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In vivo Techniques for Clinical Studies of Glucose Metabolism

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Abstract The use of radioactive tracers is inevitable for in vivo clinical studies outside Japan. In the field of research in diabetes mellitus, glucose metabolism is a major concern and has been studied in humans using a tracer technique. The recent advances in this field and other useful *in vivo* techniques for study of glucose metabolism are reviewed with comments on their problems. Residual hepatic glucose production represents the endogenous glucose production. Blood flow or water exchange in organs has been regarded as one of the important factors for the determination of glucose utilization. Total glucose disposal is expressed as approximated glucose infusion rate plus residual hepatic glucose production. Dual tracer (3-^{3}H-glucose, U-^{14}C-glucose) technique with indirect calorimetry is useful to address intracellular glucose metabolism. While a minimal model approach has been used especially for the epidemiological research, its theoretical background and problems are discussed. A novel approach to measure glucose transport *in vivo* makes it possible to estimate intracellular functional glucose concentration. Although the ethical problem of using small doses of radioactive tracer is very difficult to solve in Japan, comprehension of tracer methods is important for the understanding of physiological aspects of glucose metabolism.

Key word:tracer, biomedicine, *in vivo*, glucose metabolism

Introduction

Despite tremendous advance in the field of molecular biology, when it comes to evaluate biochemical phenomena and to give physiological meaning to *in vitro* findings, the measurement of flux of substrate *in vivo* is still of major scientific interest for clinical investigators. In the research field of diabetes mellitus, impairment of insulin sensitivity was demonstrated *in vivo* in human and this is now emphasized as one of the causative factors for non-insulin dependent diabetes mellitus[1]. The insulin clamp technique[2] made this finding possible and recently the intracellular glucose metabolism has been studied with more sophisticated techniques of tracer infusion and indirect calorimetry[3]. This kind of *in vivo* method, especially in humans, is unfamiliar in Japan, while the method in which radioactive or stable tracers are employed routinely has been used in clinical investigations in the United States of America and European countries. In this review, tracer technique, together with other new techniques, for the study of glucose metabolism are summarized systematically. After the general discussion, the discussion consists of three parts; glucose appearance, glucose delivery, and glucose utilization.

Methods in General

Glucose is an important energy source for

Table 1 Calculation of Effective Dose Equivalent
(at the injection of 150 μCi of tritiated glucose to human)

Organ/Tissue	Weighting Factor	Organ Dose Equivalent mrem	Organ Effective Dose Equivalent mrem
Gonads	0.25	8.55	2.138
Breast	0.15	8.55	1.283
Red bone marrow	0.12	11.25	1.350
Lungs	0.12	8.55	1.026
Thyroid	0.03	8.55	0.257
Bone surfaces	0.03	7.65	0.230
Upper Intestine	0.06	8.55	0.513
Lower Intestine	0.06	8.55	0.513
Kidney	0.06	8.55	0.513
Bladder	0.06	9.15	0.549
Heart	0.06	8.55	0.513
Total	1.00		8.885

the brain; plasma glucose level is strictly regulated by hormones. Insulin is the key hormone to regulate glucose metabolism. The insulin clamp technique is regarded as the gold standard for the measurement of insulin sensitivity. In this discussion, except for the description of the minimal model approach, human subjects are considered to be in a steady state of glucose metabolism during the basal state or insulin stimulated state. For the evaluation of hepatic glucose production, insulin infusion rate is selected to be less than 20 mU/m^2/min. For the study of glucose disposal, insulin infusion rate of $\sim$ 40 mU/m^2/min preferentially is used. If this rate is more than 100 mU/m^2/min, administration of potassium has to be considered. Bolus dose of insulin is important. Usually subjects who are already receiving exogenous insulin are not studied.

The major part of glucose is metabolized into H_2O and CO_2. This means that the injected radioactive molecule in radioactive glucose comes out of the body as water or carbon dioxide. Since the specific activities of these are very small by major dilution, the influence of recycling is negligible for the estimation of turnover of substrates and this can be easily confirmed by simple calculation. An example of calculation for radiation dosage used for radiation safety is shown in Table 1. Since the dose equivalence of one chest X-ray examination is $\sim$ 8 mrem by NCRP (National Council on Radiation Protection and Measurement) Report 100, and cardiac catheterization is $\sim$ 3,000 mrem by ICRP (International Commission on Radiological Protection) Publication 34, the total dose equivalence of 8.9 mrem by using 150 μCi of ^{3}H-glucose seems to be a relatively small invasion. The retaining period of radioactive molecule in the body, which is used for the calculation of dose, is evaluated by mean resident time. This kind of calculation is made on the basis of exponential decline of the radioactivity in the tissue. In the case of administration of ^{14}C-glucose, glucose is oxidized and ^{14}C-carbon dioxide is expired before equilibration in the body. Since the rate of oxidation of plasma glucose is as high as half of total glucose disposal, more than half of radioactivity is expired rapidly during the study period of several hours, despite the biological half life of 10 days for ^{14}C. Expired ^{14}C-carbon dioxide is not likely to be fixed by humans. Although

there is possible over-estimation of dosage as described above, underestimation of radiation dosage has been pointed out by some investigators[4,5]. However if one considers the existence of natural occurring radioactive tracers, residual period of these in the human body is infinite. Furthermore, the existence of oncogenic chemical in daily life, including tobacco, and extra-violet exposure to skin etc., makes the argument of danger of dosage of small amount of radioactive tracers relatively weak. The use of radioactive tracers in children or pregnant females is strongly restricted.

