Continuous metabolic monitoring techniques

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Chapter 7

Quantitative model of glucose transfer in subcutaneous interstitial gradients assessed with oral glucose tolerance tests and ultraslow microdialysis

Chapter 7

Abstract

Introduction: The aim of this study was to test compartmental models for subcutaneous glucose kinetics in order to find which determinants contribute, and to what extent to subcutaneous glucose levels. A secondary aim was to evaluate the possibility to reverse the algorithm found.
Methods: The data of the seven subjects in chapter 6 were used to evaluate three models. Model A is a two compartments model, using the measured arterial glucose as the linear input into the connective tissue compartment. The latter compartment has one linear glucose output. Model B is like A, but modifies the output using the arterial insulin levels. Model C is like B, but adds a third compartment between the arterial and the connective tissue compartment, and modifies also the input using the arterial insulin levels. To identify the best model, first the model parameters have been estimated by weighted nonlinear least squares. Second, to make a comparison among the models in terms of parsimony, the Akaike Information Criterion was used.
Results: The parameter precision was acceptable (coefficient of variation below 100%) and the fractional standard deviation was estimated to be below 5%. Model B showed the best fit, with model C in second place. However, the results also show parameters k3 and k4 (insulin effect) to virtually disappear in some subjects, suggesting a more simple model is then sufficient.
Conclusions: The heterogeneity of the parameters found may be a reflection of the mix in the subcutis of insulin dependent adipose and insulin independent connective tissue, and the tissue concentrations varying with the distance to the nearest capillaries.
A future reversement of the best model found will need the insulin levels as input for the algorithm. This will be possible, as the previous insulin administration will be remembered by a future artificial pancreas. However, the presented model needs additional data in future studies to be validated for this purpose.

Introduction

The fate of glucose in the interstitial fluid of tissues is of importance to better understand some pathophysiologic mechanisms in diabetes mellitus. Accordingly, interstitial fluid may inform more specific on tissue metabolism than blood, because the interstitium is usually regarded as one or more kinetically separate compartments and the place of action of insulin[1]. Major pathophysiologic phenomena localised in tissue are insulin resistance[2] and increased capillary permeability related to diabetic and hypertensive complications[3]. Further, the proper functioning of pancreatic islets transplants appears to depend not only on controlling the immunologic rejection, but also on creating a close kinetic contact with the bloodstream[4]. An interstitial glucose kinetic model may help to understand the basics of metabolism inside tissue in control conditions and of diabetic pathophysiology. Such model may also improve the interpretation of interstitial glucose sensor measurements, intended to manage diabetes without blood sampling[5-7].

Glucose kinetic models have been developed previously for the entire body, e.g. the so-called "minimal model"[1]. The interstitial fluid proper can be modelled thus far only indirectly by the input-output method (arterial-venous differences measurement)[8], by the glucose clamp method, or by thoracic duct lymph measurements[9]. All these attempts to validate the characteristics of peripheral interstitial fluid compartment in a glucose kinetics model were inevitably indirect, because of hitherto technical inaccessibility of the human interstitial compartment. Thoracic duct lymph is mostly constituted by liver and intestines, which glucose metabolism differs considerably from peripheral tissues as muscle, fat, and connective tissue. So, the important insulin effect on the peripheral tissues can be modelled little with thoracic duct lymph measurements. There is also criticism on compartmental modelling concerning the assumptions of no concentration gradients within compartments, and only single transfer rates between compartments[10].

