Glucose Naturally Labeled with Carbon-13: Use for Metabolic Studies in Man

Abstract. *The ratio of carbon-13 to carbon-12 is much higher in most commerical preparations of glucose used for oral glucose tolerance tests than it is in carbon dioxide in expired air. This recent discovery provided a novel and potentially significant means of studying glucose metabolism. The changes in the ratio of carbon-13 to carbon-12 in carbon dioxide expired after oral glucose administration were determined by mass spectrometry. In six healthy male volunteers, the administration of glucose resulted in a marked, reproducible rise in the isotopic ratio in expired carbon dioxide; the ratio reached its maximum at 4 hours and then declined progressively.*

The isotopic ratio $^{13}C/^{12}C$ in CO_2 in the air breathed out by higher animals is markedly affected by phylogenetic characteristics, type of diet, and metabolic conditions, as demonstrated first by Duchesne and coworkers (*1*, *2*) and confirmed by Jacobson *et al.* (*3*).

Thanks to the discovery of a glucose of particularly high $^{13}C/^{12}C$ value, a method for following the metabolism of glucose to CO_2 has been developed. First applied to the rat (*2*), this method, using naturally labeled [^{13}C]glucose as a tracer, has now been extended to man. This report presents initial results obtained with normal human volunteers subjected to oral glucose tolerance tests (OGTT). The results reported here demonstrate the potential significance of this simple procedure, which can be readily performed in an appropriately equipped laboratory.

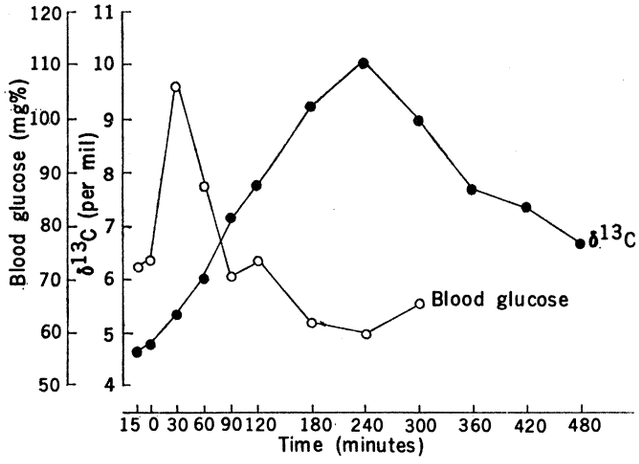
Six healthy human male volunteers, age 20 to 32 years, gave formal consent to the present study. All were of normal body weight according to standard criteria (*4*), and none had a family history of diabetes or postprandial glycosuria. Prior to testing, the subjects were fed a diet providing a minimum of 250 g of carbohydrate per day for at least 3 days. All subjects fasted overnight before the test. In the standard OGTT, 100 g of glucose (anhydrous glucose, Ludeco, Brussels) was administered in 400 ml of water. Blood samples were obtained from an indwelling catheter in an antecubital vein 15 minutes before glucose ingestion, at the time glucose was given, and 30, 60, 90, 120, 180, 240, and 300 minutes thereafter. Blood glucose was determined by the method of Hoffman (*5*) adapted to the Technicon AutoAnalyzer, and plasma insulin was measured by the method of Quabbe (*6*) with human insulin as standard. Plasma free fatty acids (FFA) were determined by the method of Dole and Meinertz (*7*). All determinations were made in duplicate. Samples of expired air were collected into 1-liter rubber balloons 15 minutes before glucose was given, at the time of glucose ingestion, and 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 minutes afterward. Water and Co_2 were separated from the air by trapping in liquid nitrogen by means of a vacuum pump. The CO_2 was then evaporated into the apparatus, while water remained trapped in a mixture of methanol and Dry Ice (*8*). The isotopic ratios of isolated CO_2 samples were determined with a Varian MAT-CH5 double-collector mass spectrometer. To improve the sensitivity of measuring $^{13}C/^{12}C$ in samples that contained very small quantities of ^{13}C, a standard source prepared from commercial CO_2 (Air Liquide, Liège, Belgium) was used for comparison (*9*). Therefore, $^{13}C/^{12}C$ values are reported relative to the standard according to the formula of Craig (*10*):

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

($\delta^{13}C$ in per mil). All determinations were made in quadruplicate. The standard deviation based on determinations under these conditions was 0.1 per mil. Isotopic ratios were determined for several glucose preparations available commercially (Table 1). For this purpose, the organic carbon of the samples was converted into CO_2 by combustion at 900°C under an atmosphere of pure O_2 and was then treated as described.

The OGTT resulted in a rise in blood glucose and plasma insulin concentrations and a fall in plasma FFA concentration (Table 2), all changes being within normal limits for subjects without any abnormality in glucose tolerance (*11*). The $\delta^{13}C$ in control samples of expired air taken after an overnight fast averaged 4.8 ± 0.2 per mil (standard error of mean), with a range of 3.6 to 5.7 per mil. In all subjects, the administration of glucose resulted in a marked rise in $\delta^{13}C$ of expired air, which reached its maximum at 4 hours. It then declined progressively, although failing to return to control values even 8 hours after the beginning of the OGTT (Fig. 1). The reproducibility of these changes in $\delta^{13}C$ in expired air in two subjects is illustrated in Fig. 2.