Plasma glucose is measured by Beckman Glucose Analyzer II (Brea, CA). Radioactivity of tracers is measured by β-counter after the separation from plasma samples by evaporation, ion-exchange column, or HPLC. Specificity for the separation to detect radioactivity is dependent on only radioactive compounds. Infusate has to be measured simultaneously. When you use 6-^{3}H-glucose as a tracer, plasma sample contains ^{3}H-lactate and separation by HPLC or ion-exchange column will be necessary for the detection of ^{3}H-glucose[6]. A hand warmer (~70°C) is used to obtain an arterialized blood sample. A warm-air box is recommended to obtain arterialized venous blood than a heated blanket[7]. Glucose specific activity in the artery is the same as that in the peripheral vein. During the basal period there are very small A-V (arterial-venous) differences of glucose concentrations, and arterialization is not an important issue. However, during the insulim clamp, the clamp level of glucose would be different. Even though the calculated Ra (rate of appearance) of glucose from the specific activity of tracer is valid, evaluation of glucose disposal depends on the glucose level.

Appearance of Glucose

In the physiologic state glucose is produced mainly by the liver. The sources for glucose production are glycogen and gluconeogenic precursors. The metabolic pathways for the production of glucose are well known; the regulation of this pathway is based on hormonal regulation. Enzyme induction now is studied by the expression of responsible genes in various conditions in the field of molecular biology. Methods to measure the rate of gluconeogenesis are not established yet. However the fraction of the glucose formed by gluconeogenesis from a certain precursor is used to evaluate the rate of gluconeogenesis in humans[8]. For example, gluconeogenesis from lactate estimated by using 3-^{14}C-lactate infusion in steady state is;

$$\text{Lactate Gng} = \frac{^{14}\text{C}-\text{glucose SA}}{\text{lactate SA } (\times 2)} \cdot \text{Ra}$$

$$\text{Ra} = \text{HGP} + \text{GIR}$$

(Lactate Gng: gluconeogenesis from lactate, Ra: rate of appearance of glucose, HGP: hepatic glucose production, GIR: glucose infusion rate, lactate SA: average lactate specific activity for lactate utilization in the liver) Since average lactate specific activity for lactate utilization in the liver is unknown, this lactate specific activity has to be estimated. When specific activity is expressed by mole, to adjust the conversion from lactate to glucose, the specific activity of lactate has to be multiplied by 2. In this kind calculation, in which conversion of molecules is involved, one has to be careful in the way the specific activity is expressed.

Another source of glucose is exogenous administration. In the experimental situation glucose administration is possible through the vein or artery as well as through the gut through oral ingestion. Infusion of glucose is controlled variably by a syringe pump (Harvard apparatus, MA), which should be well calibrated. One should keep in mind that the real concentration of 20% of glucose, which is the concentration usually used in clinical studies, is often ~ 180 mg/ml.

To know the total glucose appearance, tracer dilution method is favorably employed. 3-^{3}H-glucose preferentially is given as a bolus followed by continuous infusion (~ 0.2 μCi/min). The bolus dose is calculated from the estimation of the hepatic glucose production. This is paradoxical because the tracer is used for the estimation of the hepatic glucose production. In the diabetic state, consideration of the glucose pool (bolus / continuous infusion rate=glucose pool / hepatic glucose production estimated

from fasting plasma glucose) is not enough to estimate bolus dose, because the decline of the glucose level during the basal state also increases the specific activity. Thus the following rough calculation is acceptable. Bolus [μCi] = continuous infusion rate [μCi/min] × plasma glucose level expressed by [mg/dl] × 1 [min·dl/mg]. If you do not reach a steady state of tracer, you may employ a non-steady state equation based on mathematical models.

