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Measurement of exogenous carbohydrate oxidation: a comparison of [U-¹⁴C]glucose and [U-¹³C]glucose tracers

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Moseley, L., R. L. P. G. Jentjens, R. H. Waring, R. M. Harris, L. K. Harding, and A. E. Jeukendrup. Measurement of exogenous carbohydrate oxidation: a comparison of [U-¹⁴C]glucose and [U-¹³C]glucose tracers. *Am J Physiol Endocrinol Metab* 288: E206–E211, 2005. First published February 22, 2005; doi:10.1152/ajpendo.00423.2004.—The purpose of this study was to assess the level of agreement between two techniques commonly used to measure exogenous carbohydrate oxidation (CHO_{EXO}). To accomplish this, seven healthy male subjects (24 ± 3 yr, 74.8 ± 2.1 kg, $\dot{V}_{O_{2max}}$ 62 ± 4 ml·kg⁻¹·min⁻¹) exercised at 50% of their peak power for 120 min on two occasions. During these exercise bouts, subjects ingested a solution containing either 144 g glucose (8.7% wt/vol glucose) or water. The glucose solution contained trace amounts of both [U-¹³C]glucose and [U-¹⁴C]glucose to allow CHO_{EXO} to be quantified simultaneously. The water trial was used to correct for background ¹³C enrichment. ¹³C appearance in the expired air was measured using isotope ratio mass spectrometry, whereas ¹⁴C appearance was quantified by trapping expired CO₂ in solution (using hyamine hydroxide) and adding a scintillator before counting radioactivity. CHO_{EXO} measured with [¹³C]glucose ([¹³C]CHO_{EXO}) was significantly greater than CHO_{EXO} measured with [¹⁴C]glucose ([¹⁴C]CHO_{EXO}) from 30 to 120 min. There was a 15 ± 4% difference between [¹³C]CHO_{EXO} and [¹⁴C]CHO_{EXO} such that the absolute difference increased with the magnitude of CHO_{EXO}. Further investigations suggest that the difference is not because of losses of CO₂ from the trapping solution before counting or an underestimation of the “strength” of the trapping solution. Previous research suggests that the degree of isotopic fractionation is small (S. C. Kalhan, S. M. Savin, and P. A. Adam. *J Lab Clin Med* 89: 285–294, 1977). Therefore, the explanation for the discrepancy in calculated CHO_{EXO} remains to be fully understood.

exercise; isotopic fractionation; enrichment; specific activity

BOTH STABLE AND RADIOACTIVE TRACERS have been used in the study of human metabolism. For example, to study the oxidation of ingested carbohydrate (CHO), it is common to use a ¹³C tracer (1, 8, 13–15, 18, 23, 27, 29) or ¹⁴C tracer (4, 5, 9–11, 19, 24) to trace a CHO of interest. Whichever tracer is used, the principles and assumptions are the same, and the calculations depend on the enrichment (¹³C) or specific activity (SA; ¹⁴C) of tracer in the ingested CHO, the enrichment/SA of tracer in expired gas, and the rate of carbon dioxide production (\dot{V}_{CO_2}). Stable isotopes are measured in solids or expired gases by separation and quantification of the tracer-to-tracee ratio by mass spectrometry. Radioactive isotopes are measured after a quantity of CO₂ is trapped in solution and mixed with a

scintillation liquid. The scintillator absorbs the radiation and releases the energy as photons, which are subsequently detected by a photomultiplier. The ¹³C method is generally considered to be the gold standard, since the potential for errors would seem to be greater in the measurement of ¹⁴C SA compared with ¹³C enrichment (17).

Without data reporting the level of agreement between two different methods, comparisons between those data sets is difficult. In addition, data regarding the agreement between the two techniques would be useful in developing more complex experimental designs, e.g., quantifying the individual oxidation rates of two different CHO ingested simultaneously. It has been demonstrated that such experiments can be conducted using only ¹³C tracers but with an experimental design that requires each subject to complete three experimental trials differing in the enrichment of the ingested CHOs (1, 21). The results are then combined, and the oxidation rate of each CHO is calculated using simultaneous equations. The combination of three sets of results amplifies the effects of within-subject variation and extraneous variables, potentially increasing errors. An alternative method would be to label the ingested CHO with different tracers ([¹³C]fructose and [¹⁴C]glucose for example), allowing the simultaneous measurement of the oxidation rates in one exercise trial (12). However, this design depends on the assumption that both methods are in direct agreement; therefore, this study aims to directly compare exogenous carbohydrate oxidation rate (CHO_{EXO}) measured with [¹³C]glucose and [¹⁴C]glucose.

