Estimating Hepatic Glucokinase Activity Using a Simple Model of Lactate Kinetics

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OBJECTIVE—Glucokinase (GCK) acts as a component of the "glucose sensor" in pancreatic β-cells and possibly in other tissues, including the brain. However, >99% of GCK in the body is located in the liver, where it serves as a "gatekeeper", determining the rate of hepatic glucose phosphorylation. Mutations in *GCK* are a cause of maturity-onset diabetes of the young (MODY), and *GCKR*, the regulator of GCK in the liver, is a diabetes susceptibility locus. In addition, several GCK activators are being studied as potential regulators of blood glucose. The ability to estimate liver GCK activity in vivo for genetic and pharmacologic studies may provide important physiologic insights into the regulation of hepatic glucose metabolism.

RESEARCH DESIGN AND METHODS—Here we introduce a simple, linear, two-compartment kinetic model that exploits lactate and glucose kinetics observed during the frequently sampled intravenous glucose tolerance test (FSIGT) to estimate liver GCK activity (K_{GK}), glycolysis (K_{12}), and whole body fractional lactate clearance (K_{01}).

RESULTS—To test our working model of lactate, we used cross-sectional FSIGT data on 142 nondiabetic individuals chosen at random from the Finland–United States Investigation of NIDDM Genetics study cohort. Parameters K_{GK}, K_{12}, and K_{01} were precisely estimated. Median model parameter estimates were consistent with previously published values.

CONCLUSIONS—This novel model of lactate kinetics extends the utility of the FSIGT protocol beyond whole-body glucose homeostasis by providing estimates for indices pertaining to hepatic glucose metabolism, including hepatic GCK activity and glycolysis rate.

Diabetes Care 35:1015–1020, 2012

Liver glucokinase (GCK) is rate limiting for the phosphorylation rate of glucose and is an important determinant of glucose tolerance in vivo. It has become increasingly clear that it is important to assess the activity of hepatic GCK in vivo in humans as a key to observing changes in liver glucose phosphorylation. A novel group of GCK activators has been introduced and has been shown to reduce the blood glucose level after chronic administration, thus positioning them as possible candidates for treatment of diabetes (1). Additionally, a common variant in GCK regulatory protein (GKRP) (P446 L) has been shown to be associated with reduced fasting and 2-h glucose, elevation of triglycerides, and reduced risk of type 2 diabetes (T2D), presumably by altering the ability of the liver to adapt to hyperglycemia (2). Rare mutations in *GCKR* have also been identified in individuals with very high triglyceride levels (3). Furthermore, we have shown that the incretin analog exenatide acts to enhance liver glucose uptake, and this may reflect altered GKRP and/or GCK activity (4).

It is impractical to estimate hepatic GCK activity directly in large populations of subjects because that would require liver biopsy. Thus, for genetic studies, there is a need to complement genotype information with simpler phenotyping to evaluate the importance of specific mutations for function. Here we introduce a novel approach for estimating GCK activity in vivo. The advantage of the approach is that it exploits clinical tests to extract information regarding glucose phosphorylation in the liver. Our approach uses blood samples taken during the frequently sampled intravenous glucose tolerance test (FSIGT), which previously has been widely performed to estimate insulin sensitivity, insulin response, β-cell function (disposition index [DI]), insulin clearance rate, and glucose effectiveness (5). FSIGTs were previously applied to a subset of the participants of the Finland–United States Investigation of NIDDM Genetics (FUSION) study of T2D genetics (6), but estimates of glucose phosphorylation have not been previously attempted.

