Acute Glucose Load, Inflammation, Oxidative Stress, Nonenzymatic Glycation, and Screening for Gestational Diabetes

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Abstract

Aims: To investigate if oral glucose tolerance test (OGTT) associates with changes in maternal symptoms (ie, flushing, sweating), blood nonenzymatic advanced glycation end products (AGE), acute-phase reactive inflammatory markers, and oxidative stress. **Methods:** Prospective case-control study of patients screened for gestational diabetes mellitus (GDM). One hundred nonfasting, second-trimester consecutive pregnant women allocated to either 50 g OGTT or water. Five women who had a 3-hour fasting 100 g OGTT also enrolled. Maternal serum glucose, AGE, soluble receptor for AGE (sRAGE), interleukin (IL)-6, and C-reactive protein (CRP) were immunoassayed. Total radical-trapping antioxidant parameter (TRAP) estimated with antioxidant capacity-ferrous assay. Data corrected for gestational age and maternal body mass index. **Results:** During 50 g OGTT there was a decrease in systolic blood pressure not accompanied by the onset of adverse clinical symptoms. There was a decrease in serum glucose levels 1 hour after water ($P = .019$) but not glucose ingestion. Serum CRP ($P = .001$) but not IL-6 was increased. The AGE, sRAGE, and TRAP levels remained unchanged. Similar results were seen during 100 g OGTT, except serum glucose was significantly elevated after 1 hour. **Conclusion:** Results suggest screening tools for gestational diabetes are safe and clinically well tolerated during pregnancy. Clinical Trial Registration: ClinicalTrials.gov NCT03029546.

Keywords

pregnancy, advanced glycation end products, RAGE, diabetes, glucose

Introduction

In the past, diagnosis of gestational diabetes was based on John O'Sullivan's recommendations of a 3-hour, 100 g oral glucose tolerance test (OGTT), given to fasting mothers.¹ Because such high glucose levels were frequently associated with vomiting and invalidation of the results, a 2-step strategy was developed and adopted in the United States. Despite this approach, some pregnant women report adverse clinical side effects (ie, flushing, sweating, vomiting) following the 50 g glucose load given to nonfasting mothers for gestational diabetes screening.^{2,3} A hyperinsulinemic hypoglycemic episode is responsible for triggering similar clinical manifestations in postbariatric surgery patients.² Yet, in normal pregnant women, the cause of these symptoms remains unknown.

The receptor for advanced glycation end products (RAGEs) is found on many cell types and tissues, including fetal membranes and placenta.⁴ The RAGE is a multiligand receptor able to bind many types of ligands including advanced glycation end-products (AGEs).⁵ Activation of RAGE has been linked to pathological conditions characterized by increased oxidative and inflammatory stress including systemic lupus erythematosus,

Alzheimer disease, and diabetes-induced hypertension where formation of AGEs is highly accentuated.⁶⁻⁸ The AGEs are modified proteins, lipids, or nucleic acids that are formed through nonenzymatic reaction of reducing sugar moieties with short-chain aldehydes or certain amino acid residues in proteins.⁹ This chemical revision of endogenous biomolecules takes place under normal physiologic conditions, but is accelerated by hyperglycemia and oxidative stress.⁸ In vitro, AGE formation is possible, but synthesis depends on the concentration and reactivity of

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sugars, amount of AGE precursors, and availability of free amino groups.¹⁰

Increased circulating AGEs have been associated with adverse life-course outcomes and decreased chance of survival, in particular in the setting of acute cardiovascular events.^{11,12} The AGEs contribute to the development of cardiovascular dysfunction by protein cross-linking and binding of RAGE, which in turn can cause oxidative stress with activation of multiple proinflammatory pathways in vascular endothelial cells.¹³ Based on these prior observations, it was reasonable to propose that some adverse clinical symptoms reported by patients who ingest sizable quantities of glucose might be attributable to the general negative effects of AGEs.

Elevated AGE levels were found in women with GDM at 24 to 30 weeks of gestation.¹⁴ However, there is little information about AGEs and the state of RAGE activation in the normal pregnant population. Here, we sought to test the hypothesis that an acute glucose load from an OGTT stimulates AGE synthesis and induces systemic oxidative and inflammatory stresses, which explain the maternal symptoms commonly associated with a gestational diabetes screen. Our goal was to quantify markers of nonenzymatic glycation, RAGE activation, inflammation, and oxidative stress in women undergoing an OGTT during the second trimester of pregnancy.

