# Blood glucose patterns and appetite in time-blinded humans

# Blood glucose patterns and appetite in time-blinded humans: carbohydrate versus fat

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**Melanson, Kathleen J., Margriet S. Westerterp-Plantenga, Wim H. M. Saris, Françoise J. Smith, and L. Arthur Campfield.** Blood glucose patterns and appetite in time-blinded humans: carbohydrate versus fat. *Am. J. Physiol.* 277 (*Regulatory Integrative Comp. Physiol.* 46): R337–R345, 1999.—We assessed the extent to which a possible synchronization between transient blood glucose declines and spontaneous meal initiation would lend support to the interpretation of a preload study with isoenergetic (1 MJ) isovolumetric high-fat or simple carbohydrate (CHO) preload drinks. Ten men (18–30 yr) fasted overnight and then were time blinded and made aware that they could request meals anytime. At first meal requests, volunteers consumed a preload; ad libitum meals were offered at subsequent requests. Postabsorptively, transient declines in blood glucose were associated with meal requests ($\chi^2$ = 8.29). Subsequent meal requests occurred during "dynamic declines" in blood glucose after the peak induced by drink consumption (100%). These meal requests took twice as long to occur after high-fat than after CHO preloads (fat = 126 ± 21, CHO = 65 ± 15 min), consistent with differences in interpolated 65-min satiety scores (fat = 38 ± 8.2, CHO = 16 ± 4). Postprandially, transient blood glucose declines were associated with meal requests ($\chi^2$ = 4.30). Spontaneous meal initiations were synchronized with transient and dynamic blood glucose declines. Synchronization of intermeal interval and dynamic declines related to higher satiating efficiency from high-fat preloads than from simple CHO preloads.

glucostatic theory; food intake regulation; intermeal interval; satiety; hunger

AN UNDERSTANDING OF FOOD intake regulation in humans is of profound clinical importance, particularly in approaching the prevention and treatment of obesity and eating disorders. Physiological, psychological, emotional, social, and cultural factors interact in a very complex manner to determine an individual's food intake. These various factors have been studied extensively over the past several decades, improving the understanding of their interactions (2). Historically, blood glucose levels have been a physiological variable believed to be instrumental in regulating food intake (32). In the 1950s, Jean Mayer (17) conducted an elaborate series of experiments in rats and mice that led to the formulation of the glucostatic theory. On the basis of blood glucose concentration or arteriovenous differences, he postulated that the rate of glucose utilization by privileged brain regions determined nutrient ingestion. Extensive rat studies from the laboratory of Steffens and colleagues (28, 30) during the 1960s and 1970s, in which blood glucose was measured at discrete intervals, demonstrated that blood glucose levels decline before a meal, remain at a lower plateau until a meal starts, and rise sharply shortly after the start of a meal. Insulin injections and infusions exacerbate this pattern (28). In the 1980s, the development of continuous blood glucose monitoring facilitated data collection, adding important information to the understanding of this relationship. Data from animal studies using this technique (4, 5, 15, 16) suggested that transient declines in blood glucose reliably precede meal initiation. More recently, this was also reported to occur postabsorptively in humans (6).

Thus far, continuous blood glucose monitoring has been applied in humans to study meal initiation during the morning after an overnight fast (6). In addition to meal initiation, the study of food intake regulation involves other dependent variables such as meal size and composition, intermeal interval, and meal frequency. For these variables, preload study designs are often used (23, 24). In such a design a preload drink or food, usually of a specific macronutrient composition, is administered to the volunteer, and after a preset time interval, ad libitum consumption of a test meal or free-living food intake is quantified and/or hunger and satiety are rated. Many such studies with preloads ranging from 420 to 1,850 kJ and intermeal intervals ranging from 30 to 315 min have shown no difference between carbohydrate and fat preloads in regulating subsequent ad libitum food intake (7, 9, 23). However, some studies have found carbohydrate more satiating than fat (1, 14), whereas others have reported a stronger satiating effect of fat (12). In a study comparing high-carbohydrate or high-fat preload meals with two different intermeal intervals, it was found that subjects consumed less at 90 min after a carbohydrate preload but not at 270 min after the preload, and no suppression of intake occurred at either time point after the fat preload (23). Seldom is the intermeal interval a dependent variable in a preload study design, nor is the time point of consuming the preload determined by the subject. In the cases in which the intermeal interval was subject determined, decreasing accuracy of energy compensation with increasing intermeal interval (23) or longer intermeal interval after fat compared with carbohydrate preloads has been observed (11).

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From the observations in humans by Campfield and colleagues (6), it appeared that transient blood glucose declines coincide to a great extent with increased hunger. In this study, as we continuously monitored blood glucose patterns, we assessed the satiating effect of a preload consisting of a particular macronutrient consumed by subjects at a time point at which they indicated that they were hungry and requested something to consume. The satiating effect might be different when the food is consumed at a time point at which the subjects, isolated from time cues, indicate that they are hungry, compared with other preload studies when the subjects get their first consumption at a fixed time point. The second dependent variable here is the intermeal interval. This allows us to calculate the satiating efficiency of a particular macronutrient using the quotient of intermeal interval to energetic consumption of the previous meal (13). Additionally, after the subject-determined intermeal interval, we have assessed energy intake and food choice from a normal Dutch lunch. Here food choice relates to macronutrient selection (33), enabling assessment of possible short-term macronutrient compensation. To observe the possible synchronization of hunger, meal requests, and transient blood glucose declines in time-blinded subjects, blood glucose was monitored continuously while appetite ratings were obtained at randomized intervals. Moreover, we hypothesized that if this synchronization did occur, it might lend support to the interpretation of the outcome.