The idea behind our method to estimate liver glucose phosphorylation is as follows. During acute hyperglycemia, after intravenous glucose injection, much of the acute glucose disposal is due to liver glucose uptake, which is primarily glucose dependent (7). Although the fate of the glucose could well include hepatic glycogen synthesis by the "direct pathway", most of the glucose taken up by the liver likely traverses the glycolytic pathway. Because there is little acute energy need for increased oxidation of the resulting three-carbon moieties, much of the resultant pyruvate is converted, via lactate dehydrogenase (LDH), to lactate, which is then easily exported into the hepatic venous effluent or stored as glycogen via the gluconeogenic pathway. Thus, it is possible to develop a mathematical model of the liver response to glucose injection, based only on the phosphorylation of glucose in the liver, glycolysis, and the ultimate export of the three-carbon compounds to lactate followed by lactate clearance from the blood. It is the dynamic relationship between plasma glucose and lactate that we use in the model to estimate hepatic glucose phosphorylation, and therefore activity of the enzyme liver GCK.

RESEARCH DESIGN AND METHODS

—To build and test models, it was necessary to procure a database in

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Received 17 August 2011 and accepted 29 January 2012.
DOI: 10.2337/dc11-1540
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc11-1540/-/DC1.

which the FSIGT had been performed, and for which glucose and lactate had been measured. Although a large database with recently measured samples would have been preferable, such a database was not readily available, due to the lack of lactate measurements conducted in recent studies. However, we did have access to samples from the FUSION database, in which we had previously demonstrated that there was no systematic change in the plasma glucose levels even after 14 years of storage at −80°C in our laboratory (see below). Therefore, we could assume that lactate also would not change, as evaporation or infection of samples would affect both lactate and glucose. That there was no such event allowed us to use lactate measurements performed recently and assume that such measurements applied to tests performed some years ago.

Data for designing the model were collected as part of the FUSION study (8) from Helsinki and Kuopio, Finland, in 1998–1999. Glucose and insulin were measured in nondiabetic spouses of T2D patients and their offspring at that time (6,8), but lactate was not. Therefore, we measured lactate and remeasured glucose on plasma samples stored for ~14 years at −80°C. Glucose measurements before and after storage were remarkably similar (r = 0.98). Although we did not measure lactate in 1998–1999, it seems likely, as a small nutrient molecule, that it also survived storage intact. Thus for modeling, we used glucose and lactate values measured in 2010 as representative of measurements that could have been made at the time of the FSIGTs.

Study subjects were 142 nondiabetic volunteers (75 women and 67 men; age, 38.7 ± 12.6 years; body weight, 74.5 ± 14.1 kg; BMI, 25.9 ± 4.1 kg/m^2 [mean ± SD]) chosen at random from the FUSION study cohort.

Reduced-sample, tolbutamide-modified FSIGTs were performed in the morning after an overnight fast (9). After drawing one basal sample, glucose (0.3 g/kg, 50% dextrose; Abbott Hospital Products, Chicago, IL) was injected over 1 min. Blood samples for glucose and insulin (and now lactate) were taken at 2, 4, 8, and 15 min. At 20 min, tolbutamide (300 mg for BMI <30 kg/m^2; 500 mg for BMI >30 kg/m^2) was injected. Additional blood samples were collected at 22, 25, 30, 40, 50, 70, 100, 120, and 180 min. Samples were placed into 1.7-mL chilled tubes coated with lithium fluoride and heparin containing 50 μL EDTA. Samples were immediately centrifuged and plasma was separated. Glucose was measured in 1998–1999 with a YSI autoanalyzer; glucose and lactate were measured in 2010 with a similar YSI 2300 autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH).

Numerical methods

Model parameter estimation was performed using MLAB (Civilized Software, Bethesda, MD), which uses a Levenberg-Marquardt iterative, weighted least-squares algorithm. Prior to estimation and in an effort to dampen the random noise in the lactate observations, data points were presmoothed using three-point moving average in MLAB. Averages for observations are presented as mean ± SE unless otherwise specified. Parameter estimates are reported as medians and range unless otherwise noted. Statistical analysis was performed using STATA (StataCorp, College Station, TX).

