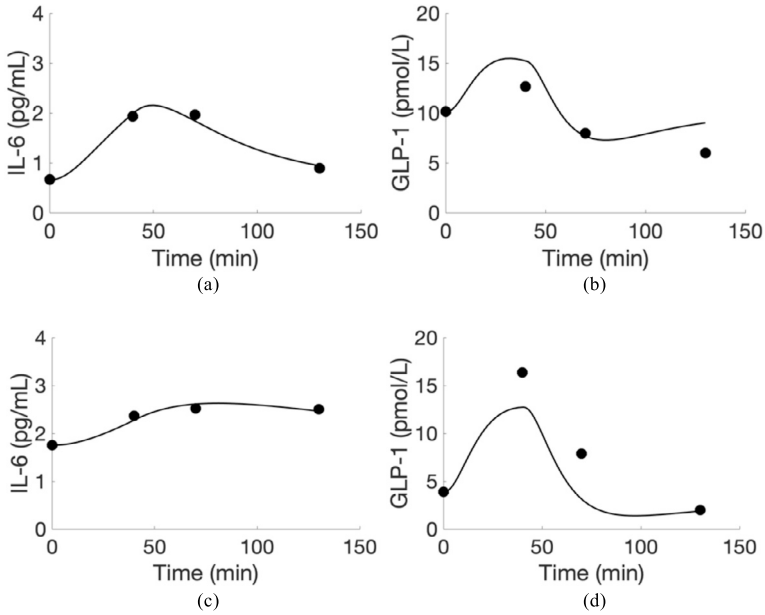

Fig. 4. Fit results for model validation over the virtual population. In detail: IL-6 for a virtual subject whose data have the maximum deviation from the IL-6 mean curve of [28] (closed circles) and model output (continuous line) (a), and related values for GLP-1 (b); similar information is reported for a virtual subject having maximum deviation from the GLP-1 mean curve: (c) and (d).

whose maximum absolute values were equal to 0.3377 pg·mL^{-1} for IL6 and 0.9845 pmol·L^{-1} for GLP-1.

IL-6 and GLP-1 in the 100 virtual subjects are shown in Fig. 3. Parameter estimates are 0.0015 [0.0011; 0.0024] pg·mL^{-1}·min^{-1}, 0.0377 [0.0257; 0.0510] min^{-1}, 0.0117 [0.0100; 0.0153] min^{-1}, 53.0236 [28.9766; 74.4232] 10^{-3}·pmol/pg for SR_{ex}, k_m, k_e and k_0, respectively. Mean residuals are between 0.0051 and 0.5493 pg·mL^{-1} for IL-6 and between 0.0133 and 4.1540 pmol·L^{-1} for GLP-1; an example of model fit for virtual subjects showing high IL-6 and GLP-1 curve (above the IL-6 and GLP-1 average curves in the virtual population) is reported in Fig. 4. Of note, a positive significant linear correlation ($r = 0.85$, $p<0.001$) was found between k_0 and $AUC_{GLP\text{-}1}$ to $AUC_{IL\text{-}6}$ ratio over the 100 virtual subjects. Results of the sensitivity analysis are reported in Fig. 5: k_0 changes equal to 10%, 25%, 50%, as compared to its estimated value on the mean experimental data, provided a change in GLP-1 model output of about 0.4%, 2.6% and 10.6%, respectively.

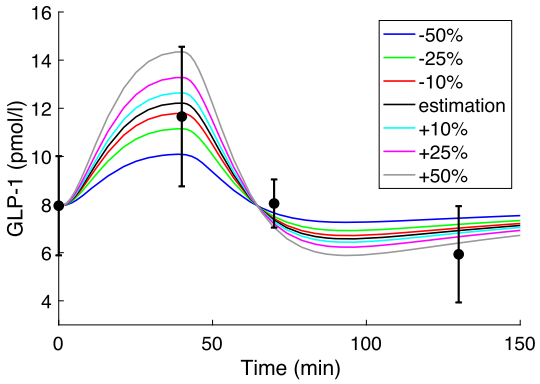

Fig. 5. Sensitivity analysis results. Considering reported mean experimental data (open circles) and the GLP-1 model prediction as a result of the parameter estimation (black line), sensitivity to changes in k_0 was evaluated by assuming for k_0 +10%, +25% +50% and -10%, -25% and -50% variations concerning the estimated value, while keeping all the other model parameters as fixed to the estimated values. The corresponding GLP-1 model predictions are described in the legend.

