Breath $^{13}$CO$_2$ background enrichment during exercise: diet-related differences between Europe and America

ANTON J. M. WAGENMAKERS, NANCY J. REHRER, FRED BROUNS, WIM H. M. SARIS, AND DAVID HALLIDAY

Nutrition Research Center, Department of Human Biology, University of Limburg, 6200 MD Maastricht, The Netherlands; and Nutrition Research Group, Clinical Research Center, Harrow HA1 3UJ, United Kingdom

WAGENMAKERS, ANTON J. M., NANCY J. REHRER, FRED BROUNS, WIM H. M. SARIS, AND DAVID HALLIDAY. Breath $^{13}$CO$_2$ background enrichment during exercise: diet-related differences between Europe and America. J. Appl. Physiol. 74(5): 2353–2357, 1993.—A traditional North American diet contains a high percentage of carbohydrates (CHO) derived from C$_4$ plants (maize, sugar cane), whereas a European diet contains primarily CHO derived from C$_3$ plants (potato, sugar beet). The natural $^{13}$C enrichment of the first type of CHO is higher than that of the latter type. $^{13}$CO$_2$ production from orally ingested C$_4$ plant-derived CHO can, therefore, be used to quantify oxidation rates of orally ingested CHO at rest and during exercise. Recently it has been shown that oxidation rates assessed this way in North Americans should be corrected for an increase in breath background $^{13}$CO$_2$ during exercise. We hypothesized that the indicated difference in metabolic origin of CHO would imply that no such correction is required for subjects on a European diet. We therefore studied changes from rest in breath $^{13}$CO$_2$ enrichment in Dutch volunteers during cycle ergometry at 65% maximal work load (experiment 1, 2 h, 6 subjects) and 70% maximal oxygen uptake (experiment 2, 90 min, 8 subjects) while ingesting water (experiment 1 and 2) and potato starch-derived glucose (experiment 2). Experiment 1 was done before and after careful instruction of the subjects to refrain from nutrient sources potentially containing CHO of C$_3$ metabolic origin. No significant changes from rest $^{13}$CO$_2$ enrichment were observed in tests with water and potato-derived glucose ingestion in subjects who excluded CHO of C$_4$ metabolic origin from their diet.

carbon-13 enrichment; stable isotopes; carbohydrate ingestion

A BELGIAN LABORATORY (10) in 1973 was the first to report that maize-derived glucose could be used to estimate oxidation rates of orally administered carbohydrates in humans. This is because of the fact that a selective isotope fractionation effect occurs during the reactions of photosynthesis, i.e., the initial fixation of atmospheric CO$_2$ (1). This isotope fractionation effect leads to so-called C$_3$ plants (maize, sugar cane) synthesizing carbohydrates with a $^{13}$C enrichment that is $\sim$15 parts per thousand (per mil) higher than in C$_4$ plants (potatoes, sugar beet). Therefore, oral administration of 100 g of maize-derived glucose to an adult normally consuming a diet consisting only beet- and potato-derived carbohydrates (those living in most Western European countries and consuming traditional diets only) will increase the enrichment of breath $^{13}$CO$_2$ by $\sim$5–8 per mil (10). Since then many studies have reported oxidation rates of orally ingested carbohydrates both at rest (11, 12, 15, 16) and during exercise (2–8, 13, 14, 17–21) in which use has been made of this difference in natural $^{13}$C enrichment of carbohydrates.

In many of these studies it was assumed that breath $^{13}$CO$_2$ enrichment at rest before intake of the naturally enriched carbohydrates could be used as background $^{13}$CO$_2$ enrichment, as suggested by Mosora et al. (15); in some studies the $^{13}$CO$_2$ enrichment during exercise without oral substrate (water ingestion) was used as the background (6, 13, 14). Recently, a paper originating from a Canadian laboratory (18) suggested that both types of background correction were probably inadequate and could lead to a substantial overestimation of the oxidation rate of orally administered carbohydrates. Most sources of nutritional fat have a lower $^{13}$C enrichment than carbohydrates, and this would lead to a higher enrichment of the body carbohydrate stores compared with that of the body fat stores. On theoretical grounds it was argued that these enrichment differences could lead to an increase in the background enrichment of $^{13}$CO$_2$ during exercise compared with at rest because of a shift in substrate oxidation (primarily fat at rest to primarily carbohydrates in the early phase of exercise at 50–70% maximal oxygen uptake [V$_{O_2 \text{max}}$]). Significant increases of 1–2 per mil in the breath background $^{13}$CO$_2$ enrichment indeed have been observed during exercise without oral substrate intake in reports in which American and Canadian subjects were studied (13, 14, 23). Furthermore, it was argued that during exercise tests in which subjects drink only water, the shift from primarily carbohydrate oxidation (early phase of exercise) back to fat oxidation (prolonged exercise) would occur sooner than when carbohydrate was ingested and would influence the breath $^{13}$CO$_2$ background enrichment, making exercise with water ingestion also an inadequate background correction. Therefore, an alternative method has been proposed in which two exercise tests were performed employing equal oral carbohydrate intakes but different $^{13}$C enrichments to estimate the oxidation of oral carbohydrates (18). It also was stated that in many previous studies substantial overestimations had been made of oxidation rates of oral carbohydrates because of a failure to compensate with an adequate background correction.

