RELATIONSHIPS AMONG GLUCOSE AVAILABILITY, SIGNALS FROM FAT DEPOTS AND BRAIN AMINO ACID PATTERNS AS FACTORS CONTROLLING FOOD INTAKE

by

Larry Lynn Meliza

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Signed Harris — 7-10-75

Clinton L. Crafton — 7-10-75

Mary C. Wright — 7-10-75

Mark S. Olson — 6/23/75

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ABSTRACT

The purpose of the present research was to determine the manner in which glucose availability, signals from fat depots, and brain amino acid patterns may interact to control food intake by evaluating the following proposals: (1) signals from fat depots override glucose availability as a factor controlling food intake in rats demonstrating long-term hyperphagia, (2) the regulation of amino acid intake is inferior to the regulation of caloric intake, and (3) the regulation of amino acid intake becomes inferior to the regulation of caloric intake by glucose availability and/or signals from fat depots with the advent of long-term hyperphagia.

Hyperphagic alloxan-diabetic rats responded to decreased glucose availability (induced via a single injection of insulin) by increasing short-term food intake and responded to increased glucose availability (induced via intragastric loads of sucrose solution and via ingestion of glucose and sucrose solutions) by decreasing daily food intake. In disagreement with previous findings, it was concluded that signals from fat depots do not influence food intake by overriding glucose availability as a factor controlling food intake. Further, it was concluded that the effect which signals from fat depots have on food intake is

mediated by the monitoring of glucose availability and not by altering behavioral sensitivity to diet palatability, since hyperphagic diabetic rats were shown to be unresponsive to sweet tastes.

Rats maintained on a protein-free diet demonstrate anorexia concurrent with a behavior change characteristic of the gluco-deprived rat (enhanced responsiveness toward sweet solutions). This finding indicates that anorexia associated with maintenance on a protein-free diet cannot be ascribed to a decrease in the amount of food required for satiety as determined by the regulation of caloric intake. Further, maintenance on a protein-free diet was found to inhibit insulin-induced hyperphagia. In a model where food intake is determined by regulation of amino acid intake and regulation of caloric intake, finding that anorexia demonstrated by rats maintained on a protein-free diet cannot be explained in terms of caloric regulation, suggests that anorexia must be ascribed to the regulation of amino acid intake. In a model where amino acid intake regulation does not contribute toward determining amount of food intake, anorexia demonstrated by rats maintained on a protein-free diet is inexplicable. The regulation of amino acid intake is not inferior to the regulation of caloric intake.

Maintenance on a protein-free diet failed to preclude the development of hyperphagia in alloxan diabetic rats, suggesting that the regulation of amino acid intake becomes

inferior to the regulation of caloric intake with the advent of long-term hyperphagia. However, combining insulin-replacement with maintenance on a protein-free diet was associated with severe food intake depressions in diabetic rats, while neither insulin-replacement nor maintenance on a protein-free diet alone were sufficient to attenuate diabetic hyperphagia. In view of recent data which show that (1) the efficacy of ingestion of a protein-free diet in altering brain free amino acid patterns is greatly attenuated in the diabetic rat and (2) exogenous insulin has a profound effect on brain free amino acid patterns, it is suggested that the ability of combined insulin-replacement and maintenance on protein-free diets to depress food intake is a more adequate test of the proposed inferiority of amino acid intake regulation than is the ability of maintenance on a protein-free diet to depress food intake in the hyperphagic diabetic rat. Therefore, it was concluded that the regulation of amino acid intake does not become inferior to the regulation of caloric intake with the advent of long-term hyperphagia.

INTRODUCTION

Although evidence exists that various metabolites have an effect on food intake, the manner in which these metabolites interact to control food intake has never been elucidated (Widdowson, 1970). Thus it is not surprising that metabolites believed to be involved in the control of food intake may not vary in the predicted direction with food intake across various perturbations of metabolism (Stevenson, 1969). The purpose of the present research was to determine the manner in which glucose availability (Mayer, 1955), signals from fat depots (Kennedy, 1952), and brain amino acid patterns (Peng, Tews, and Harper, 1972) may interact to control food intake.

Mayer and Bates (1952) proposed that a neural feeding center monitors sources of energy available to the brain. Since glucose is the primary source of energy, Mayer (1955) proposed that neural feeding centers monitor glucose levels. Mayer's proposal is valid in that (1) blood glucose levels vary in the predicted direction with food intake in rats (Tonge and Oately, 1972), (2) inhibition of glucose uptake by the non-metabolizable glucose analogue 2-Deoxy-D-Glucose (2DG) is associated with increased food intake (Smith and Epstein, 1969), and (3) hypoglycemia

induced by a single injection of insulin is associated with increased food intake (Booth and Brookover, 1968).

Booth (1972a) has suggested that any energy substrate available to the brain may act to depress food intake. Since the importance of glucose as an energy substrate decreases in association with fasting and diabetes mellitus (Krebs, 1966), different predictions may be generated from the theories of Mayer and Booth with respect to the effect that alternative sources of energy have on food intake of fasted and diabetic rats. Metabolites derived from the oxidation of ketone bodies and free fatty acids (which are alternative sources of energy in the diabetic or fasted rat) act to inhibit glycolysis and glucose transport (Newsholme, Randle, and Manchester, 1962; Blackshear and Aberti, 1974). Therefore, substances which would act to sate hunger under Booth's model would act to prevent glucose from acting as a satiety signal under Mayer's gluco-specific model. Under Mayer's model, inhibition of glucose uptake by metabolites derived from ketone body and free fatty acid oxidation could lead to a situation in which food intake is not regulated.

The models of Mayer and Booth also differ with respect to the inclusion of signals from fat depots. Mayer (1955) has proposed that long-term food intake may be controlled by signals from fat depots, while Booth (1972a) suggests that both short-term and long-term food intake are

controlled by the availability of energy substrates to neural feeding centers and that there is no evidence for supervening signals from fat depots. Under Mayer's model signals from fat depots may influence food intake by (1) increasing the amount of glucose required for satiety or (2) by overriding glucose availability as a factor controlling food intake.

The ability of rats demonstrating long-term hyperphagia to adjust food intake in response to alterations in glucose availability is critical in determining the existence of signals from fat depots and in determining the nature of the possible relationship between glucose availability and signals from fat depots as factors controlling food intake. If rats demonstrating long-term hyperphagia retain behavioral sensitivity to glucose availability (as manifested by the appropriate adjustments in food intake following alterations in glucose availability), then Booth's energostatic model would be unable to explain the phenomenon of long-term hyperphagia. However, retention of behavioral sensitivity to glucose availability in rats demonstrating long-term hyperphagia might suggest that Mayer's glucostatic theory is unable to explain short-term food intake, since inhibition of glucose transport by ketone bodies and free fatty acids is expected to block the relationship between plasma glucose levels and food intake. Retention of behavioral sensitivity to glucose availability

would suggest that short-term food intake is controlled by the availability of any source of energy as proposed by Booth (1972a) and that signals from fat depots act to increase the amounts of energy substrates required as proposed by Mayer (1955).

If behavioral sensitivity to glucose availability is not retained in rats demonstrating long-term hyperphagia, then the concept of overriding signals from fat depots would be supported. Loss of behavioral sensitivity to glucose availability would also support proposals that short-term food intake is controlled by a glucospecific mechanism and that long-term hyperphagia is due to the effectiveness of ketone bodies and free fatty acids in blocking glucose transport. Support would be offered for Booth's energostatic model in that the availability of a major source of energy, glucose, is expected to be diminished. However, support is offered for Booth's model only if various sources of energy are not equivalent in their ability to sate hunger, since alternative sources of energy are being utilized.

In the present research, the organism selected as a model of the rat demonstrating long-term hyperphagia is the hyperphagic diabetic rat. In the insulin-deficient diabetic rat, cells which require insulin for glucose transport are deprived of glucose. Metabolic adjustments to the diabetic state include the catabolism of adipose tissue and muscle

protein. Amino acids derived from the catabolism of muscle and dietary protein are utilized in the biosynthesis of glucose (Exton, 1972), thus contributing to hyperglycemia. Free fatty acids and ketone bodies derived from the catabolism of adipose tissue and from dietary intake of fats are utilized as sources of energy without being converted to glucose (Krebs, 1966). Metabolites derived from oxidation of ketone bodies and free fatty acids act to inhibit glycolysis and glucose transport (Newsholme et al., 1962; Blackshear and Aberti, 1974), thus contributing to diabetic hyperglycemia. The chronic diabetic rat demonstrates hypophagia for approximately 4 days following the induction of diabetes (Kumaresan and Turner, 1965). Diabetic rats which survive the initial period of hypophagia develop hyperphagia (Booth, 1972b). Thus the chronic diabetic rat demonstrates hypophagia then hyperphagia concurrent with hyperglycemia.

Booth (1972c) has shown that the hyperphagic diabetic rat does not depress food intake following an intragastric load of glucose solution, suggesting that either (1) diabetic hyperphagia is due to the fact that signals from fat depots override glucose or energy substrate availability as a factor controlling food intake or (2) diabetic hyperphagia is due to unregulated food intake. The purpose of the first phase of this research was to determine if hyperphagic diabetic rats respond to alterations in

extracellular glucose availability with the appropriate adjustments in food intake.

The purpose of the second phase of this research was to determine the nature of the relationship between the regulation of amino acid intake and the regulation of caloric intake by glucose availability and proposed signals from fat depots. The regulation of amino acid intake would be inferior to the regulation of caloric intake, if regulation of caloric intake may preclude the regulation of amino acid intake.

Musten, Peace and Anderson (1974) have proposed that regulation of amino acid intake is inferior to the regulation of caloric intake (regulation of caloric intake being the regulation of food intake by glucose availability, energy substrate availability or signals from fat depots). The definition of amino acid intake regulation utilized by Musten et al, was ingestion of a given amount of amino acids. The line of evidence on which the proposal of Musten et al. is based is the observation that rats maintained on low-protein diets will not increase food intake in order to ingest sufficient amounts of amino acids (presumably because increases in food intake would exceed a limit determined by the regulation of caloric intake).