4. Discussion

4.1. Novelties, relevance and clinical applications

In this study, we developed a mathematical model describing the stimulatory effect of IL-6 on GLP-1 secretion following physical exercise. To our knowledge, this is the first model describing IL-6 and GLP-1 kinetics and their interaction, thus being a step forward in the "exercise immunometabolism" perspective. The model has two applications: first, it may be applied for simulation purposes, possibly leading to improved knowledge of the underlying physiological and pathophysiological processes. Indeed, the mechanisms of IL-6 and GLP-1 interactions appear still partly debated [9]. Most importantly, the model may have direct clinical applications: in our approach, having fixed some parameters to reasonable values, the most relevant parameters can be estimated in the single individual, thus having potential clinical relevance. The model parameter of major interest is k_0, representing the link between IL-6 muscle concentration and GLP-1 intestinal secretion. Since in our model approach GLP-1 secretion depends on time variations of the IL-6 muscle concentration by a proportionality factor represented by k_0, such parameter can be considered as a sensitivity of GLP-1 gut secretion to exercise-based IL-6 increase. Thus, the potential clinical relevance of k_0 lies in its precise physiological meaning, and in the opportunity to estimate it individually. Of note, the concept of possible variability among different subjects in the sensitivity of GLP-1 gut secretion to IL-6 action appears consistent with the notion of inter-individual variability in the GLP-1 secretion by the intestinal L cells in response to identical luminal nutrient stimulation [31].

What specific clinical applications may be expected by the assessment of the GLP-1 sensitivity to exercise-based IL-6 release by muscles? It is worth noting that the benefit of a healthy lifestyle, including regular physical exercise, for the prevention of several diseases, among which T2D, has been known for a long time. However, it has been generally believed that a healthy lifestyle, and specifically physical exercise, typically improves insulin sensitivity [32–34]. At contrast, the other main factor in the maintenance of glucose homeostasis, *i.e.,* beta-cell function/insulin secretion, has been found hardly modifiable by lifestyle only, thus requiring pharmacological treatment (such as the incretin analogs [35]) or exogenous insulin administration. Interestingly, the effect of exercise on GLP-1 secretion, thanks to the mediating action of IL-6, discloses the possibility of a beneficial action of physical activity on the beta-cell axis. In our opinion, the exercise-related GLP-1 secretion increase appears as one of the most promising mechanisms for the recently shown "drug-free" improvement of beta-cell function [36], and this motivated our study.

It may be observed that our model requires measurement of both IL-6 and GLP-1, both of which are currently not common in the clinical routine, not even in diabetic populations. On the other hand, diabetes care is expected to evolve towards precision diagnostics and therapeutics [37], thus it is likely that laboratory measures currently not routinely performed become common in the near future. In addition, some advanced approaches for improved care, still not feasible in the clinical routine, may be already applied in specific clinical trials, where participants typically undergo intensive phenotyping and care.

4.2. Previous studies in the field

Comparison of the present model to previous models appears difficult, since to our knowledge no model of IL-6 and GLP-1 interactions has been previously proposed. In one of our previous studies [21], we proposed the two-compartment model approach for IL-6 kinetics, whereas the kinetic model of GLP-1 and related integration with IL-6 in the context of physical exercise is original and represents the main novelty of this study. When applying our model to human data (though limited to average data as derived by scientific literature), we selected those reported in the study by Islam et al. [28]. In that study, changes in IL-6 and GLP-1 were analyzed in relation to different intensities of physical exercise (running). In addition to Islam's study, a limited number of studies focused on IL-6 and GLP-1 interactions during physical exercise [9,10,38]. Notably, the majority of such studies were developed in the last years, showing the emerging relevance for the exercise-related IL-6/GLP-1 issue. Unfortunately, none of those studies (apart for Islam's) can be exploited to further test our model, since not involving human subjects and/or with experimental design unsuitable for our needs.

