

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

3

4

5 Shaun Sutehall¹, Borja Muniz-Pardos², Danijela Šmajgl³, Magda Mandic³, Cedric Jeglinski³,
6 Andrew Bosch¹, Stuart Galloway⁴ and Yannis Pitsiladis⁵

7

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

14

15 **Corresponding author:**

16

17 Professor Yannis Pitsiladis

18 Collaborating Centre of Sports Medicine, University of Brighton

19 Eastbourne

20 UK

21 Email: y.pitsiladis@brighton.ac.uk

22

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

25

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

27

28

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

57 **Introduction**

58

59 Mechanistic studies utilising a metabolic “tracer” (e.g., a substance containing ^{13}C or ^{14}C) are
60 often used to investigate a multitude of physiological mechanisms. Widely used methods
61 include those measuring gastric emptying (GE) (3,4), detecting the presence of certain
62 species of bacteria within the gastrointestinal tract (1) and determining the rate at which
63 exogenous carbohydrate (CHO) is oxidised during exercise (15). In such studies, an
64 ingestible source that contains a high abundance of ^{13}C is selected or, alternatively, a small
65 dose of ^{13}C enriched material is added to the ingested beverage/food stuff. Among the
66 available tracers, namely ^{13}C and ^{14}C , the use of ^{13}C is most often favoured to minimise the
67 exposure to radiation participants receive through the ingestion of the radioactive ^{14}C isotope.
68 Specifically, to assess the rate at which an exogenous source of CHO (ExCHO) is oxidised as
69 a substrate for physical work, a CHO source containing ^{13}C must be consumed by the athlete
70 at regular intervals. As exercise is initiated, both endogenous (i.e., blood glucose and
71 muscle/liver glycogen) and exogenous (i.e., ingested CHO) will be oxidised and CO_2
72 produced. As the endogenous source of CHO contains mainly ^{12}C , any ^{13}C that is released is
73 derived from the ingested source of CHO. ExCHO oxidation rate can therefore be calculated
74 by measuring the ratio of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ in expired breath, in addition to CO_2 production
75 under steady state metabolic conditions (14). This method has become increasingly popular
76 due to the relatively simple procedures required, minimally invasive nature, and safety of
77 ^{13}C -labelled CHO ingestion. Similarly, the measurement of GE requires athletes or patients to
78 ingest a beverage/test meal containing a substrate labelled with ^{13}C such as octanoic acid (11)
79 or acetate (5). These ingested tracers will empty from the stomach, rapidly absorbed through
80 the intestine, oxidised and the resultant $^{13}\text{CO}_2$, expired in breath.

81 While these study designs are generally straightforward, the quantification of $^{13}\text{CO}_2$ and
82 $^{12}\text{CO}_2$ in expired breath samples requires the use of an isotope ratio mass spectrometer
83 (IRMS).

84

85 Mass spectrometry has been suggested to be the most precise method in detecting the
86 abundance of stable isotopes (21) and is considered the “gold standard” in measuring expired
87 breath samples for the abundance of $^{13}\text{CO}_2$. While the use of mass spectrometers to measure
88 $^{13}\text{CO}_2$ enrichment in expired breath is accurate, it is associated with expensive procedures,
89 namely a high cost of sample carrier gas helium and trained technicians required to operate
90 the IRMS. Due to these expenses and lab space required, many departments within

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

95

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

104

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

110

111 **Methods**

112

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

116

117 *Participants*

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

122

123 *Experimental trials*

124 Following $\dot{V}O_2$ max tests and familiarisation with the testing procedures, all participants
125 performed four experimental trials. Each trial consisted of 105 min of prolonged running at
126 $71 \pm 4\%$ $\dot{V}O_2$ max while ingesting 175 mL of one of four experimental beverages every 15
127 min. Two beverages contained 10% CHO ($70 \text{ g} \cdot \text{hr}^{-1}$ CHO), with one containing an additional
128 0.2% sodium alginate and pectin ($70 \text{ g} \cdot \text{hr}^{-1}$ encapsulated CHO). One beverage included a 26%
129 CHO ($180 \text{ g} \cdot \text{hr}^{-1}$ encapsulated CHO) beverage with an additional 0.2% sodium alginate and
130 pectin, and the final beverage was distilled water. The addition of sodium alginate and pectin
131 has been shown to form a pH-sensitive hydrogel and encapsulate CHO within a hydrogel in
132 the stomach (9) and assessing its potential impact on ExCHO is the primary aim of the
133 current investigation's companion study (18). The addition of sodium alginate and pectin to
134 CHO beverages has been described and reviewed in detail elsewhere (7, 17). All CHO
135 beverages contained maltodextrin and fructose in a ratio of 1:0.7, with both the CHO
136 naturally enriched with ^{13}C . To further increase the enrichment of each CHO beverage, an
137 additional $50 \text{ mg} \cdot \text{L}^{-1}$ of D-glucose- $^{13}\text{C}_6$ tracer was added to each CHO beverage, with
138 resulting drink enrichments of $28.15 \pm 1.23 \text{ ‰}$ Vienna PeeDee Belemnite (VPDB) ($70 \text{ g} \cdot \text{hr}^{-1}$
139 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
140 $\text{g} \cdot \text{hr}^{-1}$ encapsulated CHO). It is important to note that in this experiment the tracer and tracee
141 were not perfectly matched, in all likelihood resulting in the tracer not following the tracee
142 exactly and as a result exogenous glucose oxidation was not computed from the results
143 provided. However, the comparison of ^{13}C enrichment ($^{13}\text{CO}_2$) between the methods of
144 measurement remains valid.

