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Reproducibility of Oral Glucose Tolerance Tests with Three Different Loads*

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Summary. Oral glucose tolerance tests (5 × 50 g, 5 × 75 g and 5 × 100 g glucose load) were carried out in 20 healthy male volunteers on 15 separate occasions at intervals of 3 or 4 days. The mean age of the group of younger men was 39.4 years and of the group of older men, 68.2 years. One and two hours after the administration of 100 g glucose the whole group showed a significantly smaller individual variation of blood sugar response than after both 50 and 75 g. When subdividing the groups of young and old men the same trend was noted. Oral glucose tolerance tests with a 100 g glucose load showed a greater reproducibility than those with a 50 and a 75 g glucose load.

Key words: Oral glucose tolerance tests, different glucose loads, variation of blood sugar response, reproducibility.

Introduction

For many years it has been known that glucose tolerance tests are not stable on repetition. Under similar conditions the same individuals may show dissimilar blood glucose levels at coincident times after glucose ingestion.

A review of the literature shows that there have been few investigations with more than duplicate determinations of blood glucose within a short time and most of the tests were only performed with a single oral glucose load (Bock, Schneider, Gilbert, 1926; Hale-White and Payne, 1926; Lennox, 1927; Glassberg, 1930; John, 1934; Freeman *et al.*, 1942; Hofstatter *et al.*, 1945; Mosenthal and Barry, 1950; Unger, 1957; McDonald *et al.*, 1965; Castro *et al.*, 1970).

This report presents a study of the reproducibility of oral glucose tolerance tests with three different loads.

Materials

20 healthy male volunteers without known diabetes or any other condition that might affect their blood sugar levels were investigated. 15 oral glucose tolerance tests were carried out on each subject at intervals of 3 or 4 days. The glucose load consisted of 50, 75 and 100 g, each dose being given five times to each subject (Table 1).

Three subjects were only tested on five occasions with a 50 g glucose load. One of them became ill, another one was excluded at his own request and a third was found to be diabetic.

The 11 younger men were administration officials of Düsseldorf with a mean age of 39.4 years. The other 9 men were living in a home for the aged, their mean age being 68.2 years. Body weight deviated from the ideal by a mean of + 15.4% for the young group and + 2.2% for the old group (Metropolitan Life Insurance Company tables, 1959; Knußmann *et al.*, 1972).

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Methods

Glucose tolerance tests were performed under comparable conditions. No drugs were allowed for the entire test period. The mean carbohydrate intake of about 250 g was ensured for the old men by communal feeding in the old peoples home. The young men were asked to adhere carefully to a prescribed diet high in carbohydrates. A light occupation in a sitting position was permitted during the trial. Smoking was prohibited. Glucose loading was given in the form of an adequate amount of "Gluco-50" test drink ("Boehringer") dissolved in a specified amount of water (50 g glucose in 220 ml, 75 g in 330 ml and 100 g in 440 ml).

Capillary blood samples were taken from the ear lobe at 8.00 a.m. following a ten-hour fasting period and 60 and 120 min after the glucose load. Furthermore, blood samples were taken 180 min after a 100 g glucose load from 10 of the young men. Blood sugar was determined by Auto-Analyzer (Technicon).

Results

Fasting blood sugar levels (Table 2)

The average of mean individual fasting blood sugar levels was lower in the young men (83.7 mg per 100 ml) than the old (91.4 mg per 100 ml). The average of individual variation coefficients[1], however, was similar for both groups (25.2 for the young and 25.8 for the old).

If one excludes the subject ("no. 20") who developed diabetes, the average of the individual variation coefficients for the old men becomes less than that for the young. The average of mean individual fasting blood sugar levels, however, remains greater than that of the younger group, though the difference is not significant.

1 The variation coefficient indicates the relation between standard deviation and mean blood sugar.

Table 1. *Group of subjects*

Number of cases	Age (years)	Mean age (years)	Percentage deviation from ideal weight (per cent)	Average percentage deviation from ideal weight (per cent)	Number of oral glucose tolerance tests. Amount glucose given: 50 g	75 g	100 g
1	34		+27.0		5	5	5
2	35		+17.8		5	5	5
3	36		+13.0		5	5	5
4	37		+17.0		5	5	5
5	37		+19.5		5	—	—
6	40	39.4	− 1.0	+15.4	5	5	5
7	40		+10.0		5	5	5
8	40		+10.0		5	5	5
9	43		+ 7.0		5	5	5
10	45		+30.0		5	5	5
11	46	52.4	+19.0	+9.5	5	5	5
12	59		+11.0		5	5	5
13	64		− 2.2		5	5	5
14	69		+23.0		5	5	5
15	69		−23.0		5	5	5
16	69	68.2	+31.0	+ 2.2	5	5	5
17	69		− 2.0		5	5	5
18	70		+ 4.0		5	5	5
19	71		−10.0		5	—	—
20	74		−12.0		5	—	—

