

Role of sugars in human neutrophilic phagocytosis^{1, 2}

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ABSTRACT This study was designed to test *a*) whether carbohydrates other than glucose decreased the phagocytic capacity of neutrophils in normal human subjects, *b*) the duration of this effect, and *c*) the effect of fasting on neutrophilic phagocytosis. Venous blood was drawn from the arm after an overnight fast and at 0.5, 1, 2, 3, or 5 hr postprandial and this was incubated with a suspension of *Staphylococcus epidermidis*. The phagocytic index (mean number of bacteria viewed within each neutrophil) was determined by microscopic examination of slides prepared with Wright's stain. Oral 100-g portions of carbohydrate from glucose, fructose, sucrose, honey, or orange juice all significantly decreased the capacity of neutrophils to engulf bacteria as measured by the slide technique. Starch ingestion did not have this effect. The decrease in phagocytic index was rapid following the ingestion of simple carbohydrates. The greatest effects occurred between 1 and 2 hr postprandial, but the values were still significantly below the fasting control values 5 hr after feeding ($P < 0.001$). The decreased phagocytic index was not significantly associated with the number of neutrophils. These data suggest that the function and not the number of phagocytes was altered by ingestion of sugars. This implicates glucose and other simple carbohydrates in the control of phagocytosis and shows that the effects last for at least 5 hr. On the other hand, a fast of 36 or 60 hr significantly increased ($P < 0.001$) the phagocytic index. *Am. J. Clin. Nutr.* 26: 1180–1184, 1973.

A major defense mechanism of higher animals is neutrophilic phagocytosis (1, 2). Phagocytosis is an energy requiring mechanism that is inhibited by an inadequate supply of glucose (3). On the other hand, there is evidence to suggest that an excess of glucose may also decrease phagocytic activity. Patients with diabetes mellitus are characterized by elevated blood glucose levels and lowered resistance to infection. Diabetics have also been found to have impaired neutrophilic phagocytosis as compared with normal subjects (4–7). Kijak et al. (8) found that the greater the blood glucose level the lower the phagocytic capacity in diabetic patients. Moreover, oral administration of increasing amounts of glucose progressively lowered the phagocytic capacity of neutrophils in normal subjects (8) or patients (9) when measured at 45 min postprandial. Thus, both the ingestion of glucose and the level of glucose in the blood seem to be implicated in the control of neutrophilic phagocytosis. There seems to be no further information relating carbohydrates with neutrophilic phagocytosis or with the duration of decreased phagocytosis following glucose ingestion.

The purpose of these studies was to *a*) test whether ingestion of carbohydrates other than glucose decreases neutrophilic phagocytosis, *b*) determine how long neutrophilic phagocytosis remains lowered after oral administration of carbohydrate, and *c*) test whether fasting (the absence of oral glucose sources) affects neutrophilic phagocytosis.

Methods

Ten subjects (6 females and 4 males) volunteered for the study. Except for one teenage boy, the subjects were between the ages of 20 and 34. In addition, eight patients with abnormal blood glucose levels following glucose loading were also studied. After an overnight fast of approximately 12 hr, each subject was given 100 g carbohydrate. This included glucose, starch, fructose, and sucrose, and the carbohydrate in honey and orange juice. The carbohydrate in honey is comprised of: glucose, 44%; fructose, 52%; sucrose, 2%; and dextrin, 2%; and that of orange juice

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is glucose, 25%; fructose, 25%; and sucrose, 48% (10). Each day of the study, the various carbohydrates were administered, with each subject receiving a different carbohydrate until he had completed the series of six carbohydrate sources. Blood was drawn at 0 (12-hr overnight fast), 0.5, 1, 2, 3, and 5 hr postprandial. The 0.5-hour time period blood was not drawn for all the subjects on all of the carbohydrates. For the 1-, 2-, and 3-hr periods, more than the 10 original subjects participated with fructose, sucrose, honey, and starch. As values for the phagocytic index were similar to those of the 10 subjects, these were also included in the analyses.

