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# Labeled $CO_2$ production and oxidative vs nonoxidative disposal of labeled carbohydrate administered at rest

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**Abstract**

Carbon isotopes (*C) have been extensively used in man to describe oxidative vs nonoxidative disposal of an exogenous load of labeled carbohydrate (*C-CHO) at rest in various experimental situations. It is hypothesized that $V*CO_2$ reflects *C-CHO oxidation. However, when glycogen is synthesized through the indirect pathway (which is responsible for ~50% of glycogen storage), *C could be lost, diluted, and exchanged in the pyruvate-lactate pool, in the pool of tricarboxylic acid cycle intermediates, as well as at the entrance of the tricarboxylic acid cycle, and along the pathway of gluconeogenesis. This could result in a lower *C/C in the glycogen stored than in the CHO administered, in an increased production of $*CO_2$, and, respectively, in an overestimation and an underestimation of the oxidative and nonoxidative disposal of the CHO load. Results from the present experiment offer a support to this hypothesis. Over a 10-hour period after ingestion of a $^{13}$C-pasta meal (313 ± 10 g dry mass or 258 ± 8 g of glucose) in 12 healthy subjects (6 men and 6 women), exogenous CHO oxidation computed from $V^{13}CO_2$ (recovery factor, 0.54) significantly exceeded total CHO oxidation computed by indirect respiratory calorimetry corrected for urea excretion: 154.2 ± 2.6 vs 133.5 ± 3.2 g. In an additional study conducted in rats, $^{13}C/^{12}C$ in glycogen stores was significantly ~50% lower than in the $^{13}$C-CHO ingested, over a wide range of enrichment. These results suggest that because of dilution, loss, and exchange of *C in the indirect pathway of glycogen synthesis, the oxidative vs nonoxidative disposal of exogenous *C-CHO cannot be accurately tracked from $V*CO_2$.


## 1. Introduction

Various types of substrates and/or foods naturally or artificially enriched in $^{13}$C or $^{14}$C have been used to describe some metabolic consequences of carbohydrate (CHO) administration in man, at rest, from the production of labeled $CO_2$ ($V*CO_2$): exogenous CHO oxidation [1,2] vs storage [3,4], changes in total and endogenous CHO oxidation, and glycogen turnover [5,6], as well as the effect of various factors on these phenomena (eg, type [3] and amount [7,8] of CHO administered, route of administration [9,10], sex [11], nutritional status [12-16], a previous exercise period [4,17,18], training [19], or prolonged bed rest [20], obesity [21-24], diabetes [25], and some drugs [21,22,25,26]). In these studies, it was assumed that $V*CO_2$, estimated from $V*CO_2$ at the mouth with or without a correction for the incomplete recovery of $*CO_2$ produced in tissues [27], accurately reflects the oxidative disposal of the CHO load. The nonoxidative disposal of the CHO load, computed by difference, is thought to mainly represent storage under the form of glycogen but could also include, in a lesser extent, incomplete absorption in the peripheral blood with, possibly, colonic fermentation [28], accumulation into the glucose space during the period of observation, urinary loss [15], and/or de novo lipogenesis [20,29].

It could be hypothesized, however, that when glycogen is synthesized from *C-CHO, $V*CO_2$ could overestimate the oxidative disposal of exogenous CHO and underestimate nonoxidative disposal, as well as endogenous CHO oxidation (computed by difference between total CHO oxidation measured using indirect respiratory calorimetry and exogenous CHO oxidation). This is because of the fact that

