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Adding Glucagon-Stimulated GH Testing to the Diagnostic Fast Increases the Detection of GH-Sufficient Children

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Key Words
Hypoglycemia · Growth hormone · Short stature · Glucagon · Testing · Stimulation

Abstract
Background/Aims: The evaluation of children with unexplained hypoglycemia may include a diagnostic fast. However, low growth hormone (GH) concentration during hypoglycemia is not specific to GH deficiency (GHD). The aim of this study was to determine if serial GH measurement following glucagon administration, in the setting of a diagnostic fast, would increase the number of children identified as not having GHD. ***Methods:*** We conducted a retrospective chart review of children who had serial GH measurements performed after glucagon administration at the end of a diagnostic fast. Glucagon was administered at the end of the fasting study, and GH was measured every 30 min for 210 min. ***Results:*** Of the 29 children in this series, only 3 (10%) had GH concentrations >7 ng/ml at the end of the fast, which increased by 16 (55%) after serial GH testing. The percentages of samples with GH concentrations >7 ng/ml were: 10% at baseline, and 25, 39, 41, 41, 33, 43, and 0% every 30 min thereafter. ***Conclusion:*** Additional GH measurements after glucagon administration following a diagnostic fast can improve the identification of children without GHD and thereby save them unnecessary GH stimulation testing and potential GH treatment.

Introduction

Growth hormone (GH) plays an important role in the regulation of substrate use in the fasting state. Prolonged fasting results in an increase in frequency and amplitude of GH bursts [1] and GH secretion increases as blood glucose falls below 60 mg/dl [2, 3]. GH promotes lipolysis [4] and reduces utilization of protein [5–7] and glucose [8]. It also causes insulin resistance through direct downstream effects on insulin signaling [9, 10], as well as indirectly by increasing nonesterified free fatty acid concentrations [4]. Consequently, hypoglycemia can be a presenting feature of GH deficiency (GHD) [11–13]. This is generally more problematic in younger infants [14, 15], although older children with GHD may have impaired fasting tolerance [13].

Severe hypoglycemia in childhood can be associated with adverse neurocognitive outcomes [16–18], and the identification and treatment of the cause of hypoglycemia

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disorders is critical in minimizing this risk. In addition to GHD, the differential diagnosis for hypoglycemia in childhood includes disorders of intermediary fuel metabolism, excess or inappropriate insulin secretion, and adrenal insufficiency. The standard approach to determining the etiology of hypoglycemia includes the measurement of the fuel and hormonal responses in the 'critical' lab sample drawn during hypoglycemia [19, 20]. Where possible, these serum markers are measured during opportunistic hypoglycemia, but a structured diagnostic fasting study may be required. Although GH increases as blood glucose concentration falls, low GH concentrations during hypoglycemia are commonly seen at the time of drawing the critical sample even in the absence of GHD [21–24].

The evaluation of GHD as a potential cause of hypoglycemia is complicated by the lack of a reliable and specific gold standard test for this condition. GH stimulation tests (GHSTs) are recommended to confirm GHD in children with clinical suspicion of GHD [25, 26]. Factors including the assay used and individual pharmacokinetics also influence the serum GH concentrations measured in these tests [27]. A large proportion of infants [28] and older children [29–31] will not have GH concentrations above arbitrary GH thresholds using these tests. Consequently, the misdiagnosis of children with GHD as the etiology of their hypoglycemia is possible and may result in the initiation of an incorrect treatment plan.

Glucagon is used clinically to assess the inappropriate availability of glucose stores (glycogen) during hypoglycemia. In a child with hypoglycemia, an inappropriate rise in glucose concentration following glucagon administration may be consistent with hyperinsulinism [19, 32] or hypopituitarism (in the newborn period) [33]. Glucagon is also a GH secretagogue, and the serial measurement of GH concentration following glucagon administration is used as one of the GHSTs of GH sufficiency in children with suspected GHD [34]. When intramuscular glucagon is used to assess GH secretion, peak GH concentrations are generally seen between 90 and 120 min following administration [28, 35]. Although glucagon is administered at the end of many fasting studies to evaluate glycogen stores, the routine serial measurement of GH following this glucagon administration is not generally performed.

