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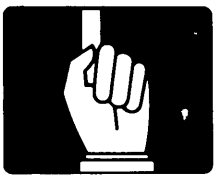

Factors Affecting Interpretation of Postprandial Glucose and Insulin Areas

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Recently there has been an increased interest in determining the circulating glucose concentration after the ingestion of various individual foods and mixed meals. The purpose of these determinations is to systematically rank foods with respect to their quantitative effect on postmeal glucose concentration. Potentially such data could be useful in designing a diet for individuals with diabetes. We believe this concept is good. However, several factors that may affect interpretation of the data used to develop this ranking need to be considered before the utility of this approach to dietary management can be assessed: *1*) duration of time over which the data are collected and analyzed; *2*) use of absolute versus incremental areas in the determinations; *3*) inclusion or exclusion of negative areas if incremental areas are used; *4*) differences in response to a given food in males compared with females; *5*) severity of diabetes; *6*) confounding effects of oral agents or insulin treatment; *7*) reproducibility of data; *8*) differences in collection of blood sample; *9*) food composition, processing, and preparation; *10*) the dose-response relationship to ingestion of a given carbohydrate; *11*) the meal being studied, i.e., first, second, or third meal of the day; and *12*) a possible effect of the composition of the previous meal, if the response is tested to any meal other than the first meal of the day. *Diabetes Care* 10:759–63, 1987

For the past several years there has been an interest in determining the circulating glucose concentrations in peripheral blood after the ingestion of individual foods (1–5). These studies have been conducted in normal and diabetic subjects. The data obtained from such studies have been analyzed by quantitating the glucose response to a known amount of carbohydrate in a particular food. A common way of calculating this has been to determine a glycemic index. The glycemic index has been defined by Jenkins et al. (3) as the area under the glucose curve after a 50-g carbohydrate test meal divided by the area under the glucose curve after a meal consisting of a standard reference food containing 50 g carbohydrate, multiplied by 100. Originally, blood glucose area was determined over a 2-h period, and 50 g glucose was used as the standard reference meal (3). Recently, blood glucose determinations of diabetic subjects have been performed over a 3-h period, and the standard reference meal has been 50 g carbohydrate in the form of bread (6). Such data have been used to assign a relative ordering of plasma glucose responses to various carbohydrate-containing foods. The ultimate purpose of this ordering is to assist the diabetic individual in meal planning. Theoretically, ingestion of foods of low glycemic index could be useful in the dietary management of diabetes.

Recently, the validity and clinical utility of this index has been called into question. When foods of varying glycemic index were incorporated into a mixed meal, the glycemic index was not useful in predicting the glucose response (7–10). However, this has not been a universal finding (11–14). In our laboratory, we have been interested in quantitating both the insulin and glucose responses to foods as accurately as possible. During these studies, we became aware of a potential source of error in determining the relative glucose area (RGA) after the ingestion of various foods. This potential source of error is the time duration over which the plasma glucose area is determined after a meal. Subsequent review of other studies has indicated several potentially confounding variables that should be considered in interpretation of glycemic index data.

In this report, we use the term RGA instead of *glycemic index*, because the time over which relative areas are determined is variable. RGA is defined as the net glucose area after the ingestion of a test meal, divided by the net glucose area after the ingestion of 50 g glucose, multiplied by 100.

This may be determined at any time point after the ingestion of a single food or a mixed meal. The fasting glucose concentration is used as a baseline. The glycemic index was originally based on 2 h of data with glucose as the reference food (3) and now is based on 2 h of data in normal subjects or 3 h of data in diabetic subjects with bread as the reference food (15).

When we began our studies of glucose and insulin responses to food ingestion, we determined the time required for peripheral plasma glucose and peripheral serum insulin concentrations to return to premeal values after the ingestion of 50 g glucose. We observed that the time required for the glucose concentrations to return to the fasting baseline concentration was ~1.5 h in normal individuals (16). Insulin concentrations returned to baseline at ~3 h. In untreated mildly non-insulin-dependent diabetic (NIDDM) male subjects, ~4–5 h was required for plasma glucose to return to the fasting concentration (17,18). Insulin concentrations were still modestly elevated 5 h after ingestion of 50 g glucose in these subjects. However, we did not consider it practical to continue the studies for a longer period.

Knowing that the rate of digestion and absorption of foods may be different, it was necessary to arbitrarily choose a time frame during which to quantitate the glucose and insulin responses to various other foods. We chose to collect and analyze data for 5 h after ingestion of the test foods in the diabetic patients, because 5 h generally was required for the glucose concentration to return to the fasting level after 50 g glucose, the standard reference meal in our study. Five hours appears to be the longest period monitored in any published study. We decided it would be useful to determine the effect on RGA when the data were quantitated over various periods up to 5 h.