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~50% of glycogen synthesis occurs through the indirect pathway, that is, after the breakdown of glucose into pyruvate-lactate and conversion of pyruvate-lactate back into glucose and glycogen through gluconeogenesis from the pool of pyruvate-lactate and from the pool of the tricarboxylic acid (TCA) cycle intermediates (Fig. 1) [30-33]. These pools are fueled not only from *C–pyruvate-lactate but also from unlabeled substrates with exchange of *C with unlabeled carbon atoms (or C, ie, carbon atoms from pools with a *C/C equal to the background *C/C) [34]. In addition, *C can also be lost as $*CO_2$ in the decarboxylation of pyruvate into acetate and of oxaloacetate into phospho*enol*pyruvate (Fig. 1). The first consequence, as shown by Newgard et al [35] and by Katz et al [34], is that *C/C in the glycogen synthesized is lower than in the CHO administered. The second consequence is that, although the flux of pyruvate-lactate from exogenous CHO actually fuels glycogen synthesis, a portion of *C appears as $*CO_2$ at the entrance of the TCA cycle, or within this cycle, as well as in the gluconeogenic pathway. Another portion could also be irreversibly lost, within the period of the experiment, in metabolic pathways fueled from the pool of TCA cycle intermediates (eg, formation of glutamate and glutamine) or in carboxylation reactions. However, it could be hypothesized that, after meal, the flux of *C–pyruvate-lactate largely exceeds those in these pathways and that most of the *C exchanged with C or lost as $*CO_2$ will appear as $*CO_2$. The result is that the oxidative disposal of exogenous CHO computed from $V*CO_2$ could be overestimated, whereas nonoxidative CHO disposal, as well as endogenous CHO oxidation, could be underestimated.

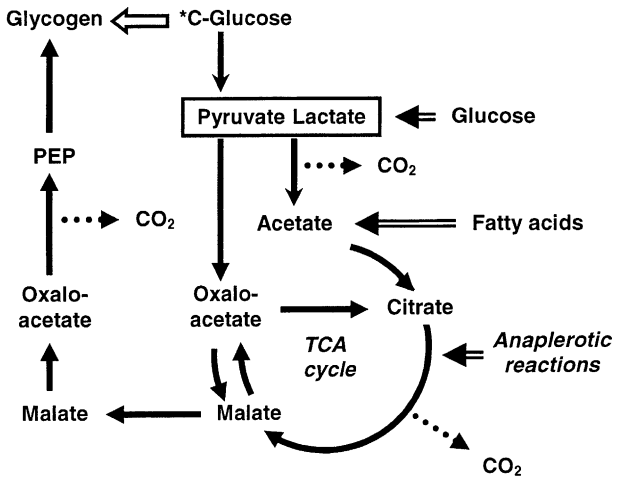


Fig. 1. Direct and indirect pathways (white and black arrows, respectively) of glycogen synthesis from labeled glucose (*C-glucose) showing loss, dilution, and exchange of *C in the pool of pyruvate-lactate, at the entrance of the TCA cycle, in the pool of TCA cycle intermediates, and in the gluconeogenic pathway with production of $*CO_2$ (dotted arrows), and synthesis of glycogen stores through the indirect pathway with a $*C/^{12}C$ lower than that in *C-glucose administered. The double arrows indicate the supply of unlabeled substrates to the pyruvate-lactate and TCA cycle intermediate pools. PEP indicates phospho*enol*pyruvate.

Results observed in our laboratory support this hypothesis and suggest that the oxidative disposal of exogenous CHO cannot be accurately tracked from *C-CHO and $V*CO_2$. Indeed, we showed in human subjects that after a large $^{13}$C-CHO meal, the oxidation rate of exogenous CHO computed from $V^{13}CO_2$ exceeded that of total CHO computed from indirect respiratory calorimetry. A reexamination of some data in the literature shows that this phenomenon was actually present in several previous studies [4,13,14,16]. As an additional verification of the hypothesis that *C is lost in the indirect pathway of glycogen synthesis, presumably under the form of $*CO_2$, we performed an experiment in rats similar to those conducted by Newgard et al [35] and by Katz et al [34]. After a near-complete depletion of liver glycogen stores by a combination of a diet poor in CHO and prolonged swimming exercise, the animals were fed with $^{13}$C-CHO at various levels of enrichment. The $^{13}C/^{12}C$ in the liver glycogen accumulated was ~40% to 50% lower than that of the CHO ingested.

## 2. Methods

### 2.1. Experiment in humans

The oxidation of exogenous $^{13}$C-CHO provided by a pasta meal was studied on a group of 12 healthy lean subjects (6 men and 6 women; aged 27.4 ± 1.2 years, body mass, 64.5 ± 3.5 kg; height, 172 ± 2 cm; maximal oxygen consumption on cycle ergometer, 39.4 ± 1.7 mL · kg$^{-1}$ · min$^{-1}$), with fasting plasma glucose concentration in the reference range (4.4 ± 0.1 mmol/L). All the subjects were lean (body mass index 22.0 ± 1.0 kg/m$^2$) and healthy, and none of them were smokers, heavy drinker, under medication, or had gained or lost weight over the past year (<1- to 2-kg changes). The subjects gave their informed written consent to participate in the study, which was approved by the institutional board on the use of human subjects in research. The women were studied between days 6 and 10 (midfollicular phase) and between 17 and 25 days after the first day of menses (midluteal phase), depending on the duration of the cycle.

