# Determination of steady state and nonsteady-state glycerol kinetics in humans using deuterium-labeled tracer

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**Abstract** Using deuterium-labeled glycerol as tracer and gas–liquid chromatography–mass spectrometry techniques for the determination of isotopic enrichment, we have developed a simple and ethically acceptable method of determining glycerol appearance rate in humans under steady-state and nonsteady-state conditions. In normal subjects, the appearance rate of glycerol in the post-absorptive state was 2.22 ± 0.20 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, a value in agreement with those reported in studies with radioactively labeled tracers. The ratio nonesterified fatty acid (NEFA) appearance rate/glycerol appearance rate ranged from 1.95 to 3.40. In insulin-dependent diabetic patients with a mild degree of metabolic control, the appearance rate of glycerol was 2.48 ± 0.29 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. The volume of distribution of glycerol, determined by the bolus injection technique, was (mean) 0.306 $l \cdot kg^{-1}$ in normal subjects and 0.308 $l \cdot kg^{-1}$ in insulin-independent diabetic patients. To evaluate the usefulness of the method for determination of glycerol kinetics in nonsteady-state conditions, we infused six normal subjects with natural glycerol and calculated the isotopically determined glycerol appearance rate using a single compartment model (volume of distribution 0.31 $l \cdot kg^{-1}$). During these tests, the expected glycerol appearance rates were successively 5.03 ± 0.33, 7.48 ± 0.39, 9.94 ± 0.34, 7.48 ± 0.39, and 5.03 ± 0.33 $\mu mol \pm kg^{-1} \cdot min^{-1}$, whereas the corresponding isotopically determined appearance rates were 4.62 ± 0.45, 6.95 ± 0.56, 10.85 ± 0.51, 7.35 ± 0.34, and 5.28 ± 0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Thus, the present method appears useful for the determination of human glycerol kinetics under steady-state and nonsteady-state conditions. — **Beylot, M., C. Martin, B., Beaufrere, J. P. Riou, and R. Mornex.** Determination of steady state and nonsteady-state glycerol kinetics in humans using deuterium-labeled tracer. *J. Lipid Res.* 1987. **28:** 414–422.

**Supplementary key words** lipolysis • glycerol • stable isotope • mass spectrometry • diabetes • free fatty acids

Glycerol is an important metabolite since, in addition to being an oxidative substrate for some tissues (1), it provides the backbone of glyceride lipids and is an important neoglucogenic substrate in both lean and obese fasting subjects (1–8). Moreover, since glycerol is an end product of triacylglycerol breakdown and cannot be reutilized by adipose tissue, its appearance rate is considered to reflect lipolysis in adipose tissue and, as such, has been used for studies of fat mobilization in vivo (1, 8–13). In most of these studies, the determination of glycerol appearance rate was performed using radioactive glycerol as tracer (9–13). Such tracers cannot be used in children or pregnant women, or for repetitive studies in humans. Moreover, the determination of glycerol radioactivity in blood or plasma samples is tedious and time consuming. Carpentier et al. (14) have presented a method using unlabeled glycerol but their method can be used only in steady-state conditions and therefore is not suitable for studies of acute regulation of fat metabolism. Stable isotope-labeled tracers are an attractive alternative to radioactive tracers. Recently, [2-$^{13}$C]glycerol and [$^{2}H_5$] or [$^{2}H_8$]glycerol have been used to measure glycerol appearance rate, respectively, in human newborns (8) or adults (15) and in rats (16). These determinations were performed only in steady-state conditions and we are presently aware of no study validating a method for the determination of glycerol appearance rate in nonsteady-state conditions. We present here the validation of a method using deuterium-labeled glycerol as tracer and a gas–liquid chromatography–mass spectrometry technique for the determination of glycerol appearance rate in steady-state and nonsteady-state conditions.

## SUBJECTS AND METHODS

### Animal studies

The validity of the use of [1,2,3-$^{2}H_5$]glycerol for the determination of glycerol flux was evaluated in six

Abbreviations: VD, volume of distribution; NEFA, nonesterified palmitic acid; FFA, free fatty acids; IRI, immunoreactive insulin.

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anesthetized adult rats that had been fasted overnight. Catheters were placed in a femoral artery for blood sampling and in a jugular vein for tracer infusion. [2-$^{13}$C]Glycerol and [1,2,3-$^{2}H_5$]glycerol were infused simultaneously at the same rate (0.378 to 0.403 $\mu$mol · kg$^{-1}$ · min$^{-1}$ and blood was sampled after 40, 50, and 60 min of infusion for the determination of glycerol concentration and of glycerol enrichment. These determinations were performed as described below except that the ion of m/z 146 was also monitored, in addition to the ions 145 and 148, in order to determine the enrichment in [2-$^{13}$C]glycerol from the ratio 146/145 and an appropriate calibration curve. Glycerol concentration was 130 ± 14 $\mu$mol · l$^{-1}$. Glycerol appearance rate determined with [1,2,3-$^{2}H_5$]glycerol was 10.98 ± 0.68 $\mu$mol · kg$^{-1}$ · min$^{-1}$, a value comparable to the result obtained with [2-$^{13}$C]glycerol (11.60 ± 0.76 $\mu$mol · kg$^{-1}$ · min$^{-1}$).

