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The Effects of Carbohydrate Variation in Isocaloric Diets on Glycogenolysis and Gluconeogenesis in Healthy Men*

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ABSTRACT

To evaluate the effect of dietary carbohydrate content on postabsorptive glucose metabolism, we quantified gluconeogenesis and glycogenolysis after 11 days of high carbohydrate (85% carbohydrate), control (44% carbohydrate), and very low carbohydrate (2% carbohydrate) diets in six healthy men. Diets were eucaloric and provided 15% of energy as protein. Postabsorptive glucose production was measured by infusion of [6,6-$^2H_2$]glucose, and fractional gluconeogenesis was measured by ingestion of ^2H_2O. Postabsorptive glucose production rates were 13.0 ± 0.7, 11.4 ± 0.4, and 9.7 ± 0.4 μmol/kg·min after high carbohydrate, control, and very low carbohydrate diets, respectively ($P < 0.001$ among the three diets). Gluconeogenesis was about 14% higher after the very low carbohydrate diet (6.3 ± 0.2 μmol/kg·min; $P = 0.001$) compared to the control diet, but was not different between the high carbohydrate and control diets (5.5 ± 0.3 *vs.* 5.5 ± 0.2 μmol/kg·min). The rates of glycogenolysis were 7.5 ± 0.5, 5.9 ± 0.3, and 3.4 ± 0.3 μmol/kg·min, respectively ($P < 0.001$ among the three diets).

We conclude that under eucaloric conditions in healthy subjects, dietary carbohydrate content affects the rate of postabsorptive glucose production mainly by modulation of glycogenolysis. In contrast, dietary carbohydrate content affects the postabsorptive rate of gluconeogenesis minimally, as evidenced by only a slight increase in gluconeogenesis during severe carbohydrate restriction. (*J Clin Endocrinol Metab* **85:** 1963–1967, 2000)

NUTRITIONAL intake is an important determinant of the rate of postabsorptive glucose production. There is a direct relation between carbohydrate intake and postabsorptive glucose production (1). Carbohydrate overfeeding increases postabsorptive glucose production (2), whereas fasting reduces glucose production (3, 4). Changes in postabsorptive glucose production reflect changes in gluconeogenesis and/or glycogenolysis, because endogenous glucose can only be derived from gluconeogenesis and glycogenolysis. Quantification of these two pathways is essential for better understanding of changes in intrahepatic glucose metabolism induced by variations in carbohydrate intake. Several studies have addressed this issue by measuring the incorporation of gluconeogenic precursors into glucose. In perfused livers of rats fed a eucaloric carbohydrate-free diet, conversion of alanine and pyruvate to glucose is increased compared to that with a control diet (5). In humans, conversion of alanine to glucose is decreased after several days of excessive carbohydrate intake (2). Although alanine is an important precursor of gluconeogenesis, extrapolation to total gluconeogenesis should be interpreted with caution.

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Recently, Landau *et al.* described the use of ^2H_2O for the measurement of gluconeogenesis (6, 7). This method allows for quantification of total gluconeogenesis regardless of the contributions of individual gluconeogenic precursors. To study the effect of dietary carbohydrate content on the contribution of gluconeogenesis and glycogenolysis to postabsorptive glucose production, we used ^2H_2O and measured the effects of variations in carbohydrate content in isocaloric diets on postabsorptive glucose production and gluconeogenesis in six healthy men. Each diet was used for 11 days and contained an identical amount of proteins of similar composition, whereas the remainder of the calories consisted of only carbohydrates (diet 1), only fat (diet 2), or an approximately equal distribution of carbohydrates and fat (diet 3).

Subjects and Methods

Subjects

Six healthy males (aged 29–55 yr; body mass index, 21–26 kg/m^2) were studied on three separate occasions after an overnight fast. All subjects were in good health and did not use any medication. All participating subjects gave written informed consent. This study was approved by the medical ethical committee of the Academic Medical Center.

