of this dissimilarity in the primary structure and physical properties of these CS samples. The pharmacokinetic properties of a proprietary CS were investigated by Conte *et al.* (1995). Significant extraction procedures were utilized to generate a low-molecular weight product that could be characterized for structure, physiochemical properties, and purity. Only a fraction with a relative molecular weight of about 14 kDa was used in their experiments. This fraction showed a sulfate-to-carboxyl ratio of 0.95 due to the high percentage of monosulfated disaccharide sequences [55% CS-A (4-O-sulfonated GalNAc) and 38% CS-C (6-O-sulfonated GalNAc)], and a low amount of disulfated disaccharide sequences (1.1%). The purity of the preparation was greater than 97% CS. This sample was radioactively labeled and orally administered to the rats and dogs. Although more than 70% of the radioactivity was absorbed and was subsequently found in urine and tissues, the radioactivity associated with an intact molecule of CS corresponding to the molecular mass of the administered CS was relatively small (approximately 8.5%), and this percentage decreased rapidly over time. The majority of the radioactivity absorbed was actually associated with molecules with a molecular mass of less than or equal in size to GalNAc residues. This radioactivity increased over time and remained elevated. Radioactivity after 24 h was highest in the small intestine, liver, and kidneys (tissues responsible for the absorption, metabolism, degradation, and elimination of the compound); however, relatively high amounts of radioactivity were also found in tissues, which utilize amino sugars such as joint cartilage, synovial fluid, and trachea. This group also orally administered CS to healthy volunteers in either a single daily dose of 0.8 g or in two daily doses of 0.4 g. Although both dosing schedules increased plasma concentration of exogenous molecules associated with CS, the results have indicated that the oral administration of one dose of 0.8 g CS was the more effective regimen. Some physiological parameters associated with GAGs, such as hyaluronan, were also analyzed to investigate whether orally administered exogenous CS affects synovial fluid in patients with OA. The results indicated that the treatment with CS might also modify these parameters. Thus, despite all of these studies, the oral bioavailability and efficacy of CS remains controversial. However, the majority of physiological benefits subsequent to administration of CS appears to be a direct result of increased availability of the monosaccharide/disaccharide residues of CS produced by the action of enzymes found in the intestine (Hong *et al.*, 2002).

V. A New Concept for Explaining the Effect of Chondroitin Sulfate in Arthritis Treatment

Polysaccharides, such as CS, are clearly poorly absorbed through the digestive system. Moreover, we have shown that the half-life of CS in the circulatory system is 3–15 min, based on the pharmacokinetic study of

intravenously administered CS (Sakai *et al.*, 2002b). Accordingly, it appears unlikely that orally administered CS is systemically distributed to connective tissues, such as cartilage and skin, and that exogenously administered CS directly stimulates chondrocyte synthesis of extracellular matrix components. This suggests that the mechanism of action of exogenously administered CS might be mediated from within the intestinal tract by other systems, such as the immunological system (Wrenshall *et al.*, 1999). Our laboratory has already shown that CS affects and upregulates the *in vitro* antigen-specific Th1 immune response on murine splenocytes sensitized with ovalbumin (OVA) and that CS suppresses the antigen-specific IgE responses (Sakai *et al.*, 2002a). These findings also suggest a therapeutic use of CS to control the IgE mediated allergic response. The number and position of O-sulfo groups varies among CS samples obtained from different sources (Alves *et al.*, 1997; Farias *et al.*, 2000; Santos *et al.*, 1992). We hypothesized that the immunological activity of orally administered CS might also be different among the several types of CS. Thus, it is important to determine the structure–activity relationship (SAR) of CS, particularly with respect to the number and position of O-sulfo groups in CS. Knowledge of the SAR of CS will be necessary to further explore its effective use as a therapeutic agent.

It is generally accepted that CD4+ T cells are subpopulations containing 2 cell types (Th1 and Th2), based on their different patterns of cytokine secretion (Mossmann and Coffman, 1989a,b). Th1 cells secrete IFN-γ, IL-2, and IL-12. Th2 cells produce IL-4, IL-5, and IL-10. IFN-γ and IL-12 induce the differentiation of Th0 cells to Th1 cells, whereas IL-4 induces the differentiation to Th2 cells (Fig. 2). Therefore, it is believed that an increase

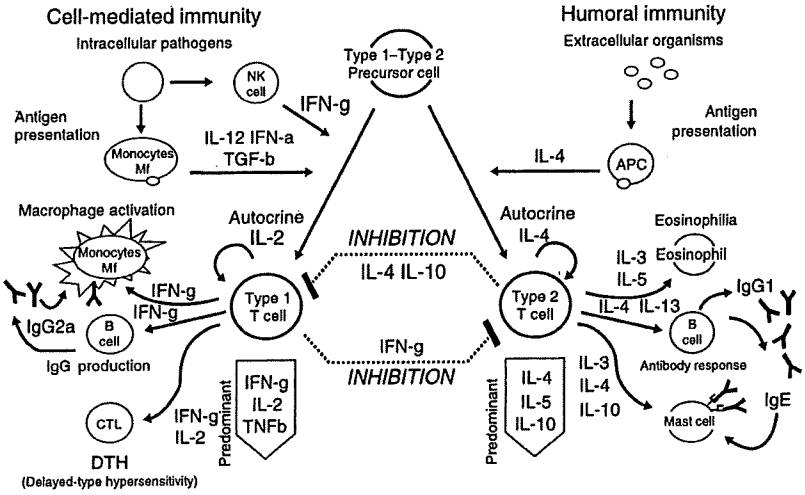

FIGURE 2 Overview of Th1/Th2 balance.

in IFN-γ and IL-12 shifts the Th1/Th2 cell balance to predominantly Th1, while an increase in IL-5 and IL-10 shifts the balance to predominantly Th2 (Akiyama *et al.*, 1999; Nagafuchi *et al.*, 2000). We have previously reported that CS induced Th1-type cytokine (IFN-γ, IL-2, and IL-12) secretion but suppressed Th2-type cytokine (IL-5 and IL-10) secretion by the OVA-sensitized splenocytes (Sakai *et al.*, 2002a). We have also already shown that both O-sulfo group content and position in CS is important for the Th1-promoted activity of murine splenocytes, in terms of the cytokine production and Th1/Th2 balance (Akiyama *et al.*, 2004). We first examined whether the activity was associated with the O-sulfo groups in CS and confirmed that the sulfation of a polysaccharide has played an important role in the activity. We have reported that CS induced the Th1-promoted activity while dextran, a neutral polysaccharide used as a control, did not (data not shown). In contrast, dextran sulfate also did not show significant effects on cytokine production by murine splenocytes (Fig. 3). These results indicate that the polysaccharide type and sulfation is critical for the Th1-promoted activity (Akiyama *et al.*, 2004). We subsequently showed the effect of the level of sulfation number and position of CS on the Th1-promoted activity (Akiyama *et al.*, 2004). While fully sulfonated CS exhibits Th1-promoted activity, intact CS-A and the partially O-sulfonated CS demonstrate higher activity CS (Fig. 1). These results strongly suggested that excess sulfo groups in CS could decrease the Th1-promoted and Th2-inhibitory activities of CS. Among the monosulfated CS, CS-A, -C, and -B, the CS-A sample showed highest activity (Figs. 1 and 3). This result suggested that the [-4)GlcA(β1–3)GalNAc4S(β1-]$_n$ sequence is more important for activity than the [-4)GlcA(β1–3)GalNAc6S(β1-]$_n$ or [-4)IdoA(β1–3)GalNAc4S(β1-]$_n$ sequences characteristic of CS-C and -B, respectively (Fig. 1). Chondroitin sulfate-B [dermatan sulfate (DS)], while nearly structurally identical to CS-A (it contains IdoA instead of GlcA), shows lower activity. This is surprising as the greater flexibility of the IdoA residue in CS-B is commonly used to explain the propensity of IdoA-containing GAGs to interact with proteins and display a large number of different biological activities (Kawashima *et al.*, 2002). Examination of the disulfated CS samples shows that the effects of CS-E on the Th2-inhibitory activity were higher than those of CS-D or -A (Fig. 3, also see structures shown in Fig. 1). These results suggested that the [-4)GlcA(β1–3)GalNAc4S6S(β1-]$_n$ sequence in CS-E is more important for high activity than the [-4)GlcA2S(β1–3)GalNAc6S(β1-]$_n$ sequence characteristically found in CS-D. Furthermore, these experiments demonstrate that the [-4)GlcA(β1–3)GalNAc4S(β1-]$_n$ and [-4)GlcA(β1–3)GalNAc4S6S(β1-]$_n$ sequences in CS are more critical for higher activity. Researchers have reported many biological activities for sulfated polysaccharides (Chaidedgumjorn *et al.*, 2002; Koyanagi *et al.*, 2003; Linhardt and Toida, 1997; Toida *et al.*, 1999). In most cases the number of sulfo groups in the polysaccharide directly correlates with the level of bioactivity (Chaidedgumjorn *et al.*, 2002; Koyanagi *et al.*, 2003; Toida *et al.*,

