Basal and Postprotein Insulin and Glucagon Levels During a High and Low Carbohydrate Intake and Their Relationships to Plasma Triglycerides

Yoshikuni Fujita, M.D., Antonio M. Gotto, D.Phil., M.D., and Roger H. Unger, M.D., Dallas and Houston

SUMMARY

The effects of acute and chronic differences in the carbohydrate content of the diet on plasma insulin, glucagon, insulin-glucagon molar ratio (I/G), and triglycerides were studied. Acute effects were studied by varying the carbohydrate content of a single test meal, while chronic effects were determined by varying the carbohydrate content of the diet for a week. A test meal containing 0.6 gm. of gelatin per kilogram plus 0.6 gm. per kilogram of glucose resulted in much higher levels of insulin and I/G ($p < 0.005$), lower glucagon levels ($p < 0.05$), and slightly higher triglycerides (N.S.) than did a meal of 1.2 gm. per kilogram of gelatin alone. One week of a 12-gm. carbohydrate, 2870-calorie diet lowered insulin ($p < 0.001$), I/G ($p < 0.05$), and triglycerides ($p < 0.001$) and increased glucagon (N.S.), whereas a 390-gm. carbohydrate, 2784-calorie intake significantly increased insulin, I/G, and triglycerides ($p < 0.005$) and lowered glucagon ($p < 0.02$) within two days; even greater changes in hormones were observed on a 510-gm. carbohydrate intake. Of those patients in whom a high carbohydrate intake induced a triglyceride rise of at least 40 mg. per deciliter, a significant correlation between the change in I/G and the change in triglycerides was noted ($r = 0.82$; $p < 0.01$). The results are compatible with but do not prove the proposal that pancreatic α and β cells play a mediating role in carbohydrate induction of hyperlipidemia. DIABETES *24*:552-58, June, 1975.

Glucose is clearly the most influential of the nutrients with respect to insulin and glucagon secretion. Not only does it directly stimulate beta cell secretion[1,2] and suppress alpha cell secretion,[3,4] but it affects profoundly the secretory responses of both these cells to the ingestion or infusion of other nutrients. For example, intravenously administered glucose greatly increases the insulin response and abolishes the glucagon response that occur during the ingestion of protein[5] or fat[6] or during the infusion of alanine.[7] Orally administered glucose also increases the insulin response to a protein meal.[8] In addition to the influence of concurrent glucose entry upon the alpha and beta cell responses to food, there is evidence that the antecedent carbohydrate intake also affects their behavior in a similar direction.[9]

While the phenomenon of carbohydrate-induced hypertriglyceridemia has been recognized for a number of years,[10] the mechanism of this effect is poorly understood. It seems possible that insulin and glucagon may be the mediators of certain metabolic processes commonly attributed to high carbohydrate intake, such as protein sparing and induction of hyperlipoproteinemia. For example, the increase in VLDL synthesis induced by a high carbohydrate intake could well be mediated through the increased secretion of insulin, which is reportedly increased in endogenous hypertriglyceridemia[11-17] and/or through the decreased secretion of glucagon, which opposes it,[18] the latter possibility having been suggested previously by Eaton and Kipnis.[19]

The present study was designed to characterize more fully the effects of antecedent and concurrent carbohydrate intake upon plasma insulin and glucagon concentrations, both in the fasting state and during a protein meal, and to look for possible relationships between the two hormones and plasma VLDL levels.

METHODS AND MATERIALS

Twenty-two normal hospital employees volunteered for this study. All but two were males. All subjects denied a personal and family history of diabetes mellitus. Ages ranged from twenty to forty-eight years and averaged twenty-nine. Weights ranged from 61 to 105 kg. and averaged 73. All

From the Veterans Administration Hospital, Dallas, Texas, and Departments of Internal Medicine, the University of Texas Southwestern Medical School at Dallas, and Baylor College of Medicine, Houston, Texas.

Address reprint requests to: Roger H. Unger, M.D., VA Hospital, 4500 S. Lancaster Road, Dallas, Texas 75216.

Accepted for publication February 25, 1975.

subjects were asked to abstain from alcohol throughout the study periods and to avoid the ingestion of sugar in drinks or in the cooking of their food. All tests were conducted on an outpatient basis with the informed consent of the individual.

