No evidence for isotope discrimination of tritiated glucose tracers in measurements of glucose turnover rates in man

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Summary. Under non-steady-state conditions, glucose turnover rates determined with tritiated glucose tracers are often underestimated. To examine whether isotope discrimination or a tracer contaminant can contribute to this, we compared the turnover rates of unlabelled and tritiated glucose under isotopic steady-state conditions. The turnover rates were measured in 20 healthy subjects at two insulin concentrations ($79 \pm 3 \text{ mU} \cdot \text{l}^{-1}$ and $704 \pm 62 \text{ mU} \cdot \text{l}^{-1}$). Euglycaemia was maintained by infusing unlabelled glucose mixed with (3^3H)- or (6^3H)-glucose. In both studies, the isotopically determined glucose disposal rate was virtually identical to the exogenous glucose infusion rate (low insulin 7.66 ± 0.48 vs $7.58 \pm 0.44 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, high insulin 13.36 ± 0.74 vs $13.55 \pm 0.98 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). The individual values were correlated in both the low ($r = 0.85$, $p < 0.001$) and high dose

insulin ($r = 0.81$, $p < 0.001$) studies. Tritiated glucose specific activities were also compared in arterialized and deep venous blood across forearm tissues during the high-dose insulin infusion. Glucose specific activities were similar in arterialized and deep venous blood when analysed with HPLC and conventional methods. In summary: (1) Under isotopic steady-state conditions the turnover rates of unlabelled and labelled glucoses are similar. (2) Unlabelled and labelled glucose are handled identically across forearm tissues. (3) We found no tracer impurity in our tritiated glucose preparations. We conclude that (3^3H)- and (6^3H)-glucose tracers can be used to reliably measure glucose turnover rates in man.

Key words: Glucose metabolism, tritiated glucose, hyperinsulinaemia, radioisotope effect.

Tritiated glucose tracers are commonly used in clinical research for measuring glucose turnover. Recently however, the validity of the use of tritiated glucose for measurement of glucose turnover rates has been questioned. During insulin-stimulation, the glucose turnover rate is frequently underestimated and impossible negative values for endogenous glucose production are observed [1, 2]. It has been suggested that the underestimation is caused by an isotope effect, i.e. that tissues metabolize tritiated glucose slower than unlabelled glucose [3–5]. These studies, however, have been performed under non-steady-state conditions or with impure tracers [4], which may introduce sources of error independent of any isotope effect. In the present study, we examined the existence of an isotope effect for (6^3H)- and (3^3H)-glucose by comparing the turnover rates of tritiated and unlabelled glucose under isotopic steady-state conditions at two insulin concentrations. In addition, we compared directly the disposal of unlabelled and labelled glucose by determining the plasma glucose specific activities across forearm tissues.

Subjects and methods

Subjects

Twenty normal subjects participated in the studies (age 31 ± 2 years, body mass index $22 \pm 1 \text{ kg/m}^2$). At least two days before the study, the subjects were asked to consume a weight maintaining diet containing at least 200 g carbohydrate per day. The nature, purpose, and possible risks of the study were explained to the subjects before they gave their voluntary consent to participate. The experimental protocol was approved by the Ethical Committee of the Helsinki University Hospital.

Design

Two studies were performed. Both studies were done after an overnight fast. At 08.00 hours, an indwelling catheter was inserted in an antecubital vein for infusion of unlabelled and labelled glucose, and insulin. A second catheter was inserted retrogradely into a hand vein for blood sampling. The hand was kept in a heated (65°C) chamber to arterialize venous blood [6]. The outline of the two studies is shown in the Figure 1.