METHODS

Subjects. Seven trained cyclists/triathletes whose characteristics [age, mass, and maximal oxygen uptake ($\dot{V}_{O_{2max}}$)] were 24 ± 3 yr, 74.8 ± 2.1 kg, 62 ± 4 ml·kg⁻¹·min⁻¹ and whose maximal aerobic power (W_{max}) was 370 ± 13 W participated in this study. Subjects were fully informed of the nature, purpose, and associated risks of the study before providing written consent. All subjects were healthy as deemed by a general health questionnaire. The South Birmingham Local Research Ethics Committee approved all procedures before the onset of the study.

General design. Subjects visited the laboratory for three exercise tests, a preliminary exercise test to determine the workload for the experimental trials and two experimental trials consisting of 120 min of steady-state cycling exercise. During the experimental trials, subjects ingested an experimental beverage (8.7% glucose solution or water) at regular intervals. The glucose solution was labeled with trace amounts of [U-¹³C]glucose and [U-¹⁴C]glucose,

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and samples of expired gas were collected to allow determination of CHO_{EXO}.

Preliminary testing. All subjects attended the laboratory ~7 days before the first experimental trial for an incremental exercise test to exhaustion on an electromagnetically braked cycle ergometer (Excalibur Sport; Lode, Groningen, The Netherlands). The test began at an intensity of 95 W, and the workload was increased by 35 W every 3 min until exhaustion. Heart rate (HR) was recorded continuously during the test using radio telemetry (Polar Vantage NV; Polar Electro, Kempele, Finland). W_{\max} was calculated from the last completed work rate, plus the fraction of time (in s) spent in the final noncompleted work rate multiplied by the work rate increment. Breath-by-breath measurements were performed throughout exercise using an on-line automated gas analysis system (Oxycon Pro; Jaeger, Wuertzberg, Germany). The volume and gas analyzers of the system were calibrated using a 3-liter calibration syringe and calibration gas (4.95% CO₂-balance N). Oxygen uptake ($\dot{V}O_2$) was considered to be maximal when at least two of the following three criteria were met: 1) a leveling off of $\dot{V}O_2$ with increasing work rate (increase of no more than 2 ml·kg⁻¹·min⁻¹), 2) an HR within 10 beats/min of the predicted maximum (220 beats/min – age), 3) a respiratory exchange ratio (RER) >1.05. $\dot{V}O_{2\max}$ was calculated as the average $\dot{V}O_2$ over the last 60 s of the test.

Experimental trials. All exercise was performed in the morning with subjects having fasted for at least 10 h and having refrained from strenuous activity and alcohol for 24 h. On arrival in the laboratory, each subject's body mass was recorded (model 708; Seca, Hamburg, Germany) and a Teflon catheter (Quickcath II; Baxter, Norfolk, UK) was inserted into an antecubital vein for blood sampling. Before the onset of exercise, resting breath samples were collected. Exercise consisted of 120 min of cycling on an electromagnetically braked cycle ergometer at 50% of W_{\max} , with breath samples collected at 15-min intervals throughout exercise. $\dot{V}O_2$ and $\dot{V}CO_2$ were measured for 4-min periods every 15 min, as described above. Recordings were made as the mean of eight breaths, and data were averaged every 30 s.

Beverages. Subjects consumed 600 ml liquid at the onset of exercise and 150 ml every 15 min for the remainder of exercise (1.65 liters in total). Beverages were either water (WATER) or a glucose solution delivering 1.2 g/min glucose (Glc); both beverages contained 20 mmol/l sodium in the form of sodium chloride. The CHO beverage was prepared with wheat-derived glucose (Amylum, London, UK) with a naturally low ¹³C enrichment [–27.5 δ¹³C‰ vs. Pee Dee Bellemnitella (PDB)]. To this 0.0748 g/l [U-¹³C]glucose (Cambridge Isotope Laboratories) and 0.45 MBq/l [U-¹⁴C]glucose (Amersham Pharmacia Biotech, Little Chalfont, UK) was added, resulting in a ¹³C enrichment of 45.2 δ¹³C‰ vs. PDB and a radiation dose rate of 0.37 MBq/h (58,963 ± 639 dpm/mmol ingested glucose). The ¹³C enrichment and ¹⁴C SA of the beverage was measured using isotope ratio mass spectrometry (EA-IRMS; Europa Scientific, Crewe, UK) and a liquid scintillation counter (LS 1800; Beckman) respectively.

