Alteration of CO_2 Production During Nonfasting Isotopic CO_2 Breath Tests: Concise Communication

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Carbon isotope breath tests are often interpreted assuming a constant endogenous production of CO_2 (some including calculations assuming a specific production of 9 mmol CO_2/body weight per hour). We have evaluated the endogenous-CO_2 production following ingestion of caloric meals varying with the range of most currently available carbon isotope breath tests. On three separate test days, fasting basal CO_2 production was 8.08 ± 0.55 , 8.00 ± 0.47, and 8.23 ± 0.48 mmol/kg-hr (mean ± s.e.m.), with a range 6–11 mmol/kg-hr. Administration of zero and 100 kcal led to no significant change from the basal CO_2 production. In contrast, administration of 200 kcal or more led to significant elevation of endogenous CO_2 production both by normal subjects and by subjects with nutrient malabsorption. This phenomenon could influence interpretation of some nonfasting isotopic CO_2 breath tests; it deserves further evaluation.

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Breath analysis for isotopic CO_2 (i.e., C-13 or C-14) following the administration of substrates labeled with isotopic carbon affords noninvasive testing of events occurring in the gastrointestinal lumen or of metabolic events following gastrointestinal absorption or parenteral administration (*1*,*2*). As noted in recent reviews of the use of breath analysis in gastroenterology, this technique allows evaluation of such diverse states as bacterial overgrowth in the small intestine, ileal malabsorption of bile salts, metabolism of fat following intestinal absorption, and hepatocyte function (*1–4*). In clinical use (and often in research using breath analysis) a constant endogenous CO_2 production of 9 mmol CO_2 per kg body weight per hour is assumed, against which changes in expired labeled CO_2 are analyzed (*2–4*). Use of this assumption eliminates actual measurement of total CO_2 of the sample, simplifying the procedure for clinical use. However, concern over the validity of this assumption has arisen because of the known variation of CO_2 production with exercise, and because the original determination of this constant was made by studying thirteen fasting healthy subjects, who themselves had a range of CO_2 production varying from approximately 7 to 12 mmol/kg-hr (*5*,*6*). This concern over possible variation in endogenous CO_2 production may be one of the reasons why breath analysis as a time- and expense-saving diagnostic procedure has not received as wide a clinical use as it deserves. In the present study we have evaluated the endogenous-CO_2 output of individuals administered caloric loads varying within the range of those used during most breath tests currently available for clinical use.

MATERIALS AND METHODS

All studies were carried out under carefully controlled conditions in a clinical research ward. Informed consent was granted by all subjects, and all investigations were approved by the University Health Center's Committee For The Protection of Human Subjects. Ten fasting subjects with malabsorption of several kinds were administered, on three separate and random days, one of the three test meals containing zero, 100, or 750 kcal. In addition, six normal subjects without malabsorption were

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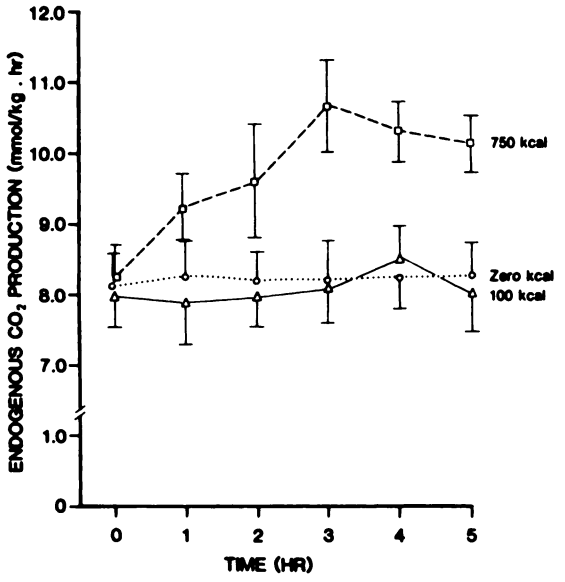

