Dietary Specific Sugars for Serum Protein Enzymatic Glycosylation in Man

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All glycoprotein sugars can theoretically derive from glucose. However, dietary specific sugars could represent preferential substrates or have regulatory roles in enzymatic glycosylation. This hypothesis was tested in man using stable isotopes. Healthy subjects ingested different amounts (150, 300, or 550 mg) of artificially ^{13}C-enriched sugar (galactose, mannose, or glucose) diluted in 200 mL water containing 50 g ^{13}C-poor sucrose. ^{13}C enrichment of expired CO_2 was monitored for 8 hours during indirect calorimetry. Serum glycoproteins were precipitated and delipidated at various intervals. Glycoprotein neutral sugars were obtained by acidic hydrolysis, purified by ion-exchange chromatography, derivatized to alditol acetates, and analyzed by gas chromatography–isotope ratio mass spectrometry. The oxidation rate for galactose and mannose was slower than the rate for glucose. Total oxidation over the 8-hour period was less than 10% of the ingested amount of galactose or mannose. Galactose and mannose were readily incorporated into glycoprotein glycans, in the native form or after interconversion, despite ingestion of a large excess of sucrose: glycoprotein sugar ^{13}C enrichment was strongly higher after ^{13}C-galactose or ^{13}C-mannose than after ^{13}C-glucose. Thus, the metabolism of these three sugars appears to be different. Specific dietary sugars could represent a new class of non essential nutrients displaying interesting metabolic roles. This could have practical consequences especially in parenteral nutrition, where glucose is currently the only sugar available for metabolism.

GLYCANNIC CHAINS of glycoproteins are involved in many important biological functions such as cell-cell interactions or glycoprotein trafficking.[1] Despite a high level of genetic control through the presence and expression of glycosyltransferases, the glycan structure in glycoproteins is not primarily encoded by the genome and thus displays some level of microheterogeneity that could depend, in part, on the availability of sugar-nucleotide, obligatory substrates for glycosyltransferases. For example, some in vitro studies demonstrated that an exogenous supply of nucleotides can modify enzymatic glycosylation.[2] However, the effects of or the need for specific sugars in glycosylation are not known. Recently, exogenous mannose has been shown to be preferred over glucose for glycosylation in cultured human fibroblasts.[3]

All glycoprotein sugars can derive from glucose: the metabolic pathways and enzymes involved have been well characterized (reviewed by Martin et al[4]). Old studies performed in animals using radioactive sugars by injection or, rarely, by ingestion demonstrated that these specific sugars are readily incorporated into glycoproteins; moreover, ingestion of a large excess of the corresponding unlabeled sugar increases catabolism and decreases incorporation of the label.[4] However, these studies had no nutritional purpose, and none were performed in man. We have previously demonstrated in the rat[5-7] that stable-isotope technology can be applied to study the metabolism of glycoprotein sugars in nutritionally relevant conditions. We report here on the application of this technology for such a study in man.

SUBJECTS AND METHODS

Subjects

Four healthy subjects participated in the study. Each subject provided written consent after being informed of the nature, purpose, and possible risks of the study. Subject characteristics are listed in Table 1. The protocol was approved by the Ethical Committee of Lyon A.

Experimental Protocol

One week before the experiment, nutrients known to be enriched in ^{13}C (maize, cane sugar, exotic fruits, and tinned foods) were eliminated from the diet. After an overnight fast, subjects ingested a tracer dose of artificially ^{13}C-enriched (>99% ^{13}C) sugar. [U-^{13}C]glucose and [1-^{13}C]galactose were obtained from Eurisotop (CEA, St Aubin, France), and [1-^{13}C]mannose was obtained from Isotec (Miamisburgh, OH). In different sessions separated by at least 2 weeks, each subject ingested three doses of the same sugar (150, 300, and 550 mg in random order) together with 50 g ^{13}C-poor sucrose in 200 mL water. Galactose and mannose were tested in two subjects. Four hours after tracer ingestion, the subjects ate a naturally labeled carbohydrate-rich meal (pasta). The experimental design is depicted in Fig 1.