Breath sample collection and analysis. Subjects exhaled via a two-way mouthpiece in a 6-liter mixing chamber for no less than 60 s before sample collection. Samples for the determination of ¹³C enrichment were then collected in duplicate in 10-ml evacuated tubes (Exetainers; Labco, Buckinghamshire, UK) and analyzed using continuous-flow isotope ratio mass spectrometry (IRMS; Europa Scientific). Briefly, the contents of samples and references were flushed and transported by helium carrier gas through a packed-column gas chromatograph, held at 75°C. The resultant chromatographic peak then entered the IRMS, where the isotopomers at mass-to-charge ratio 44, 45, and 46 for CO₂ were measured and a δ¹³C value determined. The reference gas used during analysis was 3.3% CO₂ in a helium balance with δ¹³C = –29.01 vs. PDB. The 3.3% CO₂ mixture was prepared from a CO₂ cylinder calibrated against calcite (NBS-19); (δ¹³C value of +1.95 vs. PDB), an isotope reference standard distributed by the International Atomic Energy Agency, Vienna.

Duplicates of 28 breath samples were analyzed in a second laboratory using a different mass spectrometer (MAT 252; Finnigan, Bremen, Germany) to verify the ¹³C enrichment data. The mean changes in enrichment from baseline were similar between duplicates (6.12 ± 0.37 and 6.12 ± 0.38 δ¹³C‰ vs. PDB) with no significant difference between data sets ($P = 0.234$).

For the determination of breath ¹⁴C activity, a 6-liter rubber anesthetic gas bag was filled from the mixing chamber via a two-way Hans Rudolph valve. The collected air was then passed in duplicate through a solution containing 1 ml hyamine hydroxide in 1 M methanol (Zinsser Analytic, Berkshire, UK), 2 ml of 96% ethanol (BDH Laboratory Supplies, Poole, UK), and one to two drops of phenolphthalein (Riedel-de Haën, Seize, Germany; see Ref. 10). When the breath sample was passed through this trapping solution, a color change (pink to clear) occurred when exactly 1 mmol CO₂ was trapped in the solution. Scintillation cocktail (17 ml; Ready Gel, Beckman Coulter, High Wycombe, UK) was then added to this liquid before samples were mixed thoroughly, and activity (cpm) was measured using a liquid scintillation counter (LS 1800; Beckman). Counts were automatically corrected for quench and converted to disintegrations per millimole.

Calculations. By use of the measurements of enrichment/SA of the expired air and in addition to the rate of $\dot{V}CO_2$, values for exogenous CHO oxidation rate were calculated.

¹³C calculations. The isotopic enrichment was expressed as the change (δ) per mil (‰) difference between the ¹²C-to-¹³C ratio of the sample and a known laboratory reference standard according to the formula of Craig (6):

$$\delta^{13}\text{C} = \left[\left(\frac{^{12}\text{C}/^{13}\text{C} \text{ sample}}{^{12}\text{C}/^{13}\text{C} \text{ standard}} \right) - 1 \right] \times 10^3\text{‰}$$

The δ¹³C was then related to an international standard PDB.

Exogenous glucose oxidation determined using a [¹³C]glucose tracer ([¹³C]CHO_{EXO}) was then calculated using the formula:

$$[^{13}\text{C}]\text{CHO}_{\text{EXO}} = \dot{V}CO_2 \times \left(\frac{\delta\text{Exp} - \text{Exp}_{\text{bkg}}}{\delta\text{Ing} - \text{Exp}_{\text{bkg}}} \right) \times \left(\frac{1}{k} \right)$$

where δExp is ¹³C enrichment of expired air during exercise (δ¹³C‰ vs. PDB), δIng is ¹³C enrichment of the ingested beverage (δ¹³C‰ vs. PDB), δExp_{bkg} is ¹³C enrichment of expired air in the water trial [background (δ¹³C‰ vs. PDB)], and k is equal to 0.7467 [volume of CO₂ (liters) produced by the oxidation of 1 g glucose].