Collier and Bolles (1968) have suggested that rats will eat for both calories and nutrients, while the rat demonstrating long-term hyperphagia will eat for calories

at the expense of nutrient intake. Collier and Bolles, like Musten et al. (1974), define regulation of amino acid intake in terms of amounts of amino acids ingested. The proposal of Collier and Bolles suggests that the regulation of energy intake by glucose availability is not superior to the regulation of amino acid intake, but with the advent of long-term hyperphagia, the regulation of amino acid intake becomes inferior to either (1) regulation of caloric intake by glucose or energy substrate availability or (2) regulation of caloric intake by signals from fat depots or both (depending on whether or not glucose or energy substrate availability are themselves overridden by signals from fat depots as factors controlling food intake with the advent of long-term hyperphagia).

In order to evaluate the proposals of Musten et al. (1974) and Collier and Bolles (1968) it was necessary to determine if alterations in glucose availability or long-term hyperphagia have an effect on the ability of rats to regulate amino acid intake. However, it was first necessary to determine if the definition of amino acid intake regulation utilized by Musten et al. (1974) and Collier and Bolles (1968) was valid, since an alternative definition of amino acid intake regulation was available. According to the work of Peng et al. (1972), regulation of amino acid intake would be defined as the inhibition of intake of diets which are associated with perturbations of the relationships between

the levels of free essential amino acids found in the brain. Selecting the most valid definition of amino acid intake regulation is critical in any attempt to determine the proposed inferiority of the regulation of amino acid intake.

General Methods

Maintenance

Subjects were housed in individual cages during testing and for at least 10 days before the initiation of testing. Diet was placed in a 250-ml glass beaker attached to the corner of each cage. Liquid was presented in either 100-ml or 240-ml graduated cylinders or bottles attached to the front of each cage by a metal clip. Food intake was measured to the nearest tenth of a gram and liquid intake was measured to the nearest ml. The laboratory was artificially illuminated with light onset at 7:30 a.m. and offset at 7;30 p.m. Laboratory temperature was maintained at approximately 23 degrees Centigrade.

Diets

Diets utilized in this series of experiments included Purina Laboratory Chow, a 10% casein diet and a protein-free diet. These last two diets were prepared by the experimenter using the facilities of the Department of Agricultural Biochemistry at The University of Arizona.

Contents of these diets are given in Table 1. Vitamin-Free Casein, Rogers-Harper Salt Mix and vitamins were purchased from the Nutritional Biochemicals Corporation.

Induction of Diabetes

Diabetes was induced via injections of alloxan monohydrate at a dosage of 135 mg per kilogram of body weight. The solution injected was 10% (w/v) alloxan monohydrate in isotonic saline. Subjects were fasted for 24 hours before receiving injections. Alloxan Monohydrate was purchased from Sigma.

Insulin

Insulin utilized was Illetin-100-U, purchased from Lilly Pharmaceuticals. Insulin was diluted from 100 units per cc to 10 units per cc with isotonic saline before being administered.

Table 1. Composition of vitamin mixture, 10% casein diet and protein-free diet expressed in terms of percentage of total content.

Vitamin Mix		Diets		
		Component	Protein-Free	10% Casein
Thiamine HCl	0.100%	Vitamin Mix	0.50%	0.50%
Riboflavin	0.100%	Rogers-Harper		
Niacinamide	0.500%	Salt Mix	5.00%	5.00%
D Calcium		Dextrose	44.65%	39.65%
Pantothenate	0.100%	Corn Starch	44.65%	39.65%
Pyridoxine HCl	0.100%	Corn Oil	5.00%	5.00%
Folic Acid	0.010%	Choline Chloride	0.20%	0.20%
Menadione	0.010%	Casein	0.00%	10.00%
D Biotin	0.004%			
Ascorbic Acid	1.000%			
Tocopherol				
Acetate	8.000%			
Vitamin B-12				
(0.1% in Mannitol)	0.600%			
Vitamin A Acetate				
+ Vitamin D_2	0.161%			
Sucrose	98.315%			

ADJUSTMENTS IN FOOD INTAKE ASSOCIATED WITH ALTERATIONS IN GLUCOSE AVAILABILITY IN THE HYPERPHAGIC DIABETIC RAT

Experiment 1: Increased Food Intake of Hyperphagic Diabetic Rats Associated with Insulin-Induced Hypoglycemia

In the rat, a single injection of insulin is associated with short-term hyperphagia (Booth and Brookover, 1968). Booth and Pitt (1968) have demonstrated that insulin-induced hypoglycemia precedes hyperphagia by approximately 30 minutes, thus casting some doubt as to whether or not insulin hyperphagia is a direct response to decreased glucose availability to the lateral hypothalamus (Epstein and Teitelbaum, 1967). However, Panksepp (1974) has shown that cells of the lateral hypothalamus show a depressed rate of glucose utilization in comparison with other areas of the brain so that a considerable time-lag may be expected between alterations of plasma glucose levels and changes in the rate of glycolysis within the lateral hypothalamus.

The purpose of this experiment was to determine if diabetic rats demonstrate short-term or long-term changes in food intake following a single injection of insulin.

Methods

Subjects were male Holtzman albino rats. Eight subjects were controls (weight range, 222-438 grams) and

15 subjects were hyperphagic diabetics (weight range, 136-375 grams). Diabetic subjects had been injected with alloxan 4 weeks before the initiation of testing. Subjects were randomly assigned to a sequence of insulin and saline injections with subjects receiving one injection per day. Each subject was tested under the assigned sequence twice (total of 4 observations). One day separated the initial sequence from the repetition.

Weight, food intake, and liquid intake measurements were taken at 9 a.m. each day and injections were administered at 9:30 a.m. Injected materials were insulin (20 units per kilogram of body weight) and isotonic saline. Food and water were returned to subjects following injections. Liquid and food intake measurements were taken 4 and 23 hours after injections.

Data were analyzed using an analysis of variance mixed design (between subjects variable of diabetes and within subjects variables of insulin and repetitions).

Results

Both hyperphagic diabetic rats and control rats respond to a single injection of insulin by increasing food intake (Fig. 1). Mean short-term food intake of diabetics was 7.9 grams following injections of saline and 9.8 grams following injections of insulin ($F = 4.362$, $p < 0.05$). Mean short-term food intake of controls was 3.3

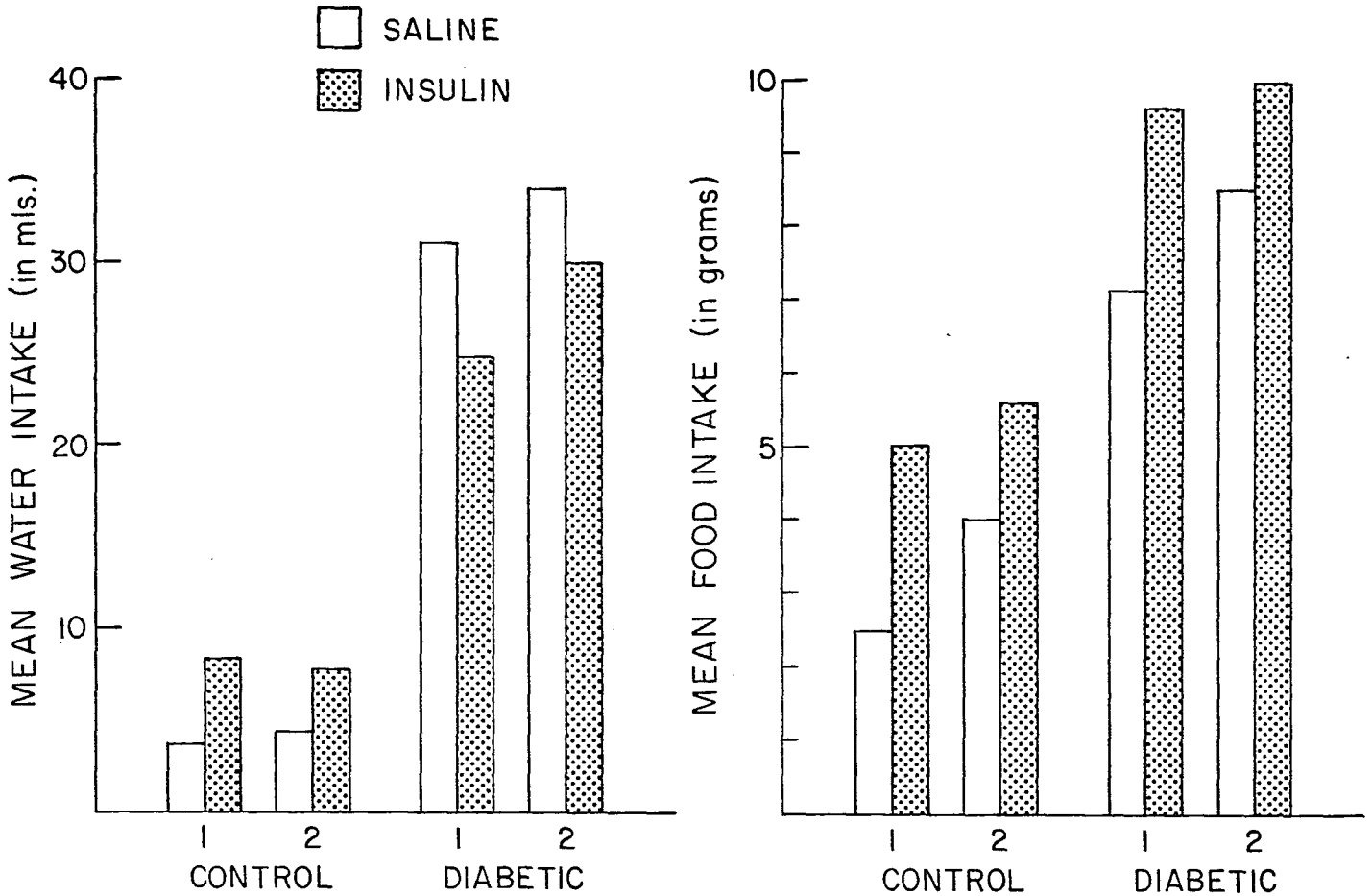

Fig. 1. Mean short-term water (a) and food (b) intake of diabetic and control rats receiving insulin (20 units/kilogram) and isotonic saline as a function of repetitions.

grams following injections of isotonic saline and 5.3 grams following injections of insulin ($F = 9.553$, $p < 0.01$). Insulin had no effect on daily food intake of either diabetic or control animals.