Fig. 1. Design of the study

Fig. 2. Plasma glucose, glucose specific activity and serum insulin concentrations during the low (left panel, $n = 10$) and high insulin infusion studies (right panel, $n = 7$). In the high insulin infusion study, glucose specific activity is shown both in the arterIALIZED (x) and deep venous plasma (o) to demonstrate the steady-state situation. All the other data are from arterIALIZED blood

Study I

In the first study, the turnover rate of 3- ^3H -glucose was compared to that of unlabelled glucose, using a technique similar to that of Finegood et al. [7], in ten subjects during a euglycaemic insulin clamp (insulin infusion rate $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). To reach isotopic steady-state, the glucose pool was labelled with a primed ($40 \mu\text{Ci}$) continuous ($0.5 \mu\text{Ci}/\text{min}$) infusion of D-(3- ^3H)-glucose, specific activity

5 Ci/mmol (Amersham, Buckinghamshire, UK) before starting the insulin infusion. At 120 min, a euglycaemic insulin clamp was started as previously described [8, 9]. The duration of the insulin clamp study was 120 min (Fig. 1). During the clamp, plasma glucose specific activity was maintained constant as follows. During the initial 10 min of insulin infusion, the basal (^3H)-glucose infusion was continued; at 10 and 20 min, the tritiated glucose infusion rate was diminished to 50% and 75% of basal respectively, and at 30 min it was discontinued. The stepwise decrease in the basal tritiated glucose infusion was used to avoid a decline in baseline steady state plasma glucose activity during the initial period of the insulin clamp. In addition, sufficient 3- ^3H -glucose was added to the exogenously infused "cold" glucose to ensure constancy of the baseline tritiated glucose specific activity during the insulin clamp. The effected basal plasma glucose specific activity was calculated by dividing the isotope infusion rate with the estimated basal glucose turnover rate ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) [1, 8]. The quantity of isotope (dpm) added to the cold 20% glucose infusate equals the estimated basal plasma specific activity (dpm/mg) $\times$ the total amount of cold glucose in the 20% glucose solution (mg). If steady state plasma glucose specific activity can be maintained during the clamp, the turnover rate of labelled glucose can be calculated by dividing the 3- ^3H -glucose infusion rate by the steady state glucose specific activity. The glucose turnover rate was calculated during the second hour of the clamp (180–240 min), under steady-state conditions as shown in Figure 2.

Study II

In this study, the aim was to achieve steady-state glucose specific activity under conditions of higher glucose turnover rate. The second euglycaemic insulin clamp study was performed using an insulin infusion rate of $5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, and the turnover rate of (^6H)-glucose ($n = 9$) or (^3H)-glucose ($n = 1$) was compared to that of unlabelled glucose. The design of study II was different from study I (Fig. 1). Between 0 and 120 min, normoglycaemia was maintained with the infusion of cold 20% glucose. After 120 min, normoglycaemia was maintained with the infusion of 20% glucose, which contained (^3H)-glucose $100\text{--}300 \mu\text{Ci}/500 \text{ ml}$. The range of the activity was used to see a dose-response effect of label, if any, on glucose disposal. The purpose of this design was to infuse cold glucose during the non-steady-state period, when hepatic glucose production is declining. Thereafter, under conditions where hepatic glucose production is totally suppressed, mixing the tracer with cold glucose has the advantage that plasma glucose specific activity can be maintained unchanged even if glucose infusion rate is changed. Thus, under these conditions, the measurements can be done in steady-state conditions. The clamp-study lasted for 4 h in seven subjects, and for 6 h in three other subjects, to exclude any doubt of the existence of isotopic steady-state (Fig. 1). The glucose turnover rate was calculated during the last hour of the clamp in each subject.

Forearm studies

To directly compare the uptake rates of labelled and unlabelled glucose by peripheral tissues, we compared the specific activities of tritiated glucose in arterIALIZED and deep venous blood. This was done during study II. ArterIALIZED venous (A) and deep venous (DV) blood samples were obtained across the forearm simultaneously at 180, 200, 220 and 240 min in seven subjects, and at 300, 320, 340 and 360 min in three subjects (Fig. 1).