RESULTS

Reduced-sample, tolbutamide-modified FSIGT

Average fasting plasma glucose before the FSIGT was 5.4 ± 0.05 mmol/L (Supplementary Fig. 1*A*). The glucose bolus at t = 0 min resulted in a rapid rise in plasma glucose concentration to an average peak of 17.7 ± 0.49 mmol/L at t = 2 min. Plasma glucose gradually returned to baseline level by 70 min (5.5 ± 0.14 mmol/L); glucose continued to decline, and by the end of the FSIGT was on average significantly lower than before glucose injection (4.4 ± 0.04 mmol/L vs. 5.4 ± 0.05; $P < 0.00001$). Fasting plasma lactate concentration was 0.84 ± 0.02 mmol/L (Supplementary Fig. 1*B*). Plasma lactate began to increase 4 min after the glucose bolus, achieved its mean peak value of 1.30 ± 0.03 mmol/L at 40 min, and then declined monotonically to near basal by 120 min (0.78 ± 0.02 mmol/L). From the average time course of lactate, it can be seen that the tolbutamide bolus at 20 min did not result in any significant aberration from the monotonic decline in plasma lactate concentration, suggesting that perhaps insulin may not acutely influence lactate kinetics. Similar to glucose, plasma lactate levels were slightly below basal by 180 min (0.73 ± 0.02 vs. 0.84 ± 0.02 mmol/L; P = 0.0004).

Model development

Rationale. The goal of the modeling effort was to estimate glucose phosphorylation ("net" GCK activity), utilizing glucose and lactate measurements obtained from FSIGTs performed some years ago. To do this, it was necessary to test constructs that were as simple as possible, but based upon known physiology. Thus, the model was not required to account for all known relationships between glucose, lactate, and insulin. In contrast, we modeled only the role of the liver to take up glucose and export lactate. Thus the model was designed to be the simplest representation able to explain plasma lactate kinetics (the "output"), with the measured plasma glucose values assumed to be known (the "input") (Supplementary Fig. 1). In the simple representations applied, the acute pattern of plasma insulin was not included in the model of the liver, as glucose uptake by the liver is "glucose dependent" and therefore independent of acute changes of insulin in the portal vein (7). The above formulation of the model allowed for uncoupling of the lactate model from the whole-body glucose metabolism, which in part is insulin dependent, using a forcing function for the plasma glucose. The forcing-function modeling construct allowed us to use the interpolated plasma glucose profile without the need to formulate the additional forces driving the glucose disposal, such as glucose itself (glucose effectiveness) and insulin action. Thus, besides the glucose-dependent uptake of glucose by the liver, other pathways of glucose disposal are implicit in the forcing-function construct.

Assumptions. Our model makes the following simplifying assumptions, which allowed us to analyze historical data (glucose and lactate, see CONCLUSIONS). *1*) During the FSIGT, liver glucose uptake and conversion to lactate is dependent upon glucose itself but independent of the *dynamic* change in insulin (i.e., insulin regulates GCK expression chronically, but not acutely) (10). *2*) Hepatic conversion of glucose-6-phosphate (G-6-P) to glycogen during the FSIGT experiment occurs via the "indirect" pathway. The "direct" pathway represents an inconsequential fraction of the liver glucose uptake during the FSIGT and may be ignored (11). *3*) G-6-P is rapidly converted to lactate, but oxidation of pyruvate during the FSIGT is small; therefore the majority of produced lactate is exported into the blood. *4*) Once exported, lactate is degraded from blood by a first-order process. *5*) The additional increase in plasma lactate is a result of hepatic lactate production and not muscle. All glucose

taken up by muscle is retained in the form of glycogen.

Model selection. To select a model, we fit the average time course of FSIGT data (glucose and lactate) (Supplementary Fig. 1*A* and *B*) for the 142 FUSION subjects.

Model I. In our first model, we envisioned glucose entering the liver, being converted immediately to lactate via phosphorylation/glycolysis, and exiting the liver into the blood lactate pool (Fig. 1*A*). For this model, we assumed phosphorylation/glycolysis was so rapid that a single compartment could represent the combined intrahepatic pool. Although Model I was able to describe the rise and fall in lactate during the FSIGT (r^2 = 0.88) (Fig. 1*B*), there was systematic deviation between the observed and predicted lactate time course. Additionally, this one-compartment model did not accurately reproduce the plasma lactate peak value at 45 min. Therefore, we rejected this one-compartment model as too simplistic.