Application of microdialysis probes placed directly in the subcutaneous interstitium for continuous sampling or implantation of glucose sensors has made local on-line measurements in principle possible. However, in current studies using classical microdialysis, sample collection time is 10 to 60 minutes, and the probe creates a concentration gradient in the surrounding tissue. Calibration needed in vivo to correct for microdialysis tissue drainage is a rather complicated and time-consuming procedure, and requires often additional blood sampling. Directly implanted amperometric glucose sensors need in vivo calibration as well. Because any physiologic glucose gradients between

subcutaneous and blood levels are thus also calibrated for, no uptake in tissue can be found, but only a rate constant for delay[7].
Recently, an ultraslow microdialysis method has been developed, which recovers completely equilibrated samples[11]. With such a continuous sampling method, glucose measurements can be done every minute using a biosensor in a coupled flow-injection analysis system[12]. So, very frequent direct measurements of absolute glucose concentrations have now become possible in the subcutaneous compartment without additional assumptions or computations. This approach allows to investigate kinetics of glucose in the subcutaneous compartment of individual subjects. In preclinical experiments in healthy volunteers to validate this method, both delays and lower glucose levels were observed in the subcutis as compared to venous blood plasma. In order to make subcutaneous sensors accurate and reliable, this difference has to be explained and, if possible, avoided or predicted. A subcutaneous kinetic model may enable to predict blood levels from subcutaneous glucose with an appropriate algorithm[7], provided the model allows inversion.
Here, we analysed glucose and insulin data from Oral Glucose Tolerance Tests (OGTTs) in seven healthy volunteers. The data were described by three models with rising complexity to select the best quantitative model to predict subcutaneous glucose levels from arteriolized blood plasma measurements, which were used as the input function for the models. We chose a new approach by comparing lumped parameters between the compartments with multiple parameters, taking into account the characteristics of two different glucose transporters: GLUT1 and GLUT4. GLUT1 plays a role in constitutive glucose uptake over the cell membrane, whereas GLUT4 can be moved by insulin from specific membrane vesicles inside the cell to its surface. Further, we individualised the parameters for each experiment, so taking into account the possibility of concentration gradients in the studied compartments. The glucose levels in the blood plasma and subcutaneous compartment were assumed to represent a homeostatic steady state immediately before the experiment. Three models were studied. In the simplest model (model A), transport from blood plasma to the interstitium (by blood filtration (solvent drag) and diffusion) was described by one linear rate. The transport from the interstitium to the blood plasma and/or into cells (by diffusion, reabsorption, and physiological glucose uptake (glucose dependent below a level of ±10 mM) was described by a second linear rate. In the model B, the second linear rate was the sum of two rates, one of these two being linearly proportionate to the blood insulin level above steady state level (insulin dependent glucose uptake). Model C is like model B with addition of an insulin dependent glucose transport from blood

plasma to the interstitium (insulin dependent glucose distribution), and with a third compartment between blood plasma and interstitium , representing the capillary blood filtration. The closest and simplest quantitative description of the set of measurements is selected as the best model.
The main question of the present study is, to what extent tissue metabolism events are isolated from blood plasma in terms of number and influence of model parameters. Relevant is especially whether insulin is of importance in explaining the observed gradient and delay in glucose transfer glucose between the compartments. Insulin constitutes namely a from glucose independent factor in diabetic patients. So insulin is potentially an uncontrollable interferent in subcutaneous glucose measurements, and thus difficult to use in an inverse model for a sensor algorithm. In the discussion we will discuss the plausibility of the model parameters found and try to match them with physiological correlates.

Methods

Experimental protocol
A 100g oral glucose tolerance test was performed on the day after probe placement in seven healthy women between 23 and 45 years old, without (family) history of diabetes. Measurements included weight, height, waist circumference (at the point of minimal abdominal girth), and skinfold thickness at the probe site. The CMA 60 microdialysis probe was inserted with an introducer (l=54mm, o.d.=1.4mm) through a lifted skinfold in the direction of the umbilicus at 15 cm from the midline. The probe was placed in the loose connective tissue layer in-between the subcutaneous adipose tissue and the muscle aponeurosis. Low probe perfusion rates (range 30-59 nl/min) were applied by a stable suction pump, resulting in equilibrated concentrations of glucose in the microdialysis perfusate and the subcutaneous space. The average 10-90% response time of the probe to sudden changes of standard concentrations post-vivo was 4.2 minutes (time for the signal to change from 10 to 90% in a sigmoidal transition from one concentration level (0%) to another (100%))(Tiessen, submitted). Concurrent with the subcutaneous measurements were blood samples taken from a cubital vein catheter every 5 to 15 minutes during the OGTT and the preceding steady state. Cubital venous blood was arteriolized by keeping the hand on a warming pad under a cloth. 100 g glucose dissolved in 200 ml water was ingested at zero time. The Medical Ethical Committee of the University Hospital of Groningen approved the experimental procedures, which were in accordance with the Declaration of Helsinki.

