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# A New Oral Model to Assess Postprandial Lactate Production Rate

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## Abstract

**Objective:** Pediatric obesity predisposes children and adolescents to early onset insulin resistance and dysglycemia. In the last 20 years this has led to a rise in the prevalence of prediabetes, diabetes and fatty liver in youngsters, due to the high degree of insulin resistance experienced by these patients and the consequent high availability of glucose. As glucose accesses the liver, it is partly metabolized through glycolysis, whose main product is pyruvate that is then converted into Acetyl CoA and lactate. Therefore, lactate production rate (LPR) represents the best proxy for the assessment of glycolysis. Since to date there are not methods to estimate postprandial LPR, here we proposed a novel oral glucose-lactate model to estimate LPR during an oral glucose tolerance test and tested it in 24 youth with and without obesity.

**Methods:** The model is based on the oral glucose minimal model and assumes that LPR is a fraction ($fr$) of glucose disposal rate, proportional to glucose concentration and controlled by insulin action.

**Results:** The model well fitted the glucose and lactate data, and provided both precise parameter estimates (e.g. $fr$=22.5 [12.6–54.1]%, median [IQR]), CV=18 [13–25]%) and LPR time course.

**Conclusions:** The proposed model is a valid tool to assess LPR, and thus glycolysis, during OGTT in nondiabetic subjects.

**Significance:** The proposed methodology will allow to assess postprandial LPR in simple and cost-effective way.



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**Index Terms—**

physiological modeling; parameter estimation; identifiability; diabetes; prediabetes; obesity

## I. Introduction

The rise in obesity among youth in the last 40 years has been followed by an increased occurrence of prediabetes and type 2 diabetes in pediatrics [1]. Overt diabetes is preceded by a state of severe insulin resistance characterized by an increased glucose production and a lower uptake in the muscle, the result being an increased amount of glucose available as metabolic substrate [2]. Once glucose enters in the cells, it is converted into glucose-six-phosphate (G6P) and as such it can be stored as glycogen or serve as substrate for glycolysis [3]. It needs to be outlined that there are differences in terms of glucose uptake and kinases converting glucose into G6P between the liver and muscle [4]. In particular, in the liver the glucose transport is by facilitate diffusion and the synthesis of G6P is due mainly to the glucokinase (GCK) through a reaction that is not limited by the substrate [4]. In the muscle, glucose uptake is due to insulin action and the conversion of glucose into G6P is due to the action of the hexokinase [5].

Importantly, during the fasting state, about 50% of endogenous glucose production is due to glycogenolysis and about 50% to the gluconeogenesis in the liver [6]. During prolonged fasting, instead, hepatic gluconeogenesis becomes the sole mechanism to maintain glucose concentrations in plasma [6]. During the fed state, hepatic gluconeogenesis and glycogenolysis are inhibited by the insulin [7][8] and dietary carbohydrates become the main contributors to glycolysis.

Since there is a limited amount of glucose that can be stored as glycogen (about 5–6% of liver weight in the liver and 1–2% of muscles weight in the muscles)[9][10], in an environment rich in glucose (such as that of insulin resistant obese adolescents) [11] most of the glucose is processed through glycolysis especially in the liver, where insulin action on glucose uptake vanishes with the progression of insulin resistance [12]. Therefore, glycolysis represents an important bottleneck and a key crossroad in glucose metabolism.

The main product of glycolysis is pyruvate, which is promptly converted into alanine, oxaloacetate, acetyl-CoA and lactate. The latter is a closed pool in the body and the conversion rates of lactate to pyruvate and pyruvate to lactate are equal [13]. As consequence, measuring lactate synthesis provides an accurate estimate of the glycolytic flux.

Previously, investigators have proposed mathematical models to measure lactate synthesis during an intravenous glucose tolerance test (IVGTT)[14][15]. However, during the IVGTT, the glucose concentration pattern and levels are not physiological. Moreover, the intravenous test cannot be easily used in large-scale clinical studies. A more physiological experimental condition can be achieved with an oral glucose tolerance test (OGTT), as glucose enters in the circulation through the gastro-intestinal tract, like in normal daily life. To the best

of our knowledge, to date there are not models to estimate lactate production rate in the post-prandial state.

The aim of this study to fill this gap. Paralleling what has been done by Dalla Man et al [16], where the Bergman's Minimal Model, originally proposed to describe glucose kinetics during an IVGTT [17], was adapted to describe glucose kinetics and insulin action on glucose production and disposal during an OGTT, in this study, we set up a battery of six models that describe glucose-lactate kinetics during an OGTT. The models, described in details in the Method section, were tested on the data of 24 nondiabetic youths undergoing a 3-hour, 9-samples OGTT. The best model, selected using a parsimony criterion, assumes that only a fraction of the up-taken glucose is converted into lactate and this process depends on glucose concentration and disposal insulin action. Using this model, it is also possible to estimate post-prandial lactate production rate during an OGTT.

## II. Methods

### A. Data Base and Protocol

Twenty-four youth (16 with obesity and 8 without obesity, age=16±3 years, BMI=29±9 $kg/m^2$) enrolled in a study to determine the pathogenesis of obesity in youth (NCT03454828) underwent a 75 g OGTT. Yale IRB approved the study (HIC 20000239) on March 13$^{th}$2018. Plasma samples were drawn at t = −15, 0, 10, 20, 30, 60, 90, 120, 150, 180 minutes to measure plasma glucose, lactate and insulin concentrations (Fig 1). Plasma glucose was determined with an YSI 2700 Analyzer (YSI Inc). A colorimetric method was used to measure plasma concentrations of lactate (kit LC2389; Randox) during the study. Plasma insulin concentrations were measured by Radioimmunoassay (Linco).