## METHODS

*Subjects.* Ten healthy, weight-stable men recruited from the Maastricht University community completed this protocol. They all signed informed consents, and the protocol was approved by the Medical Ethical Committee of Maastricht University. As shown in Table 1, subjects were between the ages of 18 and 30 yr and were within the normal range of weight, height, and body mass index. Their scores on the Herman and Polivy Restraint Questionnaire (H&P) (10) and the Three-Factor Eating Questionnaire (TFEQ) (31) indicated that they were not inclined to control their food intake cognitively, except possibly one subject, who scored 20 on the H&P and 11 on *factor 1* (cognitive restraint) of the TFEQ.

*Protocol.* Before the protocol started, the possible test drinks and food items were evaluated by laboratory personnel. Special care was taken to ensure that the tastes of the high-carbohydrate and high-fat food choices were the same and that they only differed in macronutrient composition. From this preprotocol evaluation it was concluded that these items did not differ significantly in taste or hedonic value. Lemon-flavored drinks were chosen to mask the difference between a high-carbohydrate and a high-fat drink. The composition of these test drinks is shown in Table 2. Hedonic ratings for the two drinks, after being consumed completely by the volunteers, were not significantly different [carbohydrate = 72 ± 6, fat = 62 ± 10; not significant (NS)]. The foods offered for ad libitum consumption were typical Dutch lunch items according to food consumption surveys (18), chosen to permit ample selection of foods having similar sensory properties but differing in macronutrient composition. The purpose of these items was to allow volunteers to consume the macronutrient and energy content that they spontaneously preferred and to determine whether that preference differed after consumption of the two different preload drinks. Because the chosen food items are familiar to Dutch people, the volunteers would be able to choose from them according to experience. The food items consisted of sandwiches comprised of *1*) croissants with full-fat margarine and either high-fat cheese (total sandwich: 68.6% fat, 23.2% carbohydrate, 8.1% protein) or low-carbohydrate jam (total sandwich: 64.7% fat, 31.1% carbohydrate, 4.0% protein) and *2*) French bread with low-fat margarine and either low-fat cheese (total sandwich: 16.5% fat, 63.8% carbohydrate, 19.9% protein) or high-carbohydrate jam (total sandwich: 3.9% fat, 88.2% carbohydrate, 7.6% protein). Hedonic ratings after consumption of the high-carbohydrate (73.1 ± 6.0) and high-fat (74.2 ± 4.9) foods did not differ significantly. From these observations, we concluded that the drinks as well as the foods were of acceptable hedonic value to each of the subjects and that differences between hedonic values would not explain possible outcomes, because they did not differ significantly.

The protocol consisted of 2 test days separated by at least 1 wk. During both test days the volunteer was isolated from time cues to eliminate as much as possible habitual (time determined) meal patterns, enabling observation of meal responses to mainly physiological cues. Volunteers reported to the laboratory at 8:00 AM on each test day, after a 10-h overnight fast, at which point the time isolation began. No watches, clocks, radios, or televisions were in the room, and research staff did not make time-related statements. On the 2 test days the two different isoenergetic isovolumetric preload test drinks described above were administered in randomized order.

After the volunteer was settled and comfortable, an 18-gauge, 5-cm angiocath was placed in a suitable lower arm or hand vein. The blood-withdrawal end of a specially modified,

Table 1. *Subject characteristics*

| | Mean ± SD | Range |
|---|---|---|
| Age, yr | 21.5 ± 4.0 | 18–30 |
| Weight, kg | 72.1 ± 10.2 | 48.9–86.6 |
| Height, cm | 179.5 ± 8.6 | 159.2–186.7 |
| Body mass index | 22.2 ± 1.8 | 19.3–25.0 |
| Herman & Polivy score | 8.2 ± 1.6 | 4–20 |
| F1 (cognitive restraint) | 3.6 ± 2.9 | 1–11 |
| F2 (disinhibition) | 3.9 ± 1.9 | 2–7 |
| F3 (hunger) | 4.1 ± 2.8 | 1–8 |

$n$ = 10 men. F1–F3, *factors 1–3* of Three-Factor Eating Questionnaire.

Table 2. *Preload drinks*

| | Wt, g | Energy, kJ | Fat, g | CHO, g | Prot, g |
|---|---|---|---|---|---|
| | | *High carbohydrate* | | | |
| Water | 273 | 0 | 0 | 0 | 0 |
| Lemon syrup | 77 | 1,000 | 0 | 60.0 | 0 |
| Totals | 350 | 1,000 | 0 | 60.0 | 0 |
| kJ | | | 0 | 1,000 | 0 |
| Energy % | | | 0 | 100 | 0 |
| | | *High fat* | | | |
| Water | 279.5 | 0 | 0 | 0 | 0 |
| Heavy cream | 62.0 | 895 | 22.3 | 1.9 | 1.3 |
| Flavoring | 0.5 | 0 | 0 | 0 | 0 |
| Lemon syrup | 8.0 | 104 | 0 | 6.2 | 0 |
| Totals | 350.0 | 999 | 22.3 | 8.1 | 1.3 |
| kJ | | | 840.0 | 135.6 | 22.5 |
| Energy % | | | 84.1 | 13.6 | 2.3 |

CHO, carbohydrate.

