Relationship between Meal Size and Frequency and Plasma Insulin Response in Man

Clarence Cohn, M.D., Sheldon Berger, M.D., and Margaret Norton, M.S., Chicago

SUMMARY

The effect of varying the size of a meal on plasma insulin was determined in eight normal male adults. The effect of consuming one half of a meal at 0 time and one half at two hours later was also studied. Each subject was studied on four days, one day when he consumed a whole meal (580 calories) of natural foodstuffs; one day, one-half the meal; one day, one quarter of the meal, and one day when he consumed one half the meal at 0 time and the remainder two hours later. When fractions of the meals were ingested, all ingredients were decreased proportionately. As meal size was increased, a log-dose relationship was observed to characterize the comparison of meal size ingested to plasma insulin response. When on the fourth day the subjects consumed the meal in fractions, the mean sum of the plasma insulin responses was identical to that observed when the meal was eaten at one time. The results suggest that over a four-hour period, the plasma insulin response is a function of the magnitude of the load of calories requiring disposition and that the response does not appear to be influenced by the frequency with which the calories are ingested. DIABETES 17:72-75, February, 1968.

The metabolic consequences of a given diet depend, in part, on the frequency with which meals are eaten.[1,2] A decrease in the periodicity with which a day's allotment of nutrients is ingested is associated in animals not only with alterations in body composition (a decrease in body protein and water and an increase in lipid) but also in the severity of experimental atherosclerosis and regulation of diabetes mellitus.[1,3] These changes appear to be secondary to enzymatic adaptations to the load of foodstuffs requiring disposition per unit of time.

Previous studies of the contributions of the pituitary, thyroid, adrenal and gonadal hormones to these phenomena have suggested that their roles, if any, are only permissive.[4] Whether insulin, the only known lipogenic hormone, and one whose rate of secretion might be expected to depend upon the size of meals and the frequency with which they are ingested, participates in the observed metabolic changes is unclear. Fabry and coworkers[2] have reported that alternately fed and starved rats exhibited increased amounts of circulating insulin-like activity as measured by a diaphragm bioassay technic. No differences were observed, however, in the carbohydrate metabolism of perfused hearts obtained from rats that had formerly eaten ad libitum or were force fed.[4]

In light of these studies in animals relating metabolic changes to different feeding frequencies, experiments were designed to compare plasma insulin responses to different amounts of a standard meal, as well as to observe the effect of dividing this meal into two identical portions consumed two hours apart.

SUBJECTS AND METHODS

Eight normal male adults, aged eighteen to twenty-four years, served as subjects. After an overnight fast, a subject was given one of the following, each on a different day: a standard 580 calorie meal (26 gm. protein, 75 gm. carbohydrate and 20 gm. fat, in the form of two glasses of skimmed milk, four slices of bread and four pats of butter), one half of the meal (200 calories) one quarter of the meal (145 calories), or one half of the meal at time 0 and the remaining half two hours later. The sequence of consumption of the various meals was randomized. When divided, all components were proportionately decreased. Heparinized blood for estimation of plasma glucose[5] and immunoassayable insulin[6] was collected before the test meals were consumed and at half-hour intervals for four hours, after completion of food ingestion. The plasma was separated immediately and stored at —20° C. until the time of analysis. Insulin responses were calculated as the sum of the individual increments in plasma insulin concentration above the control (0) level and are expressed in microunits per milliliter (μU/ml.).

From the Division of Nutritional Sciences, Michael Reese Hospital and Medical Center, Department of Medicine, Northwestern University Medical School, Chicago, Illinois and the Evanston Hospital Association, Evanston, Illinois.

RESULTS

The average responses of plasma insulin and sugar to the different size meals are given in table 1. The plot of the insulin responses versus meal size conformed to a log-dose relationship and is shown in figure 1. As the quantity of foodstuffs eaten, presumably absorbed and assimilated, was increased, there was an apparent increase in insulin secretion. Although no significant difference between the plasma insulin response to the one-quarter meal and one-half meal was observed ($t = 1.67$, $p = > 0.1$), there was a significant difference between the results of the one-half meal and the whole meal ($t = 2.23$, $p = < 0.05$) and between the one-quarter meal and the whole meal ($t = 4.65$, $p = < .005$). An analysis of variance of the data yielded an F value of 3.86 and a p value of < 0.05 for 21 degrees of freedom.