Diets

The subjects were studied on three occasions, each time after 11 days on a different diet. The sequence of the three studies was determined by random assignment. The three diets consisted of liquid formulas and contained identical amounts (15% of the calories) and identical protein compositions. In addition to the proteins, the high carbohydrate diet

contained 85% of calories in the form of carbohydrates. The control diet contained 44% of calories in the form of carbohydrates and 41% in the form of lipids. The very low carbohydrate diet contained 2% of calories in the form of carbohydrates and 83% in the form of lipids. Caloric requirements for each subject were assessed by a dietician by means of a 3-day dietary journal. Meals with predetermined amounts of calories were taken at six fixed time points each day between 0800–2130 h for 11 days. In addition to the diets, the subjects were allowed to drink only water *ad libitum*. Subjects were seen daily to receive their diet for the next day. All subjects refrained from alcohol, and exercise was limited to normal daily activities during the experimental diets. Compliance with the diet was assessed by measuring the respiratory quotient, which reflects the ratio of carbohydrate/fat intake (8). Respiratory quotients were measured after 10 and 11 days of the experimental diet after an overnight fast of 14 h with an energy expenditure unit (model 2900, Sensormedics, Anaheim, CA) using the ventilated hood technique. For each subject the period between the beginning of two successive experimental diets was 8–10 weeks, during which the subjects consumed their habitual diet.

Protocol

The subjects were admitted to the clinical research center and studied in the supine position. At 0645 h, after an overnight fast of 10 h, a catheter was inserted into an antecubital vein in each arm. One catheter was used for sampling of arterialized blood using a heated handbox (60 C). The other catheter was used for infusion of [6,6-$^2H_2$]glucose. At 0655 h, urine and blood samples were taken for determination of background enrichments of body water and plasma glucose, respectively. From 0700–0900 h, ^2H_2O (>99.8% enriched; Cambridge Isotopes, Andover, MA) was administered orally every half-hour up to a total dose of 5 g/kg body water to achieve a deuterium enrichment of body water of approximately 0.5%. Body water was estimated to be 60% of total body weight. At 0900 h, after taking a blood sample for background enrichment of plasma glucose, a primed continuous infusion of [6,6-$^2H_2$]glucose (>99% enriched; Cambridge Isotopes) was started at a rate of 0.33 μmol/kg·min (prime, 26.4 μmol/kg). The subjects voided urine at 1100 h, which was discarded. Subsequently, a urine sample was obtained between 1100 and 1200 h for determination of body water enrichment. At 1130, 1145, and 1200 h, blood samples were taken for enrichment of [6,6-$^2H_2$]glucose and deuterium at C5 of plasma glucose, glucose concentration, and plasma levels of glucoregulatory hormones. During the study, subjects were allowed to drink only water, which was 0.5% enriched with ^2H_2O.

Analytical procedures

Plasma samples for glucose enrichments of [6,6-$^2H_2$]glucose were deproteinized with methanol (9). The aldonitril pentaacetate derivative of glucose (10) was injected into a gas chromatograph/mass spectrometer system. Separation was achieved on a J&W (J&W Scientific, Folsom, CA) DB17 column (30 m × 0.25 mm; df, 0.25 μm). Glucose concentrations were determined by gas chromatography, using xylose as an internal standard. Glucose was monitored at m/z 187,188, and 189. The enrichment of [6,6-$^2H_2$]glucose was determined by dividing the peak area of m/z 189 by the total peak area and correcting for natural enrichments.

To measure deuterium enrichment at the C5 position, glucose was converted to hexamethylenetetraamine (HMT) as described by Landau *et al.* (6). HMT was injected into a gas chromatograph-mass spectrometer. Separation was achieved on an AT-Amine (Alltech, Deerfield, IL) column (30 m × 0.25 mm; df, 0.25 μm). HMT consists of six formaldehyde molecules, originally derived from the C5 of six glucose molecules. The distribution of the different masses in HMT can be used to calculate the original deuterium enrichment at C5 by mass isotopomer distribution analysis (11). This adaptation to the method of Landau *et al.* (6) was validated in our laboratory, and the results from this adapted method were not different from those obtained using a calibration curve with [1,2,3,4,5,6,6-$^2H_7$]glucose (98%; CIL, Andover, MA; n = 18; $P > 0.9$, by paired *t* test). Quality control was incorporated at two levels. Within each series, unlabeled glucose (Merck & Co. Inc., Darmstadt, Germany) was also converted to HMT, and M+1 in this HMT was determined. If the measured M+1 was not within 3% of the theoretical value of natural abundance of M+1, the series was rejected. If the series was accepted, a second control was measured, a plasma sample with repeatedly measured deuterium enrichment at C5 [0.31%; n = 15; intraassay coefficient of variation (CV), 8%]. The series was also rejected if the measured enrichment from the second control was not within 2 SD. Deuterium enrichment in body water was measured by a method adapted from that reported by Previs *et al.* (12). All isotopic enrichments were measured on a gas chromatograph-mass spectrometer (model 6890 gas chromatograph coupled to a model 5973 mass selective detector, equipped with an electron impact ionization mode, Hewlett-Packard Co., Palo Alto, CA).

