# Dilution of the 75-g oral glucose tolerance test increases postprandial glycemia: implications for diagnostic criteria

*Research*

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## Abstract

**Background:** Dilution has been noticed to increase the glycemic response to various sugars, including glucose. This effect may contribute to the poor reproducibility of the oral glucose tolerance test (OGTT). To test this hypothesis we assessed the effect of diluting a 75-g OGTT on 2-hour postprandial blood glucose based diagnostic outcomes, incremental glycemia and area under the glucose curve.

**Methods:** On 3 different occasions, 10 subjects (mean age 40 [and standard error of the mean (SEM) 3.2] years; mean body mass index 27.2 [and SEM 1.2] kg/m$^2$) without previously diagnosed dysglycemia were given a 300-mL, 600-mL or 900-mL 75-g OGTT in random order. The protocol followed the American Diabetes Association's guidelines. Finger-prick capillary blood samples were obtained at fasting and then 15, 30, 45, 60, 90 and 120 minutes after the start of the test.

**Results:** At 30, 45 and 60 minutes, incremental glycemic concentrations were significantly higher with the 900-mL meal (means [and SEMs]: 4.9 [0.4] mmol/L, 5.1 [0.6] mmol/L and 4.6 [0.8] mmol/L, respectively) than with the 600-mL (means [and SEMs]: 4.0 [0.3] mmol/L, 4.2 [0.6] mmol/L and 3.6 [0.7] mmol/L, respectively) and the 300-mL meals (means and [SEMs]: 3.8 [0.5] mmol/L, 4.0 [0.5] mmol/L and 3.2 [0.6] mmol/L, respectively) ($p < 0.05$). The same was true for peak incremental blood glucose, regardless of time ($p < 0.05$). The area under the curve for the 900-mL meal (mean [and SEM] 404 [57] min·mmol/L) was significantly higher than for the 600-mL (mean [and SEM] 331 [51] min·mmol/L) and 300-mL meals (mean [and SEM] 280 [48] min·mmol/L) ($p < 0.05$). No other significant differences were observed.

**Interpretation:** Dilution of the 75-g OGTT will likely not affect current screening practices that use 2-h postprandial glucose levels as the basis for diagnosis. It may, however, bias the interpretation of older criteria that rely on intermediate time points because these midpoints appear to be sensitive to alterations in the total volume of the meal ingested.

Diabetes and intermediate classifications of hyperglycemia are on the rise.[1] It is important, therefore, to have a reliable and valid test to diagnose new cases. Both the Canadian and American Diabetes Associations in their most recent reports[2,3] recommended the preferential use of fasting plasma glucose values for diagnosis. The use of the oral glucose tolerance test (OGTT), which had also been previously recommended, was discouraged. One of the reasons for this was the poor reproducibility of the test compared with that of fasting plasma glucose levels.

Differences in the total volume of water ingested in the OGTT may explain some of the variability. The World Health Organization[4] and the American Diabetes Association[3] instruct that the OGTT meal be given as 75 g of glucose dis-

solved in 250–300 mL of water, whereas the National Diabetes Data Group[5] and the Canadian Diabetes Association[2] instruct that it be given as 75 g of glucose in a minimum of 300 mL; that is, it may be given at any volume over 300 mL. In addition, many of our patients have complained of the poor palatability[6] of the test and resulting nausea and dizziness[7] and have often requested additional water to increase its overall acceptability.

Glycemia may be affected by these volume differences. We recently demonstrated that a 3-fold increase in the volume of a 25-g oral glucose meal increased glycemia by 19.8%.[8] Others,[9] using a 50-g dose of glucose found that a 3-fold increase in volume raised peak blood glucose significantly, by 14% in pregnant women. However, volume increases in the 75-g OGTT have not been evaluated; we therefore chose to investigate the effects of a 2- and 3-fold increase in the volume of a 300-mL 75-g OGTT on glycemic concentrations, 2-hour postprandial glucose level diagnostic outcomes and the area under the blood glucose curve. The 600-mL and 900-mL volumes were chosen to cover a large physiological range.

## Methods

The 5 men and 5 women (means [and standard errors of the mean (SEM)]: age 40 [3.2] years; body mass index 27.2 [1.2] kg/m$^2$, fasting glucose 4.9 [0.2] mmol/L) who participated in the study were recruited from faculty and the student body at the University of Toronto and through hospital advertisements; written informed consent was obtained from each. All participants were healthy, medication free and had never been diagnosed with dysglycemia; 2 were smokers, and 6 were overweight by body mass index criteria (> 27 kg/m$^2$). The study was approved by the Research Ethics Committee at St. Michael's Hospital, Toronto, Ont.