2.5-m double-lumen catheter (MTB Medizintechnik, Amstetten, Germany) was fit into the angiocath. The catheter was heparinized by pumping sterile heparin-saline solution (500–5,000 U/ml) at a rate of ~25 μl/min through the distal lumen of the catheter to the tip of the cannula. The blood was continuously withdrawn through the proximal lumen of the cannula at a rate of ~25 μl/min. The blood-heparin-saline solution was mixed with a heparinized phosphate buffer at a 1-to-10 ratio and continuously infused into the sample chamber of a glucose analyzer (model 23A, Yellow Springs Instrument, Yellow Springs, OH). The transit time of this continuous sampling line was 3–5 min; this lag time was accounted for in the data analysis. Sampling occurred at a rate of 10 times/min, and analog data were amplified, digitized, interfaced (Data Translation Interface Board, model 1028), and displayed continuously on an IBM computer monitor. This monitor was not visible to the subject. For 30 min before the insertion of the catheter into the subject, and after the completion of testing, the system was calibrated using an IV bag of sterile saline with added glucose to bring the final glucose concentration to ~100 mg/dl. This calibration was done using the same blood-withdrawal cannula that was used in the subject that day.

Baseline blood glucose concentration was determined in the volunteer over a 30-min accommodation period. During the baseline period, and at random intervals throughout the day (to avoid time cues), the subject completed ratings of hunger, satiety, and desire to eat on 100-mm visual analog scales (VAS) anchored with "not at all" or "very much." The subject was informed that he could make a meal request at any time but that the requests might or might not be honored. The latter possibility was included so that the volunteers would not have the idea that the test could be completed after a meal request was made, but it was only carried out for 2 of 44 meal requests. Most volunteers spent the time studying; they were not permitted to sleep.

When the volunteer first requested something to eat, one of the two preload drinks was presented [1 MJ of simple carbohydrate (sucrose) or fat beverage; Table 2] and the volunteer was asked to consume it in its entirety. The volunteer completed hunger and satiety ratings before and after the preload. After the preload, the volunteer continued studying as before the preload, aware that he could request a meal at any time.

On subsequent meal requests, generous portions of the high-carbohydrate and high-fat food choices described above were presented to the volunteer for ad libitum consumption. Subjects were allowed to consume water with the meals. Hunger and satiety VAS ratings were completed before and after the meal. The total energy and macronutrient content of the foods consumed were determined by weighed differences. After the meal, continuous monitoring of blood glucose and randomized ratings of hunger and satiety continued. The volunteer was aware that he could request more food at any time. The testing lasted for a total of 6–8 h (or until clot formation prohibited further blood glucose monitoring). On completion of the testing, volunteers were requested to estimate the clock time to verify that they were blinded to the time of day.

*Statistics.* One-minute averages of blood glucose levels over time were plotted for each volunteer's 2 test days using the programs Microsoft Excel 4.0 and Cricket Graph 1.3 for Macintosh (Apple Computers, Cupertino, CA). The data set was shifted forward by the exact transit time of the catheter used for sampling. An analysis program was written in Microsoft Excel to scan the blood glucose values and to determine whenever there was a period of stable baseline glucose (standard deviation $< 1$ mg/dl) that lasted 5 min or longer. Transient declines in blood glucose have been defined in the literature as a drop of at least 5% below this stable baseline glucose level (standard deviation $> 2$ mg/dl) lasting at least 5 min (5) using 5-min running averages. During our analysis, we also noticed the presence of rapid ($0.85 \pm 0.18$ $mg \cdot dl^{-1} \cdot min^{-1}$) declines in blood glucose that followed a peak induced by consumption of the macronutrient preloads. Because these declines did not originate from a stable baseline, as do transient declines, but rather from a peak, we defined these as "dynamic declines." Transient and dynamic blood glucose declines were tallied for each test day, and the number of times that meal requests occurred in the presence or absence of a decline in blood glucose was quantified. Satiety quotients were calculated for the two preloads as intermeal interval in minutes divided by energy consumed in megajoules, as described by Langhans (13).

"Postabsorptive" refers to the state when all the previously ingested food has been absorbed from the digestive tract, whereas "postprandial" refers to the state when the digestive tract contains ingested food. Because in the present protocol, testing started at least 10 h after the last nutrient ingestion, the postabsorptive state was defined as the period from the beginning of the testing until the first macronutrient consumption (preload); the postprandial state was defined as the period from the point of first macronutrient consumption until the end of the testing. Comparisons between the results from the two different preload test drinks were made using paired *t*-tests and ANOVA with repeated measures on the computer software program Statview 2.0 for Macintosh. Statistical significance was accepted as $P < 0.05$. Associations between changes in blood glucose and meal requests were tested using the $\chi^2$-test for $2 \times 2$ contingency tables (20). Data presented are means ± SE unless otherwise specified.

## RESULTS

The average duration of continuous blood glucose sampling was 423 ± 19 min, ranging from 287 to 484 min. This duration did not differ between the days when a simple carbohydrate preload was given and those on which a fat preload was given (carbohydrate = 423 ± 18, fat = 422 ± 20 min). The testing was predetermined to last 6–8 h, but in 13.6% of tests the measurement was terminated earlier because of blood clotting in the line. The average volunteer estimation of clock time at the end of the testing was −45 ± 34 min, ranging from −178 to +24 min, thus verifying that the subjects were blinded to the time of day. Average baseline blood glucose was 81.0 ± 3.4 mg/dl. During each test, one to four meal requests occurred, but there was no difference in the number of meal requests that occurred on the carbohydrate-preload test day (2.3 ± 0.3) versus the number that occurred on the fat-preload test day (2.2 ± 0.2). Ratings of feelings of pleasantness during meal consumption did not differ between the days when the simple carbohydrate (79.3 ± 7.7) and high-fat (74.9 ± 5.2) drinks were consumed (NS).