The plasma insulin concentration was determined by RIA (Insulin RIA 100, Pharmacia Biotech, Uppsala, Sweden; intraassay CV, 3–5%; interassay CV, 6–9%; detection limit, 15 pmol/L). C Peptide was determined by RIA (RIA-Coat C-Peptide, Byk-Sangtec Diagnostica GmbH & Co., Dietzenbach, Germany; intraassay CV, 4–6%; interassay CV, 6–8%; detection limit, 50 pmol/L). Cortisol was measured by enzyme immunoassay on an Immulite analyzer (Diagnostic Products, Los Angeles, CA; intraassay CV, 2–4%; interassay CV, 3–7%; detection limit, 50 nmol/L). Glucagon was determined by RIA (Linco Research, Inc., St. Charles, MO; intraassay CV, 3–5%, interassay CV, 9–13%; detection limit, 15 ng/L). Norepinephrine and epinephrine were determined by an in-house high performance liquid chromatography method (norepinephrine: intraassay CV, 6–8%; interassay CV, 7–10%; detection limit, 0.05 nmol/L; epinephrine: intraassay CV, 6–8%; interassay CV, 7–12%; detection limit, 0.05 nmol/L). Serum free fatty acids were measured by an enzymatic method (NEFAC, Wako Chemicals GmbH, Neuss, Germany; intraassay CV, 2–4%; interassay CV, 3–6%; detection limit, 0.02 mmol/L).

Calculations and statistics

The rate of endogenous glucose production (R_a) was calculated by dividing the infusion rate of [6,6-$^2H_2$]glucose by the resulting M+2 enrichment of plasma aldenotril pentaacetate glucose. The fractional rate of gluconeogenesis was calculated by dividing deuterium enrichment at C5 of plasma glucose by deuterium enrichment in body water. The absolute rates of gluconeogenesis were calculated by multiplying fractional gluconeogenesis with endogenous glucose production. Only absolute rates of gluconeogenesis are reported unless stated otherwise. Glycogenolysis was calculated by subtracting the absolute rate of gluconeogenesis from endogenous glucose production.

The results of the three diets were analyzed with ANOVA for randomized block design and Fisher's least significant difference test when appropriate. $P < 0.05$ was considered statistically different. Data are presented as the mean ± SE.

Results

Dietary compliance was assessed by measuring the postabsorptive respiratory quotient after 10 and 11 days of the experimental diets. The respiratory quotient increased with increasing dietary carbohydrate content from 0.73 ± 0.01 to 0.81 ± 0.01 to 0.86 ± 0.02 ($P \leq 0.014$ for differences between each diet). The postabsorptive (14-h fast) concentrations of plasma glucose (Table 1) were not different between the high carbohydrate and the control diets, but were lower after the very low carbohydrate diet compared to the control diet ($P < 0.05$). The rate of postabsorptive glucose production depended on the carbohydrate content of the diets: 13.0 ± 0.7, 11.4 ± 0.4, and 9.7 ± 0.4 μmol/kg·min after 11 days of high carbohydrate, control, and very low carbohydrate diets, respectively ($P < 0.001$ among the three diets).

Baseline deuterium enrichments of body water and on the C5 position of glucose at the beginning of each infusion protocol were not different between the diets and equaled natural abundance. Therefore, there was no underestimation of fractional gluconeogenesis due to deuterium label on the C5 position of glucose derived from previous experiments.

