

Prior Carbohydrate Consumption Affects the Amount of Carbohydrate that Rats Choose to Eat¹

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ABSTRACT Consumption of protein-rich, carbohydrate-restricted reducing diets has been associated anecdotally with an increased appetite for carbohydrate. We have tested the effect of such a diet on carbohydrate intake by rats. Rats were given either a calorie-restricted ketogenic diet containing protein and fat or a control diet containing carbohydrate along with the protein and fat. When allowed to choose from a pair of isocaloric, isonitrogenous diets containing 25 or 75% dextrin, ketotic rats ate significantly more carbohydrate and total food than control animals during the first 30 minutes of feeding, apparently requiring more of the carbohydrate to obtain an increase in brain tryptophan similar to controls. Ketotic rats ate a significantly higher proportion of total calories as carbohydrate. Similar results were obtained when sucrose replaced dextrin. When ketotic and control rats chose between two diets differing in proportions of fat or protein, no differences were observed between the groups in total food intake nor in the amounts or proportions of fat or protein eaten. We also compared the effects of a small, isocaloric premeal containing only carbohydrate (1.4 g dextrose) or mixed nutrients on subsequent carbohydrate consumption in otherwise untreated rats allowed to choose from 25 and 75% dextrin diets. Rats eating the carbohydrate premeal subsequently ate as much total food as the mixed-nutrient controls, but significantly less carbohydrate. These observations suggest that carbohydrate intake is influenced by prior nutrient consumption and that prolonged deprivation of carbohydrate can lead to overconsumption of this nutrient when it is reintroduced into the diet. *J. Nutr.* 113: 70-78, 1983.

INDEXING KEY WORDS appetite • obesity • carbohydrate • ketosis

The proportions of protein and carbohydrate in a meal determine the plasma amino acid patterns during its digestion and absorption, and thereby affect the synthesis and release of brain serotonin. When rats ingest a carbohydrate-rich, protein-free meal, the resulting insulin secretion reduces the plasma levels of the large neutral amino acids (LNAA) (1, 2). Plasma tryptophan levels do not change, and as a consequence, the ratio of the tryptophan concentration to that of the LNAA (Trp:LNAA) increases. This facilitates tryptophan uptake into the brain, increasing the saturation of tryptophan hydroxylase and accelerating serotonin synthesis (1, 2). The

ingestion of a meal rich in protein produces opposite effects: plasma levels of the LNAA increase proportionately more than those of tryptophan, thus brain tryptophan and serotonin levels fail to increase and can actually fall (1, 2).

The changes in serotonin release after food intake may be involved in mechanisms that control appetite for protein (3) and carbo-

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hydrates (4, 5) and may ensure that the daily relative intakes of carbohydrate and protein fall within a desired range. This hypothesis is supported by evidence that drugs enhancing serotonin's release from brain neurons or blocking its reuptake decrease voluntary carbohydrate intake by rats (4) and humans (6). Serotonin-releasing neurons may be involved in a short-term negative feedback mechanism that diminishes the likelihood that animals will follow one predominantly carbohydrate meal with another one.

If high plasma Trp:LNAAs ratios tend to suppress appetite for carbohydrate, then it might be anticipated that animals eating diets that decrease this ratio would tend to eat more carbohydrate when given the opportunity. One such diet is the low-carbohydrate, ketogenic diet widely used to facilitate weight loss (i.e., the protein-sparing, modified fast diet). This high-protein diet supplies very large amounts of LNAAs and relatively little tryptophan (the scarcest amino acid in protein). Moreover, such diets elicit the secretion of relatively little insulin (7), causing plasma LNAAs to be elevated (Burckhardt, P., Harief, E., Wurtman, R. J., Wurtman, J. J., & Mauron, C., unpublished observations).