The subjects were studied for 10 hours after ingestion of a large pasta meal after a period of rest or exercise. During the 3 days preceding each test, the subjects refrained from exercising and from ingesting alcohol, and they were provided with prepackaged meals providing 35 kcal · kg$^{-1}$ · d$^{-1}$ (~21% proteins, ~56% CHO, and ~23% lipids). On the day of the experiment, after a 12-hour overnight fast, the subjects ingested a standardized breakfast (6 kcal/kg: ~13% proteins, ~45% CHO, and ~42% lipids) between 7:00 and 7:30 AM and then rested for 150 minutes or exercised from 8:00 to 9:30 AM on a cycle ergometer (Ergomeca, La Bayette, France) at a workload corresponding to 50% of the maximal aerobic workload (90.5 ± 6.0 W). The order of presentation of the 2 situations was randomized among the subjects. Between 10:00 and

11:00 AM, the subjects ingested a large starch meal (5 g dry weight pasta per kg body mass; Panzani, Marseille, France) boiled for 7 minutes in water (100 g pasta per liter with 7 g of table salt per liter). The pasta was administered in 5 equal portions at 15-minute intervals and was served with steamed onions and tomatoes with salt and pepper (60 mL/100 g dry weight pasta). The total amount of pasta ingested was 313 ± 10 g, containing 232 ± 7 g of starch (74%) corresponding to 258 ± 8 g of glucose (162 g of starch = 180 g of glucose). Approximately 0.55% of the semolina in the pasta was derived from durum wheat grown in an atmosphere containing 0.1% $CO_2$ artificially enriched in $^{13}C$ ($^{13}CO_2/CO_2$, ~11%; Euriso-top, France; actual $^{13}C/C$ of the grains, 11.1%). The average final $^{13}C/^{12}C$ in the cooked pasta was 26.7 ± 3.2‰ $\delta^{13}C$ versus the $PDB_1$ Chicago Standard (n = 36).

Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) (Tissot Spirometer, Warren-Collins Inc, Braintree, Mass; oxygen and carbon dioxide analyzers MGA-1100, Marquette Electronics Inc, Milwaukee, Wis) were measured for 20 minutes every 60 minutes after the meal. In addition, immediately before ingesting the pasta and at 60-minute intervals after the meal, a sample of expired gas was collected in vacutainers (Becton Dickinson, Franklin Lakes, NJ) for the measurement of $^{13}C/^{12}C$ in expired $CO_2$ using mass spectrometry (Prism, Micromass, Manchester, UK), after cryodistillation, as previously described [29]. The isotopic composition was expressed in per mill difference by comparison with the $PDB_1$ Chicago Standard: ‰ $\delta^{13}C\ PDB_1 = [(R_{spl}/R_{std}) - 1] \times 1000$, where $R_{spl}$ and $R_{std}$ are the $^{13}C/^{12}C$ ratio in the sample and standard (1.1237%), respectively.

Protein oxidation was computed from the amount of urea excreted in urine taking into account that 1 g of urea excreted corresponds to 2.9 g of proteins oxidized [36]. CHO and lipid oxidation were then computed from $\dot{V}O_2$ and $\dot{V}CO_2$ [37], corrected for the volumes of $O_2$ and $CO_2$ corresponding to protein oxidation (1.010 and 0.843 L/g, respectively) [36].

The amount of $^{13}C$-CHO that was oxidized (exogenous CHO oxidation, in grams of glucose per minute) was computed as follows:

$$\text{exogenous CHO oxidation} = \dot{V}CO_2\left[\left(R_{exp} - R_{ref}\right)/\left(R_{exo} - R_{ref}\right)\right]/[k_1 \times k_2]$$