Deuterium enrichments on the C5 position of glucose between 1130–1200 h were constant within each experiment. Actual enrichments of body water and on the C5 position of glucose are shown in Table 2. The postabsorptive rates of gluconeogenesis and glycogenolysis are presented in Fig. 1. Gluconeogenesis was not affected by the high carbohydrate diet compared to the control diet, but was approximately 14% higher ($P = 0.001$ *vs.* both other diets) after 11 days of a very low carbohydrate diet. The rate of glycogenolysis was related to dietary carbohydrate content, with the highest rate after high carbohydrate and the lowest rate after very low carbohydrate intake ($P < 0.001$ among the three diets). After 11 days of eucaloric, very low carbohydrate feeding, the rate of glycogenolysis was 3.4 ± 0.3 μmol/kg·min or about 35% of postabsorptive glucose production.

Plasma insulin and C peptide concentrations were lower after the very low carbohydrate diet compared to the other diets. Other glucoregulatory hormones were not different between the diets (Table 1). Plasma concentrations of free fatty acids were higher after the very low carbohydrate diet compared to the control diet (0.78 ± 0.12 *vs.* 0.36 ± 0.05 mmol/L; $P = 0.001$), but were not different between the control and high carbohydrate diets (0.36 ± 0.04 mmol/L).

Discussion

This study describes the effects of modulation of carbohydrate content in isocaloric diets on postabsorptive glucose production. The data indicate that the postabsorptive rate of glucose production is a reflection of dietary carbohydrate content. The main mechanism involved is modulation of the rate of glycogenolysis. High dietary carbohydrate intake results in high postabsorptive rates of glycogenolysis without any change in the rate of gluconeogenesis. After very low carbohydrate intake, the rate of glycogenolysis is low compared to that after control feeding, and gluconeogenesis is slightly stimulated.

In the present study ^2H_2O was used to quantify gluconeogenesis. The ratio of deuterium enrichment at the C5 position of glucose over the enrichment in body water was used to quantify fractional gluconeogenesis. Chandramouli *et al.* showed that deuterium enrichment in body water equals that at the C2 position of glucose in the same study design of isotope administration that we used in the present study (7). Chandramouli *et al.* also showed that deuterium enrichment at C2 and in body water was essentially at steady state approximately 1 h after completion of ^2H_2O intake (7). Previously we found that deuterium enrichment in body water was at a steady state within 1 h after completion of ^2H_2O intake under conditions identical to those in the present study (unpublished data). As samples for determination of gluconeogenesis in the present study were taken 2.5 h after completion of ^2H_2O, it is unlikely that steady state was not achieved. However, under other conditions, for instance in diabetes mellitus, a longer period between ^2H_2O administration and sampling might be required.

Another methodological issue may be raised, in that we

TABLE 1. Postabsorptive concentrations of plasma glucose and glucoregulatory hormones after 11 days on high carbohydrate, control, and very low carbohydrate diets

	High carbohydrate	Control	Low carbohydrate
Glucose (mmol/L)	5.11 ± 0.11	5.17 ± 0.17	4.65 ± 0.21[a]
Insulin (pmol/L)	38 ± 3	37 ± 3	25 ± 4[b]
C Peptide (pmol/L)	362 ± 35	435 ± 73	195 ± 55[c]
Glucagon (ng/L)	60 ± 4	57 ± 3	65 ± 7
Cortisol (nmol/L)	224 ± 14	217 ± 21	265 ± 26
Epinephrine (nmol/L)	0.31 ± 0.07	0.31 ± 0.05	0.24 ± 0.05
Norepinephrine (nmol/L)	1.94 ± 0.49	1.88 ± 0.29	1.85 ± 0.08

Results are expressed as the mean ± SE.
[a] $P < 0.05$ *vs.* control diet.
[b] $P < 0.01$ *vs.* control diet.
[c] $P < 0.001$ *vs.* control diet.