FIG. 1. Endogenous-CO_2 production (mean ± s.e.m.) of ten subjects before, and at hourly intervals following, administration of the three "test meals" of zero, 100, and 750 kcal, the latter as 200 kcal at time zero and 550 kcal at 2 hr. Endogenous-CO_2 production increased significantly with administration of 200 kcal or more ($p < 0.01$ at 2 hr, $p < 0.001$ after 2 hr).

administered the zero- and the 750-kcal meal. The zero and 100 kcal doses were in the form of 1 g and 25 g of D-xylose, respectively, administered in 500 ml of water (*7*). The 750-kcal meal was in the form of liquid nutrient administered as 200 kcal at time zero and 550 kcal 2 hr later, which is similar to the protocol that we and others use, during a bile-acid breath test, to stimulate flow of endogenous bile acids from the biliary tree (*8–10*). Using the drum displacement technique, we made a timed spirometric collection of exhaled breath in the basal state and every 30 min for 5 hr after ingestion of the test meal. Percentage of carbon dioxide in the specimen was determined in an infrared carbometer. Standard CO_2 gas for calibration of the carbometer was analyzed manometrically with the Scholander 0.5 ml apparatus (*11*). All data were evaluated with the paired Student's *t*-test (*12*).

RESULTS

Fasting basal endogenous CO_2 production for the ten subjects with malabsorption (three separate test days) and for the six normal subjects was similar, averaging between 8 and 8.2 mmol CO_2/kg body weight per hr. Range of the basal endogenous CO_2 production (6.0–11.0 mmol/kg-hr, Fig. 1) was similar to that of normal subjects studied by Winchell (*6*). On test days on which zero and 100 kcal were administered, no significant change from the basal level of CO_2 output was seen. In contrast, administration of 200 kcal, for the first part of the 750-kcal meal, led to a significant increase ($p < 0.01$) in endogenous CO_2 within 2 hr, compared with the basal state for the same test day or with the same time period of the other two test days (Fig. 1). Administration of an additional 550 kcal (after the 2-hr determination) led to an additional rise in endogenous CO_2 production, with a mean peak increase of 30% over the basal state ($p < 0.001$) in paired comparison with both the basal state and the endogenous CO_2 production during the third hour of testing on the other two test days.

A similar level of basal CO_2 production and a significant increase with the administration of the 750-kcal, but not zero-kcal meal, was seen in a separately studied group of normal controls (Fig. 2). At no point after the administration of the 750-kcal or zero-kcal meals was there a significant difference between the normal group and the group with malabsorption given the same meal. Thus the change in endogenous CO_2 production seen with the administration of 200 kcal or more probably involves intraluminal factors (e.g., secretion by the stomach, pancreas, and/or small intestine; acid-base reactions; etc.) as well as possible postabsorptive metabolism of the meal.

DISCUSSION

The use of breath excretion of volatile carbon isotopes following the administration of substrates labeled with isotopes of carbon has the potential to simplify the time-consuming and expensive evaluation of certain gastrointestinal disorders (*1–4*). The present study validates the simplifying step of assuming a constant endogenous CO_2 production during those tests in which limited calories are administered either before or as part of the test. We did note that significant elevation of endogenous CO_2 occurs when 200 kcal or more were administered. Any rise in endogenous CO_2 production would tend to dilute the pool of isotopic CO_2 produced as a result of metabolism of the labeled substrate. This dilution of labeled CO_2, if unaccounted for by actual

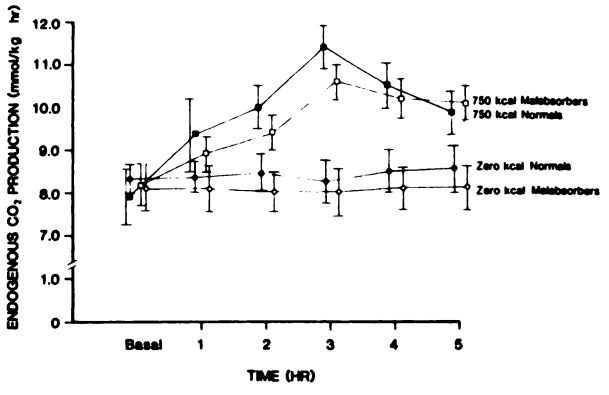

FIG. 2. Comparison of endogenous-CO_2 production (mean ± s.e.m.) of six normal subjects and ten subjects with malabsorption, given both a zero-kcal and a 750-kcal test meal. No significant difference between subject groups for any time period occurred with either of the two test meals. Variance from basal endogenous-CO_2 production with 750-kcal regimen was similar to that shown in Fig. 1.