### B. Models

The models available in the literature to describe glucose conversion into lactate are those proposed by Stefanovski et al. [14] and Watanabe et al. [15]. They were both tested on data of an intravenous glucose tolerance tests (IVGTT).

As a first attempt to estimate lactate production rate during an oral test, we coupled the models proposed by Stefanovski et.al [14] and Watanabe et al. [15] to the description of the meal glucose rate of appearance (Ra) included in the Oral Glucose Minimal Model (OGMM) [16][18]. As discussed in the Results, the performance of these models was not satisfactory. Therefore, we formulated and tested four new models, which incorporate either the insulin action, as in the OGMM, or a model of glucose kinetics and production (the Single Tracer Oral Minimal Model, STOMM) recently proposed by Visentin et al. [19] (Fig. 2).

**Model 1.—**This model is inspired by the model proposed by Stefanovski et.al. [14]. Model equations are mostly taken from [14], but at variance with the original work, in which glucose data were used as forcing function, here glucose dynamic is included in the model, so that a model of the rate of glucose absorption from the gastro-intestinal tract, Ra(α,t) had to be added:

$$\begin{cases} \dot{Q}_g(t) = Ra(\boldsymbol{\alpha}, t) - k_{pg}\left(Q_g(t) - V_g G_b\right) & Q_g(0) = V_g G_b \\ \dot{Q}_p(t) = -k_{lp} Q_p(t) + 2k_{pg}\left(Q_g(t) - V_g G_b\right) f_{switch} & Q_p(0) = 0 \\ \dot{Q}_l(t) = -k_l(Q_l(t) - Q_{lsb}(t)) + k_{lp} Q_p(t) f_{switch} & Q_l(0) = V_l L_b \\ G(t) = \dfrac{Q_g(t)}{V_g} \\ L(t) = \dfrac{Q_l(t)}{V_l} \end{cases} \quad (1)$$

where $Q_g$ is plasma glucose mass, G is plasma glucose concentration, $G_b$ its basal value, $Q_p$ is the mass of an intermediate substrate (likely pyruvate, P), $Q_l$ is the plasma lactate mass, L(t) is lactate concentration and $L_b$ its basal value. $Q_{lsb}$ is the sliding baseline for plasma lactate [14]:

$$Q_{lsb} = \frac{t(L_{max} - L_b)V_l}{t_{max}} + L_b \quad (2)$$

where $L_{max}$ is the value of plasma lactate at the end of the experiment (here 180 min) and $t_{max}$=180 min. Finally, $f_{switch}$ is a function controlling the influx to $Q_p$(t) and $Q_l$(t) compartments:

$$f_{switch} = \begin{cases} 1 \; if \; G(t) \geq G_b \\ 0 \; if \; G(t) < G_b \end{cases} \quad (3)$$

In other words, if glucose concentration is above $G_b$, then $f_{switch}$ is equal to 1 and $Q_g$ can be converted in $Q_p$ (with rate constant $k_{pg}$ - the factor 2 accounts for the fact that one molecule of glucose is transformed in two pyruvate molecules) and $Q_p$ in $Q_l$ (with rate constant $k_{lp}$), otherwise such conversions are inhibited. $Q_l$ is then cleared from plasma with rate constant $k_l$. $V_g$ and $V_l$ are the volumes of distribution of glucose and lactate, respectively.

As already observed, at variance with the original IVGTT model [14], here, glucose enters in the circulation with rate Ra($\alpha$,t), which, according to [18], is described by a piecewise-linear function with known break-point $t_i$ and unknown amplitude $\alpha_i$, followed by an exponential decay:

$$Ra(\boldsymbol{\alpha}, t) = \begin{cases} \alpha_{i-1} + \dfrac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}}(t - t_{i-1}) \;\; for \; t_{i-1} \leq t \leq t_i, i = 1 \ldots 5 \\ \alpha_5 e^{-\frac{(t - t_5)}{T}} \qquad\qquad for \; t > t_5 \end{cases} \quad (4)$$

with $\alpha$ denoting [$\alpha_1$, $\alpha_2$, … $\alpha_5$] and $t_5$=180 min.

**Model 2.—**This model is similar to the previous one but, at variance with that and similarly to [16]–[18], it accounts for insulin action on glucose conversion into lactate:

$$\begin{cases} \dot{Q}_g(t) = Ra(\boldsymbol{\alpha}, t) - (p_1 + X(t))Q_g(t) + p_1 V_g G_b & Q_g(0) = V_g G_b \\ \dot{X}(t) = -p_2 X(t) + p_2 S_I[I(t) - I_b] & X(0) = 0 \\ \dot{Q}_p(t) = -k_{lp} Q_p(t) + 2(p_1 + X(t))\big(Q_g(t) - V_g G_b\big) f_{switch} & Q_p(0) = 0 \\ \dot{Q}_l(t) = -k_l(Q_l(t) - Q_{lsb}(t)) + k_{lp} Q_p(t) f_{swit} & Q_l(0) = V_l L_b \\ G(t) = \dfrac{Q_g(t)}{V_g} & \\ L(t) = \dfrac{Q_l(t)}{V_l} & \end{cases} \tag{5}$$

where, like in [13][15], $Q_g$ is plasma glucose mass, G is plasma glucose concentration, I plasma insulin concentration (suffix "b" denotes basal values), X net insulin action on glucose production and utilization, $Q_p$ is the mass of an intermediate substrate (likely pyruvate, P), $Q_l$ is the plasma lactate mass, L(t) is lactate concentration and $L_b$ its basal value, $V_g$ and $V_l$ the glucose and lactate distribution volumes respectively, $p_1$ the fractional (i.e., per unit distribution volume) glucose effectiveness measuring glucose ability, per se, to promote glucose disposal and inhibit glucose production, $p_2$ is the rate constant describing the dynamics of insulin action and $S_I$ net insulin sensitivity, i.e. the ability of insulin to inhibit glucose production and enhance glucose utilization. As reported in [17], $p_1$ is linked to $S_I$ through the glucose effectiveness at zero insulin, GEZI:

$$p_1 = \frac{GEZI}{V_g} + S_I I_b \tag{6}$$

Glucose is first converted into the intermediate substrate (the factor 2 accounts for the fact that one molecule of glucose is transformed in two pyruvate molecules), and this conversion is controlled by insulin effect (X). Then, pyruvate is converted into lactate with the rate $k_{lp}$, which is then degraded with the constant rate $k_l$.