The rates of oxygen consumption and carbon dioxide production were measured at regular intervals with a Deltatrac metabolic monitor (Datex, Helsinki, Finland). Breath samples were collected in a 5-L rubber balloon and immediately transferred to two 10-mL glass vacutainers.

Preparation of Glycoprotein Neutral Sugars

The sample preparation has been reported in detail.[5,8] Briefly, glycoproteins from 2 mL serum were precipitated (trichloroacetic acid 20%), delipidated (chloroform:methanol 2:1 vol/vol), and hydrolyzed (HCl 1N for 2 hours at 100°C). After addition of 100 μg xylose as an internal standard, neutral sugars were purified by ion-exchange chromatography (Dowex 50 × 2, H^+ form; Dowex 1 × 2, formate form; Sigma, St Louis, MO) and concentrated by lyophilization. Neutral sugars dissolved in 0.2 mL 1N ammonia were reduced by 2% $NaBH_4$ in 1 mL dimethylsulfoxide. After stopping the reaction with 0.1 mL acetic acid, derivatization was performed by reaction with 2 mL acetic anhydride using 0.2 mL 1-methylimidazole as a catalyst. Alditol acetates were extracted by 2 mL dichloromethane and dried. The $^{13}C/^{12}C$ ratio in alditol acetates was determined in duplicate by gas chromatography–isotope ratio mass spectrometry (Hewlett-Packard 5890, Les Ulis, France; Sira 12 VG Isogas, Middlewich, UK) as previously described.[5-7,9] ^{13}C isotopic enrichment of the ingested solution was determined using an elemental nitrogen and carbon

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Table 1. Subject Characteristics

Subject No.	Sex/ Age (yr)	Height (cm)	Weight (kg)	BMI (kg/m²)
1	M/35	171	71	24.3
2	F/28	172	64	21.6
3	F/25	166	48	17.4
4	M/27	172	68	23.0

Abbreviations: M, male; F, female; BMI, body mass index.

analyzer (Carlo Erba, Massy, France) on-line with an isotope ratio mass spectrometer (Sira 10 VG Isogas).[9]

Calculations

The $^{13}C/^{12}C$ ratio of samples and standard was used to calculate the δ per 1,000 value after correction for ^{17}O content[10] with the equation, $\delta\ ^{13}C\% = [(^{13}C/^{12}C_{sample} - {}^{13}C/{}^{12}C_{standard})/(^{13}C/^{12}C_{standard})] \times 10^3$, where the international standard is the Pee Dee Belemnite (PDB) standard. The $\delta\ ^{13}C\%$ measurement was transformed to ^{13}C atom percent (AP) with the formula, $AP = 100R(0.001\ \delta\ ^{13}C_S + 1)/1 + R(0.001\ \delta\ ^{13}C_S + 1)$, where R is the $^{13}C/^{12}C$ of PDB ($R = 0.0112372$) and $\delta\ ^{13}C_S$ is the $\delta\ ^{13}C$ value of the sample. The calculated AP was transformed to mole percent excess (MPE) with the formula, $APE = AP_S - AP_B$, where AP_s is the AP of the sample and AP_B is the AP in the basal state before ingestion of the labeled sugar. APE was transformed to MPE to take into account the different enrichment of labeled sugars[11] with the formula, $MPE = y \cdot APE$, where y is the ratio of the number of carbons in the derivatized molecule to the number of labeled carbons in the native molecule ($y = 18/1$ for mannose and galactose and 18/6 for glucose).

The amount of ^{13}C sugar incorporated into glycoproteins was calculated with the formula (11), mg ^{13}C sugar incorporated = $MPE_{glycoprotein\ sugar}/MPE_{ingested\ sugar} \times$ (mg ingested ^{13}C sugar), where the MPE value of glycoprotein sugars corresponds to the highest labeling value.