The present studies examine the hypothesis that chronic consumption of carbohydrate-deficient diets by rats results in an increased appetite for carbohydrates and that this effect is independent of their sweetness. We also demonstrate the reverse relationship, i.e., that consumption of a carbohydrate premeal selectively reduces mealtime carbohydrate intake when rats are allowed to choose between two diets of different carbohydrate content.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were obtained at weights of approximately 250 g (60 days of age). They were housed singly in suspended wire-mesh cages at 23° and maintained on a reverse lighting schedule (dark period: 0900 to 2100). Rats were placed immediately on the ketogenic or control diets; control animals were given ad libitum access to food. Experimental

animals were given either ad libitum or restricted access to food as described below. All rats were given ad libitum access to water.

Rats were weighed on arrival and every 3 days thereafter. Rats were always weighed on the day of the feeding experiment, and food and nutrient intake adjusted for the differences in weight.

Induction of ketosis. Ketosis was produced by allowing experimental animals to eat a single, carbohydrate-free diet containing 30% protein, 37% fat and 33% cellulose. Control animals received an isocaloric diet containing 60% carbohydrate, 30% protein and 10% fat. Both diets contained 2.2% vitamin mix, 4% Rogers-Harper salt mix and 4% agar (table 1). In the first set of experiments, both groups were given unlimited access to food and consumed the same numbers of calories daily. Ketosis was invariably present after 3 weeks of consuming the unrestricted, ketogenic diet. In the later experiments, the food intake of the ketotic rats was reduced to 75% of that eaten by the control group in order to accelerate the onset of ketosis (10–14 days). After 2 weeks of caloric restriction, the ketotic animals weighed about 75 g less than control animals. Since both types of experimental animals behaved similarly in the subsequent food choice studies described below, we adopted caloric restriction as part of our standard procedure for preparing ketotic rats.

Measurement of food intake and nutrient choice. All experiments examining the effect of ketosis on food intake and nutrient choice followed the same procedure. The ketotic or control diets were removed from the cages at the onset of the dark period. Four hours later, a pair of preweighed experimental diets was put into each cage. Food intake measurements were made 0.5, 1.0, 3, 8 and 24 hours after presentation of the diets. Each feeding experiment was repeated at least once; the experiment using the dextrin diets was done 4 times. Data were analyzed to determine total food, calorie, and test nutrient intake, and the percentage of total calories consumed as the test nutrient. All data were subjected to a one-way ANOVA and the Tukey test (8).

Diets. The compositions of the test diet pairs are shown in table 1. Since the test diets

TABLE 1
Composition of test diets

Test diets		Components ¹			
		CHO	Protein	Fat	Cellulose
		%			
Dextrin	High	75	20	5	0
	Low	25	20	27	28
Sucrose	High	75	20	5	0
	Low	25	20	27	28
Protein	High	45	45	10	0
	Low	45	5	28	22
Fat ²	High	50	25	25	0
	Low	50	25	5	20
Dextrin ²	High	70	20	10	0
	Low	25	20	10	45

¹ In addition all diets contained 4% agar, 4% Rogers-Harper salt mix, and 2.2% vitamin mix (ICN Pharmaceuticals, Inc., Cleveland, OH) and 1 liter water per kilogram dry diet. The Rogers-Harper salt mix consists of the following ingredients (grams/kilogram salt mix): ammonium molybdate, 0.025; calcium carbonate, 292.9161; calcium phosphate (dibasic), 4.3002; cupric sulfate, 1.5601; ferric citrate (19.7% Fe), 6.2302; magnesium sulfate, 99.8055; manganese sulfate, 1.2101; potassium iodide, 0.005; potassium phosphate, 343.1189; sodium chloride, 250.6138; zinc chloride, 0.20; sodium selenite, 0.015. Vitamin mix contains the following ingredients (gram/kilogram vitamin mix): vitamin A concentrate (200,000 U/g), 4.5; vitamin D concentrate (400,000 U/g), 0.25; α -tocopherol, 5.0; ascorbic acid, 45.0; inositol, 5.0; menadione, 2.25; *p*-aminobenzoic acid, 5.0; niacin, 4.5; riboflavin, 1.0; pyridoxine hydrochloride, 1.0; thiamin hydrochloride, 1.0; calcium pantothenate, 3.0; and biotin, 20 mg; folic acid, 90 mg; vitamin B-12, 1.35 mg. All diets used vitamin-free casein as the source of protein and vegetable shortening as a source of fat. ² Nonisocaloric.