$Q_{lsb}$ and $f\_switch$ are defined in equations (2) and (3).

**Model 3.—**This model is an extension of the model proposed by Watanabe et.al. [15] to the oral test case:

$$\begin{cases} \dot{Q}_g(t) = Ra(\boldsymbol{\alpha}, t) - (p_1 + X(t))Q_g(t) + p_1 V_g G_b & Q_g(0) = G_b V_g \\ \dot{X}(t) = -p_2 X(t) + p_2 S_I[I(t) - I_b] & X(0) = 0 \\ \dot{Q}_p(t) = 2fr[p_1 + X(t)]Q_g(t) - \big(k_p + k_{lp}\big)Q_p(t) + k_{pl} Q_l(t) & Q_p(0) = Q_{pb} \\ \dot{Q}_l(t) = -\big(k_l + k_{pl}\big)Q_l(t) + k_{lp} Q_p(t) & Q_l(0) = \mathrm{L_b} V_g \\ G(t) = \dfrac{Q_g(t)}{V_g} & \\ L(t) = \dfrac{Q_l(t)}{V_l} & \end{cases} \tag{7}$$

where $Q_g$ is plasma glucose mass, G is plasma glucose concentration, I plasma insulin concentration, X net insulin action on glucose production and utilization, $Q_p$ is the mass of

an intermediate substrate, $Q_l$ is the plasma lactate mass, L(t) is lactate concentration and $L_b$ its basal value, $V_g$ and $V_l$ the glucose and lactate distribution volumes respectively, $p_1$ the fractional glucose effectiveness $p_2$ is the rate constant describing the dynamics of insulin action and $S_I$ insulin sensitivity

Differently to Models 1 and 2 and according to [15], only a fraction of glucose (*fr*) is converted into pyruvate, $Q_p(t)$ (the factor 2 accounts for the fact that one molecule of glucose is transformed in two pyruvate molecules), while the remaining (1-*fr*) follows other metabolic paths. Pyruvate is then converted in lactate, $Q_l(t)$ with rate constant $k_{lp}$ or degraded with rate constant $k_p$. Finally, $Q_l(t)$ is either re-converted in pyruvate, with rate constant $k_{pl}$ or cleared with rate constant $k_l$. The model assumes that the lactate volume of is equal to the glucose one.

According to [15], to make the model *a priori* identifiable (see section Model Identification), one has to re-parameterize the third and fourth equations of (7) and substitute them with a second order differential equation:

$$\ddot{Q}_l(t) = A_3[p_1 + X(t)]Q_g(t) - A_2Q_l(t) - A_1\dot{Q}_l(t); \quad Q_l(0) = L_bV_l \tag{8}$$

with

$$\begin{cases} A_1 = k_l + k_{lp} + k_p + k_{pl} \\ A_2 = k_lk_{lp} + k_pk_{pl} + k_lk_p \\ A_3 = 2frk_{lp} \end{cases} \tag{9}$$

**Model 4.—**Differently from Model 3, in which X represent the net insulin action on glucose production and utilization, here, these two components are separately described, as in the STOMM [19], and the rate of glucose into lactate is assumed to be a fraction, *fr*, of the utilization only. Another difference consists in the fact that this model does not include the metabolic intermediate compartment. Model equations are:

$$\begin{cases} \dot{Q}_g(t) = EGP(t) + Ra(\boldsymbol{\alpha}, t) - [p_{1D} + X_D(t)]Q_g(t) & Q_g(0) = G_bV_g \\ \dot{Q}_l(t) = 2fr[p_{1D} + X_D(t)]Q_g(t) - k_lQ_l(t) & Q_l(0) = L_bV_l \\ G(t) = \dfrac{Q_g(t)}{V_g} \\ L(t) = \dfrac{Q_l(t)}{V_l} \end{cases} \tag{10}$$

where $Q_g$, G, $Q_l$, L, Ra, *fr* and $k_l$ are defined like in Model 3, $p_{1D}$ is the fractional glucose effectiveness on glucose disposal only, $X_D$ is insulin action on glucose disposal, related to insulin concentration through a first order differential equation:

$$\dot{X}_D(t) = -p_{2D}X_D(t) + p_{2D}S_{ID}[I(t) - I_b] \quad X_D(0) = 0 \tag{11}$$

with $p_{2D}$ the rate constant describing the dynamics of disposal insulin action and $S_{ID}$ disposal insulin sensitivity. Note that, paralleling what done for Model 2, $p_{1D}$ is linked to $S_{ID}$ through the disposal glucose effectiveness at zero insulin, $GEZI_D$:

$$p_{1D} = \frac{GEZI_D}{V_g} + S_{ID} I_b \tag{12}$$

EGP is the endogenous glucose production (mainly occurring in the liver), described, like in [19], by the equations:

$$\begin{cases} EGP(t) = EGP_b - p_3[G(t) - G_b] - X_L(t) - X_{Der}(t) & \\ \dot{X}_L(t) = -p_{2p} X_L(t) + p_{2p} X_p(t) & X_L(0) = 0 \\ \dot{X}_p(t) = -p_{2p} X_p(t) + p_{2p} p_4 [I(t) - I_b] & X_p(0) = 0 \end{cases} \tag{13}$$