Determination of Exogenous Sugar Oxidation

CO_2 in expired gas was separated from the other components and analyzed for ^{13}C content by a gas chromatograph coupled with an isotope-ratio mass spectrometer.[12] Sugar oxidation was calculated from the isotopic enrichment of expired carbon dioxide, and carbon dioxide production was determined by indirect calorimetry using the equation,[13]

$$\%\ \text{exogenous sugar oxidized per min} = \frac{\text{mmol/L } CO_2 \times f \times \text{MPE } ^{13}C \times 1.25}{\text{mmol/L } ^{13}C \text{ ingested}}$$

where the amount of CO_2 is given by indirect calorimetry, f equals

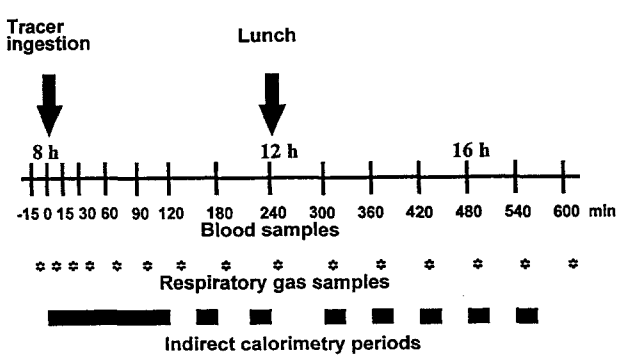

Fig 1. Experimental design.

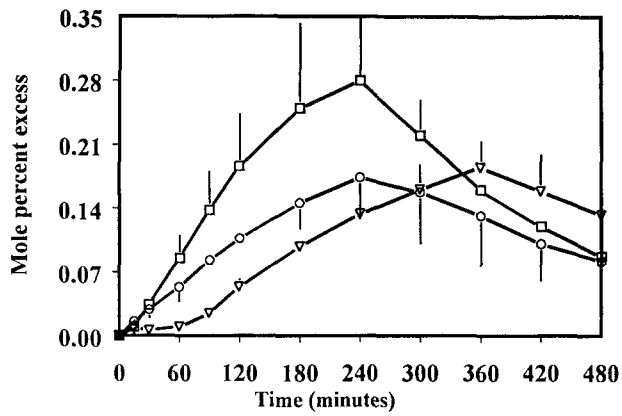

Fig 2. Oxidation kinetics of exogenous ^{13}C-labeled sugars after ingestion at time 0 of 550 mg labeled sugar together with 50 g sucrose. Results are the mean ± SD for 2 tests for each sugar. □, ^{13}C-glucose; ○, ^{13}C-galactose; ▽, ^{13}C-mannose.

12/44 (proportion of carbon in carbon dioxide), and 1.25 is the retention factor for CO_2 in the serum bicarbonate pool.

The total amount of exogenous sugar oxidized is calculated from the area under the curve according to the method of Wolever and Jenkins.[14]

RESULTS

Oxidation of Exogenous Labeled ^{13}C Sugars

The kinetics of ^{13}C enrichment in expired carbon dioxide are shown in Fig 2 for the highest dose of 550 mg. Oxidation of labeled galactose and mannose was lower than for glucose. Mannose oxidation was consistently delayed at 360 minutes compared with galactose and glucose, which peaked at 240 minutes. The sharp decline in ^{13}C-glucose oxidation after 240 minutes might correspond to ingestion of the glucose-rich meal: indeed, the respiratory coefficient was low at 240 minutes (0.812 ± 0.027) and then regularly increased to 0.876 ± 0.048 at 330 minutes (mean ± SD for 18 tests). Total oxidation of exogenous labeled sugars for the 8-hour session is shown in Fig 3. The amount of oxidized sugar is proportionate to the ingested dose.

