Effect of Age, Hours Since Last Food, Time of Day, and Ketonuria on 1-Hour Glucose Tolerance

NANCY PHILLIPS, PhD, and THOMAS DUFFY, MD

AGE, time interval since last food, and time of day have been shown to affect the results of the 1-hour glucose tolerance test (*1–3*). Decreased glucose tolerance in nondiabetic persons with ketonuria has also been reported (*4*). As part of an effort to establish screening norms, the effect of these variables on 1-hour glucose tolerance was further analyzed in a population of women having multiphasic health examinations. The results of this analysis confirm and enlarge upon the findings of the earlier studies.

The data in this report were generated during the 13-month period December 1968–December 1969 by the Automated Multitest Laboratory (AML) at the Kaiser Foundation Hospital in Walnut Creek, Calif. This multiphasic testing facility was especially built for a longitudinal study on the medical effects of the oral contraceptive drugs. The vast majority of examinees, therefore, are women of reproductive age.

Dr. Phillips is an epidemiologist, formerly with the contraceptive drug study, and Dr. Duffy is a clinical researcher and physician, Department of Internal Medicine, Kaiser-Permanente Medical Center, Walnut Creek, Calif.

This study was supported by the Department of Health, Education, and Welfare, National Institute of Child Health and Human Development, Public Health contract No. PH 43-67-1346. Tearsheet requests to Dr. Nancy Phillips, Cardiovascular Research Institute, University of California School of Medicine, San Francisco, Calif. 94122.

Materials and Methods

Study subjects. The study subjects were white female subscribers to the Kaiser Foundation Health Plan residing in the Walnut Creek service area. This area comprises a number of suburban communities within commuting distance of Oakland and San Francisco. None of the women included in the analysis were known to be pregnant at the time of the multiphasic examination, and all took and retained the full glucose challenge. Known diabetics were excluded from the analysis. Four previously undiagnosed but overt diabetics were also excluded because their extremely high postchallenge values tended to distort the summary statistics.

The mean age of the 6,692 women studied was 37.9 years. Although the womens' ages ranged from 18 through 72 years, all but 1.5 percent were 20–54 years of age.

One-third of the women were taking an oral contraceptive at the time of the examination. The 1-hour postchallenge serum glucose levels of users

of oral contraceptives were approximately 10 mg. per 100 ml. higher, on the average, than those of nonusers of the same age. This difference between users of contraceptive drugs and nonusers, however, had no effect on the relationship of 1-hour glucose tolerance with age, interval since last food, time of day, or ketonuria that is described in this report.

The prevalence of ketonuria and the distribution in the times of challenge and the hours since last food were the same for both users and nonusers. Although the proportion of the women who were contraceptive drug users was inversely related to age, the coefficients of the regression of 1-hour serum glucose on age, computed separately for users and nonusers, were not discernibly different from that computed for the two groups combined.

Glucose tolerance screening test. Each woman was challenged with 75 grams of glucose in a 6-ounce aqueous solution of artificial lemon-lime flavor, to which 2 ounces of crushed ice had been added. The examinations were scheduled between 9 a.m. and 1 p.m., and the glucose load was administered right after the patient registered for her examination. It usually took less than 5 minutes to drink the glucose. The time of the challenge was recorded at the last swallow.

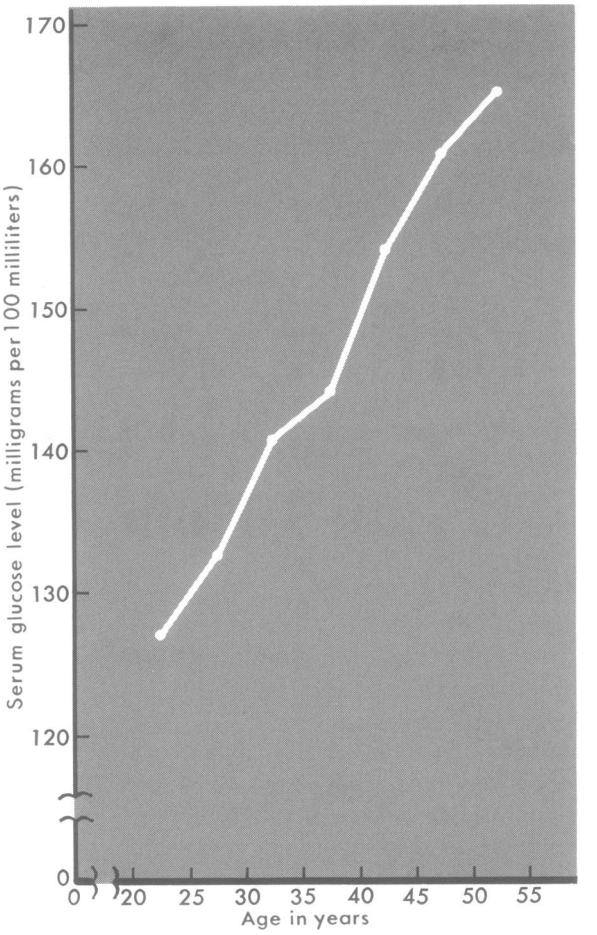
Table 1. The mean and standard deviation of 1-hour serum glucose levels (mg. per 100 ml.), by age

