

The validity and reliability of a novel isotope ratio infrared spectrometer to quantify ^{13}C enrichment of expired breath samples in exercise

Shaun Sutehall¹, Borja Muniz-Pardos², Danijela Šmajgl³, Magda Mandić³, Cedric Jeglinski³, Andrew Bosch¹, Stuart Galloway⁴ and Yannis Pitsiladis⁵

¹Division of Exercise Science and Sports Medicine, University of Cape Town, Cape Town, South Africa; ²GENUD (Growth, Exercise, Nutrition and Development) research group, University of Zaragoza, Zaragoza, Spain; ³Thermo Fisher Scientific, Bremen, Germany; ⁴PENRG (Physiology, Exercise, Nutrition Research Group), Faculty of Healthy Sciences and Sport, University of Stirling, Stirling, Scotland; ⁵Collaborating Centre of Sports Medicine, University of Brighton, Eastbourne, United Kingdom

Corresponding author:

Professor Yannis Pitsiladis
Collaborating Centre of Sports Medicine, University of Brighton
Eastbourne
UK
Email: y.pitsiladis@brighton.ac.uk

Keywords: Isotope ratio mass spectrometry, Isotope ratio infrared spectrometry, expired breath ^{13}C enrichment, exercise

Running title: The validity and reliability of a novel Isotope Ratio Infrared Spectrometer

29 **Abstract**

30

31 **Rationale.** The traditional method to measure $^{13}\text{CO}_2$ enrichment in breath involves isotope
32 ratio mass spectrometry (IRMS) and has several limitations such as cost, extensive training
33 and large space requirements. Here we present the validity and reliability data of an isotope
34 ratio infrared spectrometer (IRIS) based method developed to combat these limitations.

35 **Methods.** Eight healthy male runners performed 105 min of continuous running on a
36 motorised treadmill while ingesting various carbohydrate beverages enriched with ^{13}C and
37 expired breath samples obtained every 15 min in triplicate. A total of 213 breath samples
38 were analysed using both methods, while 212 samples were repeated using IRIS to determine
39 test-retest reliability. Bland-Altman analysis was performed to determine systematic and
40 proportional bias, and intraclass correlation coefficient (ICC) and coefficient of variation
41 (CV) to assess level of agreement and magnitude of error.

42 **Results.** The IRIS method demonstrated a small but significant systematic bias to
43 overestimate $\delta^{13}\text{CO}_2$ (0.18‰; $p<0.05$) compared with IRMS, without any proportional bias or
44 heteroscedasticity and a small CV% (0.5%). There was a small systematic bias during the
45 test-retest of the IRIS method (-0.07‰; $p<0.05$), no proportional bias, an excellent ICC
46 (1.00) and small CV% (0.4%).

47 **Conclusions.** The use of the Delta Ray IRIS to determine ^{13}C enrichment in expired breath
48 samples captured during exercise has excellent validity and reliability when compared with
49 the gold standard IRMS.

50

51 **New & Noteworthy statement**

52

53 The use of IRIS to determine ^{13}C enrichment in expired breath samples captured during
54 exercise to determine exogenous glucose oxidation during exercise has excellent validity and
55 reliability when compared with the gold standard IRMS.

