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Brief Report—Endocrine Care

Validation of a Novel Method for Determining the Renal Threshold for Glucose Excretion in Untreated and Canagliflozin-treated Subjects With Type 2 Diabetes Mellitus

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Context: The stepwise hyperglycemic clamp procedure (SHCP) is the gold standard for measuring the renal threshold for glucose excretion (RT_G), but its use is limited to small studies in specialized laboratories.

Objective: The objective of the study was to validate a new method for determining RT_G using data obtained during a mixed-meal tolerance test (MMTT) in untreated and canagliflozin-treated subjects with type 2 diabetes mellitus (T2DM).

Design: This was an open-label study with 2 sequential parts.

Setting: The study was performed at a single center in Germany.

Patients: Twenty-eight subjects with T2DM were studied.

Interventions: No treatment intervention was given in part 1. In part 2, subjects were treated with canagliflozin 100 mg/d for 8 days. In each part, subjects underwent an MMTT and a 5-step SHCP on consecutive days.

Main Outcome Measures: For both methods, RT_G was estimated using measured blood glucose (BG) and urinary glucose excretion (UGE); estimated glomerular filtration rates were also used to determine RT_G during the MMTT. The methods were compared using the concordance correlation coefficient and geometric mean ratios.

Results: In untreated and canagliflozin-treated subjects, the relationship between UGE rate and BG was well described by a threshold relationship. Good agreement was obtained between the MMTT-based and SHCP-derived RT_G values. The concordance correlation coefficient (for all subjects) was 0.94; geometric mean ratios (90% confidence intervals) for RT_G values (MMTT/SHCP) were 0.93 (0.89–0.96) in untreated subjects and 1.03 (0.78–1.37) in canagliflozin-treated subjects. Study procedures and treatments were generally well tolerated in untreated and canagliflozin-treated subjects.

Conclusions: In both untreated and canagliflozin-treated subjects with T2DM, RT_G can be accurately estimated from measured BG, UGE, and estimated glomerular filtration rates using an MMTT-based method. **(*J Clin Endocrinol Metab* 98: E0000–E0000, 2013)**

Plasma glucose (PG) is filtered by the glomerulus and reabsorbed in the proximal tubule via the sodium-dependent glucose transporters, SGLT2 and SGLT1 (1). The relationship between PG and renal glucose filtration, reabsorption, and excretion is generally described as a threshold-type relationship (2) and the renal threshold for

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Abbreviations: BG, blood glucose; CI, confidence interval; $CrCl_{12h}$, measured 12-hour creatinine clearance; eGFR, estimated glomerular filtration rates; GMR, geometric mean ratio; MMTT, mixed-meal tolerance test; PG, plasma glucose; RT_G, renal threshold for glucose excretion; SGLT, sodium-dependent glucose transporter; SHCP, stepwise hyperglycemic clamp procedure; T2DM, type 2 diabetes mellitus; UGE, urinary glucose excretion.

glucose excretion (RT_G) is often reported as 180–200 mg/dL (10–11 mM) in healthy subjects (2–4).

SGLT2 inhibitors are emerging as potential antidiabetic therapies (5, 6). In diabetic rats, the SGLT2 inhibitor canagliflozin lowered mean RT_G from 415 to 94 mg/dL (23–5 mM) (7).

The availability of a simple method to estimate RT_G would facilitate investigation of factors regulating renal glucose transport. The gold-standard stepwise hyperglycemic clamp procedure (SHCP) method can only be applied in specialized laboratories. A new method for estimating RT_G using measurements obtained under standard clinical trial conditions has been used to characterize the effects of canagliflozin on RT_G (8, 9). This study compared RT_G values obtained using the new method during a mixed-meal tolerance test (MMTT) with those obtained using SHCP in untreated and canagliflozin-treated subjects with type 2 diabetes mellitus (T2DM).

Materials and Methods

Subjects

Eligible subjects were men and women aged 18 to 65 years with T2DM, body mass index of 20 to 39.9 kg/m^2, glycated hemoglobin of 7.0% to 10.0%, on stable metformin dose or no antihyperglycemic medications, with fasting blood glucose (BG) of 144 to 270 mg/dL (8–15 mM). Subjects participated in either part 1 or part 2 (not both).