4.3. Study limitations and related comments

Our modelling approach has some limitations. First, the model was tested only on values related to moderate exercise intensity. This is due to the reason that the part of the model concerning oxygen uptake may be inadequate in high-intensity exercise, likely affecting the whole cascade of results. On the other side, our specific interest in moderate exercise is due to some reasons. First, the data by Islam et al. [28] indicates that the other exercise types are not superior to the moderate type in stimulating GLP-1 secretion. In addition, with the moderate exercise the GLP-1 increase was faster. It is also worth noting that some studies documented some practical difficulties in performing vigorous physical activity, in parallel with a higher risk of drop-out (at least in people with diabetes [39]). Besides, performing moderate rather than high-intensity exercise does not prevent reaching remarkable clinical results, for instance, in terms of reduction of the risk for cardiovascular mortality [40]. It also has to be noted that we validated our modelling approach on data of active GLP-1, as reported in the study by Islam et al. [28]. Some previous studies indicated total GLP-1 rather than active GLP-1 as more appropriate to describe GLP-1 secretion [41,42]. However, we were more interested in the effects of GLP-1 on insulin secretion rather than in GLP-1 secretion, and the former may be better represented by active GLP-1 [42]. It should also be emphasized that the accuracy and reliability of our modelling approach should mainly depend on the kinetics of IL-6 and GLP-1 rather than on their absolute values. Based on some previous studies where both active and total GLP-1 were measured in the same subjects and related temporal patterns were displayed [43–54], we did not identify an indication of remarkable differences in the kinetics of active and total GLP-1, which allows us to claim that our modelling approach may be properly applicable to both active and total GLP-1 data. On the other side, the

investigator possibly using our model on total GLP-1 data has to be aware that we specifically validated it only on active GLP-1 data.

Our model focuses on the possible effects on GLP-1 of IL-6 released during physical exercise. However, it has to be acknowledged that there are other cytokines released during exercise that may affect insulin secretion, such as irisin [55]. Such stimulation of insulin secretion by those further cytokines may be assessed in future studies with different models. Indeed, cytokines with possible effects on GLP-1 secretion (if any) may be considered in more complex models, although it is worth noting that models requiring several input variables have fewer chances to be adopted in the clinical context.

In our model approach, it was necessary to set some model parameters to reasonable but fixed values. This is a limitation, but it was mandatory to preserve the identifiability of the model parameters of major relevance (especially k_0, as discussed). It cannot be excluded that future studies may allow determining improved values for such fixed parameters, possibly specific for populations with different characteristics (such as lean and obese, diabetic and nondiabetic, young and elderly, *etc.*). Furthermore, despite the precision of parameter estimates in the present study is not high in its absolute sense, it is however perfectly in line with typical ranges of its domain. Indeed, as reviewed by Clewell et al. [56], CV% in human metabolism studies typically ranges between 30 and 70%.

The model did not show an excellent fit for the IL-6 peak value. However, the shape of the IL-6 curve was correctly caught, thus results are acceptable. To overcome the problem, it would be necessary to increase the model complexity (likewise, the number of compartments for plasma IL-6), but again this may translate into a non-identifiable model. In addition, the accuracy in the fit of the experimental data may have been partly affected by the measurement error on IL-6 and GLP-1 [57,58].

Finally, Islam's study [28] included only healthy participants, and hence it is possible that the IL-6 and/or GLP-1 patterns may be different in other populations, such as in obesity or type 2 diabetes [54]. On the other side, we do not expect such possible differences to be critical for our model approach: in type 2 diabetes, lower fluctuations may be expected for the variables of interest (especially for GLP-1), this being favorable in our model approach. Nonetheless, we acknowledge that this has to be proved in future studies.

It also has to be acknowledged that we currently cannot provide any evidence of possible different results in our modelling approach when applied to men and women separately, which we may expect to observe in consideration of the sex-related differences in incretin release following nutrient stimulation [59].

5. Conclusions

This study addresses for the first time the problem of modelling the IL-6/GLP-1 interaction during physical activity, whose interest relies on the observation that under certain conditions GLP-1 secretion undergoes an increase triggered by exercise-related IL-6 stimulation. Since GLP-1 enhances insulin secretion, GLP-1 stimulation during physical exercise may have remarkable potential clinical applications. Indeed, it may become an option for insulin secretion improvement without pharmacological intervention, despite being currently considered hard to obtain. The proposed model may have a role in this purpose, as it provides a parameter related to the sensitivity of GLP-1 secretion to exercise-related IL-6 action, which is computable in the single individual. Future studies have to better assess model potential by analysis of individual data from people with different clinical characteristics.

CRediT authorship contribution statement

Micaela Morettini: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Writing – original draft. **Maria Concetta Palumbo:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. **Alessandro Bottiglione:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. **Andrea Danieli:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. **Simone Del Giudice:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft. **Laura Burattini:** Investigation, Resources, Validation, Writing – review & editing. **Andrea Tura:** Investigation, Supervision, Validation, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Simulink model representation

Simulink representation of the whole model and of the GLP-1 and IL-6 subsystems is provided in Fig. A.1, Fig. A.2 and Fig. A.3, respectively. All options of the Simulink model blocks have been retained at the default value.