56

Introduction

Mechanistic studies utilising a metabolic “tracer” (e.g., a substance containing ^{13}C or ^{14}C) are often used to investigate a multitude of physiological mechanisms. Widely used methods include those measuring gastric emptying (GE) (3,4), detecting the presence of certain species of bacteria within the gastrointestinal tract (1) and determining the rate at which exogenous carbohydrate (CHO) is oxidised during exercise (15). In such studies, an ingestible source that contains a high abundance of ^{13}C is selected or, alternatively, a small dose of ^{13}C enriched material is added to the ingested beverage/food stuff. Among the available tracers, namely ^{13}C and ^{14}C , the use of ^{13}C is most often favoured to minimise the exposure to radiation participants receive through the ingestion of the radioactive ^{14}C isotope. Specifically, to assess the rate at which an exogenous source of CHO (ExCHO) is oxidised as a substrate for physical work, a CHO source containing ^{13}C must be consumed by the athlete at regular intervals. As exercise is initiated, both endogenous (i.e., blood glucose and muscle/liver glycogen) and exogenous (i.e., ingested CHO) will be oxidised and CO_2 produced. As the endogenous source of CHO contains mainly ^{12}C , any ^{13}C that is released is derived from the ingested source of CHO. ExCHO oxidation rate can therefore be calculated by measuring the ratio of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ in expired breath, in addition to CO_2 production under steady state metabolic conditions (14). This method has become increasingly popular due to the relatively simple procedures required, minimally invasive nature, and safety of ^{13}C -labelled CHO ingestion. Similarly, the measurement of GE requires athletes or patients to ingest a beverage/test meal containing a substrate labelled with ^{13}C such as octanoic acid (11) or acetate (5). These ingested tracers will empty from the stomach, rapidly absorbed through the intestine, oxidised and the resultant $^{13}\text{CO}_2$, expired in breath.

While these study designs are generally straightforward, the quantification of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ in expired breath samples requires the use of an isotope ratio mass spectrometer (IRMS).

Mass spectrometry has been suggested to be the most precise method in detecting the abundance of stable isotopes (21) and is considered the “gold standard” in measuring expired breath samples for the abundance of $^{13}\text{CO}_2$. While the use of mass spectrometers to measure $^{13}\text{CO}_2$ enrichment in expired breath is accurate, it is associated with expensive procedures, namely a high cost of sample carrier gas helium and trained technicians required to operate the IRMS. Due to these expenses and lab space required, many departments within

Universities and institutions do not own an IRMS and prefer to send samples to an external laboratory. Additionally, analysis of samples using IRMS requires an experimenter to load / analyse each sample and therefore represents a significant time burden to experimenters whose time could be better spent collecting data.

The Thermo Scientific™ Delta Ray™ Isotope Ratio Infrared Spectrometer with the Universal Reference Interface Connect (Delta Ray IRIS) has been recently developed as a more economical and portable instrument to assess isotope ratios and concentrations of CO₂ in air. Delta Ray IRIS is a laser-based instrument and has recently been used in various applications like monitoring of the atmospheric ¹³CO₂:¹²CO₂ within caves (19), around volcanic sites (6), studying biosphere-atmosphere CO₂ exchange processes in the beech forest (4) and monitoring of the coral reef metabolism (16). While promising, its validity and reliability measuring isotope ratios in expired breath in humans is unknown.

Therefore, the aim of the present study was to determine the validity and reliability of the Delta Ray IRIS analytical technique when compared to the gold standard IRMS analysis method (i.e., gas chromatography isotope ratio mass spectrometer) to assess ¹³CO₂ enrichment of breath samples obtained during steady state treadmill running exercise in trained athletes.

Methods

This present investigation is a companion study to a larger project investigating the use of CHO beverages during prolonged running, with the full details of methods available elsewhere (18).

Participants

Eight well-trained male runners were recruited for this study (age; 28 ± 9 yr, height; 178 ± 7 cm, body mass; 69.0 ± 9.1 kg, maximal oxygen consumption [$\dot{V}O_{2max}$]; 69.9 ± 8.1 mL·kg⁻¹·min⁻¹). Written informed consent was collected prior to initiation of data collection and this study was approved by the University of Stirling ethics committee.