The data in table 2 indicate that similar mean plasma insulin responses occurred when a 580 calorie meal was consumed at one time or as two equal portions two hours apart. Furthermore, it may be noted (table 1) that the mean plasma insulin levels returned to their control values four hours after the first ingestion of the meal, irrespective of whether it was all consumed at once or in divided portions. Under the latter conditions, plasma insulin concentrations attained fasting levels within two hours after the second portion of food had been eaten (table 2).

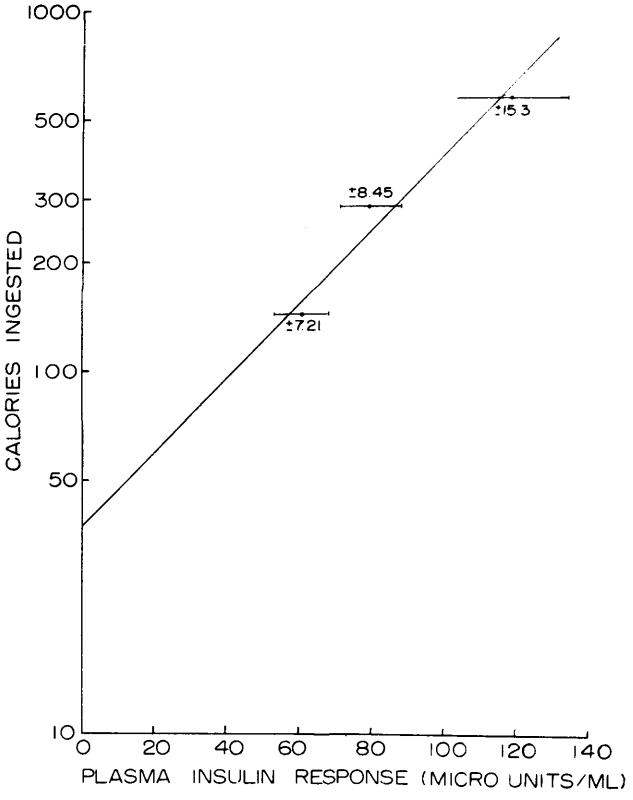

FIG. 1. Comparison of plasma insulin responses to quantity of calories ingested. The whole meal (580 calories) consisted of 26 gm. protein, 75 gm. carbohydrates and 20 gm. fat. When the caloric intake was reduced in amount, all constituents of the meal were decreased proportionately.

DISCUSSION

The findings demonstrate that as the quantity of mixed foodstuffs in a meal is increased, there are concomitant increases in the levels of plasma insulin which are proportional to the logarithm of the number of calories ingested. Since the insulin response conforms to a log-dose relationship, this observation lends additional

TABLE 1

Effect of meal size and feeding frequency on plasma glucose and immunoassayable insulin levels*

Meal size†	¼ of meal		½ of meal		Whole meal		½ meal at time 0 ½ meal at 120 minutes	
Time (minutes) after ingestion of nutrients	Plasma levels of							
	Glucose	Insulin	Glucose	Insulin	Glucose	Insulin	Glucose	Insulin
Fasting	103±7.0	16.3±2.6	98± 6.4	17.3±2.6	101±5.7	19.6±2.5	95±3.7	17.6±2.8
30	114±7.0	43.3±4.1	108± 6.4	43.6±5.8	102±5.8	54.9±3.5	111±5.5	46.8±5.8
60	92±4.7	27.3±2.9	104± 2.0	37.6±2.8	95±4.0	46.0±4.9	91±9.2	38.1±2.5
90	96±5.3	24.5±2.8	100± 5.4	25.5±2.9	98±3.3	37.6±3.9	85±7.6	28.4±2.4
120	89±5.1	20.1±1.9	102±15.0	26.1±4.8	93±2.3	37.6±3.2	94±9.3	27.1±3.9
150	91±5.4	20.8±1.7	97± 8.5	24.9±3.4	97±3.4	29.3±3.6	95±9.3	41.0±5.1
180	95±4.7	19.1±1.3	86± 4.3	21.4±2.7	97±3.9	25.5±3.4	87±4.7	34.8±4.3
210	95±4.7	19.8±1.5	107±15.9	21.1±4.3	100±8.9	26.1±2.9	87±5.9	26.3±3.1
240	95±4.7	16.0±2.2	95± 5.3	17.2±2.0	98±6.6	19.8±3.0	94±9.6	17.4±3.3