From the results obtained for two subjects with three doses, the percentage of oxidized sugar for galactose and mannose, respectively, within 1 hour was 0.46 ± 0.11 and 0.1 ± 0.08 and

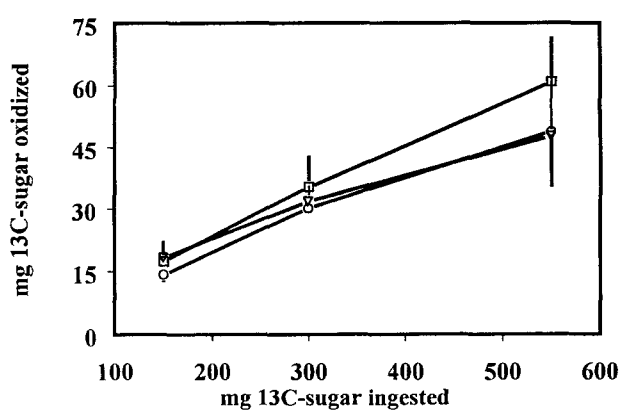

Fig 3. Total oxidation of exogenous labeled sugars over the 8-hour period as a function of the ingested dose. Symbols are as in Fig 2.

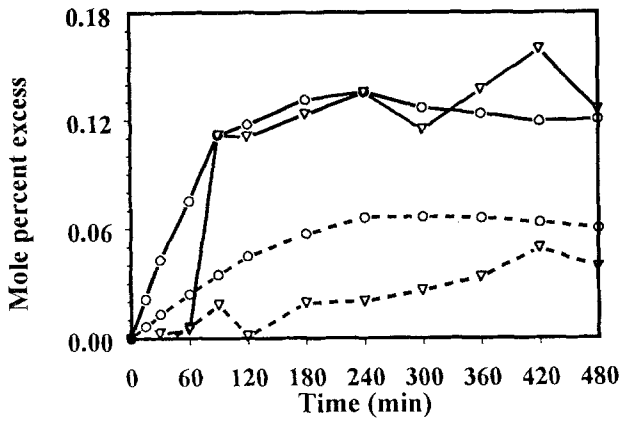

Fig 4. Incorporation of exogenous ^{13}C sugars into glycoprotein sugars for subject no. 1. ○, From ingested galactose; ▽, from ingested mannose; ———, into glycoprotein galactose; -------, into glycoprotein mannose.

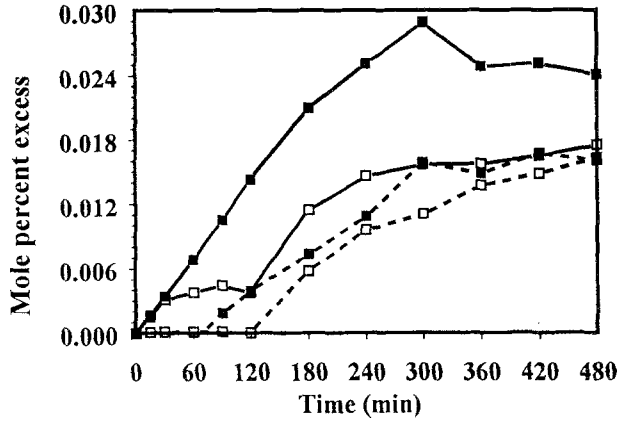

Fig 6. Incorporation of exogenous ^{13}C-glucose into glycoprotein sugars. ———, into glycoprotein galactose; -------, into glycoprotein mannose; ■, subject no. 3; □, subject no. 4.

within 5 hours 6.8 ± 1.2 and 4.85 ± 0.85. Oxidation within the whole 8-hour experimental session was less than 10% of the ingested dose.