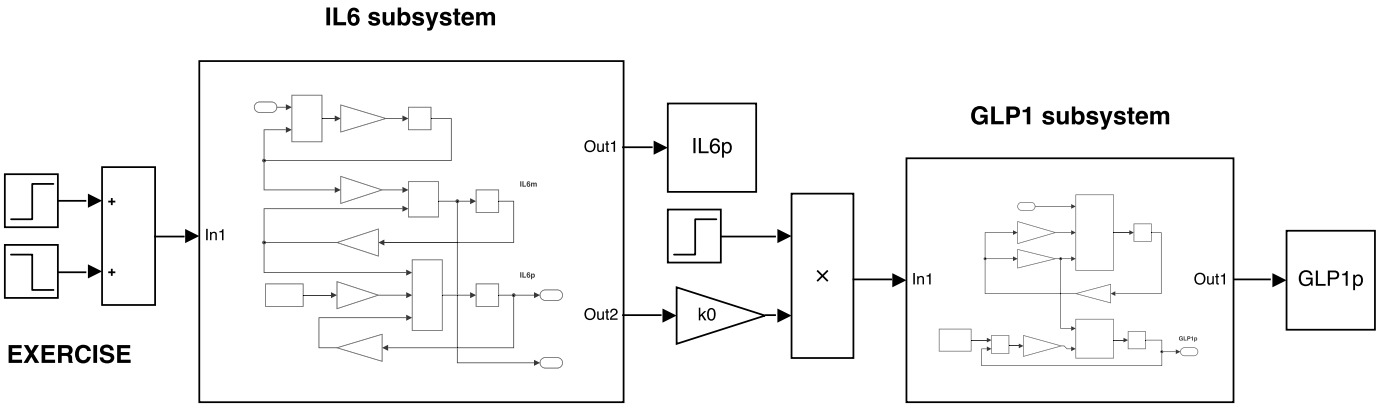

Fig. A.1. General Simulink model, composed by the GLP-1 and IL-6 subsystems.

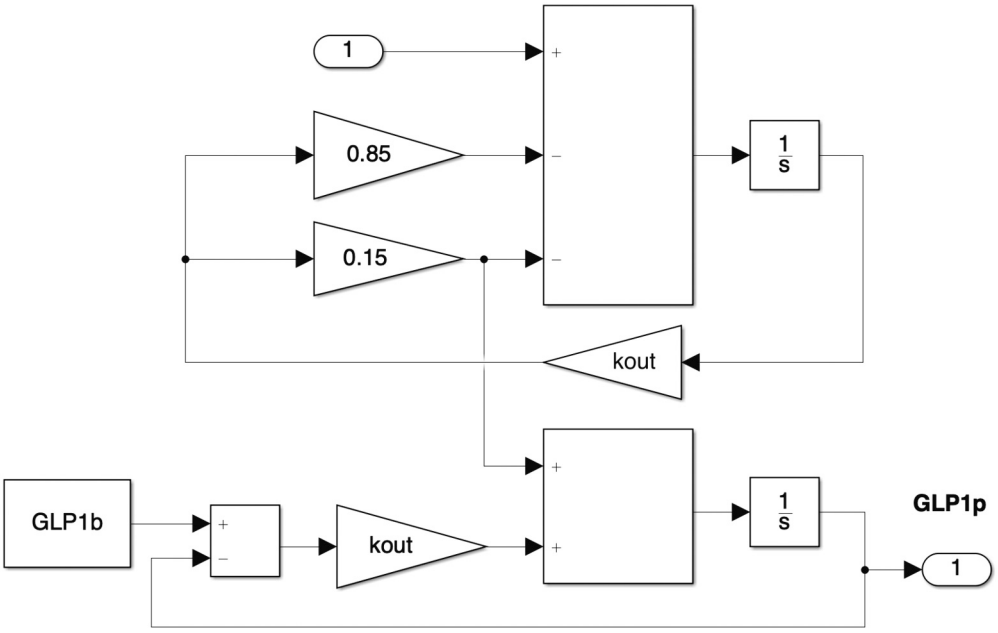

Fig. A.2. Simulink model of the GLP-1 subsystem.