Age in years	Number of women	Serum glucose	
		Mean	S.D.
20–24	558	127.1	30.9
25–29	919	132.8	35.1
30–34	1,042	140.4	38.1
35–39	1,133	144.5	40.9
40–44	1,156	154.4	41.8
45–49	1,060	160.7	41.1
50–54	731	165.3	43.7

Table 2. The mean and standard deviation of 1-hour serum glucose levels (mg. per 100 ml.), by hours since last food when challenged

Hours since last food	Number of women	Serum glucose	
		Mean	S.D.
Less than 1	58	142.3	43.8
1	85	135.5	41.8
2	137	136.7	43.3
3	152	144.7	42.9
4	1,377	161.2	41.0
5	689	169.8	39.3
6	129	168.4	42.0
7–8	27	144.6	40.0
Fasting overnight	3,991	139.3	38.6

Figure 1. Mean 1-hour serum glucose level by age

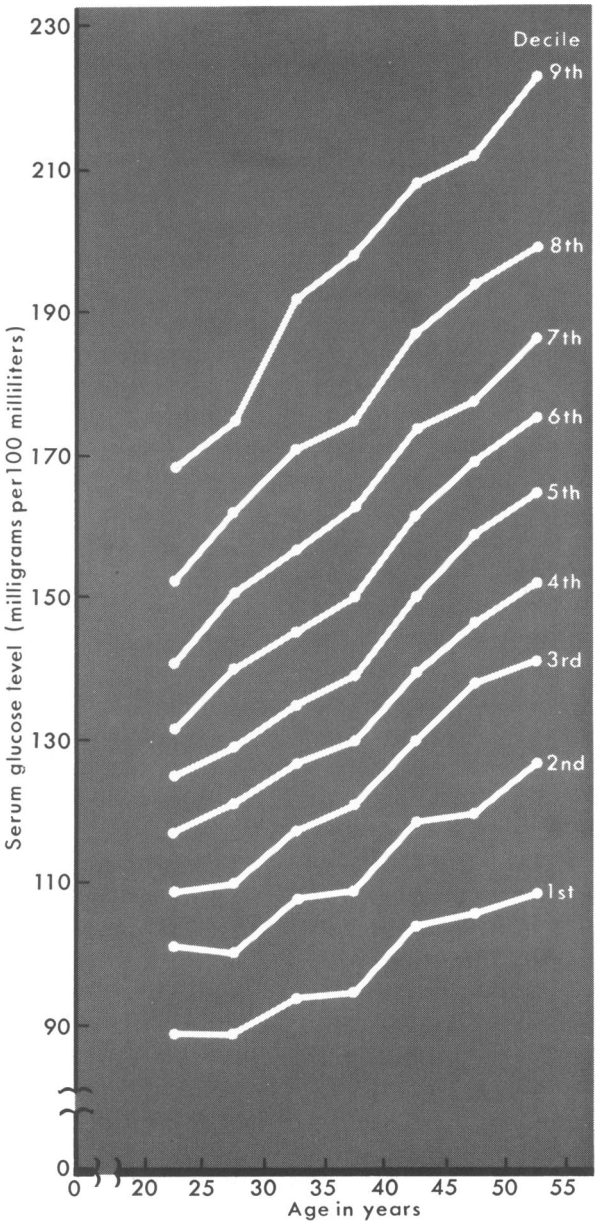


Examinees received no instruction on a preparatory diet. They were, however, instructed to take nothing by mouth except water for at least 4 hours preceding their appointment. Approximately 6 percent of the women did not heed this instruction. Because the examinations were scheduled for the morning hours, 60 percent of the women were tested after an overnight fast.

Sixty minutes after the challenge, a sample of venous blood was drawn. The serum was separated within 30 minutes of the venepuncture, and the glucose concentration was determined by the ortho-toluidine method. This method is based on a color reaction given by aldosaccharides with ortho-toluidine in glacial acetic acid containing thiourea.

Urine ketones. Immediately following the venepuncture, the patient was asked for a urine specimen. The presence of ketone bodies in the specimen was then determined by a Ketostix reagent strip. The test was read as positive only

Figure 2. Deciles of 1-hour serum glucose levels by age



if there was a decided color change. Trace reactions, therefore, were considered negative.