Incorporation of Exogenous ^{13}C-Labeled Sugars Into Serum Glycoproteins

The results for all subjects at the highest dose (550 mg) are reported in Figs 4 to 6 and summarized in Fig 7 for the three doses of galactose and glucose. The level of glycoprotein sugar labeling is significantly lower from ingested ^{13}C-glucose versus ingested ^{13}C-galactose or ^{13}C-mannose (Figs 4 to 6). Moreover, for ingested galactose, the amount of ^{13}C label incorporated into glycoproteins was proportionate to the ingested dose, whereas for ingested glucose, the amount of ^{13}C label incorporated into glycoproteins was similar for the doses of 300 and 550 mg (Fig 7)

Although oxidation patterns were similar between subjects, there were large interindividual variations in the handling of exogenous sugars for glycoprotein synthesis. First, for the amount of label incorporated into glycoproteins, subject no. 1 (Fig 4) showed incorporated labeling at a level twice that of subject no. 2 (Fig 5). Second, for the nature of the labeled glycoprotein sugar, subject no. 1 (Fig 4) showed strong and preferential incorporation of label into glycoprotein galactose from either ingested mannose or galactose. Moreover, this incorporation into glycoprotein galactose was delayed and apparent only at 90 minutes, as compared with the rapid and linear direct incorporation of ingested galactose into glycoprotein galactose. Subject no. 2 (Fig 5) showed preferential incorporation of label into glycoprotein galactose from ingested galactose and into glycoprotein mannose from ingested mannose. By contrast, labeling from ingested glucose was more equally incorporated into glycoprotein mannose and galactose, especially for subject no. 4 (Fig 6). Combining results obtained at the three doses for two subjects, the incorporation level was proportionate to the ingested dose for galactose, whereas it plateaued at the same level at 300 and 550 mg for glucose (Fig 7). Mannose cannot be represented on Fig 7, due to the large interconversion to galactose in subject no. 1.

The level of glycoprotein labeling remained high or plateaued through the end of the 8-hour session, although at 240 minutes after tracer ingestion, the subjects ate a ^{13}C-poor meal

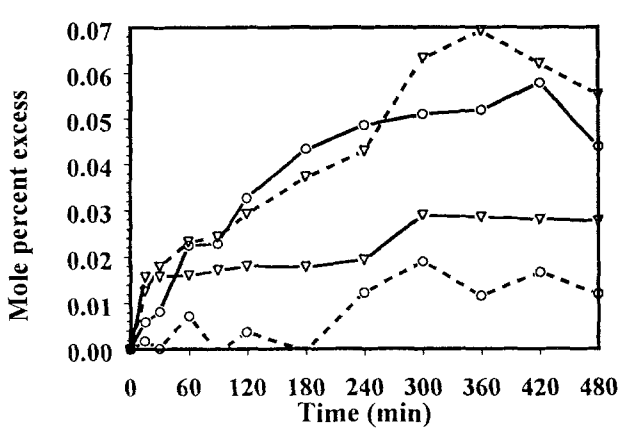

Fig 5. Incorporation of exogenous ^{13}C sugars into glycoprotein sugars for subject no. 2. Symbols are as in Fig 4.

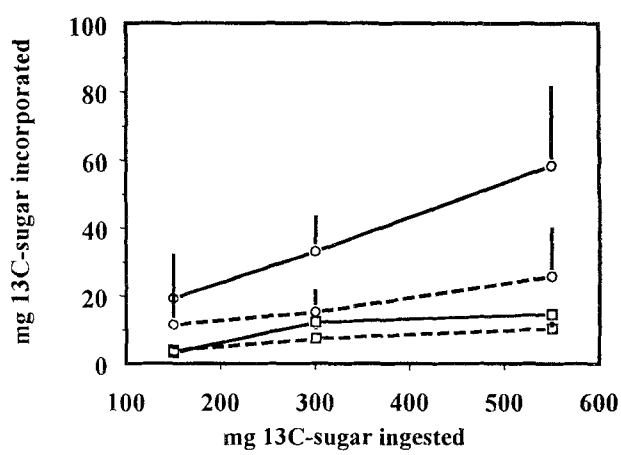

Fig 7. Maximum incorporation of exogenous ^{13}C-galactose and ^{13}-glucose into glycoprotein sugars according to the ingested dose. □, From ingested ^{13}C-glucose; ○, from ingested ^{13}C-galactose; ———, into glycoprotein galactose; -------, into glycoprotein mannose.