Three dietary regimens were employed: A low carbohydrate diet consisting of 12 gm. of carbohydrate, 260 gm. of protein, 190 gm. of fat, and 2870 calories; a moderately high carbohydrate diet made up of 390 gm. of carbohydrate, 36 gm. of protein, 120 gm. of fat, and 2784 calories; and a very high carbohydrate diet composed of 510 gm. of carbohydrate, 72 gm. of fat, 44 gm. of protein, and 2843 calories. All meals were calculated and carefully explained to the subjects by a hospital dietitian. The high carbohydrate diet preceded the low carbohydrate diet in seven patients and followed it in ten. Calculations of calories were based on the tables of the United States Department of Agriculture in Turner's *Handbook of Diet Therapy*.[20]

To permit the study of the influence of a fat-free, sugar-free protein meal on the endogenous lipoproteins, gelatin was selected. It was given in the form of 20 per cent warm semiliquid solution flavored with chicken broth. A dose of 1 gm. of protein per kilogram of body weight was consumed over a thirty-minute period. Although an incomplete protein with respect to its amino acid composition, gelatin was regarded as an ideal protein test meal for these studies since it is an effective stimulus of glucagon secretion, and its effect on insulin and glucagon levels was not different from more commonly ingested forms of protein.[5]

During each of the three diet regimens a single fasting blood sample was obtained between 8 and 10 a.m. on days 2, 4, and 7, and the subject was weighed. Gelatin meal experiments were conducted on the seventh day of a dietary regimen after an overnight fast. An 18-gauge butterfly needle was placed in the antecubital vein and experiments were begun after a twenty-minute rest period. Nine milliliters of blood was collected at frequent intervals for glucose, insulin, and glucagon assays in chilled tubes containing 5,000 KI units of Trasylol and 12 mg. Na-EDTA. An additional 3 ml. of blood was collected separately in chilled tubes containing 3 mg. of Na-EDTA for measurement of plasma triglycerides, which after an overnight fast were considered to reflect the endogenous lipoprotein level. None of the subjects had visibly detectable chylomicrons after the sample was stored overnight at 4° C.

Glucose was measured by the glucose oxidase method using the Technicon AutoAnalyzer. Plasma triglycerides were measured by the method of Kessler and Lederer[21] using the Technicon AutoAnalyzer Fluorometer II. Insulin was measured by the Herbert modification[22] of the method of Yalow and Berson.[23] Glucagon was measured in duplicate by a modification[24] of the previously described radioimmunoassay,[25] using antiserum G58, which is highly specific for pancreatic glucagon. The assay system measured changes of 20 pg. per millimeter with 95 per cent confidence.

Urine ketones were measured daily during the low carbohydrate regimen with Acetest tablets (Ames Company, Elkhart, Indiana).

RESULTS

Comparison of Plasma Glucose, Insulin, Glucagon and Triglycerides After Gelatin Alone and Gelatin plus Glucose

To compare the insulin and glucagon responses to the ingestion of protein with and without glucose, nine subjects on an ad lib diet were given either 1.2 gm. per kilogram of gelatin or 0.6 gm. per kilogram of gelatin plus 0.6 per kilogram of glucose in random order on two occasions three days apart. As shown in figure 1, gelatin alone caused a significant rise in mean insulin, from 8 ± 0.8 μU. per milliliter to a peak level of 24 ± 3 μU. per milliliter at forty-five minutes ($p<0.005$), and glucagon rose gradually from 64 ± 10 pg. per milliliter to a maximum level of 165 ± 24 pg. per milliliter at 180 minutes ($p<0.005$). By contrast, after gelatin plus glucose, insulin rose to a peak of 63 ± 12 μU. per milliliter, significantly greater than with gelatin alone ($p<0.005$), while the increase in plasma glucagon was significantly less ($p<0.05$), rising to a maximum level of only 130 ± 17 pg. per milliliter at ninety minutes and declining thereafter. The mean insulin: glucagon molar ratio (I/G) declined slightly following the ingestion of gelatin alone, from 3.2 ± 0.3 to 2.3 ± 0.2 at 120 minutes ($p<0.05$); with gelatin plus glucose it rose to a peak of 14.8 ± 2.3 at thirty minutes ($p<0.001$). Glucose, unchanged by gelatin alone, rose after the mixed meal from 78 ± 3 to a peak of 104 ± 4 mg. per 100 ml. at thirty minutes.

A small, progressive rise in plasma triglycerides from 99 ± 10 mg. per 100 ml. to a peak of 113 ± 8 mg. per 100 ml. was noted forty-five minutes following the glucose-gelatin mixture, but this did not differ significantly either from the baseline values or from the triglyceride levels observed after gelatin alone.