This study was conducted at 1 center in Germany. The protocol and amendment were approved by an Independent Ethics Committee. All subjects gave written informed consent, in accordance with the Declaration of Helsinki, following institutional guidelines, and in compliance with Good Clinical Practices and regulatory requirements.

Design

This was an open-label study in untreated (part 1) or canagliflozin-treated (part 2) subjects. In part 1, subjects entered the clinical research unit on day −1 and 12-hour creatinine clearance ($CrCl_{12h}$) was measured. Following an overnight fast, subjects underwent an MMTT on day 1 and SHCP on day 2. In part 2, canagliflozin 100 mg was given once a day for 8 days. Subjects entered the clinical research unit on day 6 and $CrCl_{12h}$ was measured; MMTT was performed on day 7 (10 min after canagliflozin dosing), and SHCP was performed on day 8 (canagliflozin was dosed after the lowest glycemic target was reached).

Procedures

The MMTT contained approximately 700 kcal (including 100 g carbohydrates) and was given at t = 0 (0800 hours). BG was measured at t = −15, 0, 30, 60, 90, 120, 180, and 240 minutes. Urine was collected over 0 to 2 hours and 2 to 4 hours. SHCP was performed using Biostator (Life Science Instruments, Elkhardt, Indiana) through retrograde catheterization in a hand vein heated to 55°C to measure arterialized venous BG. In part 1, BG targets were 126, 171, 216, 261, and 306 mg/dL (7–17 mM). BG was reduced to 126 mg/dL using iv regular insulin infusion and maintained there for approximately 2 hours. Subsequent clamp steps were achieved using 20% glucose infusion with bolus infusions to reach BG targets quickly; each step was maintained for 2.5 hours. Part 2 used BG targets of 72, 117, 162, 207, and 252 mg/dL (4–14 mM). Urine was collected over the first hour and last 1.5 hours of each step.

Bioanalytical

Blood and urine glucose were determined by the Biostator; a glucose oxidase-based reference method (Super GL Glucose Analyzer; Hitado GmbH, Möhnesee, Germany) was used for confirmation. GFR was estimated using MDRD formula (estimated glomerular filtration rates [eGFR]) (10) and $CrCl_{12h}$.

Determining RT_G

The relationship between urinary glucose excretion (UGE) and BG was approximated by an idealized threshold relationship:

$$\text{rate of UGE (mg/min)} = \begin{cases} 0 & \text{if BG} \le RT_G \\ \text{GFR (dL/min)} \times (\text{BG (mg/dL)} - RT_G\text{ (mg/dL)}) & \text{if BG} > RT_G \end{cases} \quad (1)$$

as used previously (11, 12). For SHCP, $RT_{G:SHCP}$ was determined using robust nonlinear regression (*nlinfit* in Matlab [13]) with equation 1 and measured UGE and BG during the last 1.5 hours of the 5 clamp steps. Best-fit values of RT_G and GFR were obtained for all subjects except for 1 subject in part 1, who had too little UGE during several steps for both RT_G and GFR to be estimated, and for 2 subjects in part 2 for whom no physiologically reasonable RT_G value could be determined. For the subject in part 1 with low UGE, GFR was set to $CrCl_{12h}$ and regression was used to determine RT_G.

For the MMTT, $RT_{G:MMTT}$ was calculated from equation 1 using measured BG, UGE, and eGFR ($CrCl_{12h}$ was used for comparison), as previously described (8, 9). Because the true BG vs UGE relationship is not a perfect threshold and even normoglycemic subjects (where BG $\ll RT_G$) have small amounts of UGE, $RT_{G:MMTT}$ was only estimated for subjects with UGE > 600 mg. This value was chosen based on previous studies in nondiabetic subjects where 98% of subjects had 24-hour UGE < 600 mg (9) and because the 3 subjects in part 1 whose BG remained below their $RT_{G:SHCP}$ values during the entire MMTT had UGE of 0 to 589 mg, whereas all other subjects had UGE > 1 g. In part 2, $RT_{G:MMTT}$ was not determined for 1 subject due to incomplete urine collection.