The microdialysis perfusate glucose was analyzed every minute in a flow-injection analysis with a biosensor as described previously[12]. Blood glucose concentrations were measured in plasma with the Vitros 750 analyser (Ortho-Clinical Diagnostics, Illkirch Cedex, France) after centrifugation of arteriolised blood. Insulin levels were determined in blood plasma by radio immuno assay (Pharmacia & Upjohn, Uppsala, Sweden).
Anthropometric data and parameters of model B were analysed using multiple linear regression, with the model parameters as the dependent variables and body mass index, waist-hip ratio and skinfold thickness as the independent variables.

Kinetic models
Model A
Glucose modelling was done by considering only the glucose measurements, i.e. plasma glucose as the known input and the subcutaneous interstitial data as the output of a linear compartment model. The first model, Model A, is shown in Fig.1 and described the glucose exchange between plasma and interstitial space by the following differential equation:

$$\dot{C}_{sc}(t) = k_{21}C_{plasma}(t) - k_{02}C_{sc}(t) \qquad C_{sc}(0) = C_{scb} \qquad (1)$$

where C_{plasma} is the plasma glucose concentration, C_{sc} is the glucose interstitial concentration and C_{scb} represents the steady state value of the subcutaneous glucose. Parameter k_{21} (min^{-1}) is the rate constant between plasma and interstitial space, parameter k_{02} (min^{-1}) is a rate constant describing both the exchange between interstitial space and plasma and the irreversible loss into the tissue.

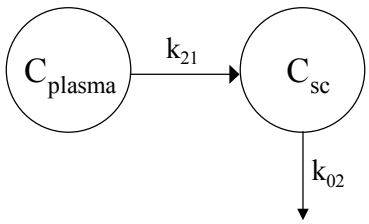

Fig.1 Model A

Considering that before the OGTT experiment one has the steady state relation:

$$k_{21}C_p - k_{21}C_{sch} = 0 \tag{2}$$

one can obtain the following steady state relation:

$$k_{21} = \frac{k_{02}C_{scb}}{C_p} \tag{3}$$

where C_p is the plasma glucose value at the basal state. Thus k_{02} was the only parameter to identify of Model A.

The second model, Model B, is shown in Fig.2. It assumes as Model A that glucose distribution is represented by two compartments. Glucose disappearance from the interstitial space is now linearly dependent on plasma insulin concentration above the basic level Ib. In particular the Model B is described by the following differential equation:

$$\dot{C}_{sc}(t) = k_{21}C_{plasma}(t) - [k_2 + k_3(I(t) - I_b)]C_{sc}(t) \qquad C_{sc}(0) = C_{sc} \tag{4}$$

where C_{sc} and C_{plasma} have the same meaning above, and I and I_b represent insulin concentration and the steady state value of the plasma insulin respectively. Considering that before the OGTT experiment one has the steady state relation:

$$\begin{aligned} k_{21}C_p - k_2C_{scb} &= 0 \\ k_{21} &= \frac{k_2C_{scb}}{C_p} \end{aligned} \tag{5}$$

where C_p has the same meaning above. Thus k_2 and k_3 were the two parameters to identify.