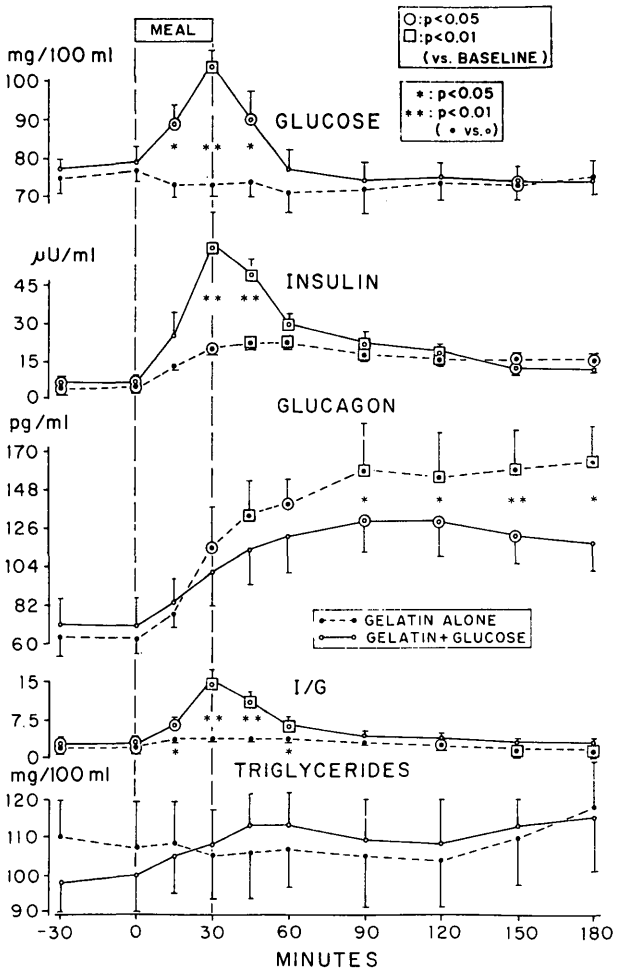

FIG. 1. Mean plasma glucose, insulin, glucagon, molar I/G, and triglyceride responses to 1.2 gm. per kilogram of gelatin or 0.6 gm. of gelatin plus 0.6 gm. of glucose in nine subjects. Tests were given in random order three days apart.

Effects of a Low Carbohydrate Diet on Fasting Glucose, Glucagon, Insulin, and Triglycerides

To determine the effects of a low carbohydrate intake on fasting insulin, glucagon and triglyceride levels, seventeen normal subjects were placed on a diet containing 12 gm. of carbohydrate for seven days. The results appear in table 1A.

After two days on the low carbohydrate diet, mean glucose had declined approximately 10 mg. per deciliter ($p<0.001$) and remained in this range through the seventh day. Mean insulin was significantly decreased ($p<0.001$) after seven days of the diet, while glucagon rose slightly within two days and averaged 80 ± 6 (N.S.) at the seventh day (groups A and B of table 1 combined). The I/G declined from 3.7 ± 9.3 to 2.4 ± 0.3 after two days ($p<0.01$) and remained significantly reduced thereafter. Plasma triglycerides had declined within forty-eight hours from 89 ± 1 to 72 ± 6 ($p<0.005$) mg. per deciliter and after six days averaged 58 ± 5 mg. per deciliter ($p<0.001$). These changes are compared with other diets in figure 2.

During the period of carbohydrate restriction, all but one of the seventeen patients lost weight, ranging from 0.3 to 2.9 kg., suggesting general adherence to the diet. However, correlations between weight change and triglycerides were not meaningful because of the fact that in some subjects the period of carbohydrate restriction preceded and in others followed the high carbohydrate regime. All but one subject was ketonuric on the seventh day of the diet.

Effects of a 390-gm. Carbohydrate Diet on Fasting Insulin, Glucagon, and Triglycerides

Nine of the seventeen patients received a 390-gm. carbohydrate diet for seven days. On the second day of this diet mean plasma glucose had risen significantly above the level on the seventh day of the low carbohydrate diet ($p<0.005$) and remained at approximately this level throughout the week (table 1A). Insulin had also risen significantly during the first two days ($p<0.01$), and glucagon had declined significantly ($p<0.02$). The mean I/G was significantly higher on the second day ($p<0.001$), and triglycerides had risen from 55 ± 8 to 95 ± 12 mg. per deciliter ($p<0.005$), reaching a peak of 120 ± 17 mg. per deciliter on the fourth day. These changes are compared with the other diets in figure 2.

