Are Peristaltic Pumps as Reliable as Syringe Pumps for Metabolic Research? Assessment of Accuracy, Precision, and Metabolic Kinetics

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Syringe pumps are traditionally used to infuse tracers in metabolic research because they are perceived to be more accurate and precise than peristaltic pumps. This study evaluated the accuracy (actual *v* programmed infusion rate) and precision (reproducibility of infusion) of a peristaltic pump (Gemini PC 2; IMED, San Diego, CA) and a syringe pump (Model 22; Harvard Apparatus, Natick, MA) for metabolic research. In one protocol, saline delivery was measured in vitro in 5 trials at 4 flow rates: 3, 30, 150, and 300 mL/h. In the second protocol, basal glycerol rate of appearance (Ra) was determined in vivo in 5 women on 2 consecutive days. On day 1, [2-^{13}C]glycerol was infused with 1 pump and [1,1,2,3,3-$^{2}H_5$]glycerol with the other. On day 2, the opposite pattern was used. The accuracy of the 2 pumps was the same (error ~2%). In addition, both the syringe and the peristaltic pumps were very precise, with coefficients of variation (CV) <1% at all flow rates. Glycerol Ra values were the same when tracer was infused with either a syringe or peristaltic pump on day 1 and day 2: 4.1 ± 1.7 (syringe pump) and 4.2 ± 1.9 (peristaltic pump) μmol · kg fat mass (FM)$^{-1}$ · min^{-1} on day 1; 4.2 ± 1.2 (syringe pump) and 4.2 ± 1.3 (peristaltic pump) μmol · kg FM^{-1} · min^{-1} on day 2. These data demonstrate that both syringe and peristaltic pumps are very accurate and precise across a large range of flow rates. Moreover, the assessment of in vivo substrate kinetics in human subjects is the same when either pump is used to infuse isotope tracers.

ISOTOPE TRACER dilution techniques are often used to assess substrate kinetics (ie, substrate rate of appearance [Ra]) into the bloodstream and substrate rate of disappearance (Rd) from the bloodstream) in vivo. This approach involves measuring the dilution in plasma of a labeled substrate tracer that has been infused into the systemic circulation. Both radioactive and stable isotope labeled tracers, including glycerol,[1,2] fatty acids,[3,4] glucose,[5,6] and amino acids[7,8] have been used to determine substrate kinetics in human subjects. Accurate calculation of substrate Ra and substrate Rd requires the use of a reliable infusion pump and knowing the exact rate of tracer infusion.[9]

Traditionally, syringe pumps have been used to infuse tracers in metabolic studies; these pumps are considered by many investigators to be the "gold standard" for achieving reliable infusion rates. Syringe pumps contain a small-angle stepping motor that drives a lead screw to progressively compress the syringe plunger, and thereby smoothly delivers a precise volume of the tracer solution per unit of time. However, there are several limitations in using these pumps for research studies conducted in human subjects. First, syringe pumps cannot deliver large volumes of fluid (usual maximum of 60 mL for a single syringe pump and 360 mL for a multiple-syringe pump), unless many pumps are used simultaneously or the syringe is refilled or replaced during an infusion study. Refilling or replacing syringes during a study can be difficult, because of the potential for disturbing isotopic steady state and the considerable time and effort needed to fill syringes for large volume infusion studies, such as a high-dose hyperinsulinemic clamp procedure.[10] Second, syringe pumps do not contain a pressure monitoring system. Thus catheter migration into subcutaneous tissue or partial flow obstruction may not be noticed until visible tissue swelling is present or infusate flow is completely stopped. Third, syringe pumps are not approved by the Food and Drug Administration (FDA) for use in human subjects, and most pump manufacturers explicitly state that their pumps are not intended for use in human subjects to avoid regulatory requirements. Although, syringe pumps have been widely used in many research studies without adverse effects, the lack of FDA certification and hospital approval can make it difficult to get permission to use syringe pumps for patient-oriented research.

Peristaltic pumps are commonly used to infuse medications, fluids, and nutrients in outpatient and inpatient clinical settings. These pumps contain a rotating rollbar, which intermittently compresses the infusion tubing generating a controlled pulsatile flow. The advantages of peristaltic pumps are that they are readily available to investigators working in a clinical environment, allow the delivery of large fluid volumes, contain a pressure monitoring system that allows the investigator to detect early changes in obstruction of flow, and are approved for use in patients by the FDA and hospital administrative boards. Nonetheless, these pumps are not usually used in metabolic research because of the perception that the pulsatile flow can affect the assessment of substrate kinetics, and peristaltic pumps do not infuse solutions as accurately and precisely as syringe pumps.