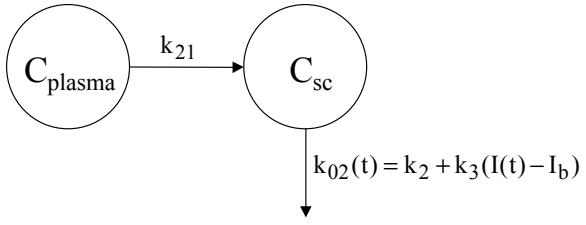

Fig.2 Model B

The third model, Model C, considers the presence of a delay between plasma glucose and interstitial glucose and of two nonlinear parameters, i.e. $k_{21}(t)$ and $k_{02}(t)$, controlled directly by the arterial plasma insulin kinetics. By introducing a third compartment, we obtained the model in Fig.3 where C_{plasma} is the plasma glucose concentration, C_r is the delayed plasma glucose concentration and C_{sc} is the glucose interstitial concentration. The differential equations describing the model are:

$$\begin{aligned} \dot{C}_{sc}(t) &= [k_1 + k_4(I(t) - I_b)]C_r(t) - [k_2 + k_3(I(t) - I_b)]C_{sc}(t) = 0 \qquad (6) \\ \dot{C}_r(t) &= k_r C_{plasma}(t) \\ C_{sc}(0) &= C_{scb} \\ C_r(0) &= C_{rb} \end{aligned}$$

where C_{scb} and C_{rb} are the steady state values of plasma glucose and delayed plasma glucose respectively. By considering that before the OGTT experiment one has the steady state relation:

$$k_1 C_p - k_2 C_{scb} = 0 \qquad (7)$$

one has:

$$k_2 = \frac{k_1 C_p}{C_{scb}} \qquad (8)$$

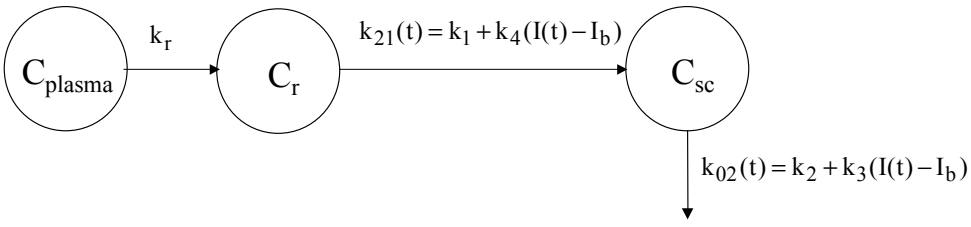

Fig.3 Model C

Model Identification

Model parameters have been estimated by weighted nonlinear least squares. Note that there is no equation for the first compartment for both the models, because $C_{plasma}(t)$ is assumed to be known and used as the input for model identification. The interstitial glucose data are described by:

$$C_{sc}^{obs}(t_j) = C_{sc}(t_j) + e(t_j) \qquad j=1, 2 \ldots N \tag{9}$$

where $e(t_j)$ is the measurement error at time t_j and N the number of data.
Measurement error was assumed to be additive, uncorrelated, gaussian, zero mean and with a constant fractional standard deviation (FSD=10% of the measurement) with an unknown proportionality factor γ:

$$\sigma^2(t_j) = \gamma\left[0.1 * C_{sc}^{obs}(t_j)\right]^2 \tag{10}$$

The scale factor γ was estimated a posteriori as:

$$\gamma = \frac{WRSS(\hat{\mathbf{p}})}{N - P} \tag{11}$$

where $WRSS(\hat{\mathbf{p}})$ is the value of the cost function evaluated at the minimum, i.e. for $\mathbf{p}$ equal to the vector of estimated model parameters $(\hat{\mathbf{p}})$:

$$WRSS(\hat{\mathbf{p}}) = \sum_{j=1}^{N} \frac{1}{\sigma^2}\left[C_i^{obs}(t_j) - C_i(\hat{\mathbf{p}}, t_j)\right]^2 \tag{12}$$

Note that $\gamma \approx 1$ would indicate that the assumption FSD=10% is reasonable.
The precision of parameter estimates was expressed as FSD% and obtained from the inverse of the Fisher information matrix $\mathbf{M}$ by:

$$COV(\hat{\mathbf{p}}) = \gamma \mathbf{M}^{-1} \tag{13}$$

To make a comparison among the models in terms of parsimony, the Akaike Information Criterion (AIC) was used:

$$AIC = N \ln WRSS(\hat{\mathbf{p}}) + 2P \tag{14}$$

where $WRSS(\hat{\mathbf{p}})$ is the weighted residual sum of squares, P is the number of parameters and N is the number of the data points.