Seven subjects ranging from 23 to 51 years old volunteered in the study to test the effect of fasting on phagocytosis. After the 12-hr fast (as above), the subjects drank approximately 8 oz cold water and thereafter they were allowed only water ad libitum for an additional 48 hr, or a total of 60 hr. At the end of 12 hr, instead of receiving 100 g carbohydrate these subjects drank 8 oz cold water; blood samples were collected at the times coinciding with the 0-, 2-, and 5-hr sampling periods of the carbohydrate-fed subjects.

Blood was drawn from the arm between 7 and 8 AM with a heparinized tube for the phagocytic determinations and with an oxalated tube for determinations of plasma glucose (alkaline ferricyanide reducing sugar method), total white cell count, neutrophil count, total red blood cell count, hemoglobin, and hematocrit.

A sample of 0.9 ml freshly drawn blood was mixed in siliconized tubes and stoppers with 0.1 ml saline suspension of *Staphylococcus epidermidis*. This was rotated mechanically end to end at 37 C for 30 min. The phagocytic index, the mean number of bacteria in the first 50 neutrophils viewed under the microscope in slides stained with Wright's stain, is dependent on the relative number of bacteria available for ingestion by the leukocytes. The bacteria concentration was adjusted to give a phagocytic index of approximately 15 on the control fasting blood samples by diluting

the saline suspension to 18% transmittance at a wave length of 420 nm. Each subject served as his own control and the 12-hr fasting value was the control for each subject.

The general linear hypothesis model (11) and linear regression (12) were used for statistical analysis.

Results and discussion

Table 1 shows the total white cell count, neutrophil count, total red blood cell count, hemoglobin, and hematocrit. These values were within the laboratory normal range for both males and females during each of the time periods in which blood was drawn. There were no apparent trends in these parameters following the ingestion of carbohydrates.

Table 2 shows the phagocytic index and plasma glucose of normal human subjects fed different carbohydrates after an overnight fast. The highest phagocytic index was found before ingestion of each carbohydrate. The lowest phagocytic index occurred between 1 and 2 hr postprandial with all the sugars. This drop of approximately 50% in phagocytic activity was significant ($P < 0.001$) as compared with fasting values. In contrast, starch ingestion did not lower the phagocytic activity of leukocytes as did the other carbohydrates and the difference between starch and sugars during the 5-hr postprandial period was highly significant ($P < 0.001$). The results with starch contrasted with the mean averages \pm SE of the other carbohydrates are shown in Fig. 1. Our findings with glucose as shown graphically in Fig. 2 support

TABLE 1
Mean red blood cell count (RBC), white blood cell count (WBC), hemoglobin, hematocrit, and number of neutrophils at each time interval for men and women on the various carbohydrate groups

Parameter measured	Normal range	Fasting	30 min	1 hr	2 hr	3 hr	5 hr
Females (6)^a							
RBC ($\times 10^6$)/ml	4.2-5.5	4.1 \pm 0.1	4.1 \pm 0.1	4.1 \pm 0.1	4.1 \pm 0.1	4.0 \pm 0.1	4.0 \pm 0.1
HGB, g/100 ml	12-15	12.1 \pm 0.2	12.1 \pm 0.2	12.2 \pm 0.2	12.1 \pm 0.1	11.9 \pm 0.2	12.0 \pm 0.2
HCT, %	37-47	36.5 \pm 0.5	36.8 \pm 0.5	37.0 \pm 0.6	36.5 \pm 0.6	35.8 \pm 0.6	36.6 \pm 0.6
WBC ($\times 10^3$)/ml	4.5-10	5.3 \pm 0.2	5.5 \pm 0.3	5.7 \pm 0.2	5.5 \pm 0.2	5.4 \pm 0.2	6.4 \pm 0.3
Neutrophils, % WBC	40-60	43 \pm 6	35 \pm 6	52 \pm 6	50 \pm 7	44 \pm 7	55 \pm 7
Males (4)^a							
RBC ($\times 10^6$)/ml	4.5-6	5.2 \pm 0.0	5.2 \pm 0.0	5.1 \pm 0.1	5.2 \pm 0.1	5.2 \pm 0.1	5.1 \pm 0.1
HGB, g/100 ml	14-16.5	15.1 \pm 0.1	14.8 \pm 0.1	15.9 \pm 0.1	15.0 \pm 0.2	14.7 \pm 0.2	14.8 \pm 0.2
HCT, %	45-52	44.6 \pm 0.3	44.3 \pm 0.3	44.0 \pm 0.3	44.5 \pm 0.5	44.1 \pm 0.5	43.8 \pm 0.5
WBC ($\times 10^3$)/ml	4.5-10	4.7 \pm 0.2	4.4 \pm 0.2	4.5 \pm 0.2	4.7 \pm 0.2	4.9 \pm 0.2	5.4 \pm 0.3
Neutrophils, % WBC	40-60	59 \pm 5	45 \pm 7	49 \pm 6	50 \pm 5	46 \pm 6	53 \pm 6