Analytical methods

Plasma glucose concentration during the clamp was measured with the glucose oxidase method (Beckman Glucose Analyzer II, Beckman Instruments, Fullerton, Calif., USA). Serum insulin was

measured radioimmunologically after precipitation with polyethylene glycol [10]. ^3H -glucose radioactivity (dpm/ml) was determined after deproteinisation of plasma with $\text{Ba}(\text{OH})_2\cdot\text{ZnSO}_4$, and evaporation of tritiated water as previously described [11]. The specific activity of glucose (dpm/mg) was calculated by dividing the ^3H -glucose radioactivity with the plasma glucose concentration (mg/ml). For determination of ^6H -glucose specific activity, 4 ml of plasma was deproteinized by addition of an equal volume of chilled 7% perchloric acid. Following centrifugation, the supernatant was neutralized with 4 mol/l KOH and transferred to stacked polypropylene columns (Biorad, Richmond, Calif., USA). The top column contained 9 ml of AG1 anion exchange resin (formate form, 100–200 mesh, Biorad), and the bottom column 6 ml of AG50 cation exchange resin (100–200 mesh, Biorad). For elution of glucose, the columns were washed with 35 ml of water. The neutral eluate was collected, dried in vacuum at 25°C , resuspended in 2 ml of H_2O , and a 200 μl aliquot was taken for glucose determination (Beckman Glucose Analyzer). The remaining 1.8 ml aliquot was counted for glucose radioactivity in a scintillation counter (LKB Wallac, Turku, Finland) after addition of 10 ml scintillation solvent (Aquasol, New England Nuclear, Boston, Mass., USA) with correction of quenching.

HPLC analysis of tracer purity

Since commercially available isotope preparations might contain non-glucose contaminants [4], glucose specific activity was measured in some samples ($n = 24$) both by ion-exchange chromatography and HPLC. Plasma samples were deproteinized, neutralized and run through anion and cation exchange columns as described above. After drying, the neutral fraction was resuspended in 1 ml water, transferred into 1.5 ml tubes, dried again, resuspended in 200 μl of 0.05 mol/l H_3PO_4 , filtered through a 0.22 μm Millipore filter (Millex-GV₄, Bedford, Mass., USA) and shot on an organic acid HPLC column (Aminex HPX-87, Biorad). The HPLC system consisted of an LKB HPLC pump, controller and a programmable Hellirac fraction collector (LKB, Bromma, Sweden), sample injector (Reodyne, Cotati, Calif., USA), absorbance detector (Spectroflow 783, Kratos Analytical, Ramsey, NJ, USA) and data processor (Shimadzu C-R34, Chromatopac, Shimadzu Co., Kyoto, Japan).

The eluent was monitored at a wavelength of 194 nm. The flow rate was 0.6 ml/min, pressure 45–50 bar, and mobile phase 0.05 mol/l H_3PO_4 . Glucose standards and glucose added to plasma eluted at 9.5 min. A 2 min fraction around the glucose peak was collected, 1 ml taken for measurement of radioactivity, and the remaining aliquot used for measurement of the glucose concentration [12].

Calculations

During the last 60 min of the insulin clamp (180–240 min, $n = 17$, or 300–360 min, $n = 3$), the glucose disposal rate was calculated (a) from the mean unlabelled glucose infusion rate; and (b) by dividing the infusion rate of (^3H)- or (^6H)-glucose (dpm/min) by the tritiated glucose specific activity (dpm/mmol). The disposal rates of labelled and unlabelled glucose were compared by linear regression analysis. We also calculated, whether there was a correlation between the % deviation of the tracer-determined glucose turnover rate, calculated using Steele's equation [13] from the cold glucose infusion rate, and the % deviation of observed from the predicted steady-state specific activity. The predicted specific activity was calculated by dividing the isotope infusion rate with the cold glucose infusion rate. Thus, $100 \times (\text{SA}_{\text{ss}} - \text{SA}_{\text{obs}}) / \text{SA}_{\text{ss}}$ was divided by $100 \times (\text{Ra} - \text{Ginf}) / \text{Ginf}$, where SA_{ss} and SA_{obs} are the theoretical and observed specific activities, respectively, Ra is the isotopically determined glucose turnover rate, and Ginf is the infusion rate of cold glucose. The residual endogenous glucose production was calculated during the

last 60 min of the insulin clamp by subtracting Ginf from the total glucose turnover rate (Rt) determined with the labelled glucose,

$$\text{Rt} = \frac{\text{Ginf}}{\text{SA}}.$$

Statistical analysis

Statistical comparisons were done with paired *t*-tests [14]. All results are given as mean $\pm$ SEM.

