Physiological Modulation of Plasma Free Fatty Acid Concentrations by Diet

Metabolic implications in nondiabetic subjects

THOMAS M.S. WOLEVER, MD, PHD
ANITA BENTUM-WILLIAMS, MSC
DAVID J.A. JENKINS, MD, PHD

OBJECTIVE — To determine the effect of varying the amount of carbohydrate and glycemic index (GI) of breakfast test meals on plasma free fatty acid (FFA) responses of nondiabetic subjects and to see whether the glycemic response at lunch was related to the plasma FFA response to breakfast.

RESEARCH DESIGN AND METHODS — We studied eight subjects over a 6-h period on four separate occasions using a randomized Latin-square design. They received isocaloric breakfast test meals that were either high (84 g) or low (41 g) in carbohydrate and had either a high (~100) or a low (~70) GI, followed by a standard lunch 4 h later.

RESULTS — The initial fall in plasma FFAs after breakfast was similar for all four test meals, but the extent of rebound differed significantly. The mean plasma FFA concentration just before the start of lunch (4 h) was highest after the low-GI, low-carbohydrate breakfast (418 ± 42 μmol/l), followed by high-GI, low-carbohydrate (277 ± 48 μmol/l), high-GI, high-carbohydrate (227 ± 32 μmol/l), and low-GI, high-carbohydrate (149 ± 23 μmol/l) ($P < 0.01$). The concentration of plasma FFAs at 4 h was directly related to the total area under the glycemic response curve to lunch ($r = 0.691$, $n = 32$, $P < 0.0001$).

CONCLUSIONS — In nondiabetic subjects, the type and amount of carbohydrate eaten at breakfast influences the plasma glucose, insulin, and FFA responses to breakfast and also affects the glucose, insulin, and FFA responses to a subsequent standard lunch. The glycemic responses after lunch were closely related to the plasma FFA concentration 4 h after breakfast, which we speculate is due to the inhibitory effect of FFAs on insulin action.

From the Department of Nutritional Sciences, Faculty of Medicine, and Clinical Nutrition and Risk Factor Modification Centre and Division of Endocrinology and Metabolism, St. Michael's Hospital, University of Toronto, Ontario, Canada.

Address correspondence and reprint requests to T.M.S. Wolever, MD, Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Received for publication 22 November 1994 and accepted in revised form 16 March 1995.

ANOVA, analysis of variance; FFA, free fatty acid; GI, glycemic index; NIDDM, non-insulin-dependent diabetes mellitus.

There has been much interest in determining how diet influences postprandial plasma glucose and insulin responses (1), which may make an important contribution to overall plasma glucose and insulin concentrations. Increased plasma glucose and insulin may be related, at least in part, to the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM) (2) and atherosclerosis (3), and high plasma glucose level to the microvascular complications of diabetes (4). Reducing postprandial glucose and insulin responses with soluble fiber (1), low–glycemic index (GI) foods (5), increased meal frequency (6), and α-glucosidase inhibitors (7) reduces mean blood glucose and insulin concentrations over the course of the day. Less attention has been paid to dietary factors that affect postprandial plasma free fatty acids (FFAs). Nevertheless, plasma FFA concentrations are raised in NIDDM subjects (8). Raised plasma FFA concentrations, at least in some circumstances, may contribute to insulin resistance (9,10), reduce glucose oxidation (11), and increase hepatic glucose (9,10) and triglyceride production (12,13).

If nondiabetic subjects ingest 50 g oral glucose, plasma FFA concentrations fall to reach a nadir ~2 h after the start of glucose consumption and then rebound above the baseline level by 4 h (14). Previous studies have shown that slowing of glucose absorption by adding soluble fiber (15) or by sipping glucose slowly (14) prevents the rebound of FFAs and is associated with improved carbohydrate tolerance 4 h after the start of glucose consumption. Low-GI starchy foods (16) and high-carbohydrate breakfasts (17) are associated with improved second-meal carbohydrate tolerance, but it is unknown whether this is related to differences in the plasma FFA response to breakfast.

The primary purpose of this study, therefore, was to determine whether the type and amount of carbohydrate eaten at breakfast influences postprandial plasma FFA concentrations. We

Table 1—*Foods in the four different breakfast test meals*

	High-carbohydrate		Low-carbohydrate	
	High-GI	Low-GI	High-GI	Low-GI
White bread (g)	44	40	20	20
Cornflakes (g)	60	—	20	—
Oat cereal (g)	—	83	—	29
Whole milk (g)	—	—	100	100
2% milk (g)	100	100	—	—
Margarine (g)	—	3	13	16
Cheddar cheese (g)	23	10	45	36
Orange juice (g)	100	100	100	100

hypothesized: *1*) that low-GI and high-carbohydrate breakfasts would reduce the plasma FFA rebound before lunch compared with high-GI and low-carbohydrate breakfasts; and 2) that the glycemic response to a standard lunch would be directly related to the plasma FFA concentration at the start of lunch.

RESEARCH DESIGN AND METHODS

— Eight subjects (four men, age 25 ± 1 years, weight 78.0 ± 6.6 kg, body mass index 24.9 ± 0.9 kg/m^2; four women, age 28 ± 1 years, weight 57.7 ± 2.1 kg, body mass index 21.9 ± 1.4 kg/m^2) were studied on four separate occasions separated by at least a 1-week interval, using a randomized Latin-square design. The subjects were instructed to maintain their normal diet throughout the course of the study. The protocol for the study was reviewed and approved by the human subjects review committee of the University of Toronto.