In this equation, (1) $\dot{V}CO_2$ is the production of $CO_2$ at the mouth (L/min, not corrected for protein oxidation), (2) $R_{exp}$ is the observed isotopic composition ($^{13}C/^{12}C$) of expired $CO_2$, (3) $R_{ref}$ (−23.5‰ ± 0.2‰ $\delta^{13}C$ $PDB_1$, n = 36) is the isotopic composition of expired $CO_2$ before ingestion of the meal, (4) $R_{exo}$ is the isotopic composition of the CHO ingested, (5) $k_1$ is the volume of $CO_2$ provided by the oxidation of glucose (0.7426 L/g), and (6) $k_2$ is the fractional recovery at the mouth of the $*CO_2$ produced in tissues. For the measurement of the $^{13}C/^{12}C$ ratio in the pasta ingested, 0.5- to 0.8-g samples were combusted for 60 minutes at 400°C in the presence of copper oxide, and the $CO_2$ recovered was analyzed by mass spectrometry (Prism, Micromass) after cryodistillation. The value of $k_2$ used in the computation of exogenous CHO oxidation, which is a matter of debate (range, 0.43 [3] to 1.0 [11,14,25]), was set at 0.54, as suggested by Schneiter et al [5] and as used in several recent studies [9,10,26,29]. However, the effect of choosing higher values will be discussed. Exogenous CHO oxidation was also corrected for possible contamination of $V^{13}CO_2$ from $^{13}CO_2$ produced from the oxidation of $^{13}C$-proteins, assuming that 31% of the proteins present in the meal (13.5 g/100 g) were oxidized [29,38]. Endogenous CHO oxidation was computed by difference between CHO oxidation, which was calculated by indirect respiratory calorimetry, and exogenous CHO oxidation.

The detailed values of protein, lipid, and total CHO oxidation have been reported elsewhere for each group (men, women in the luteal and follicular phase of the cycle) in the 2 experimental situations (postrest and postexercise) [17]. However, for the sake of the present report, the purpose of which was to compare total and exogenous CHO oxidation, the observations made in the 2 experimental conditions, in women in both phases of the cycle and in men, were pooled (n = 36).

### 2.2. Experiment in rats

The $^{13}C/^{12}C$ in ingested CHO and in liver glycogen stores was compared on adult male Sprague-Dawley rats (312 ± 9 g) (Anilab, Ste-Foy, Québec, Canada). The animals were cared for and handled in accordance with the recommendations from the Animal Care Committee of the Université du Québec à Trois-Rivières, which approved the protocol.

The rats were housed in individual cages in a room maintained at 20°C to 23°C with a 12/12-hour light-dark cycle and were fed ad libitum with regular rat chow (Rat Chow 5012, Agribrands Purina Canada, St Romuald, Québec, Canada) and tap water. One week before the experiment, the rats were accustomed to swim (10 minutes on day 1, 20 minutes on day 2, and 30 minutes subsequently) 4 at a time in a 60 × 90-cm tank filled with 50 cm of water at 37°C. To deplete liver and muscle glycogen stores, the animals were submitted to a 3-hour swimming exercise beginning at 7:00 AM, after an overnight with access to foods poor in CHO (lard). A first group of rats (n = 7) was killed immediately after the exercise session. The other groups (n = 7 each) were deprived of food for the remainder of the day and were then fed overnight with lard (−20.5‰ $\delta^{13}C$ $PDB_1$), $^{13}C$-enriched glucose at 1.7‰ or 97.8‰ $\delta^{13}C$ $PDB_1$ (provided as a 45% solution in water), or corn (−10.2‰ $\delta^{13}C$ $PDB_1$). The glucose derived from corn was purchased from Biopharm (Laval, Québec, Canada) and was artificially enriched in $^{13}C$ using U $^{13}C$-glucose ($^{13}C/C$ >99%, Isotec, Miamisburg, Ohio). The following morning, between 8:00 and 9:00 AM, the animals were deeply anesthetized (sodium

pentobarbitate 50 mg/kg, IP), and the liver was removed, rapidly frozen in liquid nitrogen, and stored at −80°C until analysis. The animals were then killed by sectioning the aorta.

Liver glycogen concentration was determined using the phenolsulfuric acid reaction [39]. Glycogen in liver samples was also hydrolyzed into glucose after homogenization in HCl (0.03 N) by boiling for 150 minutes in HCl (1 N) [40]. After centrifugation at 3000*g*, the supernatant was collected and neutralized with NaOH. The glucose was then isolated by double-bed ion-exchange chromatography, as previously described [19]. For the measurement of $^{13}C/^{12}C$ in the glucose, lard, and corn ingested, as well as in the glucose isolated from liver glycogen in rats, 0.5- to 0.8-g samples were combusted for 60 minutes at 400°C in presence of copper oxide, and the $CO_2$ recovered was analyzed by mass spectrometry (Prism, VG, Manchester, UK) after cryodistillation.