In Fig. 1, representative curves from four different subjects are presented, depicting examples of the different types of responses observed. Data from a subject on the fat-preload test day are shown in Fig. 1*A*. The first meal request occurred in association with a transient decline in blood glucose, and the second occurred at the nadir after the decline in blood glucose that followed the preload-induced rise. As shown in Fig. 1*B*, another subject's first meal request came in the absence of

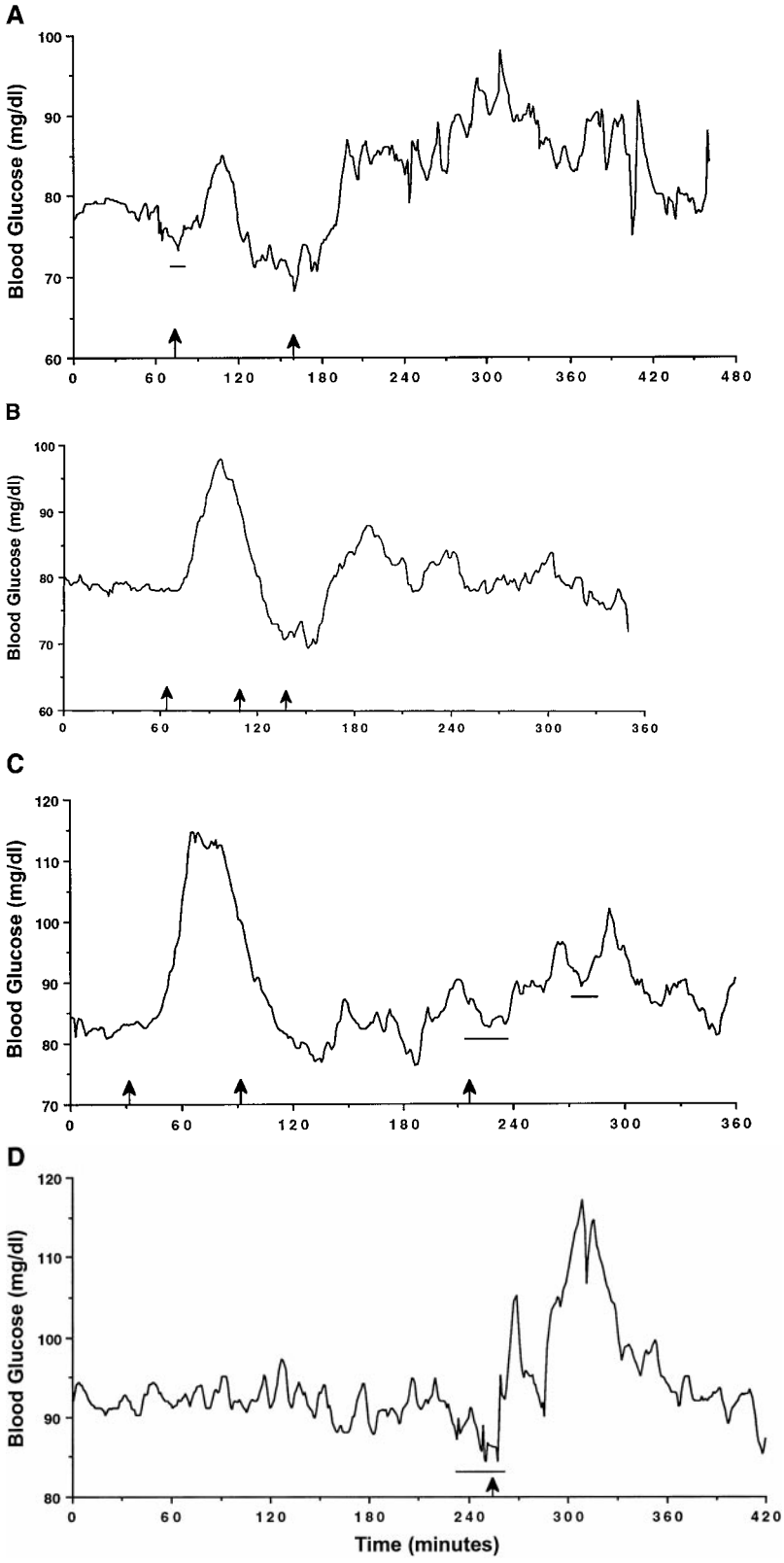


Fig. 1. *A*: blood glucose over time in a volunteer monitored continuously on day when fat preload test drink was consumed. *B*: blood glucose over time in a volunteer monitored continuously on day when carbohydrate preload test drink was consumed. *C*: blood glucose over time in a volunteer monitored continuously on day when carbohydrate preload test drink was consumed. *D*: blood glucose over time in a volunteer monitored continuously on day when carbohydrate preload test drink was consumed. Arrows indicate spoken meal requests; horizontal bars indicates transient decline in blood glucose.

significant changes in blood glucose, the carbohydrate drink was then consumed, blood glucose peaked, and the next meal request occurred during the rapid drop in blood glucose that followed that peak. This meal request was denied, and the subsequent meal request occurred after the nadir of the decline. A typical biphasic response in blood glucose after the ad libitum meal can be seen in this curve. Figure 1*C* depicts a volunteer's carbohydrate-day data, in which the first meal request occurred in the absence of a transient decline in

blood glucose. The second meal request occurred during the fall in blood glucose that had followed the rapid postdrink rise, and the third meal request occurred in association with a transient decline in blood glucose. A subsequent transient decline in blood glucose was not associated with a meal request. Data from the volunteer who scored highest on the H&P and TFEQ are shown in Fig. 1*D*. On both of his test days, this subject's blood glucose hovered around baseline without significant (>5%) declines for >4 h, and then a transient decline occurred, during which he made his first and only meal request of the test day.