Statistical analysis. The distribution of 1-hour glucose tolerance, like many physiological variables, was skewed to the right. The skewness was too slight, however, to invalidate the use of the arithmetic mean as a description of central tendency or of the standard deviation to describe dispersion. Furthermore, the degree of skewness, as evaluated by the difference between the mean and median, was unrelated to age or the other variables studied. Certain quartiles are also used to describe changes in the location and dispersion of the glucosemia distribution associated with increasing age.

Findings

Age. As noted earlier, the effective range of ages covered by these data was 20–54 years. Within this range, 1-hour serum glucose values increased linearly with age at the rate of 1.3 mg. per 100 ml. per year. The age-specific mean values are given in table 1 by 5-year age intervals. These values are also plotted in figure 1.

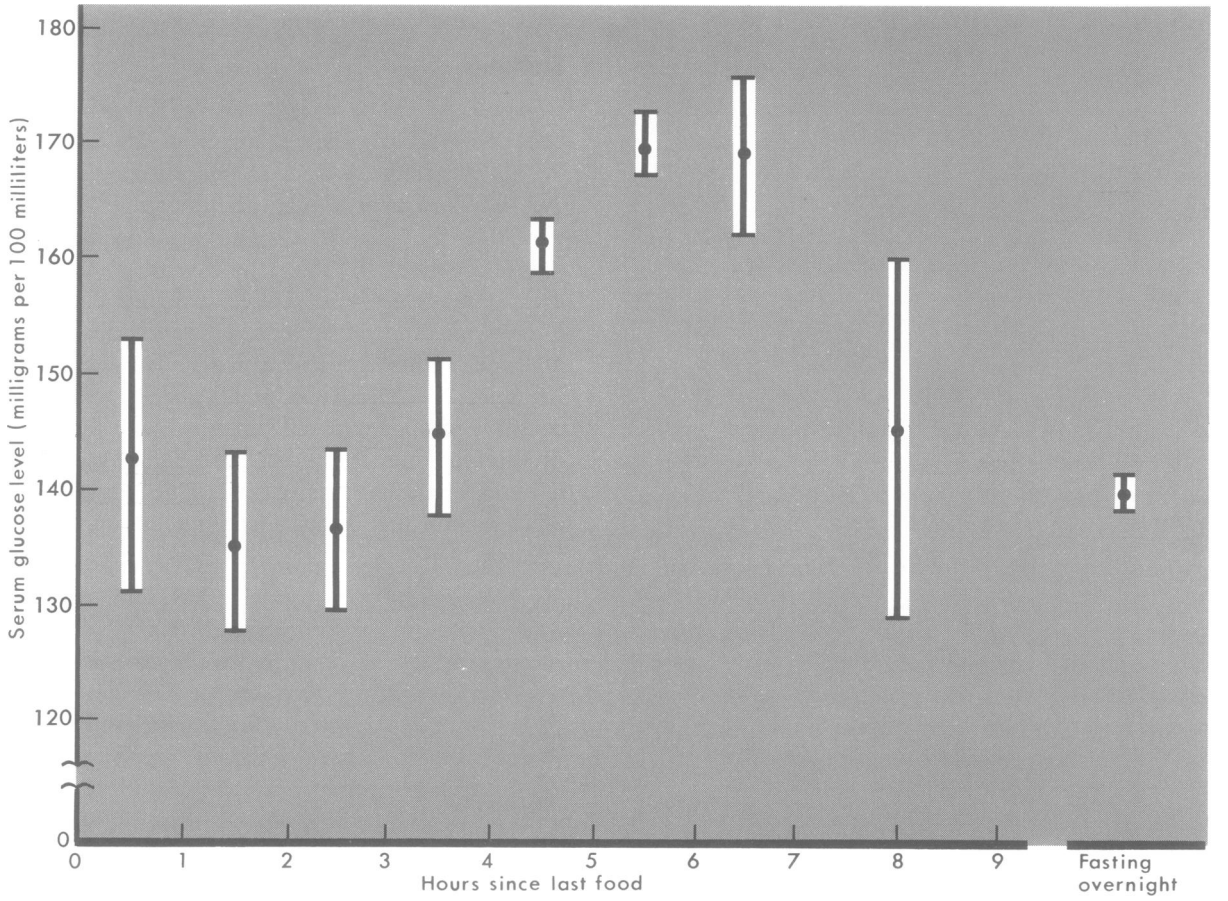
Not only the mean, but the entire distribution of values shifted upward with age. Thus, as shown in figure 2, the location of all deciles of the distribution also increased linearly with age, but at different rates. In general, the higher the decile, the greater the rate of increase. The regrssion coefficients of the decile locations on age are as follows:

Decile	*Average increase in location per year of age (mg. per 100 ml.)*
1st	0.7
2d	1.0
3d	1.2
4th	1.2
5th	1.4
6th	1.5
7th	1.5
8th	1.6
9th	1.8

Table 3. The mean and standard deviation of 1-hour serum glucose levels (mg. per 100 ml.), by time of challenge and hours since last food