Model II. In our second model (Fig. 1*C*), glucose enters the liver and is phosphorylated by GCK to G-6-P. Phosphorylation is controlled by the parameter K_{GK}, which represents absolute GCK activity. A second parameter, K_{12}, represents flux through glycolysis and conversion of six- to three-carbon moieties. The lactate pools in the liver and blood are assumed to be in equilibrium and so are represented by a single compartment. Fractional clearance of plasma lactate is represented by parameter K_{01}. Our three-parameter Model II accurately accounted for the lactate data (r^2 = 0.95) (Fig. 1*D*) and estimated the peak time and value with no apparent significant deviation model estimates from the data. Model fitting to the average time course of lactate observations also provided estimates of the three model parameters with fractional SD (FSD) of <5%. FSD is a ratio of the SD σ and the mean μ (FSD = σ/μ). Equations for Model II and analysis details are described in the Supplementary Data.

Kinetic analysis of tolbutamide-modified FSIGTs

We applied Model II to individual data from 142 FUSION subjects who underwent a tolbutamide-modified FSIGT (Fig. 2 and Supplementary Table 1). The median is reported for each parameter, as distributions of values were skewed. Mean FSDs of the estimates were all <50%, consistent with the model being identifiable from the data. Residuals of the fit of the model to the lactate data were small and random, suggesting an excellent fit of the model to the lactate data.

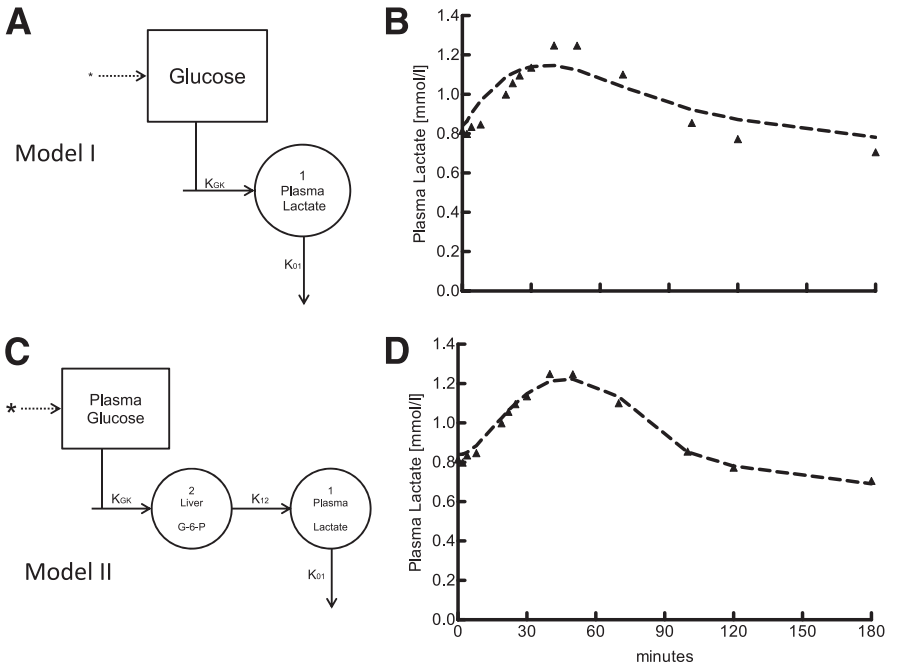

Figure 1—*One-compartment model depiction* (A) *and corresponding fit to the average time course of plasma lactate* (B). *Two-compartment model* (C) *and fit of the model to the observed average data* (D).