Results

Tracer purity

The ^3H -glucose specific activities obtained with ion exchange and HPLC were similar (696 ± 82 and 704 ± 87 dpm/ μmol , $n = 24$, respectively) and correlated ($r = 0.97$, $p < 0.001$). Analysis of neutral eluates of the (^6H) and (^3H) glucose tracers after ion-exchange chromatography with HPLC revealed no contaminants, even after rerunning the isotopes on the HPLC column (data not shown).

Study I

Plasma glucose concentration remained unchanged at 5.0 ± 0.2 mmol/l with a coefficient of variation (CV) of $5 \pm 2\%$ throughout the study (Fig. 2). Plasma (^3H)-glucose specific activity was constant during the last 60 min and averaged 116 ± 3 dpm/ μmol^{-1} (CV $6 \pm 1\%$, Fig. 2). The mean serum insulin concentration was 79 ± 3 mU $\cdot\text{l}^{-1}$ (CV $6 \pm 1\%$). The isotopically determined rate of glucose disposal (7.66 ± 0.48 mg $\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was virtually identical to the exogenous glucose infusion rate (7.58 ± 0.44 mg $\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) during the last 60 min of insulin clamp (from 180 to 240 min, Fig. 1). The individual values of all 20 subjects during the last 60 min of steady-state hyperinsulinaemia for glucose disposal as determined with the unlabelled or labelled glucose, are given in Figure 3. The correlation coefficient between the two measurements for the 10 subjects was 0.85, $p < 0.001$ (Fig. 3). The equation between the turnover rates of labelled and unlabelled glucose was: labelled glucose turnover rate = $0.93 \times$ unlabelled glucose turnover rate + 0.63, where 0.93 is not significantly different from 1, and 0.63 is not statistically different from 0. The remaining endogenous glucose production rates during the last three 20 min periods of the last hour of hyperinsulinaemia (180–240 min, Fig. 2) were 0.07 ± 0.18 mg $\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, 0.07 ± 0.15 mg $\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and -0.03 mg $\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, respectively.

Study II

In the seven subjects studied during the fourth hour of the insulin clamp (from 180 to 240 min, Fig. 1), the steady state plasma glucose concentration was 5.2 ± 0.1 mmol $\cdot\text{l}^{-1}$ (CV $5 \pm 1\%$), serum insulin concentration 704 ± 62 mU $\cdot\text{l}^{-1}$.

Fig. 3. The relationship between the disposal rate of unlabelled glucose and glucose labelled with (^3H) or (^6H)-glucose. Solid circles indicate subjects studied during the low dose insulin infusion and (^3H)-glucose, x = subjects studied during the high dose insulin infusion and (^6H)-glucose for 4 hours, circled, x = subjects studied during the high dose insulin infusion and (^6H)-glucose for 6 hours, and filled square a subject studied during the high dose insulin infusion and with (^3H)-glucose as the tracer

Fig. 4. Plasma (^3H)- and (^6H)-glucose specific activities in arterialized and deep venous blood in subjects studied during the high dose insulin infusion between 180–240 min (x) or between 300–360 min (o)

l^{-1} (CV $7 \pm 2\%$), and glucose specific activity $620 \pm 135 \text{ dpm} \cdot \mu\text{mol}^{-1}$ (CV $11 \pm 3\%$, Fig. 2). In the three subjects studied from 300 to 360 min, the individual mean plasma glucose and insulin concentrations were $5.5 \text{ mmol} \cdot l^{-1}$, $5.3 \text{ mmol} \cdot l^{-1}$, $4.8 \text{ mmol} \cdot l^{-1}$, and $568 \text{ mU} \cdot l^{-1}$, $712 \text{ mU} \cdot l^{-1}$, $562 \text{ mU} \cdot l^{-1}$, respectively. The glucose specific activities were 895, 1110 and $1185 \text{ dpm} \cdot \mu\text{mol}^{-1}$, respectively. In these ten subjects, the glucose infusion rate was $13.36 \pm 0.74 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, which was virtually identical to that determined with tritiated glucose