### 2.3. Statistics

Data are reported as mean ± SE. Comparisons were made using 1- or 2-way analysis of variance (ANOVA) for independent or repeated measures, and Tukey post hoc tests, when needed, with $P < .05$ (Statistica package, StatSoft, Tulsa, Okla).

## 3. Results

Table 1 shows urea excretion, protein oxidation, respiratory exchanges, and lipid and total CHO oxidation over the 10-hour observation period after the pasta meal. Exogenous CHO oxidation computed from $\dot{V}CO_2$ and $^{13}C/^{12}C$ in expired $CO_2$ (Fig. 2) with $k_2 = 0.54$ and endogenous CHO oxidation are also shown. An increase in the oxidation rate of total CHO was observed after ingestion of the pasta meal, with a peak in the interval 120 to 240 minutes (Fig. 2). This was because of a high oxidation rate of exogenous CHO, which peaked in the interval 240 to 360 minutes. In contrast, the oxidation rate of endogenous CHO progressively decreased over the observation period and, with $k_2 = 0.54$, was close to zero in the interval 120 to 240 minutes. Beyond 240 minutes, the rate of exogenous CHO oxidation became significantly larger than that of total CHO oxidation, and the rate of endogenous CHO oxidation became negative. Over the 10-hour observation period after the meal, with $k_2 = 0.54$, total CHO oxidation computed from indirect respiratory calorimetry corrected for protein oxidation was significantly 16.8% ± 1.9% lower than exogenous CHO oxidation computed from $V^{13}CO_2$, and the total amount of endogenous CHO oxidized was thus negative (Table 1). As shown in Fig. 2, with increasing value of $k_2$ (from 0.43 to 1.0), the rate of exogenous CHO oxidation decreased for simple arithmetic reasons. For the highest recovery factor of $^{13}CO_2$ at the mouth ($k_2 = 1$), the rate of exogenous CHO oxidation remained lower than that of total CHO oxidation. In contrast for $k_2 = 0.43$ [3], the rate of exogenous CHO oxidation was about twice the rate

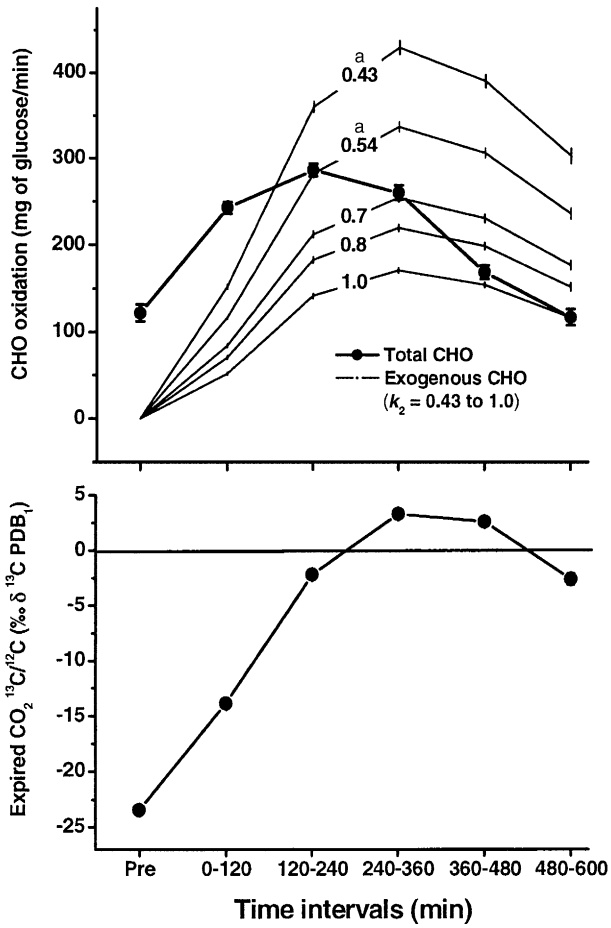


Fig. 2. Changes in $^{13}C/^{12}C$ in expired $CO_2$ (bottom) and rate of total and endogenous CHO oxidation after the $^{13}C$ pasta meal computed with $k_2$ ranging from 0.43 to 1.0 (top): mean ± SE (some error bars lie within the symbols); [a]exogenous CHO oxidation significantly higher than total glucose oxidation; 2-way ANOVA for repeated measures (exogenous vs total glucose, and time intervals), and Tukey post hoc test, $P < .05$.