Short-term water intake was increased for controls receiving insulin and decreased for diabetics receiving insulin (Fig. 1). The interaction between diabetes and insulin was significant ($F = 9.525$, $p < 0.01$). Mean short-term water intake of diabetics was 32.4 ml following saline injections and 27.3 ml following insulin injections ($p < 0.05$). Mean short-term water intake of controls was 4.1 ml following saline injections and 8.1 ml following insulin injections ($p < 0.05$). The effects of insulin injections on water intake of diabetic and non-diabetic rats have previously been reported and were utilized as a measure of the effectiveness of insulin injections in the present experiment.

Experiment 2: Decreased Daily Food Intake of Hyperphagic Diabetic Rats Following Intragastric Loads of Sucrose Solution

The ability of rats to respond to intragastric loads by decreasing food intake has been utilized as a measure of the ability to regulate food intake in terms of the availability of energy substrates (Epstein and Teitelbaum, 1962). This test has recently been used to show that rats with bilateral lesions of the ventromedial hypothalamus

retain the ability to regulate food intake in response to energy substrate availability (Panksepp, 1971a). The purpose of the present experiment was to determine if the hyperphagic diabetic rat retains the ability to adjust food intake in response to intragastric loads of sucrose solution.

Methods

Subjects were 14 male Holtzman albino rats over 60 days of age (7 hyperphagic diabetics and 7 controls). Subjects were adapted to the force-feeding procedure with 3 feedings of dilute sucrose solution given over a 3-day period. Subjects were not anesthetized during the force-feeding procedure. Solutions force-fed were 5 ml of (1) 100% (w/v) sucrose solution, (2) 50% (w/v) sucrose solution, and (3) tap water. Sequence of solutions were randomly assigned for each subject. Subjects were force-fed at 11:30 a.m., and food and liquid measurements were taken at 11 a.m.

Data were analyzed with an analysis of variance mixed design (between subjects variable of diabetes and within subjects variable of solution).

Results

Diabetic and non-diabetic rats depress daily food intake following intragastric loads of sucrose (Fig. 2). The effect of solution was significant ($F = 15.850$, p less

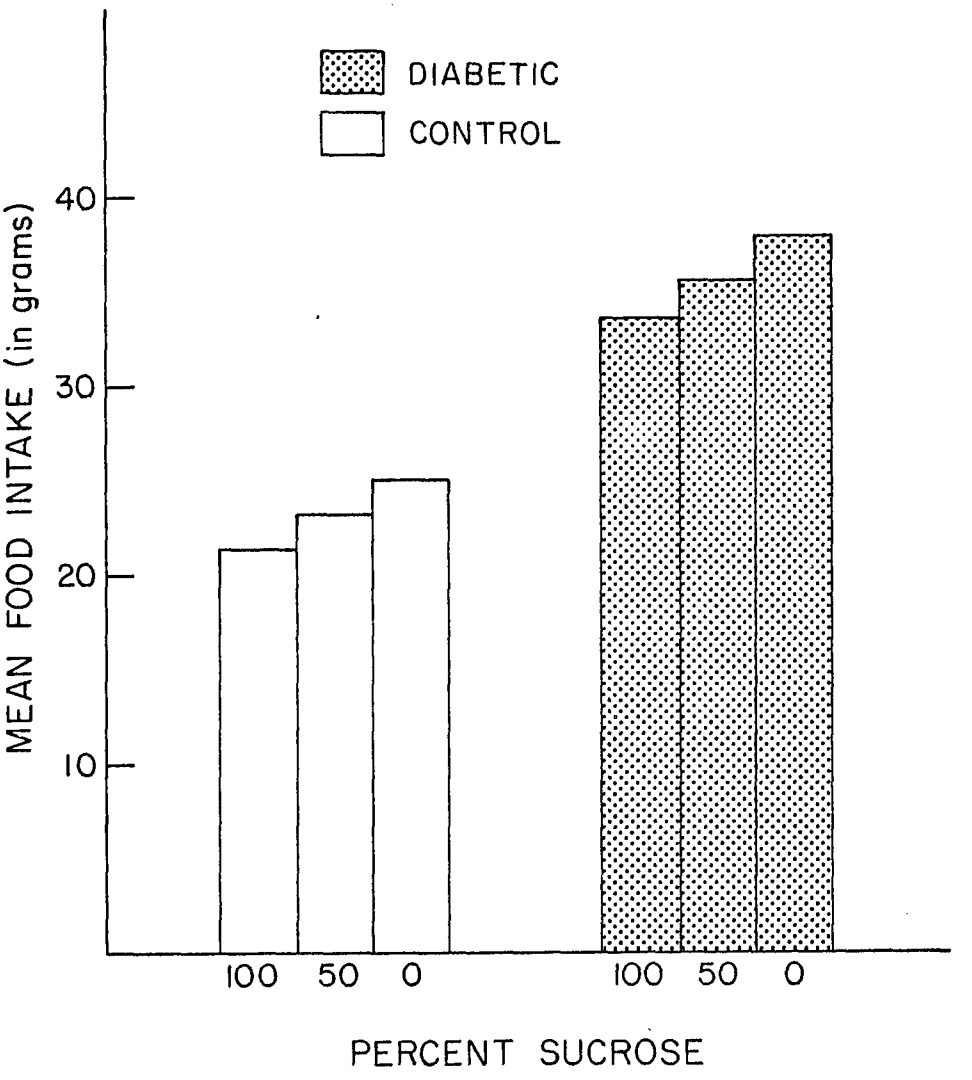

Fig. 2. Mean daily food intake of diabetic and control rats forcefed 5 ml of (1) 100% (w/v) sucrose solution, (2) 50% sucrose solution, and (3) tap water.

than 0.05). Food intake associated with 100% (w/v) sucrose solution was significantly less than that associated with tap water (p less than 0.05). Intake associated with 50% (w/v) sucrose solution was not significantly less than that associated with tap water or significantly greater than that associated with 100% (w/v) sucrose solution. Mean daily food intake of diabetics was 33.2 grams following 100% (w/v) sucrose solution, 35.1 grams following 50% sucrose solution and 37.5 grams following tap water. Mean daily food intake of controls was 21.8 grams following 100% sucrose solution, 23.6 grams following 50% sucrose solution, and 27.6 grams following tap water.

Experiment 3: Decreased Daily Food Intake of Hyperphagic Diabetic Rats Associated with Ingestion of Sucrose Solution

Food intake of rats is depressed in association with ingestion of sucrose solutions (Collier and Bolles, 1968; Hsiao and Pertsulakes, 1970). Since ingestion of calories in non-feeding situations is compensated for by the appropriate dimunitions in food intake from feeding situations, depressed food intake in association with ingestion of sucrose solution is a measure of the rats' ability to regulate daily caloric intake. The importance of nutritive value of solutions in depressing daily food intake is shown by the fact that ingestion of saccharin solution is not associated with depressions in daily food intake (Hsiao and

Pertsulakes, 1970). In addition, bulk of material ingested in solution cannot be responsible for depressions in daily food intake associated with the ingestion of sucrose solution, since bulk has only a short-term effect on food intake (Snowdon, 1975). If diabetic rats display hyperphagia because their food intake is unregulated, then diabetic rats should not compensate for the ingestion of sucrose solution by depressing daily food intake.

Methods

Subjects were 14 male Holtzman albino rats over 60 days of age. Seven of the subjects were hyperphagic diabetics and 7 were controls. Daily food intake, liquid intake and weight measurements were made for each subject for a period of 2 days. Liquid presented on one test day was 9% sucrose solution and that on the second was tap water. The sequence of liquids was randomly assigned for each subject. Data were analyzed with an analysis of variance mixed design (between subject variables of diabetes and within subject variable of solution). Significant interactions were analyzed with Duncan's new multiple range test.