Hours since last food and time of challenge	Number of women	Serum glucose	
		Mean	S.D.
Less than 4 hours:			
9:00–10:29	143	127.9	38.0
10:30–11:59	170	142.0	44.0
12:00–1:30	119	151.8	43.7
Overall	432	140.0	43.0
4 hours:			
9:00–10:29	82	154.0	46.8
10:30–11:59	651	159.9	41.2
12:00–1:30	644	163.4	40.0
Overall	1,377	161.2	41.1
5 or 6 hours:			
9:00–10:29	22	161.1	36.0
10:30–11:59	207	169.7	41.4
12:00–1:30	589	169.8	39.2
Overall	818	169.6	29.7
Fasting overnight:			
9:00–10:29	1,922	133.9	27.8
10:30–11:59	1,548	143.6	39.0
12:00–1:30	521	146.3	38.2
Overall	3,991	139.3	38.6

Figure 3. Mean 1-hour serum glucose (with 95 percent confidence interval) by hours since last food when challenged

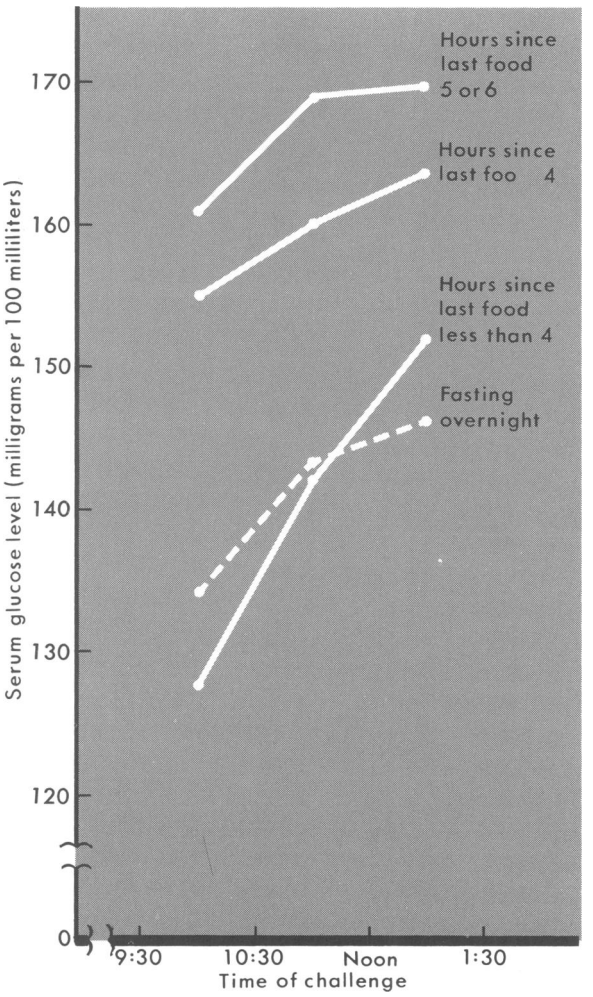


The heterogeneity of the decile regression coefficients reflects the increased dispersion of the glucosemia distribution with age. The interquartile range, for example, widened from 40 mg. per 100 ml. at ages 20–24 years to 58 mg. per 100 ml. at 50–54 years. Thus, the variability among examinees in 1-hour serum glucose also tended to increase with age.

Hours since last food. The mean 1-hour values are shown in table 2 and figure 3 by hours since last food when challenged. As can be seen, the postchallenge levels increased as the interval since the last food lengthened from 1 to 6 hours, and then they began to decrease. Because the number of examinees who last ate 7 or 8 hours earlier was small, these data are insufficient to describe exactly what happens as the interval lengthens beyond 6 hours. It is clear, however, that the postchallenge values of women tested after an overnight fast were much more similar to those of women who ate less than 4 hours before the test than to those of women who ate 4, 5, or 6 hours earlier.

The mean 1-hour values by hours since last food and the time of day when challenged are shown in table 3. Women who ate less than 4 hours before the challenge were grouped together because their mean 1-hour values were not significantly different from one another. Women challenged 5 or 6 hours after eating are also grouped together because they, too, are relatively homogeneous in glucose response. As shown in figure 4, 1-hour serum glucose levels tended to increase as the morning progressed into early afternoon; but, regardless of the time of challenge, the 1-hour values of fasting women were much lower than those of women who had eaten 4 or 5 to 6 hours before the challenge. The postchallenge levels of fasting women averaged 16 to 20 mg. per 100 ml. lower than those of women who ate 4 hours before

Figure 4. Mean 1-hour serum glucose by time of challenge and hours since last food (HSLF)



the challenge and were tested at the same time of day. The difference between fasting women and those who ate 5 to 6 hours earlier was somewhat greater, averaging approximately 25 mg. per 100 ml. Examinees challenged less than 4 hours after food, however, were not discernibly different from fasting women in glucose response regardless of the time of challenge.