The aim of this study was to determine if the serial measurement of GH concentration following glucagon administration, in the setting of a diagnostic evaluation of a child with unexplained hypoglycemia, would increase the number of children identified as not having GHD.

Methods

Given the poor specificity of GH measurement during hypoglycemia for GHD [22], a clinical protocol for incorporating serial GH measurement after glucagon administration during hypoglycemia was developed. The only additional intervention in this clinical protocol was the serial measurement of GH following glucagon administration in children with unexplained hypoglycemia and clinical suspicion of GHD. This protocol was implemented in 2012 for patients in whom GHD was considered clinically as a likely etiology of their hypoglycemia, and medical records were retrospectively reviewed in March 2015. This study was approved by the Institutional Review Board at The Children's Hospital of Philadelphia.

Protocol

At The Children's Hospital of Philadelphia, the diagnostic evaluation of hypoglycemia includes a standard diagnostic fasting study. The maximum fasting time is age dependent. Children aged <1 month are fasted for up to 18 h, those between 1 and 12 months for up to 24 h, and children over 1 year for up to 36 h. The monitoring protocol depends on the clinical scenario, but generally includes blood glucose monitoring during the fast using a bedside glucose meter (Nova StatStrip point-of-care glucose monitor; Nova Biomedical Corporation, Waltham, Mass., USA) every 3 h until blood glucose is <70 mg/dl, hourly until it is <60 mg/dl, and every 30 min until it is <50 mg/dl. β-Hydroxybutyrate is also measured at the bedside every 3 h using a handheld meter (PrecisionXtra; Abbott Laboratories). The study is ended when a confirmatory glucose of <50 mg/dl is recorded, if β-hydroxybutyrate concentration exceeds 2.5 mmol/l, or if the maximum predetermined fasting time is reached.

A full critical blood draw is taken at the end of the fast. This includes the measurement of glucose, free fatty acids, β-hydroxybutyrate, insulin, ammonia, lactate, basal metabolic profile, acylcarnitine, C-peptide, carnitine, insulin-like growth factor-binding protein (IGFBP)-1, cortisol, and GH. At the end of the diagnostic fast, glucagon is administered intravenously at a dose of 1 mg for all patients, and glucose is measured every 10 min by bedside glucose meter. Dextrose is administered if blood glucose does not rise by at least 20 mg/dl within 20 min. If, however, glucose concentration rises by at least 20 mg/dl, then blood glucose checks are continued for a further 20 min before the fast is ended. If at any point during the glucagon test the child is unwell, dextrose is administered. The administration of dextrose at the end of the fast consists of a 2-ml/kg bolus of 10% dextrose, and the child is provided a meal containing 40 g of carbohydrates.

In addition to this standard protocol, additional GH concentrations were measured in patients in whom there was clinical suspicion of GHD. GH concentrations were measured at 30, 60, 90, 120, 150, 180, and 120 min following glucagon administration (fig. 1). Where there were clinical limitations to the blood volume that could be drawn, the 90- and 120-min specimens were prioritized.

Laboratory Measurement

GH was measured by a solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000; Siemens, Berlin, Germany), plasma glucose by an oxidase colorimetric reaction (Vitros 5600; Ortho Clinical Diagnostics, Raritan, N.J., USA) and betahydroxybutyrate by a D-3 hydroxybutyrate dehydrogenase colorimetric reaction (Vitros 5600; Ortho Clinical Diagnostics). IGF-I was measured by radioimmunoassay after acid ethanol extraction (Esoter-

