

The effect of extremely high glucose concentrations on 21 routine chemistry and thyroid Abbott assays: interference study

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Abstract

Introduction: Extremely high glucose concentrations have been shown to interfere with creatinine assays especially with Jaffe method in peritoneal dialysate. Because diabetes is the fastest growing chronic disease in the world, laboratories study with varying glucose concentrations. We investigated whether different levels of glucose spiked in serum interfere with 21 routine chemistry and thyroid assays at glucose concentrations between 17-51 mmol/L.

Materials and methods: Baseline (group I) serum pool with glucose concentration of 5.55 (5.44-5.61) mmol/L was prepared from patient sera. Spiking with 20% dextrose solution, sample groups were obtained with glucose concentrations: 17.09, 34.52, and 50.95 mmol/L (group II, III, IV, respectively). Total of 21 biochemistry analytes and thyroid tests were studied on Abbott c8000 and i2000sr with commercial reagents. Bias from baseline value was checked statistically and clinically.

Results: Creatinine increased significantly by 8.74%, 31.66%, 55.31% at groups II, III, IV, respectively with P values of < 0.001. At the median glucose concentration of 50.95 mmol/L, calcium, albumin, chloride and FT4 biased significantly clinically (-0.85%, 1.63%, 0.65%, 7.4% with P values 0.138, 0.214, 0.004, < 0.001, respectively). Remaining assays were free of interference.

Conclusion: Among the numerous biochemical parameters studied, only a few parameters are affected by dramatically increased glucose concentration. The creatinine measurements obtained in human sera with the Jaffe alkaline method at high glucose concentrations should be interpreted with caution. Other tests that were affected with extremely high glucose concentrations were calcium, albumin, chloride and FT4, hence results should be taken into consideration in patients with poor diabetic control.

Key words: assay interference; glucose interference; preanalytical phase; creatinine; Jaffe kinetic assay; thyroid function tests

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Introduction

According to the World Diabetes Foundation, diabetes is the world's fastest growing chronic disease that affects 6.4% of the world's adult population (1), hence laboratories study with high glucose levels at varying concentrations. However, extremely high glucose concentrations have been shown to interfere with creatinine assays, especially with the Jaffe method in peritoneal dialysate (2-5).

The accuracy of the clinical laboratory tests is important for patient care, and control of the whole testing process is the responsibility of laboratory professionals. Though the errors in the analytical phase are under strict control by the improved technology and control materials, body fluid compounds are known to interfere with some analytical reaction steps that are not yet solved by the

manufacturers. The most common analytical assay method affected in clinical chemistry is the Jaffe's reaction for creatinine determination.

The interference is defined as "the effect of a substance present in the sample that alters the correct value of the result, usually expressed as concentration or activity, for an analyte" (6). The major endogenous substances that interfere with the laboratory analyses are hemoglobin, bilirubin, and lipids (6-8).

Glucose is cited as an endogenous interferent factor for creatinine assay with the Jaffe method in several articles (9,10) especially in the peritoneal dialysate and this reaction is known to be affected by bilirubin, hemoglobin, lipid, and protein (11,12). Studies have been published comparing the Jaffe and enzymatic methods about the reliability for creatinine determination in serum and body fluids, however there is no consensus.

Because the magnitude of interference varies from method to method and according to the concentration of the interferent (11,13), we aimed to experiment the interfering effect of glucose at different levels on routine biochemistry analytes and thyroid function tests in human sera.

Materials and methods

Subjects

This study was conducted in the clinical laboratory of Abant İzzet Baysal University, Bolu, Turkey in 2015. We assessed the study with consecutive serum samples collected for routine clinical care. No additional blood was taken from patients. The study protocol was conducted in accordance with the ethical standards of the institutional committee on human experimentation and with the Helsinki Declaration. The study was approved by the review board of Ordu University, Turkey with the project number: 16 /13.April.2015.