We assessed relationships between the parameter estimates from the lactate model with metabolic indices from the minimal model of glucose and insulin metabolism (Table 1). We found that fasting glucose was weakly, but significantly, negatively correlated with estimated K_{GK} (ρ = −0.24; P = 0.005). Estimated K_{12} was weakly, but significantly, positively correlated with DI (ρ = 0.30; P = 0.0005), suggesting that a decrease in DI would be accompanied by a decrease in lactate appearance. Additionally, we found a weak, but significant, negative correlation between estimated fractional lactate clearance (K_{01}) and DI (ρ = −0.31; P = 0.0003).

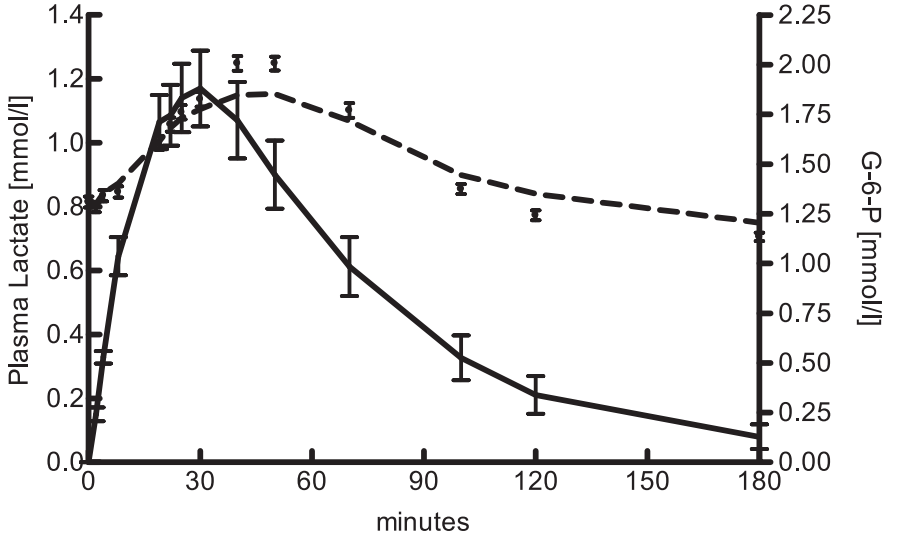

Figure 2—*Average time course of lactate (solid dots), model fit (dashed black line), and predicted time course of change in hepatic G-6-P concentration (solid black line).*

Table 1—*Spearman correlations and P values between minimal model indices of glucose metabolism*

	K_{GK}	K_{12}	K_{01}
AIRg	0.1519	**0.2805**	**−0.2672**
P	0.0776	**0.0009**	**0.0017**
S_I	−0.0515	0.0013	−0.0519
P	0.5592	0.9884	0.5559
Sg	0.1416	**0.2649**	**−0.3315**
P	0.1067	**0.0022**	**0.0001**
DI	0.0945	**0.298**	**−0.3131**
P	0.283	**0.0005**	**0.0003**
Fasting FFA	0.118	−0.0557	−0.0737
P	0.1746	0.523	0.3975
Fasting glucose	**−0.24**	−0.104	0.0849
P	**0.0051**	0.23	0.3277
Fasting insulin	−0.0074	0.0431	−0.0416
P	0.9321	0.6193	0.6316

Indices of glucose metabolism: insulin sensitivity (S_I), the ability of insulin to promote glucose disposal; glucose effectiveness (Sg), ability of glucose to promote its own disposal; acute insulin response to glucose (AIRg), quantitative measure of ability of pancreas to secrete insulin as a response to the bolus of glucose; DI, overall index of β-cell function and the state of glucose homeostasis; and the three parameters of the novel lactate model (K_{GK}, K_{12}, and K_{01}). Statistically significant numbers are shown in boldface.

CONCLUSIONS—The enzyme GCK (hexokinase IV) is a key regulator of carbohydrate metabolism. In pancreatic β-cells, it is accepted that this enzyme acts as a "glucose sensor", as the rate of β-cell glucose phosphorylation by GCK is rate limiting for glucose metabolism, and hence signaling to insulin secretion. Knocking out β-cell *GCK* in mice results in extreme diabetes, leading to ketoacidosis and death (12). In humans, 250 mutations in the *GCK* gene on chromosome 7 have been identified as being responsible for maturity-onset diabetes of the young (MODY) (13), and certain rare but severe forms of diabetes are due to mutations in *GCK* (14).