Materials and Methods

Patients and Study Design

Following approval by the institutional review board (#2015H-0236), we performed a case-control study at The Ohio State University Wexner Medical Center from November 2015 to April 2016. The trial was registered at ClinicalTrials.gov (NCT03029546). Participants were recruited in the outpatient clinic and provided written informed consent. At enrollment, information on maternal demographics, gestational age (GA), and medical and social history was obtained. All women were followed prospectively until delivery. Maternal and neonatal outcome data were abstracted at discharge following delivery. Eligible women were ≥ 18 years of age, had regular prenatal care visits, were in their second trimester, did not undergo an OGTT during the current gestation, and planned to deliver at our medical facility.

One hundred nonfasting, consecutive pregnant women were prospectively allocated during the second trimester to either 50 g OGTT (study group, $n = 50$) or an equivalent volume of water (control, $n = 50$). Based on ethical consideration, women presenting between 24 and 28 weeks could be enrolled only as part of the 50 g OGTT group, because based on the institutional review board recommendations patients should have been subjected to a minimal number of blood retrievals.¹⁵ The blood pressure and intensity of maternal clinical symptoms (ie, dizziness, hot flushes, and tachycardia) were recorded before intake and monitored after glucose ingestion at 15-minute intervals for 1 hour. Blood samples were collected immediately before the OGTT and 1 hour thereafter.

To determine whether a more prolonged exposure to higher glucose levels impacts on blood analytes, in addition to our initial study and control groups, we enrolled 5 consecutive pregnant women who did not pass the 50 g OGTT and presented for the 3-hour fasting 100 g OGTT. Our institutional threshold for passing the 50 g OGTT is <140 mg/dL in a non-fasting mother.¹⁴ Clinical symptoms and blood pressure were monitored using the same protocol. Blood samples were collected immediately before and at 1-hour intervals for 3 hours. In our medical center, the protocols for screening and diagnosis of gestational diabetes were in accordance to the Coustan-Carpenter criteria.¹⁶

Maternal Blood Collection and Storage

All maternal blood samples were retrieved by venipuncture using BD Vacutainer blood collection tubes. The blood was allowed to clot after which the tubes were spun at $3000 \times g$ at 4°C for 20 minutes. The supernatant (serum) was aliquoted and immediately stored at -80°C .

Measurement of Maternal Serum Analytes

Serum AGEs were measured using a competitive immunoassay (OxiSelect Advanced Glycation End ELISA; Cell Biolabs, San Diego, California) that collectively quantifies 2 main AGEs: N_ϵ -(carboxymethyl) lysine (CML) and pentosidine.¹⁷ Serum soluble RAGE (sRAGE) was measured as marker of RAGE pathway activation (Human RAGE Quantikine ELISA kit; R&D systems, Minneapolis, Minnesota). C-reactive protein (Human CRP Quantikine ELISA; R&D systems), and Interleukin (IL)-6 (human IL-6 Quantikine HS ELISA; R&D systems) were measured as indicators of inflammation. Serum glucose was measured using the Trinder method (glucose LiquiColor enzymatic test; Stanbio Laboratory, Boerne, Texas). The minimal detectable dose for glucose was 0.3 mg/dL. The intraassay coefficients for all analytes varied from 3% to 5%.

Serum total reactive antioxidant potential (TRAP) was quantified using the total antioxidant capacity-peroxyl assay (Northwest Life Science Specialties, Vancouver, Washington) according to the manufacturer's protocol. Luminescence readings were taken every 112 seconds for 48.5 minutes on a CLARIOstar microplate reader (BMG Labtech, Cary, North Carolina) using $20\times$ diluted serum samples. The intraassay coefficient of variation was 3.2%. For all assays, values were interpolated against a standard curve run within each assay and the average of technical duplicates reported as the final result.