Differently from Model 3, the endogenous glucose production (EGP) is variable, with $k_1$ glucose effectiveness on glucose production, $X_L$ insulin action on EGP, $p_{2P}$ the rate constant describing the dynamics of liver insulin action, $p_4$ a parameter governing liver insulin sensitivity ($S_{IL}$), which can be calculated as:

$$S_{IL} = \frac{p_4}{G_b} \tag{14}$$

as previously reported [19], and $X_{Der}$ the EGP suppression component proportional to glucose rate of change through $k_{gr}$ as:

$$X_{Der}(t) = \begin{cases} k_{gr} \dfrac{dG(t)}{dt} & if \dfrac{dG(t)}{dt} \geq 0 \\ 0 & if \dfrac{dG(t)}{dt} < 0 \end{cases} \tag{15}$$

**Model 5.—**Model 5 is based on the same assumption of Model 4 on insulin action (eqs 11–13) but, differently from Model 4, here lactate production rate is controlled by lactate concentration itself, though a Langmuir model:

$$\begin{cases} \dot{Q}_g(t) = EGP(t) + Ra(\boldsymbol{\alpha}, t) - [p_{1D} + X_D(t)] Q_g(t) & Q_g(0) = G_b V_g \\ \dot{Q}_l(t) = \begin{cases} 2\left(1 - \dfrac{L(t)}{\beta}\right)[p_{1D} + X_D(t)] Q_g(t) - k_l Q_l(t) & if\ L(t) < \beta \\ -k_l Q_l(t) & if\ L(\,|\,t) \geq \beta \end{cases} & Q_l(0) = L_b V_l \\ G(t) = \dfrac{Q_g(t)}{V_g} & \\ L(t) = \dfrac{Q_l(t)}{V_l} & \end{cases} \tag{16}$$

In other words, the model assumes that, when lactate concentration overcomes a given threshold β, the influx in lactate compartment is blocked and glucose follows other metabolic paths.

**Model 6.—**This model is a combination of Model 3 and 4. Like Model 4, it considers the insulin effect on glucose production and utilization separately and, like Model 3, it assumes that glucose is first converted in pyruvate, $Q_p(t)$, and this one is converted in lactate, $Q_l(t)$. Similarly to Model 3, the differential equations describing the bi-directional pyruvate-lactate transformation are coupled in a single second-order differential equation (8).

## C. Model Identification

Like the OGMM and the STOMM model, on which they are based, the above models are not a priori identifiable. Therefore, their identification requires a number of assumptions that were discussed in detail [16][18][19]. Briefly, to ensure the *a priori* identifiability, one has to assume a value for $V_g$. Here, we fixed $V_g$ to its population value ($V_g$=1.45 dl/kg [19]) and assumed that $V_l$ is equal to $V_g$. To improve numerical identifiability of the remaining parameters, a Gaussian *Bayesian* prior was applied to parameters GEZI and $p_2$ or $p_3$, $GEZI_D$ and $p_{2D}$ with values taken from [16] and [19], respectively.

Finally, a constraint was imposed to guarantee that the area under the estimated Ra(α,t) equals the 90% of the amount of ingested glucose: this constraint provided an additional relationship among the unknown parameters $\alpha_i$, thus reducing the number of unknowns by one.

It is worth noting that that, at variance with [19], here the oral glucose load was unlabeled. Nevertheless, models 4, 5 and 6, still allowed to segregate glucose production from disposal, by exploiting the additional measurement of lactate, whose production is a fraction of glucose utilization only.

The models were identified simultaneously on plasma glucose and lactate concentrations, using a *Bayesian* Maximum a Posteriori (MAP) estimator [21], with the exception of Model 1 which was identified using the weighted nonlinear least squares (WNLLS).

Glucose and lactate measurements errors were assumed to be independent, Gaussian with zero mean and known SD [coefficient of variation (CV) = 2%]. Insulin concentration was the model forcing function and was assumed to be known without error.

Parameter estimation was performed in Matlab™ (R2019b) [22], using the *lsqnonlin* routine to run the optimization and the *ode45* solver to integrate the model differential equations.

## D. Model Assessment

The best model was selected by comparing model performances on the basis of different criteria. Once calculated the weighted residuals as (data-model prediction)/SD, where SD is the standard deviation of measurement error, we tested their independence using Wald-Wolfowitz Run Test [24], and assessed the model ability to describe the data (weighted residual sum of squares, WRSS), the precision of parameters estimates (expressed as percent

coefficient of variation, CV%), and model parsimony, by the Bayesian Information Criterion (BIC) [23]:

$$BIC = Nln(M) + 2\ln(WRSS) \tag{17}$$

where N is the total number of the parameters of the model, and M is the number of samples. In particular, we first discarded those models that did not fit the data well (more than 10% of subjects not passing the Run Test), second, we discarded those models that were providing imprecise parameter estimates (CV>100% in more than 30% of the subjects) and, finally, we applied the parsimony criterion only to the remaining models to select the best one.

### E. Statistical Analysis

Data are presented as mean±SE for normally distributes variable and median [IQR] for not normally distributed ones. Gaussianity was verified using Lilliefors test. Comparisons were done using Wilcoxon Signed Rank Test with significance level set at p=0.05 [25].