The purpose of the present study was to evaluate the suitability of using a peristaltic pump for metabolic studies by comparing the performance of a commonly available peristaltic pump with that of a standard syringe pump. In vitro studies were performed to determine the accuracy (volume delivered relative to target volume) and precision (reproducibility of

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infusion volumes) of these pumps across a range of infusion rates commonly used in metabolic studies. In vivo studies were performed to determine the calculated Ra value for glycerol, a rapidly turning over substrate that demonstrates minute-to-minute variability in Ra, when the peristaltic and syringe pumps were used to infuse labeled glycerol in human subjects.

RESEARCH DESIGN AND METHODS

In Vitro Study to Assess Accuracy and Precision

A syringe (Model 22; Harvard Apparatus, Natick, MA) and a peristaltic (Gemini PC 2; IMED, San Diego, CA) pump were used to deliver a 0.9% NaCl solution into paraffin-covered, preweighed containers at each of 4 flow rates: 3, 30, 150, and 300 mL/h. These rates were chosen to span the range of rates often used in metabolic research (eg, from a very low tracer infusion rate to a very high infusion rate required to infuse dextrose spiked with tracer during the hyperinsulinemic-euglycemic clamp procedure). In all trials, standard infusion tubing and a 0.2-μm inline filter were used to mimic typical experimental conditions of studies performed in human subjects. A constant flow was achieved before the collection of saline was started.

To evaluate the syringe pump, 5 trials were performed at each flow rate on separate days. Sixty milliliter syringes (internal diameter, 26.7 mm; Becton Dickinson, Franklin Lakes, NJ) were filled with saline to the 50 mL mark for all trials. One syringe held the volume infused for 1 hour for flow rates of 3 mL/h and 30 mL/h, whereas 3 and 6 syringes were needed to hold the volume infused for 1 hour at flow rates of 150 mL/h and 300 mL/h, respectively. The syringe pump was also tested with a single syringe at high flow rates, by infusing at 150 mL/h and 300 mL/h for 20 and 10 minutes, respectively.

To evaluate the peristaltic pump, 5 trials were performed at each flow rate on separate days. The proximal tubing was inserted into a standard 1 L polyvinyl chloride bag containing a 0.9% NaCl solution. The peristaltic pump was also tested by connecting the proximal tubing to a single 60-mL syringe filled with 0.9% NaCl solution and infusing for 1 hour at 3 mL/h and 30 mL/h, for 20 minutes at 150 mL/h, and for 10 minutes at 300 mL/h.

In Vivo Study to Assess Substrate Kinetics

Five women, 2 lean and 3 obese, (age, 30 $\pm$ 8 years; body mass index, 32 $\pm$ 9 kg/m^2; and 66% $\pm$ 19% fat-free mass [FFM], determined by dual energy x-ray absorptiometry [Hologic QDR 1000/W, Waltham, MA]) participated in this study. All subjects were considered healthy, except for obesity, after completing a comprehensive medical examination, which included a history and physical examination, a 12-lead electrocardiogram, and standard blood tests. No subject was taking regular medications or smoked tobacco. Written informed consent was obtained from all subjects before their participation in the study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine in St. Louis, MO.