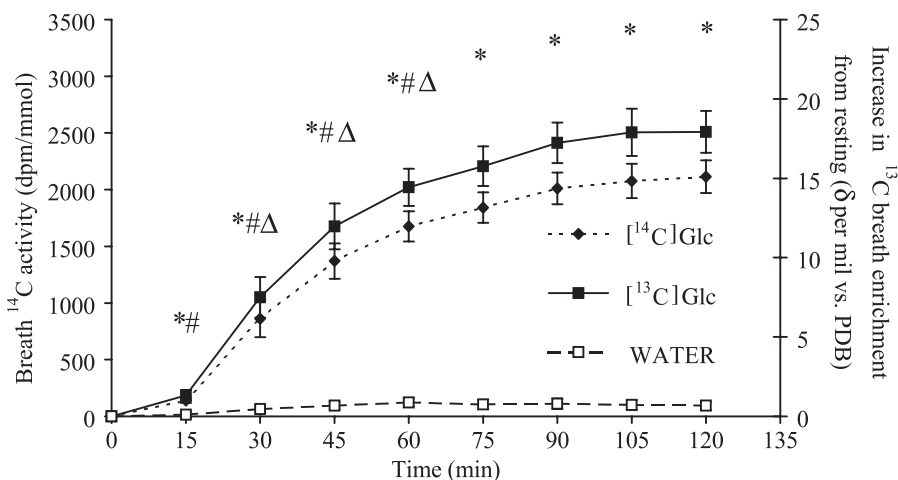
¹⁴C calculations. Exogenous glucose oxidation determined using a [¹⁴C]glucose tracer ([¹⁴C]CHO_{EXO}) was calculated using the formula:

$$[^{14}\text{C}]\text{CHO}_{\text{EXO}} = \dot{V}CO_2 \times \left(\frac{\text{SA}_{\text{CO}_2} \times 6}{\text{SA}_{\text{Glc}}} \right) \times \left(\frac{1}{k} \right)$$

where units for $\dot{V}CO_2$ are liters per minute, SA_{CO₂} is the radioactivity of 1 mmol expired CO₂ (dpm/mmol), and SA_{Glc} is the radioactivity of the ingested drink (dpm/mmol) (note: The radioactivity of the expired CO₂ is multiplied by a factor of 6 because there are 6 carbon atoms in one glucose molecule).

It is possible that a proportion of the CO₂ produced by CHO oxidation during exercise may be temporarily trapped in the bicarbonate pool. Under resting conditions, the turnover of this pool is slow, and equilibrium may take several hours to achieve (26). As a result, the appearance of labeled CO₂ may not represent the production of CO₂ within the exercising musculature. During exercise, however, the turnover rate is accelerated and thus it is likely that an equilibrium will be obtained after ~60 min (20, 26). Therefore, calculations of CHO_{EXO} in the first 60 min of exercise are likely to underestimate the actual rate of oxidation. However, both [¹⁴C]CHO_{EXO} and [¹³C]CHO_{EXO} are likely to be equally affected by CO₂ trapping; therefore, comparisons between methods are valid at all time points.

Fig. 1. Mean corrected breath ¹⁴C activity (dpm/mmol) and increase in ¹³C breath enrichment [δ‰ vs. Pee Dee Bellemnitella (PDB)]. Data are means ± SE. *Glucose (Glc) ¹³CO₂ enrichment significantly greater than water. #Glc ¹⁴CO₂ enrichment significantly greater than previous time point. Δ¹³CO₂ enrichment significantly greater than previous time point.



Dietary controls. During exercise, the relative amounts of endogenous fat and CHO oxidized will change. Because CHO stored as glycogen generally has a higher ¹³C enrichment than fat, changes in substrate oxidation can affect background ¹³C enrichment (28). These shifts in background ¹³C enrichment have the potential to lead to erroneous data (22). To decrease the magnitude of the shift in ¹³C enrichment during exercise, steps were taken to reduce the enrichment of the endogenous CHO stores. Subjects were instructed to undertake a glycogen-exhausting exercise bout 5 days before each trial and to avoid foodstuffs with a naturally high ¹³C enrichment until the experimental trial. Subjects were given guidance to do this (28), and this method has previously been shown to be effective in reducing the magnitude of the background shift in ¹³C enrichment during exercise (25, 29).