^a Number of subjects indicated in parentheses.

TABLE 2
Neutrophilic phagocytosis and plasma glucose of human subjects fed different carbohydrates after an overnight fast

Treatment and parameter measured	Time after 100-g carbohydrate oral dose					
	Fasting ^a (10-13) ^b	30 min (5-10)	1 hr (10-13)	2 hr (9-11)	3 hr (9-11)	5 hr (6-10)
Glucose						
Phagocytic index ^c	16.2 ± 0.9 ^{d,e}	13.8 ± 1.3	11.7 ± 0.8	9.6 ± 0.6	12.9 ± 0.6	13.5 ± 0.7
Plasma glucose, mg/dl	86 ± 2 ^d	117 ± 9	97 ± 7	88 ± 7	67 ± 4	77 ± 2
Fructose						
Phagocytic index	15.5 ± 1.0	13.3 ± 2.0	8.5 ± 0.9	9.7 ± 1.2	11.0 ± 0.9	12.8 ± 1.1
Plasma glucose, mg/dl	89 ± 3	96 ± 5	96 ± 2	89 ± 3	87 ± 2	81 ± 3
Sucrose						
Phagocytic index	15.2 ± 1.0	12.1 ± 1.7	8.6 ± 0.9	9.3 ± 0.7	12.5 ± 1.1	15.0 ± 1.3
Plasma glucose, mg/dl	90 ± 2	110 ± 7	88 ± 5	82 ± 2	79 ± 3	80 ± 3
Honey						
Phagocytic index	15.9 ± 0.9	15.9 ± 1.7	9.7 ± 0.9	9.7 ± 0.9	12.4 ± 1.1	12.7 ± 1.0
Plasma glucose, mg/dl	89 ± 1	115 ± 15	89 ± 5	75 ± 3	81 ± 4	87 ± 2
Orange juice						
Phagocytic index	16.6 ± 1.2	12.6 ± 1.0	10.1 ± 1.3	9.6 ± 0.8	13.2 ± 1.4	11.7 ± 0.8
Plasma glucose, mg/dl	89 ± 2	114 ± 9	93 ± 4	86 ± 4	86 ± 2	81 ± 2
Starch						
Phagocytic index	15.7 ± 0.8	17.1 ± 1.3	14.4 ± 0.9	14.1 ± 1.1	14.6 ± 1.3	13.6 ± 0.7
Plasma glucose, mg/dl	88 ± 2	98 ± 7	96 ± 4	83 ± 3	83 ± 3	85 ± 2

^a After an overnight fast of approximately 12 hr. ^b Numbers in parentheses indicate subjects per group for the various time intervals and different carbohydrates. ^c Mean number of bacteria (*Staphylococcus epidermidis*) viewed within each neutrophil. ^d Mean ± standard error. ^e Statistical analyses are given in the text. The significant reduction in phagocytic index ($P < 0.001$) was statistically indistinguishable after feeding each of the various simple carbohydrates and the phagocytic index with the sugars was significantly less ($P < 0.001$) than with starch.

earlier findings that glucose ingestion lowered the phagocytic index (8).