It was apparent that, at least for some foods, RGA was not constant with time (Fig. 1), and the differences in RGA often were quite large. For example, after the ingestion of sucrose, the RGAs at 1, 3, and 5 h were 116, 67, and 44, respectively (Fig. 1A); i.e., when the glucose area after the ingestion of sucrose was determined at 1 h and compared with the glucose area after the ingestion of glucose at 1 h, the postsucrose area was 116% that of glucose. Decreases in RGA with time also were observed after fructose and milk ingestion (Fig. 1A), and similar results were obtained when the data for apples, apple juice, and orange juice were analyzed (data not shown). Thus, sucrose-, fructose-, and lactose-containing foods generally resulted in a decrease in RGA

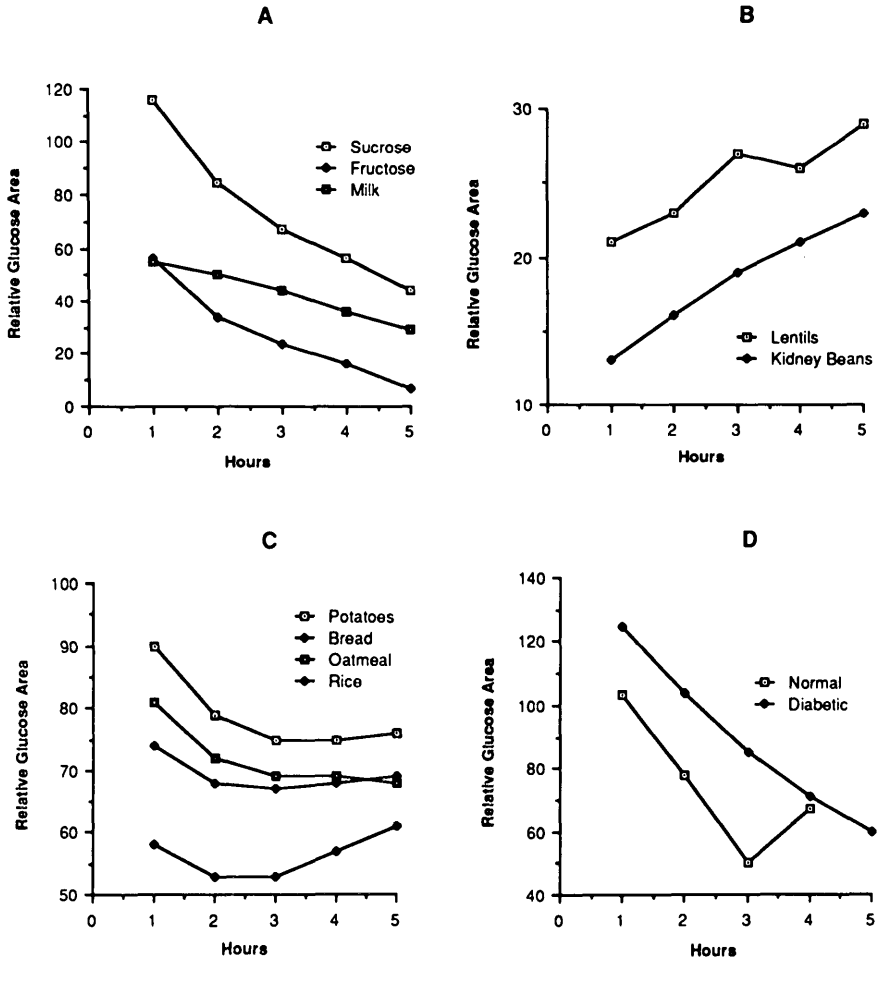

FIG. 1. Relative glucose area (RGA) after ingestion of various foods. *A*: decreasing RGA with time after ingestion of 50 g CHO in form of sucrose, fructose, or milk in untreated non-insulin-dependent diabetic (NIDDM) male subjects (n = 7) (17). *B*: increasing RGA with time after ingestion of 50 g CHO in form of lentils or kidney beans in untreated NIDDM male subjects (n = 7) (18). C: little change in RGA with time after ingestion of 50 g CHO in form of potatoes, bread, oatmeal, or rice in untreated NIDDM male subjects (n = 7) (18). *D*: decreasing RGA with time after ingestion of glucose plus protein meal. Normal subjects (n = 8) ingested 50 g glucose plus 50 g protein (lean beef) (16); diabetic subjects (n = 6) ingested 50 g glucose plus 25 g protein (cottage cheese) (35).

with time. On the other hand, with slowly digested foods such as legumes and kidney beans, RGA increased with time (Fig. 1B; 18).