Table 2. *Fasting blood sugar levels*

Number of subjects	Number of fasting blood sugar determinations	Average of mean individual fasting blood sugar levels mg per 100 ml	Student's test P	Average of individual variation coefficients	Student's test P
1	15				
2	15				
3	15				
4	15				
5	5				
6 "young men"	15	83.7±0.9		25.2±2.3	
7	15				
8	15		5−10%		> 50%
9	15		5−10%		
10	15				> 50%
11	15	87.0±2.0		25.5±1.8	
12	15				
13	15				
14	15	87.3±1.8		24.5±2.9	
15	15				
16 "old men"	15	91.4±4.5		25.8±3.0	
17	15				
18	15				
19	5				
20	5				

Values ± behind the mean values are standard errors of mean.
P means probability.
The variation coefficient indicates the relation between standard deviation and mean blood sugar.

Blood sugar levels 60 min after glucose administration (Table 3)

The average of mean individual blood sugar levels *increases* with increasing glucose load 60 min after glucose ingestion. On the contrary, the average of individual variation coefficients *decreases* with increasing glucose amounts.

The differences between the mean glucose response after the various glucose amounts (50 and 75, 50 and 100 and 75 and 100 g) are not significantly different by Student's test. However, the average of individual variation coefficients after the 50 and 100 g glucose loads differed with a probability of 1−2 per cent.

If one separates the young group from the old

(Table 4) the same trend is noted; but a significant difference between average variation coefficients due to the 50 and 100 g glucose loads is only found in the old men.

glucose ingestion. The average of individual variation coefficients, however, *decreases* from 50 to 100 and from 75 to 100 g glucose load. There are significant differences between average variation coefficients due

Table 3. *Blood sugar levels 60 min after glucose ingestion (all subjects)*

Amount glucose given g	Average of mean individual blood sugar levels (5 determinations for each subject) mg per 100 ml	Student's test P		Average of individual variation coefficients	Student's test P		Number of subjects
50	152.44±10.89			30.78±1.88			20
		> 50%			> 50%		
75	153.91±10.64		> 50%	28.18±3.51		1–2%	17
		> 50%			20–25%		
100	160.51± 9.30			22.81±2.64			17

Explanations see Table 2.

Table 4. *Blood sugar levels 60 min after glucose ingestion for the two age groups*

Amount glucose given g	Average of mean individual blood sugar levels mg per 100 ml	Student's test P		Average of individual variation coefficients	Student's test P		Number of subjects
young men							
50	121.53± 8.02			31.91±3.40			11
		> 50%			> 50%		
75	130.24± 8.39		20–25%	30.25±4.92		25–50%	10
		25–50%			25–50%		
100	142.56± 8.89			25.84±3.17			10
old men							
50	190.22±14.24			29.40±3.01			9
		> 50%			25–50%		
75	187.71±16.09		> 50%	25.23±5.05		5%	7
		> 50%			25–50%		
100	186.14±14.39			18.49±4.30			7

Explanations see Table 2.

Table 5. *Blood sugar levels 120 min after glucose ingestion (all subjects)*

Amount glucose given g	Average of mean individual blood sugar levels (5 determinations for each subject) mg per 100 ml	Student's test P		Average of individual variation coefficients	Student's test P		Number of subjects
50	92.96±7.00			33.88±4.09			20
		2–5%			> 50%		
75	116.02±8.00		< 0.1%	34.30±3.82		5%	17
		10–20%			2–5%		
100	130.20±5.53			23.34±2.97			17

Explanations see Table 2.

Blood sugar levels 120 min after glucose administration (Table 5)

Glucose given in a dose of 50 g produces a different response than the 75 and the 100 g loads. The differences are significantly in the whole group two hours after the glucose load.

The average of mean individual blood sugar levels *increases* with increasing glucose load 120 min after to amounts of 50 and 100 as well as 75 and 100 g glucose.

The separate groups of young and old men (Table 6) show the same trend in comparison with the whole group; but there are significant differences between average blood sugar responses after 50 and 100 g glucose load and between average variation coefficients due to 75 and 100 g glucose load only in the young group.

Variances for the various groups of different glucose loads compared by F-test (Table 7)

If one compares the variances[2] for the groups given different glucose loads, a lower result for the variances is found with the 100 g glucose load than with 50 and 75 g glucose.

There is no significant association between the variances of the 50 and 75 g glucose loads; but there is a significant relation between the variances due to 50 and 100 and to 75 and 100 g glucose loads 60 and 120 min after glucose administration. This was found for the whole group.

Comparing the separate groups of young and old men (Table 8), the results for the old men are similar to those of the whole group. For young men there are significant differences only between the variances of 50 and 75 and of 50 and 100 g glucose 120 min after the glucose load.