However, 99% of the GCK in the body resides in the liver, where it catalyzes glucose phosphorylation (1). The expression of hepatic *GCK* is chronically regulated by insulin. In the fasting state, GCK is coupled with GKRP (the product of the *GCKR* gene) and sequestered in the nucleus. Increased intracellular glucose, as well as fructose-1-phosphate, causes the release of GCK from the GCK-GKRP complex, allowing GCK to be transported into the cytoplasm to enable glucose phosphorylation, and subsequent catabolism and storage as glycogen. Thus, GCK and its regulator GKRP play a central role in the ability of the liver to adjust to availability of carbohydrate, and act as the gatekeeper for liver glucose catabolism. In fact, a common variant in *GKRP* reduces the risk of T2D (3).

Although most genetic studies have focused on GCK as the "glucose receptor" in the β-cells of the pancreas, GCK in the liver is also important for glucose homeostasis. Reduced glucose tolerance has been demonstrated in a liver-specific GCK mouse knockout (15).

Given this background, it is important to assess GCK activity in human subjects. However, such assessment traditionally requires liver biopsy, which is not feasible in larger studies. Thus, we set out to assess GCK activity using a modeling approach based on data from the FSIGT. The advantages of such an approach are obvious: using readily obtainable data and mathematical modeling to assess important but otherwise difficult to determine physiological parameters. Given that the samples used were 14 years old, our work further demonstrates the applicability of our modeling approach retrospectively, to studies performed in the past, as long as the samples are stored appropriately. The method will also be useful for investigative groups who have likewise used the FSIGT (16,17).

The model in this study (Model II) is simpler than the one we introduced some years ago (18). That earlier two-compartment model used glucose, insulin, and lactate data obtained during an FSIGT to estimate kinetic indices such as glucose effectiveness, insulin sensitivity, relative contribution of glucose to plasma lactate changes, and descriptive lactate kinetics parameters (18). That approach was complex and required multiple concurrent observations of glucose, insulin, and lactate. The complexity resulted from an effort to account simultaneously for glucose and lactate kinetics, and invoke the effects of insulin. The approach discussed here is a simplification based on the concept of partition analysis and uncoupling (19). Rather than modeling all the interactions in glucose/insulin/lactate homeostasis, we simply treated the liver as a single "input-output" system. We used the forcing-function construct to introduce plasma glucose as the input to the system. Such a formulation permitted us to focus on the estimated hepatic glucose uptake and lactate production/clearance without the need to estimate other forces that drive the clearance of glucose from plasma, which are implicit in the forcing-function construct. This allowed us to model only the liver, exposed to changes in plasma glucose during the FSIGT, and predict the plasma time course of lactate, not accounting for plasma glucose and insulin. Thus we simplified the modeling process, yet yielded the variable we were searching for: liver phosphorylation of glucose, i.e., GCK activity.

As described above, this simplified approach requires certain assumptions. First, we imposed the well-documented concept (7) that glucose itself acutely regulates hepatic GCK activity. In fact, activation of GCK under hyperglycemic conditions is glucose dependent, and independent of ambient insulinemia (20). Therefore, we assumed that blood lactate changes and relative conversion of glucose to lactate could be quantified independent of acute changes in insulin. This assumption does not violate insulin's demonstrated chronic regulation of *GCK* expression (21); it simply required us to assume that the changes in activity of GCK during the short time period of the FSIGT were not related to the change in plasma insulin.

Our second assumption relates to the locus of the conversion of glucose to lactate. Due to the speed of the reaction and the notion that almost 99% of GCK is located in the liver (22), we assumed that during the FSIGT, the majority of glucose that eventually gets converted to lactate is captured by the liver. There is good evidence that glucose conversion to lactate under hyperglycemic conditions is a hepatic process, with little contribution of skeletal muscle, wherein plasma glucose is captured as glycogen (20).