A glucose tracer is supposed to disappear once it is utilized and should not come out in the same tracer form, otherwise recycling of tracer gives an underestimation of the rate of appearance. Tritium in 3-^{3}H-glucose ends up in glycogen or ^3H_2O. Since tritium radioactivity in plasma is basically ^3H_2O and ^{3}H-glucose with the use of 3-^{3}H-glucose, the separation of ^{3}H-glucose radioactivity can be easily achieved by evaporation. With the constant infusion of 3-^{3}H-glucose, specific activity in plasma can be modified only by cold glucose appearance. The specific activity of glucose is the same after mixture in right ventricle and utilization occurs, keeping the specific activity constant. Thus a tracer can predict the glucose appearance by the specific activity. A compartment model predicts the glucose rate of appearance in the following way[9];

$$Ra_1 = \frac{Ra_1^*}{a_1} - \frac{q_1}{a_1} \cdot \frac{da_1}{dt} - \sum_{j \in S} k_{1j} \cdot \left(1 - \frac{a_j}{a_1}\right) \cdot q_j$$

(q: mass, a: specific activity, c: concentration, kij: kinetic parameters, Ra: rate of appearance of tracee, Ra*: rate of appearance of tracer, S: system)

The first term on the right is the same as the steady-state term in Steele's equation[10]. The second term is the time-dependent term for the accessible (first) compartment; the third is time and space dependent. If the specific activity of each compartment is close enough to the specific activity of the first compartment, the first and the second terms describe the rate of appearance of glucose to the plasma compartment (Ra_1) (independent of compartment model structure). When insulin infusion is started and the infusion rate of tracer is fixed, the specific activity will decline according to time because of cold glucose infusion. The dilution of tracer occurs primarily in the first compartment and specific activity in other compartments is higher than that of the first compartment. Specific activity in the first compartment could be increased by the equilibration process from other compartments. During the bolus dose, if this very high specific activity is trapped in some remote organ or glycogen, the same story will increase the specific activity in the first compartment.

In a non-steady state of tracer, the second and the third term is not negligible, and a reduced compartment model is used for the calculation. After the validation, Radziuk's two compartment model[11] is one of the best models for this purpose. The kinetic constant and initial values for this model can be predicted from the calculation during the basal state by Steele's equation. From the observation of change of tracer concentration, it is not possible to determine the kinetic constant as a constant in a non-steady state in the model which has inaccessible compartments. This feature paradoxically makes this model flexible. Adjustment of tracer disappearance automatically takes account of the shift of kinetic constant according to time.

To keep the steady state of tracer, the hot glucose infusion technique was proposed[12]. The original method only was good to reach a steady state during the last period of insulin infusion, and they did not stop the tracer infusion that was used during the basal period. This made a major problem when this method was applied to the estimation of HGP during the early period of the insulin clamp to assess the suppression of HGP[13,14]. If you want keep the specific activity constant, first one must estimate the specific activity of glucose in plasma during the basal equilibration period in order to prepare the hot glucose infusate. Second, to keep the specific activity constant during the early period of insulin infusion, one must estimate the suppression of hepatic glucose production and reduce the tracer infusion rate according to this suppression. Exogenous tracer infusion during the basal period corresponds to hepatic glucose production. Since the tracer

method itself is employed to estimate hepatic glucose production, those disadvantages are crucial, paradoxical flaws in this method. To estimate the specific activity during the basal period, total radioactivity in the plasma or its increment can give information about the glucose specific activity. To estimate the suppression of hepatic glucose production, glucose A-V balance, which reflects muscle glucose uptake, might be helpful.

In the steady state of tracer, glucose appearance is calculated by tracer infusion rate divided by specific activity. However, the specific activity of glucose in the liver is diluted by cold glucose production by the liver. Even in the steady state of tracer, the underestimation of glucose appearance is theoretically predicted. The previous discussion was made from the hypothesis of the compartment model. However, the position of the liver is in the circulation. Liver is one of the active organs which use glucose. When average liver specific activity is SA_a/f ($f>1$: definition) during steady state;

$$HGP=\frac{Ra^*}{SA_a}-GIR+\frac{(f-1)}{SA_a}\cdot(SA_a\cdot[G]-SA_{hv}\cdot[G]_{hv})\cdot LFlow$$

$$>\frac{Ra^*}{SA_a}-GIR$$

$$f-1>0$$

$$(SA_a\cdot[G]-SA_{hv}\cdot[G]_{hv})\cdot LFlow=Hguptake^*>0$$

(HGP: Hepatic glucose production, GIR: glucose infusion rate, Ra*: rate of appearance (infusion rate) of tracer, SA_a: specific activity of glucose in the artery (and portal vein), SA_{hv}: specific activity in hepatic vein, LFlow: liver flow, $[G]_a$: artery (and portal vein) glucose concentration, $[G]_{hv}$:glucose concentration in the hepatic vein)

During the basal state, f could be much higher than 1 because of the existence of hepatic glucose production. During insulin clamp, HGuptake* (liver tracer uptake) is high and the presence of glycogen turnover keeps $f>1$[15]. Thus both during the basal and during the insulin clamp, Ra*/SA-GIR gives underestimation of hepatic glucose production by the magnitude given by the equation;

$$\frac{(f-1)}{SA_a}\cdot HGuptake^*$$

Practically, while tracer methods can underestimate Ra and HGP becomes negative by other causes of overestimation of plasma specific activities, residual HGP (>0) is accepted to express HGP. In this case, HGP is assumed to be zero. The concept of residual hepatic glucose production is justified in this way.