To determine if a statistically significant relationship between the changes in I/G and triglycerides could be established, the differences in I/G on the seventh day of the high and low carbohydrate diets were plotted as a function of the triglyceride difference on these days for each subject. As shown in figure 3, a high degree of correlation between the differences in I/G and plasma triglycerides was observed in this particular group ($r=0.73$, $p<0.01$).

The remaining eight of the seventeen subjects received the 510-gm. carbohydrate diet for seven days (table 1B). There was no statistically significant gain in weight during this week. This is attributed to the wide variation in weight change resulting from the fact that in some patients the high carbohydrate diet followed a free diet while in others it followed a period of carbohydrate restriction. However, poor adherence to this particular dietary regime could not be excluded in some subjects. Compared to the values on the final day of the low carbohydrate diet, glucose, insulin, and I/G were all significantly increased on the second day ($p<0.005$), and the mean triglyceride level had doubled. Glucagon declined, but not significantly. All changes became statistically significant thereafter; on

TABLE 1

Fasting plasma glucose, insulin, glucagon, I/G, and triglyceride levels in normal subjects on diets of varying carbohydrate content

A	Diet	Ad Lib*	12 Gm. Carbohydrate				390 Gm. Carbohydrate			
Subject	Day		1	2	4	7	1	2	4	7
Mean ± S.E.M.	Glucose	84.8±1.8	—	76.3± 3.0	75.1± 2.3	70.2±3.3	—	86.4± 2.7	84.4± 2.5	84.2± 2.5
N=9	Insulin	9.7±1.0	—	8.8± 1.9	9.0± 1.6	6.9±0.7	—	12.5± 1.9	12.3± 2.1	8.5± 0.7
	Glucagon	75.1±6.6	—	86.3±13.2	89.2±11.1	83.4±8.5	—	68.3± 7.7	76.1± 9.8	65.4± 5.5
	I/G	3.2±0.3	—	2.7± 0.6	2.6± 0.5	2.2±0.3	—	4.4± 0.6	3.9± 0.7	3.1± 0.3
	TG	81.3±7.7	—	67.1± 7.8	72.7± 7.8	54.8±8.2	—	95 ±11.7	120.1±16.8	111 ±16.0
vs. Baseline*	Glucose			<0.05	<0.005	<0.005		N.S.	N.S.	N.S.
(*t* test for	Insulin			N.S.	N.S.	<0.01		N.S.	N.S.	N.S.
2 groups)	Glucagon			N.S.	N.S.	N.S.		N.S.	N.S.	N.S.
P	I/G			N.S.	N.S.	<0.05		N.S.	N.S.	N.S.
	TG			N.S.	N.S.	<0.05		N.S.	N.S.	N.S.
12 gm. CHO vs.	Glucose			<0.05	N.S.	<0.005				
390 gm. CHO	Insulin			<0.025	N.S.	<0.005				
(paired *t*	Glucagon			<0.05	<0.001	<0.001				
test)	I/G			<0.005	<0.02	<0.001				
P	TG			<0.01	<0.005	<0.001				

B							510 Gm. Carbohydrate			
Mean ± S.E.M.	Glucose	87 ±2	84 ± 5	74 ± 2.0	74 ± 4	76 ±2	85 ± 4	82 ±2	87 ± 3	89 ± 2
N=8	Insulin	11.7± 1.4	7.3± 0.6	8.1± 1.0	8.2± 0.8	6.5±0.7	12.8± 4.1	12.4± 1.2	9.9± 1.0	9.8± 0.7
	Glucagon	69.1± 2.9	107.5±25.9	118.3±34.5	95 ±13.1	75.6±7.3	65.5±10.7	58.8± 3.8	52.1± 6.1	51.1± 7.4
	I/G	4.3± 0.4	1.9± 0.4	2.1± 0.4	2.3± 0.3	2.3±0.3	4.9± 1.4	5.2± 0.7	4.7± 0.5	4.5± 0.4
	TG	98 ±12	78 ±22	79 ± 8	69 ± 4	62 ±5	112 ±23	148 ±34	129 ±21	120 ±15
vs. Baseline*	Glucose		N.S.	<0.001	<0.05	<0.001	N.S.	N.S.	N.S.	N.S.
(*t* test for	Insulin		N.S.	<0.05	<0.05	<0.005	N.S.	N.S.	N.S.	N.S.
2 groups)	Glucagon		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
P	I/G		<0.05	<0.01	<0.01	<0.01	N.S.	N.S.	N.S.	N.S.
	TG		N.S.	N.S.	N.S.	<0.05	N.S.	N.S.	N.S.	N.S.
12 gm. CHO vs.	Glucose		N.S.	<0.001	<0.05	<0.001				
510 gm. CHO	Insulin		N.S.	<0.005	N.S.	<0.005				
(paired *t*	Glucagon		N.S.	N.S.	<0.01	<0.005				
test)	I/G	N.S.	<0.005	<0.01	<0.005					
P	TG		N.S.	<0.05	<0.02	<0.01				