By the use of a general linear hypothesis model that included patient and diet effects, it was found that the phagocytic activity was still significantly lower ($P < 0.001$) at 5 hr postprandial than before feeding and that the return to the control fasting values was similar for each of the sugars. Statistical analyses also provided no evidence that the number of neutrophils are related to the phagocytic index. This would lead to the conclusion that a decreased phagocytic index is associated with impaired function rather than the number of leukocytes. Data from Bybee and Rogers (7) support this conclusion by showing that leukocytes with normal activity phagocytized normally when placed in serum from ketoacidotic diabetic patients, but leukocytes from ketoacidotic patients had impaired phagocytosis even when placed in serum from non-ketoacidotic patients. Evidently, there is a decreased chemotaxis of leukocytes of diabetic patients

that is reversed by insulin (9), but the mechanism of action is not known.

Table 2 also shows the plasma reducing sugar concentrations. Comparable but lower values were obtained by use of the glucose oxidase method. The fasting (zero) values were essentially the same for all groups. This might be expected because 10 values in each group were from the same 10 subjects, taken on different days. The plasma glucose concentration was elevated within 0.5 hr postprandial. The rate of return to fasting levels, including overshoot, appears to vary according to the type of carbohydrate ingested. This may be related to varying rates of absorption of carbohydrates from the gastrointestinal tract and/or varying rates of conversion of ingested carbohydrates to glucose. The degree of overshoot of the blood glucose control mechanisms may also play a role according to the specific carbohydrate ingested.

No significant relationship was found between plasma glucose levels and phagocytic

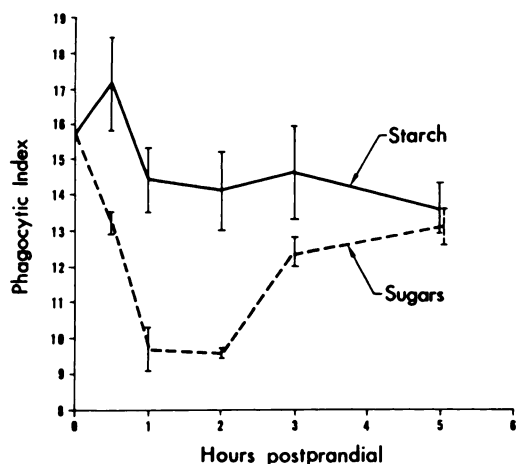


FIG. 1. Comparison of the effects of sugars and starch on the phagocytic index after an overnight fast in normal human subjects ingesting 100 g carbohydrate. The values for starch \pm standard error (solid line) and for the sugars \pm standard error of their means (broken line) are derived from Table 2. The sugars include glucose, fructose, sucrose, honey, and orange juice.

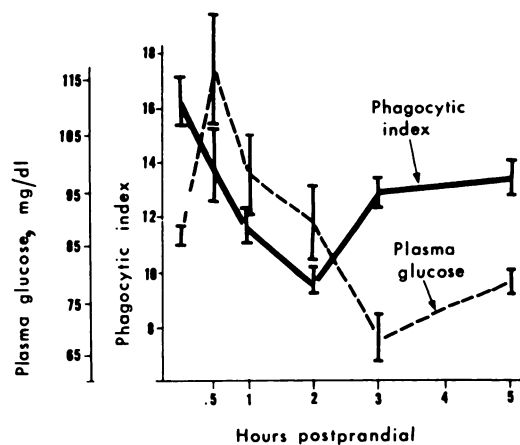


FIG. 2. The phagocytic index of neutrophils (solid line) and blood glucose concentration (broken line) in blood of normal human subjects after ingesting 100 g glucose after an overnight fast. The vertical lines represent the standard deviations.

index in the normal subjects by use of a general linear hypothesis that tested the 30-min to 1-hr glucose level changes (highest values) with changes in phagocytic index between fasting and 2 hr (lowest values) after feeding. Kijack et al. (8) reported an inverse relationship between plasma glucose and phagocytic activity in diabetic patients. In our studies, 100-g doses of

carbohydrate were employed and these amounts may be above the threshold response levels for glucose and phagocytic index in normal subjects.