($13.55 \pm 0.98 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). During the three consecutive 20 min periods from 180 to 240 min, the glucose disposal rates were $13.24 \pm 1.04 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $13.41 \pm 0.78 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, and $13.54 \pm 0.98 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (NS), respectively, indicating constancy of glucose metabolism during the last 60 min. The individual values for all 20 subjects during the last 60 min of steady-state hyperinsulinaemia are shown in Figure 3. The correlation between the two determinations was $r = 0.81$, $p < 0.001$. The equation between the turnover rates of labelled and unlabelled glucose was: the turnover rate of labelled glucose = $1.03 \times$ the turnover rate of unlabelled glucose + 0.42 . In this equation, 1.03 and 0.42 were not significantly different from 1, and 0, respectively.

When all 20 studies were combined, the mean turnover rates for unlabelled and labelled glucose were $10.47 \pm 0.78 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and $10.61 \pm 0.86 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively. The correlation coefficient for all data was $r = 0.93$, $p < 0.001$, and the equation relating the two was as follows: turnover rate of labelled glucose = $0.98 \times$ the turnover rate of unlabelled glucose + 0.18 . There was a close correlation between the % underestimation of the rate of glucose appearance determined with labelled glucose using the Steele equation, and the % deviation of observed plasma glucose specific activity from the calculated target specific activity ($r = 0.85$, $p < 0.001$).

Forearm studies

To analyse directly the existence of isotope discrimination for tritiated glucose, we determined glucose specific activities in arterialized and deep venous blood in the ten subjects participating in study II. The individual values were correlated (Fig. 4), and the mean values in arterialized ($621 \pm 109 \text{ dpm} \cdot \mu\text{mol}^{-1}$) and deep venous blood ($640 \pm 111 \text{ dpm} \cdot \mu\text{mol}^{-1}$) were similar indicating that forearm tissues handle unlabelled and tritiated glucose identically.

Discussion

In recent years it has become apparent that use of tritiated glucose may underestimate the rate of glucose disposal, especially under hyperinsulinaemic conditions, when the rate of glucose flux is high [1, 3, 15]. Since the rate of endogenous glucose production is calculated by subtracting the exogenous glucose infusion rate from the rate of total glucose appearance, it follows that negative values for hepatic glucose output may be observed. Obviously, a negative number for hepatic glucose production can have no physiological meaning and raises concern about the validity of the methodology. There are several possibilities which may contribute to the underestimation of glucose turnover and negative values for hepatic glucose production.

First during the insulin clamp, if some glucose were first incorporated into liver glycogen, and subsequently released while retaining its label, glucose turnover could

be underestimated [16]. This could occur if the clamp were performed in the usual manner, i.e. the constant rate isotope infusion in the basal state is continued during insulin infusion. During the equilibrium period when tritiated glucose is infused under basal conditions of insulinaemia, a high plasma tritiated glucose specific activity is achieved and this labelled glucose could be incorporated into glycogen. During an insulin clamp, the plasma glucose specific activity declines dramatically due to infusion of cold glucose. During a $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ euglycaemic insulin clamp the tritiated glucose specific activity can be expected to decrease to 10–20% of basal levels [17]. If significant cycling of glycogen occurs and this follows the first in first out principle [18], tritiated glucose could be released from liver glycogen during the later stages of the insulin clamp despite net glycogen synthesis. This could falsely elevate plasma tritiated glucose specific activity. Such a sequence, of course, can only occur if glucose is incorporated into glycogen via the direct pathway, since (^3H)-glucose would lose its label if incorporated into glycogen via the indirect pathway. If, however, the basal tritiated glucose specific activity is maintained constant throughout the insulin clamp, as was done in the present study, glucose incorporated into glycogen and subsequently released would have the same specific activity as that of plasma and an underestimation of glucose disposal would not be observed.