Ingestion of some foods resulted in little change in the RGA with time, because the peak response and time required for plasma glucose to return to the fasting concentration was similar to that after ingestion of pure glucose (Fig. 1C; 18). Protein ingestion does not increase plasma glucose concentration but stimulates insulin secretion (19,20). Thus, the additional insulin secreted in response to protein when given with glucose could influence RGA. Indeed, this was the case. In both normal and NIDDM individuals, RGA after ingestion of glucose and protein decreased with time (Fig. 1*D*). This primarily was due to a more rapid return of the glucose concentration to baseline when 50 g protein in the form of lean beef was ingested with 50 g glucose (16). Thus, the protein in a mixed meal is likely to influence RGA, and the effect would be more prominent at later time points.

We and others have demonstrated little effect of dietary fat on the plasma glucose response after the first meal of the day in normal subjects (21) and insulin-dependent diabetic patients (22). However, the 1-h postprandial glucose area was significantly less after the ingestion of 50 g fat given with 50 g carbohydrate compared with 50 g carbohydrate alone in normal subjects (23). In NIDDM patients, the presence of fat in the meal modestly decreased the postprandial glucose area as well (22). Furthermore, the presence of a large amount of fat in the meal resulted in a delay of the glucose peak of the second and third meals of the day (21).

Theoretically, all studies should be conducted until RGA remains essentially unchanged with time. However, conducting studies over the time necessary to achieve this may be impractical. A compromise may be to select a time at which the rate of change approaches a minimum. Alternatively, the glucose response to various foods may be studied with the usual duration of time between meals in that population. In our society, meals are generally ingested 4–6 h apart.

Diabetes presents a particular problem because it affects the duration of time over which the postmeal glucose concentration remains elevated, even though it does not significantly influence the rate of digestion or absorption of foods. Both the duration and magnitude of elevation may be influenced by the severity of the insulin insufficiency. Theoretically, the severity of diabetes also could affect the metabolism of fructose and galactose. Therefore, the response to various foods should be standardized in the study population. In addition, it may be advisable to test the effect of ingestion of pure monosaccharides and foods containing those monosaccharides on the study population (17). In diabetic patients receiving insulin or oral hypoglycemic agents, the effect of the medication may vary from day to day (24), which also could complicate interpretation of the data.

Other factors should be considered in interpreting RGA data. The area under the glucose curve has been determined with the absolute values obtained for plasma glucose (2,25) or by determining the incremental glucose area above the fasting concentration (3,6,11–13,15–18). As pointed out by Wolever and Jenkins (11), any differences between responses to foods will appear smaller if the absolute areas are determined. In addition, differences in fasting glucose values may strongly influence absolute areas but have little effect on incremental area unless the glucose exceeds the renal threshold for glucose excretion. An elevated glucose concentration should stimulate more non-insulin-dependent glucose uptake by muscle (26). Theoretically, this could affect RGA. Whether this is quantitatively important has not been determined, but it probably is not of major importance except in poorly controlled diabetic subjects.

When incremental areas are used, inclusion or exclusion of negative values also may have a considerable effect on RGA of a particular food. With incremental areas, we have observed values below baseline on several occasions, particularly after foods containing fructose or milk. Thus, the method of analysis should be clearly stated.

When the blood glucose response is determined after the ingestion of oral glucose loads given ~4 h apart to normal subjects, the response to the second meal is smaller than to the first. When glucose loads are given in succession, the facilitated disposal of the second and subsequent glucose loads is the so-called Staub-Traugott effect (27–29). Thus, the time of day and the meal sequence may influence the response. In most studies the subjects are given the test meal for breakfast. However, in one study a mixed meal was given for lunch, and the composition of the breakfast was not stated (2). Amplification of a glucose second-meal effect also may occur in normal subjects and NIDDM patients when protein is included in the meal (30).

The glycemic index for single foods has been determined based on an amount of food that contains a defined quantity of carbohydrate, usually 50 g. Whether the glucose response to ingestion of a particular food is linearly related to the amount of food ingested (and thus the quantity of carbo-

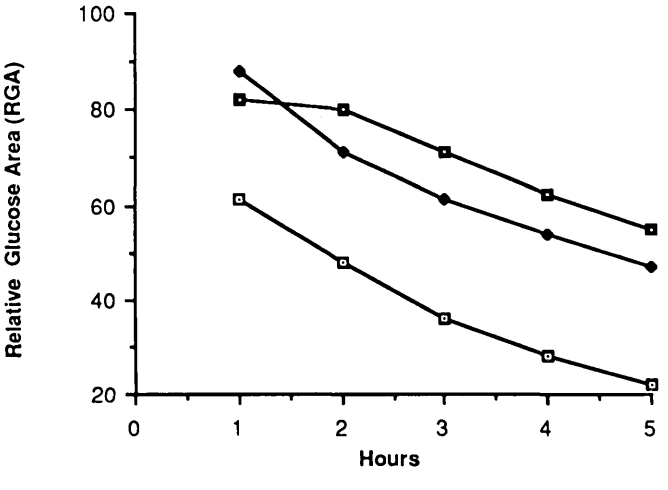

FIG. 2. Relative glucose area after ingestion of various amounts of glucose in untreated non-insulin-dependent diabetic male subjects (n = 7). ▫, 15 g glucose; ♦, 25 g glucose; ▪, 35 g glucose.