Study protocol

Fasting venous blood was collected in the morning into plastic evacuated tubes (BD Vacutainer SST II Advance, Becton- Dickinson, Franklin Lakes,

New Jersey, USA). All were left in the upright position for 30 min at room temperature for complete clot formation, and then centrifuged at 2000 x g for 10 minutes. Sera of each blood were checked visually for hemolysis, lipemia and bilirubinemia. Totally 16 serum pools were prepared with glucose concentration of 5.55 (5.44 - 5.61) mmol/L as baseline (group I). Remaining sera were aliquoted into four tubes which were spiked with a dextrose solution (20% dextrose in distilled water, Neofleks, Turktipsan, Ankara, Turkey) to obtain final median glucose concentrations of 17.09 mmol/L (990 µL sera + 10 µL dextrose), 34.52 mmol/L (975 µL sera + 25 µL dextrose), 50.95 mmol/L (960 µL sera + 40 µL dextrose).

The following parameters were studied:

- Routine parameters: glucose, urea nitrogen (BUN), creatinine, calcium, direct and total bilirubin, magnesium, phosphorus, uric acid, albumin, total protein, CRP, triglycerides, cholesterol, ALP, ALT, AST, GGT, LD, sodium, potassium, chloride.
- Immune parameters: TSH, FT3, free thyroxine (FT4).

Routine biochemistry tests were studied on an Abbott Architect c8000 autoanalyzer (Abbott Laboratories, IL, USA), immunoassays were studied on the Architect i2000sr immunochemistry system (Abbott Laboratories, IL, USA) with commercial reagents. All measurements were performed on the same day. The analytical methods used are listed in Table 1.

Intra-assay CV's were performed with three levels of control materials [Clin Chem: Multichem S Plus, lot: 14005140 (Technopath, Tipperary, Ireland)], CRP: Archem Romatoid control, lot: 1311120 (Archem Diagnostics, Istanbul, Turkey), immunoassay: Technopath Multiimmunoassay Control lot: 33709140 (Technopath, Tipperary, Ireland). Intra-assay variation was assessed from 20 determinations at two-or three levels on the same day. The between run quality control data and methods are listed in Table 1.

Statistical analysis

The dilutional bias correction for the added volume of the dextrose solution was performed with

TABLE 1. Methods and intra-assay coefficient of variations.

Analyte (units)	Method	Level 1	CV1 (%)	Level 2	CV2 (%)	Level 3	CV3 (%)
ALT (U/L)	UV without P5P	29.4	0.1	110.7	0.43	233.5	0.24
Albumin (g/L)	Bromcresol green	28	1.3	44.2	1.2	55.6	0.72
ALP (U/L)	PNP	66	1.4	184	0.8	370	0.3
AST (U/L)	UV without P5P	44.85	0.1	135	0.5	241.5	0.45
Bilirubin, direct (µmol/L)	Diazotization	7.18	0.1	19.84	1.06	53.18	0.4
Bilirubin, total (µmol/L)	Diazonium ion	14.88	0.87	37.11	0.32	108.93	0.21
Calcium (mmol/L)	Arsenazo III	1.52	1.35	2.37	0.93	3.23	0.7
Chloride (mmol/L)	Ion selective electrode	84	0.1	93.7	0.1	105.4	0.1
Cholesterol (mmol/L)	CHOD-PAP	2.63	0.87	4.11	0.1	6.30	0.34
CRP (mg/L)	Immunoturbidimetry	177	1.45	460	0.98		
Creatinine (µmol/L)	Alkaline picrate	58.34	1.31	183.87	1.31	566.64	0.99
Glucose (mmol/L)	Hexokinase	2.86	0.99	7.03	1.17	16.53	0.91
GGT (U/L)	γ-glutamyl-carboxy nitroanilide	25.45	0.1	76.5	0.77	152.5	0.7
LD (U/L)	Lactate-pyruvate, UV	107.65	5.95	223.5	1.7	476	1.07
Magnesium (mmol/L)	Enzymatic	0.54	3.1	1.08	2.04	1.80	0.84
Phosphorus (mmol/L)	Phosphomolybdate	0.69	2.32	1.25	1.06	2.27	0.1
Potassium (mmol/L)	Ion selective electrode	2.51	0.1	3.89	1.17	6.74	0.1
Protein, total (g/L)	Biuret	46.2	0.96	67.9	0.65	87.6	0.5
Sodium (mmol/L)	Ion selective electrode	119.5	0.37	143	0.31	157.5	0.52
Triglyceride (mmol/L)	GPO-PAP	0.63	0.93	1.44	0.84	2.37	0.76
Urea (mmol/L)	Urease, UV	3.06	4.26	13.93	1.04	22.93	1.26
Uric acid (µmol/L)	Uricase, PAP	142.16	1.96	359.85	0.76	475.84	0.7
FT3 (pg/mL)	Chemiluminescence	2.65	2.04	4.21	2.05	9.67	2.88
FT4 (ng/dL)	Chemiluminescence	0.62	3.27	1.68	3.56	2.9	3.77
TSH (µU/mL)	Chemiluminescence	0.09	2.39	3.58	1.28	18.9	2.76