We agree with the theoretical considerations of
Péronnet et al. (18) but wish to stress that the absolute differences in $^{13}$C content of fat and carbohydrate will be greater in North American than in European subjects because of the fact that traditional European diets contain very little or no $C_4$ plant- (maize, cane sugar) derived carbohydrates. Most of the dietary carbohydrates in Western European countries are $C_3$ plant (potato and sugar beet) derived. Therefore, the differences in enrichment of the body fat and carbohydrate stores and the shift in background $^{13}$CO$_2$ enrichment during exercise also should be smaller. Here, therefore, we investigate changes in $^{13}$C enrichment of breath CO$_2$ in Dutch subjects during exercise with ingestion of water, maize-derived maltodextrin, and potato-derived glucose.

METHODS

In experiment 1 six healthy trained male Dutch amateur cyclists were studied. The subjects exercised on electromagnetically braked cycle ergometers (Lode, Groningen, The Netherlands) at a freely chosen power output-independent pedaling rate (80-120 revolutions/min). After their maximal work load attained during incremental cycle exercise had been determined as described previously (9), the subjects were studied during 2 h of cycle exercise at 65% maximal work load. All subjects were studied on three occasions, twice while ingesting water and once while drinking a 4.5% maize-derived naturally enriched maltodextrin solution (drinks were given as specified in METHODS). Second exercise test with only water ingestion (water 2) was done after careful instruction of subjects to refrain for ≥1 wk from dietary sources potentially containing naturally enriched carbohydrates (corn flakes, candy bars, commercial sport drinks). Mean enrichment of rest samples was $-24.14 \pm 0.49$ (mean ± SD; n = 18) per mil vs. PDB. Enrichment of maltodextrin was $-9.5$ per mil vs. PDB.

FIG. 1. $^{13}$C enrichment (per mil vs. rest sample taken before warming up) of breath CO$_2$ in 6 Dutch subjects during 2 h of cycling exercise at 65% maximal work load. Subjects were studied on 3 occasions, twice while drinking water and once while drinking a 4.5% maize-derived naturally enriched maltodextrin solution (drinks were given as specified in METHODS). Second exercise test with only water ingestion (water 2) was done after careful instruction of subjects to refrain for ≥1 wk from dietary sources potentially containing naturally enriched carbohydrates (corn flakes, candy bars, commercial sport drinks). Mean enrichment of rest samples was $-24.14 \pm 0.49$ (mean ± SD; n = 18) per mil vs. PDB. Enrichment of maltodextrin was $-9.5$ per mil vs. PDB.
two occasions during 80 min of cycle exercise at 70% \( V_O_{2\text{max}} \), once while ingesting water and once during intake of a 4.5% potato starch-derived glucose solution (Avebe, Groningen, The Netherlands; \(^{13}\)C enrichment \(-24.0\) per mil vs. PDB). Subjects received careful instruction to refrain for \( \geq 2 \) wk from dietary sources potentially containing carbohydrates of \( C_4 \) metabolic origin. Drinks were given, and breath samples were collected and analyzed as described in experiment 1.

**Statistical analysis.** The Wilcoxon signed rank test was used to analyze whether the observed breath \(^{13}\)CO\(_2\) enrichments during exercise were different from the rest sample that was taken before warming up and to compare breath \(^{13}\)CO\(_2\) enrichments at given time intervals in different treatments. Significance was set at \( P < 0.05 \).

**RESULTS**

Figures 1 and 2 clearly show that in experiments in which the Dutch subjects drank only water during exer-
TABLE 1. \(^{13}C\) enrichment of breath CO\(_2\) during exercise in subject
habitually consuming a Dutch diet

<table>
<thead>
<tr>
<th>Exercise Time, min</th>
<th>Water 1</th>
<th>Water 2</th>
<th>Maltodextrin (maize)</th>
<th>Water</th>
<th>Glucose (potato)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>-0.14±0.25</td>
<td>-0.4±0.52</td>
<td>0.3±0.24</td>
<td>-0.11±0.43</td>
<td>-0.12±0.42</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>30</td>
<td>0.76±0.042</td>
<td>0.15±0.58</td>
<td>2.02±0.29</td>
<td>0.29±0.44</td>
<td>0.26±0.36</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>45</td>
<td>0.78±0.40</td>
<td>0.15±0.44</td>
<td>2.83±0.37</td>
<td>0.11±0.44</td>
<td>0.26±0.27</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>60</td>
<td>0.62±0.31</td>
<td>0.12±0.41</td>
<td>3.51±0.27</td>
<td>0.27±0.47</td>
<td>0.36±0.21</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>75</td>
<td>0.68±0.34</td>
<td>0.04±0.48</td>
<td>3.62±0.54</td>
<td>0.26±0.57</td>
<td>0.29±0.16</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>90</td>
<td>0.46±0.33</td>
<td>0.04±0.52</td>
<td>3.90±0.18</td>
<td>End of test</td>
<td>End of test</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>0.22±0.23</td>
<td>-0.1±0.62</td>
<td>3.98±0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.01±0.26</td>
<td>-0.24±0.57</td>
<td>3.99±0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD in per mil v. rest sample of 6 subjects (experiment 1) or 8 subjects (experiment 2). Protocols of experiments 1 and 2 are as described in legends to Figs. 1 and 2. Wilcoxon signed rank test was used to analyze whether observed breath \(^{13}CO_2\) enrichments were different from those of rest sample that was taken before warming up. Significance was set at \(P < 0.05\).