Table 3 shows the phagocytic index and plasma glucose of eight patients having a glucose tolerance curve of the diabetic type. The lowest phagocytic index values of the entire study were obtained with this group for the fasting through the 3-hr postprandial period. The highest blood glucose levels were also obtained with this group. Therefore, decreased phagocytosis may be the result of elevated blood glucose levels. Our results are in agreement with Kijack et al. (8) relating the decreased phagocytic activity to the increased glucose concentration in diabetic patients. These observations are consistent with those of others (4-7) who found lower phagocytic activity in neutrophils of diabetic patients as compared with normal subjects.

In the fasting study the mean red blood cell count, hemoglobin, hematocrit, white blood cell count, and number of neutrophils at each fasting time interval for men and women were variable but mostly within laboratory normal limits. There were no obvious trends with fasting in any of these parameters.

Figure 3 graphically shows the mean phagocytic index and blood glucose levels for the seven subjects at the various time intervals during fasting. During the 5-hr period in the morning of the 1st day of fast, there was no significant change in either the phagocytic index or the blood concentration. This time period corresponds to the same 5-hr period after ingestion of carbohydrates as shown in the experiments above. This shows that the drop in phagocytic activity after carbohydrate administration was due to dietary effects and not to a

TABLE 3
Plasma glucose level and neutrophilic phagocytosis in eight patients given an oral 100-g dose of glucose

Time interval	Plasma glucose	Phagocytic index
Fasting	102 \pm 5 ^a	12.4 \pm 0.7 ^a
30 min	187 \pm 17	9.1 \pm 0.9
1 hr	231 \pm 26	7.2 \pm 0.8
2 hr	185 \pm 30	5.3 \pm 0.7
3 hr	126 \pm 23	9.7 \pm 1.1

^a Mean \pm standard error of the mean.

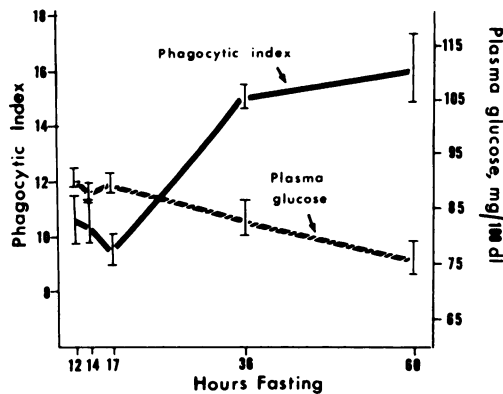



FIG. 3. The phagocytic index of neutrophils (solid line) and glucose concentration (broken line) in blood of human subjects during a 60-hr fast. The 12-hr fasting period corresponds to the overnight fast (or 0 hours) in Figs. 1 and 2.

phenomenon normally occurring during this time of the day.

Statistical analysis of the entire fasting period shows that the within groups regression coefficient was significantly greater than zero ($P < 0.001$), which indicated a significant increase in phagocytic index due to the 36- and 60-hr fast. The glucose concentration during this time obviously did not drop to such a low level as to inhibit phagocytosis by depleting the leukocytes of their energy source. The maintenance of lowered blood glucose levels in the lower portion of the normal fasting range by limited fasting may provide a clinical method of enhancing the body's defenses against infection by increased phagocytosis.

Our studies were intended to determine the effects of carbohydrates on neutrophilic phagocytosis and the duration of these effects. We found that sugars impaired the neutrophils to engulf bacteria. The greatest reduction in phagocytosis occurred at approximately 2 hr postprandial but the effects were still evident 5 hr after ingestion. In contrast, a fast of 36 to 60 hr enhanced the phagocytic capacity of neutrophils. This implicates carbohydrates in the enhancement and inhibition of neutrophilic phagocytosis. These observations become meaningful when it is recognized that phago-

cytosis is the rate limiting step in the reduction of viable organisms (3). Thus, diet may play a key role in the control of resistance to infection.

We are presently studying the effects of various levels of carbohydrates as well as the possible role of protein, fat, and ordinary meals on neutrophilic phagocytosis. Another unanswered question is whether the effects on phagocytosis are due to the specific carbohydrate directly or to generalized mechanisms that control glucose levels. 

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