Immune parameters (TSH, FT3 and FT4) were analyzed on Abbott Architect i2000, remaining (routine parameters) were studied on Abbott Architect c8000.

Intra-assay variation, assessed from 20 determinations, was measured on a single assay.

ALP - alkaline phosphatase; ALT - alanine aminotransferase; AST - aspartate aminotransferase; CHOD-PAP - cholesterol oxidase-phenol aminophenazone; CRP - C reactive protein; FT3 - free triiodothyronine; FT4 - free thyroxine; GGT - gamma-glutamyl transferase; GPO-PAP - glycerol-3-phosphate oxidase-phenol aminophenazone; LD - lactate dehydrogenase; PAP - phenol aminophenazone; P5P - Pyridoxyl-5-Phosphate; PNP - p-Nitrophenyl phosphate; TSH - thyroid stimulating hormone.

the result multiplied by 0.01, 0.025, 0.04, respectively, for group II, III and IV then by adding to the value obtained.

For samples supplemented with the interfering effect of glucose, the clinical significance was calculated as: $[(C_x - C_1) / C_1] \times 100$; where C_1 represents

the median of the baseline sample and C_x represents the median of the experimented sample.

The criterion for the presence of a bias specific for that analyte was defined according to the desirable bias updated in 2014 (14). Results are expressed as a median (min-max) as the number of subjects

were small (N = 16). Interference effects were checked by Wilcoxon signed rank test for paired data when concentrations were compared with baseline. Bonferroni correction was applied and P value of $0.05 / 4 = 0.0125$ was considered as significant. All statistical analyses were carried out with SPSS version 15.0 (SPSS, Chicago, IL, USA).

Results

The interference of glucose on creatinine showed an increasing trend with increasing glucose concentrations. The creatinine bias percentages at median glucose concentrations of 17.09, 34.52, and 50.95 mmol/L in serum pools were 8.74%, 31.66%, and 55.31%, respectively, with P value of < 0.001 at each level (Figure 1, Table 2).

Serum calcium concentration decreased, albumin, chloride and FT4 concentration increased with percent biases of -0.85%, 1.63%, 0.65%, 7.4% at glucose concentrations of 50.95 mmol/L where

the interferences were clinically significant with P values 0.138, 0.214, 0.004, and < 0.001 , respectively. Although triglycerides, BUN and direct bilirubin biased at different levels of glucose significantly statistically, none of them reached to a clinical significance. Remaining parameters AST, ALT, ALP, LD, GGT, total bilirubin, cholesterol, phosphorus, magnesium, sodium, potassium, total protein, uric acid, CRP, TSH, FT3 were detected as free of interference at each level of glucose in sera.

Discussion

This study aimed to assess the influence of glucose on routine chemistry and thyroid assays in serum by high glucose concentrations. As a result, a numerous biochemistry parameters were found as unaffected. All the same we observed the interference on creatinine measurement with the Jaffe kinetic method at high glucose concentrations. Additionally, calcium, albumin, chloride, and FT4 measurements interfered with glucose at 50.95 mmol/L.

Glucose has been reported to interfere with the measurement of creatinine concentrations in some creatinine assays where mostly the Jaffe method (3-5,9). The kinetic Jaffe reaction is still the predominantly used assay method in creatinine determination where creatinine reacts with picric acid to form a yellow-red creatinine-picric acid complex in an alkaline pH. The rate of increase of the complex formation in absorbance at 500 nm is directly proportional to creatinine concentration in the sample. Many substances interfere with this assay that positive as well as negative biases have been demonstrated with bilirubin, dopamin, glucose, drugs and ketoacids (11). The interfering effect is between the reaction creatinine and alkaline picrate (15). Bilirubin interferes this assay with the same reaction under alkaline conditions which is oxidized to biliverdin causing a decrease in absorbance at 520 nm that result in false lower creatinine concentrations (15).