*The results are the mean, ± S.E. in mg./100 ml. and μU./ml., respectively.
†The entire meal consisted of 26 gm. protein, 75 gm. carbohydrate and 20 gm. fat. When divided, all components were reduced proportionately.

credence to the validity of the data. No significant difference in plasma insulin response was found between the 145 and 290 calorie meals, but this may be attributed to either the relatively small number of subjects studied, the large standard error of the mean insulin responses, to the relatively small difference between the size (stimulus) of these meals or to a combination of all factors. However, when the data from all three meals were subject to analysis of variance, the distribution of the results was significant to better than 5 per cent.

In contrast to the increase in insulin concentrations, when meal size was increased, no corresponding change was observed in plasma glucose levels (table 2).

When a comparison is made of the insulin response to the whole meal and to the divided meal, the results suggest that the quantity of insulin that is released after the consumption of a meal, under the conditions employed, is independent of the rate at which the food is eaten. A difference in feeding frequency likewise was not associated with any significant modification of the plasma glucose responses.

Previous studies have examined plasma insulin responses to the various foodstuffs utilizing either "pure" foods or artificial combinations of dietary ingredients.[7-10] Hence it is difficult to compare the insulin responses observed in the present study with those reported by others.

The relatively normal amounts and distribution of administered nutrients used in this investigation were associated with rather small changes in insulin and glucose levels. Since insulin levels increased in spite of unchanging glucose levels, the principal stimulus for insulin secretion in the present subjects may not have been glucose. The absence of a significant elevation in plasma glucose concentrations after food is not unusual.[11]

The use of plasma insulin responses to infer changes in the rate of insulin secretion is based on the observations of Yalow et al.[12] that insulin disappears from plasma at a constant rate in vivo, independent of concentration. Hence, increases in plasma insulin responses may be assumed to reflect an increased rate of insulin release into the circulation. Several methods [13,14] were used to estimate plasma insulin responses and all indicated that larger meals evoke a greater response than small ones and that the same number of calories eaten in divided portions results in an insulin secretion similar to that observed after ingestion of the entire amount at once.

TABLE 2

Manner of food ingestion and plasma insulin response*

Subject	Entire meal at time 0	½ meal at time 0 ½ meal at 120 minutes
	Plasma insulin response† (μU./ml.)	
K	175	116
N	130	149
W	82	128
R	158	173
T	77	65
H	161	109
S	104	94
C	62	117
Mean response‡	119	119
S.E.	±15.3	±11.6

*The entire meal consisted of 26 gm. protein, 75 gm. carbohydrate and 20 gm. fat. When divided, all components were divided equally.

†Calculated as the sum of the increments in plasma insulin concentrations above the fasting level.

‡Paired comparison of the values yielded p value of 0.03.

ACKNOWLEDGMENT

This research was supported in part by the following grants from the National Institutes of Health: AM-00193, AM-05686 and HE-05528, and by a grant from the Thomas J. Lipton Foundation.

We wish to thank Mrs. Betty Rutschmann, Mrs. Louisea Bell and Mrs. Dorothy Joseph for technical assistance.

REFERENCES

1 Cohn, C.: Meal-eating, nibbling and body metabolism. J. Amer. Diet. Assoc. *38*:433-36, 1961.

2 Fabry, P., Petrasek, R., Kujalova, E., and Holeckova, E.: Adaptace na zmeney prijen potravy. Statni zdravotnicke nakladatelstvi. Prague, 1962.