A summary of the responses related to patterns of blood glucose is presented in Table 3. In the postabsorptive state, the interdependence between meal requests and transient declines in blood glucose was statistically significant ($\chi^2 = 8.29$, $P < 0.010$).

The decline in blood glucose after the rise induced by the consumption of the preload drink could not be defined as a transient decline as previously described in the literature because it did not occur immediately after a stable baseline (5, 6). Because these declines occurred after the rise induced by the preload drink, we termed them dynamic declines. In the postdrink situation, 18 of these dynamic declines were observed, 14 of which were associated with a meal request. Of those that were not associated with a meal request, two occurred in the volunteer who scored as a restrained eater: he never made a meal request after his drink. The postdrink declines in this volunteer lasted the longest of any volunteer: 78 min after the carbohydrate drink and 111 min after the fat drink. In two other volunteers, no meal requests were made during the dynamic decline but were made in association with a subsequent transient decline in blood glucose. Thus, in 100% of the cases, these meal requests came in association with declines in blood glucose, so $\chi^2$ analysis was not applicable to this condition. Dynamic declines were more rapid (1.27 $mg \cdot dl^{-1} \cdot min^{-1}$ for CHO, 0.41 $mg \cdot dl^{-1} \cdot min^{-1}$ for fat; $P = 0.02$), constituted a larger change in blood glucose (41.6 mg/dl for carbohydrate, 22.3 mg/dl for fat; $P = 0.00$ for the difference between the carbohydrate or fat preload effect), and were shorter in duration (42 min for carbohydrate, 67 min for fat; $P = 0.01$)

Table 3. *Responses observed in relationship between blood glucose patterns and meal requests*

| | Declines in Blood Glucose Associated With Meal Requests | | | Meal Requests Associated With Declines in Blood Glucose | | |
|---|---|---|---|---|---|---|
| | Yes | No | Total | Yes | No | Total |
| Postabsorptive transient* | 11 (84.6%) | 2 (15.4%) | 13 | 11 (55.0%) | 9 (45.0%) | 20 |
| Postprandial dynamic | 14 (77.8%) | 4 (22.2%) | 18 | 14 | 0 | 14 |
| Postprandial transient† | 7 (50.0%) | 7 (50.0%) | 14 | 7 (70.0%) | 3 (30.0%) | 10 |
| Total‡ | 32 (71.1%) | 13 (28.9%) | 45 | 32 (72.2%) | 12 (27.3%) | 44 |

$n = 10$ men. *$\chi^2 = 8.29$, $P < 0.010$; †$\chi^2 = 4.30$, $P < 0.050$; ‡$\chi^2 = 7.31$, $P < 0.010$.

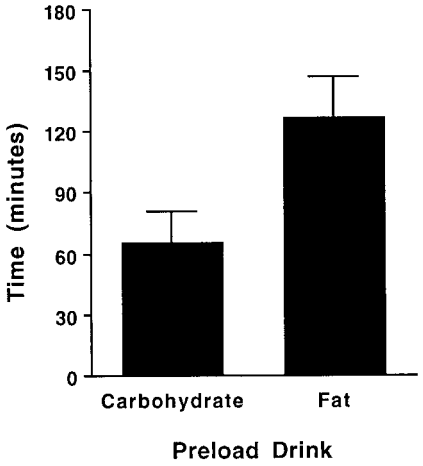


Fig. 2. Intermeal interval between each preload drink and second meal request ($n = 10$ subjects). Second meal requests occurred at 65 ± 15 min after carbohydrate preload drink and at 126 ± 21 min after fat drink. Values are means + SE ($P = 0.017$).

after the simple carbohydrate preload compared with those after the fat preload. Peak blood glucose was 120.6 ± 6.6 mg/dl after the carbohydrate preload and was 105.2 + 7.5 mg/dl after the fat preload. In the postprandial state, transient declines in blood glucose were also interdependent with meal requests, as can be seen in Table 3 ($\chi^2 = 4.30$, $P < 0.050$).

In total, 71.1% of all declines in blood glucose, including both transient and dynamic declines, were associated with meal requests. Of all spoken meal requests, 72.2% were associated with either a transient or dynamic decline in blood glucose. Collectively, $\chi^2$ analysis revealed that the interdependence of these variables was significant ($\chi^2 = 7.31$, $P < 0.010$).

As illustrated in Fig. 2, the intermeal interval after the carbohydrate preload drink was about one-half as long as that after the fat preload drink ($P = 0.017$). A second meal request occurred 65 ± 15 min after the simple carbohydrate drink, whereas it occurred 126 ± 21 min after the fat drink. Thus the satiety quotients were 65 and 126 for the simple carbohydrate and fat preloads, respectively.

Hunger scores during the interval between each of the preload drinks and subsequent meal requests are plotted in Fig. 3*A*. Because the ratings were obtained at randomized intervals to maintain time blinding, intermediate scores were interpolated to 30, 65, and 126 min for each subject and averaged for the group. Hunger scores immediately after the consumption of the two drinks were almost identical (carbohydrate = 58.2 ± 6.1; fat = 55.9 ± 7.7; NS). Although the slopes diverged after 30 min, this did not reach statistical significance. Regression equations for hunger scores over the intermeal interval were significant for both the carbohydrate ($R = 0.435$, $P = 0.007$) and fat ($R = 0.408$, $P = 0.007$) preloads.