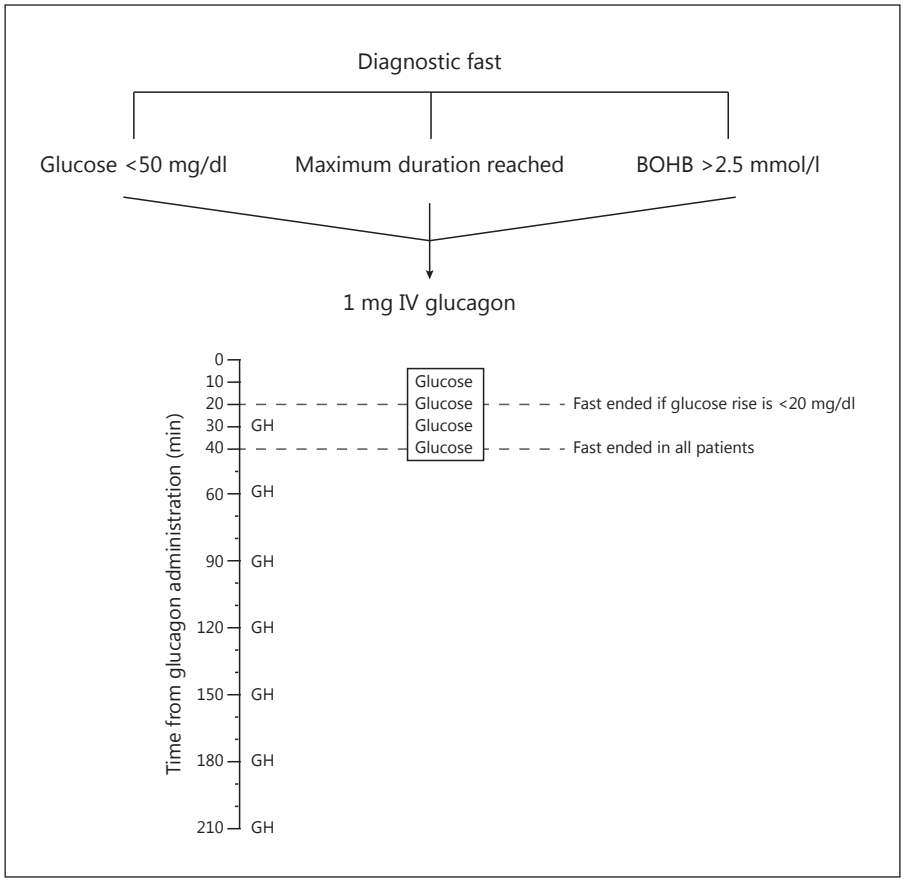

Fig. 1. Protocol for additional GH measurements after glucagon administration, in the context of a diagnostic fasting study. BOHB = β-Hydroxybutyrate.

ix Laboratories, Austin, Tex., USA) and IGFBP-3 was measured by radioimmunoassay (Esoterix Laboratories). Where relevant, assay-specific Z-scores for age and gender were reported.

Statistical Analysis

The threshold GH concentration considered to represent GH sufficiency varies between centers and generally ranges from 5 to 10 ng/ml [36, 37]. In this study, we use 7 ng/ml as the threshold for GH sufficiency. We also describe overall data if thresholds of 5 or 10 ng/ml were used.

Height and weight Z-scores at the time of the stimulation test were generated using the World Health Organization standards [38]. Unless otherwise stated, continuous variables were presented as the median (interquartile range). All data analyses were performed using SPSS 22.0 (IBM, N.Y., USA). Figures were generated using Prism 5.0 (GraphPad Software Inc., Calif., USA) and Adobe Illustrator 16.0 (Adobe Systems Inc., Calif., USA).

Results

Between July 2013 and March 2015, 29 patients with unexplained hypoglycemia and suspected GHD (largely due to coexisting short stature) had serial GH measurement following diagnostic fast and glucagon administration. The median (interquartile range) height Z-score was –2.3 (–3.3, –1). Of these patients, 6 had a final diagnosis of GHD and were treated with GH. The remaining patients had a diagnosis of hyperinsulinism (n = 9), ketotic hypoglycemia (n = 13), or mitochondrial disorder (n = 1). Out of all children included in this study, 4 (14%), 3 (10%), and 1 (3%) had GH concentrations above thresholds of 5, 7, and 10 ng/ml, respectively, at the end of the diagnostic fast. The additional GH measurement after glucagon administration identified 24 (86%), 19 (66%), and 15 (52%) children with GH concentrations exceeding these thresholds. The demographics of the patients included in this study are shown in table 1.