3 Cohn, C.: Feeding patterns and some aspects of cholesterol metabolism. Fed. Proc. *23*:76-81, 1964.

4 Cohn, C.: Neuroendocrine aspects of feeding behaviour and obesity. Assoc. for Research in Nervous and Mental Disease. Baltimore, Williams & Wilkens, Vol. 43, 1966, p. 212.

5 Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. *153*: 375-80, 1944.

6 Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: two antibody system. Diabetes *12*:115-26, 1963.

7 Berger, S., and Vongaraya, N.: Insulin response to ingested protein in diabetes. Diabetes *15*:303-06, 1966.

8 Floyd, J. C., Jr., Fajans, S. S., Conn, J. W., Knopf, R. F., and Rull, J.: Insulin secretion in response to protein ingestion. J. Clin. Invest. *45*:1479-1502, 1966.

9 Rabinowitz, D., Merimee, T. J., Maffezzoli, R., and Burgess, J. A.: Patterns of hormonal release after glucose, protein, and glucose plus protein. Lancet *2*:454-57, 1966.

10 Estrich, D., Ravnik, A., Schlierf, G., Fukayama, G., and Kinsell, L.: Effects of co-ingestion of fat and protein upon carbohydrate-induced hyperglycemia. Diabetes *16*:232-37, 1967.

[11] Sindoni, A., Jr.: Fasting blood sugar vs. post prandial blood sugar as observed in normal individuals, medical (non-diabetic) patients and patients with diabetes. Amer. J. Dig. Dis. *13*:178-92, 1946.

[12] Yalow, R. S., Glick, S. M., Roth, J., and Berson, S. A.: Plasma insulin and growth hormone levels in obesity and diabetes. Ann. N.Y. Acad. Sci. *131*:357-73, 1965.

[13] Perley, M., and Kipnis, D.: Insulin secretion and biological effectiveness of endogenous hormone in normal, obese-diabetic and non-obese diabetic subjects (abstract). Clin. Res. *13*:331, 1965.

[14] Farquhar, J., Frank, A., Gross, R. C., and Reaven, G. M.: Glucose, insulin and triglyceride responses to high and low carbohydrate diets in man. J. Clin. Invest. *45*:1648-56, 1966.

Bile Salts Inhibition of Cholesterol Synthesis

Direct evidence that certain bile salts may be involved in regulation of cholesterol synthesis has now been reported (G. M. Fimognari and V. W. Rodwell, Science *147*:1038, 1965). In the course of attempts to increase the solubility of mevalonic acid oxidoreductase from the particulate fraction of rat liver these workers observed inhibition of mevalonate synthesis from acetate upon treatment of the homogenates with bile salts. Their interest in this reaction stems from the likelihood that this step is the site of inhibition in cholesterol fed animals, as discussed above.

They measured the rate of incorporation of C^{14}-acetate into mevalonic acid as catalyzed by a rat liver homogenate preparation. Cholesterol at a level of 2.5 mMolar inhibited from 0 to 15 per cent, whereas cholate at this concentration caused a 95 per cent inhibition. Cholate at a level of 0.25 mMolar still inhibited 38 per cent. Deoxycholate at this lower concentration resulted in a 97 per cent reduction in rate. Taurocholate was about as effective as cholate, and taurodeoxycholate inhibited to about the same degree as deoxycholate.

Fimognari and Rodwell considered the possibility that the inhibition might be due to a detergent-like effect of disrupting the particulate enzyme involved in catalyzing the conversion of acetate to mevalonate. Triton X-100 at a level of 40 μg. per ml. failed to inhibit, and this would argue against the possibility that the effects of the bile salts were nonspecific. Even stronger evidence against this view is the finding that cholate and deoxycholate competitively inhibited a soluble mevalonic acid oxidoreductase of bacterial origin.

These experiments, coupled with the other evidence presented above, would seem to suggest that at least one of the physiologic regulators of cholesterol synthesis is the bile salts. With this possibility in mind it might be hoped, and indeed anticipated, that a clearer understanding of normal biosynthetic routes and regulatory devices should lead to at least some new insight into the still mysterious relationship between arteriosclerosis and cholesterol.

From *Nutrition* Reviews, Vol. 23, No. 6, June 1965, p. 188