Subjects came to the Clinical Nutrition and Risk Factor Modification Centre at 0730 after a 10- to 12-h overnight fast. An indwelling cannula was inserted into a forearm vein, a fasting blood sample was withdrawn, and the cannula was kept patent by flushing with normal saline. The subjects then ate one of four different isocaloric test breakfasts over a 20-min period and remained seated over the next 4 h, during which time further blood samples were taken 1/2, 1, 1 1/2, 2, 3, and 4 h after the start of breakfast. Immediately after the 4-h blood sample, the subjects ate a standard lunch, the composition of which was exactly the same on each occasion. Further blood samples were taken 1/4, 1/2, 3/4, 1, 1 1/2, and 2 h after the start of the lunch meal.

The compositions of the breakfast test meals are shown in Tables 1 and 2. The four breakfast meals were isocaloric but varied in the amount and type of carbohydrate they contained according to a 2 × 2 Latin-square design. Two of the meals were high in carbohydrate (~84 g; ~66% of energy), and two were low in carbohydrate (~41 g; ~33% of energy) with the reduction in carbohydrate being accomplished by an isocaloric exchange with fat. Two of the meals had a high GI (~100), and two had a low GI (~70), with the difference being due to an exchange of breakfast cereal (high GI, cornflakes, GI = 121; low GI, psyllium oat cereal, GI = 61). Thus, the four breakfast meals tested were high-carbohydrate, high-GI; high-carbohydrate, low-GI; low-carbohydrate, high-GI; and low-carbohydrate, low-GI. A constant amount of milk and orange juice was given with each of the four breakfast meals to keep constant the amount of simple carbohydrate. The GI is defined as the incremental area under the blood glucose response curve for a food expressed in percent of that for an equal carbohydrate portion (50 g of carbohydrate) from white bread tested by the same subjects (18). The GI of cornflakes was taken to be 121, which is the previously published average from four separate studies (18). The low-GI cereal, a psyllium-enriched oat cereal, was developed as a prototype, low-GI breakfast cereal. Based on tests in 13 nondiabetic subjects (4 male and 9 female, only 1 of whom participated in the studies reported here), the incremental area under the glycemic response curve for the oat cereal was significantly less than that of white bread (Fig. 1), with a GI of 61 ± 8.

The standard lunch meal consisted of 90 g white bread, 10 g margarine, 45 g cheddar cheese, 50 g tomato, 14 g raisins, 100 g apple juice, and 30 ml whole milk (575 kcal), 68 g (47% of en-

Table 2—*Composition of breakfast test meals*

	High-carbohydrate		Low-carbohydrate	
	High-GI	Low-GI	High-GI	Low-GI
Energy (kcal)	504	503	501	504
Protein (g)	18.5	20.0	18.9	18.8
Carbohydrate (g)	84.3	83.3	41.1	41.3
Simple sugars (g)	19.2	20.7	15.9	16.6
Fat (g)				
Total	10.6	9.7	30.1	29.9
Saturated	6.1	3.6	14.2	12.5
Monounsaturated	3.4	3.5	10.0	10.3
Polyunsaturated	0.3	2.1	4.8	6.2
GI	102.0	69.7	92.5	70.0

Meal GI was calculated from the GI values of the individual foods in the test meals.

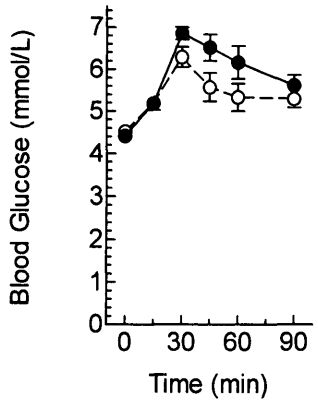

Figure 1—*Capillary blood glucose responses of 50-g carbohydrate portions of white bread (●) and the psyllium oat cereal (○) used in this study in the low-GI breakfasts. The values are means ± SE for 13 nondiabetic subjects.*

ergy) carbohydrate, 26 g (40%) fat, and 20 g (14%) protein. Breakfast and lunch test meals were served with a cup of water, coffee, or tea (plus artificial sweetener and/or 30 ml 2% milk if desired) according to the subject's choice. The drinks chosen by each subject were kept standard for all four tests. A nonhydrogenated, canola oil margarine was used for all breakfast and lunch test meals (Becel, Lipton, Toronto, Canada).

Blood samples were taken into 3-ml sodium fluorocitrate Vacutainers (Becton Dickinson, Rutherford, NJ). After centrifugation at 2,000 rpm for 5 min, plasma was removed and aliquots were frozen at −20°C before analysis. Plasma glucose was measured using an automatic glucose analyzer (model 2300 STAT, YSI, Yellow Springs, OH). Plasma FFA and triglyceride concentrations were measured enzymatically using commercially available kits (FFA: Wako, Osaka, Japan; triglyceride: GPO-Trinder, Sigma, St. Louis, MO]). Plasma insulin was measured by radioimmunoassay using a commercially available kit (Insulin RIA 100, Pharmacia, Dorval, Canada).

Results are expressed as means ± SE. Areas under the curve were calculated geometrically in two ways. The incremental area under the curve, ignoring the area below fasting, was used to indicate the overall rise of plasma glucose or insulin above the baseline for 2 h after the start of the meal. For breakfast, the fasting level was taken to be the baseline; for lunch, the value at 4 h was taken as the baseline. The total area under the curve was used to indicate the average concentration of plasma glucose, insulin, FFAs, and triglycerides after the breakfast and lunch meals. For breakfast, the total area was calculated over the 4 h after the start of the meal (0–4 h), and for lunch the total area was calculated over the 2 h (4–6 h) for which blood was sampled.