*Mean of -3 day and -2 day fasting values.

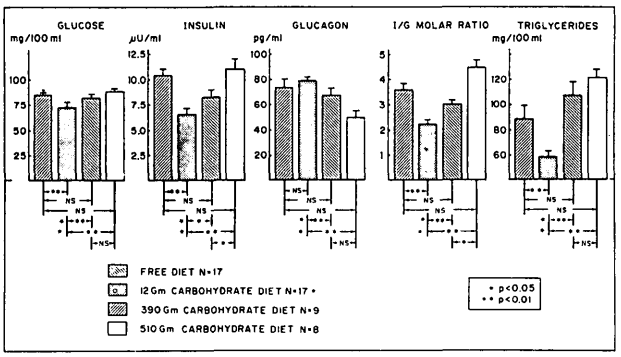

FIG. 2. Comparison of mean morning fasting plasma glucose, insulin, glucagon, molar I/G, and triglyceride levels after a free diet or after seven days of diets of varying carbohydrate content. *While the mean level for the 12 gm. carbohydrate diet includes all seventeen subjects, the statistical comparison with the high carbohydrate groups consists of paired analysis of the groups of nine and eight subjects who received, respectively, 390 and 510 gm. of carbohydrate.

the seventh day the I/G was almost twice as high as on the seventh day of carbohyrate restriction ($p<0.001$), and triglycerides had risen from 62 ± 6 to 120 ± 15 mg. per deciliter ($p<0.01$). These changes are compared with other diets in figure 2.

As indicated in figure 3, the differences in I/G and in plasma triglycerides on the seventh day of the

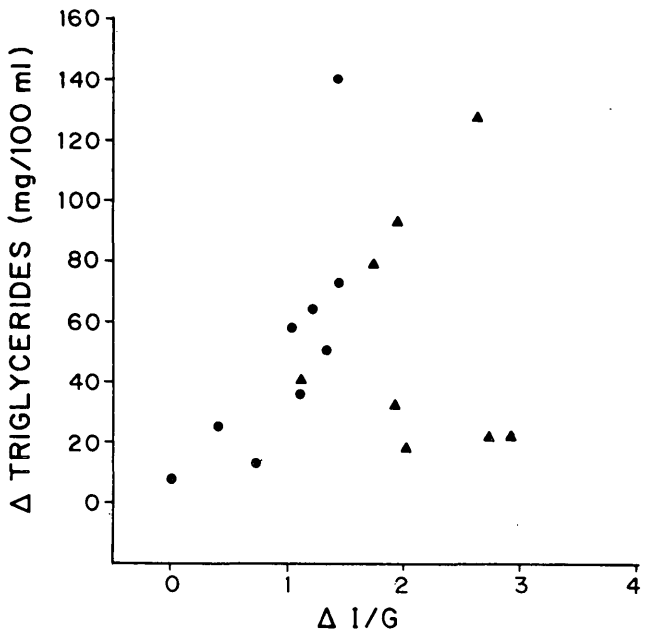

FIG. 3. Difference in molar I/Gs on the seventh day of a high and low carbohydrate diet plotted as a function of the differences in plasma triglycerides on those days. • represents the nine subjects on the 390-gm. diet ($r=0.73$; $p<0.01$) and ▲ the seven subjects on the 510-gm. diet ($r=0.02$). If one were to omit from the calculation all patients whose triglyceride levels failed to rise by at least 40 mg. per deciliter on the high carbohydrate diet, $r=0.82$ for the entire group ($p<0.01$).