### Human studies

Thirteen normal subjects (nine men and four women, aged 21 to 36 years (mean 28), body weight 53 to 83 kg (mean 55)) and five insulin-dependent diabetic subjects (three men and two women, aged 36 to 64 years (mean 50), body weight 53 to 72 kg) were studied. Each subject gave consent to the study after being informed about its nature, purpose, and possible risks.

### Procedures

All tests were performed in the postabsorptive state. On the morning of the test, indwelling catheters were placed into veins of one forearm for the infusions. To obtain arterial blood samples, another catheter was placed in a retrograde manner in a vein of the opposite hand kept at 60°C. Catheters were kept patent by a slow infusion of isotonic saline. After 30 min of bed-rest and the collection of three initial blood samples, the following tests were performed.

*Normal subjects.* In an initial study, the apparent volume of distribution (VD) of glycerol was determined in eight subjects by an intravenous bolus injection of [1,2,3-$^{2}H_5$]glycerol (5.0 to 8.5 $\mu$mol · kg$^{-1}$). Blood was collected 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 20, 22, and 25 min after the bolus. A primed (10 times the infusion rate over 1 min) continuous intravenous infusion (0.036 to 0.085 $\mu$mol · kg$^{-1}$ · min$^{-1}$) of [1,2,3-$^{2}H_5$]glycerol was then initiated and blood samples were collected at 70, 80, 90, and 100 min for the determination of glycerol appearance rate in the postabsorptive state. Then, in six subjects, infusion of the tracer was continued for an additional period of 120 min and natural glycerol was infused at increasing (2.5, 5.0, and 7.5 $\mu$mol · kg$^{-1}$ · min$^{-1}$) and next decreasing (5.0 and 2.5 $\mu$mol · kg$^{-1}$ · min$^{-1}$) rates. Each period of infusion of natural glycerol lasted 20 min except the highest infusion rate period which lasted 40 min. Blood samples were collected during each infusion period for the determination of various metabolites and hormones. Aliquots of natural and labeled glycerol were collected at the end of each test in order to check the concentration. The actual infusion rates obtained were determined from this concentration and the volume of infusate that was delivered.

In a second study, the appearance rates of glycerol and of palmitate were determined simultaneously in five subjects by a primed continuous infusion of [1,2,3-$^{2}H_5$]glycerol and by the infusion of albumin-bound [1-$^{13}$C]palmitate (0.035 to 0.045 $\mu$mol · kg$^{-1}$ · min$^{-1}$) for 100 min.

*Diabetic patients.* Five insulin-dependent diabetic patients were studied. Each subject had his or her usual insulin injection the day before the study, but no insulin was injected the morning of the test. In each patient the apparent VD of glycerol and its appearance state in steady-state condition were determined, as described in the normal subjects, by a bolus injection and then a primed continuous infusion of the tracer.

### Preparation of infusates

[1,2,3-$^{2}H_5$]Glycerol (76% atom % $^{2}H_5$) and [1-$^{13}$C]palmitic acid (99% atom % 1-$^{13}$C] were purchased from Merck Isotopes (Dorval, Quebec, Canada). Natural glycerol was from Merck (Darmstadt, West Germany). Natural and labeled glycerol were diluted with sterile water, and [1-$^{13}$C]palmitic acid was bound to human serum albumin as described previously (8). The actual concentration and enrichment of the tracers were determined in each preparation. All infusates were shown to be pyrogen-free and before infusion they were passed through a 0.22 $\mu$m Millipore filter.

### Analytical procedures

Plasma glucose (17), glycerol (18), and total nonesterified free fatty acids (19) were determined by enzymatic methods. The recovery of added glycerol was 95 to 100% and the intraassay coefficient of variation was 6.1 ± 1.2%. Immunoreactive insulin was determined on plasma kept frozen until the day of the assay (20).

For the determination of glycerol enrichment, blood samples were immediately centrifuged at 4°C and the plasma was kept frozen (−20°C) until the assay. On the day of the assay, plasma proteins were precipitated by perchloric acid (6% w/v). After centrifugation, the supernatant was neutralized and subjected to sequential anion and cation exchange chromatography (AG 1 × 8 and AG 50w × 4, Bio-Rad, Richmond, CA). The neutral eluate was lyophilized before derivatization. The glycerol 1,2,3 triacetate ester was then prepared by adding 20 $\mu$l of pyridine (Pierce Chemical Company, Rockford, IL) and 20 $\mu$l of acetic anhydride (Sigma, St. Louis, MO) at room temperature. Aliquots (1 $\mu$l) were injected into a gas chromatograph (Fractovap 4160, Carlo Erba, Massy, France) coupled with a mass spectrometer (Quadrupole R 10-10, Nermag, Rueil Malmaison, France) and equipped with a