Statistical Analysis

The sample size calculation was based on a prior study of serum AGE levels and variability at midpregnancy, which reported standard deviations ~ 5 ng/mL.¹⁸ Considering a type I α error of 0.05 and statistical power of 80%, enrolling 50 patients in each group would allow the detection of a 2 ng/mL difference in AGE levels. Statistical analyses were

Table 1. Demographic Clinical and Outcome Characteristics of Women Undergoing 50 g OGTT and Controls.

Variable	50 g OGTT (Study Group), n = 50	Water (Control Group), n = 50	P Value
Clinical characteristics at the time of enrollment and during pregnancy ^a			
Maternal age, years	26 [23-30]	25 [22-30]	0.594
Parity	2 [1-3]	1 [0-2]	0.011
Gravidity	4 [2-5]	2 [1-4]	0.002
Gestational age, weeks	26 [25-27]	22 [20-29]	0.001
History of GDM, n (%)	2 (4)	1 (2)	1.0 ^b
BMI, %	29 [26-35]	30 [26-35]	0.975
Time from last meal to testing, minutes	165 [60-555]	210 [83-720]	0.275
Race			
Non-Hispanic white, n (%)	26 (52)	14 (28)	0.199 ^b
African American, n (%)	21 (42)	32 (64)	
Other, n (%) ^b	3 (6)	4 (8)	
50 g OGTT result			
Passed, n (%)	32 (64)	43 (86)	0.021 ^b
No-pass, n (%)	18 (36)	7 (14)	
GDM diagnosis this pregnancy, n (%)	0 (0)	2 (4)	0.495 ^b
Clinical characteristics at the time of delivery ^a			
GA at delivery, weeks	38 [37-39]	39 [37-39]	0.113
BMI, %	31 [27-38]	32 [27-37]	0.82
Neonatal birth weight, g	2986 [2665-3337]	3110 [2708-3386]	0.561
Delivery mode			
Vaginal, n (%)	30 (64)	37 (77)	0.493 ^b
Cesarean, n (%)	17 (36)	11 (23)	
Neonatal sex			
Male, n (%)	29 (58)	28 (56)	1.0 ^b
Female, n (%)	21 (42)	22 (44)	

Abbreviations: BMI, body mass index; GA, gestational age; GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test.

^aData are median [interquartile range] and analyzed by Mann-Whitney rank-sum test, unless noted.

^bAnalyzed by χ^2 tests.

performed with SigmaPlot, version 12.5 (Systat Software, San Jose, California). Data distribution was tested using Shapiro-Wilk test. Statistical differences in analytes measured by immunoassay were tested following logarithmic transformation of data. Differences between the 2 groups were compared using Mann-Whitney rank-sum test or 2-way repeated analysis of variance (before and after OGTT). χ^2 or Fisher exact test was used for comparisons between proportions. Spearman correlations were used to measure colinearity between selected independent variables. Multiple linear regression was used to correct for differences in GA and glucose levels between the groups. Data are presented as median and interquartile range unless otherwise specified. A *P* value <.05 was considered significant throughout the analysis.

Results

Characteristics of Patients at the Time of Atrial Fibrillation Sampling

Demographic and pregnancy characteristics are presented in Table 1. Women who received a 50 g OGTT were of significantly higher parity and gravidity and were recruited as a group at a higher GA compared to control women who received water (*P* = .001). During their prenatal care, more women in our study group did not pass the 50 g OGTT compared with what

the control group. However, overall there was no difference in prevalence of gestational diabetes mellitus (GDM) between groups, above what is expected in the general population.¹⁴ Only 2 women were diagnosed with GDM, both in the control group. One woman who did not pass the 3-hour OGTT was classified as type 1 GDM and had a normal obstetrical outcome. The second woman received insulin throughout pregnancy and also had a normal pregnancy course.