Results

Diabetic and control rats demonstrate a depression in daily food intake when given continuous access to 9% sucrose solution in the absence of tap water (Fig. 3). The

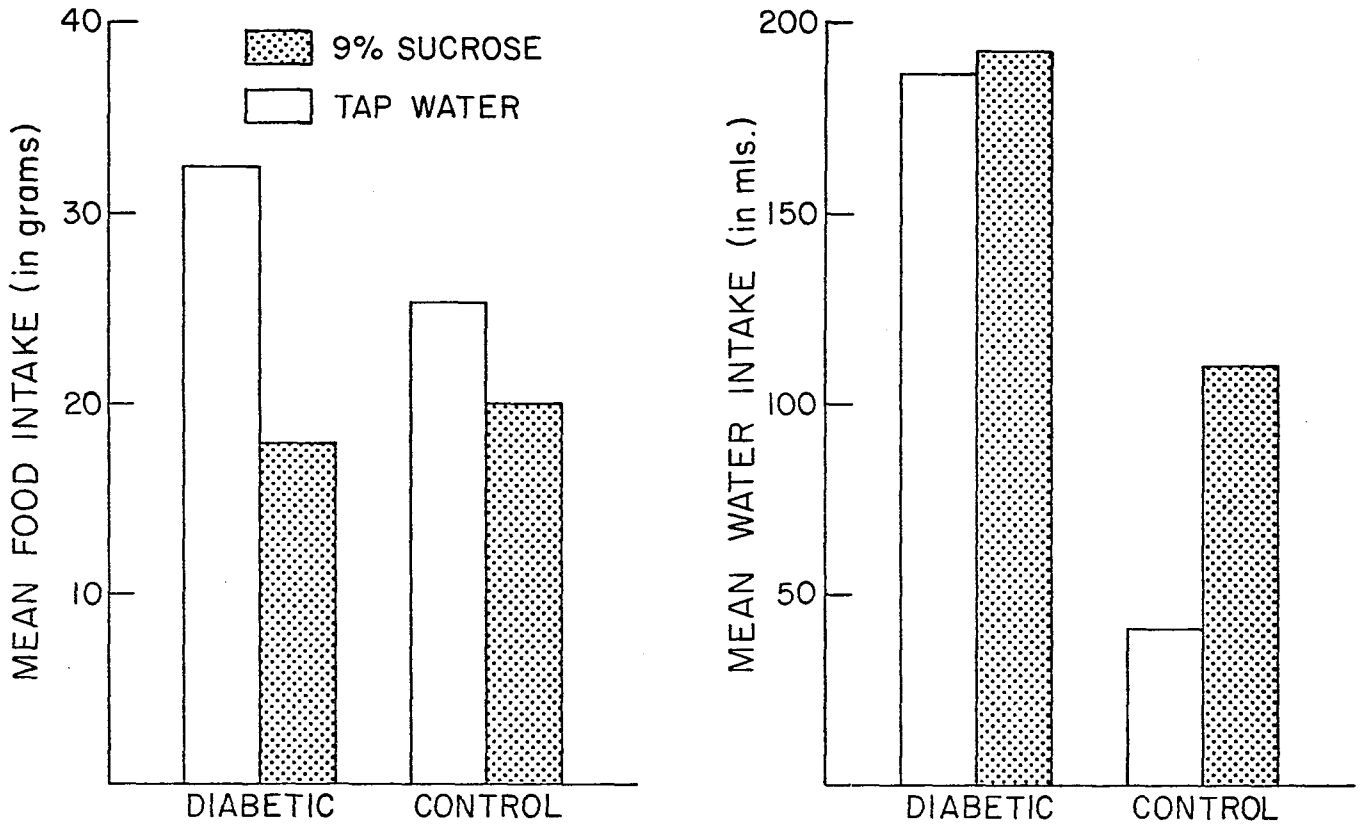

Fig. 3. Mean daily food (a) and liquid intakes (b) of diabetic and control rats given continuous access to 9% sucrose solution or tap water.

interaction between diabetes and solution was significant ($F = 13.522$, $p < 0.005$). Mean daily food intake of diabetic rats was 33 grams with tap water and 18.2 grams with sucrose. Mean daily food intake of controls was 25.8 grams with tap water and 20.2 grams with sucrose. Both diabetic and control rats ate significantly more with tap water present than with 9% sucrose solution present ($p < 0.05$), and diabetics ate significantly more than controls when both were presented with tap water; however, difference in food intake between diabetics and controls given continuous access to 9% sucrose solution was not significant.

Diabetic rats, unlike control rats, fail to respond to the sweet taste of sucrose solution by increasing liquid intake. The interaction between diabetes and solution was significant ($F = 20.500$, $p < 0.001$). Diabetic rats drank more than controls when given access to 9% sucrose solution (182.6 ml versus 121 ml) and tap water (168.6 ml versus 39.7 ml). However, while liquid intake of controls was greater with 9% sucrose solution than with tap water ($p < 0.05$), solution had no effect on liquid intake of diabetic rats.

Experiment 4: Decreased Responsiveness Toward Sweet Tastes in the Hyperphagic Diabetic Rat

The hyperphagic diabetic rat fails to respond to the sweet taste of sucrose solution by increasing liquid intake in a manner comparable to controls. Attenuated responsiveness toward sucrose solution could be due to the tendency for rats to limit intake of sucrose solutions when diets are present which fulfill nutritive as well as caloric requirements (Collier and Bolles, 1968). Previous studies in this research have shown that the hyperphagic diabetic rat regulates daily food intake; therefore there is a limit in the amount of calories ingested each day and an increase in the amount of calories derived from sucrose solutions must be associated with a decrease in the amount of calories derived from dry diet. However, the diabetic rat is a hyperglycemic animal and nondiabetic hyperglycemic rats demonstrate an attenuated preference for sweet solutions (Booth, Lovett, and McSherry, 1972). Diminished intake of sucrose solutions by diabetic rats could be due to an avoidance of sweet tastes.

In order to discriminate between decreased responsiveness to sucrose solution as a manifestation of (1) an attempt to maintain high nutrient intake or (2) the tendency of hyperglycemic animals to depress intake of sweet solutions, liquid and food intake were compared when diabetic and non-diabetic subjects were presented with a

sweet non-nutritive solution (0.4% saccharin) or a non-sweet nutritive solution (9% glucose).

The possibility of diminished responsiveness to sweet tastes in the diabetic rat is directly related to the study of the relationship between glucose availability and signals from fat depots as factors controlling food intake. In the introduction to this research, it was implied that signals from fat depots must influence food intake by either (1) overriding glucose or energy substrate availability as a factor controlling food intake or (2) increasing the amount of glucose or total energy substrates required for satiety. However, an alternative possibility has been set forth. Jacobs and Sharma (1968) have suggested that long-term hyperphagia is mediated by increased sensitivity to diet palatability and gives rise to a situation in which animals eat amounts of food which exceed limits determined by metabolic signals. Although behavioral sensitivity to sweet tastes and amount of food intake usually vary together, it is legitimate to maintain that sensitivity to sweet tastes and glucoprivation are separate factors controlling food intake since the onset of behavioral sensitivity to sweet tastes precedes the onset of glucoprivation-induced eating in the development of the rat (Jacobs, 1964a; Houpt and Epstein, 1973).

Methods

Subjects were 15 male Holtzman albino rats (8 hyperphagic diabetics and 7 controls) over 90 days of age. Diabetes was induced 4 weeks before the onset of the experiment. Subjects were randomly assigned to sequences of the following solutions (with each solution presented individually for a 23-hour period): (1) 9% glucose solution, (2) 0.4% saccharin solution, and (3) tap water. Food intake and liquid intake measurements were taken at 11 a.m. Data were analyzed with an analysis of variance mixed design (between subject variable of diabetes and within subjects variable of solution). Significant interactions were analyzed with Duncan's new multiple range test.

Results

Diabetic and non-diabetic rats differ with respect to responsiveness to the sweet taste of saccharin, but do not differ with respect to responsiveness to non-sweet glucose solution or with respect to the effect that ingestion of saccharin and glucose solutions have on food intake. Hyperphagic diabetic rats appear to be unresponsive to sweet tastes.

Food Intake. The interaction between diabetes and solution was significant ($F = 4.421$, $p < 0.05$). Food intake of diabetics given tap water (32.2 grams) was greater than that of controls given tap water (22.2 grams).

Food intake of diabetics given saccharin solution (27.1 grams) was greater than that of controls given saccharin solution (20.9 grams). However, food intake did not differ between diabetic and control rats given continuous access to 9% glucose solution.

Diabetic rats respond to 9% glucose solution by increasing liquid intake but fail to respond to 0.4% saccharin solution by increasing liquid intake (Fig. 4). Since the interaction between diabetes and solution was not significant, Duncan's test could not be utilized to determine if the effects of various solutions on liquid intake differed as a function of diabetes.

Experiment 5: Effects of Glucose on Food Intake of Diabetic Rats Due to Nutritional and not Osmotic Value of Glucose

Diabetic rats presented with glucose solution respond by increasing liquid intake and decreasing food intake. A question arises as to whether or not the previously observed effectiveness of glucose and sucrose solutions in depressing food intake is due to the nutritive value of these solutions. Increased intake of glucose solutions and decreased intake of food are expected if glucose solutions are unable to sate thirst of diabetic rats. Although the osmotic effects of glucose on food intake of non-diabetic rats may be negligible (Jacobs, 1964b; Hsiao and Langenes, 1971), the polydipsia demonstrated by diabetic rats is a function of blood glucose