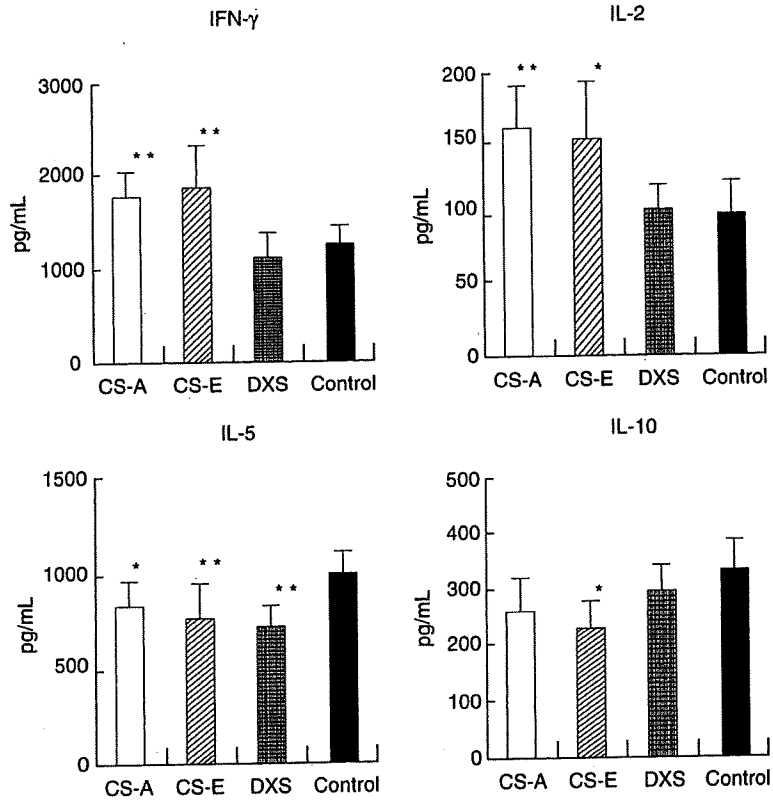

FIGURE 3 Effect of CS on the cytokine production of murine splenocyte *in vitro*. BALB/c mice ($n = 5$) were intraperitoneally injected on day 0 and 13 with 20 μg of ovalbumin (OVA) and 2 mg of $Al(OH)_2$ at a total volume of 400 μl. Spleen cells (5.0×10^6 cells/ml) were collected on day 14 and were cocultured with OVA (final 100 μg/ml). The amounts of cytokines in the supernatant were measured by ELISA. Asterisk indicates significance of difference from control value ($*p < 0.05$, $**p < 0.01$). Bars represent mean values (±S.D.) for six wells.

1999). Koyanagi *et al.* (2003), have shown that by increasing the number of sulfo groups in fucoidans (sulfonated fucans), its antiangiogenic and antitumor activities can be potentiated. Our laboratory has also reported the many biological activities of the chemically fully sulfated poly- and oligosaccharides (Chaidedgumjorn *et al.*, 2002; Suzuki *et al.*, 2001; Toida *et al.*, 1999, 2000). Chondroitin sulfate has been found in many tissues (Suzuki *et al.*, 1968) and cells (Ohhashi *et al.*, 1984; Petersen *et al.*, 1999; Stevens *et al.*, 1988), and has been reported to interact with various biologically important molecules and regulate their functions. We have demonstrated the importance of the content, position, and number of O-sulfo groups in

CS for immunological activity of the OVA-stimulated murine splenocytes *in vitro* (Akiyama *et al.*, 2004; Sakai *et al.*, 2002a). It was also shown that Th1-promoted and Th2-inhibitory activity of CS on murine splenocytes could be associated with binding to L-selectin. It has been reported that a large CS/DSPG interacts through its CS/DS chains with the adhesion molecules L- and P-selectin, CD44, and chemokines. Kawashima *et al.* (1999, 2000) and others (Capila and Linhardt, 2002; Hirose *et al.*, 2001) reported that oversulfated CS/DS, containing [-4)GlcA(β1-3)GalNAc4S6S(β1-]$_n$ sequences, interacts with L-selectin, P-selectin, and chemokines. Our findings may indicate that these same [-4)GlcA(β1-3)GalNAc4S6S(β1-]$_n$ sequences in CS would be associated with the strongest effects on the promotion of the Th1-type cytokine production and the inhibition of the Th2-type cytokine production. The present structural characterization of CS to Th1-promoted, and Th2-inhibitory activity is consistent with the high-affinity binding of CS, containing the [-4)GlcA(β1-3)-GalNAc4S(β1-]$_n$ and [-4) GlcA(β1-3)GalNAc4S6S(β1-]$_n$ sequences, to L-selectin (Kawashima *et al.*, 2002). These findings also may support our hypothesis that such an immunological activity could be associated with the binding of CS-A to L-selectin on T cell surface. These results also may indicate that differences in the content and position of O-sulfo groups in CS could markedly influence Th1-promoted and Th2-inhibitory activities, as do differences between GlcA and IdoA residues in CS. In these observations, however, the inhibition of CS binding to L-selectin could not be shown by FACS analysis using labeled antiL-selectin monoclonal antibody (Akiyama *et al.*, 2004). This result may suggest that the epitope region on L-selectin to antiL-selectin monoclonal antibody might not be located in the lectin region that binds to CS. We have not yet established the relationship between the various CS samples from natural products and the immunological activities. Thus, further studies are required on the relative L-selectin binding affinity of different types of CS to fully elucidate the importance of L-selectin-CS binding. The effect of heparin was also examined on Th1/Th2 balance and found to demonstrate the same level of activity as CS at identical doses (results not published). We are considering future studies to assess the effects of heparin and its derivatives on these activities to fully elucidate the SAR of GAG.

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The American Journal of Clinical Nutrition

Availability, fermentability, and energy value of resistant maltodextrin: modeling of short-term indirect calorimetric measurements in healthy adults[1–3]

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ABSTRACT

Background: Determination of the metabolizable (ME) and net metabolizable (NME) energy of total carbohydrate requires estimation of its available (AC) and fermentable (FC) carbohydrate content. Modeling of indirect calorimetric observations (respiratory gas exchange) and breath hydrogen would appear to make it possible to estimate noninvasively these nutritional quantities and the approximate time-course of availability.

Objective: We assessed the time-course of metabolism and energy availability from resistant maltodextrin (RMD) by modeling of respiratory gases after a single oral dose.

Design: Seventeen healthy adults (13 M, 4 F; aged 25–46 y) were randomly assigned to treatments (water, maltodextrin, or RMD) in a multiple-crossover, single-blinded trial with ≥7 d washout. We monitored 8-h nitrogen-corrected oxygen and carbon dioxide exchanges and breath hydrogen. All treatment groups took low-carbohydrate meals at 3 and 6 h.

Results: Indirect calorimetry alone provided only qualitative information about the nutritional values of carbohydrate. In contrast, modeling of gaseous exchanges along with the use of central assumptions showed that 17 ± 2% of RMD was AC and 40 ± 4% was FC. As compared with 17 kJ gross energy/g RMD, mean (±SE) energy values were 7.3 ± 0.6 kJ ME/g and 6.3 ± 0.5 kJ NME/g. The fiber fraction of RMD provided 5.2 ± 0.7 kJ ME/g and 4.1 ± 0.6 kJ NME/g.

Conclusions: Modeling with the use of this noninvasive and widely available respiratory gas–monitoring technique yields nutritional values for carbohydrate that are supported by enzymatic, microbial, and animal studies and human fecal collection studies. Improvement in this approach is likely and testable across laboratories. *Am J Clin Nutr* 2006;83:1321–30.

KEY WORDS Indirect calorimetry, available carbohydrate, fermentable carbohydrate, energy value, modeling, humans

INTRODUCTION

Dietary fibers can contribute appreciably to the availability of energy from foods. This capability has nutritional importance and also receives interest from the food industry (1). Variation in the fermentability of different fiber sources has led to the recommendation of specific food energy conversion factors for added, novel, or functional fibers intended for specific dietary needs related to energy requirements, weight control, and regulation via food codes (2). Evaluation of energy availability from fiber in humans has resulted in the successful application of detailed indirect calorimetry (IDC) to quantify heat production (1, 3–6). This quantification shows a predictable rise in heat production that results from fermentation in humans, as occurs in animals. The rise is greater than that for available carbohydrate (AC), and, consequently, fiber has a net metabolizable energy (NME) value that is less than its metabolizable energy (ME) value (1, 2). Whether IDC can also be used to assess carbohydrate availability or fermentability (or both), from which energy values would be calculable by using established general energy conversion factors (2), is a question left open by studies conducted to date.