25-m fused silica capillary column (OV 1701, Chrompack, Bridgewater, NJ). The operating conditions were: injector temperature, 200°C; oven temperature, 165°C; and source temperature, 220°C. Fragmentation of the molecules was performed by electron impact (70 eV) and the ions of m/z 145 (natural glycerol) and 148 (labeled glycerol) were selectively monitored. These ions result from the fragmentation of the molecule between the carbons 2 and 3 or 1 and 2 giving identical fragments which lack (for labeled glycerol) two of the five deuterium atoms (**Fig. 1**). The peak areas ratio 148/145 was calculated and the corresponding molar ratios were determined from standard curves prepared by mixing weighed amounts of natural and labeled glycerol and injected before plasma samples. Enrichment as low as 0.4% could be accurately measured. The intraassay coefficient of variation was 5.6 ± 1.6% and the interassay coefficient of variation was 7.4 ± 1.9%.

For the determination of the concentration and enrichment of plasma palmitate, known amounts of heptadecanoic acid were added to plasma before extraction of nonesterified fatty acids as described by Bougneres and Bier (21). The extracts were dried over $N_2$ and the *t*-butyldimethylsilyl derivatives of NEFA (22) were prepared by adding 20 μl of pyridine and 20 μl of N-methyl-N (*t*-butyldimethylsilyl)trifluoroacetamide (Regis Chemical Company, Morton Grive, IL) at room temperature. Gas-liquid chromatographic resolution was obtained on a 25-m fused silica capillary column (OV 1701) maintained at 220°C. Fragmentation was obtained by electron impact and ions of m/z 313 and 314 (natural and labeled palmitate) and 327 (heptadecanoate) were monitored. Palmitate enrichment and concentration were determined respectively from the peak area ratios 314/313 and 313/327 and from corresponding standard curves.

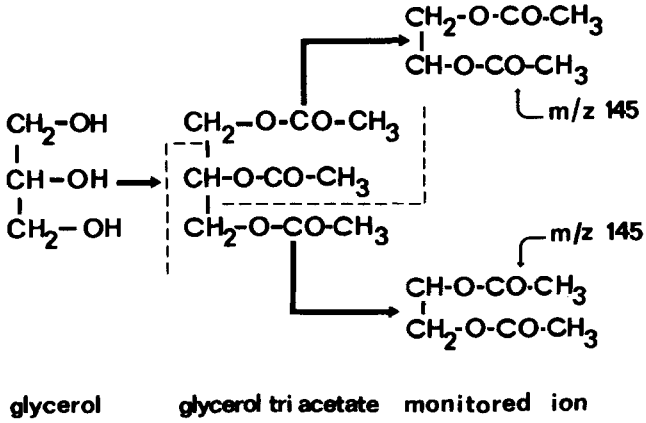


**Fig. 1.** Fragmentation scheme of the triacetate ester of glycerol. The fragmentation between carbons 1 and 2 or 2 and 3 of glycerol gives two symmetrical fragments of m/z 145 for natural glycerol. For [1,2,3-$^2H_5$]glycerol, there is a loss of two deuterium atoms during this fragmentation and the fragments monitored have an m/z ratio of 148.

## Calculations

*Appearance rate in the postabsorptive state.* Since stable levels of concentration and enrichment were obtained, the appearance rates were calculated using formula for steady-state conditions:

$$Ra = \frac{F}{MR} \qquad Eq.\ 1)$$

where F is ithe infusion rate of the tracer and MR is the molar ratio in plasma of labeled to natural glycerol or palmitate. Total NEFA appearance rate was calculated by multiplying the palmitate appearance rate by the ratio of total NEFA concentration/palmitate concentration since palmitate is a representative tracer for circulating fatty acids as a group (23).

*VD of glycerol.* The concentration of plasma [1,2,3-$^2H_5$]glycerol attained after the bolus injection was determined from the concentration of total plasma glycerol and the glycerol enrichment. A sum of two exponential functions was fitted to the data:

$$C[1,2,3\text{-}^2H_5]\text{glycerol} = A_1e^{-\alpha 1t} + A_2e^{-\alpha 2t} \qquad Eq.\ 2)$$

where C is the concentration of labeled glycerol, t is the time, $A_1$ and $A_2$ are the intercepts ($\mu mol \cdot l^{-1}$) at zero time, and $\alpha 1$ and $\alpha 2$ are the rate constants. From this equation it is possible to calculate the VD of glycerol by dividing the dose of tracer injected by the extrapolated concentration of [1,2,3-$^2H_5$]glycerol at zero time. Knowing the appearance rate, the VD, and the concentration of glycerol, it is then possible to calculate the pool of glycerol (M = VD × C), the fractional turnover rate (K = Ra/m), the mean transit time (t = M/Ra) and the half-life time of plasma glycerol ($t_{1/2}$ = 0.693/K).