A third critical assumption is that glucose taken up by liver, and phosphorylated, is not immediately converted to liver glycogen. We assume that G-6-P in liver passes through glycolysis, and that contribution of the so-called "direct pathway" (11), G-6-P to G-1-P to uridine diphosphate glucose (UDP-glucose) to glycogen, is minor. This assumption is consistent with the demonstrated importance of the indirect pathway (reversal of glycolysis) in glycogen synthesis (23,24), and the slow appearance of liver glycogen during hyperglycemic clamps (25). Nevertheless, several groups contend that direct pathway has relatively higher contribution compared with the indirect pathway of hepatic glycogen formation (26,27). Therefore, it will be critical to examine the relative importance of the direct pathway versus glycolysis and export of lactate during the abrupt hyperglycemic period of the FSIGT. Another alternative explanation we offer as support to the

Table 2—*Comparison of unit transformed average parameter values with previously published data*

Parameter	Units	Model estimated value	Reference value	Reference	Species
Hepatic glucose uptake	μmol/min/mL[1]	0.01	0.023	Viljanen et al., 2009[5]	Human
			0.035	Iozzo et al., 2004[6]	Human
Hepatic lactate production	μmol/kg/min[2]	2.58	3.98	Davis et al. (32)	Dog
Hepatic lactate production	μmol/min[3]	201	140	van Hall (31)	Human
Whole-body fractional lactate clearance rate	L/min[4]	0.034	0.037	Menzies et al. (33)	Human
			0.020, 0.029	Beneke et al. (34)	Human

[1]Hepatic rate of substrate uptake per unit of time and per mL of liver volume. [2]Standard units of whole-body substrate production rate of amount per kg of whole-body mass per unit of time. [3]Whole-organ substrate production rate per unit of time. [4]Standard fractional clearance rate with units fraction per unit of time. [5]Viljanen AP, Iozzo P, Borra R, et al. Effect of weight loss on liver free fatty acid uptake and hepatic insulin resistance. J Clin Endocrinol Metab 2009;94:50–55. [6]Iozzo P, Lautamaki R, Geisler F, et al. Non-esterified fatty acids impair insulin-mediated glucose uptake and disposition in the liver. Diabetologia 2004;47:1149–1156.

importance of hepatic lactate production in the process of hepatic glycogenesis is Jungermann's hypothesis (28). According to Jungermann, the hepatic tissue is of a heterogeneous nature and the hepatocytes close to the portal vein (exogenous glucose entry point to the liver) are rich with gluconeogenic enzymes converting lactate to glucose. Subsequently this "new" glucose gets taken up by the hepatocytes in the perivenous region, which are rich with glycolytic enzymes, and ultimately it is directly converted to glycogen in this more central population of hepatocytes.

A final, potentially controversial assumption is that during the FSIGT, little of the pyruvate produced in the liver is oxidized for energy, and most is therefore exported as lactate. Previously, it has been established that, in general, LDH flux direction depends on the pyruvate/lactate ratio and oxidation state of the system. Nevertheless, it has been shown that the LDH isoforms present in muscle and liver have a characteristic low K_m and high V_{max}, resulting in lactate production regardless of the redox state (29,30). Additionally, because the test is generally performed under fasting, resting conditions, there is little need for additional oxidative energy in liver, and under such conditions, it is reasonable to assume that the tricarboxylic acid cycle handles little of the substrate emanating from the glycolytic pathway.