Of the 29 patients in this series, only 3 (10 %) had GH concentrations >7 ng/ml at the end of the fasting study, and all 3 of these children also had GH measurements above this threshold again on serial testing. The percentage of samples with GH concentrations above 5, 7, and 10 ng/ml at each time point is shown in figure 2. These data suggest that samples at 60, 90, and 120 min after glucagon

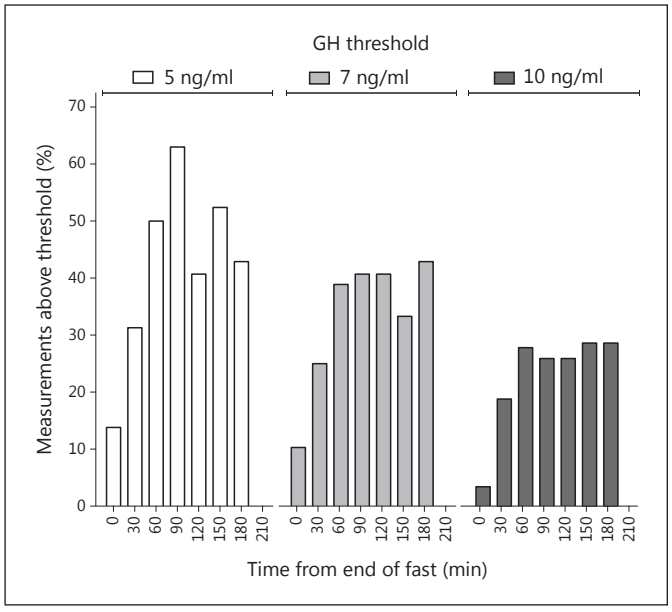

Fig. 2. The percentage of children with GH concentrations greater than or equal to thresholds of 5, 7, or 10 ng/ml at the time of glucagon administration at the end of the diagnostic fasting study (n = 29), or 30 (n = 16), 60 (n = 18), 90 (n = 27), 120 (n = 27), 150 (n = 21), 180 (n = 7) or 210 min (n = 3) later.

Table 1. Demographic data, diagnoses, critical sample measurements, and serial GH concentrations following glucagon administration

Males, n (%)	16 (55)
Age, years	1.8 (0.7, 3.4)
Height, Z-score	–2.3 (–3.3, –1)
Weight, Z-score	–1.2 (–2.3, –1)
Duration of fast, h	15 (9.8, 20)
Final diagnosis, n (%)	
Hyperinsulinism	9 (31)
Ketotic hypoglycemia	13 (45)
Mitochondrial disorder	1 (4)
GHD	6 (21)
Lab tests at the end of the fast	
Glucose, mg/dl	45 (42, 51.5)
Cortisol, μg/dl	16 (10.3, 20.5)
β-Hydroxybutyrate, mmol/l	2.4 (1.5, 2.75)
GH measurements[1], ng/ml	
Baseline (n = 29)	2.6 (1, 3.5)
30 min (n = 16)	2.5 (1.6, 8.2)
60 min (n = 18)	4.9 (2.4, 10.7)
90 min (n = 27)	5.6 (4.2, 12.9)
120 min (n = 27)	4.4 (2.5, 10.2)
150 min (n = 21)	5.1 (2.2, 11)
180 min (n = 7)	3.2 (1.2, 11.6)
210 min (n = 3)	3.6 (2, 4.9)
Subjects who exceeded threshold on testing	
GH >5 ng/ml at baseline	4 (14)
GH >5 ng/ml after serial measurements	25 (86)
GH >7 ng/ml at baseline	3 (10)
GH >7 ng/ml after serial measurements	19 (66)
GH >10 ng/ml at baseline	1 (3)
GH >10 ng/ml after serial measurements	15 (52)

Data are presented as the median (interquartile range), unless otherwise indicated. [1] Note that n represents the number of data points available for GH measurement at each time point.

administration should be prioritized where frequent sampling is not possible.

Of the 26 (90%) patients with GH concentrations <7 ng/ml during hypoglycemia, 10 (34%) also had peak GH concentrations below this threshold on serial measurement after glucagon administration. Of these 10 children without GH concentrations >7 ng/ml, 9 underwent additional GH stimulation testing using arginine and clonidine. Six were diagnosed with GHD and treated with GH. Two children did not have peak GH concentrations >7 ng/ml following arginine and clonidine stimulation testing, but their clinical picture was considered to be more consistent with hyperinsulinism than GHD, and they were not treated with GH. Both of these children were treated with diazoxide, with a good clinical response.