145

146 Every 15 min over the 105 min run, an end-tidal expired breath sample was collected into a
147 750 mL discard bag, with the initial 400 mL of the breath removed through a discard bag. A
148 10 mL sample was then drawn into a syringe and injected into a 10 mL Exetainer tube (Labco
149 Ltd, High Wycombe, UK) in triplicate.

150

151

152 *Analysis*

153 All breath samples were analysed for $^{13}\text{CO}_2$: $^{12}\text{CO}_2$ carbon isotope ratio using a gas
154 chromatography isotope ratio mass spectrometer (GC-IRMS, Europa Scientific, Crew, UK).
155 Specifically, each sample was flushed into a packed column gas chromatograph which was
156 held at $60 \text{ }^\circ\text{C}$, with the resulting chromatographic peak passed into the GC-IRMS (Hydra
157 2020 IRMS, Europa Scientific, Crewe, England) where isotopomers at 44, 45 and 46 m/z for

158 CO₂ were measured and the $\delta^{13}\text{C}$ value determined. Samples of the international standard IA-
159 CO₂-7 were measured prior and during sample measurement to ensure correct calibration,
160 this standard has $\delta^{13}\text{C}$ value of -38.48 ‰ vs VPDB. The reference material used during the
161 $\delta^{13}\text{C}$ analysis was IA-R005 (beet sugar), with an $\delta^{13}\text{C}$ of -26.03 ‰ VPDB. In order to ensure
162 quality control, check samples of IA-R005, IA-R006 (cane sugar, $\delta^{13}\text{C} = -11.64$ ‰ VPDB)
163 and IA-R071 (sugar, $\delta^{13}\text{C} = -19.26$ ‰ VPDB) were analyzed during batch analysis of the
164 samples. Both the international standard and references were supplied by the International
165 Atomic Energy Agency, Vienna.

166

167 The method by which the Delta Ray IRIS measures the abundance of ^{12}C and ^{13}C is
168 fundamentally different to an IRMS. While IRMS is based on mass separation of charged
169 ionic species, the Delta Ray IRIS is laser-based absorption spectrometer that employs a mid-
170 infrared laser with a power of approximately 2 μW and operates at 4.3 μm . The laser scans
171 the spectral region containing four CO₂ absorption lines: $^{12}\text{C}^{16}\text{O}^{18}\text{O}$ ($\lambda=4.3286\mu\text{m}$), $^{13}\text{C}^{16}\text{O}^{16}\text{O}$
172 ($\lambda=4.3283\mu\text{m}$), $^{12}\text{C}^{16}\text{O}^{16}\text{O} - \text{CO}_2$ (1) ($\lambda=4.3280\mu\text{m}$) and $^{12}\text{C}^{16}\text{O}^{16}\text{O} - \text{CO}_2$ (2) ($\lambda=4.3277 \mu\text{m}$),
173 with a scanning frequency of 500 Hz. The concentration and isotopic composition of the gas
174 sample is simultaneously measured by direct laser absorption through temperature and
175 pressure controlled multiple-pass absorption cell. To correct for linearity, the reference CO₂
176 gas is adjusted to match the sample gas concentration in addition to an interface which
177 determines the nonlinearity by diluting the reference gas with CO₂-free synthetic air. A two-
178 point calibration is used based on gas samples with higher (“Ambient” $\delta^{13}\text{C} = -9.86$ ‰
179 VPDB) and lower (“Bio” $\delta^{13}\text{C} = -25.5$ ‰ VPDB) isotopic values, both gases were supplied
180 by Thermo Fisher Scientific, (Bremen, Germany). A full description of the Delta Ray IRIS
181 functioning is described elsewhere (20). To determine the validity of the Delta Ray IRIS, the
182 results assessed through the Delta Ray IRIS were compared to those determined by a GC-
183 IRMS. To determine the test-retest reliability assessment of the Delta Ray IRIS, a second
184 analysis was conducted using the third exetainer seven days after the first Delta Ray IRIS
185 analysis, with these two samples being compared with each other.