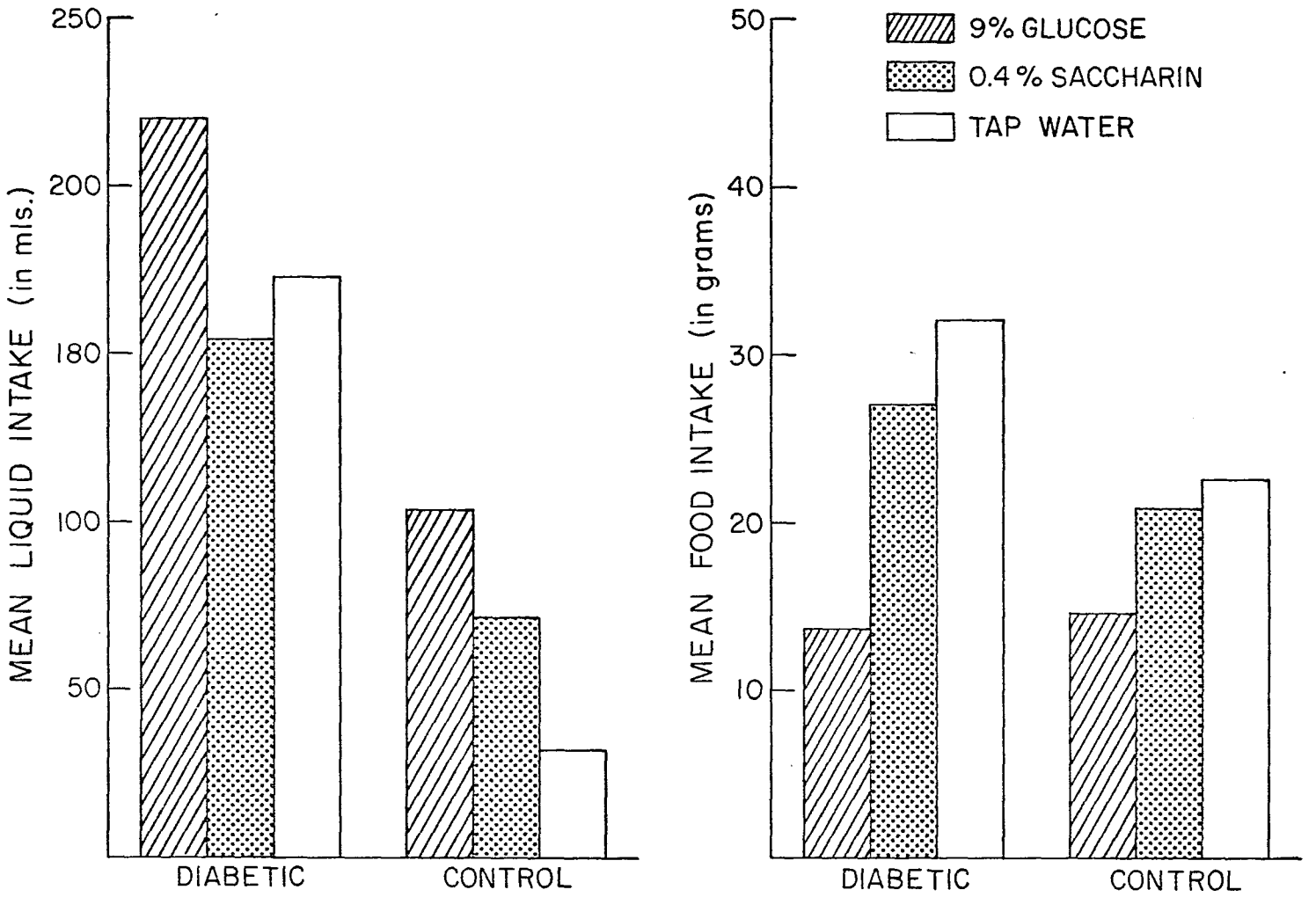

Fig. 4. Mean daily liquid (a) and food (b) intake of diabetic and control rats given continuous access to 9% glucose solution, 0.4% saccharin solution, or tap water.

levels (Hernandez and Briese, 1972). Pilot studies had demonstrated that providing the diabetic rat with continuous access to isotonic saline in the absence of tap water was associated with severe depressions in food intake and an increase in liquid intake. The possibility therefore existed that the effects of glucose and sucrose solutions on food intake of diabetic rats is dependent on the osmotic value of solutions not on the nutritive value.

The purpose of the following experiment was to determine if the effects of ingestion of isotonic saline on liquid intake and food intake of diabetic rats can be equated with the effects of ingestion of glucose solution. Two-bottle preference tests combined with food intake measurements were utilized, with subjects receiving 9% glucose solution paired with tap water on one day and isotonic saline paired with tap water on a second day.

Methods

Subjects were 14 naive Holtzman male albino rats (7 controls and 7 hyperphagic diabetics) over 90 days of age. Diabetes had been induced 4 weeks before the onset of the experiment. Subjects were randomly assigned to sequences of the following solutions: (1) tap water, (2) isotonic saline + tap water, and (3) 9% glucose solution + tap water. Food and liquid intake measurements were taken at 9 a.m.

Data were analyzed using an analysis of variance mixed design.

Results

Food intake of diabetic and control rats is d depressed when 9% glucose solution and tap water are presented but not when isotonic saline and tap water are presented. The interaction between diabetes and solution was significant ($F = 5.387$, $p < 0.025$). Food intake of diabetics was greater than that on controls when either tap water alone or tap water with isotonic saline were provided ($p < 0.05$); however, food intake did not differ between diabetic and control rats given continuous access to both 9% glucose solution and tap water.

Both diabetic and control rats preferred 9% glucose solution over tap water; however, while diabetic rats preferred tap water over isotonic saline, control rats preferred isotonic saline over tap water. The interaction between diabetes and solution was significant ($F = 12.339$, $p < 0.005$). Diabetic rats drank more tap water than isotonic saline ($p < 0.05$) and more 9% glucose solution than tap water ($p < 0.05$). Control rats drank more isotonic saline than tap water ($p < 0.05$) and more 9% glucose solution than tap water ($p < 0.05$) (Fig. 5).

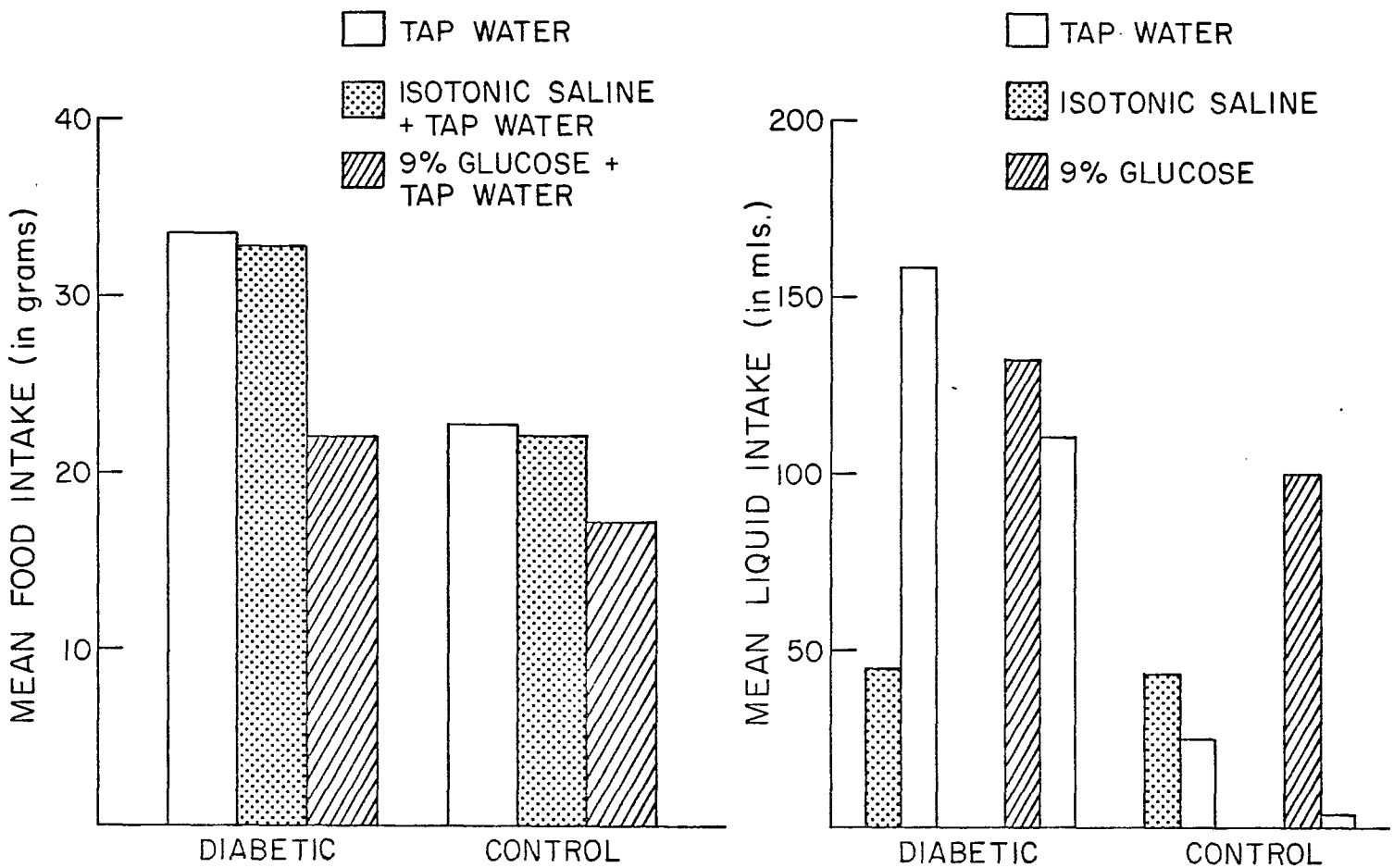

Fig. 5. Mean daily food (a) and liquid (b) intake of diabetic and control subjects given continuous access to water and water paired with isotonic saline or 9% glucose solution in two-bottle preference tests.

Hyperphagic diabetic rats do not respond to 9% glucose solution in the same manner that they respond to a second osmotically active solution, isotonic saline. Therefore, the observed effects of glucose on food intake of hyperphagic diabetic rats appears to be due to the nutritive value of glucose and not to effects of glucose on thirst.

Discussion

Hyperphagic diabetic rats respond to decreases and increases in extracellular glucose availability with the appropriate adjustments in food intake. The diabetic rat appears to be capable of regulating daily food intake in response to alterations in glucose availability and proposals which ascribe diabetic hyperphagia to a loss of the ability to regulate food intake appear to be unwarranted (Panksepp and Nance, 1972; Booth, 1972c).

Although decreased food intake and increased liquid intake in association with ingestion of glucose solutions could be due to the possible effects of glucose on thirst in the diabetic rat, preference behavior of diabetics suggests that they respond to the nutritive value of glucose solutions. Further, the amount of glucose solution which replaces one gram of dry diet in thediabetic rat was 11.75 ml when 9% glucose solution was provided alone and 11.33 ml when 9% glucose solution and tap water were

available. Ingestion of considerable amounts of water with glucose solution fails to diminish the food intake depressing effects of glucose solution in the diabetic rat.

Diabetic rats respond to non-sweet glucose solution by increasing liquid intake. The only organism which fails to respond to glucose solutions by increasing liquid intake is the rat with bilateral lesions of the lateral hypothalamus (Mufson and Wampler, 1972). Therefore, the hyperphagic diabetic rat does not behave as would be expected if high basal blood glucose levels or glycolytic inhibition acted to block behavioral sensitivity to glucose availability mediated by lateral hypothalamic feeding centers.