To gain insight into this possibility and to compare the human response in the short term to resistant maltodextrin (RMD) and maltodextrin (MD), we monitored carbohydrate oxidation by using the well-recognized noninvasive IDC technique (1,7). Stored glycogen is also calculable as AC intake in excess of oxidation. We described the principles of IDC elsewhere along with details of the corrections needed when different types of carbohydrate, fat, protein, or fiber are ingested (1, 7).

The complete combustion of RMD yields carbon dioxide in amounts equal to the amount of oxygen consumed [respiratory quotient (RQ) = 1], which is the same as IDC assumes for carbohydrate. However, the general assumptions do not hold during fermentation because combustion is incomplete with the excretion of the combustible end-products hydrogen, methane, and microbial biomass. In addition, there is unquantified temporal accumulation of short-chain organic acid intermediates in the colon (1). Here, for the first time, a post hoc account is given of carbohydrate oxidation measurements acquired by using IDC under these circumstances, with the use of breath-hydrogen

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measurement to monitor fermentation. Also for the first time, a separate estimation of carbohydrate utilization by absorption, fermentation, and likely excretion in feces, as distinct from the commonly reported oxidation, is given. Moreover, we make an evaluation of the assumptions used, the possible errors in calculated outcomes, and ways to minimize these errors in the future.

We used commercial RMD that has Generally Recognized as Safe status in the United States (21 CFR 184.1444, maltodextrin) and that was certified as approved for Foods for Specified Health Uses in Japan (8). The RMD is an aggregate of glucose polymers, averaging 12 degrees of polymerization (range: 2–62) and containing currently unspecified 1–2 and 1–3 glucosidic linkages (9). Animals digest and absorb ≈10% of RMD in the small intestine (10), whereas ≈40% of RMD appears in feces (11).

SUBJECTS AND METHODS

Subjects

Seventeen adults (13 M, 4 F) with a mean (±SD) age of 33 ± 6 (range: 25–46) y, body weight of 61.7 (range: 46–74) kg, and body mass index (BMI; kg/m^2) of 21.7 ± 2.0 (range: 17.3–24.8) participated in a principal 8-h study monitoring both carbohydrate oxidation and fermentation. Two preparatory studies were also undertaken, one focusing on carbohydrate oxidation in 5 (2 M, 3 F) subjects for 3 h, and another focusing on fermentation marked by breath hydrogen in 10 (8 M, 2 F) subjects for up to 24 h; each study was within the range of subject characteristics for the principal study. All subjects were from the Osaka district, habitually consumed a typical Japanese diet, and were healthy. None had taken medication likely to affect substrate metabolism or antibiotics that may affect fermentation for ≥1 mo.

All volunteers gave written informed consent. The study was approved by the Ethics Committee of the University of Shizuoka.

Study protocols

In the principal study, we investigated both carbohydrate oxidation (kJ/min) and breath hydrogen (ppm). The study was a multiple-crossover single-blinded design, and we familiarized the subjects with the procedures beforehand in a dummy run. Seventeen participants (13 M, 4 F) were randomly assigned by computer program, rested, and fasted overnight (11–12 h), and they took a single oral dose of water (3 mL/kg body wt) containing maltodextrin (0.3 g/kg) or RMD (0.6 g/kg) or water alone as control. Breath oxygen consumption and carbon dioxide production while supine were monitored hourly by IDC for 15-min periods over the next 8 h. During this time, we collected urine for nitrogen measurement, after storage at −20 °C. At 180 and 360 min after oral treatments, we provided low-carbohydrate meals, which were eaten completely. Both meals consisted of eggs, tuna fish canned in oil, and soybean curd; contained 22 g fat, 30 g protein, and 4.3 g AC; and provided 1437 kJ and ≤0.6 g fiber. For each subject, we made observations 9 times at intervals of ≥7 d. These observations comprised 3 repeated measurements (on different days) and 3 different treatments (control, MD, and RMD) for each of the 17 participants, for a total of 153 observations.

We undertook 2 preparatory studies whose general design was similar to that of the principal study. The first assessed only carbohydrate oxidation for 3 h but after different oral doses of maltodextrin providing 0, 0.15 or 0.3 or 0.6 g/kg body wt. Observations were made 8 times for each participant with intervals ≥ 7 d in a rotary multiple crossover design. The 8 observations comprised 2 repeat measurements (on different days) and the 4 doses, on each of the 5 participants, totaling 40 observations.

The second preparatory study assessed the breath-hydrogen response to RMD hourly except for a break from 15 to 21 h after the ingestion. The study had a sequential design in which 0.6 g RMD in 3 mL water/kg body wt was provided on the first occasion, and water alone was provided on the next. Thus, we made observations twice for each participant with an interval of ≥7 d. With 10 participants, this totaled 20 observations.

Materials

The RMD and maltodextrin preparations were from the Matsutani Chemical Industry Co (Itami, Hyogo, Japan). They were defined as Fibersol-2 and TK-16, respectively.

Indirect calorimetry

Respiratory exchange measurements were made hourly in the principal study by using a $V_{max}29$ (SensorMedics, Yorba Linda, CA). After stabilization was confirmed (≈7 min), data were averaged for 8 min. In the preparatory study, minute-by-minute measurements were made. Calibration used standard gas of oxygen (26%) and carbon dioxide (4%) accurate to within 0.1%. We measured nitrogen in urine by using the Dumas (combustion) method in an NC-80 nitrogen analyzer (Sumitomo Chemical Industry Co, Tokyo, Japan). Carbohydrate oxidation was calculated by the method of Weir (12), according to the following equation. Adjustments for incomplete oxidation due to fermentation were made post hoc as described in Results.

$$C(\text{kJ/min}) = 71.80 \times \dot{V}CO_2(\text{L/min}) - 50.8 \times \dot{V}O_2(\text{L/min}) - 44.4 \times N(\text{g/min}) \quad (1)$$

where C = carbohydrate oxidation, $\dot{V}CO_2$ = carbon dioxide uptake, and $\dot{V}O_2$ = oxygen uptake.

Breath hydrogen

To mark changes in the rate of fermentation, we measured breath hydrogen. End-expired air (200 mL) was sampled after calorimetric measurements, and the air sample was tested in a TGA-2000HC hydrogen-gas analyzer (Teramecs, Kyoto, Japan).

Statistical analysis

Data are given as means (±SE) or as means (95% CI). Linear (regression), nonlinear (exponential), and dynamic (delay) models were implemented with consideration for measurement error in the determinants (error-in-variables regression). All models used mean values for treatment groups with least-squares estimates weighted for SEs. In addition, estimation with error-in-variables regression used reliability estimates for the measured determinants (13). Carbohydrate oxidation was adjusted for covariation between initial rates at time zero (t_0) and the rate at the i^{th} time of measurements (t_i). These adjustments were toward the initial rate averaged across all treatment groups. The principal

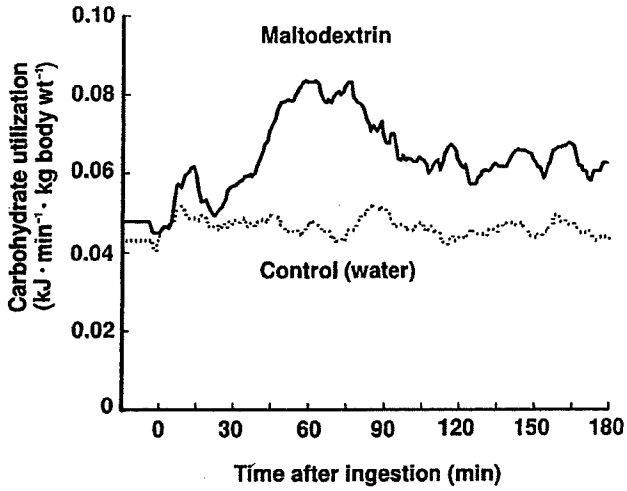

FIGURE 1. Oxidative utilization of maltodextrin in a human volunteer; minute-by-minute measurement was done with indirect calorimetry.

study had a multiple crossover design with 9 periods, 6 sequences, and 3 entries. Each was without significant effect, so these factors were dropped from the analysis to maximize df; the same process was followed in the preliminary studies. Modeling, described as needed in Results, was explored initially in MS EXCEL with subsequent fitting and parameter estimation in STATA software (version SE9; Stata Corp, College Station, TX). Because the data on each occasion derive from a time series, we applied Durbin-Watson tests for positive and negative autocorrelations (14).