contained varying amounts of cellulose, we performed preliminary experiments to determine whether their cellulose contents affected their palatability, and thus the amounts that rats consumed. Adult (150 g) Sprague-Dawley rats were given simultaneous access to one of 6 pairs of isocaloric diets in which both carbohydrate and cellulose contents were varied. The content of cellulose in individual diets ranged from 1.8% to 34%. Food intake and weight gain were measured daily for 5 days after a 10-day period of accommodation to the diets. Average daily food intake for each group of rats ranged from 39.8 g of diet for the diet pair with the lowest

cellulose contents (1.8 and 4.5%) to 41.6 g for the diet pair with the highest cellulose contents (14.1 and 34%). No difference in growth was found among the groups. This experiment was repeated twice with similar results. Since the cellulose content of the diets had no effect on food intake or growth, we felt justified in using cellulose in the present study to keep the nutrient densities in test diets constant.

Confirmation of ketosis. The appearances of acetoacetate and β -hydroxybutyrate in the urine were used as ketotic markers. Urine was tested daily for acetoacetate with Ketostix (Ames Co., Division Miles Laboratories, Elkhart, IN), and 6-hour urine collections were tested for β -hydroxybutyrate levels (9). Rats were considered ketotic when the Ketostix registered moderate amounts of acetoacetate (40 mg/dl) and β -hydroxybutyrate was detected in the urine. Control animals never had measurable amounts of either ketone in the urine.

RESULTS

Effect of ketosis on food and carbohydrate intakes

Dextrin diets (25 and 75%). Twelve ketotic and control rats were given simultaneous access to a pair of isocaloric, isonitrogenous diets containing 25 or 75% dextrin (table 1). During the first 30 minutes of food intake, the ketotic group consumed significantly more food than control rats [4.3 ± 0.03 g/100 g body weight vs. 3.0 ± 0.20 g/100 g body weight, $P < 0.01$ (fig. 1)]. The selection of the diets was not random; the ketotic animals chose to consume more of their food from the high carbohydrate diet such that their total carbohydrate intake was significantly higher than that of controls [2.3 ± 0.3 g/100 g body weight vs. 1.0 ± 0.2 g/100 g body weight, $P < 0.01$ (fig. 2)] as was the percent of calories that they consumed as carbohydrate [$51 \pm 3.0\%$ vs. $37 \pm 4.0\%$, $P < 0.001$ (fig. 3)]. Measurements made during the remaining 23-hour period did not reveal significant differences in the amounts of food or carbohydrate consumed by the two groups.

Sucrose diets (25 and 75%). A parallel series of experiments performed as above for dextrin, was carried out to see whether the

taste of the carbohydrate would affect its consumption by the ketotic animals. The sweet-tasting carbohydrate, sucrose, was substituted for the starch, dextrin, in a pair of 25 and 75% carbohydrate diets (table 1), and ketotic and control rats were given simultaneous access to these diets. (Control and ketotic groups each included 12 rats, and the experiment was replicated three times.) During the first 30 minutes of food intake, the ketotic rats consumed significantly more food than the control animals [7.0 ± 0.5 g/100 g body weight vs. 3.0 ± 0.4 g/100 g body weight, $P < 0.001$ (fig. 4)]. As in the study with the 25 and 75% dextrin diets, the ketotic rats also chose to consume significantly more total carbohydrate than controls [4.3 ± 0.3 g/100 g body weight vs. 1.5 ± 0.4 g/100 g body weight, $P < 0.001$ (fig. 5)] and significantly more of their total calories as carbohydrate [$61 \pm 2.0\%$ vs. $50 \pm 5.0\%$, $P < 0.05$ (fig. 6)].