The inability of hyperphagic diabetic rats to reliably diminish food intake following an intraperitoneal injection of 0.3 grams of glucose (Booth, 1972c) is not necessarily proof that behavioral sensitivity to glucose availability is less in diabetic rats than in non-diabetic rats, since variance in food intake within diabetic rats may be in excess of the small effect that such an injection has on the food intake of non-diabetic rats. When Mayer and Bates (1952) utilized repeated injections of glucose solution, they found food intake of diabetic rats to be significantly depressed.

In disagreement with Booth (1972a) it must be said that there is evidence for supervening signals from fat

depots controlling long-term food intake. Since behavioral sensitivity to the availability of energy substrates does not appear to be diminished in diabetic rats, supervening factors are required to explain the fact that a hyperglycemic animal is hyperphagic. Signals from fat depots are the only proposed supervening factors of a metabolic nature which have face validity. Injections of the lipid metabolite prostaglandin E_1 (PGE_1) into the lateral hypothalamus (but not other areas of the brain) are associated with depressions in food intake (Baile, Simpson, Bean, McLaughlin, and Jacobs, 1973).

Additional studies are required to determine if PGE_1 is a natural inhibitor of food intake and if PGE_1 is responsible for diabetic hyperphagia. These additional studies must demonstrate that availability of PGE_1 to the lateral hypothalamus is decreased in animals demonstrating long-term hyperphagia. Further, the onset of decreased availability of PGE_1 to the lateral hypothalamus must be concurrent with the onset of diabetic hyperphagia in young rats, since Meliza and Zimmer (1975, in press) have shown that onset of factors responsible for diabetic hyperphagia are unlikely to be obscured by malaise in young rats. Demonstrating that delayed onset of diabetic hyperphagia in young rats is unlikely to be due to malaise, suggests that prolonged insulin deficiency and not insulin deficiency per se is required for diabetic hyperphagia to develop.

That insulin-replacement failed to attenuate diabetic hyperphagia in Experiments 1 and 10 also suggests that insulin deficiency per se is not a sufficient stimulus for diabetic hyperphagia. Baile et al. (1973) have proposed that levels of PGE_1 may covary with the lipogenesis/lipolysis ratio; however, the profound alteration found in the alteration of this ratio concurrent with the onset of insulin deficiency is not associated with increased food intake as would be expected if rates of lipogenesis and lipolysis per se were important in controlling food intake.

A question arises as to the manner in which effects of signals from fat depots on food intake are mediated. Effects of signals from fat depots may be mediated by modulating the amount of glucose or energy substrate availability required for satiety or by modulating behavioral sensitivity to other factors which influence food intake. The primary nonmetabolic factor expected to be responsible for diabetic hyperphagia is diet palatability (Hernandez and Briese, 1972).

Jacobs and Sharma (1968) have proposed that the advent of long-term hyperphagia is associated with increased behavioral sensitivity to diet palatability such that amount of food intake is controlled to a greater extent on the basis of diet palatability and to a lesser extent on the basis of physiological signals than before the onset of long-term hyperphagia. The proposal of Jacobs and Sharma is

based on the enhanced sensitivity to diet palatability demonstrated by VMH-lesioned rats (Levison, Frommer, and Vance, 1973) and the enhanced intake of sweet solutions demonstrated by fasted rats (Bacon, Snyder, and Hulse, 1954; Collier and Bolles, 1968). However, the VMH-lesioned obese rat (which is not hyperphagic) demonstrates behavioral hypersensitivity to diet taste, while the VMH-lesioned hyperphagic rat is grossly insensitive to diet taste (Teitelbaum, 1955). Since food intake and behavioral sensitivity to diet palatability do not covary in the predicted direction in the VMH-lesioned rat, ascribing VMH-hyperphagia to increased responsiveness to palatable diets appears unwarranted.

Booth (1972d) has provided evidence which suggests that increased behavioral sensitivity of fasted rats to diet taste may be an artifact. Measures of behavioral sensitivity to diet taste involve determining the effect on daily food intake of increasing or decreasing diet palatability. Booth has shown that greater responsiveness of fasted rats to diet palatability is a product of the interaction between sensitivity to diet palatability and the amount of glucose required to sate hunger.

Panksepp (1971b) has proposed that the ventromedial hypothalamus is critical for long-term control of food intake, since increased food intake following food deprivation is not found in VMH-lesioned rats. Utilizing enhanced

food intake as a criteria for long-term regulation of food intake suggests that the hyperphagic diabetic rat is unable to regulate long-term food intake (Panksepp and Nance, 1972). The present findings suggest that the hyperphagic diabetic rat is capable of regulating food intake and thus call into question the validity of Panksepp's criteria.

Since the brain as a whole does not require insulin for glucose transport (Crone, 1965), experimenters have suggested that Mayer's (1955) glucostatic theory would be consistent with the hyperphagia demonstrated by hyperglycemic diabetic rats only in an instance where a neural feeding center was shown to be insulin-dependent (Andersson, 1971; Hernandez and Briese, 1972; Nance and Gorski, 1973). Debons, Krimsky, Likuski, From, and Cloutier (1968) have provided evidence which suggests that glucose transport in the VMH-satiety-center is insulin-dependent, leading Hernandez and Briese (1972) to ascribe diabetic hyperphagia to a functional lesion of the ventromedial hypothalamus. However, evidence suggests that diabetic hyperphagia cannot be ascribed to a functional lesion of the ventromedial hypothalamus. VMH-lesioned weanling rats do not develop hyperphagia until maturity is reached (Frohman, Goldman, Schnatz, and Bernardis, 1971; Gold and Kapatos, 1975), while diabetic weanling rats display hyperphagia (Goldman, Schnatz, Bernardis, and Frohman, 1972).

INFERIORITY OF THE REGULATION OF AMINO ACID INTAKE IN COMPARISON WITH REGULATION OF CALORIC INTAKE

Experiment 6: Invalidity of Ascribing Anorexia Associated with Maintenance on a Protein-Free Diet to the Regulation of Caloric Intake

Musten et al. (1974) have proposed that the regulation of amino acid intake is inferior to the regulation of caloric intake. These experimenters define the regulation of amino acid intake in terms of ingestion of given amounts of amino acids. Therefore, under the definition of amino acid intake regulation utilized by Musten et al., rats maintained on a protein-free diet are precluded from regulating amino acid intake. However, rats maintained on a protein-free diet demonstrate anorexia (Harper, 1967) and this anorexia must be explained in terms of a regulatory activity. Under the proposal of Musten et al., anorexia demonstrated by rats maintained on a protein-free diet must be due to the fact that the amount of diet required for satiety as determined by the regulation of caloric intake has decreased.

An alternative definition of the regulation of amino acid intake is available. Data suggest that regulation of amino acid intake is an attempt to maintain a given relationship between the amounts of free essential amino acids found in the brain by inhibiting the intake of diets

which perturb the free amino acid patterns of the brain (Peng et al., 1972). Utilizing this definition of amino acid intake regulation, rats demonstrating anorexia while maintained on a protein-free diet are regulating amino acid intake, since ingestion of protein-deficient diets is associated with severe depressions in the levels of methionine and tyrosine found in the brain (Peng, Meliza, Vavich, and Kemmerer, 1974).

Questions about the inferiority of the regulation of amino acid intake in comparison with the regulation of caloric intake cannot be answered until one of the proposed definitions of amino acid intake regulation is shown to be invalid. It is possible to determine the validity of these definitions because the validity of the definitions are dependent on the validity of 2 different models of the relationship between amino acid intake regulation and caloric regulation as factors controlling total food intake. Under the model of Musten et al. (1974), total food intake is determined by the regulation of caloric intake. Under the model of Peng et al. (1972), total food intake is determined by the regulation of both amino acid and caloric intake. The definition of amino acid intake regulation utilized by Musten et al. (1974) would be invalid if it is shown that anorexia associated with maintenance on a protein-free diet is not due to the regulation of caloric intake.

Rats maintained on food-deprivation schedules or presented with a limited amount of food demonstrate enhanced intake of sweet solutions (Hsiao and Pertsulakes, 1970). If anorexia associated with maintenance on a protein-free diet is due to some factor acting to prevent rats from eating to satiety (namely the regulation of amino acid intake), then intake of sweet solutions should increase. However, if anorexia associated with maintenance on a protein-free diet is due to a decrease in the amount of food intake required for satiety, then rats maintained on a protein-free diet should not be any more responsive to sweet solutions than are rats maintained on an adequate protein diet. Therefore, the purpose of the present experiment was to determine if responsiveness toward sweet solutions is increased when rats are maintained on a protein-free diet.

Methods

Subjects were 12 male Holtzman rats with weights ranging between 230 and 260 grams. All subjects received both protein-free and 10% casein diets during the course of the experiment with diets being alternated daily. One-half of the subjects were randomly assigned to receive 0.2% saccharin solution paired with protein-free diet and 9% sucrose solution paired with 10% casein diet. The remainder of the subjects were assigned to the opposite diet-solution

pairings (protein-free diet paired with 9% sucrose solution and 10% casein diet paired with 0.2% saccharin solution). Diet was presented at 8 p.m. each day and removed for testing purposes betweel 11 a.m. and 1 p.m. on the following day. Flavored solutions were presented between 11:30 and 11:45 a.m. Diet was alternated daily between protein-free and 10% casein diets for a period of 8 days.

Data were analyzed with an analysis of variance mixed design (between subject variable of solution-diet pairing group and within subjects variable of diet and repetitions). Significant interactions were further analyzed using Duncan's new multiple range test.