Experimental trials

Following $\dot{V}O_{2\max}$ tests and familiarisation with the testing procedures, all participants performed four experimental trials. Each trial consisted of 105 min of prolonged running at $71 \pm 4\%$ $\dot{V}O_{2\max}$ while ingesting 175 mL of one of four experimental beverages every 15 min. Two beverages contained 10% CHO ($70 \text{ g} \cdot \text{hr}^{-1}$ CHO), with one containing an additional 0.2% sodium alginate and pectin ($70 \text{ g} \cdot \text{hr}^{-1}$ encapsulated CHO). One beverage included a 26% CHO ($180 \text{ g} \cdot \text{hr}^{-1}$ encapsulated CHO) beverage with an additional 0.2% sodium alginate and pectin, and the final beverage was distilled water. The addition of sodium alginate and pectin has been shown to form a pH-sensitive hydrogel and encapsulate CHO within a hydrogel in the stomach (9) and assessing its potential impact on ExCHO is the primary aim of the current investigation's companion study (18). The addition of sodium alginate and pectin to CHO beverages has been described and reviewed in detail elsewhere (7, 17). All CHO beverages contained maltodextrin and fructose in a ratio of 1:0.7, with both the CHO naturally enriched with ^{13}C . To further increase the enrichment of each CHO beverage, an additional $50 \text{ mg} \cdot \text{L}^{-1}$ of D-glucose- $^{13}\text{C}_6$ tracer was added to each CHO beverage, with resulting drink enrichments of $28.15 \pm 1.23 \text{ ‰}$ Vienna PeeDee Belemnite (VPDB) ($70 \text{ g} \cdot \text{hr}^{-1}$ CHO), $26.90 \pm 1.54 \text{ ‰}$ VPDB ($70 \text{ g} \cdot \text{hr}^{-1}$ encapsulated CHO) and $4.04 \pm 0.32 \text{ ‰}$ VPDB ($180 \text{ g} \cdot \text{hr}^{-1}$ encapsulated CHO). It is important to note that in this experiment the tracer and tracee were not perfectly matched, in all likelihood resulting in the tracer not following the tracee exactly and as a result exogenous glucose oxidation was not computed from the results provided. However, the comparison of ^{13}C enrichment ($^{13}\text{CO}_2$) between the methods of measurement remains valid.

Every 15 min over the 105 min run, an end-tidal expired breath sample was collected into a 750 mL discard bag, with the initial 400 mL of the breath removed through a discard bag. A 10 mL sample was then drawn into a syringe and injected into a 10 mL Exetainer tube (Labco Ltd, High Wycombe, UK) in triplicate.

Analysis

All breath samples were analysed for $^{13}\text{CO}_2$: $^{12}\text{CO}_2$ carbon isotope ratio using a gas chromatography isotope ratio mass spectrometer (GC-IRMS, Europa Scientific, Crew, UK). Specifically, each sample was flushed into a packed column gas chromatograph which was held at $60 \text{ }^\circ\text{C}$, with the resulting chromatographic peak passed into the GC-IRMS (Hydra 2020 IRMS, Europa Scientific, Crewe, England) where isotopomers at 44, 45 and 46 m/z for

CO₂ were measured and the $\delta^{13}\text{C}$ value determined. Samples of the international standard IA-CO₂-7 were measured prior and during sample measurement to ensure correct calibration, this standard has $\delta^{13}\text{C}$ value of -38.48 ‰ vs VPDB. The reference material used during the $\delta^{13}\text{C}$ analysis was IA-R005 (beet sugar), with an $\delta^{13}\text{C}$ of -26.03 ‰ VPDB. In order to ensure quality control, check samples of IA-R005, IA-R006 (cane sugar, $\delta^{13}\text{C}$ = -11.64 ‰ VPDB) and IA-R071 (sugar, $\delta^{13}\text{C}$ = -19.26 ‰ VPDB) were analyzed during batch analysis of the samples. Both the international standard and references were supplied by the International Atomic Energy Agency, Vienna.