Ketotic rats continued to consume significantly more of their calories as sucrose during the next 30-minute period of food intake; however, neither their total food nor their sucrose consumption differed significantly from those of controls during this period. No

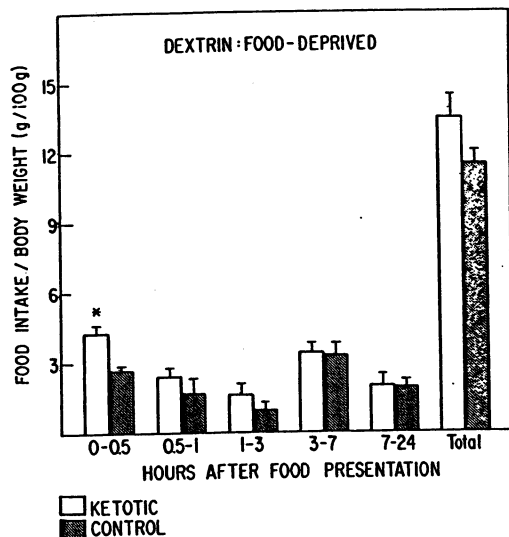


Fig. 1 The effect of ketosis on food intake among ketotic and control rats given simultaneous access to a pair of isocaloric, isonitrogenous diets containing 25 or 75% dextrin. Data represent grams food intake/100 g body weight over 24 hours. Data are given as means and SEM. Ketotic rats consumed significantly more food than controls, $P < 0.01$.

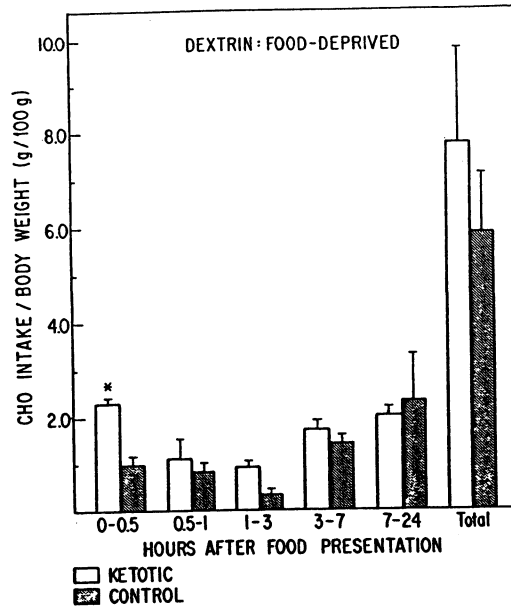


Fig. 2 The effect of ketosis on carbohydrate intake among ketotic and control rats given simultaneous access to a pair of isocaloric, isonitrogenous diets containing 25 or 75% dextrin. Data represent grams carbohydrate intake/100 g body weight over 24 hours. Data are given as means and SEM. Ketotic rats consumed significantly more than controls, $P < 0.01$.

differences in food or carbohydrate intake or in calories consumed as carbohydrate were detected during the rest of the 23-hour measurement period.

Effect of ketosis on fat intake. The preference shown by the ketotic rats for the 75% carbohydrate diet could be interpreted as an aversion to fat rather than a preference for carbohydrate: the high carbohydrate diet contained 5% fat; the low, 27%. Experiments were carried out to distinguish between these two possibilities. In the first study, ketotic and control animals were given simultaneous access to a pair of diets that contained similar amounts of protein and carbohydrate but different amounts of fat (5 and 25% fat, table 1). (The fat contents of this diet pair were similar to those used in the carbohydrate diets.) We anticipated that if the rats had an aversion to fat, they would select more of the low fat diet. Food intake measurements made after the first 30 minutes of feeding demonstrated no preference for either of the diets by the ketotic rats: they consumed 2.1