Results

Intake of protein-free diet is associated with an increase in the intake of flavored solutions paired with this diet in relation to the intake of flavored solutions paired with the intake of 10% casein diet (Fig. 6). The effect of diet was significant ($F = 83.796$, $p < 0.001$). Liquid intake was significantly greater on days when protein-free diet was presented than on days when 10% casein diet was presented ($p < 0.05$). It was concluded that caloric regulation cannot be responsible for anorexia demonstrated by rats maintained on a protein-free diet, since such animals demonstrate a behavior

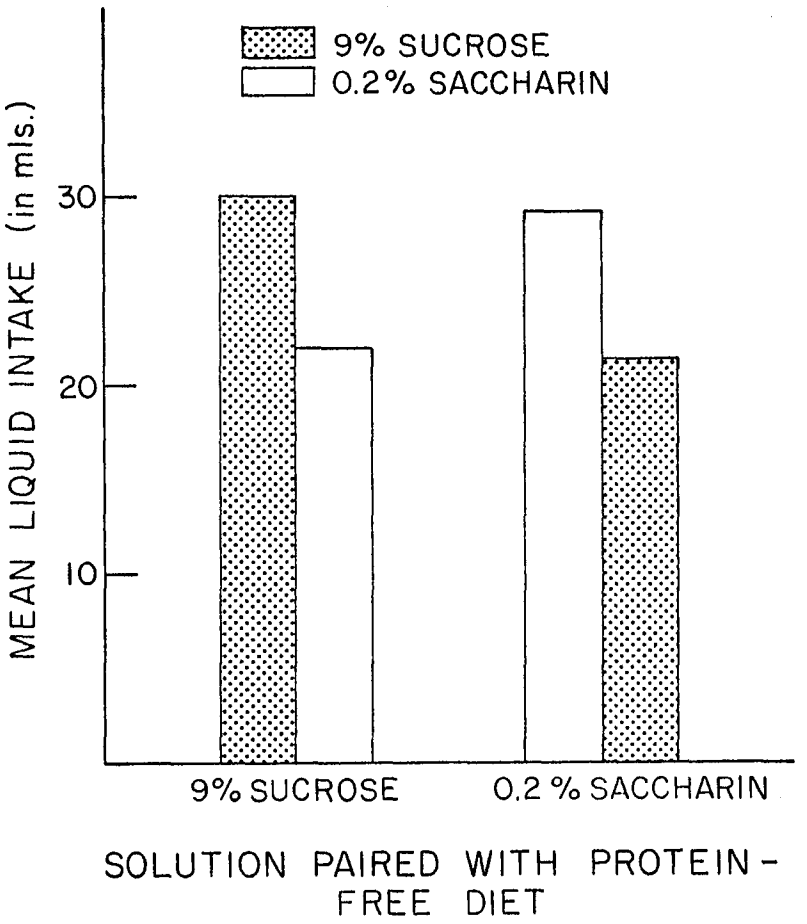

Fig. 6. Mean liquid intake as functions of type of solution presented and type of solution paired with protein-free diet.

characteristic of the gluco-deprived rat (enhanced intake of sweet solutions).

The effect of repetitions was significant ($F = 23.032$) in that the amount of each solution drank increased during the experiment. However, increased intake of sweet solutions paired with protein-free diet in comparison with the intake of solutions paired with 10% casein diet was manifested during the first 2 days of the experiment. Hence, the effect that maintenance on a protein-free diet has on responsiveness to sweet solutions does not require prolonged maintenance on a protein-free diet, and thus avoids the problem of confounding caloric regulation by glucose availability with proposed regulation of caloric intake by signals from fat depots. Therefore it was suggested that the regulation of amino acid intake may act to preclude the regulation of caloric intake by glucose availability.

Experiment 7: Maintenance on a Protein-Free Diet Precludes Insulin-Induced Hyperphagia

The results of the previous experiment suggest that glucoprivation may not be a sufficient stimulus to override the regulation of amino acid intake, since anorexia associated with maintenance on a protein-free diet is concurrent with a behavior characteristic of the gluco-deprived rat. The purpose of the present experiment was to determine if

insulin hypoglycemia is a sufficient stimulus to enhance food intake of rats maintained on a protein-free diet.

Methods

Subjects were 12 Holtzman male albino rats over 90 days of age. One-half of the subjects were randomly assigned to maintenance on a protein-free diet with remaining subjects being assigned to maintenance on a 10% casein diet. Subjects were randomly assigned to sequences of insulin (20 units per kilogram of body weight, i.p.) and isotonic saline injections. Subjects were adapted to the assigned diets for 1 day before the initiation of testing. Injections were administered at 9 a.m. and food intake was determined 4 hours later. Each subject received insulin on one day and isotonic saline on a second day. Results were analyzed using an analysis of variance mixed design (between subject variable of diet and within subjects variable of insulin). Significant interactions were analyzed using Duncan's new multiple range test.

Results

Injections of insulin fail to increase food intake when subjects are maintained on a protein-free diet, while increasing food intake of subjects maintained on a 10% casein diet. Mean intake of subjects maintained on a protein-free diet was 0.70 grams, while mean food intake of subjects maintained on a 10% casein diet was 2.38 grams

(F = 33.008, p less than 0.001). Mean food intake following saline injections was 1.18 grams, while mean food intake following insulin injections was 1.90 grams (F = 26.96, p less than 0.001). The interaction between diet and insulin was significant (F = 30.803, p less than 0.001). Mean intake of subjects maintained on a protein-free diet was 0.73 grams following saline and 0.68 grams following insulin. Mean intake of subjects maintained on a 10% casein diet was 1.63 grams following saline and 3.13 grams following insulin (p less than 0.05).

Experiment 8: Maintenance on a Protein-Free Diet Fails to Preclude the Development of Diabetic Hyperphagia

Collier and Bolles (1968) have proposed that the regulation of amino acid intake becomes inferior to the regulation of caloric intake with the advent of long-term hyperphagia. The purpose of the present experiment was to determine if maintenance on a protein-free diet precludes the development of diabetic hyperphagia.

Methods

Subjects were 16 male Holtzman albino rats over 90 days of age. Subjects were randomly assigned to one of 4 treatment groups: (1) diabetic, 10% casein diet; (2) diabetic, protein-free diet; (3) control, 10% casein diet; and (4) control, protein-free diet. Subjects were maintained on Purina Laboratory Chow until the initiation of testing.

Experimental diets were first presented immediately following injections of either alloxan monohydrate or isotonic saline. Food intake was measured for a period of 8 days following injection, with intake measurements taken at 9 a.m. Data were analyzed with an analysis of variance mixed design (between subject variables of diabetes and diet and within subjects variable of days). Significant interactions were further analyzed using Duncan's new multiple range test.

Results

Maintenance on a protein-free diet does not preclude the development of diabetic hyperphagia (Fig. 7). Intake of diabetic subjects maintained on protein-free diet exceeded that of controls maintained on protein-free diet beginning with day 4 ($p < 0.05$) and exceeded that of controls maintained on 10% casein diet beginning with day 6 ($p < 0.05$).

Experiment 9: Enhanced Ability of Maintenance on a Protein-Free Diet to Depress Food Intake of Hyperphagic Diabetics Receiving Insulin

VMH-lesioned rats depress food intake when maintained on amino acid imbalanced diets (Leung and Rogers, 1970). Therefore, finding that the hyperphagic diabetic rat demonstrates hyperphagia while maintained on a protein-free diet suggests that the mechanisms behind VMH-hyperphagia and diabetic hyperphagia may differ. However, the attenuated

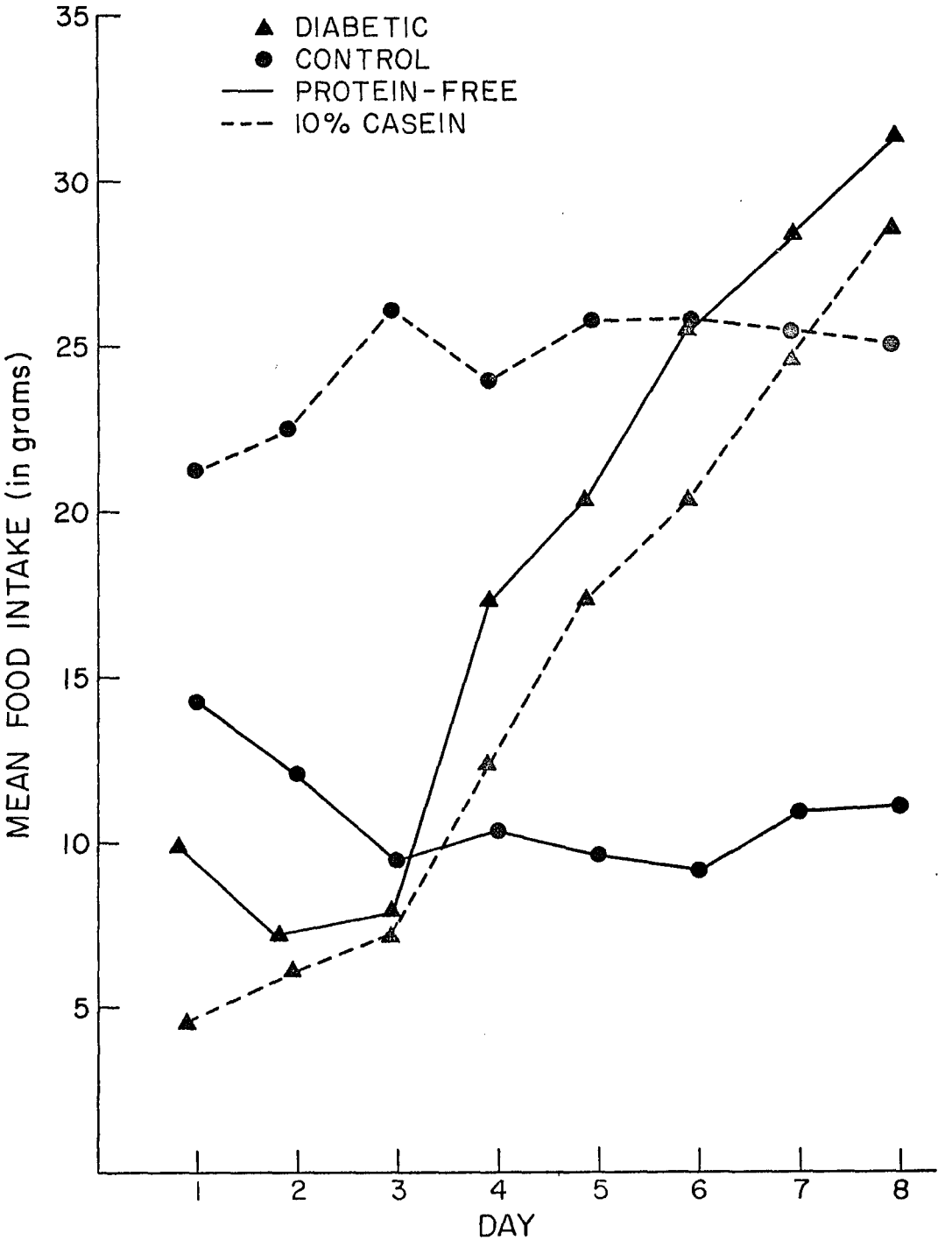

Fig. 7. Mean daily food intake of diabetic and control rats maintained on either protein-free diet or 10% casein diet.