Interpolated satiety scores during the interval between each of the preload drinks and subsequent meal requests are plotted in Fig. 3*B*. Satiety scores immediately after the consumption of the two drinks were almost identical (carbohydrate = 44.8 ± 7.5; fat =

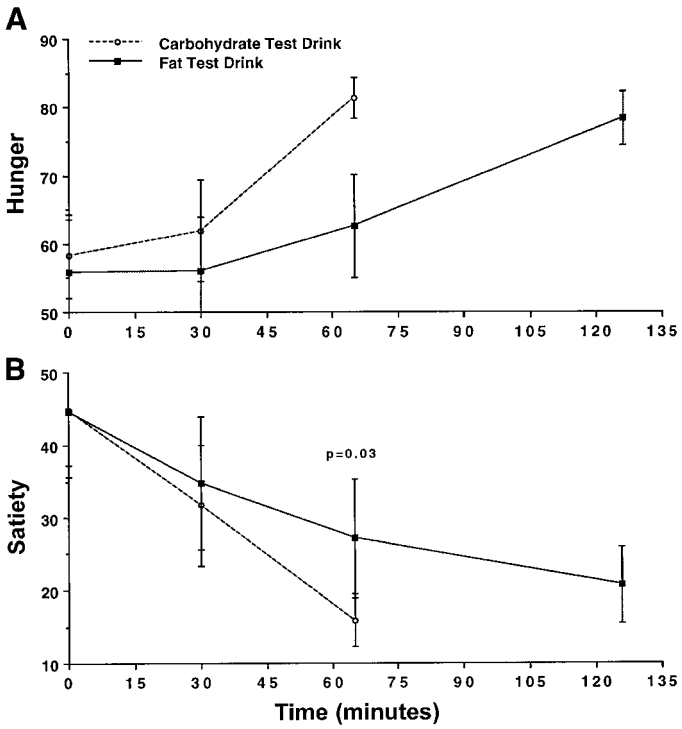


Fig. 3. *A*: interpolated hunger scores during intermeal interval between preload drinks and second meal requests (65 min after carbohydrate drink, 126 min after fat drink; $n = 10$ subjects). *B*: interpolated satiety scores during intermeal interval between preload drinks and second meal requests (65 min after carbohydrate drink, 126 min after fat drink; $n = 10$ subjects). *Time zero* indicates completion of consumption of preload drink.

44.5 ± 8.8; NS). Furthermore, at the time of the second meal request after the two drinks, there was no significant difference in satiety ratings (carbohydrate = 15.9 ± 3.7 at 65 min, fat = 20.8 ± 5.3 at 126 min; NS). However, at the point in time when the second meal request occurred after the carbohydrate drink (65 ± 15 min), there was a statistically significant difference in satiety scores between the two drinks (carbohydrate drink = 15.9 ± 3.7, fat drink = 38.4 ± 8.2; $P = 0.03$). Regression equations for satiety scores over the intermeal interval were significant for both the carbohydrate ($R = 0.460$, $P = 0.004$) and fat ($R = 0.453$, $P = 0.002$) preloads. Although the duration of the intermeal interval differed after the two preload drinks, there was no significant difference in the total energy or macronutrient intakes once the ad libitum food items were consumed, as seen in Table 4.

The cumulative satiety index was calculated as (ImI 1/ingested MJ 1) + (ImI 2/ingested MJ 2), where ImI 1 and ImI 2 are the intermeal intervals between the preload drink and *meal 1* and between *meal 1* and *meal 2*, respectively, and ingested MJ 1 and ingested MJ 2 are the totals of energy, in megajoules, ingested before the interval (for the preload drinks and the first ad libitum meal, respectively). This would permit observation of preload effects on satiety quotient over the whole test day. The cumulative satiety index for the group was 128.7 ± 21.4 after the fat preload and 73.9 ± 15.2 after the carbohydrate preload. Excluding test days on which one meal request was made (4 trials in 3 volunteers), the cumulative satiety index was significantly higher for fat (126.0 ± 27.5) than for carbohydrate (70.5 ± 17.1), by paired *t*-test ($P = 0.017$).

Table 4. *Ad libitum energy and macronutrient intake after 2 preload drinks*

| Nutrient | Carbohydrate Drink | Fat Drink |
|---|---|---|
| Energy, kJ | 4,013 ± 789 | 4,519 ± 677 |
| Carbohydrate, g | 89.9 ± 16.4 | 118.6 ± 15.1 |
| Fat, g | 55.1 ± 13.4 | 55.4 ± 10.8 |
| Protein, g | 23.7 ± 4.5 | 26.4 ± 3.6 |

Values are means ± SE.

## DISCUSSION

The observations of spontaneous meal initiations synchronized with postabsorptive and postprandial transient blood glucose declines or dynamic blood glucose declines were possible because the habitual meal pattern according to time cues was prevented; physiological signals were permitted to prevail. We demonstrated that two types or classes of deviations in blood glucose concentration were associated with spoken meal requests and a relatively high level of hunger. The first was the endogenous transient decline in blood glucose that was observed in both the postabsorptive and postprandial states. The second was the falling phase of the perturbation in blood glucose induced by a liquid meal high in simple carbohydrate or fat (postpreload). In both experimental situations, the majority of spoken meal requests occurred after or during the two distinct patterns of blood glucose concentration. Therefore, these studies demonstrate that the association among hunger, meal requests, and deviation in blood glucose concentration was larger than would be expected if it were accidental. Also, the slope of VAS satiety ratings over time was different between the fat and carbohydrate drinks. In this situation, when the physiological timing of food intake occurred, a different effect of the macronutrient composition of the preload was observed.