hydrate) is another important consideration. This is particularly relevant when applying the glycemic index to mixed meals in which the amount of a food ingested is less than that used to determine the glycemic index. As indicated in Fig. 2, RGA is not constant with time when 15, 25, and 35 g glucose were ingested by untreated NIDDM patients. In addition, the slope of RGA with time was not constant from one dose to another, indicating that as a greater quantity of carbohydrate is ingested, plasma glucose concentration tends to remain elevated longer. These confounding factors should be considered when comparing data from various laboratories and mixed-meal responses with those anticipated from the glycemic index of various carbohydrate-containing foods in the mixed meal.

The variability of an individual's response to replicate testing also must be considered. We have very little information on this subject, but our available data from NIDDM patients indicate that the standard error can be quite large. We have no information on replicate testing of other foods. Others reported that when five normal subjects ingested two rice meals ~2 yr apart, glucose areas differed by 0–23% (15).

Use of capillary versus venous blood sampling also may result in problems in comparing data. Capillary blood is used directly for glucose determination with enzyme-impregnated plastic strips. Venous blood is centrifuged, and the serum or plasma is used for enzymatic glucose determination. Both assay methods are specific for glucose, but the absolute glucose concentration measured may differ. Although glucose is freely diffusible between plasma and erythrocytes and the glucose concentration is the same in both plasma water and erythrocyte water, the water content of the erythrocyte is ~72%, whereas that of plasma is ~94% (31). Thus, the true glucose concentration in whole blood is a function of the hematocrit. For 100 mg/dl plasma glucose, whole-blood glucose would be 89 mg/dl based on a hematocrit of 45; i.e., plasma would be higher by 11%. On the other hand, the glucose concentration may be lower in venous blood drawn from an antecubital vein compared with capillary blood from a finger because of removal of glucose by skin and skeletal muscle in the forearm.

We have determined another potential problem in interpreting the glucose response to foods. The glucose response to a mixed meal was different in normal men and women (21). In women the plasma glucose was transiently elevated for 1 h after breakfast. Over the subsequent 3 h, glucose concentration was below the fasting baseline. This biphasic response was not present in normal men. Consequently, 4-h glucose area after a mixed meal was -10 mg · h · dl^{-1} in women but $+70$ mg · h · dl^{-1} in men. Whether this sex difference exists in diabetic individuals has not been determined. Also, we are not aware of studies designed to determine a potential sex difference in the glucose response to individual foods.

For starch-containing foods, food processing and preparation may affect RGA. For example, if starch is extracted from beans, it is rapidly digested, whereas in ordinary cooked beans, digestion is slow and incomplete (32). When 50–75 g of raw starch was administered by mouth to normal subjects, no change in blood glucose was observed. However, when cooked starch was administered, blood glucose clearly increased (33). Starch exists in plants in the form of granules, the structure of which is characteristic for each plant species. Before starch can be hydrolyzed in vivo, the granule must be disrupted. This generally is done by heating in water, which causes the granules to swell, resulting in disruption of the structure. However, the ease with which disruption is accomplished varies considerably. Generally, starches with a high amylose-to-amylopectin ratio are more rigid and resistant to disruption than are starches with a low amylose-to-amylopectin ratio. For example, ingestion of a 50-g carbohydrate meal of muffins made from low-amylose cornstarch results in an increase in plasma glucose that is even greater than with 50 g pure glucose (117%), whereas ingestion of a 50-g carbohydrate meal of muffins made from high-amylose cornstarch was only 62% of that obtained with 50 g pure glucose (19). The only difference in the two meals was the type of cornstarch used to make the muffins. This clearly demonstrates that starch structure has effects on the postmeal plasma glucose response. In fruits such as bananas, the degree of ripeness may be a significant factor in the response (34).

In summary, there are numerous factors that may influence RGA and thus the interpretation of glycemic-index data. The concept of systematically ranking foods with respect to the quantitative effect on blood glucose is good and should prove useful in designing a diet for diabetic individuals and in educating patients. However, problems that may affect interpretation of such data must be recognized. These fall into three broad categories: the method of determining the glucose area, the patient population and method of sample collection, and the foods tested. From the number of factors identified that may influence the interpretation of postprandial glucose and insulin areas, additional, carefully designed research is necessary before single-food RGA data may be logically applied to meal planning for individuals with diabetes.

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