When you use a fixed insulin infusion rate, adjusting cold glucose infusion to the same level after the oral glucose administration, you can evaluate the parenteral glucose uptake[16]. In a more physiologic state, glucose uptake from the gut is estimated from ^{14}C-glucose ingestion[17].

Since NMR method enables the measurement of the decay of liver glycogen, gluconeogenesis is estimated by HGP-glycogenolysis[18]. However, as discussed above, HGP which is estimated from tritiated glucose is an underestimation and this formula gives underestimation of gluconeogenesis. Furthermore NMR is based on image and all the signal from liver glycogen may not be detected. Other techniques for the estimation of glucose production are local catheterization method[17] and isotopomer analysis for gluconeogenesis[19]. Some compounds are used for the study of carbohydrate metabolism in the liver[20]. Glucuronide formation has been used to sample hepatic uridine diphosphate glucose for the study of glycogen metabolism and the pentose pathway. Phenylacetate has been used to sample hepatic α-ketoglutarate for the estimation of relative flux through the Krebs' cycle. Acetylation has been used to sample hepatic acetyl-CoA.

Glucose Delivery

Glucose is delivered by the circulation of blood. A major factor for whole body glucose delivery is the glucose concentration itself. Since the relationship between hypertension and insulin resistance has been a topic[21], the modulation of hemodynamics by insulin and its effect on glucose metabolism are also of interest for investigators.

Blood flow is monitored locally by several method. Dye dilution technique is the golden standard for the measurement of blood flow.

Constant infusion of indocyanine green day (ICG) and measurement of its dilution give information about blood flow; Blood flow= Infusion rate of ICG / diluted concentration (or difference of A-V concentration). Instead of using dye, a double-lumen thermodilution catheter is used for the local thermodilution technique[22].

A bolus dose of mannitol or L-glucose is used for the measurement of extracellular space and blood flow. In addition to the blood flow, water distribution space accessible by glucose is evaluated by 3-O-methylglucose. In this kind of wash-out curve analysis the following formula is applied;

$$\text{flow}=\frac{\text{Dose}}{\int_0^{\infty} c(t)\,dt} \qquad c(t)\text{:concentration at outlet}$$

Instead of measuring the outlet, image analysis by PET scanner enables the measurement of the water flow by using $H_2{}^{15}O$[23]. Also, straingauge is used for the measurement of flow by venous occlusion plethysmography[24]. When flow is high (or low), certain parameters in those methods will be high (or low). However, when those parameters are high (or low), there is no confidence that the flow is high (or low) in both methods. If the water distribution space is increased, false increases of flow may be reported by PET technique. If the elasticity of organ is decreased, the increment of strain-gauge could stay low with the increase of flow. In both methods the water exchange during the insulin-stimulated state can be reflected in their results. Insulin has been shown to increase local blood flow and the effect of insulin on blood flow in insulin resistant states has been reported to be impaired using the thermodilution technique[25]. Although there is a general agreement about the effect of insulin on hemodynamics, its physiologic meaning is still controversial[26].

Glucose Utilization

Whole Body Analysis

Glucose is transported into cells by a glucose transporter. A major regulatory factor is insulin. Total glucose disposal is the sum of glucose disposal of three different tissues in the body; insulin sensitive (and glucose sensitive), glucose sensitive (and insulin non-sensitive), and glucose non-sensitive (and insulin non-sensitive) tissues. Insulin sensitive tissue is usually glucose sensitive. Excretion from kidney is also included in glucose disappearance but is usually excluded from total glucose disposal or M value. Rate of appearance (Ra) of glucose is known from the tracer method. When glucose concentration is the same, glucose disappearance is equal to Ra. The specific activity of the tracer is not changed by the change of glucose utilization. Thus, even in the metabolically non-steady state, specific activity can be constant and glucose appearance can be calculated by a simple steady state equation. However, the glucose disposal has to be evaluated from the decline or increase of glucose level. Adjustment or approximation of glucose utilization by the difference of glucose level (space correction) is as follows[2];

$$\text{SC in 20 minutes}=\frac{(C(t+20)-c(t))\cdot A}{BW}\times 379.5$$

$$SC\approx 190\times\frac{\triangle C}{\triangle t}$$

(SC: space correction [mg/kg BW/min], t: time:, A: surface area [m^2], BW:body weight [kg], C(t): glucose concentration at time t [mg/ml])

When glucose distribution volume is 25% of body weight (250 ml/kg BW), 250 can be used instead of 190. However, the plasma accessible immediate space is only ~ 45 ml/kg and the time which is necessary for the homogenous distribution of glucose is very different during the basal state and during the insulin stimulated state. In Steele's equation in the non-steady state, ~ 162.5 (65% of 250) is employed instead of 190. Thus it is difficult to evaluate the difference of plasma glucose concentration in a short period of time. Total glucose disposal (TGD) from plasma is expressed as follows;

TGD=GIR−space correction+residual HGP−urine excretion

In non-insulin dependent diabetes mellitus, subjects have higher fasting plasma glucose

levels during the basal state. In this case a major concern during the basal state is the evaluation of hepatic glucose production. However, metabolic clearance of glucose (glucose disposal / glucose level) is often employed to evaluate the glucose disposal. During 40 mU/m²/min infusion of insulin, after two or three hours' of duration, glucose level usually reaches ~ 5 mM.