Comparison of the estimates of our three model parameters to previously published estimates revealed reasonable concordance (Table 2). It is worth noting that the literature values were obtained from a variety of experimental protocols. Therefore, it is understandable that there is a lack of complete overlap of our estimates and published literature values. In fact, there is not even a clear consensus for some of the values across different references. In our search for comparable published indices, we were looking for the most direct experimentally measured values. Oftentimes, specific physiological processes, such as rate of glycolysis or hepatic glucose uptake, are difficult to directly assess in humans due to the invasive nature of the estimation methods. In such cases where we were not able to obtain direct estimates, we reported hypothesized expected values or animal estimated values instead. Our estimated value for GCK activity, $\widehat{K_{GK}} = 0.0020\ \text{min}^{-1}$ (Supplementary Table 1), is equivalent to 0.01 μmol/min/mL for hepatic glucose uptake, comparable to basal hepatic glucose uptake of 0.023 and 0.035 μmol/min/mL in humans (Table 2) . In a recent review, van Hall (31) states that the uptake and potential release of lactate from liver is very difficult to directly assess in humans. Therefore, only the net uptake of lactate by the liver (200 μmol/min) has been estimated based on measurements of concentration differences across the splanchnic bed. The actual release and uptake of lactate by the liver remain unknown. Nevertheless, van Hall (31) states that there is a net release of 140 μmol/min lactate across the splanchnic bed that remains unaccounted for. We believe that our estimate of hepatic lactate release of 201 μmol/min is comparable to the abovementioned net release of lactate from the splanchnic bed. Therefore, we speculate that the unaccounted quantity may represent the hepatic release of lactate. Our glycolysis parameter estimate of $\widehat{K_{12}} = 0.0623\ \text{min}^{-1}$ (Supplementary Table 1) is equivalent to the average hepatic lactate production of 2.58 μmol/kg/min, comparable to the basal hepatic lactate production for dogs of 3.98 μmol/kg/min (32) (Table 2). Finally, our estimate of whole-body fractional clearance rate of lactate, $\widehat{K_{12}} = 0.034\ \text{min}^{-1}$, was in reasonable agreement with previously published values of 0.02–0.037 min^{-1} in humans (33,34) (Table 2).

Previously, it has been shown that different activating or deactivating mutations in *GCK* or *GKRP* are associated with fasting glucose levels (35,36), consistent with GCK being a key regulator of glucose storage and disposal. Lovejoy et al. (37) suggest that the state of insulin resistance and T2D is associated with impaired lactate appearance. To confirm this notion, we examined the correlation between the DI for each subject and appearance of lactate in plasma (K_{12}). In fact, we discovered a correlation between lactate disappearance parameter and DI (Table 1), suggesting that in subjects with reduced DI, lactate appearance is diminished, similar to the results in those with diabetes. Although not a direct validation of our modeling approach, the results suggest that the model may indeed represent glucose/lactate physiology during the FSIGT. Furthermore, we intend to design novel studies in which we will validate our estimates of in vivo hepatic GCK activity by comparing them to in vitro measurements.

In conclusion, we describe a novel two-compartment model of plasma lactate kinetics during FSIGT. The model is simple and uses easily obtainable glucose and lactate data from the FSIGT to obtain estimates of GCK activity, fractional lactate appearance in plasma, and lactate clearance from plasma. The parameters of the model are well resolved with FSIGT data on single individuals. The model is concordant with previous observations regarding hepatic GCK activity and whole-body lactate metabolism. We believe this novel model of lactate metabolism will give new insight into the influence of genetic alterations of *GCK* and *GCKR* in vivo and extend the usability of the FSIGT protocol by providing specific information about the status of hepatic glucose homeostasis, including estimates of hepatic lactate production and whole-body lactate clearance. Additionally, our model can be used to test the in vivo

effectiveness of pharmacological activators of GCK, a potential new class of antidiabetic drugs.

Acknowledgments—This work was supported by research grants from the National Institutes of Health (DK027619, DK029867, and DK062370).

No potential conflicts of interest relevant to this article were reported.

D.S. planned the study, analyzed data, and drafted the manuscript. J.H.Y. analyzed data and drafted the manuscript. M.R. drafted and reviewed the manuscript. R.M.W. analyzed data. M.A., V.I., M.B., and F.S.C. reviewed the manuscript. A.U.J. collected data. R.N.B. conceived the modeling idea, planned the study, analyzed data, and drafted the manuscript. D.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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