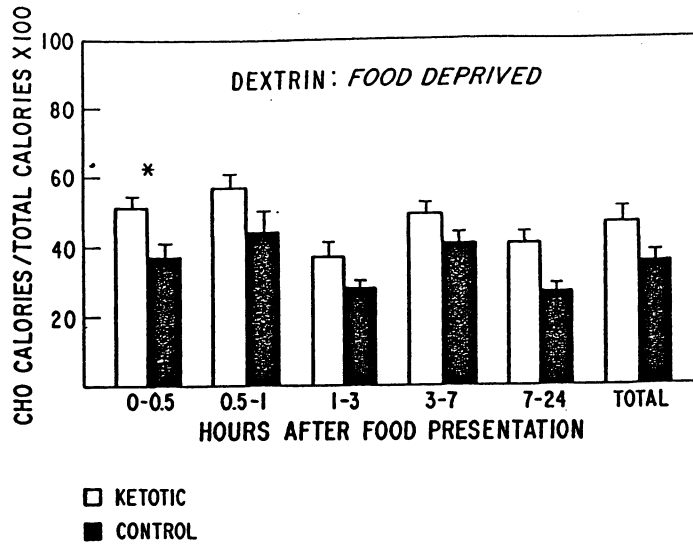


Fig. 3 The effect of ketosis on the percent of total calories consumed as carbohydrate among ketotic and control rats given simultaneous access to a pair of isocaloric, isonitrogenous diets containing 25 or 75% dextrin. Data represent percent of total calories consumed as carbohydrate over 24 hours. Data are given as means and SEM. Ketotic rats consumed significantly more than controls, $P < 0.001$.

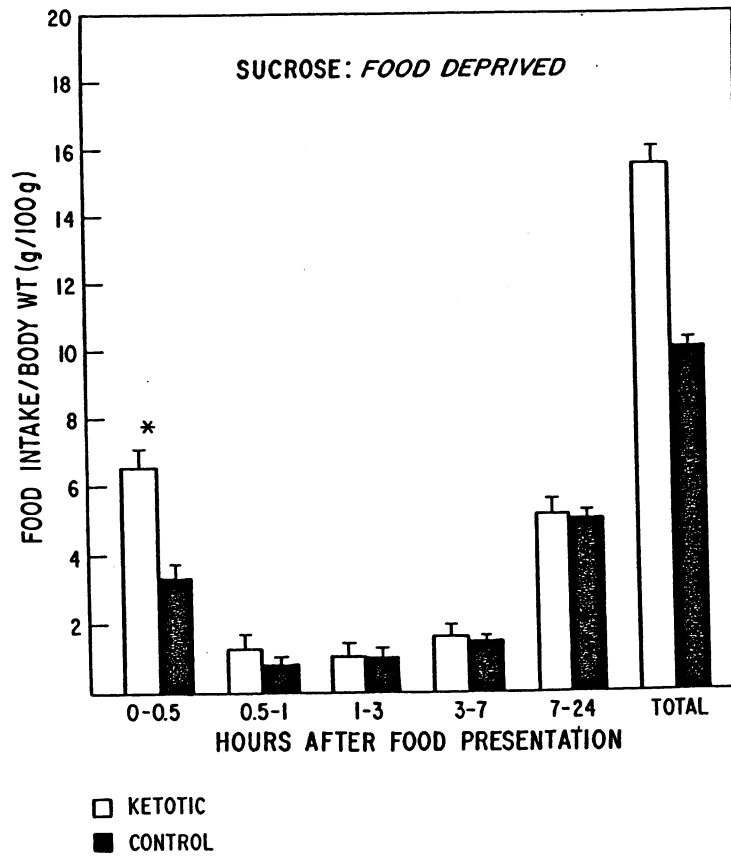


Fig. 4 The effect of ketosis on food intake among ketotic and control rats given simultaneous access to a pair of isocaloric, isonitrogenous diets containing 25 or 75% sucrose. Data represent grams food intake/100 g body weight over 24 hours. Data are given as means and SEM. Ketotic rats consumed significantly more than controls, $P < 0.001$.