Since the experiment was designed as a 2 × 2 Latin square, the data were subjected to analysis of variance (ANOVA), and we examined for the main effects of carbohydrate level and GI and interaction between carbohydrate level and GI. For the main effect of carbohydrate, the means of the two high-carbohydrate treatments (i.e., high and low GI) were compared with the means of the two low-carbohydrate treatments, with the F value from the ANOVA indicating whether the differences were statistically significant. Similarly, for the main effect of GI, the means of the two low-GI treatments (i.e., high- and low-carbohydrate) were compared with the means of the two high-GI treatments. To determine the significance of differences between individual means for the four treatments, the Neuman-Keuls procedure was used to adjust for multiple comparisons. Linear regression analysis was used to determine the relationship between FFA concentration at 4 h and the glycemic response to lunch, expressed as the total area under the curve using the individual values for eight subjects on four test occasions. Relationships between other variables were also determined in the same way.

RESULTS

Responses to breakfast (0–4 h)

The mean fasting levels of plasma glucose were virtually identical before each of the four different breakfast meals. There was a significant main effect of carbohydrate on the total and incremental areas under the plasma glucose curves. The total area under the plasma glucose curve after the two low-carbohydrate meals (mean of high and low GI) was significantly lower than that for the two high-carbohydrate meals (20.0 ± 0.5 vs. 22.2 ± 0.8 mmol · h · l^{-1}, $F = 30.23$, $P < 0.001$; Table 3). Similarly, the incremental area after the two low-carbohydrate meals (mean of high and low GI), 1.1 ± 0.2 mmol · h · l^{-1}, was significantly less than after the two high-carbohydrate meals, 2.9 ± 0.4 mmol · h · l^{-1} ($F = 68.04$, $P < 0.001$; Table 3).

The main effect of GI was significant for the incremental area but not for the total area under the glucose curve. The mean incremental area under the plasma glucose curve after the low-GI meals (mean of high- and low-carbohydrate, 1.7 ± 0.3 mmol · h · l^{-1}) was significantly less than that after the high-GI meals (2.3 ± 0.3 mmol · h · l^{-1}) ($F = 9.41$, $P < 0.05$; Table 3). There was a significant interaction between carbohydrate level and GI. The incremental area under the plasma glucose curve after the high-carbohydrate, low-GI breakfast (2.3 ± 0.3) was 34% less than that after the high-carbohydrate, high-GI breakfast (3.5 ± 0.5, $P < 0.05$; Table 3); but GI had no effect on the glycemic response to the low-carbohydrate breakfasts. Four hours after the start of breakfast, the mean plasma glucose concentration after the low-GI breakfasts (mean of high- and low-carbohydrate, 5.1 ± 0.1 mmol/l) was significantly greater than the mean after the two high-GI breakfasts (4.7 ± 0.1 mmol/l) ($F = 12.73$, $P < 0.01$; Table 3, Fig. 2). At 4 h, plasma glucose was highest after high-carbohydrate, low-GI and lowest after high-carbohydrate, high-GI breakfast (5.2 ± 0.1 vs. 4.6 ± 0.1 mmol/l), with the values for the low-carbohydrate breakfasts being intermediate.

The pattern of plasma insulin responses was similar to that of glucose responses, and the significant main effects

Table 3—*Areas under the curve and plasma concentrations at 4 h for plasma glucose, insulin, FFAs, and triglycerides*

	High-carbohydrate		Low-carbohydrate		*F* values (df)		
	High GI	Low GI	High GI	Low GI	Carb	GI	Carb × GI
Glucose (mmol/l)					(1,7)	(1,7)	(1,21)
B-IAUC	3.5 ± 0.5†‡§	2.3 ± 0.3*‡§	1.1 ± 0.2*†	1.1 ± 0.3*†	68.04‖	9.41¶	7.07¶
B-TAUC	22.0 ± 1.0‡§	22.3 ± 0.6‡§	19.8 ± 7.0*†	20.1 ± 0.5*†	30.23‖	0.48	0.01
B-4 h	4.6 ± 0.1†§	5.2 ± 0.1*‡	4.8 ± 0.1†	5.0 ± 0.1*	0.01	12.73‖	2.18
L-IAUC	2.4 ± 0.4†	1.1 ± 0.2*‡§	2.7 ± 0.5†	3.0 ± 0.4†	11.83¶	1.51	6.28¶
L-TAUC	11.6 ± 0.4§	11.3 ± 0.3‡§	12.2 ± 0.4†§	13.1 ± 0.4*†‡	12.91‖	0.96	3.72
Insulin (pmol/l)							
B-IAUC	511 ± 147‡§	376 ± 77	180 ± 36*	182 ± 41*	10.48¶	1.90	1.22
B-TAUC	786 ± 194‡§	665 ± 110‡§	399 ± 58*†	427 ± 70*†	10.45¶	0.73	1.01
B-4 h	46 ± 9†§	72 ± 9*‡	54 ± 8†	65 ± 13*	0.00	38.84‖	4.09
L-IAUC	308 ± 69§	252 ± 43§	353 ± 63	467 ± 110*†	8.00¶	1.58	4.12
L-TAUC	400 ± 86§	396 ± 57§	461 ± 74§	597 ± 132*†‡	7.88¶	5.77¶	2.37
FFAs (μmol/l)							
B-TAUC	485 ± 49§	555 ± 62§	608 ± 64§	1015 ± 116*†‡	29.96‖	10.41¶	6.39¶
B-4 h	227 ± 32†§	149 ± 25*‡§	277 ± 48†§	418 ± 42*†‡	13.31‖	0.73	8.75‖
L-TAUC	298 ± 34‡§	220 ± 36‡§	450 ± 76*†	501 ± 60*†	15.95‖	0.22	2.41
Triglycerides (mmol/l)							
B-TAUC	3.40 ± 0.81	3.61 ± 0.75	4.50 ± 0.89	3.94 ± 0.80	5.06	0.19	1.05
B-4 h	0.89 ± 0.28	0.95 ± 0.24	1.24 ± 0.21	1.21 ± 0.28	13.92‖	0.05	0.15
L-TAUC	2.14 ± 0.70	2.00 ± 0.39	2.69 ± 0.49	2.46 ± 0.48	12.18¶	0.54	0.03