*Appearance rate of glycerol in the nonsteady-state condition.* As did previous investigators (6.9-11, 13), we calculated the Ra of glycerol by the modified Steele equation:

$$Ra = \frac{F - [pV(C1 + C2)/2]\,[(E2 - E1)/(t2 - t1)]}{(E2 + E1)/2} - F \qquad Eq.\ 3)$$

where C2 and C 1 are the concentrations of plasma glycerol at sampling times t2 and t1, E2 and E1 are the corresponding enrichments of plasma glycerol in tracer (ratio of tracer present in plasma to total tracer plus natural glycerol). Vd is the volume of distribution and p is the "functional pool fraction." To determine the optimal pool fraction, Ra was calculated in each test using a series of p values from 0.4 to 1.6 in increments of 0.1. The optimal pool fraction was determined as the p value that provided the best fit of calculated and expected Ra values.

The data are shown as individual values or mean and standard error of the mean (SEM). Comparisons were performed using Student's *t* test for nonpaired data.

## RESULTS

### Appearance rate of glycerol in the postabsorptive state

Reasonably stable levels of glycerol concentrations and enrichments were obtained in the normal and insulin-dependent subjects. The appearance rate of glycerol was 2.22 ± 0.20 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the control subjects and 2.48 ± 0.29 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in the diabetic patients, a value slightly but not significantly higher than in the normal subjects (**Table 1** and **Table 2**). There was in the whole group of subjects a positive linear relationship between the appearance rate and the concentration of glycerol ($y = 0.0223 x + 1.167$, $r = 0.6095$, $P < 0.01$). The plasma clearance rate of glycerol was comparable in the control (51.0 ± 6.4 $ml \cdot kg^{-1} \cdot min^{-1}$) and the diabetic subjects (41.2 ± 3.6 $ml \cdot kg^{-1} \cdot min^{-1}$) (Tables 1 and 2).

In the five subjects where the turnover rates of glycerol and of NEFA were determined simultaneously, the ratio Ra FFA/Ra glycerol ranged from 1.95 to 3.40 (**Table 3**).

### Volume of distribution of glycerol

In all the subjects studied, the enrichment of plasma glycerol could be followed from 1 min to 25 min (**Fig. 2**). In order to obtain a measurable enrichment level at the last sampling times, it was necessary to inject a relatively large amount of labeled glycerol which significantly increased the glycerol concentration during the first 5 to 8 min of the test (**Fig. 3**). Therefore, for each sample we calculated the concentration of labeled glycerol that was attained (**Fig. 4**). There was a remarkable agreement between these values of labeled glycerol and the increase over basal values of the concentration of total glycerol. NEFA levels were not modified by the bolus injection of deuterated glycerol. The evolution of the concentration of labeled glycerol after the injection could be fitted to a sum of two exponentials (Fig. 4). The VD of glycerol ranged from 0.22 to 0.41 $l \cdot kg^{-1}$ (mean 0.306) in the control subjects and from 0.21 to 0.42 $l \cdot kg^{-1}$ (mean 0.308) in the diabetic patients. Thus, there was a good agreement between the results obtained in these two groups of subjects (Tables 1 and 2).

Knowing the turnover rate and the VD of glycerol we calculated the pool, the mean transit time, the fractional turnover rate, and the half-life time of glycerol in normal and diabetic subjects (Tables 1 and 2). For the five subjects in which the VD of glycerol was not determined, we assumed for the calculation this VD to be equal to 0.31 $l \cdot kg^{-1}$. The results obtained with this value were in agreement with those obtained in the eight other control subjects. Although the $\bar{t}$ and $t_{1/2}$ were slightly increased and the fractional turnover rate was slightly lower in the diabetic patients, there were no significant differences between the normal subjects and the diabetic patients.

TABLE 1. Concentrations of glucose, NEFA, and glycerol and data on glycerol metabolism determined in the postabsorptive state in 13 normal subjects