The method by which the Delta Ray IRIS measures the abundance of ^{12}C and ^{13}C is fundamentally different to an IRMS. While IRMS is based on mass separation of charged ionic species, the Delta Ray IRIS is laser-based absorption spectrometer that employs a mid-infrared laser with a power of approximately 2 μW and operates at 4.3 μm . The laser scans the spectral region containing four CO₂ absorption lines: $^{12}\text{C}^{16}\text{O}^{18}\text{O}$ ($\lambda=4.3286\mu\text{m}$), $^{13}\text{C}^{16}\text{O}^{16}\text{O}$ ($\lambda=4.3283\mu\text{m}$), $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ - CO₂ (1) ($\lambda=4.3280\mu\text{m}$) and $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ - CO₂ (2) ($\lambda=4.3277\mu\text{m}$), with a scanning frequency of 500 Hz. The concentration and isotopic composition of the gas sample is simultaneously measured by direct laser absorption through temperature and pressure controlled multiple-pass absorption cell. To correct for linearity, the reference CO₂ gas is adjusted to match the sample gas concentration in addition to an interface which determines the nonlinearity by diluting the reference gas with CO₂-free synthetic air. A two-point calibration is used based on gas samples with higher (“Ambient” $\delta^{13}\text{C}$ = -9.86 ‰ VPDB) and lower (“Bio” $\delta^{13}\text{C}$ = -25.5 ‰ VPDB) isotopic values, both gases were supplied by Thermo Fisher Scientific, (Bremen, Germany). A full description of the Delta Ray IRIS functioning is described elsewhere (20). To determine the validity of the Delta Ray IRIS, the results assessed through the Delta Ray IRIS were compared to those determined by a GC-IRMS. To determine the test-retest reliability assessment of the Delta Ray IRIS, a second analysis was conducted using the third exetainer seven days after the first Delta Ray IRIS analysis, with these two samples being compared with each other.

Statistical analysis

Bland-Altman plots were performed to evaluate the systematic bias and random errors for ^{13}C enrichment as assessed by the Delta Ray IRIS, with IRMS used as the reference method or “gold standard”. Proportional biases were assessed by linear regression models between the ^{13}C enrichment mean and difference between systems, indicating the potential presence of

heteroscedasticity (2). The Bland Altman method was also used for the test-retest reliability to determine bias for ^{13}C enrichment between the two measurements determined using the Delta Ray IRIS. Additionally, the intraclass correlation coefficient (ICC) was assessed for the reliability test. ICC lower than 0.5 indicated a poor reliability, values between 0.5 and 0.75 indicate a moderate reliability, values between 0.75 and 0.9 show a good reliability and values above 0.9 indicate an excellent reliability (8). The coefficient of variation (CV) was also determined to measure the degree of variation from both the validity the test-retest reliability, considering an acceptable CV in sports science has been described as 10% or less (2). A Pearson's correlation was performed to determine the correlation between the Delta Ray IRIS and IRMS breath ^{13}C enrichment measurement.

Results

A total set of 213 breath samples were collected during the exercise trials and analysed for ^{13}C enrichment using the Delta Ray IRIS and GC-IRMS. No significant differences were observed between ^{13}C enrichment as assessed by GC-IRMS and Delta Ray IRIS ($-19.56 \pm 5.71 \text{ ‰}$ and $-19.74 \pm 5.71 \text{ ‰}$, $p > 0.05$, respectively), which is reflected in the distribution of the data in Figure 1.

Fig 1 here

Bland Altman analysis revealed a significant systematic bias (0.18 ‰ , $p \leq 0.05$, Fig 1), but no significant proportional bias ($p > 0.05$), indicating that the Delta Ray IRIS slightly overestimates breath ^{13}C enrichment. The CV observed between IRMS and the Delta Ray data was 0.5 ‰ , with an ICC of 1.00. A very strong positive correlation was found between the breath ^{13}C enrichment ($R^2 = 0.99$, $p < 0.01$, Fig 2).

Fig 2 here

A total of 212 breath samples were measured a second time using the Delta Ray IRIS. The test-retest reliability assessment for the Delta Ray IRIS revealed a significant systematic bias with the second measurement (-0.07 ‰ ; $p \leq 0.05$), with no significant proportional bias. The CV and ICC were 0.4 ‰ and 1.00, respectively.

Discussion

The increasing use of stable isotopes in applied physiology and exercise science demands the development of new methods to measure breath ^{13}C that are affordable, and available to laboratories unable to access to a “traditional” IRMS system, while also demonstrating good validity and reliability is essential. This is the first study to systematically determine both the validity and reliability of Delta Ray IRIS compared to the “gold-standard” IRMS. It was found that the Delta Ray IRIS is both a valid and reliable instrument to measure breath ^{13}C enrichment, showing slight significant systematic biases for both validity and reliability tests (i.e., 0.18 ‰ and -0.07 ‰ respectively), with no proportional biases (i.e., no heteroscedasticity). Since the tracer and tracee were not perfectly matched in this experiment, exogenous glucose oxidation was not computed from the results. However, the comparison of ^{13}C enrichment ($^{13}\text{CO}_2$) between the two methods being investigated, is valid.