cise, only small changes from the resting \(^{13}C\) enrichment are observed. The mean difference from rest for all samples from all subjects obtained during exercise with only water ingestion was 0.19 ± 0.52 (SD) per mil (n = 136). Figure 1 also shows that careful instruction of the subjects to refrain for ≥1 wk from nutrient sources potentially containing carboxydrates of C\(_4\) metabolic origin led to a substantial decrease in the background \(^{13}C\) enrichment in three of six subjects; no difference was observed in the other 3 subjects.

Figure 2 shows that there are only marginal differences between the \(^{13}C\) enrichment of breath CO\(_2\) during an exercise test with water ingestion and with potato-derived glucose ingestion. The mean difference (water ingestion − glucose ingestion) for all samples from all subjects was -0.026 ± 0.369 (SD) per mil (n = 40).

Mean values per time period and data of the statistical analysis are given in Table 1. In experiment 1, careful instruction of the subjects to refrain from nutrient sources potentially containing carbohydrates of C\(_4\) metabolic origin reduced the maximal increase of the \(^{13}C\) enrichment of breath CO\(_2\) in the tests with only water ingestion from 0.79 per mil (water 1) to 0.15 per mil (water 2). A significant increase of breath \(^{13}CO_2\) enrichment from rest was only observed in the water 1 test. Mean differences from rest did not exceed 0.3 per mil and were not significant in the tests with only water ingestion after the indicated instruction was given in both experiment 1 and experiment 2. Differences from rest in the tests of experiment 2 are small in comparison to the increase in breath \(^{13}CO_2\) enrichment observed in the test with ingestion of a 4.5% maize-derived maltodextrin solution in experiment 1 (<4.0 per mil). In the test with ingestion of potato starch-derived glucose in experiment 2, the difference from rest was statistically significant, although small (<0.3 per mil). The intersubject variation was smaller in this test than in the test with only water ingestion. No significant differences were observed in experiment 2 between breath \(^{13}CO_2\) enrichments measured in the test with only water ingestion and with ingestion of potato starch-derived glucose.

DISCUSSION

The results described in this paper clearly show that, in Dutch subjects that refrain from dietary sources potentially containing naturally enriched carbohydrates of C\(_4\) metabolic origin, both the \(^{13}C\) enrichment of breath CO\(_2\) at rest and during exercise with water ingestion can be used as a valid background correction to calculate the oxidation of such naturally enriched carbohydrates taken orally during exercise. During exercise at intensities of ~70% \(V_{O_2,max}\), increases of breath \(^{13}CO_2\) enrichment from rest do not exceed 0.3 per mil, are statistically nonsignificant, and are small in comparison to increases observed during exercise with ingestion of carbohydrates of C\(_4\) metabolic origin (+4.0 per mil). For comparison, highly significant mean differences between 1.0 and 2.0 per mil have previously been reported for subjects from the US (23) and Canada (13, 14) and would lead to substantial overestimates of carbohydrate oxidation when ignored in the computation procedure (18). Care has to be taken when European athletes are studied, who are used to drinking commercial sport drinks and who regularly consume candy bars. These products may contain maize- and sugar cane-derived carbohydrates, and the same precautions may have to be taken in that case in Europe as suggested by Péronnet et al. (18) for studies in North America.

Therefore, studies of oral carbohydrate oxidation are much easier to perform in Europe given the fact that only one exercise test is required to obtain all the data necessary to calculate the oxidation rate of oral carbohydrates (when the rest \(^{13}C\) enrichment before exercise and carbohydrate intake is used as background correction). Furthermore, it is to be expected that the protocol suggested by Péronnet et al. (18), in which oxidation rates of orally ingested carbohydrates are calculated from the differ-
ence in $^{13}$CO$_2$ enrichments observed in two sequential exercise tests with an equal oral carbohydrate intake but with different $^{13}$C enrichments, will lead to a less accurate value than a protocol depending on a single test only. Daily variations in oxidation rates cannot be excluded and will increase the variability of the obtained estimates in the first case.

We conclude that studies of oral carbohydrate oxidation during exercise can be performed in Europe according to the method and computation procedure originally proposed by Mosora et al. (15) and that the caution summoned by Pérignon et al. (18) with respect to data from previous studies on oxidation of naturally enriched carbohydrates of C$_6$ metabolic origin taken orally during exercise applies more data to North American than to data from European North America.

Address for reprint requests: A. J. M. Wagenmakers, Dept. of Human Biology, Univ. of Limburg, PO Box 616, 6200 MD Maastricht, The Netherlands.

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