Clinical Signs and Symptoms

None of the women enrolled in our study displayed sweating, or complained of palpitations, abdominal distension, or anxiety. There were no differences in baseline blood pressures between the 2 groups and this result maintained after adjusting for differences in parity and GA. Percent change in systolic and diastolic blood pressures for both groups is displayed in Figure 1A and B. There was a significant percentage decrease from baseline for systolic blood pressure in both the groups during the 50 g OGTT. Change in systolic blood pressure varied with time of observation and not with group allocation. Moreover, there were no significant correlations between the changes in systolic blood pressure and changes in maternal blood glucose levels after the test in either control (*r* = 0.111, *P* = .440) or study group (*r* = -0.174, *P* = .225). There were no changes

Figure 1. Graphic representation of the percent change in systolic (A, SBP) and diastolic (B, DBP) blood pressures in women who had a 50 g oral glucose tolerance test (OGTT₅₀) or an equivalent volume of water (CRLs, $n = 50$). Values represent mean $\pm$ standard error at each time. Data were analyzed by 2-way repeated-measures ANOVA. * $P < .05$ versus baseline (time 0) in CRLs; # $P < .05$ versus OGTT₅₀ (time 0). ANOVA indicates analysis of variance.

observed in diastolic blood pressure between groups or with timing of observation.

Effect of OGTT on Glucose Concentration

Maternal blood levels of the measured analytes are presented in Table 2. In the controls, we observed a significant decrease in maternal serum glucose levels 1 hour after water ingestion ($P = .019$). In women who received the 50 g glucose load, the decrease in glucose levels did not reach statistical significance ($P = .223$). However, 1 hour after the test, the difference in serum glucose concentration between the study and control groups was significant ($P = .001$) and remained significant following correction for GA and body mass index (BMI; $P = .043$).

Effect of 50 g OGTT on Markers of Inflammation

One hour after the test, the 50 g glucose load was associated with significantly increased CRP levels ($P = .024$), which maintained significance after correction for GA and BMI ($P = .031$; Table 2). This phenomenon was not observed in the controls. No change was noted in maternal blood IL-6 levels in either OGTT or control groups (Table 2).

Effect of 50 g OGTT on AGEs, sRAGE, and Antioxidant Capacity

Maternal serum levels of AGEs remained unchanged 1 hour after 50 g glucose load (Table 2). Levels of sRAGE decreased significantly 1 hour after glucose load, but at a similar extent to that observed in women receiving water ($P < .001$ for both, Table 2). Pregnant women undergoing a 1-hour 50 g OGTT maintained intact their serum antioxidant capacity (Table 2).

Responses in Women Who Did Not Pass the 1-Hour OGTT

Among the study group, 36% (18/50) did not pass the 1-hour OGTT (finger-stick glucose level passed: 115 [99-112] mg/dL vs no-pass: 155 [142-163] mg/dL, $P < .001$). Women who did not pass the test started from significantly higher baseline glucose levels ($P = .035$). Despite this, we saw no differences in concentrations of CRP, IL-6, AGEs, and sRAGE between women who did not pass or passed their OGTT. This conclusion was maintained after corrections for GA, BMI, and time from last meal.

Effect of 100 g OGTT on Glucose, CRP, and AGEs Concentration

To test for a potential dose-time effect, we further evaluated the responses to the 100 g glucose load generally indicated in women who did not pass the 50 g OGTT. Serum glucose levels of women who had the 100 g OGTT were significantly increased 1 hour after glucose ingestion ($P = .012$). Glucose levels returned to baseline thereafter. No change was observed in CRP levels in women with a 3-hour OGTT. Similar results were observed for AGEs.

Discussion

Many investigators have focused their attention on initiation of diverse physiologic and biochemical responses following an oral glucose load in patients at risk for or with an established diagnosis of diabetes.¹⁹⁻²¹ However, more research was needed to understand the consequences of an acute glucose load in healthy pregnant women undergoing screening for gestational diabetes. In our study, a 50 g OGTT was associated with a

Table 2. Changes in Maternal Serum Analyte Levels Among Pregnant Women in Response to Administration of a 50 g Second-Trimester OGTT.^a