| Subjects | Glucose | NEFA | Glycerol | VD | Ra | P.CR | $\bar{t}$ | k | $t_{1/2}$ |
|---|---|---|---|---|---|---|---|---|---|
| | $mmol \cdot l^{-1}$ | $\mu mol \cdot l^{-1}$ | | $l \cdot kg^{-1}$ | $\mu mol \cdot kg^{-1} \cdot min^{-1}$ | $ml \cdot kg^{-1} \cdot min^{-1}$ | min | | min |
| 1 | 4.1 | 650 | 46 | 0.411 | 3.36 | 73.0 | 5.72 | 0.1746 | 3.97 |
| 2 | 3.5 | 621 | 58 | 0.363 | 2.38 | 40.4 | 8.97 | 0.1115 | 6.22 |
| 3 | 4.0 | 625 | 46 | 0.275 | 3.04 | 66.1 | 4.23 | 0.2362 | 2.94 |
| 4 | 4.1 | 331 | 18 | 0.308 | 1.32 | 79.3 | 4.20 | 0.2380 | 2.90 |
| 5 | 3.9 | 492 | 36 | 0.237 | 2.10 | 58.2 | 3.80 | 0.2583 | 2.70 |
| 6 | 4.3 | 663 | 61 | 0.240 | 2.95 | 48.2 | 4.92 | 0.2034 | 2.41 |
| 7 | 4.1 | 320 | 48 | 0.240 | 2.20 | 45.8 | 5.24 | 0.1908 | 3.63 |
| 8 | 3.6 | 558 | 49 | 0.367 | 2.44 | 49.8 | 7.40 | 0.1351 | 5.13 |
| | | | | 0.306 ± 0.024[b] | | | 5.56 ± 0.63[b] | 0.1935 ± 0.0181[b] | 3.86 ± 0.43[b] |
| 9[a] | 4.2 | 495 | 43 | | 2.60 | 61.0 | 4.23 | 0.236 | 2.94 |
| 10[a] | 4.1 | 365 | 48 | | 1.40 | 29.2 | 8.85 | 0.113 | 6.13 |
| 11[a] | 3.9 | 424 | 55 | | 1.79 | 32.5 | 7.97 | 0.1255 | 5.52 |
| 12[a] | 4.3 | 581 | 58 | | 1.81 | 31.2 | 8.39 | 0.1192 | 5.81 |
| 13[a] | 4.1 | 383 | 31 | | 1.49 | 47.5 | 5.41 | 0.1886 | 3.67 |
| | 4.02 ± 0.07 | 501 ± 35 | 46 ± 5.3 | | 2.22 ± 0.20 | 51.0 ± 6.4 | 5.10 ± 0.86 | 0.1792 ± 0.0239 | 4.23 ± 0.60 |

VD, volume of distribution; Ra, appearance rate; PCR, plasma clearance rate; $\bar{t}$, mean transit time, k, fractional turnover rate, $t_{1/2}$, half-life time.
[a]For these 5 subjects, t, k, and $t_{1/2}$ were calculated assuming a value of 0.31 $l \cdot kg^{-1}$ for the VD of glycerol.
[b]Mean ± SEM for n = 8; all other results are calculated for the whole group of 13 subjects.

TABLE 2. Concentration of glucose, NEFA, and glycerol and data on glycerol kinetics determined in the postabsorptive state in five insulin-dependent diabetic subjects

| Subject | Glucose | NEFA | Glycerol | VD | Ra | PCR | $\bar{t}$ | k | $t_{1/2}$ |
|---|---|---|---|---|---|---|---|---|---|
| | $mmol \cdot l^{-1}$ | $\mu mol \cdot l^{-1}$ | | $l \cdot kg^{-1}$ | $\mu mol \cdot kg^{-1} \cdot min^{-1}$ | $ml \cdot kg^{-1} \cdot min^{-1}$ | min | | min |
| 1 | 7.00 | 710 | 70 | 0.328 | 2.50 | 35.7 | 8.99 | 0.1112 | 6.23 |
| 2 | 12.1 | 790 | 98 | 0.210 | 3.61 | 36.8 | 5.82 | 0.1719 | 4.03 |
| 3 | 9.3 | 740 | 66 | 0.284 | 2.25 | 34.1 | 8.33 | 0.1200 | 5.77 |
| 4 | 15.1 | 850 | 41 | 0.42 | 2.05 | 50.0 | 8.40 | 0.1191 | 5.82 |
| 5 | 10.6 | 680 | 40 | 0.299 | 2.00 | 50.0 | 5.78 | 0.1730 | 4.00 |
| Mean ± SEM | 10.8 ± 1.4[b] | 754 ± 30[b] | 63.4 ± 11.0 | 0.308 ± 0.031 | 2.48 ± 0.29 | 41.2 ± 3.6 | 7.46 ± 0.69 | 0.1390 ± 0.0137 | 5.17 ± 0.46 |

VD, volume of distribution; Ra, appearance rate; PCR, plasma clearance rate; $\bar{t}$, mean transit time, k, fractional turnover rate, $t_{1/2}$, half-life time.
[a] Significantly different from the values for control subjects, $P < 0.001$.
[b] Significantly different from the values for control subjects, $P < 0.01$.

TABLE 3. Appearance rate of NEFA and of glycerol determined simultaneously in the postabsorptive state in five normal subjects

| NEFA Ra | Glycerol Ra | NEFA Ra/Glycerol Ra |
|---|---|---|
| $\mu mol \cdot kg^{-1} \cdot min^{-1}$ | | |
| 5.07 | 2.60 | 1.95 |
| 4.04 | 1.81 | 2.23 |
| 3.42 | 1.40 | 2.44 |
| 5.40 | 1.79 | 3.02 |
| 5.08 | 1.49 | 3.40 |

### Appearance rate of glycerol in nonsteady-state conditions

During the infusion of exogenous glycerol, plasma glycerol concentration remained within the range of values usually found in adult humans (**Fig. 5, Table 4**). Glucose, NEFA, or IRI concentrations were not modified (Table 4). Assuming that endogenous glycerol appearance rate was not modified by the infusion of exogenous glycerol (an assumption supported in the present results by the absence of variation of either IRI or NEFA levels), we determined the functional pool fraction of glycerol as the value that provided the best fit between the calculated and the expected (sum of basal endogenous production and of exogenous infusion rates) appearance rates of glycerol. The pool fraction obtained was 1.13 ± 0.10, a value not significantly different from 1. The functional VD of glycerol obtained (0.331 ± 0.017 $l \cdot kg^{-1}$) was not different from the value obtained from the results of the bolus injection (**Table 5**).