Subjects were admitted to the inpatient unit of the GCRC for 2 nights and 2 days. At 6 PM on the day of admission, subjects were fed a standard meal (55% carbohydrate, 30% fat, and 15% protein) containing 12 kcal/kg body weight for lean subjects (n = 2) and 12 kcal/kg adjusted body weight for obese subjects (n = 3). Adjusted body weight was calculated as ideal body weight + [(actual body weight $-$ ideal body weight) $\times$ 0.25]; ideal body weight was based on the medium-frame weight for height of the Metropolitan Life Insurance Table.[11] At 8 PM, subjects ingested a liquid formula (Ensure; Ross Laboratories, Columbus, OH) containing 40 g of carbohydrates, 6 g of fat, and 9 g of protein and then fasted until completion of the first isotope infusion study the next day. On the morning of the study, 20-gauge catheters were placed in a forearm vein for isotope infusion and in a hand vein for blood sampling; the hand was heated to 55°C for 20 minutes before sample collection to obtain arterialized blood.[12] Catheters were kept patent by slow, controlled infusion of 0.9% NaCl solution (20 mL/h). At 8 AM, primed (1.2 μmol/kg) constant (0.08 μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}) infusions of [1,1,2,3,3-$^2H_5$]glycerol and [2-^{13}C]glycerol (99% atoms percent excess; Cambridge Isotopes, Andover, MA) dissolved in 0.9% NaCl solution were started and maintained for 150 minutes. Subjects remained in bed for the entire duration of the isotope infusion. The type of pump (syringe or peristaltic) used to infuse each tracer for the first infusion study was randomly assigned. The type of pump used to infuse each tracer was reversed for the second infusion study, performed the following morning.

Blood samples were obtained before tracer infusion to determine background enrichment (tracer-to-tracee ratio [TTR]), and every 10 minutes from 9:50 AM to 10:30 AM (ie, 110 minutes to 150 minutes of labeled glycerol infusion) to determine glycerol TTR during physiologic and isotopic steady-state conditions. Blood samples were collected in chilled tubes containing EDTA and placed on ice. Plasma was separated by centrifugation within 30 minutes of collection and stored at $-$70°C until analyzed for glycerol TTR as previously described.[13] After the last blood sample was obtained, the tracer infusion was stopped, catheters were removed, and lunch was served. Subjects were then allowed to move around the GCRC freely or go home for the remainder of the day. They then returned to the GCRC in the evening, the same evening meal and snack consumed the previous day were provided, and the isotope infusion protocol was repeated the next day.

Calculations

In vitro study. For each infusion trial, actual flow rate was determined by dividing the volume of 0.9% NaCl solution in the collection container (calculated as the mass of 0.9% NaCl solution, determined to the nearest 0.001 g, divided by the density of 0.9% NaCl solution at 20°C [ie, 1.007 g/mL]) by the duration of the infusion. The accuracy of each pump was determined by comparing the actual flow rate with the theoretical, programmed flow rate [(actual infusion rate–programmed infusion rate)/programmed infusion rate $\times$ 100%). Precision was assessed as the CV of the calculated flow rate for each of the 5 trials at each flow rate.

In vivo study. Steele's equation for steady-state conditions[9] was used to calculate whole-body glycerol kinetics. Glycerol Ra was calculated by dividing the rate of glycerol tracer infusion (in μmol $\cdot$ kg fat mass [FM]$^{-1}$ $\cdot$ min^{-1}) by the average glycerol TTR in plasma during the last 40 minutes of tracer infusion when isotopic equilibrium was achieved.

Statistical Analyses

The in vitro accuracy of the pumps was compared using a 2-way (flow rate $\times$ pump) analysis of variance (ANOVA), whereas precision was assessed using a *t* test. In vivo data were analyzed using a 2-way (day $\times$ pump) ANOVA with repeated measures to test for significant differences in glycerol Ra. Any significant *F* ratios from the ANOVA were followed with Tukey's post hoc analysis. Data are reported as mean $\pm$ standard deviation (SD) and statistical significance was accepted at a *P* value $\leq$ .05.

RESULTS

In Vitro Study

Infusion rate accuracy of the peristaltic and syringe pumps was the same at 3, 30, 150, and 300 mL/h flow rates (Fig 1). Both pumps delivered approximately 98% of programmed value. Infusion rate precision was high, and infusion rate CVs were less than 1% for both pumps (Fig 2). Similar syringe

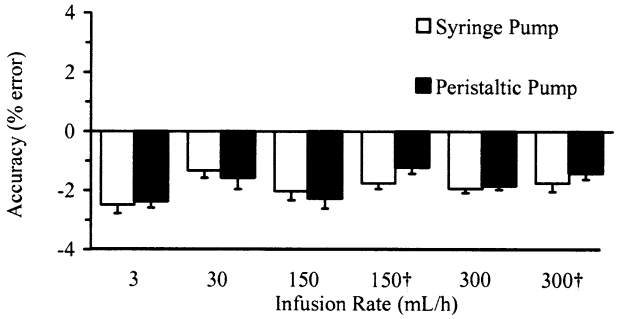

Fig 1. Accuracy [(actual infusion rate–programmed infusion rate)/programmed infusion rate × 100%] of syringe (white bars) and peristaltic (black bars) pumps. Data are mean ± SD. †Single syringe in syringe pump.