Characteristics and diagnostic evaluation of the 10 patients with suboptimal peak GH concentrations are shown in table 2. Additional diagnostic information on cases 7 and 8 from this series is provided, as the diagnosis may be unclear from the data presented in this table. Although case 7 had normal growth factor concentrations, it should be noted that IGF-I concentrations are sensitive to nutrition [39] and, in infancy, do not reliably identify infants with GHD due to the wide range of normal concentrations [40]. This infant also had low random GH measurements on days 3 (1.11 ng/ml) and 14 (0.334 ng/ml) of life, also supporting the diagnosis of GHD [41]. Case 8 had a diagnosis of focal hyperinsulinism based on a known pathogenic *ABCC8* mutation and previous fasting evaluations consistent with hyperinsulinism. The diagnostic evaluation presented in table 2 was from an evaluation after the focal lesion had been removed and the hyperinsulinism had resolved. Although his peak GH concentration did not exceed the threshold of 7 ng/ml, his linear growth pattern was not consistent with GHD, and a decision was made to observe his growth rather than initiate treatment.

Table 2. Details of the 10 children without peak GH concentrations >7 ng/ml after the fasting study and glucagon administration

Patient:	1	2	3	4	5	6	7	8	9	10
Age, years	7.5	1.2	5.3	1.8	1.4	5.1	0.1	2.6	6.1	0.7
Sex	male	male	female	male	female	female	male	male	female	male
Height, Z-score	–1.2	–0.81	–2.33	–2.2	–4.1	–0.75	–0.02	–2.47	–3.6	–0.94
Midparental height, Z-score	–0.43	NA	–0.68	0.24	–0.87	–0.46	NA	NA	–0.48	0.25
MRI brain and pituitary	normal	NP	NP	normal	NP	NP	abnormal corpus callosum	NP	ectopic neurohypophysis	normal
Fast duration, h	24	15	20	17	10	16	4	13	14	10
Critical Sample										
Glucose, mg/dl	41	45	49	47	43	51	46	53	45	52
β-Hydroxybutyrate, mmol/l	2.9	1.2	2.2	2.9	2.5	4	0.1	2.5	0.8	2.3
GH, ng/ml	3.6	1	1.1	2.4	3.5	3.5	0.8	2.4	0.1	3.2
Cortisol, μg/dl	20.8	14.4	28.9	9.3	14.3	40.7	14.9	16.3	6	20.6
Peak GH after glucagon, ng/ml	5.4	5.2	5.5	5.1	4.7	5.7	4.3	4.7	0.3	6.6
Peak GH on repeat GH stimulation test, ng/ml	7.1	4.6		6.5	3.7	8.2	1.9	6.2	0.4	1.5
Thyroid function tests	normal	NP	normal	normal	normal	NP	normal	normal	normal	normal
IGF-I, Z-score	–1.4	NP	0.1	–0.9	–3.1	NP	0.7	–1.5	–2.6	–0.7
IGFBP-3, Z-score	–1.6	NP	–0.74	–0.18	–1.6	NP	0.77	–0.73	NP	–2
Diagnosis	GHD[1]	HI	KH	GHD	GHD	KH	GHD	resolved focal HI	GHD, AI	GHD

NA = No data available; NP = test not performed; HI = diazoxide-responsive hyperinsulinism; KH = ketotic hypoglycemia; AI = adrenal insufficiency. [1] Additional clinical data supporting the diagnosis of GHD: height velocity 3 cm/year, bone age delayed by 2 years (–2.3 SD from the mean), improved growth, and no further hypoglycemia following GH treatment.

Discussion

We have shown that the serial measurement of GH following the administration of glucagon in the context of a fasting study can be a useful adjunct in children suspected of having GHD. GH measurement during hypoglycemia has poor specificity for GHD and, by adding serial GH measurements following glucagon administration, the number of children identified with peak GH concentrations above the arbitrary threshold of 7 ng/ml increased by 16 (55%). This resulted in a more focused evaluation of GH secretion in a smaller number of children than would otherwise have been performed.