ability of maintenance on a protein-free diet to depress food intake of diabetic rats is associated with an attenuated ability of maintenance on a protein-free diet to alter brain amino acid patterns (Peng, Meliza, Vavich, and Kemmerer, 1975, in press). Therefore, the apparent inability of maintenance on a protein-free diet to severely depress food intake in the diabetic rat may be due to the already perturbed plasma amino acid pattern (Felig, Marliss, Ohman, and Cahill, 1970), or to the high rates of utilization of plasma amino acids in the biosynthesis of glucose (Exton, 1972).

The problem of finding an amino acid imbalanced diet which would have an effect on brain amino acid patterns in the diabetic rat may be circumvented by utilizing an additional method of altering brain amino acid patterns. Injections of insulin drastically alter brain amino acid patterns (McIllwain and Bachelard, 1971). The purpose of the present experiment was to determine if repeated injections of insulin act to depress food intake of diabetic rats maintained on a protein-free diet.

Methods

Subjects were 18 Holtzman male albino rats over 90 days of age. Diabetes had been induced in all subjects 4 weeks before the onset of the experiment. Subjects were randomly assigned to maintenance on a protein-free diet or

a 10% casein diet (9 subjects per group). Six subjects from each diet group were randomly assigned to receive insulin-replacement. Insulin-replacement consisted of 3 subcutaneous injections daily (at 8 hour intervals) for a period of 5 days. Dosage of insulin was 15 units per kilogram. Remaining subjects received injections of isotonic saline. At the end of 5 days, one-half of the subjects receiving insulin-replacement and maintained on a protein-free diet were switched to 10% casein diet and the other half were withdrawn from insulin-replacement. Data were analyzed using an analysis of variance mixed design (between subject variable of diet and insulin and within subject variable of days). Significant interactions were analyzed using Duncan's new multiple range test.

Results

Neither maintenance on a protein-free diet nor the method of insulin-replacement utilized were sufficient to depress food intake of hyperphagic diabetic rats. However, combining insulin-replacement with maintenance on a protein-free diet was associated with a severe depression in food intake of diabetic rats, which was terminated by either withdrawing insulin-replacement or switching subjects to a 10% casein diet. The interaction between diet, days and insulin was significant ($F = 11.729$, p less than 0.001). Daily food intake of diabetics receiving insulin and

maintained on a protein-free diet was depressed below that of all other groups on days 3 through 5 ($p < 0.05$).

Discussion

The proposed inferiority of amino acid intake regulation in comparison with the regulation of caloric intake by glucose availability is based on the finding that weanling rats will eat a given percentage of daily caloric intake in the form of amino acids unless dilution of the source of amino acids by non-protein sources of energy is so great as to require ingestion of excessive amounts of calories from non-protein sources. According to Musten et al. (1974), increasing intake of a low-protein diet in order to ingest sufficient amino acids would exceed a proposed caloric intake to protein intake ratio. However, the results of experiment 6 suggest that defining the regulation of amino acid intake in terms of ingesting a sufficient amount of amino acids is invalid, since this definition leaves anorexia associated with maintenance on a protein-free diet as an inexplicable phenomenon.

Data suggest that the regulation of amino acid intake must be defined as the ability to depress intake of diets which act to perturb brain free amino acid patterns and to select diets which may correct deviant brain amino acid patterns. Anorexia associated with maintenance on an amino acid imbalanced diet is reversed by infusions of the

limiting amino acid into the carotid artery but not into the jugular vein (Leung and Rogers, 1969). Further, infusions of amino acids other than the limiting amino acid fail to reverse anorexia. Therefore, amounts of amino acids per se is not a critical factor controlling food intake. Regulation of amino acid intake must be defined as an attempt to maintain a given relationship between the levels of the various free essential amino acids found in the brain. Under such a definition, rats which depress food intake when presented with a protein-deficient diet are manifesting the ability to regulate amino acid intake and not failing to manifest such an ability (as suggested by Musten et al., 1974).

The lack of efficacy of a protein-free diet in diminishing food intake of diabetic rats initially suggested that Collier and Bolles (1968) were correct in asserting that the advent of long-term hyperphagia is associated with a state in which rats will eat for calories at the expense of nutrient value. However, the effect of a given diet on the regulation of amino acid intake is a function of factors which influence plasma amino acid patterns. Injections of cortisol, previous maintenance on a high-protein diet and diabetes are each associated with enhanced gluconeogenesis and a change in preference toward amino acid imbalanced diets over protein-free diets (Leung, Rogers, and Harper, 1968; Peng et al., 1975, in press). Aversive effects of

maintenance on amino acid imbalanced diets appear to be attenuated by the rapid utilization of plasma free amino acids via gluconeogenesis (Felig et al., 1970; Exton, 1972). Hence a diet which has a profound effect on brain amino acid patterns in normal rats may have a very minor effect when plasma amino acid patterns are perturbed by gluconeogenesis.

GENERAL DISCUSSION

The explicit purpose of this research has been fulfilled. It has been shown that (1) signals from fat depots do not override glucose availability as a factor controlling food intake in animals demonstrating long-term hyperphagia, (2) the regulation of amino acid intake is not inferior to the regulation of caloric intake, and (3) the regulation of amino acid intake does not become inferior to the regulation of caloric intake with the advent of long-term hyperphagia. Clarifying the relationship between brain amino acid patterns, glucose availability and signals from fat depots as factors controlling food intake will hopefully hasten the death of attempts to prove that only one of these factors is controlling food intake in a given organism by demonstrating that one factor influences food intake in a given organism, while a second does not.

Demonstrating that a factor does not influence food intake in a given organism gives rise to two possible interpretations: (1) the organism is insensitive to the factor being tested, or (2) the method of testing behavioral sensitivity to a factor is itself not sensitive to the effects which that factor may have on behavior. Booth (1972c) in finding that i.p. injections of glucose solution fail to depress food intake of hyperphagic diabetic rats,

concluded that diabetic rats are less sensitive to glucose availability as a factor controlling food intake than are non-diabetic rats; however, it was equally likely that the method utilized to measure behavioral sensitivity to glucose availability is itself an insensitive measure in the hyperphagic diabetic rat. Similarly, in the present research, finding that maintenance on a protein-free diet did not preclude diabetic hyperphagia could have led to the conclusion that long-term hyperphagia overrides the regulation of amino acid intake. However, additional findings suggested that the method of testing the proposed inferiority of the regulation of amino acid intake utilized was a negatively biased measure in the diabetic rat.

Demonstrating that an organism is behaviorally sensitive to a factor controlling food intake can also give rise to problems in interpretation. If an organism demonstrates behavioral sensitivity to a factor controlling food intake, it may not be for the same reasons that other organisms demonstrate behavioral sensitivity to such a factor. This problem was encountered in the present research. The possibility existed that the effects of glucose on food intake of diabetic rats was mediated by thirst and not by the nutritional value of glucose. However, the results of preference tests suggest that the effects glucose has on food intake of hyperphagic diabetic rats is not thirst mediated.

The present data suggest that the regulation of amino acid intake is relatively independent of the regulation of caloric intake by glucose availability and signals from fat depots as a factor controlling food intake (only relatively independent in that amino acid intake regulation cannot be manifested unless an organism eats). The proposed independence of the regulation of amino acid intake is in agreement with the finding that rats with bilateral lesions of the lateral hypothalamus retain sensitivity to dietary amino acid content (Scharrer, Baile, and Mayer, 1970), while losing sensitivity to glucose availability (Epstein and Teitelbaum, 1967) and losses in body weight (Boyle and Keesey, 1975).

The present research was undertaken in an attempt to determine why children deprived of carbohydrates, lipids and proteins demonstrate hyperphagia, while children selectively deprived of proteins develop anorexia. The first type of diet gives rise to marasmus and the second to kwashikior. The difference in food intake between victims of marasmus and victims of kwashikior is not a matter of dietary protein content per se, because dietary protein content is depressed for both types of victims. Differences in food intake between victims of marasmus and victims of kwashikior appears to be due to an interaction between dietary protein content, carbohydrate content and fat content, suggesting that the difference in food intake might be due to an

interaction between brain amino acid patterns, glucose availability and signals from fat depots as factors controlling food intake. However, the present data do not support such a proposal in that regulation of amino acid intake and regulation of caloric intake have been shown to be independent factors controlling food intake. The purpose of this research may have been fulfilled, since the diminished effectiveness of maintenance on a protein-free diet to depress food intake of diabetic rats suggests the importance of gluconeogenesis as a factor which may act to attenuate the effects of deficient protein consumption on food intake of children with marasmus but not kwashikior,

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