pump infusion accuracy and precision values were obtained when either single or multiple-syringes were used to infuse the 0.9% NaCl solution at 150 mL/h and 300 mL/h (Figs 1 and 2). Infusion accuracy and precision were the same when the peristaltic pump was connected to either a polyvinyl chloride bag or a syringe infusate reservoir. The accuracy when a syringe was used as a reservoir was within 2% of the programmed value at all flow rates (−1.16% ± 0.30% at 3 mL/h, −1.39% ± 0.27% at 30 mL/h, −1.25% ± 0.10% at 150 mL/h, and −1.52% ± 0.38% at 300 mL/h). The infusion rate CVs were less than 1% at all flow rates (0.30%, 0.27%, 0.10%, and 0.39% for 3, 30, 150, and 300 mL/h flow rates, respectively).

In Vivo Study

Plasma [$^2H_5$]glycerol and [^{13}C]glycerol TTRs were the same in each of the 5 subjects during the same infusion study. Therefore, glycerol Ra was the same when calculated by using either the [$^2H_5$]glycerol or [^{13}C]glycerol TTR, even though the 2 tracers were infused with different pumps. On day 1, glycerol Ra was 4.1 ± 1.7 μmol · kg FM^{-1} · min^{-1} (syringe pump) and 4.2 ± 1.9 μmol · kg FM^{-1} · min^{-1} (peristaltic pump). On day 2, glycerol Ra was 4.2 ± 1.2 μmol · kg FM^{-1} · min^{-1} (syringe pump) and 4.2 ± 1.3 μmol · kg FM^{-1} · min^{-1} (peristaltic pump). In individual subjects, glycerol Ra values obtained by using the syringe pump were highly correlated with glycerol Ra values obtained by using the peristaltic pump (Fig 3).

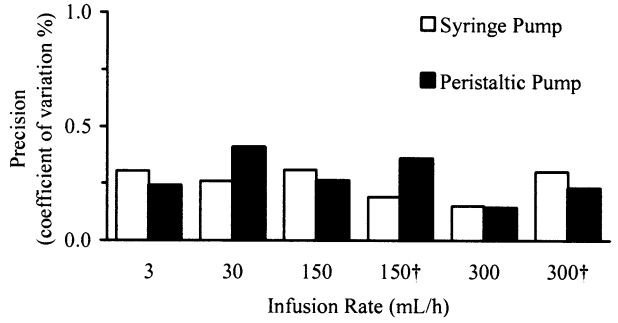

Fig 2. Precision (CV) of actual infusion rate of normal saline infused with syringe (white bars) and peristaltic (black bars) pumps at different programmed infusion rates. †Single syringe in syringe pump.

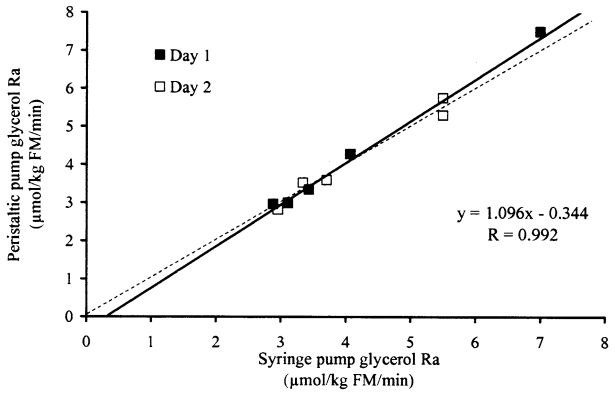

Fig 3. Relationship between glycerol Ra determined by infusing [$^2H_5$]glycerol and [^{13}C]glycerol tracers in individual subjects on 2 consecutive days (solid line). On day 1 (■), 1 tracer, chosen at random, was infused with a syringe pump and the other tracer with a peristaltic pump. On day 2 (□), the specific pump (syringe or peristaltic) used to infuse each tracer was reversed. Broken line represents the line of identity.