Glucose forms an interfering chromogen with picrate where that chromogen has a smaller absorption in the measuring wavelength. That effect is

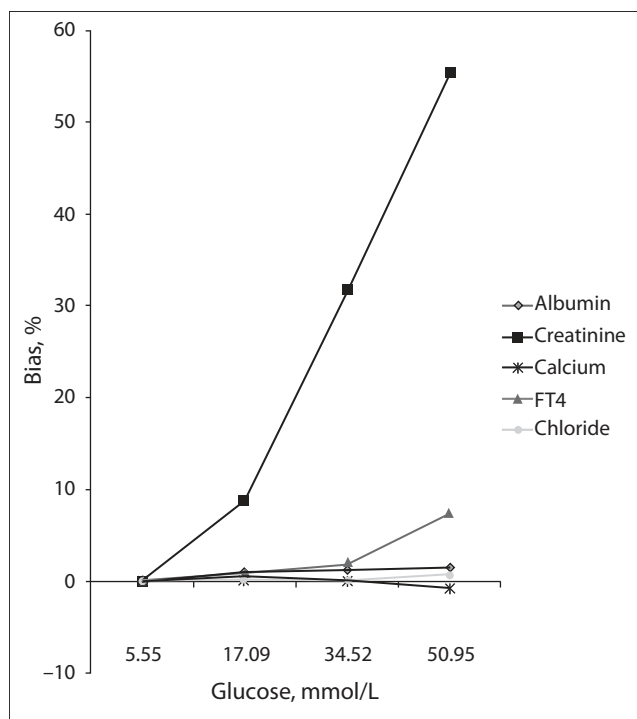


FIGURE 1. Interferogram for glucose and measured parameters: albumin, creatinine, calcium, chloride and free thyroxine (FT4).

TABLE 2. Median values of the analytes and glucose concentrations for each group, % bias of analyte concentrations in comparison to baseline values and desirable bias (14).

Analyte / units	Glucose (mmol/L)				Desirable Bias (%)
	Group I (Baseline; N = 16)	Group II (N = 16)	Group III (N = 16)	Group IV (N = 16)	
	5.55 (5.44-5.61)	17.09 (16.15-17.93)	34.52 (31.75-37.07)	50.95 (48.40-54.61)	
ALT (U/L)	18 (9-32)	18 (9-31)	17 (9-32)	19 (9-31)	
P		0.649	0.551	0.570	
%		0	-5.5	5.5	± 11.48
Albumin (g/L)	43.0 (31.0-46.0)	43.4 (30.3-46.5)	43.6 (30.8-46.1)	43.7 (31.2-46.8)	
P		0.604	0.004	0.214	
%		0.93	1.40	1.63	± 1.43
ALP (U/L)	75 (51-116)	76 (50-116)	75 (51-117)	75 (51-115)	
P		0.959	0.642	0.088	
%		1.33	0	0	± 6.72
AST (U/L)	19 (12-25)	18 (11-25)	18 (12-26)	18 (11-25)	
P		0.959	0.337	0.876	
%		-5.26	-5.26	-5.26	± 6.54
Bilirubin, direct (µmol/L)	3.68 (1.71-5.81)	3.63 (1.71-5.81)	3.68 (1.88-5.81)	3.82 (1.71-5.99)	
P		0.121	0.044	0.008	
%		-1.4	0	3.8	± 14.2
Bilirubin, total (µmol/L)	10.94 (4.96-19.15)	10.97 (4.96-19.15)	10.78 (5.30-19.15)	11.12 (4.96-19.15)	
P		0.756	0.717	0.642	
%		0.27	-1.46	1.65	± 8.95
Calcium (mmol/L)	2.36 (2.25-2.65)	2.37 (2.20-2.55)	2.36 (2.18-2.56)	2.34 (2.21-2.55)	
P		0.042	0.053	0.138	
%		0.42	0	-0.85	± 0.82
Chloride (mmol/L)	108.5 (104.0-112.0)	108.9 (104.0-112.1)	108.7 (104.6-111.7)	109.2 (105.0-112.3)	
P		0.006	0.015	0.004	
%		0.37	0.18	0.65	± 0.5
Cholesterol (mmol/L)	4.94 (3.74-6.00)	4.94 (3.73-6.04)	4.92 (3.70-6.02)	4.96 (3.76-6.03)	
P		0.234	0.865	0.816	
%		0	-0.4	0.4	± 4.1
CRP (mg/L)	30.6 (22.1-40.5)	30.2 (21.6-41.2)	30.1 (21.9-42.3)	29.9 (21.0-42.0)	
P		0.433	0.532	0.932	
%		-1.3	-1.7	-2.3	± 21.8
Creatinine (µmol/L)	69.8 (63.7-119.3)	75.9 (71.6-131.0)	91.9 (83.1-150.3)	108.5 (99.0-165.3)	
P		< 0.001	< 0.001	< 0.001	
%		8.74	31.66	55.31	± 3.96
FT3 (pg/mL)	3.06 (2.21-4.05)	3.01 (2.16-4.12)	3.01 (2.19-4.23)	2.99 (2.10-4.20)	
P		0.408	0.469	0.796	
%		-1.6	-1.6	-2.3	± 4.8