186

187 *Statistical analysis*

188 Bland-Altman plots were performed to evaluate the systematic bias and random errors for ^{13}C
189 enrichment as assessed by the Delta Ray IRIS, with IRMS used as the reference method or
190 “gold standard”. Proportional biases were assessed by linear regression models between the
191 ^{13}C enrichment mean and difference between systems, indicating the potential presence of

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

202

203 **Results**

204

205 A total set of 213 breath samples were collected during the exercise trials and analysed for
206 ^{13}C enrichment using the Delta Ray IRIS and GC-IRMS. No significant differences were
207 observed between ^{13}C enrichment as assessed by GC-IRMS and Delta Ray IRIS ($-19.56 \pm$
208 5.71 ‰ and $-19.74 \pm 5.71 \text{ ‰}$, $p > 0.05$, respectively), which is reflected in the distribution of
209 the data in Figure 1.

210

211

Fig 1 here

212

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

218

219

Fig 2 here

220

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

225

226

227

228 **Discussion**

229

230 The increasing use of stable isotopes in applied physiology and exercise science demands the
231 development of new methods to measure breath ^{13}C that are affordable, and available to
232 laboratories unable to access to a “traditional” IRMS system, while also demonstrating good
233 validity and reliability is essential. This is the first study to systematically determine both the
234 validity and reliability of Delta Ray IRIS compared to the “gold-standard” IRMS. It was
235 found that the Delta Ray IRIS is both a valid and reliable instrument to measure breath ^{13}C
236 enrichment, showing slight significant systematic biases for both validity and reliability tests
237 (i.e., 0.18 ‰ and -0.07 ‰ respectively), with no proportional biases (i.e., no
238 heteroscedasticity). Since the tracer and tracee were not perfectly matched in this experiment,
239 exogenous glucose oxidation was not computed from the results. However, the comparison of
240 ^{13}C enrichment ($^{13}\text{CO}_2$) between the two methods being investigated, is valid.

241

242 The CV in the measured breath ^{13}C enrichment between the Delta Ray IRIS and IRMS was
243 good, at 0.5%. This finding is in agreement with that of van Geldern et al (20), who reported
244 differences in $\delta^{13}\text{C}$ ranging from 0.04 to 1 ‰ in atmospheric ^{13}C when comparing the Delta
245 Ray IRIS with a traditional IRMS. Notably, in their study, when comparing nine atmospheric
246 samples collected at the test site, the Delta Ray IRIS on average, measured the delta $\delta^{13}\text{C}$ as
247 0.25 ‰ higher than IRMS (-22.50±2.36 ‰ vs -22.75±2.28 ‰, respectively). This reported
248 bias is very similar to the 0.18 ‰ systematic bias reported in the present study, reiterating
249 that the Delta Ray IRIS overestimates ^{13}C enrichment by ~0.2 ‰ when compared with the
250 gold standard IRMS. The Delta Ray IRIS demonstrated a test-retest CV of 0.4 ‰, which is
251 within the typical precision requirement for exercise science research. The CV% for
252 analytical techniques used in exercise science varies widely, however, the measurement
253 techniques for assessment of blood metabolites are typically considered acceptable when CV
254 is $\leq 3\%$.

255

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

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

267

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

274

275 An important advantage of this instrument, besides the reduced cost and its portability, is that
276 it also can monitor changes in the isotopic composition of expired breath data in real time, a
277 technique used previously to measure changes in atmospheric ^{13}C enrichment (20). This
278 could be applied to exercise science, allowing for the determination of breath-by-breath
279 ExCHO oxidation in real time in the exercising athlete. This will aid in the advance of
280 research into CHO ingestion during exercise and will allow the identification of potential
281 perturbations in ExCHO in the periods between CHO ingestion boluses (typically every 15-
282 20 min). This technology will also allow for the individualisation of CHO intake strategies to
283 elevate and maintain a high ExCHO oxidation rate with optimal precision and accuracy. For
284 example, recent research has suggested that a higher ExCHO oxidation rate is achieved when
285 beverages are provided every 20 min in a larger bolus (200 mL) rather than with repeated
286 smaller boluses every 5 min (50 mL; (10)). Similarly, this function could be applied in a
287 clinical setting when an investigation of gastric emptying using an isotopic tracer is required.
288 Currently, samples are taken every ~10 min in order to closely capture the emptying
289 characteristics of the ingested test meal/beverage (i.e. (5)) which requires a researcher present
290 to collect and transition the sample into a exetainer. If this process was automated, with the
291 patient wearing a face mask, the Delta Ray IRIS could collect and analyse expired breath
292 samples continually for the study duration, providing instantaneous feedback to researchers.