Statistical analyses

Values reported are mean ± SD. Comparisons used all subjects with RT_G values for both methods (n = 11 in each part) using a mixed-effects ANOVA model. Least-squares geometric means and 90% confidence intervals (CIs) of log-transformed RT_G values were calculated. The concordance correlation coefficient was calculated using Lin's approach in SAS (14). Similarity was assessed using the following 2 prespecified criteria: 1) estimated concordance correlation coefficient ≥ 0.7, and 2) 90% CI for the geometric mean ratio (GMR) of $RT_{G:MMTT}/RT_{G:SHCP}$ within 0.8 to 1.25.

Table 1. Demographic and Baseline Characteristics[a]

Parameter[b]	Part 1: No Treatment (n = 14)	Part 2: Canagliflozin 100 mg (n = 14)
Age, y	57 (45–63)	58 (38–66)
Gender, n		
Male	10	10
Female	4	4
Race, n		
White	14	14
BMI, kg/m^2	31 (24–36)	29 (20–36)
eGFR,[c] mL/min/1.73 m^2	88 (71–121)	89 (74–126)
$CrCl_{12h}$,[d] mL/min/1.73 m^2	121 (22)	116 (27)
Glycated hemoglobin, %	8.4 (7.1–9.4)	7.8 (7.0–9.6)
Fasting serum glucose, mg/dL	203.6 (144.1–252.3)	198.2 (144.1–252.3)
Fasting serum glucose, mM	11.3 (8–14)	11.0 (8–14)
Subjects taking metformin,[e] n	14	12

Abbreviations: BMI, body mass index; $CrCl_{12h}$, measured 12-hour creatinine clearance; MDRD, modification of diet in renal disease.

[a] All values except for $CrCl_{12h}$ were measured at the screening visit.

[b] Values shown are median (range) except for gender, race, $CrCl_{12h}$, and subjects taking metformin.

[c] Calculated using the MDRD formula (10).

[d] Mean (SD) values measured on day −1 in part 1 and day 6 in part 2.

[e] Subjects in this study were allowed to be on either a stable dose of metformin or no antihyperglycemic medications.

Results

Subjects

Twenty-eight subjects were enrolled and completed the study. Baseline characteristics are summarized in Table 1.

BG and UGE during MMTT and SHCP

Figure 1 depicts BG and UGE during the MMTT and SHCP in untreated subjects (Figure 1, A–D) and canagliflozin-treated subjects (Figure 1, E–H). UGE rates during each clamp step and in the MMTT were higher in canagliflozin-treated subjects than in untreated subjects.

BG vs UGE relationship during SHCP

In untreated subjects, the BG vs UGE relationship was well-described by the idealized threshold model (equation 1), as shown for a representative individual subject (Figure 1I) and for all untreated subjects (Figure 1J), and $RT_{G:SHCP}$ = 216.2 ± 23.4 mg/dL (12.0 ± 1.3 mM) in untreated subjects. The UGE vs BG relationship was left-shifted in canagliflozin-treated subjects, with $RT_{G:SHCP}$ = 48.6 ± 19.8 mg/dL (2.7 ± 1.1 mM) in canagliflozin-treated subjects (Figure 1K).

Comparison of the MMTT and SHCP methods

RT_G values obtained by the 2 methods were highly correlated (Figure 1L), with an overall concordance correlation coefficient of 0.94, above the prespecified similarity criterion of 0.7. There was also good agreement when assessing the GMRs for $RT_{G:MMTT}/RT_{G:SHCP}$: GMRs (90% CIs) of 0.93 (0.89–0.96) in part 1 and 1.03 (0.78–1.37) in part 2. When considering the concordance correlation coefficients for part 1 and part 2 separately, values of 0.71 and 0.49 were obtained, respectively. Potential reasons for some within-subject differences in $RT_{G:MMTT}$ and $RT_{G:SHCP}$ values observed within each part are described in the *Discussion*.

The comparisons described above are for analyses performed with eGFR used to estimate GFR during the $RT_{G:MMTT}$ calculations. Good agreement between MMTT and SHCP-derived RT_G values was also obtained when $CrCl_{12h}$ was used to determine $RT_{G:MMTT}$ in untreated subjects (GMR [90% CI] = 0.97 [0.94–1.01] for part 1), but the $RT_{G:MMTT}$ values obtained using $CrCl_{12h}$ overestimated the clamp-derived values in canagliflozin-treated subjects (GMR [90% CI] = 1.86 [1.40–2.47] for part 2).