During this test, the expected Ra values were successively 5.03 ± 0.33, 7.48 ± 0.39, 9.94 ± 0.34, 7.48 ± 0.33, and 5.03 ± 0.33 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, whereas the isotopically determined values for Ra were at the end of each infusion step 4.62 ± 0.45, 6.95 ± 0.56, 10.85 ± 0.51, 7.35 ± 0.34, and 5.28 ± 0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Examination of the individual values showed a highly significant relationship between the expected and the calculated values ($r = 0.89$, $P < 0.001$, $y = 0.993\ x - 0.1234$).

## DISCUSSION

Our aim was to validate a simple, reliable, and ethically acceptable method of determining glycerol appearance rate in steady-state and nonsteady-state conditions in human beings. In spite of the more important potential risks of isotopic effects with deuterium than with $^{13}C$, we decided to use deuterium-labeled glycerol since the high cost of $^{13}C$-labeled glycerol would at the present time limit its use, at least in adult subjects. Absolute validation of the use of [1,2,3-$^2H_5$]glycerol as tracer in human beings would have needed a direct comparison of the results with values obtained in the same subjects with radioactive tracers.

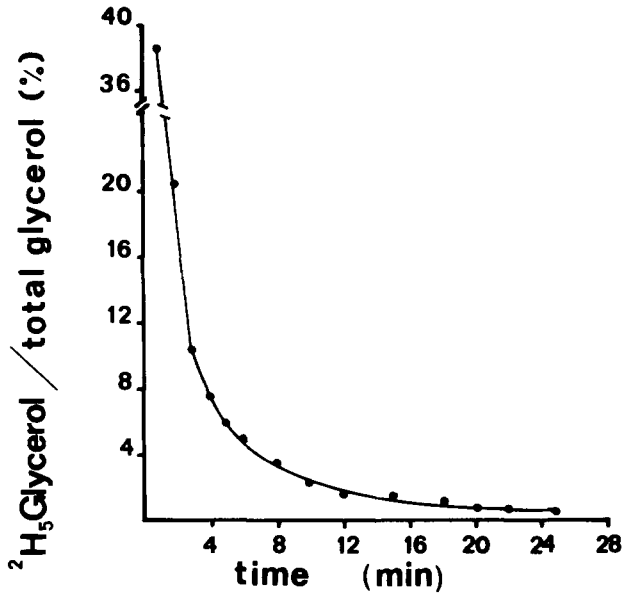


**Fig. 2.** The ratio of [1,2,3-$^2H_5$]glycerol/total glycerol after the bolus injection of labeled glycerol in one subject.

Such comparison was not possible since the use of radioactive tracers for metabolic studies in humans is not authorized in France. However, the values that we obtained are consistent with previous results obtained with radioactive tracers. Using the data of Björntorp et al. (12) ([2-$^3$H]glycerol) and Bortz et al. (1) ([$^{14}$C]glycerol), the mean values of glycerol Ra were, respectively, 1.80 and 1.50 $\mu$mol · kg$^{-1}$ · min$^{-1}$. Moreover, the ratios of NEFA Ra/glycerol Ra that we found in five subjects are consistent with results obtained in humans (12, 13) or animals (9, 13) with radioactive tracers. Lastly, since the results obtained in rats with [1,2,3-$^2H_5$]glycerol or with [2-$^{13}$C]glycerol were comparable, there is no evidence that labeling glycerol with as many as five deuterium atoms results in a significant in vivo isotopic effect.

For the determination of glycerol kinetics in nonsteady-state conditions, we have used a one-pool model with a correction for the rapidly mixing fraction of the pool. This approach is comparable to that previously used with radioactive glycerol (6, 9–11, 13) and has been previously validated for other metabolites such as glucose (24) or ketone bodies (25, 26). In this model the determination of Ra is dependent on the VD value used for the calculations. With the exception of Shaw, Issekutz, and Issekutz (6, 9), who used a value of 0.325 l · kg$^{-1}$, this VD of glycerol was assumed to be 0.65 l · kg$^{-1}$, a value near to the total body water volume. The choice of this value was based on the experiments of Larsen (27) in cats, Holst (28) in rats, and of Shafrir and Gorin (29) in man and rabbit. In these studies massive loads of natural glycerol were administered resulting in considerable elevation of plasma glycerol far above the physiological range. As pointed out by Wade (30), such massive loads of glycerol may have disrupted normal gradients of glycerol concentrations in the tissue and saturated the metabolic pathways of glycerol disposal, leading thus to erroneous results. Using [2-$^3$H]glycerol as a tracer and avoiding these possible artefacts, Wade (30) found the VD of glycerol in rabbit to be 0.341 ± 0.220 l · kg$^{-1}$ instead of 0.65 as found by Shafrir and Gorin (29) who used massive loads of glycerol. In the present experiments the variations of plasma glycerol induced by the bolus injection of deuterated glycerol were small and within the physiological range. In these conditions we found the VD of glycerol to be 0.306 l · kg$^{-1}$ in normal subjects, a value in fair agreement with the results of Wade (30). Moreover, in the experiments with staged infusion of natural glycerol, we found a good agreement between the expected Ra and the isotopically determined Ra of glycerol when using a VD value (mean) of 0.33 l · kg$^{-1}$, whereas a VD of 0.65 l · kg$^{-1}$ would have led to an overestimate of the variations of glycerol Ra. In these experiments we assumed that the release of endogenous glycerol was not depressed by the infusion of exogenous glycerol. There is experimental support for that assumption (14) and, in the present work, the lack of variation of NEFA is an additional support. In any case, if some depression of endogenous glycerol release occurred during the infusion of exogenous glycerol, the overestimate of the variations of glycerol Ra would be even greater if one used a VD of 0.65 l · kg$^{-1}$, thus detracting even more from the usefulness of this value.