Analytes and Time Periods	Water (Control), n = 50	50 g OGTT (Study), n = 50	P Values			
			Study/Control ^c	Time ^c	Study/Control × Time ^c	Study/Control
Glucose, mg/dL ^b						
Baseline	61.6 [54.1 to 76.9]	71.2 [58.3 to 81.3]	.002	.082	.051	.043 ^d
1 hour	57.9 [50.9 to 65.7]	69.5 [52.1 to 96.0]				
Percent change	−5.2 [−16.8 to 4.5]	−4.1 [−20.1 to 30.6]				
CRP, μg/mL						
Baseline	6.1 [2.4 to 11.3]	7.2 [2.9 to 12.9]	.199	.872	.024	.031 ^e
1 hour	4.4 [2.4 to 11.5]	7.2 [4.2 to 13.0]				
Percent change	−8.8 [−26.6 to 15.5]	14.2 [−1.4 to 38.7]				
IL-6, pg/mL						
Baseline	0.72 [0.55 to 0.90]	0.91 [0.63 to 1.55]	.139	.369	.184	.916 ^e
1 hour	0.69 [0.52 to 0.96]	0.90 [0.63 to 1.45]				
Percent change	6.38 [−17.71 to 15.15]	−7.69 [−19.89 to 19.29]				
AGEs, μg/mL						
Baseline	9.7 [7.0 to 11.3]	11.1 [7.4 to 13.4]	.158	.290	.344	.987 ^e
1 hour	10.0 [7.9 to 11.8]	10.7 [8.5 to 13.7]				
Percent change	2.8 [−10.2 to 20.6]	0.3 [−9.8 to 12.1]				
sRAGE, pg/mL						
Baseline	789.5 [587.5 to 1007.1]	781.5 [539.2 to 1228.3]	.688	<.001	.203	.258 ^e
1 hour	714.3 [554.0 to 960.8]	774.9 [514.2 to 1141.2]				
Percent change	−5.9 [−8.9 to −0.6]	−3.5 [−8.9 to −1.1]				
TRAP, Trolox Eq, μM ^f						
Baseline	222.5 [152.9 to 250.8]	213.7 [182.5 to 300.9]	.136	.717	.114	.496 ^e
1 hour	324.6 [223.2 to 393.1]	237.4 [201.1 to 291.0]				
Percent change	12.2 [−11.0 to 33.1]	−18.2 [−34.8 to 7.3]				

Abbreviations: AGEs, advanced glycation end products; BMI, body mass index; CRP, C-reactive protein; IL-6, interleukin 6; OGTT, oral glucose tolerance test; sRAGE, soluble receptor for advanced glycation end products; TRAP, total radical-trapping antioxidant potential.

^aData are median [interquartile range].

^bGlucose concentration measured in serum separated in laboratory.

^cTwo-way repeated-measures analysis of variance (study group × time) after logarithmic transformation.

^dAdjusted in multivariable regression for maternal age, gestational age, race, parity, and BMI.

^eAdjusted in multivariable regression for gestational age, BMI, and change in glucose concentration.

^fBased on 18 women, OGTT (n = 9), control (n = 9).

significant decrease in systolic blood pressure, but without significant changes in serum nonenzymatic glycation or oxidative stress markers. After correction for GA, maternal serum levels of CRP were significantly increased 1 hour after administration of glucose, although this inflammatory change was minor considering that IL-6 levels remained unchanged. These findings are broadly supportive of the safety of the OGTT for screening pregnant women for GDM.

The most frequently cited adverse events during an OGTT are abdominal bloating, nausea, and vomiting.²² Following surveillance of the women enrolled in our study, we believe adverse symptoms occur extremely rarely during the 1-hour OGTT. However, if adverse symptoms arise, most probably they cannot be attributed to acute changes in maternal blood pressure levels. Indeed, during the 1-hour OGTT maternal systolic blood pressure decreased significantly. Because blood pressure change was temporal and not associated with treatment group alone, the observed variation was probably part of a “white-coat” syndrome phenomenon.^{1,23} However, our blood pressure observation does not exclude the possibility that acute hyperglycemia

induces vascular endothelium dysfunction as previously demonstrated in vivo using brachial artery flow Doppler.²⁴ A 75 g glucose load reduced by 2% to 3% the artery flow-mediated vasodilatation. The impact of this reduction on arterial blood pressure remains unknown and thus further studies are needed.

The maternal symptoms previously reported to occur during an OGTT are largely similar to those of patients suffering from early dumping syndrome after gastric bypass surgery.²⁵ In the dumping syndrome, abdominal distension, sweating, and accelerated heart rate happen within minutes to 1 hour after rapid ingestion of glucose or food.²⁶ It is believed that these symptoms are the consequence of fast evacuation of the hyperosmolar glucose load into the small bowel, followed by intravascular volume contraction and hemoconcentration. Although we have not seen this in our study, it is possible in patients reporting sweating, abdominal distension, nausea, and tachycardia following an OGTT, a similar underlying subclinical phenomenon to occur, even in the absence of bypass surgery.