Results

By using Model A, results have been obtained in all the 7 subjects. Parameter values and their CV are shown in Table 1. CV are acceptable (i.e. <100%) in all the identified subjects. Note that the mean γ value often is different from 1, consequently the fractional standard deviation, FSD, of the measurement error has to be considered less than 5% instead of our original assumption FSD=10%.

Table 1. Model A results

Subjects	k_{21} min^{-1}		k_{02} min^{-1}		**AIC**	**γ**
a	0.13	(9)	0.12	(9)	0.79	0.39
b	0.07	(5)	0.06	(5)	1.30	0.76
c	1.51	(57)	1.54	(57)	1.27	0.39
d	0.88	(75)	0.92	(75)	0.88	0.82
e	0.09	(17)	0.09	(17)	1.94	4.83
f	0.10	(6)	0.10	(6)	0.40	0.29
g	0.15	(5)	0.15	(5)	0.24	0.27
mean	0.42		0.43		0.98	1.11
SE	0.21		0.22			

The results obtained in all the original 7 by using Model B are shown in Table 2. It was not possible to estimate the parameter related with the insulin stimulation for the remaining 2 subjects, because k3 was estimated equal to zero. Parameter values and their CV are shown below. CV are acceptable (i.e.

Table 2. Model B results

Subjects	k_{21} min^{-1}		k_2 min^{-1}		k_3 L/mU/min		**AIC**	**γ**
a	0.15	(7)	0.13	(7)	0.00014	(11)	0.42	0.20
b	0.07	(5)	0.06	(5)	0.00004	(20)	1.26	0.74
c	1.44	(54)	1.48	(54)	0*	-	1.27	0.39
d	0.71	(71)	0.74	(71)	0.00009	(188)	0.87	0.82
e	0.08	(16)	0.07	(16)	0*	-	1.94	4.67
f	0.11	(6)	0.11	(6)	0.00002	(87)	0.36	0.28
g	0.15	(5)	0.15	(5)	0.00003	(35)	0.22	0.26
mean	0.39		0.39		0.00006		0.90	1.05
SE	0.20		0.20		0.00002			

* parameter estimate close to zero value

<100%) in all the identified subjects except for the k3 of the subject 'd'. FSD was estimated a posteriori to be less than 5% instead of the assumed FSD=10%. The model fit to the interstitial data resulted better than the previous ones obtained by using the Model A.
Model C parameter estimates have been obtained in 6 of the original 7 subjects. Parameter values and their precision are shown in Table 3. Parameter precision are acceptable (i.e. CV<100%) in all the identified subjects with the only exception for the k4 estimate of subject 'e'. FSD was estimated a posteriori to be less than 5% instead of the assumed FSD=10%.