### F. Estimated Lactate Production Rate

All the models allow to estimate both the fractional (FLPR [$min^{-1}$]) and the total lactate production rate (LPR [mmol/kg/min]). For instance, using Model 4, one has:

$$FLPR = 2fr[p_{1D} + X_D(t)] \tag{18}$$

$$LPR = 2fr[p_{1D} + X_D(t)]Q_g(t) \tag{19}$$

## III. Results

### A. Model Assessment

Models weighted residuals are showed in Fig 3. Model comparison, in terms of percentage of subjects passing the Run Test, median WRSS, CV% and BIC, is summarized in Table I. Specifically, Model 1 was rejected, because the randomness of glucose and lactate weighted residuals was poor, and the estimated parameters did not always exhibit satisfactory precision. Model 2 provided a prediction of lactate concentration similar to Model 1 but, since it accounted for the insulin action on glucose metabolism, it better predicted glucose prediction with respect to Model 1. However, the model was rejected because the precision of the estimated parameters was not satisfactory in 50% of the subjects. Models 3 and 6 provided a better fit of the data, with respect to Model 1 and 2, but they were also discarded since the precision of the estimates was poor in a significant percentage of the subjects (58% and 54%, respectively). Models 4 and 5 provided a good fit of the data and, overall, good CV%. However, parameter β of Model 5 was very difficult to estimate with precision in almost half of the subjects. Therefore, Model 4 was selected as the best one to describe glucose-lactate kinetics during an OGTT. This choice was supported by BIC that was numerically, despite not significantly, lower in Model 4 than Model 5 (p=0.46).

### B. Key Parameters and Lactate Production Rate

The identification of Model 4 provided precise estimates of all parameters (Table II), among which, the most informative are: disposal insulin sensitivity $S_{ID}$ = 6.9 [1.7–11.1] (median [IQR]) $10^{-4}$ dl/kg/min per μU/ml, liver insulin sensitivity $S_{IL}$ = 6.5 [5.6–7.5] $10^{-4}$ dl/kg/min per μU/ml, fraction of glucose actually converted into lactate *fr* = 22.5 [12.6–54.1]% and fractional lactate clearance rate $k_l$ = 0.076 [0.053–0.117] $min^{-1}$.

The estimated FLPR and LPR time courses are reported in Fig 4. During an OGTT, FLPR rose from 0.009 [0.006–0.017] $min^{-1}$ at t=0 to a peak of 0.018 [0.013–0.034] $min^{-1}$ at t~90 min; it then descreased and reached the value of 0.013 [0.009–0.030] $min^{-1}$ at the end of the experiment. Similarly, LPR was 0.00623 [0.0043–0.0114] mmol/kg/min at basal, peaked at t~30 min reaching a value of 0.0143 [0.0108–0.0256] mmol/kg/min; it then slowly descreased to 0.0086 [0.0056–0.0155] mmol/kg/min at the end of the experiment.

## IV. Discussion

In this study we described the first model to assess LPR after oral intake of glucose, in youth with and without obesity. This is extremely useful as it allows to assess an important and often overlooked metabolic pathway such as glycolysis. In fact, as recently pointed out, “Because in mammalian systems lactate is the inexorable product of one metabolic pathway (glycolysis), and the substrate for another pathway (mitochondrial respiration), lactate is the link between glycolytic and aerobic pathways” [26]. Therefore, lactate kinetics represents the best proxy for the assessment of glycolysis. Assessing glycolysis in individuals at risk for diabetes, hypertriglyceridemia and fatty liver may provide important physiologic and clinical insight to understand the fine mechanisms underlying the development of insulin resistance and its complications in the context of pediatric obesity.

The current method used to assess LPR employs mathematical models, like those proposed by Stefanovski et.al [14] and Watanabe et.al [15]. These have been validated to describe glucose-lactate conversion during an IVGTT. We first tested if one of the two models, properly modified, could be applied to OGTT data. In order to do that, we coupled them with a parametric description of the post-prandial meal rate of appearance (Ra) that previously proved to be effective to describe post-prandial glucose kinetics [16][18].

Unfortunately, none of them provided satisfactory results in terms of model ability to fit the data as well as for the poor precision of the parameter estimates. We hypothesized that the poor performance of Model 1 could be due to the different dynamic of the glucose and insulin signals observed during IVGTT and OGTT. However, we also argued that this can be partly due to the fact that, while in [14] Stefanovski and coworkers used glucose as forcing function to predict lactate concentration, here we simultaneously fit glucose and lactate data. Therefore, we also tested Stefanovski model on our OGTT data, using glucose concentration as forcing function, but results in terms of model fitting to the data was not satisfactory (data not shown). Finally, the assumption that the glucose-lactate conversion is not controlled by insulin seems to be not supported by the data in these experimental conditions. We thus tested if adding insulin control to the Stefanovski-like model might improve the data fit (Model 2). This was the case for the glucose, as expected to some extent, but not for the

lactate time course. For what concerns Model 3, as the original paper by Watanabe et al. [15], the critical assumption is that lactate production rate is controlled by the net insulin action on both glucose production and disposal (X).

To overcome the above limitations, we tested three new models sharing the assumption that LPR is a fraction (*fr*), to be estimated, of the glucose disposal and is thus controlled by the disposal insulin action ($X_D$). The superiority of Model 4 upon the more physiological Model 6, which, like Watanabe's model, accounts for glucose conversion into pyruvate and pyruvate conversion into lactate, is likely due to the slower dynamics of the OGTT vs IVGTT and the low frequency of the sampling schedule used in this study which may mask the fast transit through the compartment chain. Model 4 and Model 5 provided similar performance overall, but parameter β, representing the lactate concentration at which LPR is saturated, was very high (134.5 [101.0–187.1] mmol/l) and estimated with poor precision (CV=1539 [920–2741]%) in a significant percentage of the subjects (10 among 24), while it was 2.5 [2.1–3.4] mmol/l (CV=14 [7–17]%) in the remaining 14. Unfortunately, this was apparently not related to the measured lactate peak (1.4 [1.2–1.9] mmol/l in the first group and 1.4 [1.3–1.4] in the second group) nor to any other subject characteristic.