Ketonuria. The prevalence of ketonuria is shown in table 4 by the time of day when the urine specimen was collected. Testing for urine ketones was not started until 2 months after the multiphasic screening program began, so that Ketostix test data are missing on approximately 1,000 women. Among those tested, 7.5 percent had ketone bodies in their urine. The prevalence of ketonuria, however, was strongly related to the time of day, increasing from less than 1 percent to more than 16 percent as the morning progressed into early afternoon. This increase in prevalence with time of day appeared to accelerate during the late morning and early afternoon hours (fig. 5).

Variation in the prevalence of ketonuria with the time of day has not, to our knowledge, been described before. The reason for the observed variation is not immediately apparent, but it cannot be attributed to the lengthening fast of women tested later in the day who had not eaten since the previous evening. The same curvilinear increase in the prevalence of ketonuria with time of day was seen regardless of the interval since last food. The prevalence of ketonuria was unrelated to age.

The mean postchallenge serum glucose levels for women with and without ketonuria by hours since last food and time of challenge are shown in table 5. In all time of challenge categories and hours since last food categories, the postchallenge values of women with ketonuria were, on the average, markedly higher than those of women with negative Ketostix tests. The observed overall mean difference was 29 mg. per 100 ml. The mean difference in the serum glucose response of ketonurics and nonketonurics, however, tended to be less marked in midday tests than in morning tests.

Time of day. The mean 1-hour values of women with negative Ketostix tests shown in table 5 are plotted in figure 6 against time of challenge by the interval since last food. Although the effect from the increasing prevalence of ketonuria has been removed, the postchallenge values still tended to increase somewhat between early morning and midday regardless of the interval since the last

Table 4. Prevalence of ketonuria, by time of day

Time of day	Number of women	Positive Ketostix test	
		Number	Percent
10:00–10:29	329	5	1.5
10:30–10:59	664	20	3.0
11:00–11:29	727	24	3.3
11:30–11:59	717	25	3.5
12:00–12:29	772	45	5.8
12:30–12:59	738	64	8.7
1:00–1:29	704	75	10.6
1:30–1:59	632	106	16.8
2:00–2:29	317	53	16.7
Overall	[1] 5,644	422	7.5

[1] Includes 44 women for whom time of challenge and hours since last food are unknown.

Figure 5. Prevalence of ketonuria (with 95 percent confidence interval) by time of day