measurement of total CO_2 output, would tend to blunt the distinction between normal and abnormal isotopic CO_2 concentrations, or of calculated (as opposed to measured) isotopic CO_2 output. The potentially reduced difference between concentrations of exhaled CO_2 in normals as against abnormals is shown graphically in Fig. 3, in which hypothetical curves are plotted with the "assumed" constant-CO_2 curves reflecting a degree of dilution of isotopic CO_2 approximately equal to the percentage rise of endogenous CO_2 at the various time points plotted in Fig. 1. Note that the two "assumed" curves in Fig. 3 are closer together than the two hypothetical "measured" curves—the end point of this concept being a diminished sensitivity for separating normals from abnormals.

Both the individual variability of basal endogenous CO_2 production, and the significant rise of endogenous CO_2 when 200 kcal or more are administered, are factors that potentially could alter the sensitivity of a carbon isotope breath test. In our recent study comparing the C-14 xylose and C-14 bile-acid breath tests in detecting small-intestinal bacterial overgrowth, failure to account for changes in endogenous CO_2 production during the bile-acid breath test would have led to misinterpretation of one out of twelve tests, adding to the already present 30% false-negative rate (*10*). Likewise, variation of isotopic CO_2 concentration, occurring with a change in the carrier meal, has been noted in two studies evaluating use of $^{14}CO_2$ analysis as a measure of absorption of C-14-labeled triglyceride (*13,14*). Although this change could have been related to real changes in the production rate of isotopic CO_2 (for example, due to delayed gastric emptying, small-bowel absorption, and/or hepatic metabolism of the labeled meal), it could also have been related to the occurrence of increased endogenous CO_2 production with increased caloric intake, as noted in the present report.

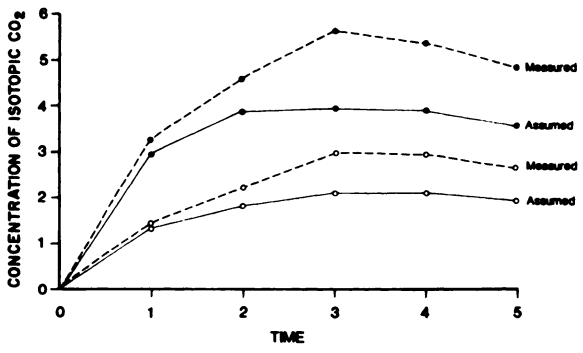

FIG. 3. Graph showing effect that unaccounted endogenous-CO_2 increases would have on labeled-CO_2 concentrations. Values for hypothetical "measured endogenous CO_2" mean have been diluted by same approximate percentages as seen with endogenous CO_2 increase at various time periods for the 750-kcal meal in Fig. 1, to generate hypothetical "assumed constant CO_2" curves. Upper and lower sets of curves represent hypothetical normal and abnormal populations. The closer opposition of two "assumed" curves, contrasted with the two "measured" curves, would correlate with a greater tendency for overlap of normal with abnormal values, and thus lower sensitivity of test.

Although variability of endogenous-CO_2 production during a nonfasting carbon isotope breath test may be partially allowed for in the establishment of the normal limits of that test, the effect that this variation has on the test, and the diagnostic capability of labeled CO_2 concentration (as contrasted to measured isotopic CO_2 output), should preferably be part of the validation procedure for that test (*7*). Some tests may require actual total-CO_2 measurement as part of the procedure. This validation is especially important for the generation of confidence in use of these time- and expense-saving tests, particularly since the use of more expensive stable-isotope breath tests is being contemplated to extend the benefits of radiocarbon breath analysis to children and to women in the reproductive age (*1*).

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