RESULTS

Carbohydrate oxidation after maltodextrin ingestion

Carbohydrate oxidation peaked at 60–80 min after maltodextrin (md) ingestion (**Figure 1**). The area under the curve above that for water (w) alone from t_0 to the end of measurements (t_{max}) was summarized as the difference in incremental (i) area under the 2 carbohydrate (C) oxidation curves, denoted by the integral $\int_{t_0}^{t_{max}} iC_w^{md} \cdot dt$. This outcome was related linearly to the amount of maltodextrin ingested (**Figure 2**A) in the range 0 to 0.6 g/kg body wt ($\bar{x}$ range: 0–36 g maltodextrin in our participants). Similar results were obtained by integration over 1-min and 1-h intervals (Figure 2B).

IDC monitors carbohydrate irretrievably used in oxidation (15). Carbohydrate used in de novo lipogenesis is portrayed in IDC as negative fat oxidation, which equals carbohydrate oxidation in IDC. Its use for glycogen storage or as replacement of endogenous glucose is not detected because no respiratory exchange of oxygen or carbon dioxide is involved. However, the linearity found (Figure 2) implies constancy in the ratio of monitored carbohydrate oxidation to unmonitored carbohydrate storage across the dose range examined.

The integrated appearance of oral carbohydrate in blood when assessed by using stable isotopes can follow a lag-rising exponential curve (16, 17) and thus approximate first-order kinetics.

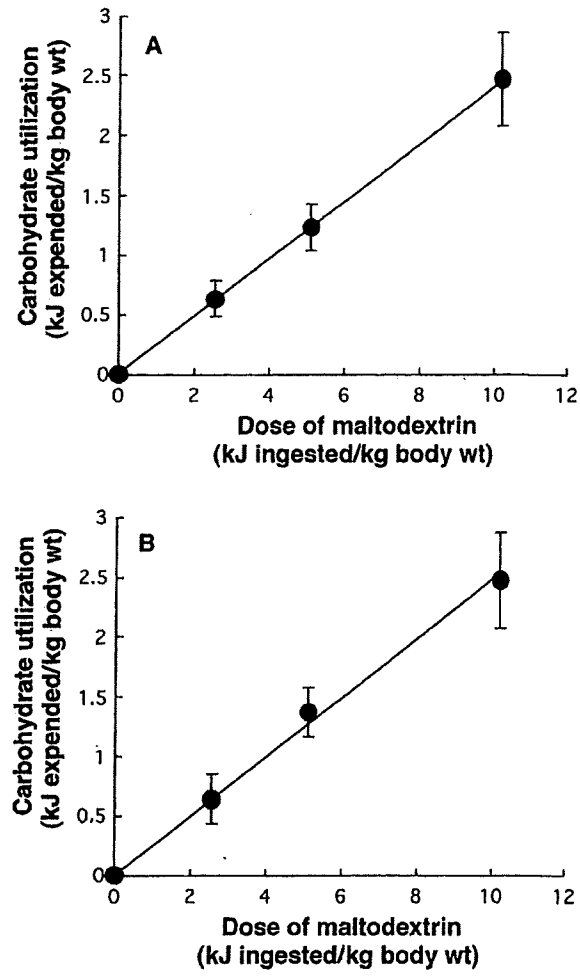

FIGURE 2. Dose-dependent oxidative utilization of maltodextrin by using indirect calorimetry in 5 human subjects up to 3 h. (A) Areas after 1-min integrations, $y = 0.26 \times x$ ($r^2 = 1.000$, $P < 0.001$). (B) Areas after 1-h integrations, $y = 0.26 \times x$ ($r^2 = 0.998$, $P < 0.001$).

Such a curve was apparent for carbohydrate oxidation, assessed here also by using IDC (**Figure 3**), as in the following equation:

$$\int_{t_0}^{t_{max}} iC_w^{md} \cdot dt = C_{max} \cdot [1 - e^{-\lambda(t - t_{lag})}] \quad (2)$$

where t is time elapsed since ingestion, t_{lag} is a lag period between carbohydrate ingestion and breath carbon dioxide excretion, C_{max} is a plateau marking the amount of ingested carbohydrate used in the oxidative process, and λ is a fractional rate constant that is related to the process half-life, as in the following equation:

$$t_{1/2} = \ln(2)/\lambda \quad (3)$$

Normalization of $\int_{t_0}^{t_{max}} iC_w^{md} \cdot dt$ by the amount of carbohydrate ingested gives the approach to C_{max} as a fraction. No significant difference occurred between the trend for all doses and that for any one dose (Figure 3).

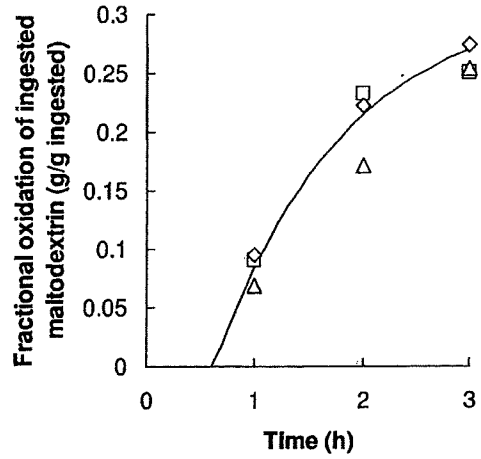

FIGURE 3. Mean (±SE) estimated lag time for oxidative utilization of maltodextrin at 3 doses [0.15 (◇), 0.30 (△), and 0.60 (□) g/kg] fitted to a common lag-rising exponential curve. The estimated lag time was 0.64 ± 0.06 h; in this study, it reached the estimated plateau (C_{max}) at 0.33 ± 0.03 g/g maltodextrin ingested.

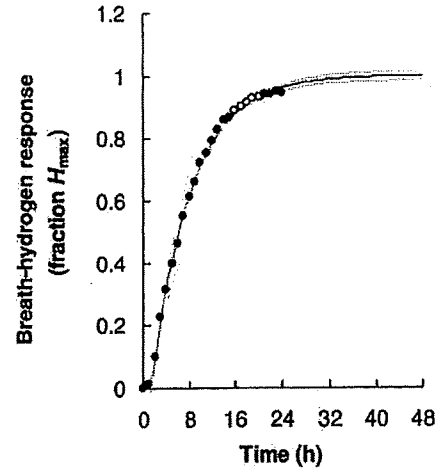

FIGURE 5. Observed means (●), interpolated overnight means (○), and SE (—) for the commulative area under the curve for the breath-hydrogen response to resistant maltodextrin. Values are normalized by the endpoint maximum (H_{max}). The central curves show the mean and SE extended toward the normalized mean and SE of H_{max}.

C_{max} occurred at 0.33 ± 0.03 g/g (or kJ/kJ). Thus, on average, approximately one-third of the maltodextrin was "seen" to be used by IDC during this small study. In the more definitive principal study, this value was 0.46 ± 0.05 g/g or ≈45%; ther-emainder entered storage, presumably as glycogen or free glucose.

The breath-hydrogen response to RMD

The breath-hydrogen concentration is a well-accepted indicator of the presence of fermentable carbohydrate in the colon. Its appearance after RMD ingestion (0.6 g/kg, averaging 36 g) in the second of the preparatory studies was rapid, after an initial lag, and it persisted up to 24 h (**Figure 4**). Observations not made overnight (15–21 h) were interpolated by fitting a second-order polynomial to each subject's results to enable integration over 24 h. Integration gave the cumulative incremental area under the curve for RMD (rmd), above that for water, toward completion of RMD fermentation (ie, $\int_{t_0}^{t_{max}} iH_w^{rmd} \cdot dt$). This quantity also fitted a lag-rising exponential curve (**Figure 5**), which was used to summarize the progress and endpoint (H_{max}) of RMD fermentation, as shown in the following equation:

$$\int_{t_0}^{t_{max}} iH_w^{rmd} \cdot dt = H_{max} \cdot [1 - e^{-\lambda(t - t_{lag})}] \qquad (4)$$

Carbohydrate utilization after resistant maltodextrin ingestion

Rates of carbohydrate oxidation from RMD or maltodextrin in the principal study are compared in **Figure 6**. These observations followed an analysis of covariance to account for often-substantial correlations with initial rates (**Table 1**). Lower rates were obtained from RMD during the first 3 h, which reflects less AC from RMD than from maltodextrin. Later, oxidation was higher for RMD than formaltodextrin, which is attributable to the

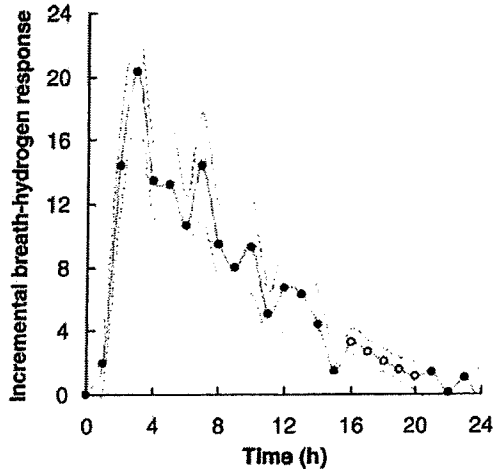

FIGURE 4. Mean (●), SE (—), and interpolated overnight (○) values for the breath-hydrogen response to resistant maltodextrin (0.6 g/kg body wt).