Data are means ± SE. B, breakfast; L, lunch; IAUC, incremental area under the curve; TAUC, total area under the curve; 4 h, concentration at 4 h (i.e., just before the patient started to eat lunch). *F* values from ANOVA for main effects of carbohydrate (Carb), GI and interaction between carbohydrate and glycemic index (Carb × GI). See text for explanation of statistical analysis. *Significantly different from high-carbohydrate, high GI: $P < 0.05$. †Significantly different from high-carbohydrate, low GI: $P < 0.05$. ‡Significantly different from low-carbohydrate, high GI: $P < 0.05$. §Significantly different from low-carbohydrate, low GI; $P < 0.05$. Significance of main effects: ¶$P < 0.05$; ‖$P < 0.01$.

of carbohydrate were found on the total ($F = 10.45$) and incremental areas ($F = 10.48$) under the plasma insulin curves ($P < 0.05$; Table 3 and Fig. 2). The main effect of GI on the incremental area under the insulin response curve did not reach statistical significance. However, for the high-carbohydrate breakfasts, the difference in plasma insulin between low- and high-GI treatments was significant at 1 h (Fig. 2). A main effect of GI on the plasma insulin concentration was noted at 4 h ($F = 38.84$, $P < 0.001$) with low-GI meals resulting in higher insulin concentrations at 4 h than high-GI meals (Table 3 and Fig. 2).

Plasma FFA concentrations were suppressed initially to an equivalent extent after each of the four meals. However, between 2 and 4 h after breakfast, there were significant differences in FFA responses. Low carbohydrate produced a marked rebound of FFAs, with the mean concentration for high and low GI at 4 h (347 ± 91 μmol/l) being 85% higher than the mean for the two high-carbohydrate breakfasts (188 μmol/l) ($F = 13.31$, $P < 0.01$; Table 3). There was a significant carbohydrate × GI interaction ($F = 8.75$, $P < 0.01$), indicating that the effect of the GI differed depending on the level of carbohydrate. For the low-carbohydrate breakfasts, a low GI was associated with a significantly higher FFA rebound than a high GI. However, for the high-carbohydrate breakfasts, a low GI was associated with a significantly lower plasma FFA concentration than a high GI (Fig. 2 and Table 3). Indeed, there was almost no rebound of plasma FFA concentrations after the high-carbohydrate, low GI breakfast, with the mean concentration at 4 h being only 1.8 times greater than that at its nadir (1 h), compared with a high-carbohydrate, high-GI meal for which the mean FFA concentration at 4 h was 7.6 times greater than that at its nadir (1.5 h).

There was a significant main effect of carbohydrate on the plasma triglyceride concentrations at 4 h ($F = 13.92$, $P < 0.01$; Table 3) with higher levels after the low- than the high-carbohydrate breakfasts. GI had no effect on triglyceride concentrations.

Responses to the standard lunch (4–6 h)

There was a significant main effect of carbohydrate on the total and incremental glycemic response areas after the standard lunch. After the low-carbohydrate breakfasts (mean of high- and low-GI), the mean