Analyte / units	Glucose (mmol/L)				Desirable Bias (%)
	Group I (Baseline; N = 16)	Group II (N = 16)	Group III (N = 16)	Group IV (N = 16)	
FT4 (ng/dL)	1.08 (0.82-1.37)	1.09 (0.81-1.42)	1.10 (0.85-1.43)	1.16 (0.93-1.47)	
P		0.286	< 0.001	< 0.001	
%		0.9	1.9	7.4	± 3.3
GGT (U/L)	20 (11-45)	20 (11-44)	19 (11-45)	19 (10-46)	
P		0.234	0.679	0.325	
%		0	-5	-5	± 11.06
LD (U/L)	179 (166-339)	176 (167-338)	181 (164-341)	178 (165-345)	
P		0.301	0.438	0.660	
%		-1.7	1.1	-0.6	± 4.3
Magnesium (mmol/L)	0.86 (0.77-0.91)	0.85 (0.78-0.93)	0.85 (0.78-0.92)	0.86 (0.77-0.94)	
P		1.000	0.836	0.679	
%		-1.2	-1.2	0	± 1.8
Phosphorus (mmol/L)	1.24 (0.97-1.45)	1.22 (1.01-1.47)	1.23 (1.00-1.46)	1.24 (1.01-1.48)	
P		0.679	0.776	0.534	
%		-1.61	-0.81	0	± 3.38
Potassium (mmol/L)	4.60 (3.90-5.20)	4.60 (3.94-5.15)	4.61 (3.90-5.12)	4.63 (3.95-5.20)	
P		0.043	0.276	0.733	
%		0	0.22	0.65	± 1.81
Protein, total (g/L)	72.5 (55.0-77.0)	72.4 (54.5-75.8)	72.8 (55.4-75.8)	72.8 (54.1-77.0)	
P		0.438	0.379	0.255	
%		-0.14	0.41	0.41	± 1.36
Sodium (mmol/L)	141.1 (139-143)	141.4 (138-143)	141.4 (138-144)	141.4 (138-144)	
P		0.677	0.299	0.406	
%		0.21	0.21	0.21	± 0.23
TSH (mIU/L)	1.06 (0.51-6.98)	1.05 (0.51-6.86)	1.05 (0.52-7.02)	1.07 (0.52-6.45)	
P		0.605	1.000	0.605	
%		-0.94	-0.94	0.94	± 7.8
Triglyceride (mmol/L)	1.45 (0.87-2.47)	1.48 (0.86-2.48)	1.48 (0.86-2.51)	1.47 (0.87-2.50)	
P		0.004	0.019	0.066	
%		2.07	2.07	1.38	± 9.57
Urea nitrogen (mmol/L)	5.18 (3.57-7.85)	5.23 (3.61-8.29)	5.12 (3.66-8.05)	5.20 (3.71-8.17)	
P		0.005	0.569	0.698	
%		0.97	-1.16	0.39	± 5.57
Uric acid (µmol/L)	303 (226-405)	300 (229-403)	302 (226-396)	300 (223-408)	
P		0.059	0.047	0.649	
%		-0.99	-0.33	-0.99	± 4.87

Values in brackets represent medians (min-max).