Safety and tolerability

Study procedures and treatments were well-tolerated. A higher incidence of adverse events was reported for canagliflozin-treated (n = 11) vs untreated (n = 2) subjects. This was primarily due to increased osmotic diuresis-related events (ie, pollakiuria, polyuria; n = 6 for canagliflozin vs 0 for untreated); these were generally mild and did not cause any discontinuations. No clinically significant clinical chemistry parameter changes were observed.

Discussion

This study validated a recently developed method for estimating RT_G from measurements commonly collected in clinical trials (8, 9). Although the method for calculating

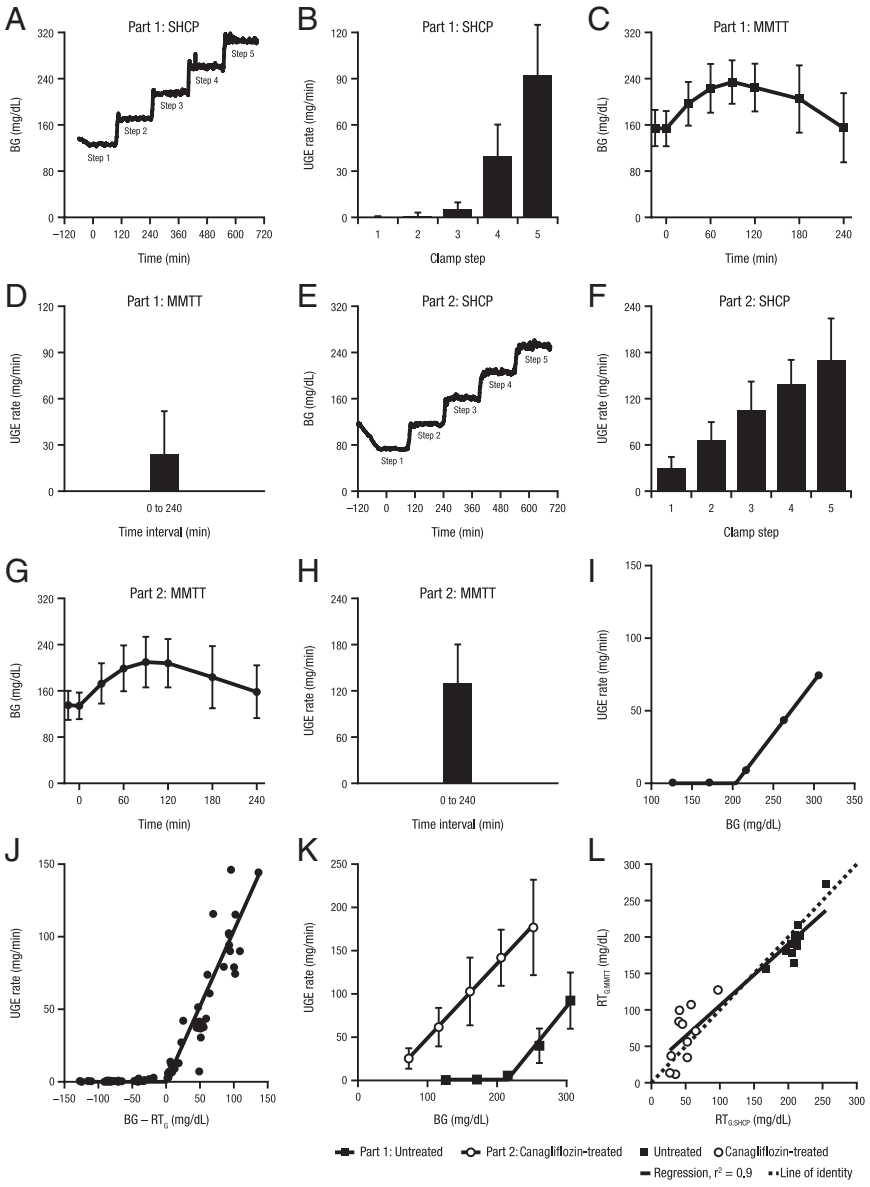

Figure 1. (A–H) BG concentrations and UGE during the SHCP and MMTT procedures in part 1 (untreated subjects; A–D) and part 2 (canagliflozin-treated subjects; E–H). Results shown are mean ± SD. UGE rates shown are the average rates measured during the last 1.5 hours of each hyperglycemic clamp step (B and F) or during the time interval shown from the MMTT (D and H). (I and J) Determination of RT_G from the SHCP. (I) Data from an individual subject. Measured UGE rate and mean BG concentration in each of the 5 clamp steps (dots) and the best fit obtained to equation 1 (line) are shown; the fit value of RT_G = 203.6 mg/dL (11.3 mM) was obtained for this subject. (J) Data from all 14 subjects in part 1. Each dot represents data from an individual subject during 1 of the 5 clamp steps, where the UGE rate is shown on the y-axis and the difference between the BG concentration in the clamp step and the subject's RT_G is shown on the x-axis. As in equation 1, subjects have virtually no UGE when BG < RT_G and the rate of UGE increases in proportion to BG-RT_G when BG > RT_G. (K) BG vs UGE relationship in untreated and canagliflozin-treated subjects. Values shown are mean ± SD. (L) Relationship between RT_G values determined by the MMTT and SHCP methods. Individual subject values (n = 11 each in part 1 and part 2) are shown as filled squares (part 1) or open circles (part 2); the dotted line represents the line of identity (exact agreement between the 2 methods).

RT_G using dynamic plasma and urine data is novel, the formulas used are straightforward generalizations of the established method for phosphate excretion (15, 16) and account for dynamic BG changes and possible times when BG < RT_G. This new method is much more generally applicable than the SHCP due to the far simpler experimental procedure. Strong agreement between RT_G values obtained by the 2 methods was observed, with an overall concordance correlation coefficient of 0.94 and GMRs of 0.93 in untreated subjects and 1.03 in canagliflozin-treated subjects.