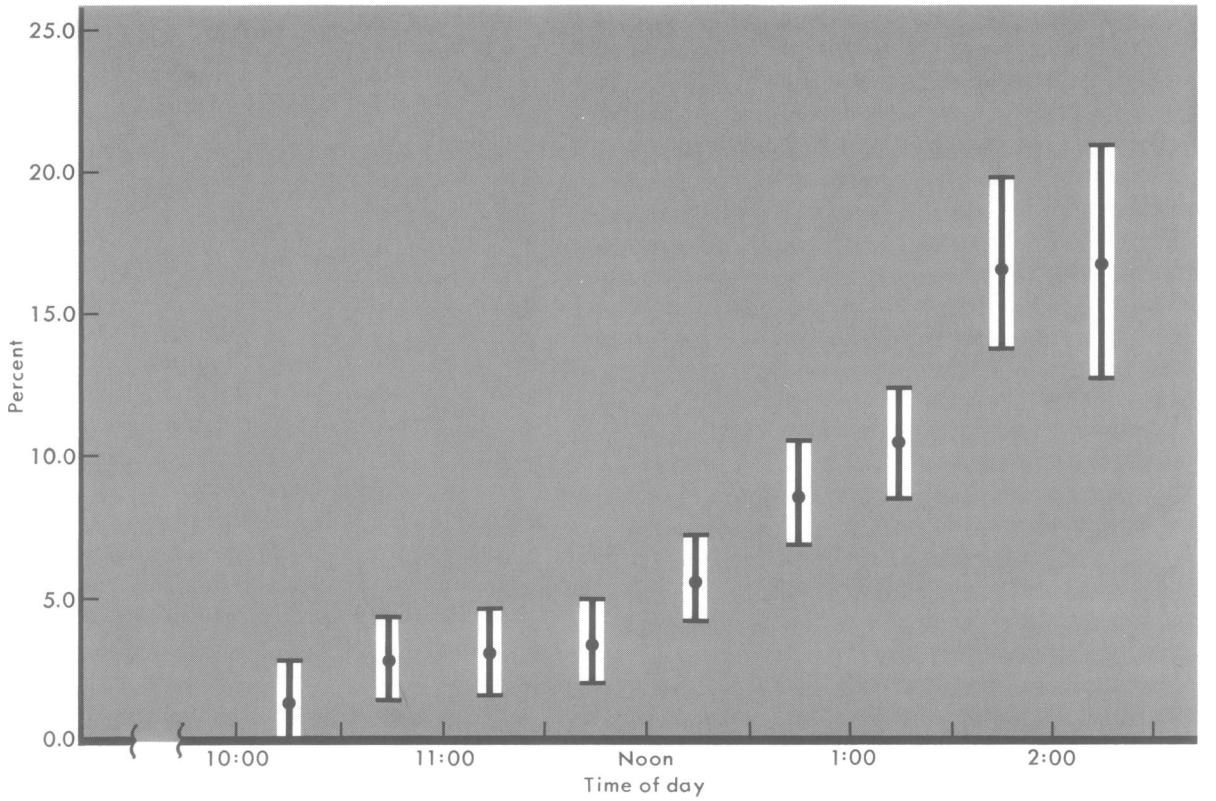
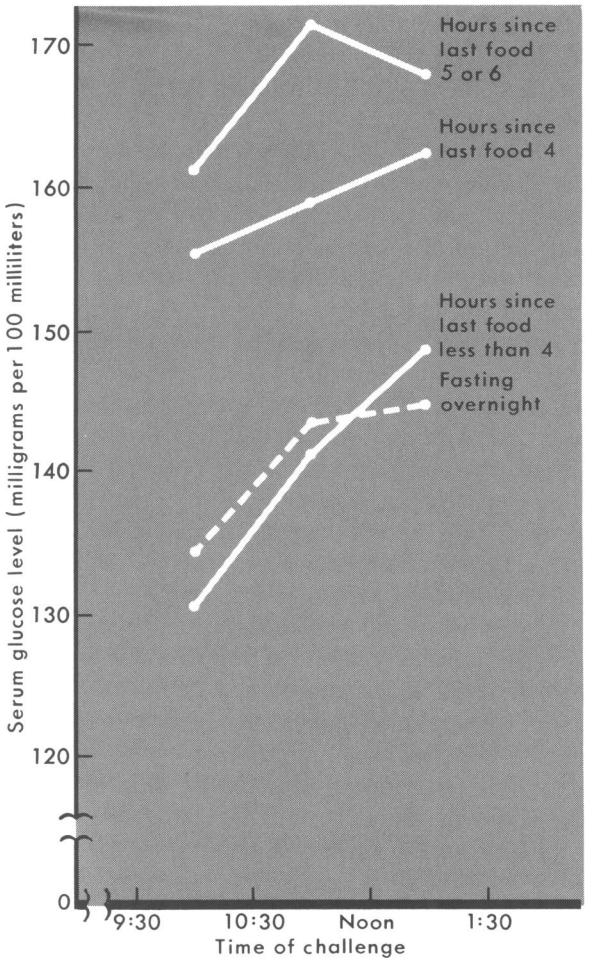


Table 5. The mean and standard deviation of 1-hour serum glucose levels (mg. per 100 ml.), by result of Ketostix test, and the mean difference between ketonurics and nonketonurics (plus or minus 95 percent confidence interval), by time of challenge and hours since last food

Time of challenge and hours since last food	Positive Ketostix test			Negative Ketostix			Difference between ketonurics and nonketonurics		
	Number of women	Serum glucose		Number of women	Serum glucose				
		Mean	S.D.		Mean	S.D.	Mean	±	95 percent confidence interval
9:00–10:29									
<4	0	.....		98	130.6	38.1	.....		
4	1	.....		70	155.7	48.0	.....		
5 or 6	0	.....		17	161.4	26.3	.....		
Fasting	48	164.4	41.4	1,457	135.4	38.3	29.0	±	11.9
10:30–11:59									
<4	4	202.0	45.2	153	141.0	43.9	61.0	±	44.8
4	38	190.8	36.2	554	159.2	40.8	31.6	±	12.0
5 or 6	20	192.2	40.9	154	171.1	40.8	21.1	±	19.0
Fasting	72	171.4	48.0	1,224	143.8	38.4	27.6	±	11.3
12:00–1:30									
<4	12	178.2	48.8	123	148.7	43.3	19.5	±	28.6
4	70	181.1	38.4	488	162.4	40.4	18.7	±	9.1
5 or 6	82	182.0	37.2	439	168.6	39.8	13.4	±	8.9
Fasting	58	166.9	34.7	383	144.3	39.0	22.6	±	9.7
All examinees	422	176.8	40.8	5,222	147.7	41.2	29.1	±	4.0

Figure 6. Mean 1-hour serum glucose by time of challenge and hours since last food (HSLF) for women with negative Ketostix tests



food. The mean difference between the early morning and midday tests results, adjusted for hours since last food, is 9 mg. per 100 ml.

Discussion

Reports of population surveys consistently show that glucose levels among examinees tend to increase as the age of the persons tested increases (*1, 2, 5–9*). The accumulated evidence suggests that the age gradient is more marked at the 1-hour sampling point than at any other, particularly when a standard but unphysiological load of glucose is used rather than a test meal.