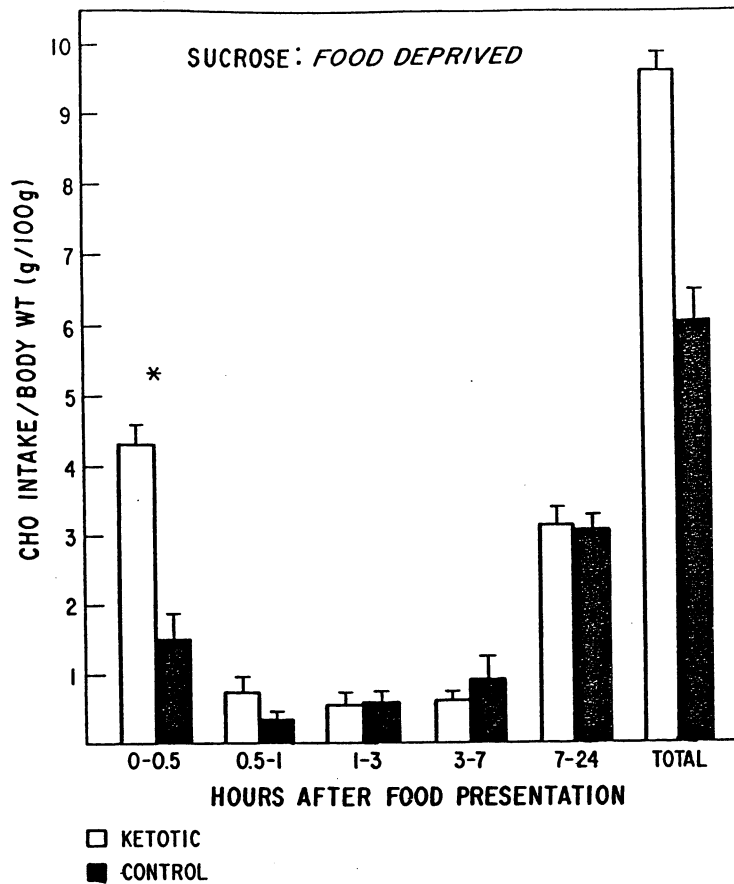


Fig. 5 The effect of ketosis on carbohydrate intake among ketotic and control rats given simultaneous access to a pair of isocaloric, isonitrogenous diets containing 25 or 75% sucrose. Data represent grams carbohydrate intake/100 g body weight over 24 hours. Data are given as means and SEM. Ketotic rats consumed significantly more than controls, $P < 0.001$.

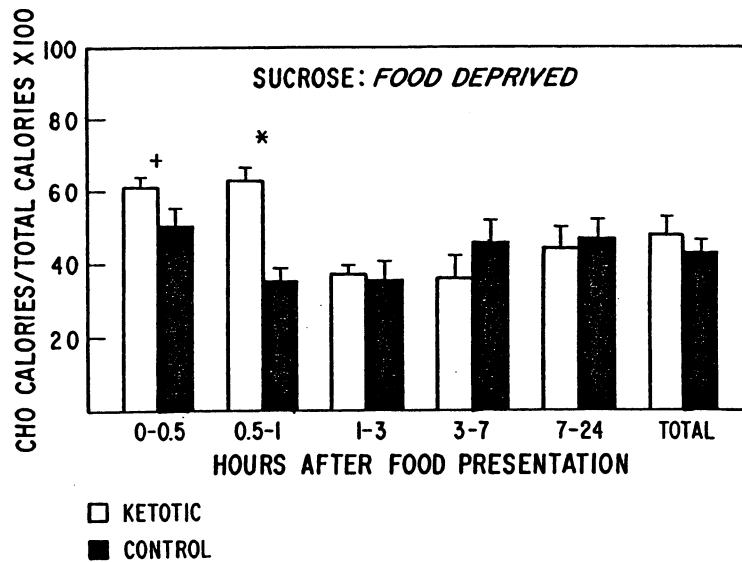


Fig. 6 The effect of ketosis on the percent of total calories consumed as carbohydrate among ketotic and control rats given simultaneous access to a pair of isocaloric, isonitrogenous diets containing 25 or 75% sucrose. Data represent percent of total calories consumed as carbohydrate over 24 hours. Data are means and SEM. Ketotic rats consumed significantly more than controls: $^+P < 0.05$; $^*P < 0.001$.

± 0.8 g of the 25% fat diet and 2.2 ± 0.7 g of the 5% fat diet. Measurements made during the remaining 23-hour period of food intake revealed this same lack of preference.

To determine whether the ketotic rats would maintain a preference for the high carbohydrate diet if the fat contents of both the high and low carbohydrate diets were similar, we allowed rats simultaneous access to 25 and 70% dextrin diets containing similar amounts of fat and protein (table 1).