12-gm. and 510-gm. diets were not significantly correlated in this group ($r=0.02$). In four of these subjects plasma triglycerides after seven days of the 510-gm. carbohydrate diet were less than 40 mg. per deciliter above the level observed after a week of the 12-gm. carbohydrate diet; if one omits from the calculation these four patients in whom the high carbohydrate intake did not seem to increase plasma triglycerides, possibly because of poor adherence to the prescribed diet, a high degree of correlation is observed in the remaining four ($r=0.995$, $p<0.01$). Thus, if one considers only those patients in whom the high carbohydrate diets induced a rise in serum triglycerides of at least 40 mg. per deciliter, a not unreasonable maneuver in a study of carbohydrate inducible triglyceride increments, a correlation coefficient of 0.82 is obtained ($p<0.01$).

Effect of Antecedent Carbohydrate Intake on the Plasma Glucagon, Insulin, Glucagon, and Triglyceride Response to a Gelatin Meal

To examine the influence of antecedent carbohydrate intake upon the alpha and beta cell response to a protein meal, the effects of a gelatin meal taken after seven days on the 12-gm. per day carbohydrate diet were compared in seven subjects with the same meal given after seven days on the 510-gm. per day diet. The results are shown in figure 4.

After carbohydrate restriction the gelatin meal was followed by a rise in glucose from a fasting level of 76 ± 2 to 84 ± 3 mg. per 100 ml. at ninety minutes ($p<0.05$) and 82 ± 2 mg. per 100 ml. at 120 minutes ($p<0.025$), while after the high carbohydrate diet no significant protein-induced change in glucose was observed. After the low carbohydrate diet insulin rose to a peak of only 16 ± 2 μU. per milliliter ($p<0.005$), in contrast to a peak of 28 ± 4 μU. per milliliter ($p<0.001$) after the high carbohydrate diet, a significant difference ($p<0.01$). After carbohydrate restriction, glucagon rose to 254 ± 65 pg. per milliliter ($p<0.02$) at 150 minutes after the gelatin, but to only 147 ± 25 after the high carbohydrate diet ($p<0.05$). The I/G before and during the gelatin meal was significantly lower at every point after the low carbohydrate diet than after the high carbohydrate regimen.

The ingestion of gelatin did not change the triglyceride concentration in either circumstance, although after the high carbohydrate regimen triglycerides were significantly higher at every point.

DISCUSSION

Plasma insulin levels have previously been reported

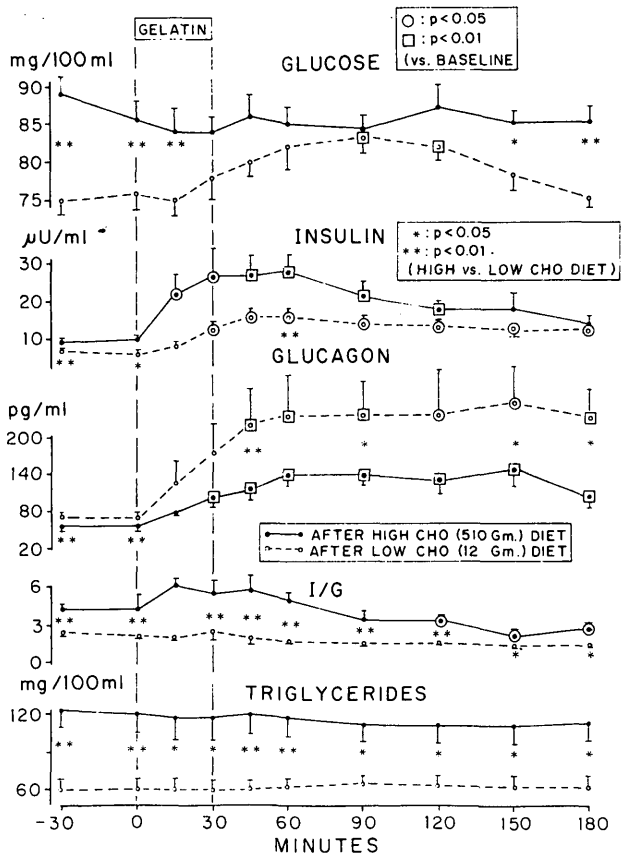

FIG. 4. Comparison of the effects of a gelatin meal on the mean glucose, insulin, glucagon, molar I/G, and triglyceride levels of subjects after one week of a 12-gm. per day or a 510-gm. per day carbohydrate diet (N=7).