Campfield and Smith (5) also proposed that it is the pattern of blood glucose over time, i.e., the transient decline in blood glucose, rather than glucose concentration per se that signals meal initiation, indicating a need for fuel ingestion to the organism. In previous studies (4, 5, 6, 16), the transient decline in blood glucose was defined as a deviation (>5%) from a stable baseline blood glucose concentration, lasting at least 5 min. In the present study, we have also observed, in most but not all situations in our volunteers, these patterns in blood glucose related to meal initiation that have been described by other investigators in both animal (4, 16, 28) and human (6) studies.

During the postabsorptive state, meal requests were related to transient declines in blood glucose; an association was observed in 84.6% of the cases ($\chi^2 = 8.29$, $P < 0.010$). This result is in accordance with a recent human study in which blood glucose was monitored in fasting humans for an average of 3.5 h and association

was reported in 83% of the subjects (6). In that study, 2 of the 18 subjects (11%) experienced transient declines in blood glucose in the absence of meal requests or expressions of hunger. In the present study, blood glucose was monitored continuously for an average of 7 h in humans, starting in the fasting state, and recorded after their meal intakes throughout the day. In 2 of 13 cases (15.4%), postabsorptive transient declines in blood glucose were observed without meal requests and 7 of 14 (50.0%) postprandial transient declines occurred in the absence of meal requests. Thus the relationship between postabsorptive transient declines in blood glucose concentration and meal requests in the present study is consistent with the previous human study that observed subjects in the postabsorptive state (6). The postprandial data from the current study provide new information to this field of research. The somewhat less consistent relationship between postprandial transient declines in blood glucose and meal requests could be caused by the differences in the ad libitum meals consumed, individual responses in postprandial blood glucose and insulin, other satiety signals present postprandially (e.g., cholecystokinin, corticotropin-releasing factor, serotonin, somatostatin, or suppressed neuropeptide Y), or cognitive factors that may have inhibited some individuals from making a third meal request. However, the interrelationship between transient declines in blood glucose and meal requests was still statistically significant ($\chi^2 = 4.30$, $P < 0.050$) in this postprandial state.

In the present study, blood glucose concentration was in a dynamic state of change throughout the postpreload interval, so that a steady baseline could not be defined during this time. Thus the deviations in blood glucose concentration could not be classified as "transient declines" in blood glucose unless a prior stable baseline blood glucose concentration was defined. Therefore, the dynamic decline, the rapid fall in blood glucose after a rise induced by the ingestion of a drink or a meal, had to be classified as another, distinct pattern of blood glucose that was related to meal requests. These declines averaged 1.27 $mg \cdot dl^{-1} \cdot min^{-1}$ for 42 min after the peak induced by the carbohydrate preload, and 0.41 $mg \cdot dl^{-1} \cdot min^{-1}$ for 67 min after the fat preload. A consistent finding in the relationship between patterns of blood glucose and meal initiation in the present study was that during the rapid dynamic decline from the test drink-induced rise in blood glucose, a meal request occurred in 77.8% of the cases. It is possible that rapid changes in blood glucose are particularly strong signals for meal initiation, thus causing a tight coupling between these variables. As suggested by Mayer (17), this may be related to changes in rates of fuel utilization, particularly by privileged brain regions. Because this is the first study that has measured glucose continuously during this intermeal interval in acutely time-blinded humans, the association of spontaneous meal requests during this pattern of dynamic change in humans is a new observation. Determining other blood parameters would be necessary in the future to elucidate the mechanisms involved in this association more completely.

Blood glucose concentrations have been experimentally manipulated using exogenous infusions of insulin and glucose in rats to further understand the roles that insulin and glucose may play in food intake regulation (4, 28). Preliminary data from infusion studies in four humans (6) show that insulin-induced transient declines in blood glucose are associated with increased feelings of hunger. In humans, ingestion of glucose has also been suggested to stimulate food intake (22). In rat studies in which declines in blood glucose were blocked by glucose infusion, meal initiation was delayed until an endogenous transient decline in blood glucose subsequently occurred (5). This suggests that the pattern of glucose, rather than insulin, provides the signal (5). In the present human protocol, we manipulated blood glucose by the physiologically relevant means of macronutrient ingestion and observed a relationship between hunger and dynamic declines in blood glucose, as measured continuously in a time-blinded situation. Thus the first important observation in this study implied synchronization between transient declines and meal requests and between postpreload dynamic declines and meal requests. The second observation concerns the dependent variable in intermeal interval.

It was shown that the intermeal interval between the drink and the meal was consistently longer after the fat-rich drink compared with the simple carbohydrate drink and that total subsequent food intake did not differ in energy content or macronutrient composition. Thus, in this case when timing was the dependent variable, it showed that subsequent food intake was quite constant, whereas the usual preload experiments show that when timing is fixed, people differ in food intake (23). With differences in fixed intervals, certain amounts were eaten by volunteers, not only depending on the characteristics of the preload but also depending on the interval (11, 23). In our design the preload is also the independent variable, but timing as well as subsequent food intake are dependent. Therefore, the design concentrates on meal interval apart from meal size. We may suggest a contribution of the data to a possible ecologically relevant insight, i.e., that macronutrient composition may influence intermeal interval and thus meal frequency, which is another food intake variable in addition to the amount eaten.