(pasta) providing a large disposal of unlabeled glucose. In addition, glycoprotein mannose maximum labeling generally appeared later (2- to 3-hour delay) than glycoprotein galactose maximum labeling.

DISCUSSION

The experimental conditions were chosen to be relevant for current nutrition, in which galactose (with the exception of galactose from dairy products) and mannose are quantitatively minor sugars in the diet compared with glucose or fructose. Thus, together with ingestion of the tracer, subjects ingested 50 g sucrose, which additionally leads to suppressed hepatic glucose production and "normalized" endogenous synthesis of specific sugars. Moreover, subjects ingested a standardized meal 4 hours after tracer ingestion.

In healthy fasting subjects, ^{13}C-galactose ingested alone at similar doses (7 mg/kg body weight) is oxidized 3% to 6% within 1 hour and 21% to 47% within 5 hours,[15] confirming old studies with larger doses and radioactive tracers.[16] The oxidation kinetics reach a maximum at 90 to 120 minutes.[15] For the same intervals in our experimental conditions, oxidation was found to be strongly lower. Since saturation of the oxidation capacity for galactose appears only at high doses,[16] it is concluded that the concomitant ingestion of sucrose together with galactose delays and decreases galactose oxidation. This might be partly explained by competition for intestinal absorption, since galactose and glucose share common intestinal transporters. Although the presence of glucose accelerates the removal of galactose from the blood[17] performed essentially by the liver,[18] this acceleration does not direct galactose to oxidation. Urinary elimination of labeled galactose was not monitored, although there is no renal threshold for this elimination.[19] However, the elimination remains low, from 3% for injected tracer doses to 18% for an injected 20-g dose[16]; thus, it seems unlikely that urinary elimination accounts for the large decrease in galactose oxidation after coingestion of sucrose.

In healthy subjects, at doses of 70 to 200 mg/kg body weight, mannose absorption was not delayed by coingestion of sucrose and serum mannose peaked at 90 minutes.[20] Thus, the delay in mannose oxidation (peak at 360 minutes) after coingestion with sucrose does not rely on competition for intestinal absorption. It could be explained by the slow clearance of mannose from the blood (half-life, 4 hours),[20] despite the existence of a glucose-insensitive high-affinity carrier at the surface of most human cells.[21]

Despite ingestion of a large excess (100-fold) of sucrose, ingested specific ^{13}C sugars, mannose and galactose, are readily incorporated into serum glycoproteins. Obviously, the label from ingested ^{13}C-glucose is diluted by unlabeled glucose, and thus quantitatively, a large part of glycoprotein sugars might effectively derive from glucose. However, the label from ingested specific sugars can also be diluted by unlabeled endogenously produced sugar. Endogenous sugar production has been studied only for galactose: it is about 12 to 24 mg/kg/d in the fasting state.[22] This endogenous production is in the range of the dose we used, but the in vivo regulation of this production is poorly understood, especially in the presence of exogenous galactose. The presence of galactose and glucose activates one of the major enzymes for the metabolic utilization of galactose, galactose-1-phosphate uridyltransferase, which produces galactosyltransferase substrate, UDP-galactose.[23] Thus, in the interpretation of these relative rates of label incorporation for various sugars, it must be kept in mind that the dilution of the label into endogenous pools such as body glucose or into direct precursor pools (sugar nucleotides) has not been determined in this study, so precise quantitative conclusions cannot be made.