Table 3. Model C results

Subjects	Cr mM/l	k_r min^{-1}	k_1 min^{-1}	k_{21} min^{-1}	k_3 L/mU/min	k_4 L/mU/min	AIC	γ
a	2.65 (10)	0.140 (14)	0.07 (8)	0.06 (8)	0.00008 (8)	0.01489 (26)	0.12	0.12
b	11.31 (3)	0.005 (12)	0.01 (10)	0.01 (10)	0.00006 (4)	0.00090 (5)	0.42	0.13
c	2.35 (80)	2.544 (78)	1.00 (39)	1.02 (39)	0* -	1.49162 (139)	1.23	0.33
d								
e	10.29 (2)	0.004 (7)	0.01 (6)	0.01 (6)	0.00006 (4)	0.00123 (4)	0.22	0.11
f	0.60 (31)	0.805 (36)	0.09 (8)	0.10 (8)	0.00003 (49)	0.57049 (121)	0.35	0.27
g	5.26 (6)	0.264 (9)	0.26 (9)	0.26 (9)	0.00008 (25)	0.00092 (50)	0.27	0.29
mean	**5.41**	**0.627**	**0.24**	**0.24**	**0.00006**	**0.34668**	**0.45**	**0.21**
SE	**1.82**	**0.402**	**0.16**	**0.16**	**0.00001**	**0.24695**		

* parameter estimate close to zero value

Correlation of anthropometric data and the parameters of model B
Regression analyses between the anthropometric data and the parameters of model B were all negative, but the tests lacked power (P=0.05, α<0.8).

Conclusions

The central goal of the present study was to determine a model closely describing the subcutaneous measurements as a prediction from blood plasma glucose levels in healthy volunteers. The successful identification of such a model, despite considerable variability of glycemia profiles, underlines the power of the here chosen new approach. The appliance of individualised parameters may have contributed to the strength of the model in the sense that it allows for physiological gradients. The use of ultraslow microdialysis to obtain absolute interstitial glucose concentrations allows modelling of glucose disposal

inside tissue, which was previously impossible[7;9]. Especially interesting is the first appliance of multiple parameters between compartments, allowing to model both glucose and insulin dependent glucose disposal. In the current study no correlations were found between the model parameters and the body measures, but this may well be a matter of increasing the number of experiments.

Results have been obtained for all three proposed models. All three models can be accepted as possible models as the assumptions of CV<100% and FSD□10% are met. The FSD can be estimated a posteriori to be less than 5% in these models.

Comparing the models in terms of parsimony, the AIC were higher for Model A fits than for Model B and Model C fits. Model B or Model C provided a slightly but significantly better fit than the Model A. Considering only Model B and Model C, the AIC were higher for Model B fits. However the lack of identifiability associated with the fits were higher for the Model C (one full non-convergence in a subject plus a parameter close to zero for Model C; only two parameter close to zero for Model B),the high CV associated with two parameters of the Model C (i.e. k4 of subjects c and f) and high variability of the estimates of k4 allow to select the Model B as the best to describe the data.

So, both models (B and C) with an insulin dependent glucose disappearance from the interstitium are better than the model (A) without insulin effects. The quantitative effect of the maximum insulin concentration (about 100mU/L) on total glucose disappearance can be estimated to be maximal ~60% in model C. This relatively small effect of insulin may be related to the low rate of glucose uptake in connective tissue as compared with adipose or muscle tissue[13]. The model parameters found here correspond also with the generally accepted assumption that transmembrane transport is the rate limiting step in the chain to glucose metabolism.

The largest impact on all models is made by the parameters which are independent from insulin. Within each experiment, the parameters k_{21} and k_{12} are almost the same as a mathematical reflection of the C_{plasma} to C_{sc} steady-state ratio being close to one in all the experiments. Between experiments, the parameters k_r, k_{21}, k_{12} and k_4 display a high (co-)variability. This variability reflects differences in speed at which the subcutaneous interstitium catches up with glucose level changes in the blood compartment. The explanation of these differences may be physiological or due to local reaction on the probe implantation. Both the mass-transfer at the blood-tissue interface, and the tissue-probe interface are generally assumed to be determined by diffusion[10]. The present data confirm this assumption, with a subdivision in diffusion and