To evaluate whole body glucose utilization, water space is used for normalization, because water space provides space for active metabolic processes. This space is related to lean body mass, which has relatively linear relationship with body surface area. The index of obesity is BMI or %IBW, which usually is not related to lean body mass. Since water space has relatively constant ratio of 73.2% of lean body mass, ~ 80 μCi of tritiated water (Total Dose [dpm]) is injected to measure lean body mass. In 30 minutes the decline of tritiated water concentration is almost stopped and homogenous dilution will be achieved. Usually the (Average [dpm/L water]) of tritium count at 90, 105, 120 minutes is used for the calculation of lean body mass (=Total Dose/Average/0.732).

Double Tracer Technique

The major pathways for glucose disposal during the insulin stimulated period are glycogen synthesis and glycolysis. To evaluate *in vivo* intracellular glucose metabolism dual tracers (3-^{3}H-glucose, U-^{14}C-glucose) are employed. Since glycolysis produce ^3H_2O from 3-^{3}H-glucose, the activity of glycolysis can be measured by the production of tritiated water[27].

$$\text{Glycolysis from plasma glucose} = \frac{^3H_2O \text{ production rate}}{^3H\text{-glucose SA}}$$

$$\text{Glycogen Synthesis} = \text{TGD} - \text{Glycolysis from plasma glucose}$$

Glucose oxidation from plasma glucose is also estimated from ^{14}-carbon dioxide production. Although in a certain study[28], bolus administration of $NaH^{14}CO_3$ was missing, a bolus administration of $NaH^{14}CO_3$ is necessary to achieve steady production of ^{14}C-carbon dioxide.

$$\text{Glucose oxidation from plasma glucose} = \frac{^{14}CO_2 \text{ production rate}}{^{14}\text{C-glucose SA} \cdot f}$$

(f: correction factor ~ 0.9[29])

Since direct precursor for Krebs' cycle is pyruvate and pyruvate is known to equilibrate with lactate rapidly[30], total intracelluar glucose oxidation is calculated from 14 C-lactate specific activities. Cori-cycle is evaluated by the difference of glucose turnover, calculated by 3-^{3}H-glucose and ^{14}C-glucose.

Indirect calorimetry

Indirect calorimetry can give information about carbohydrate utilization[31]. Substrate disappearence is calculated based on the difference of RQ (respiratory ratio) of carbohydrate (1.00), protein (0.80), and lipid (0.70). During basal state, when plasma glucose level is constant, only source of carbohydrate is glycogen in the liver and muscle. Thus carbohydrate utilization during basal state is often expressed by the glycogen depletion rate. When you convert the glycogen depletion rate, expressed by weight, into glucose equivalence, you have to multiply by 180/162. In the setting of the indirect calorimetry machine (Deltatrac, Sensormedics, Anaheim, CA), the carbohydrate utilization is expressed basically as glycogen depletion. During insulin clamp, glucose is infused artificially and the carbohydrate utilization is usually expressed by the weight of glucose. The carbohydrate disappearance basically has nothing to do wiht plasma glucose disappearance. After a certain duration of starvation, when glycogen is almost depleted, carbohydrate disappearance measured by indirect calorimetry is very low, while plasma glucose oxidation which is measured by tracer still exists. In the presence of insulin which is enough to suppress hepatic glucose production, the supply of extracellular carbohydrate is exogenous glucose infusion. Under such a condition, the oxidation of the intracellular source of carbohydrate is calculated by the difference of carbohydrate disappearance, measured by indirect calorimetry, and plasma glucose oxidation, measured by tracer[3].

Model Approach

1) *Compartment Model*

During the steady state of glucose metabolism, the analysis of change of plasma glucose tracer concentration after the bolus dose of a tracer allows estimation of the compartment structure in the glucose distribution space[32]. Increase of glucose mass in insulin sensitive tissue was predicted by the three-compartment model[33].