Statistics. Data were checked to ensure parametric assumptions were not violated before an ANOVA for repeated measures was applied. In the case of significant differences, paired-samples *t*-tests were used to identify their location. Data analysis was performed using SPSS 10.0 for Windows software (SPSS). Statistical significance was set at *P* < 0.05, and all data are presented as means ± SE.

RESULTS

¹³C enrichment. There were no differences between trials in resting breath ¹³C enrichment (-26.2 ± 0.2 vs. -26.0 ± 0.2 δ¹³C‰ vs. PDB for Glc and WATER, respectively, *P* = 0.760). Corrected mean breath ¹³C enrichments rose significantly in Glc between time points 15–30, 30–45, and 45–60 min (*P* < 0.001). There were no significant increases between any time points in WATER. Breath ¹³C enrichments above resting were greater in Glc than WATER at all time points other than rest. At the cessation of exercise, breath ¹³C enrichment above resting was 17.9 ± 1.3 and 0.7 ± 0.3 δ¹³C‰ vs. PDB for Glc and WATER, respectively. All breath ¹³C enrichment data are shown in Fig. 1. The enrichment of the breath in WATER represented $5.4 \pm 0.5\%$ (range 4–8%) of the enrichment in Glc. The coefficient of variation (CV) for the continuous-flow IRMS method was 0.2% (based on 250 measurements of 1 sample), whereas the CV for the EA-IRMS method was 0.4% (9 measurements of 1 sample).

¹⁴C SA. Mean breath ¹⁴C SA increased from 44 ± 3 dpm/mmol at rest to $2,188 \pm 129$ dpm/mmol at 120 min (Fig. 1). There were significant increases in breath ¹⁴C SA between 0–15, 15–30, 30–45, and 45–60 min. The CV within samples was 1.8% (based on 50 duplicate samples).

[¹⁴C]CHO_{EXO} vs. [¹³C]CHO_{EXO}. A comparison of the absolute values of [¹⁴C]CHO_{EXO} and [¹³C]CHO_{EXO} over time is shown in Fig. 2. [¹³C]CHO_{EXO} was significantly greater than [¹⁴C]CHO_{EXO} between 30 and 120 min, with peak exogenous oxidation rates of 0.87 ± 0.04 g/min ([¹³C]CHO_{EXO}) and 0.80 ± 0.04 g/min ([¹⁴C]CHO_{EXO}). When all time points were collapsed, [¹³C]CHO_{EXO} was $15 \pm 4\%$ greater than [¹⁴C]CHO_{EXO} (Fig. 3). The absolute difference between data points is presented in a Bland-Altman plot (2, 3; Fig. 4) with the mean difference between measures being 0.08 ± 0.06 g/min. The magnitude of the difference between measurements was normally distributed, and 95% confidence intervals and limits of agreement are indicated in Fig. 4.

RER and $\dot{V}O_2$. RER fell over time in WATER (from 0.88 ± 0.02 at time 0 to 0.81 ± 0.01 at 120 min) but not in Glc (from 0.92 ± 0.01 at time 0 to 0.88 ± 0.02 at 120 min) and was significantly different between trials at 45–120 min. There were no significant differences at any time point between WATER and Glc in $\dot{V}O_2$. Mean $\dot{V}O_2$ collapsed across all time points/trials was 3.01 ± 0.03 l/min.

DISCUSSION

The purpose of this study was to directly compare the level of agreement between CHO_{EXO} rate measured with [¹³C]glu-