We concluded that Model 4 was the one to use during a 3 hour-9 sample OGTT, like the one presented here. It provides precise estimates of key model parameters, like disposal and liver insulin sensitivity and the fraction of glucose actually converted in lactate. Nevertheless, the between-subject variability observed in these parameters is quite large. However, insulin sensitivity is known to vary a lot, even in a homogeneous young populations, e.g. [27] reported $S_I$=13.8 [8.2–16.7] $10^{-4}$ dl/kg/min per μU/ml in a group of 11 non-obese youths. Therefore, it is somewhat expected to find a slightly higher variability in our data, which include both obese and non-obese youths.

The main limitation of this study is indeed the limited number of samples per subjects and the short duration of the protocol. It is possible that a more frequent and longer sampling schedule had favored a different model (e.g. Model 6). However, this is the sampling scheduling of a standard OGTT protocol, employed by most of the researchers. Adding samples or extending the protocol duration, despite feasible to some extent, would make the use of our model less appealing for the many of them. Despite that, the selected model well performed in these experimental conditions and provided good estimate of the key metabolic variables. Another limitation is that the model has been tested in nondiabetic adolescents with and without obesity, only. Further studies are needed to validate the model in subjects with diabetes and/or of different age. Incidentally, we did not find any significant difference between the obese and nonobese group neither in the LPR, FLPR, *fr* nor $k_l$ ($LPR_{peak}$=0.0143 [0.0110–0.0256] mmol/kg/min in obese and $LPR_{peak}$ = 0.0151 [0.0107–0.0344] mmol/kg/min in lean subjects; *fr*=25.2 [14.6–35.3] % in obese and *fr*=13.4 [12.6–93.5] % in lean subjects; $k_l$=0.076 [0.051–0.100] $min^{-1}$ in obese and $k_l$=0.083 [0.061–0.157] $min^{-1}$ in lean subjects), but this could be due to the fact that the study was not powered for this purpose. Therefore, this finding must be considered preliminary and not conclusive.

## V. Conclusion

In conclusion, we proposed a model, which proved to be a valid tool to assess FLPR and LPR, and thus glycolysis, during OGTT in nondiabetic subjects with and without obesity. Further studies are needed to validate the model in subjects with diabetes or other metabolic diseases.

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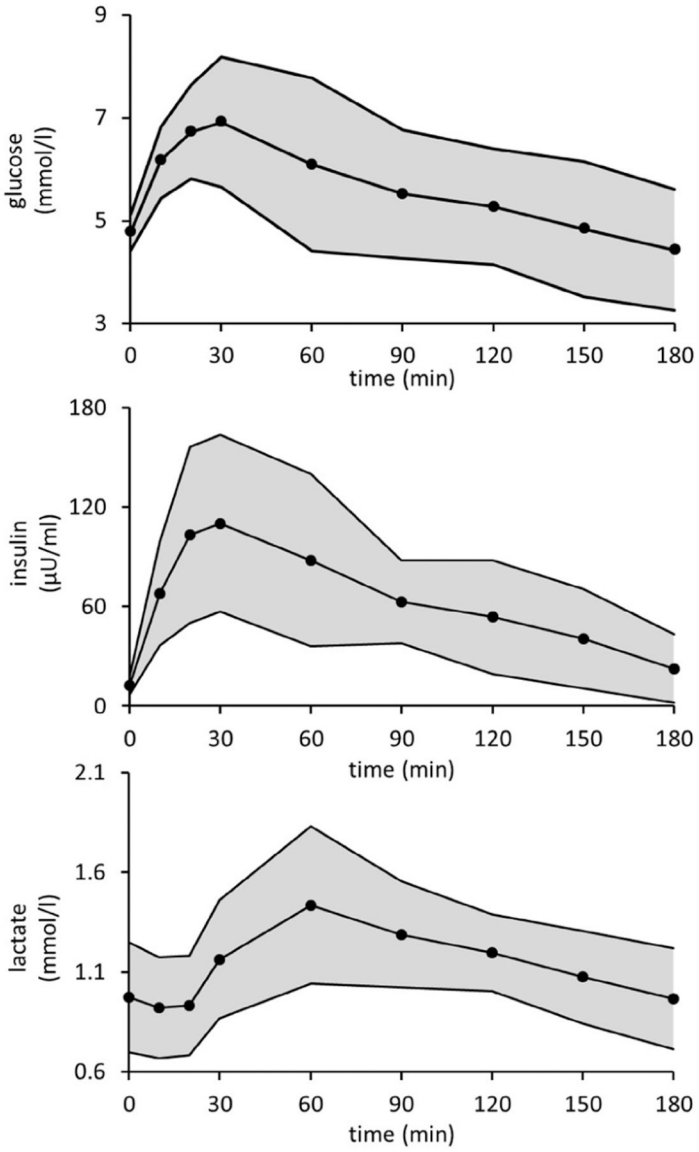


**Fig. 1.**

Mean (black line) ±SD (grey area) plasma glucose (top), insulin (middle) and lactate concentrations (bottom panel).