2) *Minimal Model Approach*

The following formula (derived from the accessible one compartment model) can describe the time course of glucose uptake when glucose concentration and insulin concentration are available as time dependent variables. When we know the time course of glucose and insulin concentration under the suppression of hepatic glucose production, we can determine constant values by the simulation process using the following formulae and non-linear fitting process.

$$\frac{dV \cdot c(t)}{dt} = -\text{Uptake} + \text{HGP} + \text{GIR}$$

$$\text{Uptake} = ia(t) \times gs(c(t)) + \gamma \times gs(c(t)) + U_b$$

$$\frac{dia(t)}{dt} = \alpha \cdot (ia_{max}(i) - ia(t)) \quad (ia(0) = ia_0)$$

$$ia_{max}(i) = I \cdot \left(1 - e^{-\ln 2 \times \frac{i(t) - I_0}{Jm}}\right) \quad (i(t) > I_0)$$

$$gs(c(t)) = C \cdot \left(1 - e^{-\ln 2 \times \frac{c(t) - C_0}{Km}}\right) \quad (c(t) > C_0)$$

V, γ, U_b, α, I, I_0, J_m, C, C_0, K_m: constant

This kind of approach has been known as a minimal model analysis. The original model which Bergman advocated in 1979[34] was a simplified description and basically the glucose concentration was described by this kind of formula.

For minimal model analysis;

$$\frac{dI(t)}{dt} = \gamma[G(t) - h]t - nI(t), \; I(0) = I_0$$

$$t > 8 \text{ min} \quad \frac{dG(t)}{dt} = -[p_1 + X(t)]G(t) + p_1 G_b, \; G(0) = G_0$$

$$\frac{dX(t)}{dt} = -p_2 X(t) + p_3[I(t) - I_b], \; X(0) = 0$$

$$\phi_1 = \frac{1}{n(G_0 - G_b)}$$

$$\phi_2 = \gamma \times 10{,}000$$

$$S_G = p_1 \times 100$$

$$S_I = \frac{p_3}{p_2} \times 10{,}000$$

Theoretically there are following problems:

1. Accessible glucose distribution volume (V) may vary according to time.
2. If V is supposed to be fixed during the calculation, the actual variability of V between the subjects has an effect on the other constants. Thus the comparison of the calculated constants between the subjects may not be valid.
3. Hepatic glucose production may not be suppressed well and may be variable according to time.
4. In the Bergman's simplified model, the glucose mass effect was not described well.
5. Reproducibility of the data was not well established.
6. Constant uptake was not defined well, because the decline of glucose (-uptake/V) has a positive constant ($p_1 \times G_b$). When one assumes the complete suppression of hepatic glucose production, this term should be a negative constant.

Interpretation of the constant values is also controversial.

1. p_1 (S_G) is the index of the metabolic clearance rate independent of insulin response. Since the analysis of the curve starts 8 minutes after the glucose load, this may be true. However, in this case the constant ($p_1 \times G_b$) could be negative. Previously this issue (including the interpretation of G_b) was controversial and the definition of the formula had been modified to clarify this term. When one starts to analyze the data from time O, $p_1 \times G_b$ could be positive and $G_b = G_0$. In this case, $p_1 \times V$ should be the basal metabolic clearance rate (=hepatic glucose production/G_0) minus constant uptake.
2. Insulin action can be determined by p_2, p_3 and Ib. To interpret those parameters, let's assume insulin steady state (like during the insulin clamp). When insulin concentration was fixed, the effect of insulin can be expressed as an exponential function. In this function $p_3 \times$ (INSULIN fixed$-I_b$) is the initial slope of the action of insulin and p_2 is the time constant and $\log_e(2)/p_2$ gives half maximum time.

While S_I which is p_3/p_2 is widely accepted as the index of insulin sensitivity, $p_3/p_2 \times$ (INSULIN fixed$-I_b$) gives the maximum response of insulin.

Since insulin resistant subjects have longer half maximum times, it may be difficult to measure the maximum. Actually it might be the case that p_3/p_2 was higher in insulin resistant subjects.

Comparison between mathematical models was reported[35]. Theoretically, the insulin clamp technique gives pure measurement of insulin sensitivity. When minimal model approach was applied to normal subjects, the correlation between the S_I and M value was ~ 0.8[36]. In non-insulin dependent diabetic subjects, this correlation is ~ 0.3[37]. If this poor correlation is caused by the misinterpretation of the constant parameters, we may apply the predicted Rd (glucose disposal rate). Insulin effectiveness (X), and Rd/V at fixed time (τ), is expressed assuming that the insulin level is constant as follows,

$I(\tau)$: constant

$$X(\tau)=\frac{p_3}{p_2}\cdot[I(\tau)-I_b]\cdot\left(1-e^{-p_2\tau}\right)$$

$$\frac{Rd(\tau)}{V}=[p_1+X(\tau)]G(\tau)-p_1G_b$$

Local Analysis

1) *Balance Technique in Steady State*

Catheterization and biopsies are basic methods for the analysis of metabolism of local organs. Forearm or leg balance technique is often used for the study of *in vivo* muscle. Biopsy enables the analysis of samples using molecular biology techniques. Muscle is the major organ responsible for glucose utilization during insulin stimulation. There is no pathway to produce glucose in the muscle, except for the small amount of degradation from branched structures of glycogen. Therefore glucose specific activity is constant. Products from the injected glucose tracer, i. e. tritiated water, is subject to the balance technique described below.