Two population studies, the National Health Examination Survey (*1*) and the Tecumseh Community Health Study (*2*), are comparable to the Walnut Creek data in that both were based on determinations from blood samples collected 1 hour following a standard challenge. The age gradient observed in the Walnut Creek data (1.3 mg. per 100 ml. per year) is the same as that reported in the Tecumseh study (13 mg. per 100 ml. per decade) which used a 100-gram glucose challenge, but somewhat greater than that noted in the national survey (10 mg. per 100 ml. per decade), which was based on a 50-gram challenge.

The rise with age in the location of the lower deciles of the glucosemia distribution, which was demonstrated in the Walnut Creek data and also reported in the Tecumseh study, suggests that some loss of glucose tolerance with age is typical of most persons. The heterogeneity of the decile regressions observed in the Walnut Creek data further suggests that the age-related changes are not uniform among individual persons. That is, as people age, they differ more and more from one another in their response to a glucose challenge. Whether this is because of differences among persons in the rate of aging or because of an increase in the prevalence of unrecognized disease with age, or both, is speculative. That some loss of glucose tolerance with age is to be expected indicates, however, that test norms for young adults may not be appropriate for older persons.

A marked increase in the 1-hour values as the interval between the last food and the challenge lengthened from 1 to 6 hours was also observed in the Tecumseh data. Because most of the tests were done in the afternoon or evening hours, the Tecumseh study provided no data on the postchallenge response of fasting examinees. Variation in the postchallenge response by hours since last food was described in the National Health Examination Survey only for persons challenged less than 4 hours after eating.

It is known that the incremental response to a glucose challenge is less when the challenge is preceded by a prior one or by a recent meal (*10,11*). Because this Staub-Traugott effect is gone within 4 hours, it is generally believed that the response of a patient challenged after this interval without food will be similar to that of one who is fasting. The data in this report indicate that this assumption is not tenable. For whatever reason, the postchallenge values of women tested 4 to 6 hours after eating were clearly elevated above those of women who had been fasting. Because the postchallenge values of women tested less than 4 hours after eating were much more similar to those obtained from fasting women, these data indicate that it is better to screen pa-

tients postprandially rather than to instruct them to eat nothing for at least 4 hours before the examination.

Aside from certain metabolic diseases, such as diabetes, ketonuria has been associated with carbohydrate deprivation (*12*). Carbohydrate deprivation has long been recognized as a cause of elevated glucose tolerance curves. To avoid misdiagnosis from carbohydrate deprivation in glucose tolerance testing, O'Sullivan has recommended routine testing for urine ketones (*4*). Information on dietary regimen was not collected in the AML (Kaiser Foundation's Automated Multitest Laboratory) questionnaire, but there is no reason to believe that the diets of AML examinees tested in the early morning differed in carbohydrate content from those of women tested later in the day. The marked increase in the prevalence of ketonuria with time of day that was observed in these data raises doubt as to what a positive test implies. The meaning of the high postchallenge values which accompanied the ketonuria, therefore, is also unclear.

Because the time of day covered by the data in this report was limited to the morning and midday hours, the effect of the time of day on the screening test cannot be fully assessed. In the National Health Examinations, the postchallenge levels were somewhat higher after the midday meal than after the morning or evening meals. No significant differences were observed in the Tecumseh survey between afternoon and evening tests. Although true diurnal periodicity is of great biological interest, it would seem of relatively little importance when screening for glucose intolerance.

Conclusion

One-hour glucose tolerance tests on 6,692 women were analyzed for variation in relation to age, hours since last food, ketonuria, and time of day. Known diabetics were excluded from the analysis. All subjects were challenged with 75 grams of glucose between the hours of 9 a.m. and 1:30 p.m. Serum glucose was determined by the ortho-toluidine method. The urine specimen was collected immediately after the 1-hour postchallenge blood specimen was drawn, and the presence of urine ketones was evaluated with Ketostix.

The age range effectively covered by the data was 20–54 years. Within this range, 1-hour serum glucose increased linearly with age at the rate of 1.3 mg. per 100 ml. per year. The location of all deciles of the distribution also rose linearly with age, but the rate of increase was greater for the higher than for the lower deciles. These cross-sectional observations suggest that some dimunition in glucose tolerance at the 1-hour sampling point may be typical of most people as they age but that the rate of deterioration varies from person to person.

The length of time between the last meal and the glucose challenge had a marked effect on the test result. Among women who had eaten on the morning of the examination, the mean 1-hour value rose more than 30 mg. per 100 ml. as the interval since the last food lengthened from 1 to 6 hours. The 1-hour values of examinees challenged after an overnight fast, however, were much more similar to those of women challenged less than 4 hours after eating than to those obtained from women whose last food had been 4 to 6 hours earlier, regardless of the time of challenge. Thus, these data indicate that a screening test 4 to 6 hours after a meal is not comparable to one administered in a fasting state.