When the volunteer determines the intermeal interval, compared with determining the amount eaten, the outcome variable is satiating efficiency. In this case, it shows that the satiating efficiency of fat lasted longer than that of simple carbohydrate. This has also been recently demonstrated in a study by Himaya and colleagues (11), in which volunteers were also blinded to time cues and intermeal intervals were determined by the subjects after the consumption of a lunch with either added butter or butter substitute. In this case, nonisoenergetic solid foods were consumed as preloads. Although this study design differed somewhat from ours, a similar effect of fat (but partly of energy) was observed, in that the intermeal interval was increased

but subsequent energy and macronutrient intakes from the ad libitum meal did not differ. In our study, regression analysis of satiety scores over time indicated similar satiety immediately after consumption of the preload drinks, reflected by similar *y*-intercepts. However, by 1 h after consumption the slopes had diverged, resulting in different satiety scores at this point after simple carbohydrate compared with fat consumption. The *R* values indicate that changes in satiety over time explained ~46% of the variance in intermeal interval. Thus one aspect of the satiating effect of a given macronutrient can be observed from the intermeal interval determined by the volunteer.

The cumulative satiety index was higher on the day on which the fat preload was consumed than the day on which the carbohydrate preload was consumed. This calculation has provided an additional approach in analyzing the effects of the macronutrient preloads on satiety and intermeal interval (13). Compensation for the consistently higher satiety index after fat intake did not occur within the observation period. However, it is not known whether there was compensation later in the day after the volunteers left the laboratory.

As mentioned in the introduction, an equal or higher satiating effect is often observed from carbohydrate compared with fat. The discrepancy between our observations and these observations may be related to timing, as discussed above, but the experimental design using preloads with 100% simple carbohydrate or 84% fat may also have some bearing. In most other studies, relatively high percentages of carbohydrate or fat are offered but often in mixed macronutrient form. It may well be that certain mixtures could have higher satiating efficiencies than the single macronutrient of which they consist, perhaps acting in a synergistic manner.

Examination of the blood glucose curves during the interval between the preload drink and ad libitum meal lends more understanding to the results of the present study and other preload studies. The rise and fall of the glucose curve after the carbohydrate drink was steep and fast, whereas that after the fat drink was more delayed, gradual, and longer. In both cases, a subsequent meal request occurred during the decline from that rise. However, the decline occurred within 1 h after consumption of the carbohydrate preload drink, and it occurred ~2 h after consumption of the fat drink. This could be caused by slower, more gradual gastric emptying of fat than of simple carbohydrate (3) and the rapid delivery of simple carbohydrate from the intestine to the bloodstream (26, 29). In humans, blood glucose response to the ingestion of four different high-carbohydrate foods has been shown to be related to the rate of gastric emptying (19). It has been suggested that rapid intestinal absorption of simple carbohydrate may lead to an abrupt termination of glucose influx, which in turn could elicit earlier and sharper food intake signals even in the presence of ample endogenous carbohydrate (8). Furthermore, the return of hunger subsequent to meal ingestion has been found to be commensurate with the gastric emptying of that meal (27). In that study, no relationship was observed between postprandial blood glucose concentration and the development of hunger. However, blood glucose was only sampled at 30-min intervals, so patterns of glucose over time may have been missed. As has been pointed out previously (1), the physiological processes occurring during the "postingestive window" have a bearing on the expression of appetite during that time. In humans, the relationship is complex, in that cognitive influences such as the memory of what has been consumed (25) may also have bearing on the intermeal interval. In the present study, the possibility exists that the volunteers may have perceived differences between the simple carbohydrate drinks and the high-fat drinks and that this may have played a role in subsequent feeding behavior independent of the postoral effects of the macronutrient differences. However, cognitive factors may be interrelated with the learning of physiological postingestive satiety signals. Given the combination of data from this laboratory paradigm designed to let physiological signals prevail, the association between the size and duration of the continuous blood glucose curves after the two preload drinks may help to explain why the subject-determined intermeal interval was about twice as long on the fat test day as on the simple carbohydrate test day. We suggest that this intermeal interval might be variable in relation to macronutrient composition and energy ingested. For instance, complex carbohydrates are likely to have different effects compared with simple carbohydrates (21).

From the combination of these data, one could hypothesize that feelings of hunger and meal initiation are caused by internal and external factors that act in concert with postabsorptive and postprandial transient declines in blood glucose levels. It is concluded that rapid declines in blood glucose are associated with hunger and meal initiation in humans. The synchronization of these rapid declines with meal requests has been shown to be related to the longer intermeal intervals after consumption of high-fat versus simple carbohydrate preloads.

### *Perspectives*

The potential source of transient declines in blood glucose, their detection by the central nervous system, and the causal link to feeding behavior have been studied extensively in rats (5, 15, 17). Correlational data in humans cannot prove causality for blood glucose declines in signaling meal initiation. However, they do provide impetus for hypotheses aimed at understanding the relationship. For example, it has been proposed (8) that transient declines in blood glucose may occur in relation to the point in time when the liver switches from retaining glucose to releasing glucose. This signal may be detected by peripheral and central nervous system glucoreceptive elements and mapped into meal initiation, as evidenced by studies in vagotomized rats (5). It could be that the organism learns over time that when blood glucose declines in this specific pattern (perhaps related to the switch from hepatic glycogenosis to glycogenolysis), which is communicated

vagally, feeding should be initiated to prevent subsequent hypoglycemia. Likewise, when blood glucose levels drop rapidly during the dynamic decline, a similar response of avoiding impending hypoglycemia may lead to meal initiation. An alternative hypothesis has been offered that transient declines may be conditioned anticipatory responses in which blood glucose is lowered in preparation for the imminent meal-induced rise in blood glucose, possibly related to cephalic phase insulin responses (34). These hypotheses remain to be substantiated, but the impact of different macronutrients, metabolic states, and subject characteristics on this relationship between blood glucose patterns and appetite could prove of interest and importance.

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