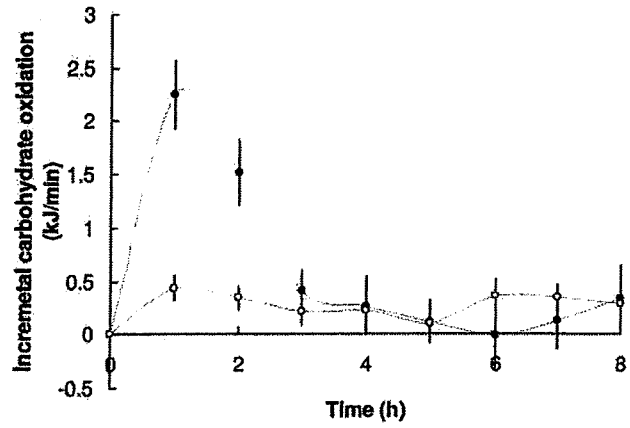

FIGURE 6. Mean (±SE) increments in oxidative utilization of maltodextrin (●, 0.3 g in 3 mL water/kg body wt) and resistant maltodextrin(○, 0.6 g in 3 mL water/kg body wt) above a water control (3 mL/kg body wt). Observations for maltodextrin are multiplied by 2 to avoid the illusion of a rate that is too low relative to that for resistant maltodextrin, which was fed at twice the dose.

TABLE 1.
Correlation of the carbohydrate oxidation rate at hourly intervals with the initial rate[1]

	Maltodextrin	RMD	Water
Time (h)			
0	1	1	1
1	0.71	0.60	0.63
2	0.44	0.55	0.35
3	0.46	0.69	0.51
4	0.49	0.75	0.44
5	0.38	0.68	0.34
6	0.61	0.49	0.38
7	0.36	0.39	0.39
8	0.18	0.53	0.09

[1] RMD, resistant maltodextrin.

sum of oxidation from the anaerobic process in the large bowel and that from aerobic processes acting on absorbed products of carbohydrate fermentation.

Modeling of carbohydrate utilization

Modeling helps to identify various assumptions and limitations and provides additional, quantitative information about the routes of carbohydrate utilization and overall energy value in a form that is conventionally useful. Thus, a model was fitted by using weighted least-squares to assess the probable fractions of RMD used as AC (absorbed via the small intestine), as FC (absorbed as short-chain organic acids via the large intestine), and overall as energy. The last was derived in amounts that can either generate heat (ME) or can replace energy spent on work and maintenance (NME) and so indicates the value in maintenance of energy balance (2).

The simplest model for the present purpose, and one that describes the increase (i) in the rate of carbohydrate oxidation at any time point [$iC(t)$], is one that includes the sum of the increase from its component parts—available carbohydrate (ac) (iC^{ac}) and fermentable carbohydrate (fc) (iC^{fc})—according to the following equation:

$$iC(t) = iC^{ac}(t) + iC^{fc}(t) \qquad (5)$$

With respect to RMD, both $iC^{ac}(t)$ and $iC^{fc}(t)$ are unknown (on the basis of the foregoing data), although they can be estimated when markers of their time-course and extent are available. The time-course of $iC^{ac}(t)$ can be approximated initially by that of maltodextrin above that of water [$iC_w^{md}(t)$]. A possible delay in the digestion and oxidation of RMD compared with those of maltodextrin is considered below. The time-course of iC^{fc} can be approximated by the breath-hydrogen concentration of RMD above that for water [$iH_w^{rmd}(t)$]; in this case, a possible delay is due to an accumulation of short-chain organic acids in the colon before their absorption and oxidation.

Assuming that there are no delays, then the adoption of the proportionality constants β_1 and β_2 allows rate estimates to be obtained by linear regression, as in the following equation, which assumes the determinants to be unbiased and reliable:

$$iC_w^{rmd}(t) = \beta_1 \cdot iC_w^{md}(t) + \beta_2 \cdot iH_w^{rmd}(t) \qquad (6)$$

The proportionality constant β_1 has meaning. Given linearity in carbohydrate oxidation via this route with dose (Figure 2), then, β_1 indicates the proportion of RMD that is available as carbohydrate, information that is used conventionally in nutrition. In contrast, β_2 simply describes the relation between the apparent rate of carbohydrate oxidation via fermentation and the appearance of hydrogen gas in breath. Equation 6 can be applied directly, although a more stable form for the purpose of regression is its integral, which replaces the rates with cumulative areas, as in the following equation:

$$\int_{t_0}^{t_{max}} iC_w^{rmd} \cdot dt = \beta_1 \int_{t_0}^{t_{max}} iC_w^{md} \cdot dt + \beta_2 \int_{t_0}^{t_{max}} iH_w^{rmd} \cdot dt \qquad (7)$$

Equation 7 simply states that the incremental area under the curve for carbohydrate oxidation between t_0 and t_{max} equals the sum of the separate incremental areas for AC and FC, which, again, assumes unbiased and reliable determinants.

Possible delays due to slow digestion of RMD or accumulation of short-chain organic acids in the colon were evaluated by using the amount entering the delay (X_{in}) and a fractional rate constant (λ) for an amount leaving the delay (X_{out}). Thus, the amount leaving the delay was assumed to follow first-order kinetics and therefore to be a fraction of the sum of the amount entering the delay in a given period of time (t_i to $t_{i+1[r]}$) and the amount remaining in the delay (D) from the prior period ending at time t_i (with D initially being zero), as shown in the following equation:

$$\int_{t_0}^{t_{max}} X_{out} \cdot dt = \int_{t_0}^{t_{max}} \lambda \cdot \left[\int_{t_i}^{t_{i+1}} X_{in} \cdot dt + D_{t_i} \right] \cdot dt \qquad (8)$$

This construct in practice was validated by a yield of $X_{out} = X_{in}$ when $\lambda = 1$ and of $X_{out} = 0$ when $\lambda = 0$ and by an equality of X_{in} and the redifferentiated X_{out} for all other values of λ. Replacement of X_{in} by iC_{wt}^{md} gave a delay for AC oxidation, whereas replacement of X_{in} by iH_{wt}^{rmd} gave a delay for oxidation via fermentation. Values of λ were used that minimized the weighted sum of squares in equation 7, when replacing inputs with delay outputs. This yielded models with 2, 3, or 4 parameters corresponding to those in equation 7 alone and to those in equation 7 with 1 or 2 delays, as shown in equation 8.

Carbohydrate availability from RMD

The estimates for β_1 and β_2, obtained by using the differential regression (equation 6), the integral regression (equation 7), and the latter with and without delays (equations 7 and 8), are shown in **Table 2**. These estimates immediately inform us that a significant fraction of RMD appeared to be available as carbohydrate; thus, β_1 was 0.16-0.17 g/g or kJ/kJ RMD—ie, ≈17% of the RMD preparation. Implementation of the delays had no significant effect on this particular result. The models so far imply high reliability for the measured determinants. Application of reliability factors (ie, 1 minus the ratio of variance among subjects to total variance in the measurement) also had no effect because the values were close to unity as assumed in ordinary least-squares estimation. Because the data derive from a time series, the residuals were examined for autocorrelation by calculation of the Durbin-Watson statistic. This showed a critical value $d = 2.66 > d_U$ ($\alpha < 0.01$, $k = 2$)—where "d_U" refers to the upper bounds, which indicated that significant positive autocorrelation was unlikely—and a critical value $4 - d = 1.33 > d_U$ ($\alpha < 0.01$, $k =$

TABLE 2.
Carbohydrate utilization from resistant maltodextrin (RMD)[1]