Table 1
Observations made in the study conducted in humans over the 10-hour period after the $^{13}C$ pasta meal

| | 0-300 min | 300-600 min | Total |
|---|---|---|---|
| Urea excreted (g) | – | – | 14.1 ± 0.5 |
| Proteins (g) | – | – | 40.8 ± 1.4 |
| $\dot{V}O_2$ (mL/min) | 314.2 ± 5.4 | 299.0 ± 4.2 | 306.6 ± 6.0 |
| $\dot{V}CO_2$ (mL/min) | 287.1 ± 4.8 | 258.6 ± 3.8 | 272.9 ± 4.1 |
| Lipids (g) | 7.5 ± 0.6 | 13.3 ± 0.9 | 20.8 ± 1.4 |
| Total CHO (g of glucose) | 78.9 ± 1.7 | 54.6 ± 1.8 | 133.5 ± 3.2 |
| Exogenous CHO (g of glucose) | 70.2 ± 1.1 | 84.0 ± 1.9[a] | 154.2 ± 2.6[a] |
| Endogenous CHO (g of glucose) | 8.72 ± 1.2 | −29.4 ± 1.3[a] | −20.7 ± 1.8[a] |

Exogenous and endogenous CHO oxidation computed with $k_2 = 0.54$. Data are presented as mean ± SE (n = 36).

[a] Exogenous CHO oxidation is significantly higher than total CHO oxidation (1-way ANOVA for repeated measures, $P < .05$), and endogenous CHO oxidation is less than 0.

Table 2
Isotopic composition ($^{13}C/^{12}C$) in the food ingested, liver glycogen content, and $^{13}C/^{12}C$, and percentage of unlabeled carbon atoms ($^{13}C/^{12}C$ = −23.7 ± 0.7‰ $\delta^{13}C$ $PDB_1$) incorporated into liver glycogen in rats

| | Food $^{13}C/^{12}C$‰ $\delta^{13}C$ $PDB_1$ | Glycogen (mg/g) | Glycogen $^{13}C/^{12}C$‰ $\delta^{13}C$ $PDB_1$ | Unlabeled C (%) |
|---|---|---|---|---|
| Fasted | – | 1.5 ± 0.1 | – | – |
| Lard | −20.51 | 4.6 ± 0.7[a] | −23.7 ± 0.7[a] | 100%[a] |
| Glucose | 1.7 | 66.6 ± 5.9[b] | −8.5 ± 0.7[b] | 50.8 ± 5.3[b] |
| Glucose | 97.8 | 57.0 ± 5.2[b] | 36.1 ± 6.5[c] | 40.1 ± 2.9[b] |
| Corn | −10.2 | 97.3 ± 10.0[c] | −16.9 ± 0.9[d] | 49.3 ± 6.9[b] |

Data are presented as mean ± SE (n = 7). Data in a given column with different superscripts are significantly different: 1-way ANOVA for independent measures and Tukey post hoc test, $P < .05$.

of total CHO oxidation beginning at ~300 minutes. The rate of exogenous CHO oxidation was higher than the rate of total CHO oxidation beginning at ~30 minutes and ~420 minutes for $k_2$ = 0.7 and 0.8, respectively.

Table 2 shows liver glycogen content and $^{13}C/^{12}C$ in the different groups of rats. As expected, liver glycogen content was very low after prolonged swimming and remained low when the rats were given a diet poor in CHO. In contrast, liver glycogen stores were high in rats provided for one night with glucose or corn after depletion of liver glycogen stores. The $^{13}C/^{12}C$ in liver glycogen, which was very low when the rats ingested the diet poor in CHO, was higher when $^{13}C$-CHO was ingested. However, the $^{13}C/^{12}C$ in liver glycogen was much lower than that of the CHO ingested, and the difference increased with the level of enrichment of the CHO (6.7 ± 1.0‰, 10.2 ± 0.7‰, and 61.8 ± 6.5‰ $\delta^{13}C$ $PDB_1$, for the corn, and for the glucose enriched at 1.7‰ and 97.8‰ $\delta^{13}C$ $PDB_1$, respectively). This is indicative of a dilution of $^{13}C$ with unlabeled carbon atoms (ie, with a $^{13}C/^{12}C$ close to the background value for $^{13}C$ enrichment, or $R_{back}$). This dilution is such that:

$$(1-x)R_{glu} + xR_{back} = R_{glyc} \quad \text{with } x = (R_{glyc} - R_{glu})/(R_{back} - R_{glu})$$

where $x$ is the fraction of unlabeled carbon atoms in the glucose stored as glycogen, and $R_{glu}$ and $R_{glyc}$ are the $^{13}C/^{12}C$ in exogenous $^{13}C$-CHO and in glycogen stores, respectively. The values of $x$ computed with $R_{back}$ = −23.7‰ $\delta^{13}C$ $PDB_1$ (ie, the $^{13}C/^{12}C$ in glycogen synthesized without CHO ingestion; Table 2) indicate that the percentages of unlabeled carbon atoms incorporated into liver glycogen were not significantly different after ingestion of corn naturally enriched in $^{13}C$ (−10.2‰ $\delta^{13}C$ $PDB_1$) and glucose artificially enriched at 1.7‰ and 97.8‰ $\delta^{13}C$ $PDB_1$.

## 4. Discussion

Results from the present experiment show that when *C-CHO is administered, the combination of indirect respiratory calorimetry corrected for protein oxidation and tracer technique to follow oxidative vs nonoxidative disposal of the CHO load at rest can provide aberrant results, with larger oxidation rate of exogenous CHO than total CHO. This is obviously impossible because there is no way that total CHO oxidation can be lower than exogenous $^{13}C$-CHO oxidation, and this suggests an error in one of the techniques used. The validity of indirect respiratory calorimetry corrected for protein oxidation to follow the oxidation rate of CHO and fat after a meal has been discussed and appears well established [41]. A systematic error in the measurement of respiratory exchanges and urea excretion cannot be totally ruled out in the present study. However, as discussed below, negative values for endogenous CHO oxidation are also found in similar studies from 3 different laboratories. The most likely explanation is thus an overestimation of exogenous $^{13}C$-CHO oxidation computed from $V^{13}CO_2$.

As hypothesized in the Introduction, this phenomenon could be because of the presence of the indirect pathway of glycogen synthesis, in which *C–pyruvate-lactate produced from *C-CHO, probably in the gut and peripheral tissues [42], is converted back into glucose in the liver. In this pathway, *C could be exchanged with C and lost as $*CO_2$ at the entrance of the TCA cycle, in the TCA cycle, as well as in the gluconeogenic pathway (Fig. 1). A first consequence of this phenomenon is that, although glycogen synthesis is actually fueled from the flux of pyruvate-lactate provided by *C-CHO, the glucose and glycogen produced are impoverished in *C vs the CHO administered. The second consequence is that a portion of *C arising from *C-CHO appears under the form of $*CO_2$, increasing $V*CO_2$ and the estimation of oxidative disposal of the CHO load. The loss, dilution, and exchange of *C in the pool of pyruvate-lactate and in the TCA cycle are well-recognized limitations for the quantitative assessment of gluconeogenesis from glucose precursors labeled with *C, such as lactate and alanine (see References [43-45] for reviews). As shown by Newgard et al [35] and by Katz et al [34], the same phenomenon is present when glycogen is synthesized from exogenous *C-glucose through the indirect pathway, which shares a portion of its reactions and intermediates with those in gluconeogenesis: *C/C in the glycogen synthesized is lower than in the glucose administered.

The negative difference observed in the present experiment between total and exogenous CHO oxidation depends on the value of $k_2$, which is a matter of debate (Fig. 2). In previous experiments, the values of $k_2$ that have been used range between 0.43 [3] and 1.0 [11,14,24,25], and sometimes different values have been used in different studies from the same laboratory (0.54 [10], 0.71 [15], 0.81 [13], and 1.0 [25]). However, there is a consensus that the recovery at the mouth of $*CO_2$ produced in tissues is not complete [27]. Higher values of $k_2$ obviously decrease the difference between total and exogenous CHO oxidation. For example, in the present experiment, over the 10-hour period of observation after the pasta meal, the rate of endogenous

CHO oxidation was positive for $k_2 = 1.0$, but this is a very unlikely value for the fractional recovery at the mouth of $*CO_2$ produced in tissues [27].