capillary filtration (fast solvent drag transport by pressure gradient directly over the capillary wall, as represented in model C by k_r). According to Fick's law on diffusion, a concentration difference is needed for mass transfer. This implies that a concentration difference is a conditio sine qua non for glucose transport from capillaries to the tissue interstitium, but this need not be steep. During a 75g OGTT, the arterial-venous glucose level difference over abdominal wall tissues increases from 1.4% in steady state[14] to ~0.7mM at 60 minutes[8;15]. Arterial-venous differences are constituted by metabolite exchanges over the capillary wall. Consequently, the gradients inside the tissue interstitium lining the capillaries will be of at least the same extent. The observed individual kinetic parameters appear to fall within the outline of this physiological gradient, and may be explained accordingly. Using individualised parameters as performed here, enables the modelling of compartments with gradients. Another possible explanation of the variability of the k parameters might be the probe response time. There was however no correlation between k_{21} in vivo and the probe response times in vitro (R^2=0.17). So, this is not a plausible explanation. With a recently developed ultrafiltration probe, we have now reduced the response time further to 1-2 minutes by reducing the internal dead volume(submitted). Also, the response curve in vitro may be subtracted mathematically from the in vivo sensor signal, which is possible by means of deconvolution[16].

Further, it cannot be excluded that the instrumental lag-time (time from actual change of concentration to 10% signal change) was underestimated in vivo. The instrumental lag-time can only be checked independently with limited precision by weighing the nanolitre flow pump on a milligram balance.

The implantation of the probe in the subcutaneous tissue can also not be excluded as a cause for the observed differences between the arterial and the tissue interstitial compartment. The probe with a diameter of 340µm increases the interstitial volume because the interstitial width between cells can be equal or less than 1µm. A larger interstitial volume lengthens the diffusional path between neighbouring cells and surrounding capillaries, and needs more time for mass transfer to equilibrate in it self. The fluid volume near the probe may be changed as well through blood shedding, colloidal attraction of the dialysis fluid and the evacuation of the fluid for measurements. Probe implantation may further decrease local diffusion by fibrin deposits from bleeding and exudation due to introduction damage by the needle, later mechanical friction, and vessel response to environmental changes.

In the present study we placed the probe in the subcutaneous loose connective tissue instead of the usual placement in the adipose tissue, trying to diminish

blood shed and cell debris from the introductory needle and probe-tissue friction. Also, the interstitium is larger in loose connective tissue than in adipose tissue and has a better diffusional capacity[17]. The steady state glucose concentration levels in this study are kinetically much closer to the blood plasma levels and less variable as compared to previous research performed in adipose tissue[12]. We interpret this favourable difference as resulting from the difference in tissue anatomy and its effect on probe implantation and functioning.

An attempt to inverse the presented model for glucose sensor calibration purposes will encounter two difficulties. The first difficulty is the interindividual variability of the parameters modelled, the second, the absence of any moment of steady state in diabetes patients. The necessity to know the insulin levels as input for the algorithm need not be a hindrance, as the insulin administration would be remembered by a future artificial pancreas. However, additional research is needed to construct a simple, generally applicable algorithm to predict blood plasma glucose from subcutaneous glucose measurements. The range of uncertainty of the blood plasma concentration may still be indicated by the range of the model parameters. This range may be useful e.g. to set the threshold for a hypoglycaemia alarm of a subcutaneous sensor. So, more work has to be done on the development of the glucose sensor and the proposed model.

The model parameters are likely to be different in diabetes patients groups from the healthy volunteers studied here. In patients with insulin resistance or microvascular complications, differences in tissue insulin sensitivity and capillary permeability are difficult to study with current techniques[3]. Such studies may be improved with the here presented direct interstitial measurements and the proposed model. The for each patient individually assessed parameters may be of diagnostic value, and the presented model may be validated for this purpose in future studies.