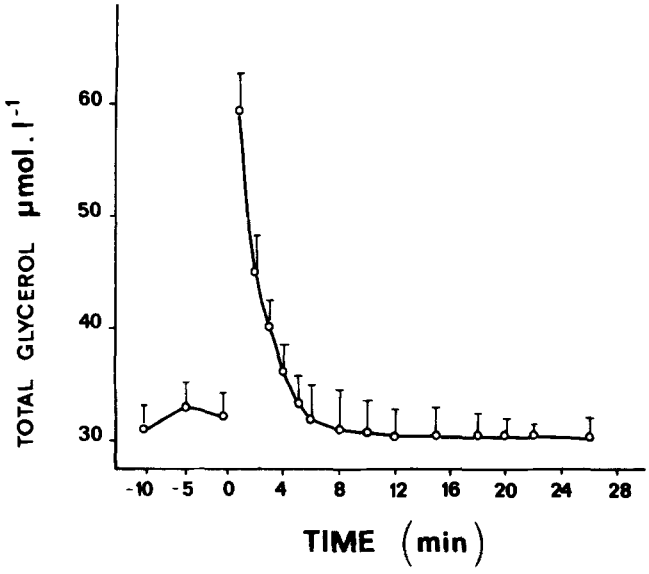


**Fig. 3.** Concentration of total plasma glycerol before (−15 min to 0 time) and after (1 to 25 min) the bolus injection of [1,2,3-$^2H_5$]glycerol at time 0.

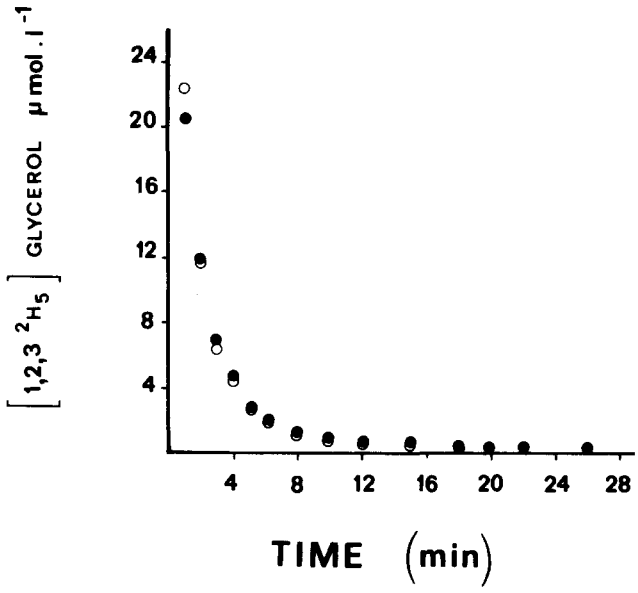


**Fig. 4.** Concentration of [1,2,3-$^2H_5$]glycerol after the bolus injection in one subject. The experimental data (○) were fitted to a sum of two exponentials ($y = 34.0e^{-0.6443t} + 3.0e^{-0.1293t}$); (●) are the points recalculated from the equation.

The mean transit time and half-life time of glycerol that we found in normal subjects are lower than previously reported (13, 31), whereas the fractional turnover rate that we found is higher (13, 31). This is not surprising since such results are also dependent on the VD value used in the calculation. Previous authors used a VD of 0.65