A reexamination of some previous studies of exogenous *C-CHO oxidation indicates that exogenous CHO oxidation was also larger than CHO oxidation. In the studies by Acheson et al [14] and by Sonko et al [4], although $k_2 = 1$, the oxidation rate of exogenous CHO exceeded by ~5% to ~25% that of total CHO oxidation between the ~6th and 12th hours in the observation periods. In the study conducted by Féry et al [16], exogenous CHO oxidation was ~55% higher than total CHO oxidation (11 vs 7 g per 5 hours). The computation was made with $k_2 = 0.71$, but the difference would remain slightly negative even with the unlikely value of 1 for $k_2$ (7.8 vs 7 g). A negative difference between exogenous glucose and total CHO oxidation could also be computed from the data reported in another study by Féry et al [13] during an hyperinsulinemic-euglycemic clamp in subjects fasting for 4 days. For example, in the group receiving the larger amount of glucose (290 mg · $m^{-2}$ · $min^{-1}$), total glucose oxidation was only 22 mg · $m^{-2}$ · $min^{-1}$, whereas exogenous glucose oxidation computed from plasma glucose oxidation (62 mg · $m^{-2}$ · $min^{-1}$) and the fraction of exogenous glucose in plasma glucose (rate of glucose infusion/glucose Ra: 290/307 = 0.94) amounted to 62 × 0.94 = 58 mg · $m^{-2}$ · $min^{-1}$. In this study, $k_2 = 0.81$, but the difference between exogenous and total CHO oxidation would remain largely negative even with $k_2 = 1$ (47 vs 22 mg · $m^{-2}$ · $min^{-1}$).

Taking together the observations made in the present experiment and this reexamination of some data in the literature provides a support to the hypothesis that $V*CO_2$ overestimates the oxidative disposal of exogenous CHO (and thus underestimates nonoxidative disposal, including glycogen synthesis) because of exchange of *C with C in the pools of pyruvate-lactate and of the TCA cycle intermediates and $*CO_2$ loss when glycogen is synthesized from exogenous *C-CHO through the indirect pathway.

A direct corollary of this phenomenon is that $*C/^{12}C$ in liver glycogen stores synthesized will be lower than that in the CHO administered. As expected, and as already reported by Newgard et al [35] and Katz et al [34], in the study conducted in rats, lower $^{13}C/^{12}C$ was observed in glycogen stores than in the $^{13}C$-CHO ingested. When no CHO was ingested during the night after the prolonged swimming exercise, liver glycogen content only slightly increased (~3 mg/g). This indicates that in the absence of dietary CHO, only a small amount of glycogen could be accumulated in the liver through gluconeogenesis. Accordingly, when corn or glucose was ingested after depletion of liver glycogen stores, it can be estimated that ~93% to 95% of the liver glycogen accumulated derived from exogenous CHO. The lower $^{13}C/^{12}C$ observed in the glycogen stores than in the $^{13}C$-CHO ingested indicates that a large percentage of glycogen synthesis took place through the indirect pathway. In addition, the percentage of $^{13}C$ incorporated into glycogen was independent from the level of enrichment of the ingested CHO and not significantly different in the 3 experimental situations (40%-50%). These observations are well in line with those reported by Newgard et al [35] and Katz et al [34] in support to the hypothesis that after glucose administration, a large portion of liver glycogen was synthesized after conversion of glucose into lactate pyruvate. This mechanism is the basis for the hypothesis suggested in the present study that the *C not recovered in glycogen stores appears as $*CO_2$ and, accordingly, that $V*CO_2$ at the mouth after administration of *C-CHO overestimates *C-CHO oxidation.

In conclusion, results from the present study indicate that $V*CO_2$ production, even when corrected for the incomplete recovery at the mouth of $*CO_2$ produced in tissues, does not adequately track oxidation vs storage of exogenous *C-CHO administered at rest. This is probably because of the presence of the indirect pathway for glycogen synthesis, along which a portion of *C is exchanged with unlabeled carbon atoms and lost, whereas $*CO_2$ is produced. These phenomena lead to an overestimation of exogenous CHO oxidation and thus an underestimation of glycogen synthesis and of endogenous CHO oxidation.

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