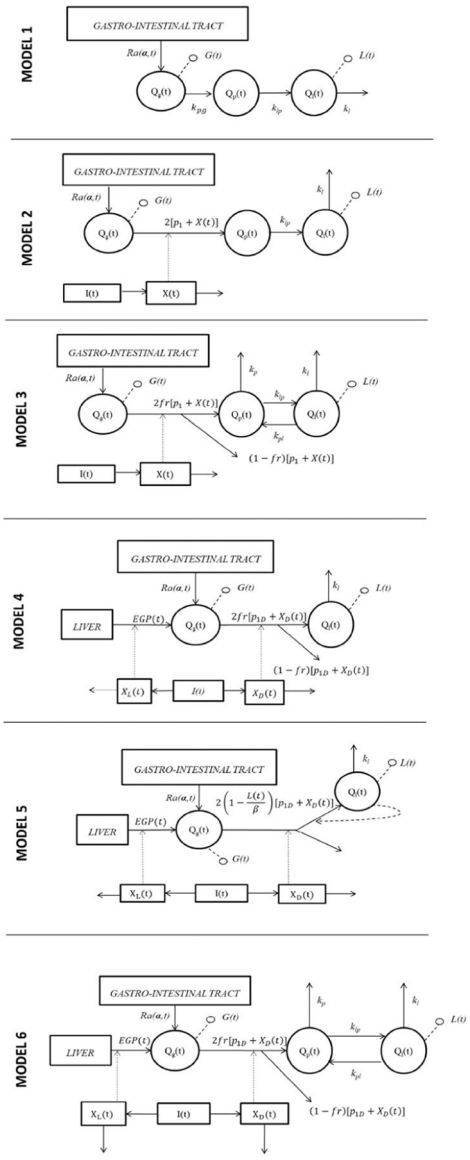


**Fig. 2:**
Graphical representation of the six tested models.

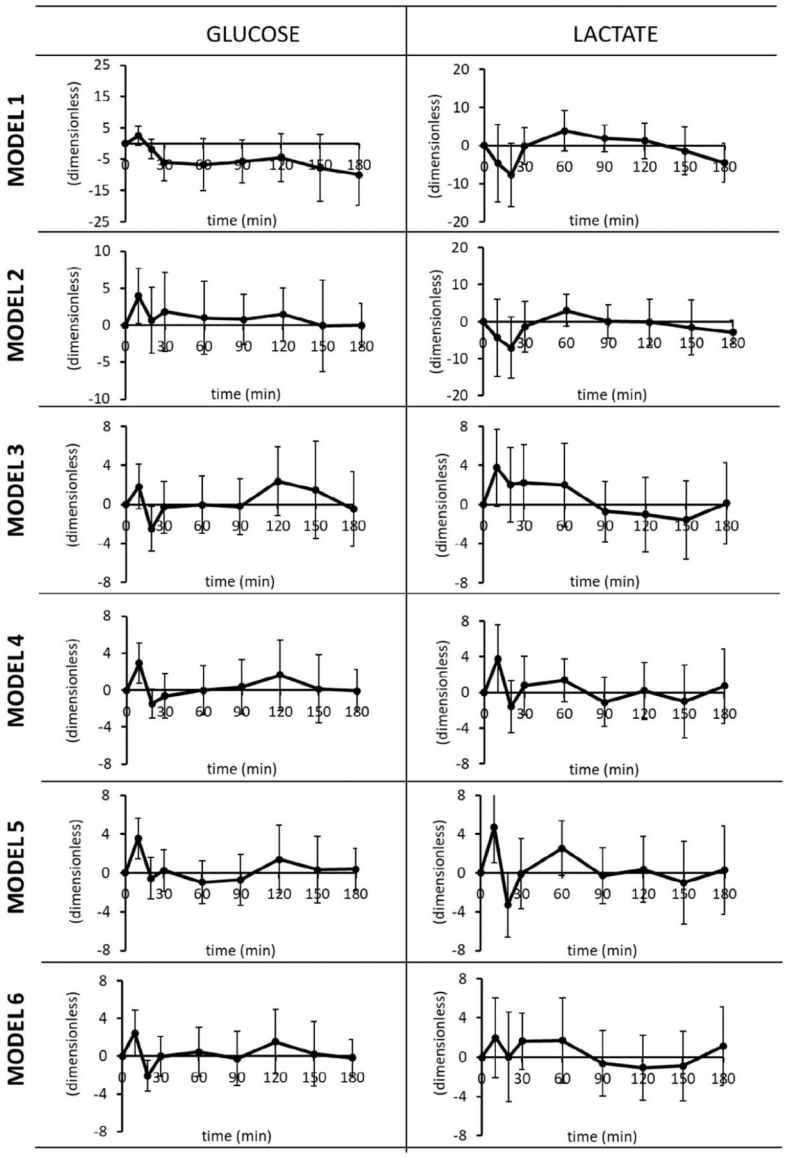


**Fig.3:**

Average glucose (*left*) and lactate (*right panels*) weighted residuals of the six tested models. Vertical bars represent ± SD.