A second potential explanation for the underestimation of glucose turnover may relate to the marked expansion of the glucose space that occurs, when glucose flux is stimulated by insulin [17]. This effect is most evident, when rates of glucose utilization exceed $5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ [17]. Under true steady-state conditions, glucose turnover can simply be calculated by dividing the infusion rate of tritiated glucose counts by the steady-state plateau of plasma tritiated glucose specific activity. Under non-steady-state conditions, compartmental analysis must be employed to calculate rates of glucose turnover and the most common approach is to use Steele's equations for a single glucose pool model with a constant pool fraction of 0.65. However, recent studies by Ferrannini et al. [17] have shown that under hyperinsulinaemic conditions the glucose pool may increase by 25–30% or more compared to the basal state. As a consequence of this, if the plasma ^3H -glucose specific activity has not truly plateaued and tritiated glucose counts are disappearing into continuously expanding glucose space, the rate of glucose disposal will be significantly underestimated. If one takes into account this expansion of the glucose space during an insulin clamp, one can accurately estimate the rate of glucose turnover and negative numbers for hepatic glucose production are completely obviated [15]. From the above considerations it also follows that if one maintains the plasma tritiated glucose specific activity constant during the insulin clamp, changes in the space of glucose distribution do not enter into calculation of glucose turnover, which simply equals the glucose infusion rate divided by the steady state plasma tritiated glucose specific activity.

A third prerequisite for the use of glucose isotopes in metabolic studies is that labelled glucose is metabolized identically to unlabelled glucose. It has recently been sug-

gested that glucose labelled with tritium may be handled differently than the native glucose molecule [1–5]. This phenomenon is known as an "isotope effect". If such an isotope effect were indeed responsible for the underestimation of glucose turnover during insulin stimulation, one would expect an underestimation of glucose disposal and negative numbers for hepatic glucose production regardless of whether or not the plasma tritiated glucose specific activity was maintained constant. In the present study, the turnover rates of unlabelled and labelled glucose were compared under isotopic steady-state conditions at two insulin concentrations. Tritiated glucose was mixed with cold glucose and infused at a variable rate throughout the clamp studies to maintain plasma tritiated glucose specific activity constant (Fig. 2). Under these steady-state conditions of constant tritiated glucose specific activity, the mean rate of glucose disposal estimated from the exogenous infusion of cold glucose closely approximated that determined with tritiated glucose. Furthermore, a close agreement between the two estimates of glucose disposal was observed (Fig. 3). Similar specific activities in arterialised and deep venous blood across forearm tissues in our subjects further indicate that unlabelled and labelled glucose are handled identically. Recent studies using similar steady-state conditions for isotopically labelled glucose have also reported identical disposal rates for labelled and unlabelled glucose [7, 19–22]. Ferrannini et al. [17] have also shown that the rate of glucose infusion during the third hour of a euglycaemic insulin clamp was nearly identical to that calculated from a bolus injection of (^3H)-glucose using a physiological three-compartmental model. These observations strongly argue against an "isotope effect" as the explanation for the underestimation of glucose disposal under insulin-stimulated conditions.

Finally, the presence of a labelled contaminant in the tracer will also result in falsely elevated glucose specific activity due to accumulation of the contaminant in the circulation [4]. In the current study, the specific activities determined with HPLC and conventional methods were similar and no contaminant was found in the tritiated glucose preparations.

In the current study, a close correlation was found between the relative deviation, albeit small, of plasma glucose specific activity from the target (i.e. steady-state), and underestimation of glucose turnover. Thus, the greater the deviation of specific activity from isotopic steady-state, the greater the underestimation of glucose turnover calculated according to the one-compartmental model of Steele. These data suggest that a likely reason for underestimation of isotopically determined glucose turnover under non-steady-state conditions is model inadequacy. However, these observations do not exclude the possibility of cycling of tritiated glucose through glycogen and back to plasma glucose [16]. It should be emphasized that these two latter explanations are not mutually exclusive.

In summary, tritiated glucose labelled in the 3 or 6 position provides an accurate estimate of glucose disposal as long as the plasma glucose specific activity is maintained constant. Thus, underestimation of glucose turnover and hepatic glucose production cannot be attributed to an iso-

tope effect of tritiated glucose. We did not find any tracer contaminant in the present study. Thus, the most likely reason for the reported underestimation of glucose disposal is model inadequacy under non-steady-state conditions.