to be increased in hypertriglyceridemia.[11-17] The results described in this communication indicate that ingested glucose, like infused glucose, increases the insulin response and reduces the glucagon response to a protein meal, and thus causes a major increase in I/G. These hormonal responses persist throughout the entire 180 minutes of observation and, on the basis of previous studies, probably continue for at least 240 minutes.[5] Hence, if one eats three meals per day, the levels of insulin and glucagon between breakfast and four hours after the evening meal, or about sixteen hours per day, may well be determined primarily by the amount and composition of each meal. A high carbohydrate intake at each of the three meals would thus be expected to maintain a high I/G ratio throughout most of the day. On the basis of the presumed actions of insulin[11-17] and glucagon[18] upon hepatic VLDL synthesis, one would expect that a high I/G would be accompanied by a higher rate of VLDL secretion than would be the case at low I/G levels resulting from carbohydrate restriction. Indeed, Eaton and Kipnis have suggested previously that glucagon suppression by carbohydrate may mediate carbohydrate induction of hyperlipemia.[17]

In our study, the effects of carbohydrate deprivation upon both the hormone concentrations and triglyceride levels are apparent within forty-eight hours; as expected, the low-insulin-high-glucagon pattern was accompanied by a decline in fasting triglyceride levels. Within two days after the addition of a high amount of carbohydrate to the diet, a high-insulin-low-glucagon pattern had appeared and plasma triglyceride levels had risen. Despite certain impressive statistical correlations between changes in fasting I/G and changes in fasting triglycerides, the rise in I/G in individual subjects was not always associated with a commensurate increase in plasma triglycerides, and vice versa. Thus, even if the hormones play a major role in the mediation of carbohydrate-induced hyperlipemia, they are not the only important determinants. On the other hand, the design of these studies is not ideally suited to establish or exclude the influence of the hormones in the control of endogenous triglyceridemia. A single daily insulin and glucagon determination may not be an adequate reflection of the hormone levels throughout the day; nor do peripheral I/G ratios necessarily mirror the I/G ratios in the portal circulation. The lack of a consistent chronologic relationship between changes in I/G and triglycerides in each subject during the first days of the regimens (table 1) does not, therefore, exclude a mediating role.

Nor does the design of this study, in which the order of high and low carbohydrate regimens was randomized, allow a satisfactory assessment of the influence of weight change on the results, since the baseline dietary conditions prior to institution of a given dietary regimen were not uniform.

The effect of antecedent carbohydrate intake upon the insulin and glucagon responses to a protein meal was clear-cut. In confirmation of earlier studies, insulin rose less and glucagon more after a week of carbohydrate restriction than after a week of carbohydrate abundance. The low I/G in the former instance was accompanied by a rise in glucose averaging 8 mg. per deciliter during the gelatin meal, suggesting that a larger share of the ingested amino acids may have entered gluconeogenic pathways at the expense of protein synthesis; the triglyceride levels were uninfluenced by the contrasting bihormonal responses to the protein. The mechanisms by which antecedent intake of carbohydrate is "remembered" by islet cells remains to be determined.

ACKNOWLEDGMENT

The work was supported by NIH Grant AM 02700-15; Pfizer Laboratories, New York, N.Y.; Bristol Myers Company, New York; Mead Johnson Center, Evansville, Indiana; Rabbit 30K Fund; Dr. Karl Thomae GmbH, Germany; Hoffman-LaRoche, Inc., Nutley, New Jersey; Hoechst Pharmaceutical Company, Somerville, New Jersey; and NIH Contract No. 71-2156.

REFERENCES

[1]Lacy, P.E., Young, P.A., and Fink, E.J.: Studies on insulin secretion in vitro from isolated islets of the rat pancreas. Endocrinology *83*:1155-61, 1968.

[2]Curry, D.L., Bennett, L.L., and Grodsky, G.M.: Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology *83*:572-84, 1968.

[3]Unger, R.H., Eisentraut, A.M., McCall, M.S., et al.: Measurement of endogenous glucagon in plasma and the influence of blood glucose concentrations upon its secretion. J. Clin, Invest. *41*:682-89, 1962.

[4]Vance, J.E., Buchanan, K.D., Challoner, D.R., et al.: Effect of glucose concentration on insulin and glucagon release from isolated islets of Langerhans of the rat. Diabetes *17*:187-93, 1968.