The CV in the measured breath ^{13}C enrichment between the Delta Ray IRIS and IRMS was good, at 0.5%. This finding is in agreement with that of van Geldern et al (20), who reported differences in $\delta^{13}\text{C}$ ranging from 0.04 to 1 ‰ in atmospheric ^{13}C when comparing the Delta Ray IRIS with a traditional IRMS. Notably, in their study, when comparing nine atmospheric samples collected at the test site, the Delta Ray IRIS on average, measured the delta $\delta^{13}\text{C}$ as 0.25 ‰ higher than IRMS (-22.50 ± 2.36 ‰ vs -22.75 ± 2.28 ‰, respectively). This reported bias is very similar to the 0.18 ‰ systematic bias reported in the present study, reiterating that the Delta Ray IRIS overestimates ^{13}C enrichment by ~ 0.2 ‰ when compared with the gold standard IRMS. The Delta Ray IRIS demonstrated a test-retest CV of 0.4 %, which is within the typical precision requirement for exercise science research. The CV% for analytical techniques used in exercise science varies widely, however, the measurement techniques for assessment of blood metabolites are typically considered acceptable when CV is $\leq 3\%$.

Despite revealing a systematic bias of 0.18‰, there was no proportional bias, indicating a consistent deviation from the gold standard IRMS through a range of breath ^{13}C enrichment values (i.e., from approximately -27 to -6.5 ‰). This is of particular importance considering the breath ^{13}C enrichment in exercise trials will typically increase during the exercise period

due to changes in enrichment and release of ^{13}C from the bicarbonate buffering pool, followed by a plateau, with the magnitude of the increase dependent on several factors such as the enrichment of the ingested beverage, oxidation rate of the ingested CHO, and time required to saturate the blood bicarbonate pool. Thus, if there was the presence of heteroscedasticity, the use of the Delta Ray IRIS within an exercise science setting would be questionable, or adjustments to equations used would be required to enable a consistent measurement of breath ^{13}C enrichment.

The need for validation of this platform is ever rising, with recently published research assessing different CHO beverages and their effect on ExCHO oxidation rate using the Delta Ray IRIS (12). Since the current investigation has demonstrated the reliability and validity of this platform, the aforementioned study (12) and future studies using the Delta Ray IRIS can be confident in their results to accurately reflect changes breath ^{13}C enrichment and therefore estimated ExCHO oxidation rate.

An important advantage of this instrument, besides the reduced cost and its portability, is that it also can monitor changes in the isotopic composition of expired breath data in real time, a technique used previously to measure changes in atmospheric ^{13}C enrichment (20). This could be applied to exercise science, allowing for the determination of breath-by-breath ExCHO oxidation in real time in the exercising athlete. This will aid in the advance of research into CHO ingestion during exercise and will allow the identification of potential perturbations in ExCHO in the periods between CHO ingestion boluses (typically every 15-20 min). This technology will also allow for the individualisation of CHO intake strategies to elevate and maintain a high ExCHO oxidation rate with optimal precision and accuracy. For example, recent research has suggested that a higher ExCHO oxidation rate is achieved when beverages are provided every 20 min in a larger bolus (200 mL) rather than with repeated smaller boluses every 5 min (50 mL; (10)). Similarly, this function could be applied in a clinical setting when an investigation of gastric emptying using an isotopic tracer is required. Currently, samples are taken every ~10 min in order to closely capture the emptying characteristics of the ingested test meal/beverage (i.e. (5)) which requires a researcher present to collect and transition the sample into a exetainer. If this process was automated, with the patient wearing a face mask, the Delta Ray IRIS could collect and analyse expired breath samples continually for the study duration, providing instantaneous feedback to researchers.