Each participant received three 75-g glucose Glucodex OGTT meals (Technilab Inc., Chambly, Que.) in random order: one at each of 300 mL (undiluted, osmolarity 1.39 mol/L), 600 mL (300 mL of tap water added, osmolarity 0.69 mol/L) and 900 mL (600 mL of tap water added, osmolarity 0.46 mol/L).

The protocol was designed to match the American Diabetes Association guidelines for the administration of the OGTT. Participants attended St. Michael's Hospital on 3 different mornings after a 10- to 16-hour overnight fast. They were instructed to maintain the same diet and exercise patterns the evening before each test and to consume a minimum of 150 g of carbohydrate each day over the 3 days prior to each test. To ensure that these instructions were followed, participants completed a questionnaire detailing information about their diets and lifestyle patterns before each session. Upon commencement of the test a Monoejector Lancet device (Owen Mumford Ltd., Woodstock, Oxon, England) was used to obtain a fasting finger-prick capillary blood sample (approximately 250 μL) from each participant. One of the 3 test meals was then given, with instructions to drink it over a period of exactly 5 minutes. Finger-prick blood samples were obtained again at 15, 30, 45, 60, 90 and 120 minutes after the start of the meal. No smoking or physical activity was permitted before or during the test.

All blood samples, collected in tubes containing fluoride oxalate, were immediately frozen at –20°C and analyzed within 3 days of collection. The glucose concentration of each was determined by the glucose oxidase method using a YSI 2300 Stat glucose/L-lactate analyzer, model 115 (Yellow Springs Instruments, Yellow Springs, Ohio).

Blood glucose curves were plotted as the incremental change in blood glucose over time, and the positive incremental area under the blood glucose curve was calculated geometrically for each participant, ignoring areas below the fasting value.[10] Incremental glucose concentrations were used to control for differences in baseline fasting levels between the treatments; 2-hour absolute blood glucose values were compared with Canadian Diabetes Association diagnostic criteria for impaired glucose tolerance (IGT) and diabetes (i.e., glucose cutoff values: diabetes mellitus ≥ 11.1 mmol/L, IGT 7.8–11.0 mmol/L, normal glucose tolerance < 7.8 mmol/L).[2] Criteria for venous plasma samples were used because the cutoff values for venous plasma and capillary whole blood glucose are the same.[4,5] Interactive and independent effects of volume dose (300, 600 and 900 mL) and time (0, 15, 30, 45, 60, 90 and 120 min) on incremental change in blood glucose concentrations were assessed with a repeated measures 2-way analysis of variance (ANOVA) adjusted for multiple pairwise comparisons with the Newman Keuls procedure. Differences in peak blood glucose rise and area under the curve between the 300-, 600- and 900-mL OGTT meals were assessed using repeated measures 1-way ANOVA adjusted for multiple pairwise comparisons with the Newman Keuls procedure. All results were expressed as means and SEMs and considered statistically significant if $p < 0.05$.

## Results

All participants were able to follow the study protocol without difficulty. Questionnaires revealed that for each subject evening dietary patterns and activities, amount of

**Table 1: Indices of blood glucose following a 75-g oral glucose tolerance test at 3 treatment dilutions**

| Treatment dilution, mL | Mean fasting blood glucose (and SEM), mmol/L | Mean incremental change in blood glucose (and SEM) at each time interval, mmol/L | | | | | | 2-h diagnostic value (and SEM), mmol/L* | Area under the curve (and SEM), min·mmol/L |
|---|---|---|---|---|---|---|---|---|---|
| | | 15 min | 30 min | 45 min | 60 min | 90 min | 120 min | | |
| 300 | 5.1 (0.2) | 2.1 (0.4) | 3.8 (0.5) | 4.0 (0.5) | 3.2 (0.6) | 1.6 (0.6) | 0.5 (0.6) | 5.5 (0.3) | 280.0 (47.9) |
| 600 | 4.9 (0.2) | 2.7 (0.4) | 4.0 (0.3) | 4.2 (0.6) | 3.6 (0.7) | 2.6 (0.8) | 0.6 (0.7) | 5.5 (0.4) | 331.1 (51.0) |
| 900 | 4.8 (0.2) | 2.9 (0.3) | 4.9 (0.4) † | 5.1 (0.6) † | 4.6 (0.8) † | 3.1 (0.7) | 0.7 (0.7) | 5.4 (0.4) | 404.3 (57.2)† |