Parameter	Parameter estimate[2]	P[3]	df (odf)[4]
β_1			
Equation 6 (kJ/kJ RMD)	0.17 (−0.04, 0.38)[5]	0.09	7 (151)
Equation 7 (kJ/kJ RMD)	0.16 (0.10, 0.22)	0.001	7 (151)
Equation 7 with delay after hydrogen (kJ/kJ RMD)[6]	0.17 (0.12, 0.23)	0.001	6 (150)
Equation 7 with delay after hydrogen + reliability (kJ/kJ RMD)[6]	0.16[7] (0.02, 0.30)	0.07	6 (160)
β_2			
Equation 6 ($J \cdot min^{-1} \cdot ppm\ H_2^{-1}$)	0.15 (0.08, 0.22)	0.002	7 (151)
Equation 7 ($J \cdot h^{-1} \cdot 60\ ppm\ H_2^{-1}$)	0.15 (0.12, 0.18)	0.001	7 (151)
Equation 7 with delay after hydrogen ($J \cdot h^{-1} \cdot 60\ ppm\ H_2^{-1}$)[6]	0.15 (0.12, 0.18)	0.001	6 (150)
Equation 7 with delay after hydrogen + reliability ($J \cdot h^{-1} \cdot 60\ ppm\ H_2^{-1}$)[6]	0.16 (0.12, 0.20)	0.001	6 (150)
t, AC in maltodextrin			
Equation 2, t_{lag} (h)	0.6 (0.5, 0.8)	0.001	5 (132)
Equation 3, $t_{1/2}$, AC oxidation (h)	0.9 (0.7, 1.1)	0.001	5 (132)
t, AC in RMD			
Equation 2, t_{lag} (h)	"0.6"		
Equation 3, $t_{1/2}$, delay in AC oxidation (h)	"0.0"[8]		
Equation 3, $t_{1/2}$, AC oxidation (h)	"0.9"		
t, FC in RMD			
Equation 2 and 7, t_{lag} (h)	2.3 (2.2, 2.5)	0.001	3 (96)
Equation 3, 7, and 8, $t_{1/2}$, absorption of accumulated SCOA (h)	1.3 (0.2, 8.5)	0.2	3 (96)
Equation 3 and 7, $t_{1/2}$, FC in RMD (h)	5.7 (4.5, 8.0)	0.002	3 (96)
Availability and energy value			
AC (g/100 g)	17 (12, 23)	0.001	6 (150)
FC (g/100 g)	40 (23, 75)	0.001	6 (151)
Fecal carbohydrate (g/100 g)	43 (13, 53)	0.02	6 (151)
Metabolizable energy			
(kJ/g)	7.3 (4.6, 12.1)	0.001	6 (151)
(kcal/g)[9]	*1.7 (1.1, 2.9)*		
Net metabolizable energy			
(kJ/g)	6.3 (4.0, 10.2)	0.001	6 (151)
(kcal/g)[9]	*1.5 (1.0, 2.4)*		

[1] t, time; t_{lag}, lag time; $t_{1/2}$, half-life; AC, available carbohydrate; FC, fermentable carbohydrate; SCOA, short-chain organic acids. Equations appear by number in Results. Quotation marks indicate values assumed to equal those for maltodextrin.

[2] Includes the error bounds for γ_1, γ_2, and γ_3 in Equation 14.

[3] Level of significance, $P > |t|$ vs $\mu = 0$ for the lowest yield after applying error bounds for γ_1, γ_2, and γ_3. Values of $t_{1/2}$ were tested in the λ transformation (Equation 3).

[4] The model residual df is 9 time points minus the number of time points for $t < t_{lag}$ and minus the number of model parameters encountered; the overall df (odf) is the product of the number of time points for $t > t_{lag}$ and the number of subjects per time point minus the number of parameters encountered in the model.

[5] $\bar{x}$; 95% CI in parentheses (all such values).

[6] *See* equation 8.

[7] Error in variance regression requires a constant term not present in the model; the estimated constant (0.065) reduces the β_1 coefficient to 0.095 and is expected to be drawn from the available carbohydrate peak.

[8] The $t_{1/2}$ for AC oxidation in RMD was nonsignificantly shorter (0.2 h) than that in maltodextrin. This parameter was therefore dropped from the analytical model.

[9] 1 kJ = 4.184 kcal.

2)—which indicated that significant negative autocorrelation also was unlikely.

The half-life (*see* equation 3) for the process of maltodextrin oxidation was 0.9 h (Table 2). The possibility of oxidation of AC from RMD sooner rather than later than that from maltodextrin was apparent and was quantified as $\lambda > 1$, which corresponded to a smaller $t_{1/2}$ for oxidation by 0.2 h. This parameter was neither significant nor practically different from zero and was therefore dropped to preserve df when other model parameters were fitted.

Time-course of carbohydrate availability from maltodextrin and RMD

Some idea of the time-course of carbohydrate availability and the potential errors involved can be obtained from the data. The fraction of maltodextrin used as AC can be assumed to be 1.0 (100% available) at t_{max}, but the time course is unknown. The fraction [here called phi, for fraction (Φ)] made available from the maltodextrin ($_{md}$) and used as carbohydrate ($_{ac}$) from t_0 to any one time (t) can be denoted by $[\phi_{ac}\ (t)]_{md}$. This value is approximately equal to the ratio of the incremental area before time t to the incremental area under the curve before t_{max}, when all of the AC (ac) has been used from the digestive tract, as shown in the following equation:

$$[\Phi_{ac}(t)]_{md} = \frac{1}{\int_{t_0}^{t_\infty} iC_w^{md} \cdot dt} \cdot \int_{t_0}^{t} iC_w^{md} \cdot dt \qquad (9)$$

The time-course for maltodextrin given by equation 9 is not exact; whereas a constant proportion of dose is used in oxidation

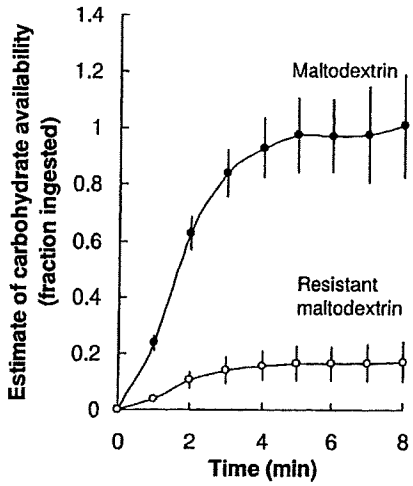

FIGURE 7. Mean (95% CI) estimates of the availability of carbohydrate from maltodextrin and resistant maltodextrin.

(Figure 2), uncertainty remains about constancy across time (although oxidation was similar with time across doses; *see* Figure 3). However, this unknown has little implication for the accuracy with which eventual carbohydrate availability and fermentability are quantified, provided the study lasts long enough to permit absorption of the AC to approach completion. By completion, 0.46 ± 0.05 g AC/g maltodextrin had been oxidized and was the denominator in equation 9.

On the basis of equation 9, an approximate time-course for carbohydrate availability is evident for maltodextrin, as shown in **Figure 7.** In this case, carbohydrate from RMD is less than fully available (Figure 7); this fraction, $[\phi_{ac}(t)]_{rmd}$ is given by the product of $[\phi_{ac}(t)]_{md}$ (from equation 9) and β_1 derived in equation 7, as shown in the following equation:

$$[\Phi_{ac}(t)]_{rmd} = [\Phi_{ac}(t)]_{md} \cdot \beta_1 \qquad (10)$$

Time-course of carbohydrate fermentability from RMD

It helps to restate in full the expression for the time-course for carbohydrate availability from maltodextrin. Expanding $[\phi_{ac}(t)]_{md}$ in equation 10 by using the righthand side of equation 9 gives the following equation:

$$[\Phi_{ac}(t)]_{rmd} = \frac{\beta_1}{\int_{t_0}^{t_{max}} iC_w^{md} \cdot dt} \cdot \int_{t_0}^{t} iC_w^{md} \cdot dt \qquad (11)$$

The fraction of RMD used via fermentation (rather than that directly available) at any time, $[\phi_{fc}(t)]_{rmd}$, can be derived in a similar way, which yields the following equation:

$$[\Phi_{fc}(t)]_{rmd} = \frac{\beta_2}{\int_{t_0}^{t_{max}} iC_w^{md} \cdot dt} \cdot \int_{t_0}^{t} iH_w^{fc} \cdot dt \qquad (12)$$

As it stands, equation 12 is biased—it lacks important features, 2 of which tend to cancel one another and must be remedied simultaneously. When the errors do not cancel, they affect the value of β_2.[r] The first error results in β_2 being too large and arises because IDC fails to represent AC used in glycogen deposition or in the replacement of endogenous glucose. We can remedy this error simply by multiplying the numerator by the fraction of AC (ac) seen by the IDC, γ_1. The second error results in β_2 being too small, because IDC fails to represent the extent to which short-chain organic acids from carbohydrate fermentation contribute to glycogen deposition or endogenous glucose during the period of calorimetry. We can remedy this error by multiplying the denominator by a similar fraction, γ_2. Both of these remedies are illustrated in the following equation:

$$[\Phi_{fc}(t)]_{rmd} = \frac{\beta_2}{\int_{t_0}^{t_{max}} \gamma_2 \cdot iC_w^{md} \cdot dt} \cdot \int_{t_0}^{t} \gamma_1 \cdot iH_w^{fc} \cdot dt \qquad (13)$$