Dose: Total dose of test material

ti: infusion time

d(t): dose rate at time t (d(t) = O for $t > t_i$)

flow(t): water flow at time t (when constant flow(t) = flow)

c(t): concentration at time t observed at outlet

R(t): residual mass in the system at time t

production(t): production rate at time t

uptake(t): uptake rate at time t

From the conservation rule of the mass,

$$\frac{dR(t)}{dt}=\text{flow}(t)\cdot d(t)-\text{flow}(t)\cdot c(t)+\text{production}(t)-\text{uptake}(t)$$

Net uptake (Netuptake (t)) and Balance (Balance (t)) are expressed;

$$\text{Netuptake}(t)=\text{uptake}(t)-\text{pruduction}(t)$$

$$\text{Balance}(t)=\text{flow}(t)\cdot d(t)-\text{flow}(t)\cdot c(t)$$

$$\text{Netuptake}(t)=\text{Balance}(t)-\frac{dR(t)}{dt}$$

$$MTT=\frac{\lim_{\Delta t\to 0}\sum_{i=0}^{\infty}t_i\cdot c(t_i)\cdot\Delta t}{\lim_{\Delta t\to 0}\sum_{i=0}^{\infty}c(t_i)\cdot\Delta t}=\frac{\int_0^{\infty}t\cdot c(t)\,dt}{\int_0^{\infty}c(t)\,dt}$$

Implication of MTT in the balance technique is same as the correction by the accumulation of mass in the local space. From the conservation rule of the mass, (when water flow is constant).

$$\frac{dR(t)}{dt}=\text{flow}\cdot d(t)-\text{flow}\cdot c(t)-\text{Netuptake}(t)$$

When MTT is defined as follows and dR(t)/dt is close to dc(t)/dt, MTT is equal to V/flow.

$$0=\text{flow}(t)\cdot d(t)-\text{flow}\cdot c(t+MTT)-\text{Netuptake}(t)$$

$$0=\text{flow}\cdot c(t+MTT)-\text{flow}\cdot c(t)$$

$$\frac{d\frac{R(t)}{V}}{dt}\cdot\frac{V}{MTT}=\text{flow}\cdot\frac{c(t+MTT)-c(t)}{MTT}$$

$$MTT=\frac{V}{\text{flow}}\cdot\frac{\frac{d\frac{R(t)}{V}}{dt}}{\frac{c(t+MTT)-c(t)}{MTT}}\approx\frac{V}{\text{flow}}$$

Blood samples are also analyzed by gas analyzer[38] and local indirect calorimetry gives information about carbohydrate metabolism.

2) *Glucose Transporter Analysis in vivo*

The analysis of wash-out curves of the tracers after the bolus dose (or during the non-steady state of tracer) determines the flux (flow). Since tracers (^{12}C-mannitol, ^{14}C-3-O-methylglucose) diffuse linearly, it seems

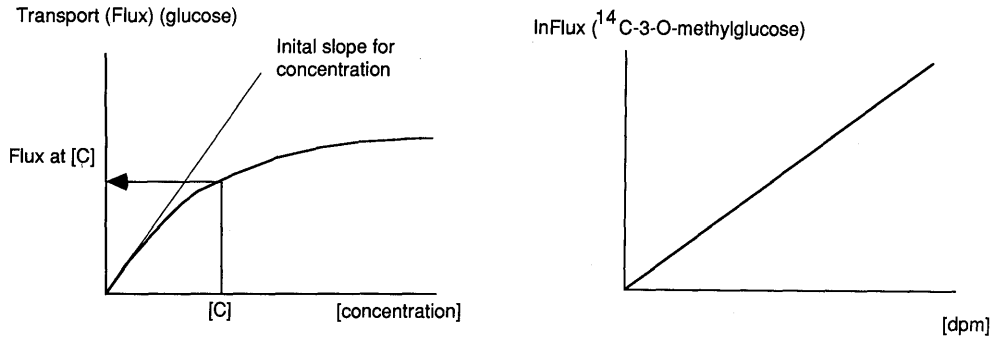
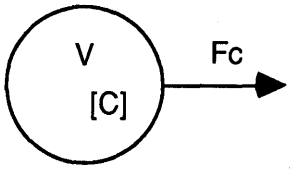


Fig. 1 The relation between glucose transport and concentration (upper panel) and the dilution of ^{14}C-3-O-methylglucose (lower panel).