It was remarkable that for the majority of tested patients, 1 hour after initiation of the 50 g OGTT, glucose levels were

slightly decreased or unchanged. Based on prior published data, this should not be surprising.^{19,27,28} In healthy adults undergoing an OGTT, the glucose and insulin peak levels occurred at 30 minutes, with a return to the baseline 1 hour after glucose ingestion.^{25,26} Interestingly, these prior observations were made following a 75 g OGTT, a 50% higher dose compared with our protocol. We were unable to identify studies that assessed maternal glucose serum levels in healthy patients 1 hour following a 100 g glucose load. Because in our study the glucose dose was double in the 5 women who were administered the 3-hour OGTT, it makes sense for maternal serum glucose concentration to be significantly elevated 1 hour after ingestion, with return to the baseline after 2 hours.

The concept that an OGTT carries potential damaging effects has biological plausibility because AGEs can induce vascular endothelial damage and impair vascular reactivity via nitric oxide and redox-induced pathways.^{29,30} Rodent studies demonstrated no change in AGEs within 2 hours following an oral glucose load.³¹ Despite these data, more information was necessary to learn if in pregnant women an OGTT has no effect on AGEs and oxidative stress, consistent with prior animal experiments. Nonenzymatic glycation is a common post-translational modification of proteins.³² Overall, synthesis of CML and other AGEs is a complex mechanism. The first step is formation of a Schiff base-adduct via interaction of monosaccharides with amino groups of various amino acids. These early glycation products are unstable. Thus, Schiff bases and Amadori products undergo a complex series of molecular rearrangements including oxidation to produce a class of irreversible adducts to proteins, the AGEs.³³ Once formed, AGEs accumulate and are removed from tissues only when the protein component is degraded. Of relevance is that under physiologic conditions, formation of the Schiff base as the initial step toward AGEs is a relatively fast process. In contrast, the slow secondary steps that lead to AGEs synthesis, and in particular to CML, could take hours or even days as demonstrated through kinetic modeling experiments.^{34,35} Collectively, the above research may explain why neither a 1-hour nor 3-hour OGTT was associated with increased maternal circulatory levels of AGEs. Our results are also consistent with the only other prior study, which reported in just 10 patients that a 50 g glucose load was associated with no change in the levels of bound CML and pentosidine.¹⁸

Multiple reactive oxygen species are implicated in diabetes. Both in vivo and in vitro experiments show that endoplasmic reticulum stress is an important contributor to glucose-induced glucotoxicity.^{24,36} In addition, in nonpregnant patients with diabetes, the neutrophil respiratory burst seems to be related to patient's glycemic control as reflected by an increase in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity.³⁷ The mechanism is complex but seems to involve among others the p47^{phox}, a cytoplasmic NADPH oxidase cytosolic subunit, which prematurely translocate to the plasma membrane in response to hyperglycemia and increases superoxide generation.³⁸ Based on the above experimental evidence, we decided to explore if maternal serum antioxidant

potential is modified during an OGTT. We found no significant decrease in serum antioxidant capacity in response to the 50 g glucose load. Our results imply that there was no increased oxidative stress, consistent with the data generated by Walsh et al who reported that in women undergoing a 75 g OGTT, plasma levels of 3-nitrotyrosine and thiobarbituric acid reactive substances (byproduct of lipid peroxidation) remained unchanged compared to the baseline.²⁴ Plasma total antioxidant activity also remained intact in healthy patients without diabetes exposed to 75 g glucose load, as reported by Ma et al.³⁹

Conclusions

From a clinical perspective, the results of our study were critically needed considering that an OGTT is universally recommended during prenatal care.¹⁴ While the search must continue to unravel changes associated with an acute glucose load, our results suggest the process of acute nonenzymatic glycation of proteins is not impacted. Although some patients may continue to report negative symptoms associated with the OGTT, these are likely short term with little impact on inflammatory markers or blood pressure. Overall, our results support the notion the 50 and 100 g OGTTs are safe and clinically well tolerated.

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Declaration of Conflicting Interests

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