These fractions (γ_1 and γ_2) are unknown at each time point. For simplicity, we can assume that both $|\gamma_1|$ and $|\gamma_2|$ are constants that vary with time between error bounds, ie, $|\gamma_1|$ and $|\gamma_2|$. As constants, they can be outside the integrals, and thus the equation below replaces equation 13. A third factor, $|\gamma_3|$, also introduced in the equation below, corresponds to another bias—ie, the overestimation of FC oxidation by IDC because of the influence of accumulating combustible gases (including hydrogen) and biomass on the process RQ (1). The γ_2 factor is reasonably well established at 0.90 for the complete process of fermentation, but it may initially be 0.97 should a lag occur in time for the generation of biomass (1), as shown in the following equation:

$$[\Phi_{fc}(t)]_{rmd} = \left(|\gamma_3| \cdot \frac{|\gamma_1|}{|\gamma_2|}\right) \cdot \frac{\beta_2}{\int_{t_0}^{t_{max}} iC_w^{md} \cdot dt} \cdot \int_{t_0}^{t} iH_w^{fc} \cdot dt \qquad (14)$$

In the current study, γ_1 was derived as the ratio of maltodextrin apparently oxidized by t_{max} to the amount of maltodextrin ingested, and it was 0.46 ± 0.05 g/g (or kJ/kJ). At any time point, the true value of γ_1 would have been more or less than 0.46, and therefore we arbitrarily applied a range ± 20% of 0.46 (ie, 0.37–0.55). The lowest limit for γ_2 must be equal to γ_1 when short-chain organic acid mixtures are assumed to be as effective as exogenous glucose in displacing endogenous glucose in oxidation or storage. The highest possible value for γ_2 is at its limit, 1.0, when carbohydrate used via fermentation has no effect on endogenous glucose metabolism and when all short-chain organic acids are oxidized after absorption, so that the utilization of fermentable carbohydrate is then fully "seen" by IDC. A more representative intermediate value of 0.67 is suggested because it corresponded both to gluconeogenic propionate's contribution to 16% of the short-chain organic acids produced (18) and to the lack of effect of acetate (and presumably butyrate) on respiratory exchange from endogenous glucose (19). Short-chain organic acids potentially could be as effective as dietary fat in saving glucose from oxidation, suppressing oxidation by as much as 20% (20). Thus, the range of values used for $|\gamma_2|$ was 0.37 to 1.0, and a suggested representative value was 0.67. The approximate rise in the amount of carbohydrate fermented with time and the

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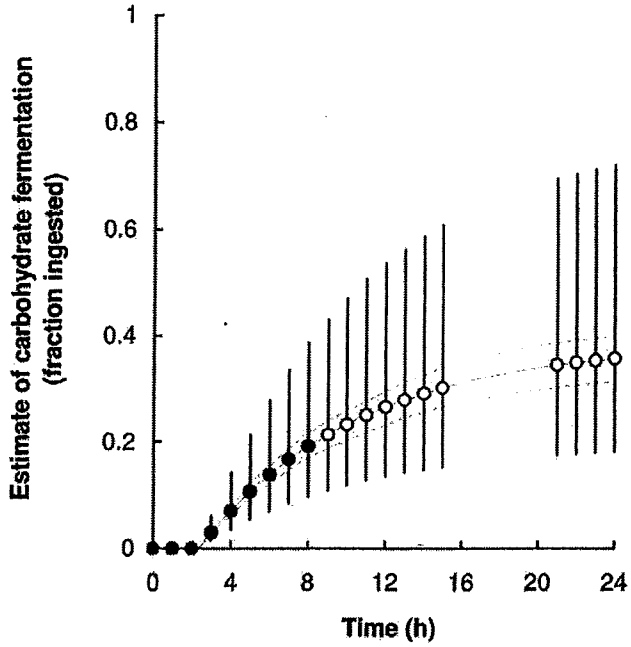

FIGURE 8. Mean (±SE; —) and 95% CI (bars) estimates of fermentable carbohydrate from resistant maltodextrin with γ_1, γ_2, and γ_3 at the upper and lower boundaries; SEs are given for assumptions of suggested values (γ_1 = 0.46, γ_2 = 0.67, and γ_3 = 0.9). The period of both indirect calorimetric and breath-hydrogen measurements, ●; the period of breath-hydrogen measurement alone, O.

errors propagated through uncertainties in β_2, γ_1, γ_2, and γ_3 are shown in **Figure 8.**

In the principal study, observations on breath hydrogen were available up to 8 h only; beyond that time, the progress and endpoint of fermentation were forecast by using parameter estimates from equation 3 (justified in the longer preparatory study; *see* Figure 5). This process allowed the application of equation 14 to quantify carbohydrate fermentation in a useful form, as shown in Figure 8. When only the experimental error is considered (ie, given the central values for γ_1, γ_2, and γ_3), the likely mean (±SE) for RMD fermentation would be 40 ± 4 g/100 g RMD. However, the additional error propagated from the bounds for γ_1, γ_2, and γ_3 yields wide CIs (95% CI: 22, 77 g/100 g RMD), so that the true value falls within the vertical bars shown (Figure 8).

Fecal carbohydrate

An AC content of 17 g/100g RMD and a fermentable carbohydrate content of 40 g/100 g RMD imply that the amount destined to reach feces would be 43 g/100 g RMD (Table 2). The wide 95% CIs for this estimate (ie, 13, 53 g/100 g RMD) indicate that direct measurement of this quantity is warranted. Nevertheless, the model indicates that a considerable proportion of RMD is highly resistant even to the fermentation process, which is difficult to discern from estimates of carbohydrate oxidation alone, as shown in Figure 6.

Estimates of energy availability

With respect to the contribution of RMD to energy balance—ie, its contribution to the NME value (2)—it is clear that RMD carbohydrate entering feces contributes no energy. At the other extreme, all of the combustible energy (gross energy) in the AC

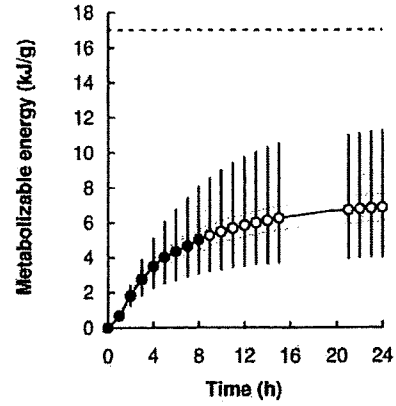

FIGURE 9. Mean (±SE; —) and 95% CI (bars) estimates of the availability of net metabolizable energy from resistant maltodextrin. γ_1, γ_2, and γ_3 are at the upper and lower boundaries, and SEs assume suggested values (γ_1 = 0.46, γ_2 = 0.67, and γ_3 = 0.9). The period of both indirect calorimetric and breath-hydrogen measurements, ●; and the period of breath-hydrogen measurement alone, O.

is available energy at 17 kJ/g. It is now well established in conventions that only half of the combustible or gross energy in FC is useful energy (2), as shown in the following equation:

$$NME_{CHO} = \Delta H_c \cdot (AC + 0.5FC) \quad (15)$$

and ME is obtained as shown in the following equation:

$$ME_{CHO} = \Delta H_c \cdot (AC + 0.65FC) \quad (16)$$

An example is given in the following equation:

$$NME_{RMD} = 17 \cdot (0.17 + 0.5 \times 0.40) = 6.3 \text{ kJ/g} \quad (17)$$

Propagation of this estimate of 6.3 kJ NME/g with time is shown in **Figure 9**, along with the experimental SE alone (given unbiased central assumptions for γ_1, γ_2, and γ_3) and with the widest 95% CIs that arise when boundary values for γ_1, γ_2, and γ_3 are used. The corresponding ME value (*see* equation 16)—ie, the amount of heat that can be generated from RMD—is obtained by replacement of 0.5 in equation 15 with 0.65 in equation 16 (2). These results are summarized in Table 2. The ME estimate for the fiber fraction of RMD was 5.2 (2.9–10.7) kJ/g RMD, whereas the NME estimate was 4.1 (2.2–8.2) kJ/g.