Note: SEM = standard error of the mean.
*Some of the 2-h diagnostic values may not equal the values given for fasting blood glucose plus the 120-min mean incremental change because all values were rounded to the nearest tenth.
†Significantly different from other treatments in the same column ($p < 0.05$).

sleep, reported feelings of health and well-being, mode of transportation to the clinic and weight were consistent between sessions. Subjects were able to consume all test meals in the time allotted, and there were no complaints about the volume of any of the tests. One exception was a subject who complained of a headache following the 300-mL OGTT. No differences were observed between men and women in response to the treatments.

The 900-mL glucose level for 1 participant (8.8 mmol/L) was diagnostic for IGT, but the 600-mL and 300-mL results for that person were not. Similarly, for another participant the 600-mL result was diagnostic for diabetes (11.8 mmol/L), but this was not confirmed by the 900-mL and 300-mL tests, both of which were diagnostic for IGT (9.9 mmol/L and 9.0 mmol/L, respectively). The results of all 3 of the OGTT tests for the remaining 8 subjects were negative for impaired glucose tolerance and diabetes. According to the criteria of the Canadian Diabetes Association and the American Diabetes Association that require 2 abnormal glucose values to confirm a diagnosis, these data indicate that only 1 of the 10 participants had impaired glucose tolerance.

Table 1 and Fig. 1 show the incremental changes in glycemic concentrations at 0, 15, 30, 45, 60, 90 and 120 minutes following the consumption of the 300-mL, 600-mL and 900-mL OGTT meals. The effects of dilution and time on blood glucose levels were significantly independent ($p < 0.01$) with no interaction ($p = 0.41$). Pairwise comparisons showed that incremental changes in glycemic concentrations at 30, 45 and 60 minutes for the 900-mL meal (means [and SEMs]: 4.9 [0.4] mmol/L, 5.1 [0.6] mmol/L and 4.6 [0.8] mmol/L, respectively) were significantly higher than both the 600-mL (means [and SEMs]: 4.0 [0.3] mmol/L, 4.2 [0.6] mmol/L and 3.6 [0.7] mmol/L, respectively) and the 300-mL (means and [SEMs]: 3.8 [0.5] mmol/L, 4.0 [0.5] mmol/L and 3.2 [0.6] mmol/L, respectively) meals ($p < 0.05$). Incremental peak blood glucose rise, calculated irrespective of time, was also significantly higher for the 900-mL meal (mean [and SEM] 5.7 [0.6] mmol/L) than the 600-mL (mean [and SEM] 5.0 [0.5] mmol/L) and 300-mL (mean [and SEM] 4.6 [0.4] mmol/L) meals ($p < 0.05$). No other significant differences in glycemic concentrations were observed at any other time interval, including the diagnostically relevant 2-hour time point.

There was a significant difference between the means of the areas under the curves for the 300-, 600- and 900-mL OGTTs ($p = 0.006$) (Table 1 and Fig. 2). Pairwise comparisons indicated that the area under the curve for the 900-mL meal (mean [and SEM] 404 [57] min·mmol/L) was significantly greater than for the 600-mL (mean [and SEM] 331 [51] min·mmol/L) and 300-mL meals (mean [and SEM] 280 [48] min·mmol/L) ($p < 0.05$). No other significant differences were observed.

## Interpretation

This preliminary study suggests that both the 3-fold dilution of the 75-g OGTT from 300 mL to 900 mL and the a 1.5-fold dilution from 600 mL to 900 mL significantly increased postprandial glycemia. The same was not true for a 2-fold dilution from 300 mL to 600 mL. These findings are consistent with those of our previous study in which we diluted 25 g of oral glucose, sucrose and fructose solutions 3-fold.[8] They are also in agreement with the findings of other studies in which both liquid[9] and solid test meals[11,12] were diluted 3-fold or greater.