Although the overall concordance correlation coefficient of 0.94 suggests strong overall agreement between the methods, the concordance was not quite as strong when considering each study part separately, particularly for the treated subjects. In untreated subjects, the between-methods difference in RT_G was <27 mg/dL (1.5 mM) (within expected precision for 45 mg/dL [2.5 mM] clamp steps) for all except 1 subject whose $RT_{G:SHCP}$ value was not consistent with the data observed during the MMTT (the subject had >3 g of UGE during the MMTT despite BG remaining below $RT_{G:SHCP}$ during the entire MMTT period, suggesting the $RT_{G:SHCP}$ value was inconsistent with MMTT observations). In canagliflozin-treated subjects, some unexpected within-subject differences in canagliflozin pharmacokinetics between the MMTT and SHCP (eg, slower absorption and delayed T_{max}) likely contributed to within-subject RT_G differences. Because the within-subject differences in RT_G values were generally small and some of the largest discrepancies were attributable to pharmacokinetic differences or to a clamp-derived RT_G value that was inconsistent with the MMTT data, the reduced concordance observed when considering the groups separately would not limit the utility of the new method.

RT_G values in canagliflozin-treated subjects in this study are modestly lower than previously reported in subjects with T2DM (8), due in part to using BG concentrations here and plasma concentrations in Ref. 8 (BG concentrations are ~15% lower than plasma concentrations [17]).

Although the new method offers a practical method for estimating RT_G, there are some limitations. The primary limitation is that subjects must have BG > RT_G to have sufficient UGE to determine RT_G; therefore, the method is not applicable in untreated normoglycemic or mildly hyperglycemic subjects with only trace amounts of UGE during an MMTT. In these cases, all that can be said is that RT_G is above the highest BG concentration measured. Consistent with this, for the 3 untreated subjects in this study with UGE < 600 mg during the MMTT, peak BG during the MMTT remained below their $RT_{G:SHCP}$ values. Another limitation is that the method assumes the BG vs UGE relationship can be approximated by a perfect threshold without splay and no information about the splay region is identified; however, very little splay was observed in the UGE vs BG relationship during the SHCP (Figure 1, I and J). Additionally, because only estimated GFR values are used, precise estimates of renal glucose reabsorption rates are not obtained from the new method.

In summary, we have developed a simple, straightforward method based on easily collected clinical data for determining RT_G in untreated and canagliflozin-treated subjects with T2DM and have demonstrated that RT_G values determined using this new method agree well with those derived using the more complicated SHCP method.