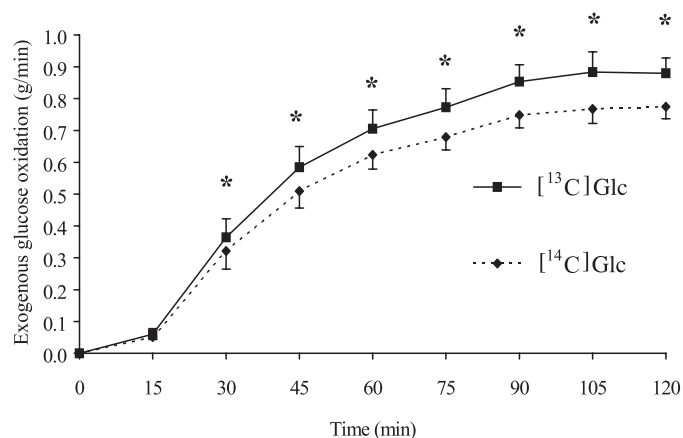


Fig. 2. Exogenous carbohydrate oxidation (g/min) as calculated with ¹³C and ¹⁴C isotopic tracers. *Significant difference between trials (*P* < 0.01).

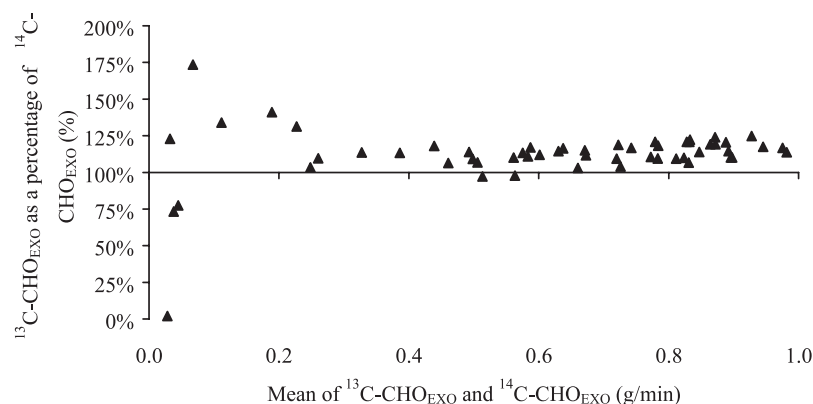


Fig. 3. Exogenous carbohydrate (CHO_{EXO}) measured with [¹³C]glucose ($^{13}\text{C}\text{CHO}_{\text{EXO}}$) as a percentage of CHO_{EXO} measured with [¹⁴C]glucose ($^{14}\text{C}\text{CHO}_{\text{EXO}}$; %); 100% represents the theoretical 1:1 relationship of the two variables.

cose and [¹⁴C]glucose. The key finding of this study is that there is a consistent ~15% difference between [¹³C]CHO_{EXO} and [¹⁴C]CHO_{EXO}. In 51 of the 56 data points, CHO_{EXO} determined with a [¹³C]glucose tracer was greater than CHO_{EXO} determined with a [¹⁴C]glucose tracer. Overall, there was a significant difference between the calculated CHO_{EXO} at seven of the eight time points. In addition, the relative difference between techniques is fairly constant at $15 \pm 4\%$, with 54 of the 56 data points lying inside the 95% limits of agreement (± 2 SD around the mean). If the data points corresponding to a mean CHO_{EXO} of 0.3 g/min or below are excluded, then the relative difference between the two techniques becomes even more consistent ($13 \pm 1\%$).

Although the ¹³C method is often considered superior because of the lower potential for errors, the use of a background correction introduces variables not used in the calculation of [¹⁴C]CHO_{EXO}. Therefore, although absolute agreement between the two methods is theoretically possible, it is unlikely. However, expressing the relationship between the two methods as though there was 100% agreement provides a useful reference point when attempting to identify an explanation for the discrepancy:

$$\dot{V}\text{CO}_2 \times \left(\frac{\text{SA}_{\text{CO}_2} \times 6}{\text{SA}_{\text{Glc}}} \right) \times \left(\frac{1}{k} \right) = \dot{V}\text{CO}_2 \times \left(\frac{\delta\text{Exp} - \text{Exp}_{\text{bkg}}}{\delta\text{Ing} - \text{Exp}_{\text{bkg}}} \right) \times \left(\frac{1}{k} \right)$$

which can be simplified to:

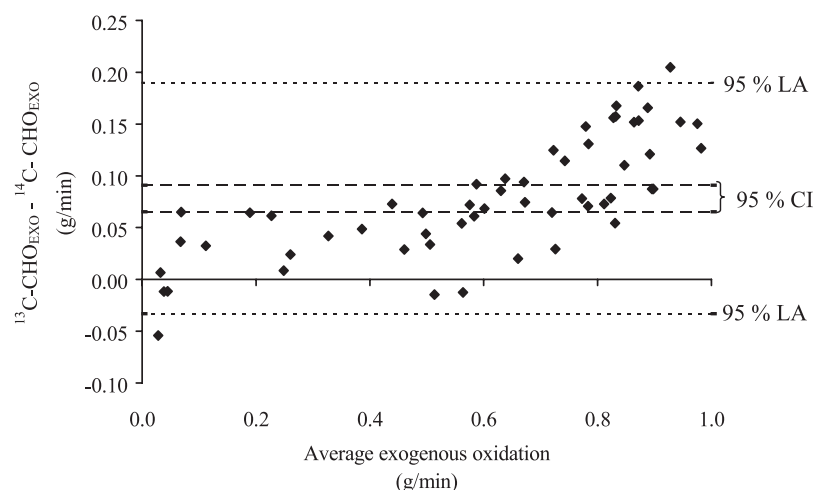


Fig. 4. Bland-Altman plot showing the agreement of the two methods. For a given pair of values, the average (x) is plotted against the difference (y); also included are 95% limits of agreement (LA) and 95% confidence intervals (CI).

$$\left(\frac{\text{SA}_{\text{CO}_2} \times 6}{\text{SA}_{\text{Glc}}} \right) = \left(\frac{\delta\text{Exp} - \text{Exp}_{\text{bkg}}}{\delta\text{Ing} - \text{Exp}_{\text{bkg}}} \right)$$

From the above equations, it can be seen that an artificial increase in [¹³C]CHO_{EXO} could be because of underestimations of δIng and Exp_{bkg} and/or an overestimation of δExp . The duplicate measurements of δIng and δExp using different mass spectrometers suggest that measurement of ¹³C enrichment is accurate (mean corrected enrichment: 6.12 ± 0.37 and 6.12 ± 0.38 $\delta^{13}\text{C}\%$ vs. PDB). In addition, two types of evacuated tubes were compared and found to give similar values for ¹³C enrichment [difference = $0.1 \pm 0.3\%$ (Exetainers; Labco, Bucks, UK, and Vacutainers; Becton-Dickinson, Drogheda, Ireland)].

The remaining explanation for an artificial increase in [¹³C]CHO_{EXO} is an underestimation of the background ¹³C enrichment, i.e., the enrichment caused by shifts in endogenous substrate metabolism. This potential problem in using ¹³C tracers during exercise has been discussed previously (22). However, the dietary instructions subjects were given have been shown to be effective in reducing background ¹³C enrichments (28). In addition, the background enrichment represented ~5.5% of the enrichment seen in the Glc trial and therefore cannot explain the observed ~15% difference seen here. The use of a water trial to calculate Exp_{bkg} is a theoretical source of error, since CHO oxidation and $\dot{V}\text{CO}_2$ were greater

after the ingestion of Glc. However, based on previous studies, it is reasonable to assume that CHO ingestion during cycling exercise did not alter muscle glycogen use (15) and will result in a small reduction in liver glycogen use (15). Only if there is a substantial shift in endogenous CHO use, there will be a shift in the background that could affect the calculations of exogenous CHO oxidation.

The authors are aware of the fact that the use of a CHO with a low natural ¹³C abundance would have been a more appropriate control trial. However, the addition of a relatively large amount of [¹³C]glucose tracer reduced the effect of a shift in background on [¹³C]CHO_{EXO} to a minimum.

Thus it seems unlikely that errors in the measurement of δIng or δExp are responsible for an overestimation of [¹³C]CHO_{EXO}.