ALP - alkaline phosphatase; ALT - alanine aminotransferase; AST - aspartate aminotransferase; CRP - C reactive protein; FT3 - free triiodothyronine; FT4 - free thyroxine; GGT - gamma-glutamyl transferase; LD - lactate dehydrogenase; TSH - thyroid stimulating hormone.

Wilcoxon signed rank test was used to compare the repeated measures with a *post-hoc* Bonferroni correction. P < 0.0125 values are considered statistically significant. Results higher than desirable bias and statistically significant differences are marked in bold.

insignificant at physiological concentrations of glucose (4), but in continuous ambulatory peritoneal dialysis (CAPD) fluid containing very high glucose concentrations, this interference becomes clinically significant. At constant creatinine concentrations, interference increased with the increasing glucose concentrations similar to our study (4,13).

Interference of creatinine measurement in CAPD fluid was found as dependent both on glucose and creatinine concentrations. At constant glucose concentrations, the higher the creatinine concentration, the more target chromogen would be formed hence would reduce the formation of glucose-picric acid interfering chromogen. That competition of creatinine and glucose for the limited amount of picric acid would reduce the interference in alkaline picric acid reaction. Conversely, in the enzymatic method, at constant glucose concentration, the magnitude of interference was higher at higher creatinine concentrations (4).

Concerning creatinine measurement in plasma and urine, both the Jaffe's reaction and the enzymatic method gave acceptable results (3,5). However in dialysate fluids there is no consensus. In the study by Ferry *et al.* (5), both methods gave false higher creatinine concentrations at high concentrations of glucose ($\geq 1.36\%$). They proposed the high performance liquid chromatography (HPLC) method as a reference to overcome that interference or to use the correction factor when used the Jaffe method. However Larpent and Verger (3) observed bias only with the Jaffe method in peritoneal dialysis solutions, hence the enzymatic method was recommended instead where the glucose interference appeared negligible like a newer study by Sjøland and Marcher (16). Also, glucose degradation product 5-hydroxymethyl furfural was detected as interfered with the Jaffe's reaction more than glucose, but not with the measurement of enzymatic creatinine (3).

The established correction equations to predict the correct creatinine value to account for the glucose interference was found as failed due to interference effect of calcium chloride, a constituent of the peritoneal dialysate (9,10). Calcium (1.75

mmol/L) enhanced the interference caused by glucose at 236 mmol/L in Jaffe creatinine method. It was considered that calcium acted as a cofactor in the reduction of alkaline picric acid to picramide in proportion to the calcium concentration (9).

Although several correction formulae for the glucose interference of creatinine measurement have been proposed, it has been considered that different analytical systems require specific formulae; besides multifactorial interference that was determined in CAPD solution requires multiple correction formulae which are impractical (4).

To our knowledge, no interference effect of glucose was reported for calcium, albumin, chloride and FT4 assays with the methods we used or others. In this study, use of Arsenazo methods of calcium measurement yielded negative bias when interfered with extremely high glucose at normal calcium levels. For FT4 assay, heterophile antibodies are considered to be the interference factors for some immunoassays (17). Use of chemiluminescence microparticle immunoassay method with a two-step design in which the patient serum and labelled FT4 are separated in a wash step. FT4 concentrations were biased positively at glucose concentration of 50.95 mmol/L with normal FT4 values.

There are some limitations to our study. As a limitation, the number of samples included was relatively small. Measurements were performed with the defined analyzer with its original reagents; therefore, these results are not universal. Additionally, the patient samples may contain various interfering substances that may be responsible for a given interference that is unknown because of the selected parameters. Our data do not identify the origin of the bias given for albumin, chloride, calcium, FT4 and also creatinine.

In conclusion, among the numerous biochemical parameters studied, only a few parameters are affected by dramatically increased glucose concentration. The creatinine measurements obtained in human sera with the Jaffe alkaline method at high glucose concentrations should be interpreted with caution as not yet solved by the manufacturers. Moreover, the interference was observed with nor-

mal creatinine concentrations that should be underlined. Calcium, albumin, chloride and FT4 assays need to be interpreted with caution in diabetics with poor glycemic control.

Potential conflict of interest

None declared.

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