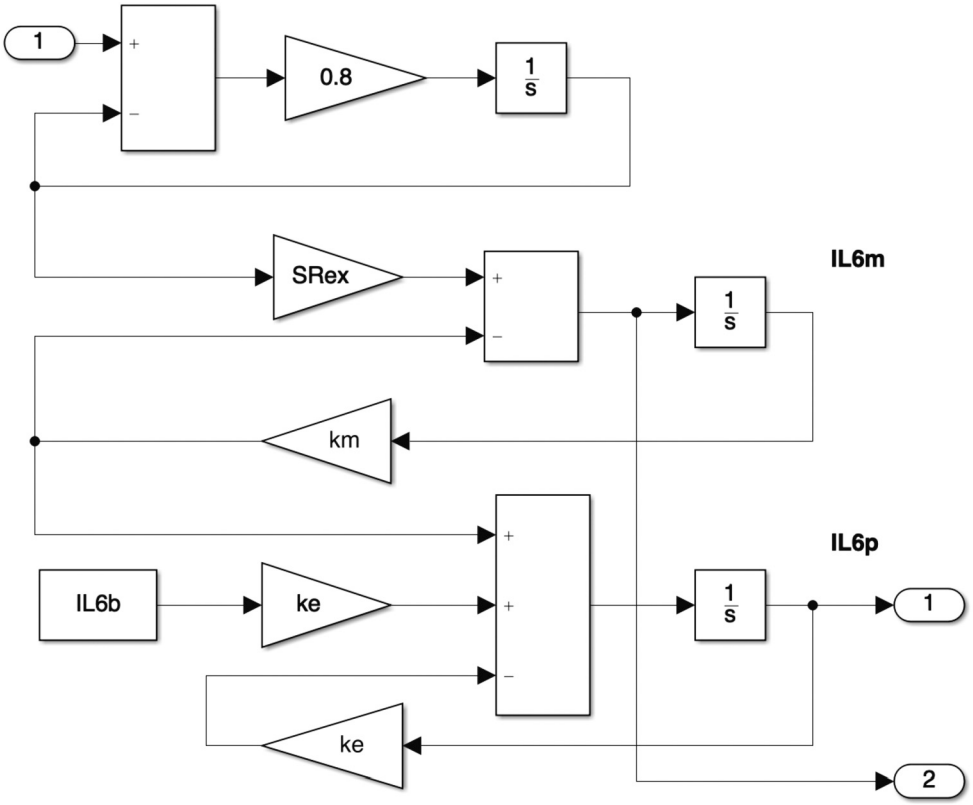

Fig. A.3. Simulink model of the IL-6 subsystem.

Appendix B. Pseudocode

The pseudocode for the main process and the function performing non-linear least squares estimate are provided.

Pseudocode 1 Main process.

Require: IL6 and GLP1 concentrations for each time point of the experiment i (with $i = 1...n$): $t_i, IL6_{exp}(t_i), GLP1_{exp}(t_i)$
Ensure: Model parameter estimates: SR_{ex}, k_m, k_e, k_0
global variables
  $IL6_b$
  $GLP1_b$
  $IL6_{exp}(t_i)$
  $GLP1_{exp}(t_i)$
  t_i
end global variables
$IL6b := IL6(0)$
$GLP1b := GLP1(0)$
$initpar := [SRex_0, k_{m,0}, k_{e,0}, k_{0,0}]$
$lb := [0, 0, 0, 0]$
$ub := [\infty, 1, 1, \infty]$
$[SR_{ex}, k_m, k_e, k_0] := \text{MinFunc}(initpar, lb, ub)$

Pseudocode 2 Function MinFunc, which performs non linear least squares estimate.

Require: par,lb,ub
function MinFunc(par, lb, ub)
  global variables
    $IL6_b$
    $GLP1_b$
    $IL6_{exp}(t_i)$
    $GLP1_{exp}(t_i)$
    t_i
  end global variables
  $w_1 := 9.1\%$
  $w_2 := 4.8\%$
  $SR_{ex} := par(1)$
  $k_m := par(2)$
  $k_e := par(3)$
  $k_0 := par(4)$
  $\Gamma_{GLP1,i} := w_1 * GLP1_{exp}(t_i)$
  $\Gamma_{IL6,i} := w_2 * IL6_{exp}(t_i)$
  $[GLP1(t_i), IL6(t_i)] :=$
  $\text{SimOut}(t_i, SR_{ex}, k_m, k_e, k_0)$
  $[SR_{ex}, k_m, k_e, k_0] :=$
  $\min_p \left(\sum_{i=1}^{n} \left(\frac{GLP1(t_i) - GLP1_{exp}(t_i)}{\Gamma_{GLP1,i}} \right)^2 + \right.$
  $\left. \sum_{k=1}^{n} \left(\frac{IL6(t_i) - IL6_{exp}(t_i)}{\Gamma_{IL6,i}} \right)^2 \right)$
  return $[SR_{ex}, k_m, k_e, k_0]$
end function

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