The ketotic rats exhibited the same preference for the high carbohydrate diet as they had shown in earlier studies using carbohydrate diet pairs with dissimilar fat contents: during the first 30 minutes of feeding, the ketotic rats consumed significantly more of the high carbohydrate diet than control animals (2.3 ± 0.3 g/100 g body weight vs. 1.2 ± 0.2 g/100 g body weight, $P < 0.01$) and approximately the same amount of the low carbohydrate diet (1.2 ± 0.2 g/100 g body weight vs. 1.4 ± 0.3 g/100 g body weight). Thereafter no differences were noted in the consumption of either diet by the 2 groups.

Effect of ketosis on protein intake. All the diet pairs used in the studies described contained the same amount of protein. Thus if ketosis alters the animal's preference for protein, this would not be detected in our food choice studies. To examine this possibility, we tested consumption of diets that were similar in calories and carbohydrate contents but contained either 5 or 45% protein (table 1).

Food intake measurements made after 30 minutes of feeding showed no effect of ketosis on protein consumption. The percent of total food eaten as protein was $19 \pm 1.6\%$ for the ketotic rats and $17 \pm 2.0\%$ for controls; total protein intake was 0.4 ± 0.17 g/100 g body weight for ketotic rats and 0.4 ± 0.07 g/100 g body weight for controls.

Effect of a carbohydrate preload on subsequent carbohydrate ingestion. The excessive overeating of carbohydrate by the ketotic rats suggested that the regulation of carbohydrate intake was impaired when this nutrient first became available to the animals. However, previous studies in which rats were allowed to choose concurrently between a high- and low carbohydrate diet indicated that normal, nonketotic rats are able to regulate carbohydrate consumption. This regu-

lation appeared to involve brain serotonin, since treatments that enhanced serotonin's release or blocked its reuptake were found to decrease the amount of carbohydrate rats elected to consume (4). Moreover, human subjects allowed ad libitum access to carbohydrate snacks also had been found to decrease carbohydrate intake after drug treatments thought to increase serotonin's release (6). Since carbohydrate intake itself increases brain serotonin synthesis, we determined whether ingestion of a small carbohydrate meal would cause normal rats to decrease their subsequent intake of this nutrient selectively, i.e., without altering their total caloric intake.

Rats maintained on the control diet (table 1) were fasted during the light period. After the onset of darkness, twelve experimental rats were given a carbohydrate pellet containing 1.4 g of dextrose, 0.4 g H₂O and 0.04 g tartaric acid. Twelve control rats were given an isocaloric, mixed nutrient pellet containing 0.25 g protein, 0.22 g carbohydrate and 0.45 g fat. One hour after the pellets had been consumed, all animals were given simultaneous access to a pair of isocaloric, isonitrogenous 25 and 75% dextrin diets (table 1). Food intake was measured 0.2, 0.5, 1 and 3 hours after these diets were placed in the cage. The effect of the carbohydrate preload was to decrease significantly the intake of carbohydrate calories without altering the intake of total calories (table 2). This result was obtained after the first 10 minutes of feeding; subsequent measurements showed no differences in nutrient or caloric intake between the two groups. Similar observations were made in three separate experiments.

DISCUSSION

These studies show that elective carbohydrate intake by animals given a choice of diets containing varying proportions of carbohydrate depends on their recent nutritional history. When animals previously restricted to a ketogenic diet containing only protein and fat are allowed to choose between 25 and 75% dextrin diets, they respond by overeating carbohydrate compared with nonketotic control rats: they increase the