The poor specificity of GH measurement during hypoglycemia has previously been described. In a study including 84 children evaluated for unexplained hypoglycemia, only 30% had peak GH concentrations >7.5 ng/ml [22]. In our study, there was a lower number (10%) of children with peak GH concentration >7 ng/ml during hypoglycemia. However, additional GH measurements were only performed in children for whom there was clinical suspicion that GHD was the etiology of their hypoglycemia, and this selection bias may have contributed to the discrepancy in results between our study and previously reported data.

One limitation to adapting our clinical protocol was the blood volume required for additional GH measurements in small infants with unexplained hypoglycemia. Serial glucose measurement during the diagnostic fast, in addition to the critical sample, can limit the blood volume that can be extracted for further tests. Previous studies suggest that the peak GH concentration following glucagon administration (glucagon stimulation test for GH reserve) generally occur after 90 and 120 min [28, 34, 42, 43]. Where necessary, GH samples were prioritized at 90 and 120 min in this study, and we have shown that these are the most useful measurements in this context to identify GH-sufficient children (fig. 2). Although not evaluated in this study, we note that cortisol responses to glucagon administration occur later, at 150 and 180 min [35, 43], and this should be considered if future studies of this test are adapted to evaluate cortisol response to glucagon in this context.

The mechanism of glucagon-induced GH secretion is not clear. Fluctuations in blood glucose following glucagon administration may contribute to GH secretion, although recent studies suggest that this may not be necessary [34, 44]. Glucagon administration increases noradrenaline secretion, [45] which may play a role in

stimulating GH secretion, but α-adrenergic blockade does not prevent glucagon-induced GH secretion [46]. Although fasting increases GH secretion [47, 48], it was not clear if allowing the patient to feed while measuring GH concentrations after glucagon secretion would affect the ability of the test to identify children with GH concentrations above the stimulation threshold. A large proportion (17/27) of children in this study had an appropriate stimulated GH response to glucagon despite having been allowed to feed.

We do not know if the serial GH response noted in this study would have been seen if glucagon was not provided. Hypoglycemia is a strong stimulus for GH secretion in the absence of additional pharmacological stimuli, and this is utilized in the commonly used insulin tolerance test of GH secretion. However, the GH response to spontaneous hypoglycemia in children is blunted in comparison to insulin tolerance testing [21]. This makes the GH concentrations seen in this study more likely to be secondary to glucagon administration rather than to hypoglycemia. We also acknowledge that an intramuscular injection of glucagon may result in higher detectable concentrations of GH relative to IV glucagon [49], possibly as a result of an additional painful stimulus [50]. As intramuscular or subcutaneous glucagon are more potent stimuli of GH secretion [49, 51, 52], it is possible that modifying the protocol to utilize these routes of administration would further improve the specificity for GHD. However, this route of glucagon administration is not routinely used in evaluating the glycemic response to hypoglycemia in our practice. Thus, we are unable to compare different routes of glucagon administration in this study.

It is important to note that many normal children will be characterized as having GHD on the GHST alone [29–31], and these results should be interpreted in the clinical context. Depending on the GH stimulus and GH concentration threshold used, the proportion of normal children who do not reach the 'sufficient' threshold can be as high as half [29]. In this study, only children suspected of having GHD at the time of the diagnostic fasting study underwent this additional serial GH measurement. Given the poor reliability of the GHST, we do not routinely perform the GHST in children with peak GH concentrations <7 ng/ml where the laboratory tests performed during hypoglycemia indicate that alternative diagnoses are more likely. In addition, GHD is not always diagnosed if peak GH concentrations on arginine and clonidine stimulation testing do not exceed 7 ng/ml in these patients. This clinical judgement and lack of robust diagnostic tools may result in children with diagnoses such as ketotic hypoglycemia being misdiagnosed as GHD.

In conclusion, we have shown that additional GH measurements after glucagon administration following a diagnostic fast can improve the identification of children with stimulated GH concentrations above stimulation test thresholds. This test can be performed in addition to the diagnostic fasting study and does not require prolongation of the fast. We also recommend that children with insufficient responses to glucagon in this setting should further have GHD confirmed by standard GHSTs if there is clinical suspicion of GHD.

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Disclosure Statement

C.P.H., V.E.D., and D.D.D.L. have no financial relationships relevant to this article to disclose. A.G. serves on the Steering Committee of the Pfizer International Growth Study Database.

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