DISCUSSION

We report on the metabolism of a carbohydrate that is mostly resistant to digestion and partially resistant to fermentation, as indicated by a short-term IDC technique and breath-hydrogen measurements. Modeling is used to estimate carbohydrate availability, fermentability, energy values, and the precision of the approach. A summary of our observations is given schematically in **Figure 10.**

We confirm that the response of carbohydrate oxidation to AC intake is linear for the quantities consumed: 0–0.6 g/kg body wt. Hence, carbohydrate availability appears predictable from gaseous exchange measurements. Whereas hourly integration is sufficient for the present, we believe that more frequent sampling

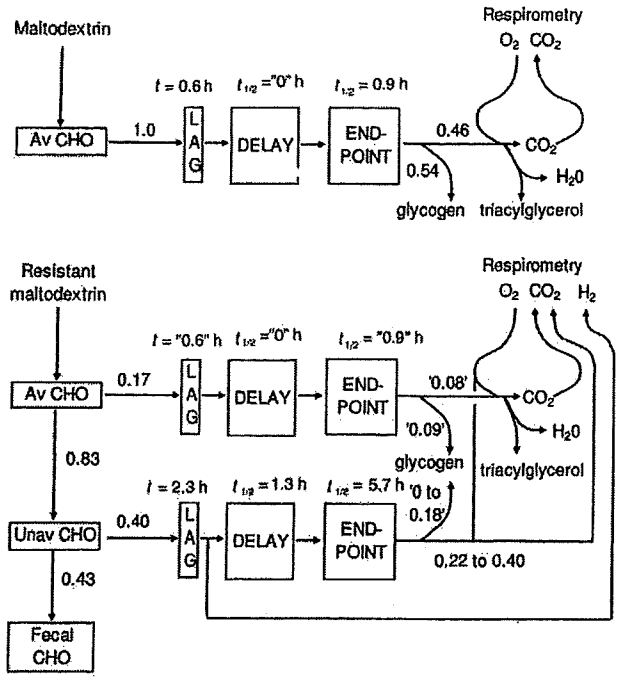

FIGURE 10. Summary of carbohydrate utilization from maltodextrin and resistant maltodextrin derived from the time course of gaseous exchanges. CHO, carbohydrate; Av, available; Unav, unavailable. Single quotes indicate values derived by calculation involving both parameter estimates and assumed values; double quotes indicate assumed values. Parameter estimates are from Table 2.

during the first 4 h would improve accuracy (**Table 3**), particularly for the parameters of time.

The linear dose response also implies a dose-independent distribution of AC between irretrievable oxidation (including, to a minor extent, de novo lipogenesis) and glycogen storage. Linearity also implies that the doses fall within the capacity of the homeostatic mechanisms for our participants. However, that may not be so at higher doses or in diabetic patients, and so we recommend standardization (Table 3), which excludes participants with abnormal carbohydrate metabolism and, possibly, aims for individuals of similar glucose tolerance.

Between studies (preparatory and principal) and in subjects (principal study), we found a trend for persons with a low fasting RQ, who tend to oxidize more lipid than carbohydrate, to oxidize less of the maltodextrin (ie, to store more maltodextrin as glycogen). The correlation of individual fasting RQs measured on different occasions is significant (P for trend = 0.013) although imperfect ($r^2 = 0.34$) and thus is a source of experimental error. Such error may be reduced by consumption of a standard high-carbohydrate meal the evening before IDC, as is often practiced. Instead, the current study adjusts carbohydrate oxidation rates for covariance with the initial (fasting) rates. This is more representative of the free-living state, but penalties may remain for intergroup comparisons. In the future, both approaches may be used simultaneously to acquire greater control (Table 3).

We found that RMD consumption produced 2 peaks of carbohydrate oxidation, one within 3 h and another ≈6–8 h after RMD ingestion. The latter is consistent with breath $^{14}CO_2$ data from nondigestible but readily fermentable [^{14}C]fructooligosaccharides (21), which were first detectable within 2 h and which reached a maximum at 6–7 h after ingestion. We consider it likely that our first peak with RMD is due in large part to the presence of AC, whereas the latter peak involves fermentation.

Breath-hydrogen appearance from RMD is similar in time-course to that of the fermentable tagatose (22). However, it occurs somewhat earlier than expected from the appearance in breath of $^{14}CO_2$ after the ingestion of fructooligosaccharides (21). This earlier appearance likely is due to the so-called "bicarbonate delay" (23), in which the $^{14}CO_2$ has to equilibrate with endogenous unlabeled bicarbonate before peaking in breath. Because of the temporal accumulation of short-chain organic acids in the colon, we expect some delay in the appearance of carbon dioxide in breath after the ingestion of FC. This accumulation likely explains the delay between breath-hydrogen appearance and the later peak of carbohydrate oxidation ($t_{1/2}$: 1.3 h; Table 2).

Our model estimate for the AC content of RMD is ≈17 g/100 g. This value accords with a glycemic (and insulinemic) response to RMD, which is ≈10% of that for maltodextrin in humans (9). Furthermore, our observation of little difference in the time-course for oxidation of maltodextrin and AC from RMD (delay $t_{1/2}$ does not differ significantly from zero) is consistent with little difference in the time-course for the glycemic (and isulinemic) response (9). Moreover, our in vivo data agree with in vitro digestion studies using a gastric juice, salivary α-amylase, pancreatic α-amylase, and intestinal mucosal enzymes, which indicate that 10.2 g/100 g RMD is available as carbohydrate (11). We cannot exclude method bias as a cause of moderately higher availabilities in vivo than in vitro. The value in vivo is sensitive to baseline information, and, thus, more time points at the start of IDC would be helpful (Table 3).

The model indicates that fermentable carbohydrate in RMD is ≈40 g/100 g. This finding is supported by a recent study of similar design in rats, which gave an identical result (T Goda et al, manuscript in preparation). RMD not accounted for as AC or FC is 43 g/100 g, and we assume that this quantity enters the

TABLE 3.
Recommendations for standardization[1]

Exclusion criterion	Abnormalities of glucose metabolism, including glucose intolerance, as identified by using an oral glucose challenge
Exclusion criterion	Abnormal or low hydrogen responders
Previous treatments	Subjects could be adapted to the trial carbohydrate
Standardization of meals the previous evening	Less variation in the ratio of carbohydrate oxidation to carbohydrate stored as glycogen, with a goal of an RQ of ≈0.85 at the start of calorimetry
≥ 2 baseline points: −0.5 h and 0 h	Greater control over variation in measurement on different occasions
More frequent time points (eg, half-hourly) in the first 4 h	Greater precision for the delays and the estimates of carbohydrate availability and fermentability
Longer period of calorimetry: ≥ 10 h	Improved association of carbohydrate oxidation with hydrogen, which would enable better modeling of the delays
Longer period of collection of breath hydrogen: ≥15 h	Improved location of the endpoint, H_{max}, for breath hydrogen
Fecal collections for unused carbohydrate	Improved time-course and estimates of carbohydrate availability and fermentability
Better definition of a priori assumed factors	More precise information on the effect of fermentation products on glycogen storage

[1] Recommendations for features in addition to those implemented in the current study. RQ, respiratory quotient.

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feces. It is interesting that Satouchi et al (24) reported that 100 g RMD consumed over 5 d causes fecal dry weight to increase by 43 g. This increase corresponds to 40 g more fecal carbohydrate and 7 g more biomass per 100 g RMD consumed [given 90% recovery of nondegradable matter in 5 d and appearance of 20 g biomass/100 g fermented carbohydrate ($\approx$ 30 kJ bioenergy/100 kJ RMD)]. Further support comes from animal studies in which 38 g/100 g RMD consumed is recovered in feces (11). The current model estimates are therefore consistent with prior studies in both humans and animals.

Our observations are also consistent with those of in vitro studies. With human fecal inocula, fermentability of RMD is 50–66 g/100 g that of 97.4% fermentable pectin (18, 25). This amount in vitro corresponds to the sum of AC and FC in RMD and is in good agreement with the current sum for AC (17 g/100 g) and FC (40 g/100 g), which is 57 g/100 g. Similarly, the sum of the nondigestible but fermentable and nonfermentable carbohydrate in RMD in the current in vivo study represents the total fiber content and is 83 g/100 g RMD. This value is comparable to, although somewhat less than the 95 g/100 g RMD assessed by the total dietary fiber method in collaborative studies of the Association of Official Analytical Chemists (10); it is more consistent with 89.8 g/100 g after the deduction of enzymatically digestible carbohydrate (11). Although such agreements support the knowledge gained from this human study, we expect to obtain stronger evidence with the use of the current method by following the general recommendations given in Table 3.

In conclusion, our observations support an earlier view (1) of the need for detail to obtain adequate interpretation of often-used IDC data when FC is present. Under such a circumstance, IDC together with breath hydrogen can give reasonably precise information about the AC content of the total carbohydrate consumed. Information on FC content, in contrast, is less precise, largely because of uncertainty in the γ factors in equation 14. Here we set particularly wide error bounds for the effect of short-chain organic acids from the colon on glucose metabolism (γ_2)—from 0% to 100% as effective as glucose in modifying glycogen storage. Otherwise, errors are quite small when γ factors that fit our current knowledge are used, and results are in good agreement with observations from a variety of in vitro, animal, and other human studies of RMD. For the present, therefore, such comparative support data remain helpful in the assessment of the nutritional value of carbohydrate, such data are essential when providing the totality of evidence, and the need still exists to assess resistance to overall digestion and fermentation directly by using fecal collection and analysis. However, fecal data alone would not provide information about the partition between AC and FC or about the time-course of in vivo availability.

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TG was responsible for the experimental design, supervision of collections and analyses, data analysis, and writing of the manuscript. YK was responsible for specimen collection, preparation, and indirect calorimetry operation. KS was manager of laboratory operations and was responsible for specimen collection and preparation. HT supervised specimen collection and gas analysis and edited the manuscript. GL was responsible for modeling and for editing of the manuscript. None of the authors had any personal or financial conflict of interest.

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