DISCUSSION

Infusions of radioactive or stable isotope labeled tracers, substrates, hormones, and pharmacologic agents are often used in experiments evaluating metabolic processes in human subjects. Syringe pumps are usually considered the "gold standard" for such infusion studies because of the belief that they are more accurate and precise than other types of pumps. However, the use of syringe pumps is laborious when large volumes of solutions need to be infused, and these pumps are not approved by the FDA for use in human subjects. Therefore, we evaluated the suitability of using a peristaltic pump, which is commonly used in clinical settings, for metabolic research. The results of our study demonstrated that actual flow rates achieved with a peristaltic pump are both accurate and precise across a large range of flow rates from 3 mL/h to 300 mL/h. Moreover, values obtained for glycerol kinetics in human subjects by using tracer dilution methods were the same when either a peristaltic or syringe pump was used to infuse a stable isotope glycerol tracer.

The results of this study demonstrate that a peristaltic pump is as accurate (ie, relationship between actual and programmed infusion rates) as a syringe pump across a wide range of infusion rates. Both pumps produced flows that were within 2% of that programmed at all rates studied. In addition, the precision (reproducibility of infusion rate) of both the syringe and peristaltic pumps was excellent, with CV values that were less than 1%.

Experiments performed in vivo indicated that the type of pump used to infuse labeled glycerol did not affect measured glycerol kinetics in lean and obese human subjects; glycerol Ra was the same whether a peristaltic or a syringe pump was used to infuse tracer. Moreover, the glycerol Ra value obtained for each subject by using one pump for tracer infusion correlated

closely with the value obtained by using the other pump. We specifically chose to evaluate glycerol kinetics to increase our ability to detect small differences in plasma glycerol TTR and calculated glycerol Ra between pumps. The rapid turnover of glycerol in plasma makes the measured value for glycerol TTR particularly vulnerable to influences from the pulsatile flow of tracer from a peristaltic pump. In contrast, substrates that turn over slowly and have a large pool size, such as glucose, would have made it more difficult to detect differences in plasma substrate TTR between experiments with different pumps.

The results of the in vivo study also demonstrated a remarkable reproducibility of measuring glycerol Ra in an individual subject when diet and physical activity are carefully controlled; glycerol Ra was nearly identical on day 1 and day 2 in each subject, despite a 2-fold range in glycerol Ra values across the entire group. In addition, our data demonstrate there is no physiologic discrimination between the 2 glycerol tracers, as evidenced by the same values for glycerol Ra (and Rd) obtained when using either [$^2H_5$]glycerol or [^{13}C]glycerol tracers (average Ra obtained in our 5 subjects: 4.1 $\pm$ 1.4 and 4.2 $\pm$ 1.5 μmol $\cdot$ kg FM^{-1} $\cdot$ min^{-1} with [$^2H_5$] and [^{13}C]glycerol, respectively).

The present study has several limitations. We only extensively tested 2 pumps, ie, 1 syringe and 1 peristaltic. Therefore, it is possible that we would not have obtained the same results with other brands or models of pumps, or even with other pumps that are identical to those that were tested in this study. However, the pumps that we tested were chosen at random, and we did evaluate other syringe and peristaltic pumps that generated similar accuracy and precision results (data not shown). It is also possible that using glass syringes with a Teflon stopper, rather than plastic syringes with a rubber stopper, would be more accurate and precise because Teflon is less compressible due to pressure build-up from the pump. Plastic syringes were tested because they are far more commonly used in metabolic research. In addition, a 60-mL syringe was the only size tested with the range of flow rates from 3 mL/h to 300 mL/h. A range of syringes, from small to large, eg, 5 mL to 140 mL, with small volume syringes tested at low flow rates and larger volume syringes tested at higher flow rates might yield more accurate results. However, both syringe and peristaltic pumps proved to be very accurate and precise across a wide range of flow rates when using a 60-mL syringe.

In summary, the results of this study demonstrate that the accuracy and precision of syringe and peristaltic infusion pumps are similar. Moreover, the assessment of in vivo substrate kinetics in human subjects is the same when either pump is used to infuse isotope tracers for metabolic studies. Therefore, investigators performing metabolic research should consider using a peristaltic pump when large volumes must be infused or when an FDA-approved medical device must be used. However, our data underscore the importance of calibrating all infusion pumps before their use in metabolic studies to ensure their accuracy at each planned rate of infusion.

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