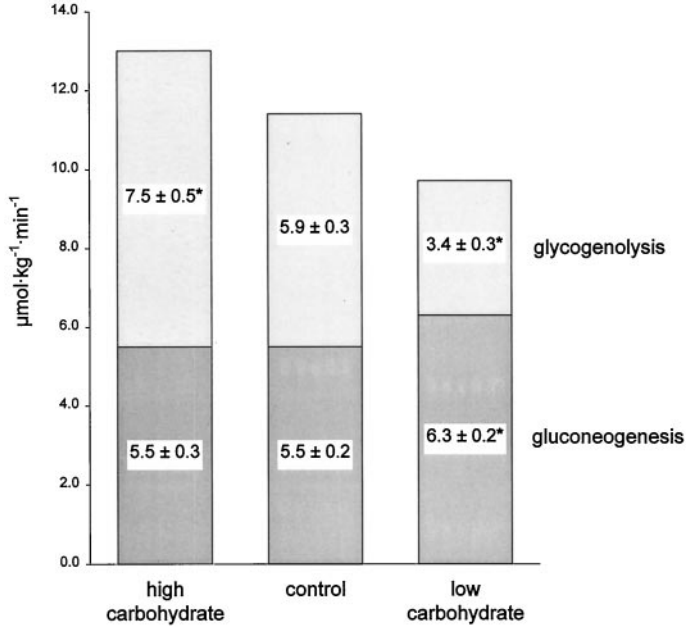

FIG. 1. Postabsorptive rates of gluconeogenesis and glycogenolysis after 11 days on high carbohydrate, control, and very low carbohydrate diets in six healthy men. Values are the mean ± SE. *, Significant difference ($P \leq 0.001$) compared to the control diet.

TABLE 2. Mean deuterium enrichments in body water and on the C5 position of glucose between 1130 and 1200 h

Subject no.	High carbohydrate		Control		Low carbohydrate	
	C5 (%)	Body water (%)	C5 (%)	Body water (%)	C5 (%)	Body water (%)
1	0.22	0.55	0.27	0.53	0.34	0.51
2	0.23	0.48	0.22	0.47	0.29	0.47
3	0.21	0.53	0.24	0.53	0.32	0.52
4	0.20	0.44	0.22	0.44	0.31	0.46
5	0.16	0.39	0.18	0.35	0.28	0.42
6	0.19	0.43	0.21	0.44	0.34	0.48

administered both ^2H_2O and [6,6-$^2H_2$]glucose, which might result in analytical interference of the isotopomers. However, administration of [6,6-$^2H_2$]glucose in the absence of ^2H_2O did not cause a detectable increase above natural abundance in M_1 at the C5 position of glucose as was observed by others (7) as well as ourselves (unpublished data). The increase due to the administration of ^2H_2O without [6,6-$^2H_2$]glucose in the enrichment in M_2 in the glucose fragment used to measure enrichment from [6,6-$^2H_2$]glucose was negligible at an infusion rate of 0.33 μmol/kg·min [6,6-$^2H_2$]glucose (unpublished data). Background enrichments for M_2 were taken 2 h after the first administration of ^2H_2O to reduce possible interference even further. Therefore, it is very unlikely that the results in the present study are subject to methodological errors.

To study the effects of varying carbohydrate intakes, two approaches are possible. Carbohydrates can be simply added to or removed from a standard diet without altering absolute amounts of fat and protein, as has been done before. These studies indicate that postabsorptive glucose production is related to carbohydrate intake (1) and that excessive carbohydrate intake reduces gluconeogenesis (2). However, this approach also affects caloric intake. To our knowledge, studies have not been carried out studying the effect of isocaloric changes in the carbohydrate to fat ratio on gluconeogenesis and glycogenolysis. Therefore, in our approach we replaced carbohydrates with fat to maintain a constant caloric intake.

In the present study the amounts and compositions of proteins in the three diets were identical, precluding any effect of protein intake on the differences observed in our study in postabsorptive glucose metabolism. Interestingly, postabsorptive glucose production still amounted to 9.7 μmol/kg·min after 11 days of carbohydrate, but not caloric, deprivation, whereas in other studies prolonged fasting resulted in lower rates of glucose production, ranging from 7.9–8.7 μmol/kg·min (3, 4, 7, 13). These data suggest that the rate of glucose production after isocaloric carbohydrate deprivation is higher than that during carbohydrate deprivation in starvation. This might be attributed to an adequate protein intake during the very low carbohydrate diet in contrast to starvation, because modulation of protein intake affects glucose production (14, 15).