Although they have similar body weight, subjects no. 1 and 2 incorporated labeled sugars at different levels. It cannot be ruled out that glycoprotein secretion may differ between the two subjects or that dilution of the label into precursor pools may vary between the subjects, but alternatively, each subject could have a maximal glycosylation rate, indicating that substrate availability would not be a limiting factor in healthy subjects. However, from studies performed in classic galactosemia due to a deficit in uridyltransferase activity, exogenous galactose could be important to maintain a correct ratio of UDP-glucose to UDP-galactose in some cells.[24] The impairment in the UDP-galactose concentration in affected subjects could be responsible, in part, for the altered synthesis of brain glycolipids in these subjects.[25]

Contrary to the results reported in animals using tracer doses of radioactive sugars,[4] there is significant interconversion of specific sugars in man in our experimental conditions. The most striking is for subject no. 1, who showed preferential incorporation of label from dietary mannose as labeled galactose into glycoproteins, while in the other cases, the level of label in derived sugar represented less than 50% of the label of sugar in the native form. This interconversion of specific sugars in the presence of a large glucose load may suggest the existence of some type of channeling of specific sugars for glycosylation or may support the hypothesis of a preferential use of specific sugars over glucose for glycosylation.[3] It has been proposed that ^{13}C-galactose could be useful to quantify hepatic secretion of glycoconjugates, since the use of acetaminophen allows trapping of UDP-glucuronic acid formed from UDP-galactose and then determination of the ^{13}C enrichment of the direct precursor of glycoconjugate biosynthesis by a simple urinary analysis.[26] There is no report of such use in man, but the existence of significant interconversion to sugars other than glucose could lead to substantial errors in some subjects.

The existence of a plateau indicates that the label is not diluted by secretion of unlabeled glycoproteins, despite continuous secretion of hepatic glycoproteins. As proposed from animal studies, this fact suggests either a recycling of specific sugars or a saturation of endogenous pools of these sugars by diet-derived labeled specific sugars.

The delay in glycoprotein mannose maximum labeling as compared with glycoprotein galactose could be due, in part, to the inner position of mannose in the glycan structure as compared with the more external galactose. Thus, this result might provide an upper limit (approximately 2 to 3 hours) for the duration of in vivo maturation of glycannic chains in the liver.

Obviously, these results must be confirmed by other tests in

different subjects. It is also necessary to study whether ingestion of specific sugars leads to modifications in glycan microheterogeneity. However, the results undoubtedly indicate that the various dietary sugars do not have the same metabolic fate. In this way, specific sugars could be nutritionally important at low doses, especially in artificial nutrition in which glucose is the only sugar provided for metabolism. Although it is sufficient for energy requirements, glucose alone is not sufficient for protein enzymatic glycosylation.

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REFERENCES

1. Dwek RA: Glycobiology: Towards understanding the function of sugars. Biochem Soc Trans 23:1-25, 1995

2. Pels Rijcken WR, Overdijk B, Van den Eijnden DH, et al: The effects of increasing nucleotide sugar concentrations on the incorporation of sugars into glycoconjugates in rat hepatocytes. Biochem J 305:865-870, 1995

3. Panneerselvam K, Etchison JR, Freeze HH: Human fibroblasts prefer mannose over glucose as a source of mannose for N-glycosylation—Evidence for the functional importance of transported mannose. J Biol Chem 272:23123-23129, 1997

4. Martin A, Rambal C, Berger V, et al: Availability of specific sugars for glycoconjugate biosynthesis: A need for further investigations in man. Biochimie 80:75-86, 1998

5. Rambal C, Pachiaudi C, Normand S, et al: Use of compounds naturally labeled with stable isotopes for the study of the metabolism of glycoprotein neutral sugars by gas-liquid chromatography–isotope-ratio mass spectrometry. Technical validation in the rat. Carbohydr Res 236:29-37, 1992

6. Rambal C, Pachiaudi C, Normand S, et al: Differential incorporation of ^{13}C label from dietary glucose into neutral sugars of rat intestine macromolecules. Ann Nutr Metab 9:143-151, 1995

7. Rambal C, Pachiaudi C, Normand S, et al: Effects of specific dietary sugars on the incorporation of ^{13}C label from dietary glucose into neutral sugars of rat intestine and serum glycoproteins. Br J Nutr 73:443-459, 1995