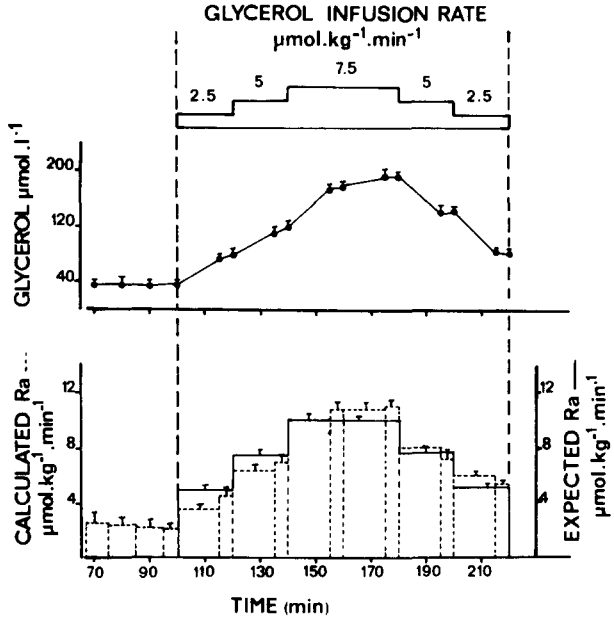


**Fig. 5.** Concentration and the isotopically determined appearance rate (calculated Ra, -----) of glycerol during the infusion of natural glycerol in six normal subjects.

TABLE 4. Concentrations of glycerol, NEFA, glucose, and immunoreactive insulin (IRI) during the infusion of natural glycerol in six normal subjects

| | | During Exogenous Glycerol Infusion | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2.5 µmol · kg$^{-1}$ · min$^{-1}$ | | 5.0 µmol · kg$^{-1}$ · min$^{-1}$ | | 7.5 µmol · kg$^{-1}$ · min$^{-1}$ | | | | 5.0 µmol · kg$^{-1}$ · min$^{-1}$ | | 2.5 µmol · kg$^{-1}$ · min$^{-1}$ | |
| | 70–100 min | 115 min | 120 min | 135 min | 140 min | 155 min | 160 min | 175 min | 180 min | 195 min | 200 min | 215 min | 220 min |
| Glycerol (µmol · l$^{-1}$) | 39 ± 7[a] | 71 ± 7 | 78 ± 6 | 109 ± 10 | 120 ± 9 | 171 ± 8 | 178 ± 7 | 192 ± 11 | 195 ± 6 | 142 ± 12 | 145 ± 11 | 102 ± 7 | 98 ± 4 |
| NEFA (µmol · l$^{-1}$) | 586 ± 64 | 670 ± 102 | 663 ± 94 | 601 ± 66 | 582 ± 59 | 579 ± 59 | 623 ± 57 | 595 ± 73 | 610 ± 73 | 549 ± 40 | 553 ± 52 | 573 ± 62 | 569 ± 78 |
| Glucose (µmol · l$^{-1}$) | 3.95 ± 0.23 | 3.93 ± 0.27 | 3.78 ± 0.38 | 3.63 ± 0.17 | 3.90 ± 0.38 | 3.97 ± 0.31 | 4.05 ± 0.30 | 4.13 ± 0.21 | 3.88 ± 0.18 | 4.05 ± 0.23 | 3.93 ± 0.24 | 3.98 ± 0.25 | 3.95 ± 0.22 |
| IRI (mU · l$^{-1}$) | 8.0 ± 0.8 | | 8.6 ± 1.1 | | 8.8 ± 1.0 | | 8.0 ± 1.0 | | 9.0 ± 1.3 | | 8.2 ± 0.9 | | 8.2 ± 0.9 |

[a] Values are means ± SEM

TABLE 5. Volume of distribution (VD) and pool fraction (p) or glycerol in six normal subjects

| | Subjects | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± SEM |
| VD | 0.410 | 0.363 | 0.275 | 0.308 | 0.237 | 0.240 | 0.303 ± 0.025 |
| p | 0.6 | 1.1 | 1.2 | 1.1 | 1.4 | 1.4 | 1.13 ± 0.10 |
| VDf | 0.25 | 0.40 | 0.330 | 0.339 | 0.322 | 0.336 | 0.331 ± 0.019 |

[a] VDf, functional volume of distribution.

$l \cdot kg^{-1}$. Recalculating their data using a VD of 0.31 $\mu mol \cdot kg^{-1}$ gave results nearer to ours.

Finally, we have determined the VD and the Ra of glycerol in five insulin-dependent diabetic patients under moderate metabolic control. We found a VD of 0.308 $l \cdot kg^{-1}$, a value nearly identical to that found in control subjects; we think that such a value will be appropriate for the determination of nonsteady-state glycerol kinetics in diabetic patients. In diabetic patients in the postabsorptive state, we found results comparable to those of control subjects, although the appearance rate and the half-life time were slightly increased and the fractional turnover rate was lower. To our knowledge, the only other study of glycerol turnover in diabetic patients is that of Pelkonen, Nikkila, and Kekki (31). These authors found an increase half-life time and a decreased fractional turnover rate of glycerol in diabetic patients. However, their diabetic subjects were in a worse metabolic state than our diabetic patients as evidenced by their higher NEFA and glycerol levels. Moreover, Pelkonen et al. (31) determined glycerol kinetics by the use of massive loads of natural glycerol and, as discussed above, their results should thus be interpreted cautiously.

In conclusion, the present study shows that [1,2,3-$^2H_5$]glycerol is a useful tracer for the determination of glycerol kinetics in humans under both steady-state and nonsteady-state conditions.

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