The mechanism by which volume amplifies postprandial glycemia is likely similar to that described previously.[11] An increase in the volume[13] or decrease in the osmolality[14] of a

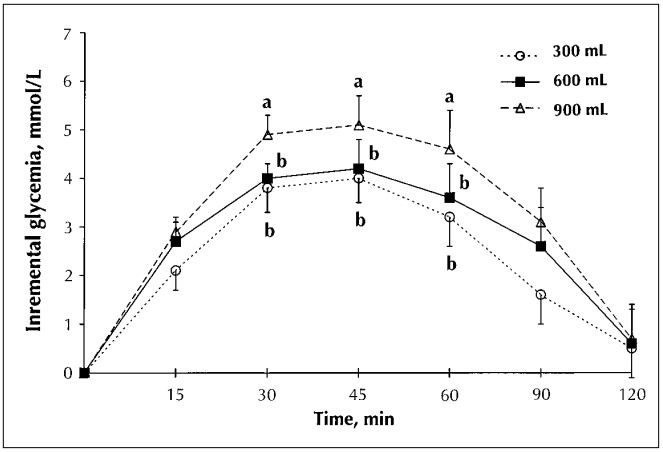


**Fig. 1: Glycemic responses to 75-g oral glucose tolerance test at 300 mL, 600 mL and 900 mL over time in 10 subjects with previously undiagnosed dysglycemia. Values are means and standard errors of the means; different letters indicate a significant difference between treatments ($p < 0.05$).**

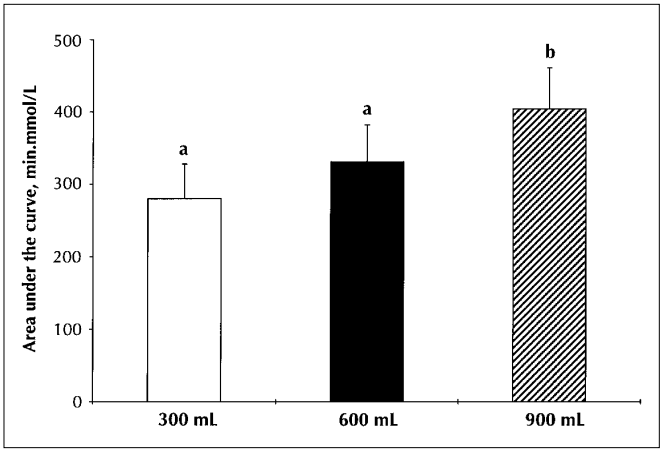


**Fig. 2: Comparison of the positive incremental area under the blood glucose curve for the 300-mL, 600-mL and 900-mL volume doses of a 75-g oral glucose tolerance test. Values are means and standard errors of the means; different letters indicate a significant difference between treatments ($p < 0.05$).**

meal may result in an increase in the rate of gastric emptying and in a subsequent increase in glycemia.[15] We believe the timing of glycemic differences observed on the present study to be consistent with this hypothesis. Findings of other related studies offer further support. It was twice observed that the faster an OGTT meal is emptied from the stomach, the higher the resulting postprandial glycemia level.[16,17] Schwartz and coworkers[9] also attributed a significant rise in glycemic concentrations at 30 minutes and fewer cases of nausea following a diluted 50-g tolerance test to a faster rate of gastric emptying.

These results, have implications for the reproducibility of the OGTT. The 30%, 14% and 19.8% differences in postprandial glucose after the dilution of 75-g (present study), 50-g[9] and 25-g[8] tolerance tests, respectively, suggest that alterations in volume may be contributing to the reported poor reproducibility of the test. Our observations that the 900-mL meal, in the case of one subject, and the 600-mL meal, in the case of another, produced false-positive 2-hour results may offer additional support. Differences in incremental changes, however, were seen only at the peak blood glucose rise and intermediate time intervals (i.e., 30, 45 and 60 min). Alterations in volume also appeared to have the least effect on incremental change in glycemic levels at 2 hours ($p = 0.97$). The likelihood, therefore, that dilution will affect the 2-hour-based diagnostic criteria and lead to misdiagnoses seems low.

It is nevertheless possible that some of the earlier reports of poor reproducibility of the test may be attributable to a volume effect. In addition to 2-hour glucose, the 1979 National Diabetes Data Group guidelines[5] relied on intermediate glycemic values for diagnosis. As we alluded, these points appear more sensitive to changes in volume than the 2-hour postprandial glycemia levels, indicating that a diagnostic vulnerability may have existed. Our data may lend support to abandoning the use of these values in subsequent established protocols for the test.[2,3,4]

Further study is required before we can be confident about exactly how much of the variation seen with the 75-g OGTT can be explained by differences in volume . Studies should be conducted to assess whether the present findings hold true in groups with different glucose tolerances and whether 1 dilution has superior reproducibility over another. Further exploration of a gastric-emptying link is also warranted.

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