8. Blackeney AB, Harris PJ, Henry RJ, et al: A simple and rapid preparation of alditol acetates. Carbohydr Res 113:291-299, 1983

9. Normand S, Pachiaudi C, Khalfallah Y, et al: ^{13}C appearance in plasma glucose and breath CO_2 during feeding with naturally ^{13}C-enriched starchy food in normal humans. Am J Clin Nutr 55:430-435, 1992

10. Craig GH: Isotopic standards for carbon and oxygen and correction factors for mass spectrometry analysis of carbon dioxide. Geochim Cosmochim Acta 12:133-149, 1957

11. Khalfallah Y, Normand S, Tissot S, et al: Determination of δ ^{13}C values of plasma lactate by gas chromatography–isotope ratio mass spectrometry. Biol Mass Spectrom 22:707-711, 1993

12. Guilluy R, Billion-Rey F, Pachiaudi C, et al: On-line purification and carbon 13 isotopic analysis of carbon dioxide in breath: Evaluation of an on-line gas chromatography–isotope ratio mass spectrometry. Anal Chim Acta 259:193-202, 1992

13. Jones PJH, Pencharz PB, Clandinin MT: Whole body oxidation of dietary fatty acids: Implication for energy utilization. Am J Clin Nutr 42:769-777, 1985

14. Wolever TMS, Jenkins DJA: The use of glycemic index in predicting the blood glucose response to mixed meals. Am J Clin Nutr 43:167-172, 1986

15. Berry GT, Nissim I, Mazur AT, et al: In vivo oxidation of [^{13}C]galactose in patients with galactose-1-phosphate uridyltransferase deficiency. Biochem Mol Med 56:158-165, 1995

16. Segal S, Blair A: Some observations on the metabolism of D-galactose in normal man. J Clin Invest 40:2016-2025, 1961

17. Williams CA, Phillips T, Mcdonald I: The influence of glucose on serum galactose levels in man. Metabolism 32:250-256, 1983

18. Williams CA: Metabolism of lactose and galactose in man. Prog Biochem Pharmacol 21:219-247, 1986

19. Harding VJ, Grant GA: Metabolism of galactose. I. Cutaneous blood sugars after galactose ingestion. J Biol Chem 99:629-646, 1932

20. Alton G, Kjaergaard S, Etchison JR, et al: Oral ingestion of mannose elevates blood mannose levels: A first step toward a potential therapy for carbohydrate-deficient glycoprotein syndrome type I. Biochem Mol Med 60:127-133, 1997

21. Panneerselvam K, Freeze HH: Mannose enters mammalian cells using specific transporter that is insensitive to glucose. J Biol Chem 271:9417-9421, 1996

22. Berry GT, Nissim I, Lin Z, et al: Endogenous synthesis of galactose in normal men and patients with hereditary galactosaemia. Lancet 346:1073-1074, 1995

23. Rogers SR, Bovee BW, Saunders SL, et al: Galactose as a regulatory factor of its own metabolism by rat liver. Metabolism 38:810-815, 1989

24. Gibson JB, Berry GT, Palmieri MJ, et al: Sugar nucleotide concentrations in red blood cells of patients on protein- and lactose-limited diets: Effect of galactose supplementation. Am J Clin Nutr 63:704-708, 1996

25. Petry K, Greinix HT, Nudelman E, et al: Characterization of a novel biochemical abnormality in galactosemia: Deficiency of glycolipids containing galactose or N-acetylgalactosamine and accumulation of precursors in brain and lymphocytes. Biochem Med Metab Biol 46:93-104, 1991

26. Hellerstein MK, Greenblatt DJ, Munro HN: Glycoconjugates as noninvasive probes of intrahepatic metabolism: I. Kinetics of label incorporation with evidence of a common precursor UDP-glucose pool for secreted glycoconjugates. Metabolism 36:988-994, 1987