Our results indicate that the rate of postabsorptive glucose production depends on the amount of carbohydrate intake, as glucose production is reduced by diminishing carbohydrate intake. This reduction in glucose production is caused exclusively by a decrease in the rate of glycogenolysis. Glucoregulatory hormones, such as glucagon, adrenaline, insulin, and glucocorticoids, have distinct modulatory effects on glycogenolysis (16). It seems unlikely that glucoregulatory hormone levels contributed to the differences in glycogenolysis, as most hormone levels were not different among the diets. Plasma insulin levels were even lower after the very low carbohydrate diet, which would favor an increase rather than a decrease in glycogenolysis. Therefore, it is likely that other factors are involved. For instance, the rate of glycogenolysis might at least in part be regulated by hepatic glycogen concentrations. In the present study glycogenolysis is defined as the rate of breakdown of glycogen molecules that were already present before administration of ^2H_2O, because formation of glycogen from gluconeogenesis and subsequent conversion to glucose during the study would be measured as gluconeogenesis. Surprisingly, glycogenolysis still accounted for about 35% of postabsorptive glucose production after 11 days of virtually absent carbohydrate intake, which indicates that glycogen stores were not fully depleted. This is supported by the observation that hepatic glycogen concentration in rats after 4 weeks of high fat feeding was still about 50% of that in carbohydrate-fed animals (17). As glycogen could not have been derived from dietary carbohydrates after 11 days of carbohydrate deprivation, the contribution of glycogen to postabsorptive glucose production must ultimately have been derived from gluconeogenesis, shuttled to glycogen.

The rate of gluconeogenesis is regulated by several factors, including glucoregulatory hormones (18). Insulin suppresses gluconeogenesis, whereas glucagon, glucocorticoids, and catecholamines enhance gluconeogenesis (19). Other factors include free fatty acids, which have been shown to stimulate gluconeogenesis (20). In the present study the rate of gluconeogenesis was not different between high and intermediate carbohydrate feedings, which is compatible with the fact that neither glucoregulatory hormones nor free fatty acid concentrations were different. The fact that eucaloric, high carbohydrate intake had no suppressive effect on postabsorptive gluconeogenesis appears to be in contrast to hypercaloric carbohydrate overfeeding, which reduces gluconeogenesis from alanine (2). This discrepancy may be due to methodological differences as well differences in study design. In the present study total gluconeogenesis was measured, *i.e.* the sum of all precursors incorporated into glucose, instead of incorporation of a single gluconeogenic precursor. Moreover, hypercaloric carbohydrate overfeeding increased glucose and insulin concentrations (2), which caused inhibition of phosphoenolpyruvate carboxykinase activity (21, 22), an enzyme that contributes to the control of gluconeogenesis.

Eucaloric low carbohydrate feeding, *i.e.* high fat feeding, stimulated gluconeogenesis, associated with increased plasma free fatty acid and decreased plasma insulin levels. A similar effect of eucaloric low carbohydrate feeding has been observed in rats (5). As has been proposed previously (5), mitochondrial acetyl coenzyme A probably plays a pivotal role, because after 11 days of virtually no carbohydrate intake, fatty acids are the main substrate for oxidation, which results in the production of large amounts acetyl coenzyme A. Acetyl coenzyme A activates pyruvate carboxylase, which might accelerate gluconeogenesis, because pyruvate carboxylase has a high flux control coefficient in gluconeogenesis (23). In addition, the decrease in plasma insulin might stimulate gluconeogenesis by induction of phosphoenolpyruvate carboxykinase. Therefore, a very low carbohydrate diet probably stimulates gluconeogenesis by enhanced fatty acid availability and reduced insulin levels.

In conclusion, carbohydrate intake affects postabsorptive glucose production mainly by modulation of glycogenolysis. The postabsorptive rate of gluconeogenesis is not affected by high carbohydrate intake, but increases after eucaloric very low carbohydrate feeding.

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