Consequently, examination of the techniques used here suggests that methodological errors in the quantification of [¹³C]CHO_{EXO} are unlikely to be the source of the discrepancy. Examination of the relationship between [¹³C]CHO_{EXO} and [¹⁴C]CHO_{EXO} shows that an overestimation of the measured SA of the ingested drink (SA_{Glc}) or an underestimation of the SA of the expired gases (SA_{CO₂}) would result in an overall underestimation of [¹⁴C]CHO_{EXO}. Samples of each subjects' beverage were analyzed in duplicate to determine SA_{Glc}, and the linearity of the scintillation counter over the range encompassed by SA_{Glc} and SA_{CO₂} was confirmed (the equation of the line describing the relationship was: $y = 1.0022x + 50.386$, $R^2 = 0.9997$). This would suggest that the trapping of the ¹⁴CO₂ in solution is the most likely source of an underestimation of [¹⁴C]CHO_{EXO}. Errors could originate in the preparation of the solution (pipette calibration and dispensing error), the absolute ability of the solution to trap CO₂, and losses of CO₂ from the solution (17) or through losses of the solution itself during trapping. Significant falls in SA from a capped sample of trapping solution + scintillation fluid have been observed previously (17). These losses were large and rapid (~15% after <5 min) and increased over time (~35% after 400 min). The authors reported that inclusion of an additional 2.5 mmol hyamine hydroxide, after adding the scintillant, reduced these losses to 5% over 400 min. However, data from this laboratory suggest this has no effect on measured SA in this case; vials were prepared in duplicate as normal, and 2.5 mmol hyamine hydroxide were added to one of the vials immediately after passing of the expired gas through the trapping solution was completed. Vials were counted repeatedly over time for 24 h. There was no significant difference between the SA of the vial pairs (mean difference = 10 ± 13 dpm) and no trend for the difference between vials to increase over time. There is a slight possibility that passing the expired gases through the trapping solution at high rates caused droplets of solution to escape from the vial. However, measurement of the mass of the vials before and after CO₂ trapping, correcting for the amount of CO₂ in solution, suggests this error is small ($2 \pm 1\%$). The remaining methodological explanation is that the "strength" of the trapping solution (i.e., the amount of CO₂ trapped in solution by 1 ml hyamine hydroxide) was less than assumed. However, titration of the hyamine hydroxide with 0.1 M hydrochloric acid (bromocresol green indicator) indicated that the strength of the trapping solution was within acceptable parameters ($99 \pm 1\%$).

The degree to which the [¹⁴C]glucose tracer used in this study was labeled should also be considered when discussing this issue, since it has the potential to cause some inconsistency between the two methods. The calculation of [¹⁴C]CHO_{EXO} assumes that six molecules of ¹⁴CO₂ are produced per ingested molecule of labeled glucose. The calculation of [¹³C]CHO_{EXO}, however, makes no such assumption because the production of ¹³CO₂ is related to the amount of labeled carbons in the ingested beverage. As a result, any shortfall in labeling the [¹⁴C]glucose will be directly reflected in the measurement of [¹⁴C]CHO_{EXO}. In this case, the [¹⁴C]glucose tracer used was $\geq 95\%$ labeled, which would therefore introduce a difference of $\leq 5\%$.

Finally, the occurrence of isotopic fractionation must be considered as an explanation. The use of tracers depends on the assumption that the tracer is metabolically indistinguishable from the tracee, and at present there is no evidence to suggest that any significant isotopic fractionation occurs when using either ¹³C or ¹⁴C. The metabolism of ¹⁴C involves greater mass displacement than the metabolism of ¹³C (30), suggesting that the tracers could be fractionated from each other during metabolism. If this were to occur then the enrichment/SA of the expired gases would give an erroneous representation of the rate of tracee oxidation. It is also possible that use of both isotopes simultaneously could magnify small variations in metabolic treatment of the isotopes. However, comparisons of ¹³C and ¹⁴C isotopes in the measurement of fatty acid turnover/oxidation (31) and glucose turnover (16) in dogs and CO₂ recovery after HCO₃ infusion in humans (7) have suggested that the level of agreement is high. For example, Kalhan et al. (16) infused both [¹³C]glucose and [¹⁴C]glucose tracers in dogs to obtain estimations of glucose turnover. Liquid scintillation was used to measure ¹⁴C SA, whereas ¹³C enrichment was measured with magnetic-deflection double-collector mass spectrometry. The authors concluded that the level of agreement between tracers was such that [¹³C]glucose could be substituted for [¹⁴C]glucose when measuring glucose turnover.

In summary, this study has directly compared the use of [U-¹⁴C]glucose and [U-¹³C]glucose tracers to measure exogenous glucose oxidation. There was a consistent and significant difference in the measured rates of exogenous oxidation between the two techniques ($15 \pm 4\%$). Although previous investigators have not found evidence of isotopic fractionation (7, 16, 31), we were unable to identify a methodological explanation for these findings.

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