The prevalence of ketonuria increased curvilinearly from less than 1 percent to more than 16 percent as the morning progressed into early afternoon. The increased prevalence of ketonuria with the time of day was independent of the interval since last food. Regardless of the time of challenge and the interval since the last food, the 1-hour postchallenge serum glucose values of women with ketonuria were considerably higher than those of women with negative Ketostix tests. The overall average difference was 29 mg. per 100 ml.

When the effect of hours since last food and of ketonuria were removed, the midday test results tended to be higher than those from the early morning. The difference, however, was small, averaging 9 mg. per 100 ml.

REFERENCES

(*1*) Gordon, T.: Glucose tolerance of adults: United States 1960–1962. Vital and Health Statistics. PHS Publication No. 1000, ser. 11, No. 2. U.S. Government Printing Office, Washington, D.C., May 1964.

(*2*) Hayner, N. S., Kjelsberg, M. O., Epstein, F. H., and Francis, T.: Carbohydrate tolerance and diabetes in a total community, Tecumseh, Michigan: 1. Effects of age, sex, and test conditions on one-hour glucose tolerance in adults. Diabetes 14: 413–423, July 1965.

(*3*) Hayner, N. S., Waterhouse, A. M., and Gordon, T.: The one-hour oral glucose tolerance test. Vital and Health Statistics. PHS Publication No. 1000,

ser. 2, No. 3, U.S. Government Printing Office, Washington, D.C., July 1963.
(4) O'Sullivan, J. B., Grant, M. E., and Francis, J. O'S.: Glucose tolerance test standardization simplified by urinary ketone testing. Public Health Rep 80: 220–224, March 1965.
(5) Jorde, R.: The diabetes survey in Bergen, Norway, 1956. Norwegian University Press, Bergen-Oslo, 1962.
(6) Nilsson, S. E., et al.: The Kristianstad survey 1963–1964—studies in a normal adult population for variation and correlation in some clinical, anthropometric, and laboratory values, especially the peroral glucose tolerance test. Acta Med Scand (supp. 428) 177: 1–42 (1965).
(7) Boyns, D. R., et al.: Oral glucose tolerance and related factors in a normal population sample: I. Blood sugar, plasma insulin, glyceride, and cholesterol measurements and the effects of age and sex. Br Med J 148: 1–4 (1969).
(8) Kaufman, B. J., Grant, D. R., and Moorhouse, J. A.: An analysis of blood glucose values in a population screened for diabetes mellitus. Can Med Assoc J 100: 692–698 (1969).
(9) Lauvaux, J. P., and Staquet, M.: Oral glucose tolerance test: study of influence of age on response to standard oral 50-gram glucose load. Diabetologia 6: 412–419 (1970).
(10) Hammon, L., and Hirschman, I. I.: Studies of blood sugar: IV. Effects upon the blood sugar of the repeated ingestion of glucose. Bull Johns Hopkins Hosp 30: 306–308 (1919).
(11) Metz, R., and Friedenberg, R.: Effect of repetitive glucose loads on plasma concentrations of glucose, insulin, and free fatty acids: paradoxical insulin responses in subjects with mild glucose intolerance. J Clin Endocrinol Metab 30: 602–608 (1970).
(12) Azar, G. J., and Bloom, W. L.: Similarities of carbohydrate deficiency and fasting. Arch Intern Med 112: 338–343, September 1963.

PHILLIPS, NANCY (Kaiser-Permanente Medical Center, Walnut Creek, Calif.) and DUFFY, THOMAS: ***Effect of age, hours since last food, time of day, and ketonuria on 1-hour glucose tolerance. Health Services Reports, Vol. 87, August–September 1972, pp. 649–657.***

One-hour glucose tolerance tests from 6,692 women having multiphasic health examinations were analyzed for variation in relation to age, interval since last food, ketonuria, and time of day. The rise with age in mean 1-hour glucose observed in earlier studies was accompanied by a linear increase in the location of all deciles of the glucosemia distribution, but the rate of increase was greater at the higher than the lower deciles.

The previously reported marked increase in 1-hour values as the interval between the last food and the challenge lengthened from 1 to 6 hours was confirmed, but women tested after an overnight fast were far more similar in glucose response to women challenged less than 4 hours after eating than to women who had eaten 4 to 6 hours earlier. As expected, women with ketonuria had elevated 1-hour serum glucose levels.

The prevalence of ketonuria, however, increased markedly with time of day, regardless of the interval since the last food. Findings on the effect of the time of day on the 1-hour glucose tolerance test have been inconsistent, although these data showed a small but definite tendency for the 1-hour values to increase between early morning and midday, a tendency which was independent of the interval since last food and the prevalence of ketonuria.