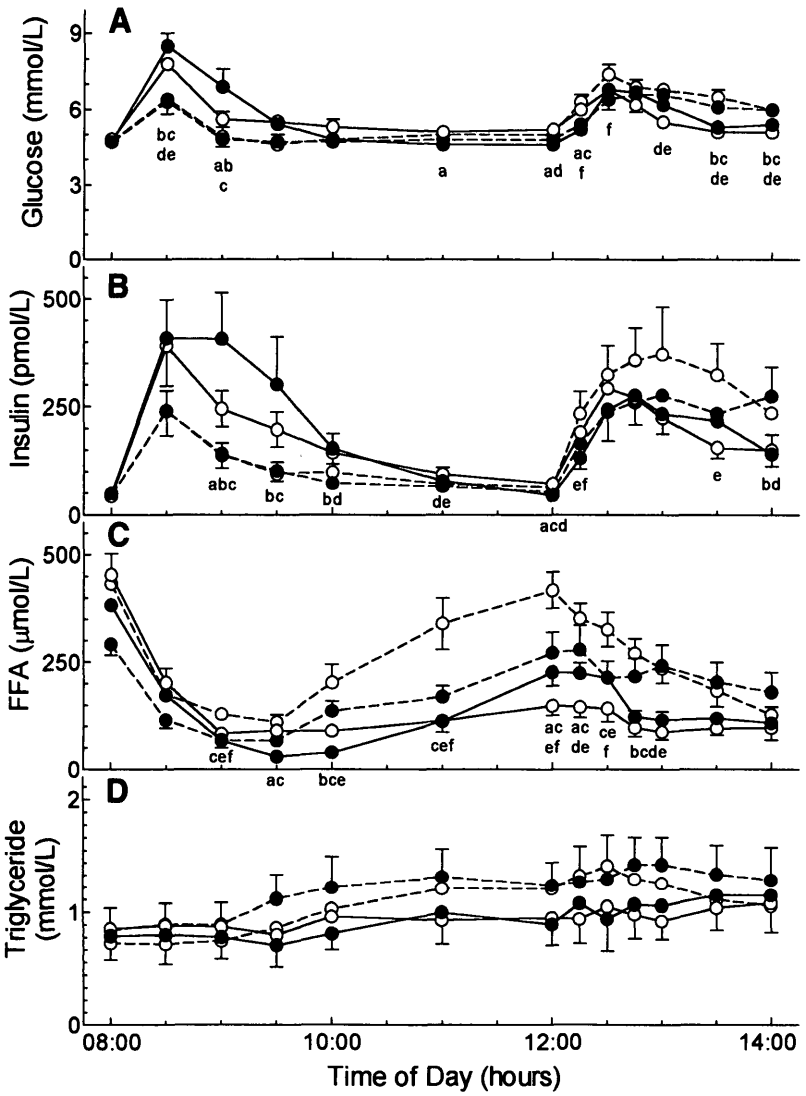

Figure 2—*Responses of plasma glucose, insulin, FFAs, and triglycerides of eight nondiabetic subjects after four different breakfasts followed by a standard lunch. The breakfast meals followed a 2 × 2 factorial design and consisted of high carbohydrate (——) or low carbohydrate (- - -) and high GI (●) or low GI (○). Values are means ± SE. Letters indicate significant differences (P < 0.05) between means as follows: a, ●——● vs. ○——○; b, ●——● vs. ●----●; c, ●——● vs. ○----○; d, ○——○ vs. ●----●; e, ○——○ vs. ○----○; f, ●----● vs. ○----○.*

total (12.7 ± 0.4 mmol · h · l^{-1}) and incremental (2.9 ± 0.4 mmol · h · l^{-1}) areas under the plasma glucose response curves after the standard lunch were significantly greater than the respective mean values after the two high-carbohydrate breakfasts (11.5 ± 0.3 mmol · h · l^{-1}, $F = 12.91$, $P < 0.01$; 1.8 ± 0.2 mmol · h · l^{-1}, $F = 11.83$, $P < 0.05$; Fig. 2 and Table 3). The highest glycemic response for lunch was seen after the low-carbohydrate, low-GI breakfast, with a total area under the curve of 13.1 ± 0.4 mmol · h^{-1} · l^{-1}, a value that was significantly greater than those for the lunches after the other three breakfasts (Table 3). The lowest glycemic response for lunch was after the high-carbohydrate, low-GI breakfast, with an incremental area under the curve of 1.1 ± 0.2 mmol · h · l^{-1}, a value that was significantly less than that after the lunches that followed the other three breakfasts (Table 3).

In general, the pattern of plasma insulin responses after the standard lunch was similar to that for plasma glucose, with significant main effects of carbohydrate on the total ($F = 7.88$, $P < 0.05$) and incremental ($F = 8.00$, $P < 0.05$) areas under the plasma insulin curves. Varying GI at breakfast did not significantly affect the insulin response after lunch, except that low-carbohydrate, low-GI meals produced the highest total area under the insulin response curve (Table 3).

There were significant main effects of breakfast carbohydrate content on the plasma FFA ($F = 15.95$, $P < 0.01$) and triglyceride ($F = 12.18$, $P < 0.02$) areas under the curve after the standard lunch, with higher levels after low- than after high-carbohydrate breakfasts. There was no significant main effect of breakfast GI on the total FFA or triglyceride areas after lunch.

The total area under the plasma glucose response to lunch was significantly related to the concentration of plasma FFAs at 4 h, just before the start of lunch ($r = 0.691$, $P < 0.0001$; Fig. 3). The FFA concentration at 4 h accounted for 48% of the variability of the total area under the glucose response curves to lunch. By contrast, the total area under the plasma insulin curve accounted for only 17% of the variability of the total area under the plasma glucose response curve to lunch ($r = 0.409$, $P < 0.02$; Fig. 4). The total area under the plasma glucose curve after lunch was not related to the plasma triglyceride ($r = 0.089$, $P = 0.63$), insulin ($r = 0.117$, $P = 0.52$), or glucose ($r = 0.166$, $P = 0.36$) concentrations just before lunch (Fig. 3). The plasma FFA concentrations before lunch were not related to the plasma triglyceride concentrations before lunch ($r = 0.25$, $P = 0.17$; Fig. 4).

CONCLUSIONS— The results show that plasma FFA concentrations in nondiabetic subjects in the middle of the day