Table 6. *Blood sugar levels 120 min after glucose ingestion for the two age groups*

Amount glucose given g	Average of mean individual blood sugar levels mg per 100 ml	Student's test P		Average of individual variation coefficients	Student's test P		Number of subjects
young men							
50	71.24±3.56			29.81±2.65			11
		5–10%			10–20%		
75	94.64±6.06		< 0.1%	38.08±4.20		10–20%	10
		10–20%			1–2%		
100	116.36±4.56			23.04±3.68			10
old men							
50	119.51±8.87			38.85±8.48			9
		25–50%			25–50%		
75	146.57±8.46		10–20%	28.91±6.98		20–25%	7
		> 50%			> 50%		
100	149.97±6.55			23.75±5.28			7

Explanations see Table 2.

Table 7. *Variances for the various groups of different glucose loads compared by F-test for all subjects*

F P	F P	Variances 60 min after glucose ingestion	Glucose load g	Variances 120 min after glucose ingestion	F P	F P
		636	50	464		
	1.07 >5%				1.12 >5%	
1.82 0.5–1%		595	75	522		1.52 2.5–5%
	1.71 1–2.5%				1.70 1–2%	
		349	100	306		

The variance of each glucose load represents a composed measure of variance by model of variance within the groups of analysis of variance.
Explanations see Table 2.

Blood sugar levels 180 min after a 100 g glucose load (Table 9)

Blood sugar determinations 180 min after a 100 g glucose dose were only carried out in 10 young men. The average of mean individual blood sugar values was 75.0 mg per 100 ml. The average of individual variation coefficients was 46.5, a considerably higher value than that 60 and 120 min after a 100 g glucose load.

Correlations between corresponding blood sugar responses of different tests

Correlations between corresponding blood sugar levels of the various tests with the same glucose load also can be a measure of reproducibility. This method was used to find out if the reproducibility changes with the number of tests.

Correlations between two successive tests do not show a trend of changing with the number of tests. No improvement of the reproducibility was apparent. The

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2 Variances were calculated by the following equation:

$$var_g = \left(\sum_{k=1}^{n_g} SAQ_k\right) / df_g$$

df_g = degrees of freedom $= n_g \times (t-1)$
g = load
k = characteristic number
n_g = number of subjects with the load "g"
SAQ_k = sum of square deviations of 5 tests with the same load of subject "k"
t = number of tests with the same load = 5.

total correlation matrix, comparing one test with another, does not show a clear trend either for the old or young men, or for the 1, 2 and 3 h values.

Conclusion

According to our results, there are smaller variation coefficients of the glucose response after a 100 g glucose load than after 50 and 75 g. Furthermore, the range of corresponding standard deviations shows generally smaller values for 100 g than for 50 and 75 g glucose loads. A comparison of the variances chiefly points to the fact that there is a smaller variation of blood sugar responses after 100 g glucose intake.

If reproducibility is considered a measure of quality for the tolerance tests, a 75 g glucose load (e.g. used by Köbberling and Creutzfeldt 1970) does not prove an advantage over a 50 g glucose load. It seems that only the 100 g dose effects an impulse of such intensity to make the organism react in a highly specific way.

This fact prompts us to recommend performing oral glucose tolerance tests with a 100 g glucose load. However, the above-mentioned results should further be confirmed on larger groups of subjects in particular women, diabetics and obese patients.

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Table 8. *Variances for the various groups of different glucose loads compared by F-test for the two age groups*

F P	F P	Variances 60 min after glucose ingestion	Glucose load g	Variances 120 min after glucose ingestion	F P	F P
young men						
	1.30	410	50	113	3.07	
1.12	> 5%				< 0.1%	2.02
> 5%	1.47	535	75	348	1.52	1–2.5%
	> 5%	365	100	229	> 5%	
old men						
	1.07	912	50	893	1.12	
1.82	> 5%				> 5%	1.52
0.5–1%	1.71	681	75	770	1.70	2.5–5%
	1–2.5%	325	100	417	1–2.5%	

Explanations see Table 7.

Table 9. *Blood sugar levels 180 min after 100 g glucose load*

Number of subjects	Average individual blood sugar levels (5 determinations for each subject) mg per 100 ml	Average of mean individual blood sugar levels mg per 100 ml	Individual variation coefficients	Average of individual variation coefficients
1	64.8 ± 8.5		29.5	
2	73.2 ± 12.9		39.5	
3	61.2 ± 8.3		30.2	
4	89.2 ± 31.3		78.5	
5	— — — — —		— —	
6 "young men"	73.2 ± 10.2	75.0 ± 3.5	31.3	46.5 ± 5.2
7	60.0 ± 9.7		36.2	
8	90.0 ± 22.5		55.9	
9	82.0 ± 16.2		44.0	
10	84.8 ± 21.0		55.3	
11	71.6 ± 20.6		64.2	

Explanations see Table 2.

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