References

1. **Bergman, R.N., Y.Z. Ider, C.R. Bowden, and C. Cobelli.** Quantitative estimation of insulin sensitivity. *Am.J.Physiol.* 236: E667-E6771979.
2. **Walton, C., I.F. Godsland, A.J. Proudler, C. Felton, and V. Wynn.** Evaluation of four mathematical models of glucose and insulin dynamics with analysis of effects of age and obesity. *Am.J.Physiol.* 262: E755-E7621992.
3. **Jager, J., P.H. Oomen, W.J. Sluiter, W.D. Reitsma, and A.J. Smit.** Improved reproducibility of the 'large-window' method of assessing transcapillary and

interstitial fluorescein diffusion in the skin in healthy subjects and in subjects with insulin-dependent diabetes mellitus. *Int.J.Microcirc.Clin.Exp.* 17: 150-158, 1997.

4. **de, V.P., S.J. Van, A.G. Nieuwenhuizen, G.M. de, R.J. Ploeg, H.B. De, and S.R. van.** Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? *Diabetes* 48: 1381-1388, 1999.
5. **Aussedat, B., A.M. Dupire, R. Gifford, J.C. Klein, G.S. Wilson, and G. Reach.** Interstitial glucose concentration and glycemia: implications for continuous subcutaneous glucose monitoring. *Am.J.Physiol.Endocrinol.Metab.* 278: E716-E7282000.
6. **Rebrin, K., G.M. Steil, A.W. van, and J.J. Mastrototaro.** Subcutaneous glucose predicts plasma glucose independent of insulin: implications for continuous monitoring. *Am.J.Physiol.* 277: E561-E5711999.
7. **Schmidtke, D.W., A.C. Freeland, A. Heller, and R.T. Bonnecaze.** Measurement and modeling of the transient difference between blood and subcutaneous glucose concentrations in the rat after injection of insulin. *Proc.Natl.Acad.Sci.U.S.A.* 95: 294-299, 1998.
8. **Frayn, K.N., S.W. Coppack, S.M. Humphreys, and P.L. Whyte.** Metabolic characteristics of human adipose tissue in vivo. *Clin.Sci.* 76: 509-516, 1989.
9. **Gastaldelli, A., J.M. Schwarz, E. Caveggion, L.D. Traber, D.L. Traber, J. Rosenblatt, G. Toffolo, C. Cobelli, and R.R. Wolfe.** Glucose kinetics in interstitial fluid can be predicted by compartmental modeling. *Am.J.Physiol.* 272: E494-E5051997.
10. **Zierler, K.** Whole body glucose metabolism. *Am.J.Physiol.* 276: E409-E4261999.
11. **Kaptein, W.A., J.J. Zwaagstra, K. Venema, and J. Korf.** Continuous ultraslow microdialysis and ultrafiltration for subcutaneous sampling as demonstrated by glucose and lactate measurements in rats. *Anal.Chem.* 70: 4696-4700, 1998.
12. **Tiessen, R.G., W.A. Kaptein, K. Venema, and J. Korf.** Slow ultrafiltration for continuous in vivo sampling: application for glucose and lactate in man. *Analytica Chimica Acta* 379: 327-335, 1999.
13. **Yeh, J.I., K.J. Verhey, and M.J. Birnbaum.** Kinetic analysis of glucose transporter trafficking in fibroblasts and adipocytes. *Biochemistry* 34: 15523-15531, 1995.
14. **Coppack, S.W., K.N. Frayn, S.M. Humphreys, P.L. Whyte, and T.D. Hockaday.** Arteriovenous differences across human adipose and forearm tissues after overnight fast. *Metabolism* 39: 384-390, 1990.
15. **Jackson, R.A., N. Peters, U. Advani, G. Perry, J. Rogers, W.H. Brough, and T.R. Pilkington.** Forearm glucose uptake during the oral glucose tolerance test in normal subjects. *Diabetes* 22: 442-458, 1973.
16. **Kawagoe, K.T. and R.M. Wightman.** Characterization of amperometry for in vivo measurement of dopamine dynamics in the rat brain. *Talanta* 41: 865-874, 1994.
17. **Harper, G.S., W.D. Comper, and B.N. Preston.** Dissipative structures in proteoglycan solutions. *J.Biol.Chem.* 259: 10582-10589, 1984.