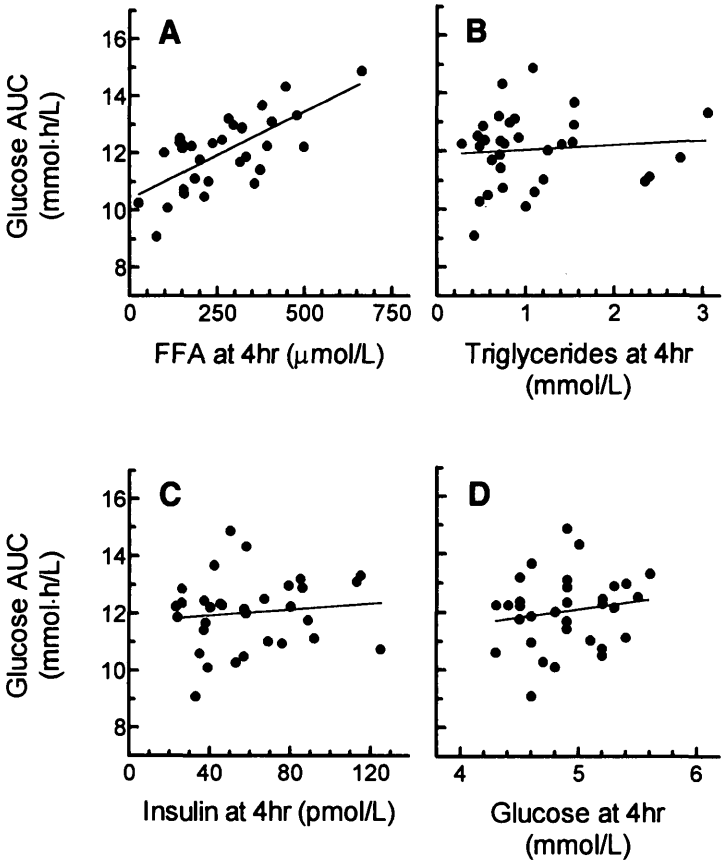

Figure 3—*Relationships between the glycemic response to the standard lunch and the concentrations of plasma FFAs (r = 0.691, P < 0.0001; A), triglycerides (r = 0.089, P = 0.63; B), insulin (r = 0.117, P = 0.52; C), and glucose (r = 0.166, P = 0.36; D) 4 h after the four different breakfast test meals. The glycemic response is expressed as the total area under the plasma glucose response curve.*

may vary markedly depending on the type and amount of carbohydrate eaten at breakfast. The blood glucose and insulin responses to a standard lunch also depend on the nature of the breakfast consumed 4 h earlier, with the glycemic response to lunch being closely related to the plasma FFA concentration just before the start of the midday meal. Low-carbohydrate breakfasts resulted in more marked FFA rebound and impaired carbohydrate tolerance to lunch compared with high-carbohydrate breakfasts. A low GI was effective only in suppressing FFAs and improving second-meal carbohydrate tolerance when present in a high-carbohydrate meal.

The present results confirm previous studies showing that high-carbohydrate and low-GI breakfasts improve blood glucose responses to the subsequent lunch, the so-called second-meal effect (16,17). In the present study, there was a clear effect of low-carbohydrate levels in impairing second-meal carbohydrate tolerance. With respect to the effect of GI, previous studies have all used high-carbohydrate breakfasts (i.e., >60% energy). In the present study, considering the high-carbohydrate breakfasts, a low GI was associated with improved second-meal carbohydrate tolerance compared with a high GI. It was of interest that the opposite was seen for the low-carbohydrate breakfasts, where the low-GI breakfast tended to impair second-meal carbohydrate tolerance compared with the high-GI breakfast. Glycemic profiles throughout the day in normal (19) and diabetic (20) subjects on high-and low-GI diets suggest that reducing dietary GI results in the expected reduction in blood glucose after breakfast and dinner but no difference after lunch. Thus, taken together with previous studies, the current results raise the interesting possibility that what is eaten for breakfast may actually have a greater effect in determining plasma glucose levels after lunch than what is eaten for lunch.

We consider our most novel and significant finding to be the demonstration that the composition of breakfast has a major effect on postprandial FFA responses with the differences persisting into the middle of the afternoon even after consumption of a standard lunch containing 68 g carbohydrate. In the literature, significant differences in plasma FFA responses after test meals of various composition have been difficult to detect and have been relatively small when seen (21–23). There may be several reasons for this. Fasting FFA concentrations tend to be quite variable, as demonstrated here, and hence it is difficult to match the fasting levels for the different treatments. In studies in which FFA responses are measured for only 2–3 h, differences between meals may not be apparent because the initial suppression of FFAs is similar for meals of markedly different composition, most likely because only a small increase in plasma insulin is required to inhibit hormone-sensitive lipase and to reduce FFA release from adipose tissue (24). In addition, some studies have examined FFA responses after lunch test meals, which may be different from those after breakfast meals.

After a rapidly absorbed carbohydrate, such as glucose, is consumed, plasma glucose and insulin concentrations rise rapidly. The high insulin concentration increases the rate of glucose disposal to such an extent that it becomes greater than the rate of glucose absorption, resulting in an undershoot of blood glucose and insulin to below their baseline levels. In the present study, after the

high-GI, high-carbohydrate breakfast, blood glucose levels fell below the baseline at 3 or 4 h in seven of eight subjects but undershot in only one subject after the low-GI, high-carbohydrate breakfast. The undershoot of glucose is associated with a small increase in counterregulatory hormones such as glucagon and catecholamines (14) which, in conjunction with the low insulin, activate FFA release from adipose tissue, causing plasma FFA concentrations to rise. Sipping glucose slowly to prevent the undershoot of blood glucose prevents the rebound of FFAs (14). Thus, the reduced FFA rebound seen after the low compared with the high-GI, high-carbohydrate breakfasts is consistent with the concept that low-GI starchy foods are slowly digested. This is further supported by the fact that plasma glucose and insulin concentrations at 4 h were significantly higher after the low- than the high-GI breakfasts. Nevertheless, even after the high-GI, high-carbohydrate breakfast, plasma FFAs rebounded significantly less then after the low-carbohydrate breakfasts.