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

305

306 **Conclusions**

307

308 In the present study, it was found that the Delta Ray IRIS is a valid and reliable method for
309 the measurement of $^{13}\text{C}:^{12}\text{C}$ in breath. Specifically, the Delta Ray IRIS showed a slight
310 overestimation of breath ^{13}C compared with the gold standard, IRMS. The slight
311 overestimation is likely to have a negligible effect on the estimation of ExCHO oxidation rate
312 and thus can be used with confidence for this application. Additionally, there was no
313 presence of heteroscedasticity and demonstrated an excellent ICC and test-retest CV% of
314 1.00 and 0.4%, respectively, far exceeding typical analytical CV% observed for some
315 analytical procedures used in the exercise sciences. Further applications of the Delta Ray
316 IRIS must be explored, such as the ability to measure mixed expired ^{13}C breath samples
317 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). $n=8$.

334 References:

- 335 1. **Atherton JC, Spiller RC.** The urea breath test for *Helicobacter pylori*. *Gut* 35: 723,
336 1994.
- 337 2. **Atkinson G, Nevill AM.** Statistical methods for assessing measurement error
338 (reliability) in variables relevant to sports medicine. *Sport Med* 26: 217–238, 1998.
- 339 3. **Bharucha AE, Camilleri M, Veil E, Burton D, Zinsmeister AR.** Comprehensive
340 assessment of gastric emptying with a stable isotope breath test. *Neurogastroenterol*
341 *Motil* 25: e60–e69, 2013.
- 342 4. **Braden-Behrens J, Yan Y, Knohl A.** A new instrument for stable isotope
343 measurements of C-13 and O-18 in CO₂-instrument performance and ecological
344 application of the Delta Ray IRIS analyzer. *Atmos Meas Tech* 10: 4537–4560, 2017.
- 345 5. **Braden B, Adams S, Duan L-P, Orth K-H, Maul F-D, Lembcke B, Hör G,**
346 **Caspary WF.** The [13C] acetate breath test accurately reflects gastric emptying of
347 liquids in both liquid and semisolid test meals. *Gastroenterology* 108: 1048–1055,
348 1995.
- 349 6. **Fischer TP, Lopez TM.** First airborne samples of a volcanic plume for $\delta^{13}\text{C}$ of CO₂
350 determinations. *Geophys Res Lett* 43: 3272–3279, 2016.
- 351 7. **King AJ, Rowe JT, Burke LM.** Carbohydrate Hydrogel Products Do Not Improve
352 Performance or Gastrointestinal Distress During Moderate-Intensity Endurance
353 Exercise. *Int J Sport Nutr Exerc Metab* 30: 305–314, 2020.
- 354 8. **Koo TK, Li MY.** A guideline of selecting and reporting intraclass correlation
355 coefficients for reliability research. *J Chiropr Med* 15: 155–163, 2016.
- 356 9. **Marciani L, Lopez-Sanchez P, Pettersson S, Hoad C, Abrehart N, Ahnoff M,**
357 **Strömf A.** Alginate and HM-pectin in sports-drink give rise to intra-gastric gelation
358 in-vivo. *Food Funct* 10: 7892–7899, 2019.
- 359 10. **Mears SA, Boxer B, Sheldon D, Wardley H, Tarnowski CA, James LJ, Hulston**
360 **CJ.** Sports Drink Intake Pattern Affects Exogenous Carbohydrate Oxidation during
361 Running. .
- 362 11. **Perri F, Pastore MR, Annese V.** 13C-octanoic acid breath test for measuring gastric
363 emptying of solids. *Eur Rev Med Pharmacol Sci* 9: 3–8, 2005.
- 364 12. **Pettersson S, Edin F, Bakkman L, McGawley K.** Effects of supplementing with an
365 18% carbohydrate-hydrogel drink versus a placebo during whole-body exercise in– 5
366 C with elite cross-country ski athletes: a crossover study. *J Int Soc Sports Nutr* 16: 46,
367 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, Myrtilinen 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