The $\delta^{13}C$ of CO_2 in expired air of



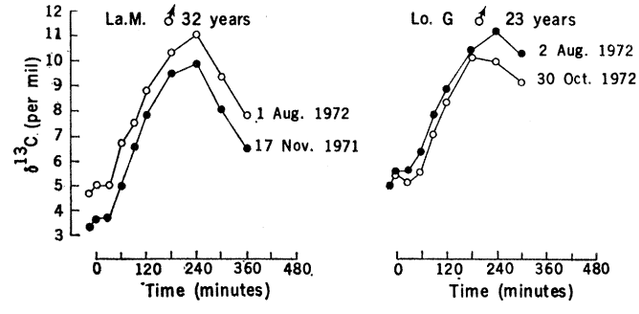


Fig. 1 (left). Changes in blood glucose (open circles) and expired air $\delta^{13}C$ (closed circles) after oral administration of glucose at zero time (mean of six subjects). Fig. 2 (right). Reproducibility of the changes in $\delta^{13}C$ in expired air after oral glucose administration in two subjects.

Table 1. The $\delta^{13}C$ in several preparations.

Preparation	Origin	$\delta^{13}C$* (per mil)
Anhydrous glucose	Merck, Darmstadt, Germany	18.5 ± 0.3
Anhydrous glucose	Glucoseries Réunies, Alost, Belgium	18.4 ± 0.3
Anhydrous glucose	Ludeco, Brussels, Belgium	17.9 ± 0.3
Raw cane sugar		15.1 ± 0.3
Crystallized saccharose (beet sugar)	Raffineries Tirlemontoises, Tirlemont, Belgium	4.0 ± 0.3

* Mean ± standard deviation (four determinations). The deviation is calculated for the whole procedure including combustion.

normal human subjects after an overnight fast, about 5 per mil, corresponds to an absolute isotope abundance of 1.085 percent for ^{13}C. For most preparations of glucose commercially available, the $\delta^{13}C$ value averaged 18 per mil, a value corresponding to a relative ^{13}C concentration of 1.100 percent. A variation in $\delta^{13}C$ of 1 per mil corresponds to a change of approximately 1×10^{-5} in the relative concentration of ^{13}C atoms. Because of the great sensitivity of double-collector mass spectrometers, such small differences can be detected with accuracy. The particularly high concentration of ^{13}C atoms in some natural sugars is probably related to an isotopic effect occurring during photosynthesis in certain types of plants. Because of a selective isotopic effect (*12*), plants in which photosynthesis proceeds according to the Calvin pathway (*13*) have relatively less ^{13}C than do those in which it proceeds by the dicarboxylic acid pathway described by Hatch and Slack (*14*); the latter plants are less common. As demonstrated by Smith and Epstein (*15*), the $\delta^{13}C$ in cane and maize is higher than that in beet, in accordance with their respective photosynthetic pathways. We made use of this property of variable natural enrichment in ^{13}C of plant sugars, used for the commercial preparation of glucose, as the basis of a new test that permitted us to follow the metabolism of glucose in man. Thus, glucose used for the OGTT was prepared industrially from maize starch, and therefore had a ^{13}C content higher than that of common foods (including meat) derived from plants in which photosynthesis generally follows the Calvin pathway. The fact that beet sugar is more widely consumed than maize or cane sugar in most West European countries contributed to the relatively low control $\delta^{13}C$ in expired air and facilitated our experimental procedure. The use of this characteristic for metabolic studies in man has not been reported previously. The use of compounds artificially enriched in ^{13}C (in contrast with naturally occurring compounds) for metabolic studies has recently been planned (*16*). The rise in $^{13}C/^{12}C$ values after oral glucose administration in our experiments might reflect not only the increased utilization of exogenous glucose but also the reduced utilization of lipids, which have a slightly lower ^{13}C content than carbohydrates and proteins (*2, 17*). This is supported by the fall in plasma FFA; the metabolism of these compounds is generally accepted to be proportional to their concentrations (*18*). Nevertheless, data for the rat, (*2, 8, 17*) as well as initial observations in man during total starvation (*8*), indicate that variations in expired air $\delta^{13}C$ due to alterations in lipid oxidation do not exceed 1 to 2 per mil. Recent studies in our laboratories demonstrate the potential of this new procedure for the study of glucose metabolism in human diabetes (*19*). It will be interesting to examine the influence of hormones (*2*) such as insulin or glucagon, and of pharmacological compounds such as sulfonylureas or biguanides.

Table 2. Changes in blood glucose, plasma FFA, plasma insulin, and expired air $\delta^{13}C$ induced by oral administration of glucose at zero time in six normal subjects. Results are given as mean ± standard error of mean.

Time (minutes)	Blood glucose (mg/100 ml)	Plasma FFA (μeq/liter)	Plasma insulin (μunit/ml)	Expired air $\delta^{13}C$ (per mil)
− 15	72.8 ± 2.1	690 ± 52	12.4 ± 2.7	4.7 ± 0.3
0	74.6 ± 2.2	630 ± 66	12.9 ± 1.2	4.9 ± 0.3
+ 30	106.8 ± 5.5*	526 ± 33	42.8 ± 10.7‡	5.4 ± 0.4
+ 60	88.0 ± 9.8*	397 ± 28†	42.9 ± 9.5‡	6.1 ± 0.3‡
+ 90	70.8 ± 5.2	349 ± 44*	42.5 ± 11.8‡	7.2 ± 0.3*
+ 120	74.5 ± 4.2	303 ± 19*	32.0 ± 3.9*	7.8 ± 0.5*
+ 180	62.5 ± 8.0	382 ± 41†	23.3 ± 4.0‡	9.3 ± 0.5*
+ 240	60.2 ± 3.1*	725 ± 201	11.8 ± 1.5	10.1 ± 0.5*
+ 300	66.3 ± 3.5	767 ± 112	11.1 ± 2.8	9.0 ± 0.5*
+ 360				7.7 ± 0.5*
+ 420				7.4 ± 0.6*
+ 480				6.7 ± 0.4†

* $P < .01$ compared to zero time value. † $P < .02$. ‡ $P < .05$.

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References and Notes

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