Postprandial plasma FFAs may also be derived from the action of lipoprotein lipase on triglyceride-rich lipoproteins such as chylomicrons derived from dietary fat. Although most of the FFAs released from lipoproteins in the circulation are taken up into adipose tissue, the glycerol and ~30% of the FFAs remain in the bloodstream to be cleared by the liver (25). The low-carbohydrate meals were higher in fat and resulted in significantly higher postprandial serum triglyceride concentrations than the high-carbohydrate breakfasts. Since hydrolysis of serum triglycerides by lipoprotein lipase is a source of plasma FFAs, the rise in triglycerides associated with the low-carbohydrate breakfasts may partly explain why these meals were associated with higher postprandial FFA levels than were the high-carbohydrate breakfasts. However, there was no relationship between plasma triglyceride and FFA concentrations at 4 h. This and the fact that the highest FFA rebound was seen after the low-GI, low-carbohydrate breakfast suggests that there is an interaction between the effects of fat and the type and quantity of carbohydrate on plasma FFA concentrations.

The present results are consistent with our previous study showing that the rebound of FFAs after bolus glucose consumption was associated with impaired disposal of an intravenous glucose load (14). In the present study, the concentrations of plasma FFA just before lunch were positively related to the mean plasma glucose concentrations (i.e., total areas under the curve) after the standard lunch. Indeed, plasma FFA concentration at 4 h was the most important determinant of the glycemic response to lunch, explaining nearly 50% of its variance. FFAs have been shown to impair insulin-mediated glucose disposal and enhance hepatic glucose output (10). Raising plasma FFA concentrations by intravenous infusions of lipid and heparin has been shown to have a rapid (within 1 h) effect in increasing fat oxidation and decreasing carbohydrate oxidation (26). However, it took 2–4 h for glucose disappearance to decrease. Boden et al. (27) have suggested that raised plasma FFAs reduce glucose uptake primarily by reducing glycogen synthesis, initially by reducing glucose transport or phosphorylation and later by reducing muscle glycogen synthase activity (27). Thus, raised plasma FFA concentrations may increase blood glucose by inducing insulin resistance at the peripheral and the hepatic level (27).

The breakfast test meals tested here were designed to be realistic in terms of the amount of energy they contained and the composition of macronutrients. The types of foods used for the high- and low-carbohydrate breakfasts were kept the same in order to introduce as few variables as possible with respect to the nature of the sugars and the fatty acid composition of the meals. In the high-carbohydrate breakfasts, there was perhaps a larger amount of cereal than might usually be eaten. This was necessary to obtain a relatively large difference in meal GI by exchanging the breakfast cereal in meals that contained a significant proportion of their carbohydrate from other foods. Milk and orange juice were added to make the meals more palatable and realistic, but the amounts were kept constant for all four meals; therefore, any differences observed could not be attributed to differences in the absolute amount of lactose or fructose in the test meals. The macronutrient composition of the low-carbohydrate breakfast was designed to be similar to that for a breakfast of bacon, scrambled eggs, hashed brown potatoes, and juice.

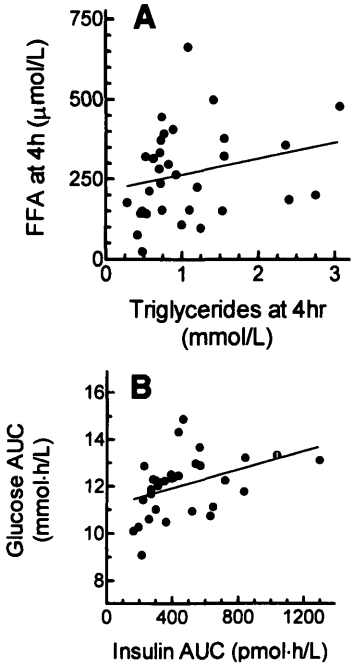

Figure 4—*Relationship between plasma concentrations of FFAs and triglycerides 4 h after the different breakfast test meals (r = 0.250, P = 0.17; A) and relationship between the plasma glucose and insulin responses after the standard lunch (r = 0.409, P = 0.020; B). Glucose and insulin responses are expressed as total area under the curve (AUC).*

It has been suggested that high plasma FFA concentrations, by causing insulin resistance and increasing hepatic glucose output, may be at least partly responsible for the high blood glucose levels seen in diabetes (9,10). Thus, break-

fast meals resulting in prolonged FFA suppression could result in improved insulin sensitivity and lower blood glucose later in the day. In support of this there is evidence that adding psyllium, a viscous fiber, to a breakfast test meal, reduces the glycemic response to the subsequent lunch in patients with NIDDM (28). It has also been shown that overall blood glucose control in diabetes can be improved by a dietary change that involves only the use of a low rather than a high-GI breakfast cereal (29). Further work will be required to determine whether plasma FFA concentrations in subjects with diabetes are sensitive to changes in the composition of breakfast.

We conclude that in nondiabetic subjects, the type and amount of carbohydrate eaten at breakfast influences the plasma glucose, insulin, and FFA responses to breakfast and also affect the glucose, insulin, and FFA responses to a subsequent standard lunch. The glycemic response to a standard lunch was directly related to the plasma FFA rebound after breakfast, which we speculate is due to the effect of FFAs on insulin action.

Acknowledgments— This work was supported by the Natural Sciences and Engineering Research Council of Canada and by Kellogg Canada, Etobicoke, Canada.