V: volume, [C]: glucose concentration



F_c:Flux at [C], d:*diffusion rate*

$$F_c = \frac{Flux_{max}}{1+\frac{K_m}{[C]}} + d \cdot [C]$$

$$F_c = k \times [C] \times V \quad \text{(definition of } k\text{)}$$

SA:specific activity (tracer / tracee)

$$F_c = \frac{F^*_c}{SA_{out\ flux}}, \quad [C] = \frac{[C^*]}{SA_{compartment}}$$

Thus

$$\frac{F^*_c}{SA_{out\ flux}} = k \times \frac{[C^*]}{SA_{compartment}} \times V \quad or \quad F^*_c = \frac{SA_{out\ flux}}{SA_{compartment}} k \times [C^*] \times V$$

only when $SA_{out\ flux} = SA_{compartment}$,

$$F^*_c = k \times [C^*] \times V$$

Fig. 2 Explanation of the k (a flow constant) which is used for the calculation to determine the structure of the multiple compartment model for glucose transporter. Although the k value is a constant for various concentrations of a tracer, the k value is not constant for concentrations of a tracee.

that it is possible to determine the kin and kout (flux) values, which are slopes of the diffusion of tracer, as constant values in the steady state of glucose metabolism (Fig. 1).

Tracer diffuses in a linear way. This slope (kin) is determined by the analysis of the washout curve of a tracer as the same kin value for cold glucose, if the efflux from a compartment has the same specific activity as the specific activity in the preceding compartment. For this end, the tracer has to behave like a tracee, when the tracer is transported into the next compartment (Fig. 2). The homogeneity in the each compartment is essential.

The flux of a tracer (diffusion) increases linearly according to the increase of tracer dpm. When flux is described by k×the mass ([C]×V), in general, the k value, which is measured by tracer ($SA_{outflux}/SA_{compartment}\times$ k), is not the same constant for cold glucose. Therefore the homogeneity of specific activity in each heterogeneous compartment is the important prerequisite in this method. If the model is good enough to satisfy this condition flux of cold glucose in a steady state is measured by this method.

The relation between the specific activity in the compartment and the specific activity in the flux is very difficult to predict. For example, after a bolus dose of a tracer the specific activity in a compartment is increasing and flux may have less specific activity, while the specific activity of the other compartment may start to decrease by the cold glucose flow and its outlet flux may have higher specific activity. If the heterogeneous system is compartmentalized well enough to assume the rapid equilibration of tracer in each compartment, this model should work. Practically, a complicated compartment model is used to establish the physiological structure of the model. Mannitol or L-glucose is used to determine the flux constant between the heterogeneous compartments of extracellular space. This determination of heterogeneity is very important.

There is a significant advantage in this method. One can identify the distribution volume (ml) of the tracer (one distribution volume for one tracer) and the mass of the glucose (mg) in each compartment. This means that one can determine the average intracellular concentration of glucose. If the influx is almost equal to the net flux into the muscle cells, one might argue that there is no use to use this complicated system. However if the prediction of intracellular glucose concentration is possible, no one doubts the significance of this method[39-41]. Kinetic constants determined by this method using only two tracers cannot determine the glucose concentration. However, with the information of glucose uptake, theoretically it is possible to determine the intracellular glucose concentration. In this way, intracellular glucose concentration can be determined *in vivo*. In the biopsy studies, increase of intracellular glucose in diabetic state has been reported[42]. Biopsy studies showed a decrease of intracellular glucose concentration[43,44] during insulin clamp in normal subjects. During the insulin-stimulated period, muscle sucks up glucose and the tissue glucose concentration declines. The decrease of average glucose concentration in the tissue does not mean the decrease of intracellular glucose concentration. When basal glucose concentration in the muscle cell is very low, intracellular glucose concentration after the increase of glucose concentration can be still low, and at least lower than extracellular glucose concentration. It is very difficult to know the average glucose concentration in the extracellular fluid as well. The intracellular glucose concentration during the insulin clamp should be more difficult to predict than the intracellular glucose concentration in the basal period and the previous calculation based on the biopsy has too many assumptions to believe. If one takes account the decrease of extra cellular glucose concentration, the shift of Km for glucose concentration[45] can be also explained. This technique is expected to elucidate the responsible defect, glucose transporter or intracellular metabolic pathway, in the diabetic state in the near future.

Afterword

So called "clinical research" is carried out by both PhDs and MDs in the USA. The department of medicine employes ~ 10% of

its faculty as PhDs and 27% of the PhDs in clinical department were principal investigators (PIs) on NIH grants in fiscal year 1987. While the largest number of PhDs have been PIs on non-clinical research projects, 45% of them were PIs on clinical research projects[46]. Since in Japan MDs have good training to be PhDs, a substantial number of scientific-minded MDs are good investigators for clinical research. Institutional review board (IRB), clinical research center (GCRC), and NIH grant system do not exist in Japan, although similar systems are found. Only MDs are involved in "clinical research" in Japan. The use of radioactive tracer on human is restricted in Japan, although diagnostic and therapeutic uses of radioactive tracer are widely applied. Despite the differences in the environment in clinical research in Japan, effort for biomedical research is intense. I hope that this introduction about *in vivo* clinical research in glucose metabolism will be an inspiration for biomedical researchers in Japan.

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