grams of carbohydrate consumed (figs. 2, 5) and the proportion of their total calories (figs. 3, 6) represented by carbohydrate. This overconsumption is not due to the need of the ketotic animals to consume more calories: when similar animals are given diets differing in fat or protein contents, they do not consume more food or calories than control animals. Nor is it due to the lower cellulose contents of the high carbohydrate diet: we previously found (see methods section) that cellulose, added in the amounts used in our test diets, had no effect on food intake. The overeating of carbohydrate by carbohydrate-deprived rats probably reflects an alteration (caused by the period of carbohydrate deprivation) in the brain mechanism that normally regulates carbohydrate intake. The mechanism responsible for this effect may involve the well-known decrease in carbohydrate-induced insulin secretion noted among rats (10, 11) or humans (12) deprived of carbohydrate for several days and then given glucose. The secretion of insulin after the initial consumption of the carbohydrate-containing test diets may have been lower in the ketotic rats than in the control animals; if so, the ketotic animals would have had to consume more carbohydrate, proportionately, than the control rats to produce a given increase in the ratio of plasma tryptophan to LNAA and in brain serotonin synthesis. In preliminary experiments, we have started to examine the effects of carbohydrate consumption on brain tryptophan levels in ketotic and control animals. When both groups consumed, after a 4-hour fast, similar amounts of carbohydrate (1.5 g/100 g body weight), brain tryptophan levels increased after 90 minutes in control animals from 1.9 ± 0.6 $\mu\text{g/g}$ brain (fasting) to 3.6 ± 0.7 $\mu\text{g/g}$ brain. However, brain tryptophan levels remained unchanged in ketotic animals, i.e., 2.4 ± 0.3 $\mu\text{g/g}$ brain in the fasting state and 2.1 ± 0.1 $\mu\text{g/g}$ brain after consumption of the carbohydrate. Moreover, when ketotic animals eating ad libitum after a 4-hour fast consumed twice as much carbohydrate as controls, their brain tryptophan levels increased only by as much as the control animals. Hence brain tryptophan levels were less sensitive to dietary carbohydrate in ketotic than in control animals. These results might also explain

TABLE 2

Effect of a carbohydrate premeal on food and carbohydrate consumption¹

Meal	Total calories	Total CHO	CHO calories
		g	%
Carbo- hydrate Mixed	26.9 ± 4.1	2.4 ± 0.2	38 ± 6.0^a
nutrient	26.2 ± 4.0	3.4 ± 0.4	58 ± 6.0

¹ Animals were given isocaloric pellets containing either 1.4 g dextrose, 0.4 g H₂O and 0.04 g tartaric acid [carbohydrate (CHO) meal] or 0.25 g protein, 0.22 g CHO and 0.45 g fat. One hour after ingestion of the pellet, all animals were given simultaneous access to a pair of isocaloric, isonitrogenous diets containing 25 or 75% dextrin. The food intake measurement shown here was made 10 minutes after the initiation of food intake. * $P < 0.01$.

why, after a sufficient amount of carbohydrate-rich diet is consumed, the ketotic animals begin to exhibit normal regulation of carbohydrate intake: sufficient insulin has, by then, been secreted to lower plasma LNAA.

That animals are able to adjust their carbohydrate intake in one meal in response to the amount of carbohydrate consumed in the previous meal was demonstrated by the finding that carbohydrate consumption was diminished, in a diet-choice situation, among rats that had received a carbohydrate premeal 1 hour earlier. The response of the animals to this carbohydrate premeal was specific; i.e., while they consumed as many total calories as the control group, they chose to eat fewer of their calories as carbohydrate. This affirms that the animals were responding to the carbohydrate and not the calories ingested, and provides further evidence that distinct mechanisms regulate appetites for total calories and for carbohydrates. Similar results have been obtained by using drugs that release serotonin or block its reuptake (4); taken together, they support the view that one function of brain serotonin is to diminish the animal's appetite for carbohydrate, and possibly increase appetite for protein after a carbohydrate-rich, protein-free meal has been consumed.

The animals' ability to regulate carbohydrate intake when allowed to eat a carbo-

hydrate-containing diet and their inability to do so when given carbohydrate-free foods should be considered in evaluating the efficacy of ketogenic weight-reducing regimes. Although weight is lost rapidly on such diets, the individual's ability to control his appetite for carbohydrates when reintroduced may be compromised, causing overconsumption of this nutrient and weight gain. The use of treatments that increase serotonergic neurotransmission might help such individuals control their carbohydrate intake and diminish the weight gain that so often accompanies the termination of ketogenic diets.

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