[5]Müller, W.A., Faloona, G.R., Aguilar-Parada, E., et al.: Abnormal alpha-cell function in diabetes: Response to carbohydrate and protein ingestion. N. Engl. J. Med. *283*:109-15, 1970.

[6]Dobbs, R., Faloona, G.R., and Unger, R.H.: The effect of intravenously administered glucose on glucagon and insulin secretion during fat absorption. Metabolism *24*:69-75, 1975.

[7]Müller, W.A., Faloona, G.R., and Unger, R.H.: The effect of alanine on glucagon secretion. J. Clin. Invest. *50*:2215-18, 1971.

[8]Rabinowitz, D., Merimee, T.J., Maffezzoli, R., et al.: Patterns of hormonal release after glucose, protein and glucose plus protein. Lancet. *2*:454-56, 1966.

[9]Müller, W.A., Faloona, G.R., and Unger, R.H.: The influence of the antecedent diet upon glucagon and insulin secretion. N. Engl. J. Med. *285*:1450-1454, 1971.

[10]Ahrens, E.H. Jr., Hirsch, J., Oette, K., et al.: Carbohydrate induced and fat induced lipemia. Trans. Assoc. Am. Physicians *74*:134-46, 1961.

[11]Reaven, G.M., Lerner, R.L., Stern, M.P., et al.: Role of insulin in endogenous hypertriglyceridemia. J. Clin. Invest. *46*:1756-67, 1967.

[12]Sailer, S., Bolzano, K., Sandhoer, F., Spath, P., and Braunsteiner, H.: Triglyceridspiegel und Insulinkonzentration in Plasma nach oraler Glukosegabe bei Patienten mit primarer kohlenhydratinduzierter Hypertriglyceridamie. Schweiz. Med. Wochenschr. *98*:1512-18, 1968.

[13]Kuo, P.T., and Feng, L.Y.: Study of serum insulin in atherosclerotic patients with endogenous hypertriglyceridemia (types III and IV hyperlipoproteinemia). Metabolism *19*:372-80, 1970.

[14]Bagdade, J.D., Bierman, E.L., and Porte, D., Jr.: Influence of obesity on the relationship between insulin and triglyceride levels in endogenous hypertriglyceridemia. Diabetes *20*:664-72, 1971.

[15]Glueck, C.L., Levy, R.I., and Fredrickson, D.S.: Immunoreactive insulin, glucose tolerance, and carbohydrate inducibility in types II, III, IV and V hyperlipoproteinemia. Diabetes *18*:739-47, 1969.

[16]Ford, S. Jr., Bozian, R.C., and Knowles, H.C., Jr.: Interactions of obesity, and glucose and insulin levels in hypertriglyceridemia. Am. J. Clin. Nutrition *21*:904-10, 1968.

[17]Eaton, R.P., and Nye, H.R.: The relationship between insulin secretion and triglyceride concentration in endogenous lipemia. J. Lab. Clin. Med. *81*:682-95, 1973.

[18]Heimberg, M., Weinstein, I., and Kobout, M.: The effects of glucagon dibutyryl cyclic adenosine 3′, 5′-monophosphate and concentration of free fatty acids on hepatic lipid metabolism. J. Biol. Chem. *244*:5131-39, 1969.

[19]Eaton, R.P. and Kipnis, D.M.: Effect of glucose feeding on lipoprotein synthesis in the rat. Am. J. Physiol. *217*:1153-59, 1969.

[20]Turner, D.: Handbook of Diet Therapy, 4th ed. University of Chicago Press, 1965.

[21]Kessler, G., and Lederer, H.: *In* Automation in Analytic Chemistry. Technicon Symposia 1965, Vol. 1: Lot, Skeggs, et al. Eds. Mediad Press, New York; p. 341.

[22]Herbert, V., Lau, K., Gottlieb, C.W., et al.: Coated charcoal immunoassay of insulin Endocrinol. Metab. *25*:1375-84, 1965.

[23]Yalow, R.S. and Berson, S.A.: Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. *39*:1157-1175, 1960.

[24]Faloona, G.R. and Unger, R.H.: Glucagon. *In* Methods of Hormone Radioimmunoassay. New York, Academic Press, 1974, pp. 316-30.

[25]Aguilar-Parada, E., Eisentraut, A.M., and Unger, R.H.: Pancreatic glucagon secretion in normal and diabetic subjects. Am. J. Med. Sci. *257*:415-19, 1969.