This will also represent a saving to both the cost and time required as the consumable cost of such studies is greatly reduced and the analysis of gas samples instantaneously, without the need to send samples to a laboratory and wait for the results. While not validated within the present study, the analysis of ambient air for ^{13}C enrichment has been explored elsewhere (20) and has shown the Delta Ray IRIS suitable for continuous measurement of ambient air, which has applications in environmental monitoring, such as within the plume gas from volcanos (13). While the Delta Ray IRIS has true potential to increase the accessibility of ^{13}C measurement, the main obstacle remains the high upfront equipment purchase cost that is significantly lower than IRMS but may remain too high for most laboratories. Finally, future research should also consider investigating the use of the Delta Ray IRIS to determine if the results presented within the present study in highly active, males are applicable over a wider range of populations.

Conclusions

In the present study, it was found that the Delta Ray IRIS is a valid and reliable method for the measurement of $^{13}\text{C}:^{12}\text{C}$ in breath. Specifically, the Delta Ray IRIS showed a slight overestimation of breath ^{13}C compared with the gold standard, IRMS. The slight overestimation is likely to have a negligible effect on the estimation of ExCHO oxidation rate and thus can be used with confidence for this application. Additionally, there was no presence of heteroscedasticity and demonstrated an excellent ICC and test-retest CV% of 1.00 and 0.4%, respectively, far exceeding typical analytical CV% observed for some analytical procedures used in the exercise sciences. Further applications of the Delta Ray IRIS must be explored, such as the ability to measure mixed expired ^{13}C breath samples continuously during exercise, which may confer a significant time and money saving benefit.

318 Legends:

319

320 *Figure 1.* Box plot of ^{13}C breath enrichment values collected during exercise and analysed
321 using either the “traditional” isotope ratio mass spectrophotometer (IRMS) or The Thermo
322 Scientific™ Delta Ray™ Isotope Ratio Infrared Spectrometer (Delta Ray IRIS) (A). Bland-
323 Altman plot illustrating the agreement between the IRMS and Delta Ray IRIS (B), indicating
324 a significant systematic bias (0.18 ‰, $p < 0.05$) but no proportional bias ($p > 0.05$). Pearson’s
325 correlation between the IRMS and Delta Ray IRIS, demonstrating a significant, strong
326 positive correlation ($r^2 = 0.99$, $p < 0.05$). $n = 213$.

327

328 *Figure 2.* Breath ^{13}C enrichment during the four exercise trials measured using the
329 “traditional” isotope ratio mass spectrometer (IRMS) or The Thermo Scientific™ Delta
330 Ray™ Isotope Ratio Infrared Spectrometer (Delta Ray IRIS). Participants provided breath
331 samples every 15 min during exercise while ingesting $70 \text{ g} \cdot \text{hr}^{-1}$ CHO (A), $70 \text{ g} \cdot \text{hr}^{-1}$ CHO and
332 sodium alginate and pectin (B), $180 \text{ g} \cdot \text{hr}^{-1}$ CHO and sodium alginate and pectin (C) or water
333 (D). $n=8$.

References:

1. **Atherton JC, Spiller RC.** The urea breath test for *Helicobacter pylori*. *Gut* 35: 723, 1994.
2. **Atkinson G, Nevill AM.** Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sport Med* 26: 217–238, 1998.
3. **Bharucha AE, Camilleri M, Veil E, Burton D, Zinsmeister AR.** Comprehensive assessment of gastric emptying with a stable isotope breath test. *Neurogastroenterol Motil* 25: e60–e69, 2013.
4. **Braden-Behrens J, Yan Y, Knohl A.** A new instrument for stable isotope measurements of C-13 and O-18 in CO₂-instrument performance and ecological application of the Delta Ray IRIS analyzer. *Atmos Meas Tech* 10: 4537–4560, 2017.
5. **Braden B, Adams S, Duan L-P, Orth K-H, Maul F-D, Lembcke B, Hör G, Caspary WF.** The [13C] acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. *Gastroenterology* 108: 1048–1055, 1995.
6. **Fischer TP, Lopez TM.** First airborne samples of a volcanic plume for $\delta^{13}\text{C}$ of CO₂ determinations. *Geophys Res Lett* 43: 3272–3279, 2016.
7. **King AJ, Rowe JT, Burke LM.** Carbohydrate Hydrogel Products Do Not Improve Performance or Gastrointestinal Distress During Moderate-Intensity Endurance Exercise. *Int J Sport Nutr Exerc Metab* 30: 305–314, 2020.
8. **Koo TK, Li MY.** A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med* 15: 155–163, 2016.
9. **Marciani L, Lopez-Sanchez P, Pettersson S, Hoad C, Abrehart N, Ahnoff M, Strömf A.** Alginate and HM-pectin in sports-drink give rise to intra-gastric gelation in-vivo. *Food Funct* 10: 7892–7899, 2019.
10. **Mears SA, Boxer B, Sheldon D, Wardley H, Tarnowski CA, James LJ, Hulston CJ.** Sports Drink Intake Pattern Affects Exogenous Carbohydrate Oxidation during Running. .
11. **Perri F, Pastore MR, Annese V.** 13C-octanoic acid breath test for measuring gastric emptying of solids. *Eur Rev Med Pharmacol Sci* 9: 3–8, 2005.
12. **Pettersson S, Edin F, Bakkman L, McGawley K.** Effects of supplementing with an 18% carbohydrate-hydrogel drink versus a placebo during whole-body exercise in- 5 C with elite cross-country ski athletes: a crossover study. *J Int Soc Sports Nutr* 16: 46, 2019.

- 368 13. **Rizzo AL, Jost H, Caracausi A, Paonita A, Liotta M, Martelli M.** Real-time
369 measurements of the concentration and isotope composition of atmospheric and
370 volcanic CO₂ at Mount Etna (Italy). *Geophys Res Lett* 41: 2382–2389, 2014.
- 371 14. **Romijn JA, Coyle EF, Hibbert J, Wolfe RR.** Comparison of indirect calorimetry and
372 a new breath ¹³C/¹²C ratio method during strenuous exercise. *Am J Physiol Metab*
373 263: E64–E71, 1992.
- 374 15. **Rowlands DS, Thorburn MS, Thorp RM, Broadbent S, Shi X.** Effect of graded
375 fructose coingestion with maltodextrin on exogenous ¹⁴C-fructose and ¹³C-glucose
376 oxidation efficiency and high-intensity cycling performance. *J Appl Physiol* 104:
377 1709–1719, 2008. doi: 10.1152/jappphysiol.00878.2007.
- 378 16. **Smajgl D, Böhm F, Eisenhauer A, Taubner I, Mandić M.** New analytical approach
379 in monitoring of CO₂ cycle in aquatic ecosystems. 2018.
- 380 17. **Sutehall S, Muniz-Pardos B, Bosch AN, Di Gianfrancesco A, Pitsiladis YP.** Sports
381 Drinks on the Edge of a New Era. *Curr Sports Med Rep* 17: 112–116, 2018. doi:
382 10.1249/jsr.0000000000000475.
- 383 18. **Sutehall S, Muniz-Pardos B, Bosch AN, Galloway SD, Pitsiladis Y.** *The impact of*
384 *sodium alginate hydrogel on exogenous carbohydrate oxidation and gastrointestinal*
385 *comfort in trained runners [Under review].* 2020.
- 386 19. **Töchterle P, Dublyansky Y, Stöbener N, Mandić M, Spötl C.** High-resolution
387 isotopic monitoring of cave air CO₂. *Rapid Commun Mass Spectrom* 31: 895–900,
388 2017.
- 389 20. **van Geldern R, Nowak ME, Zimmer M, Szizybalski A, Myrntinen A, Barth JAC,**
390 **Jost H-J.** Field-based stable isotope analysis of carbon dioxide by mid-infrared laser
391 spectroscopy for carbon capture and storage monitoring. *Anal Chem* 86: 12191–12198,
392 2014.
- 393 21. **Wolfe RR.** Measurement of Energy Substrate Metabolism. Using Stable Isotopes. .
394