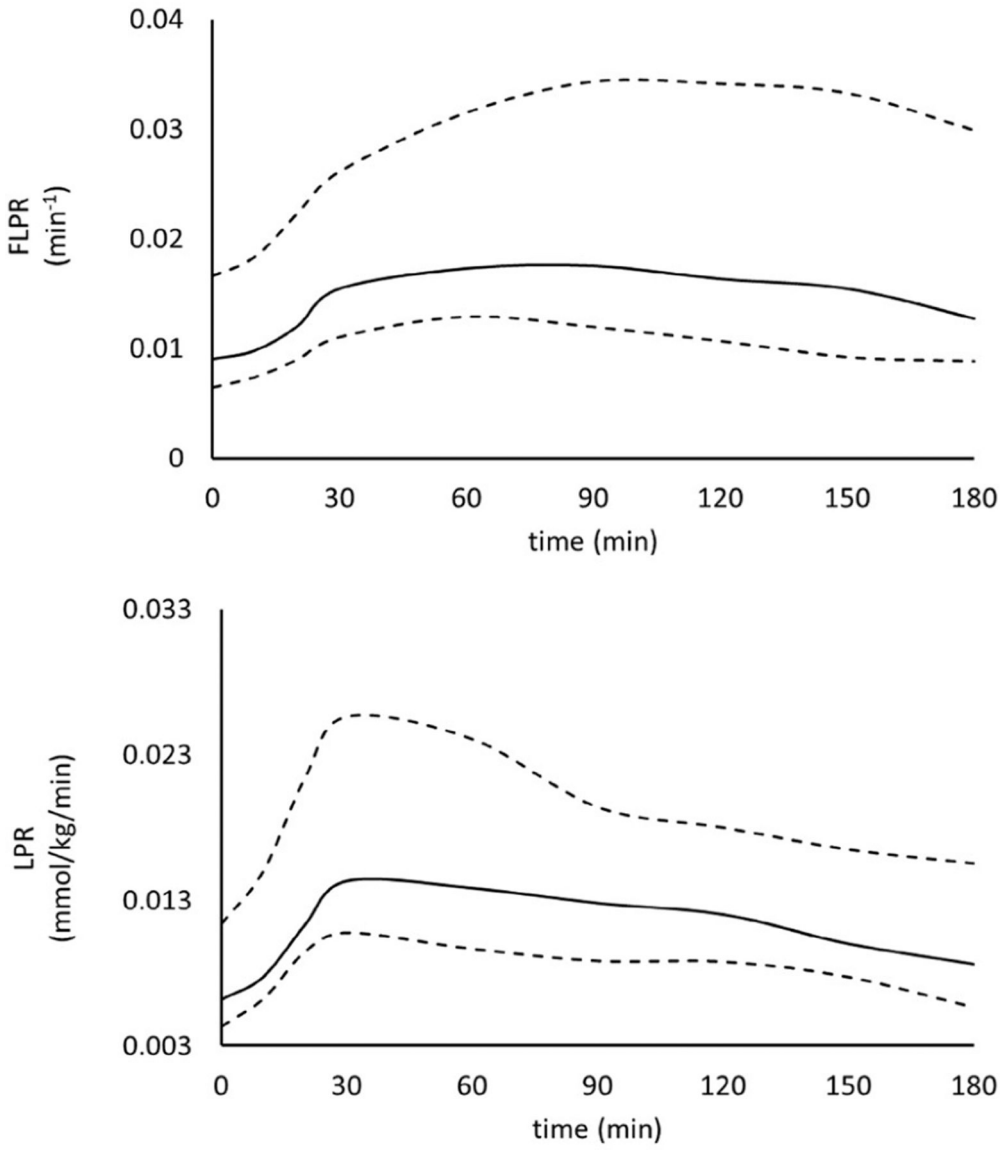


**Fig.4:**

Median (continuous) and interquartile range (dashed lines) fractional lactate production rate, FLPR, (upper) and total lactate production rate, LPR, (lower panel).

**Table I**

Model Comparison

| Model | Run Test (% of subjects passing the test) | WRSS (median [IQR]) | Precision (CV%) (median [IQR]) | BIC (median [IQR]) |
|---|---|---|---|---|
| 1 | 63% | 895 [503–1543] | 23 [6–52] % | 33.8 [32.7–34.9] |
| 2 | 92% | 280 [210–445] | 10 [6–19]% | 37.3 [36.7–38.2] |
| 3 | 92% | 194 [124–333] | 9 [5–214]% | 39.4 [38.5–40.5] |
| 4 | 96% | 163 [125–230] | 11 [8–16]% | 47.8 [47.2–48.4] |
| 5 | 96% | 193 [146–232] | 11 [7–15]% | 48.1 [47.5–48.4] |
| 6 | 96% | 142 [81–222] | 12 [8–78] % | 50.4 [49.2–51.2] |

**Table II**

ESTIMATED PARAMETERS USING MODEL 4

| Parameter | Equation | Value (median [IQR]) | CV% (median [IQR]) |
|---|---|---|---|
| $\alpha_1$ (mg/kg/min) | | 6 [4–9] | 6 [5–8]% |
| $\alpha_3$ (mg/kg/min) | *4* | 6 [4–7] | 5 [4–7]% |
| $\alpha_4$ (mg/kg/min) | | 6 [4–7] | 3 [3–5]% |
| $\alpha_5$ (ing/kg/min) | | 3 [2–5] | 9 [6–13]% |
| fr (%) | *10* | 22.5 [12.6–54.1] | 18 [13–25]% |
| $k_1$ ($min^{-1}$) | | 0.076 [0.053–0.117] | 13 [11–16]% |
| $p_{2D}$ ($min^{-1}$) | *11* | 0.023 [0.015–0.036] | 10 [8–13]% |
| $S_{ID}$ ($10^{-4}$ dl/kg/min per μU/ml) | | 6.9 [1.7–11.1] | 14 [8–22]% |
| $GEZI_D$ (dl/kg/min) | *12* | 0.025 [0.010–0.030] | 10 [8–19]% |
| $p_3$ (dl/kg/min) | | 0.017 [0.016–0.018] | 14 [13–15]% |
| $p_{2P}$ ($min^{-1}$) | *13* | 0.022 [0.020–0.027] | 12 [10–13]% |
| $p_4$ (mg/kg/min per (μU/ml) | | 0.055 [0.047–0.064] | 11 [9–12]% |
| $k_{gr}$ (dl/kg) | *15* | 0.121 [0.119–0.122] | 14 [14–15]% |