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Clinical trial registration: ClinicalTrials.gov NCT01273558.

Disclosure Summary: D.P., S.S., A.G., and P.R. are full-time employees of Janssen Research & Development, LLC. T.H. is an employee and shareholder, and L.P.-M. is an employee of the Profil Institute, which has received research support from Astellas Pharma, Bayer Health Care, Becton, Dickinson and Company, Biocon, Boehringer Ingelheim, Eli Lilly and Company, Evolva, Hoffmann LaRoche, Johnson & Johnson, Lundbeck, Novo Nordisk, Noxxon, OSI Prosidion, Sanofi-Aventis, Sirtris, and Skye Pharma. T.H. has also served on advisory panels for, and received speaker honoraria and travel grants from, Boehringer Ingelheim and Novo Nordisk.

References

1. **Bakris GL, Fonseca VA, Sharma K, Wright EM.** Renal sodium-glucose transport: role in diabetes mellitus and potential clinical implications. *Kidney Int*. 2009;75:1272–1277.
2. **Nair S, Wilding JP.** Sodium glucose cotransporter 2 inhibitors as a new treatment for diabetes mellitus. *J Clin Endocrinol Metab*. 2010; 95:34–42.
3. **Guyton A, Hall J.** *Textbook of Medical Physiology*. 11th ed. Philadelphia, PA: Elsevier Saunders; 2006.
4. **Rave K, Nosek L, Posner J, Heise T, Roggen K, van Hoogdalem EJ.** Renal glucose excretion as a function of blood glucose concentration in subjects with type 2 diabetes—results of a hyperglycaemic glucose clamp study. *Nephrol Dial Transplant*. 2006;21:2166–2171.
5. **Abdul-Ghani MA, Norton L, Defronzo RA.** Role of sodium-glucose cotransporter 2 (SGLT 2) inhibitors in the treatment of type 2 diabetes. *Endocr Rev*. 2011;32:515–531.
6. **Neumiller JJ, White JR Jr, Campbell RK.** Sodium-glucose co-transport inhibitors: progress and therapeutic potential in type 2 diabetes mellitus. *Drugs*. 2010;70:377–385.
7. **Liang Y, Arakawa K, Ueta K, et al.** Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight in normal and diabetic animal models. *PLoS ONE*. 2012;7:e30555.
8. **Devineni D, Morrow L, Hompesch M, et al.** Canagliflozin improves glycaemic control over 28 days in subjects with type 2 diabetes not optimally controlled on insulin. *Diabetes Obes Metab*. 2012;14: 539–545.
9. **Sha S, Devineni D, Ghosh A, et al.** Canagliflozin, a novel inhibitor of sodium glucose co-transporter 2, dose dependently reduces calculated renal threshold for glucose excretion and increases urinary glucose excretion in healthy subjects. *Diabetes Obes Metab*. 2011; 13:669–672.
10. **Levey AS, Coresh J, Greene T, et al and the Chronic Kidney Disease Epidemiology Collaboration.** Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem*. 2007; 53:766–772.
11. **Magni L, Raimondo DM, Bossi L, et al.** Model predictive control of type 1 diabetes: an in silico trial. *J Diabetes Sci Technol*. 2007;1: 804–812.
12. **Wong XW, Chase JG, Hann CE, et al.** Development of a clinical type 1 diabetes metabolic system model and in silico simulation tool. *J Diabetes Sci Technol*. 2008;2:424–435.
13. **Matlab®** [computer program], Version 7.10. Natick, MA: Mathworks, Inc; 2010.
14. **Lin LI.** A concordance correlation coefficient to evaluate reproducibility. *Biometrics*. 1989;45:255–268.
15. **Bagga A, Bajpai A, Menon S.** Approach to renal tubular disorders. *Indian J Pediatr*. 2005;72:771–776.
16. **Payne RB.** Renal tubular reabsorption of phosphate (TmP/GFR): indications and interpretation. *Ann Clin Biochem*. 1998;35(pt 2): 201–206.
17. **Haeckel R, Brinck U, Colic D, et al.** Comparability of blood glucose concentrations measured in different sample systems for detecting glucose intolerance. *Clin Chem*. 2002;48:936–939.