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References

1. Bergman R, Finegood DT, Ader M (1985) Assessment of insulin sensitivity in vivo. *Endocrine Rev* 6: 45–86
2. Argoud GM, Schade DS, Eaton RP (1987) Underestimation of hepatic glucose production by radioactive and stable tracers. *Am J Physiol* 252: E606–E615
3. Bell PM, Firth RG, Rizza RA (1986) Assessment of insulin action in insulin-dependent diabetes mellitus using ($^{6^{14}}\text{C}$)glucose, (^3H)glucose, and (^2H)glucose. Differences in the apparent pattern of insulin resistance depending on the isotope used. *J Clin Invest* 78: 1479–1486
4. McMahon MM, Schwenk WF, Haymond MW, Rizza RA (1989) Underestimation of glucose turnover with ($6\text{-}^3\text{H}$)- and ($6,6\text{-}^2\text{H}_2$)-but not ($6\text{-}^{14}\text{C}$) glucose during hyperinsulinemia in humans. *Diabetes* 38: 95–107
5. Bell PM, Firth RG, Rizza RA (1987) The effects of hyperglycaemia on isotopic measurement of glucose utilization using (^2H), (^3H) and ($^{6^{14}}\text{C}$) glucose in patients with Type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 30: 365–371
6. McGuire EAH, Helderman JH, Tobin JD, Andres R, Bergman M (1976) Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J Appl Physiol* 41: 565–571
7. Finegood DT, Bergman R, Vranic M (1988) Modelling error and apparent isotope discrimination confound estimation of endogenous glucose production during euglycemic glucose clamps. *Diabetes* 37: 1025–1034
8. DeFronzo RA, Tobin JD, Andres R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237: E214–E223
9. Yki-Järvinen H, Koivisto VA (1986) Natural course of insulin resistance in type 1 diabetes. *N Engl J Med* 315: 224–230
10. Kuzuya H, Blix PM, Horwitz DL, Steiner DF, Rubenstein AH (1977) Determination of free and total insulin and C-peptide in insulin treated diabetics. *Diabetes* 26: 22–29
11. Altszuler N, Barkai A, Bjerknes C, Gottlieb B, Steele R (1975) Glucose turnover values in the dog obtained with various species of labelled glucose. *Am J Physiol* 229: 1662–1665
12. Kadish AH, Little RL, Sternberg JC (1968) A new and rapid method for the determination of glucose by measurement of rate of oxygen consumption. *Clin Chim* 14: 116–131
13. Steele R, Wall JS, DeBodo RC, Altszuler N (1956) Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 187: 15–24
14. Snedecor GW, Cochran WG (1967) Statistical methods, 6th edn. Iowa State University Press, Ames, Iowa
15. Cobelli C, Mari A, Ferrannini E (1987) Non-steady state: error analysis of Steele's model and development for glucose kinetics. *Am J Physiol* 15: E679–E689
16. Shulman GI, Rothman DL, Chung Y, Rossetti L, Petit WA, Barrett E, Shulman RG (1988) ^{13}C NMR studies of glycogen turnover in the perfused rat liver. *J Biol Chem* 263: 5027–5029
17. Ferrannini E, Smith D, Cobelli C, Toffolo G, Pilo A, DeFronzo RA (1985) Effect of insulin on the distribution and disposition of glucose in man. *J Clin Invest* 76: 357–364
18. Newsholme EA (1978) Substrate cycles: their metabolic, energetic and thermic consequences in man. *Biochem Soc Symp* 43: 183–205
19. Ferrannini E, Del Prato S, DeFronzo R (1985) Glucose kinetics: tracer methods. In: Lerner J, Pohl S (eds) *Methods in diabetes research*, Vol II. John Wiley, New York, pp 19–43
20. Schwenk WF, Buttler P, Haymond MV, Rizza R (1988) Underestimations of glucose turnover corrected with HPLC purification of tritiated glucose. *Diabetes* 37: 89A
21. Molina JM, Baron A, Edelman S, Olefsky JM (1988) Variable tracer infusion rate improves determination of glucose turnover in man. *Clin Res* 36: 156A
22. Yki-Järvinen H, Consoli A, Nurhjan N, Young AA, Gerich JE (1989) Mechanism for underestimation of isotopically determined glucose disposal. *Diabetes* 38: 744–751

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