References

1. Wolever TMS, Jenkins DJA: Effect of dietary fiber and foods on carbohydrate metabolism. In *Handbook of Dietary Fiber in Human Nutrition.* 2nd ed. Spiller G, Ed. Boca Raton, FL, CRC, 1993, p. 111–152
2. Rossetti L, Giaccari A, DeFronzo RA: Glucose toxicity. *Diabetes Care* 13:610–630, 1990
3. Stout RW: Insulin and atheroma: a 20-yr perspective. *Diabetes Care* 13:631–654, 1990
4. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
5. Wolever TMS, Jenkins DJA, Vuksan V, Jenkins AL, Buckley GC, Wong GS, Josse RG: Beneficial effect of a low-glycaemic index diet in type 2 diabetes. *Diabetic Med* 9:451–458, 1992
6. Jenkins DJA, Wolever TMS, Vuksan V, Brighenti F, Cunnane SC, Rao AV, Jenkins AL, Buckley G, Patten R, Singer W, Corey P, Josse RG: "Nibbling versus gorging": metabolic advantages of increased meal frequency. *N Engl J Med* 321:929–934, 1989
7. Reaven GM, Lardinois CK, Greenfield MS, Schwartz HC, Vreman HJ: Effect of acarbose on carbohydrate and lipid metabolism in NIDDM patients poorly controlled by sulfonylureas. *Diabetes Care* 13 (Suppl. 3):32–36, 1993
8. Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YI: Measurement of plasma glucose, free fatty acids, lactate, and insulin for 24 h in patients with NIDDM. *Diabetes* 37:1020–1024, 1988
9. Fraze E, Donner CC, Swislocki ALM, Chiou Y-AM, Chen Y-DI, Reaven GM: Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: evidence for insulin resistance. *J Clin Endocrinol Metab* 61:807–811, 1985
10. Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA: Effect of free fatty acids on glucose production and utilization in man. *J Clin Invest* 72:1737–1747, 1983
11. Bevilacqua S, Buzzigoli G, Bonadonna R, Brandi LS, Oleggini M, Boni C, Geloni M, Ferrannini E: Operation of Randle's cycle in patients with NIDDM. *Diabetes* 39: 383–389, 1990
12. Havel RJ, Kane JP, Balasse O, Segel N, Basso LV: Splanchnic metabolism of free fatty acids and the production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J Clin Invest* 49:2017–2035, 1970
13. Barter PJ, Nestel PJ, Carroll KF: Precursors of plasma triglyceride fatty acid in humans: effects of glucose consumption, clofibrate administration and alcoholic fatty liver. *Metabolism* 21:117–124, 1972
14. Jenkins DJA, Wolever TMS, Ocana AM, Vuksan V, Cunnane SC, Jenkins M, Wong GS, Singer W, Bloom SR, Blendis LM, Josse RG: Metabolic effects of reducing rate of glucose ingestion by single bolus versus continuous sipping. *Diabetes* 39: 775–781, 1990
15. Jenkins DJA, Wolever TMS, Nineham R, Sarson DL, Bloom SR, Ahern J, Alberti KGMM, Hockaday TDR: Improved glucose tolerance four hours after taking guar with glucose. *Diabetologia* 19:21–24, 1980
16. Jenkins DJA, Wolever TMS, Taylor RH, Griffiths C, Krzeminska K, Lawrie JA, Bennett CM, Goff DV, Sarson DL, Bloom SR: Slow release carbohydrate improves second meal tolerance. *Am J Clin Nutr* 35: 1339–1346, 1982
17. Collier GR, Wolever TMS, Jenkins DJA: Concurrent ingestion of fat and reduction in starch content impairs carbohydrate tolerance to subsequent meals. *Am J Clin Nutr* 45:963–969, 1987
18. Wolever TMS: The glycemic index. In *World Review of Nutrition and Dietetics. Vol. 62.* Bourne GH, Ed. Basel, Karger, 1990, p. 120–185
19. Jenkins DJA, Wolever TMS, Collier GR, Ocana A, Rao AV, Buckley G, Lam KY, Meyer A, Thompson LU: The metabolic effects of a low glycemic index diet. *Am J Clin Nutr* 46:968–975, 1987
20. Brand JC, Colagiuri S, Crossman S, Allen A, Truswell AS: Low glycemic index carbohydrate foods improve glucose control in non-insulin dependent diabetes mellitus (NIDDM). *Diabetes Care* 14:95–101, 1991
21. van Amelsvoort JMM, van Stratum P, Kraal JH, Lussenburg RN, Houtsmuller UMT: Effects of varying the carbohydrate: fat ratio in a hot lunch on postprandial variables in male volunteers. *Br J Nutr* 61: 267–283, 1989
22. van Amelsvoort JMM, van Stratum P, Dubbelman GP, Lussenburg RN: Effects of meal size reduction on postprandial variables in male volunteers. *Ann Nutr Metab* 34:163–174, 1990
23. Wolever TMS, Bentum-Williams A: Effect of fixed versus weight-adjusted dose of oral glucose on plasma glucose, insulin and FFA responses: implications for the design of postprandial studies. *Nutr Res* 14:799–806, 1994

24. Nurjhan N, Campbell PJ, Kennedy FP, Miles JM, Gerich JE: Insulin dose-response characteristics for suppression of glycerol release and conversion to glucose in humans. *Diabetes* 35:1326–1331, 1986
25. Scow RO, Hamosh M, Blanchette-Mackie EJ, Evans AJ: Uptake of blood triglycerides by various tissues. *Lipids* 7:497–505, 1972
26. Boden G, Jadali F, White J, Liang Y, Mozzoli M, Chen X, Coleman E, Smith C: Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. *J Clin Invest* 88:960–966, 1991
27. Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* 93:2438–2446, 1994
28. Pastors JG, Blaisdell PW, Balm TK, Asplin CM, Pohl SL: Psyllium fiber reduces rise in postprandial glucose and insulin concentrations in patients with non-insulin-dependent diabetes. *Am J Clin Nutr* 53: 1431–1435, 1991
29. Golay A, Koellreutter B, Bloise D, Assal J-P, Würsch P: The effect of muesli or cornflakes at breakfast on carbohydrate metabolism in type 2 diabetic patients. *Diabetes Res Clin Pract* 15:135–142, 1992