Possible Role of α-Cell Insulin Resistance in Exaggerated Glucagon Responses to Arginine in Type 2 Diabetes

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OBJECTIVE — Inappropriate excessive secretion of glucagon, which contributes to postprandial hyperglycemia, is a novel target for the treatment of diabetes. In this study, we sought to determine the factors associated with exaggerated glucagon secretion in response to an arginine challenge in patients with type 1 and type 2 diabetes.

RESEARCH DESIGN AND METHODS — Changes in circulating C-peptide immunoreactivity (CPR) and immunoreactive glucagon (IRG) after an arginine challenge were investigated in 35 patients with type 1 diabetes, 130 patients with type 2 diabetes, and 35 nondiabetic control subjects.

RESULTS — No significant differences were found in the basal level and the area under the concentration-time curve (AUC) of IRG (AUC_{IRG}) among type 1 and type 2 diabetic patients and nondiabetic subjects. However, there was an inverse correlation between the AUC_{IRG} and the AUC of CPR (AUC_{CPR}) for type 1 ($r = -0.388$, $P = 0.023$) and type 2 ($r = 0.396$, $P < 0.0001$) diabetic patients, whereas AUC_{IRG} was not correlated with AUC_{CPR} in nondiabetic subjects ($r = -0.079$, $P = 0.655$). In type 1 diabetic patients, the AUC_{CPR} decreased and the AUC_{IRG} increased with increasing disease duration. In type 2 diabetic patients, both AUC_{IRG} and AUC_{CPR} increased with increasing BMI, basal CPR level, and homeostasis model assessment of insulin resistance value.

CONCLUSIONS — Our findings suggest that the pathophysiology of the exaggerated glucagon response differs between type 1 and type 2 diabetes. Intraislet insulin deficiency and α-cell insulin resistance may be the primary contributors to this condition in type 1 and type 2 diabetes, respectively.

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Diabetes is associated with increased hepatic glucose production, which is linked to fasting and postprandial hyperglycemia (1). This is caused by the reduced suppression of glucagon (2), along with the impairment of insulin secretion and insulin action. Arginine-stimulated hyperglucagonemia in patients with various forms of diabetes was first discovered by Aronoff et al. (3,4). Their results demonstrated that excess glucagon or an elevated ratio of glucagon to insulin is etiologically important in the development of endogenous hyperglycemia in diabetes through the mediation of glucose overproduction from the liver (2,5–7). Thus, glucagon and its receptor have been examined extensively in recent years as being potential targets for the treatment of diabetes (8,9). In fact, glucagon-like peptide 1 analogs, which are forthcoming antidiabetes agents, lower postprandial hyperglycemia partly by inhibiting excessive secretion of glucagon in not only type 2 but also type 1 diabetes (10). An absolute deficiency in insulin secretion has been suggested to cause an exaggerated response of glucagon to arginine in patients with diabetes (11,12). Insulin replacement therapy can correct this deficiency in patients with type 1 diabetes (11,12) but not in those with type 2 diabetes (11). The pathophysiology associated with an exaggerated glucagon response to arginine remains unclear, particularly in patients with type 2 diabetes.

Here, we comprehensively analyze factors associated with the exaggerated glucagon secretion response to an arginine challenge in patients with diabetes and show that the glucagon response to arginine reflects a distinctly different pathophysiology in type 1 versus type 2 diabetes.

RESEARCH DESIGN AND METHODS

— A total of 223 Japanese patients with diabetes who were hospitalized in the Division of Endocrinology and Metabolism at Kanazawa University Hospital (Ishikawa, Japan) were studied for the management of diabetes and for patient education. The study was conducted from April 2003 to May 2006. Patients were diagnosed according to the criteria established by an expert committee on the diagnosis and classification of diabetes (13). Type 1 diabetes was determined based on the presence of islet autoantibodies (anti-GAD antibody). In addition, all type 2 diabetic patients met the requirements for insulin therapy, as defined by the American Diabetes Association. Among the 36 patients with type 1 diabetes, we excluded one patient with Wilson's disease (supplemental Fig. 1 [available in an online appendix at http://dx.doi.org/10.2337/dc07-0066]). Among the 187 patients with type 2 dia-

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Abbreviations: AUC, area under the concentration-time curve; CPR, C-peptide immunoreactivity; HOMA-IR, homeostasis model assessment of insulin resistance; IRG, immunoreactive glucagon; QUICKI, quantitative insulin sensitivity index.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of type 1 and type 2 diabetic patients and nondiabetic subjects*

	Type 1 diabetic patients	Type 2 diabetic patients	Nondiabetic subjects
n	35	130	35
Acute/slowly progressive type 1 diabetes	26/9		
Sex (men/women)	14/21	81/49	15/20
Age (years)	49 ± 15	57 ± 13*	33 ± 6*†
BMI (kg/m^2)	22.4 ± 4.3	23.8 ± 3.6	22.7 ± 4.0
Diabetes duration (months)	99.4 ± 92.5	123.9 ± 142.8	
A1C (%)	9.7 ± 2.8	8.9 ± 2.6	5.0 ± 0.4*†
Urinary C-peptide (mg/day)	9.9 ± 13.0	43.4 ± 42.1*	
Therapy			
Insulin	35	78	
Oral antidiabetes agent	0	34	
Diet alone	0	20	

Data are *n* or means ± SD. *$P < 0.01$ vs. type 1 diabetic subjects. †$P < 0.01$ vs. type 2 diabetic subjects.

betes, we excluded 57 patients with secondary diabetes related to conditions including liver disease, pancreatic disease, and hormonal disorders (supplemental Fig. 1). Thus, 35 patients with type 1 diabetes and 130 with type 2 diabetes were enrolled in this study. Additionally, 35 nondiabetic healthy volunteers were enrolled as control subjects. The characteristics of the study subjects are shown in Table 1. Of those with type 1 diabetes, 26 patients were diagnosed with an acute-onset form and 9 with slowly progressive type 1 diabetes (14). Informed consent was obtained from all patients before study initiation, and the study was approved by the relevant ethics committee and conducted in accordance with the Declaration of Helsinki.

On admission, the patients received a standard diet of 30 kcal/kg. All patients with type 1 diabetes were treated with intensive insulin therapy. Among the patients with type 2 diabetes, 78 were treated with insulin (57 with premeal dosing of a rapid-acting insulin analog alone), 32 were treated with oral antidiabetes agents (14 with α-glucosidase inhibitors, 16 with nateglinide, 4 with metformin, and 5 with sulfonylureas), and 20 received diet therapy alone.

Arginine stimulation test

After an overnight fast, patients were kept at rest for ≥30 min, and endogenous insulin and glucagon levels, as measured by C-peptide immunoreactivity (CPR) in serum and immunoreactive glucagon (IRG) in plasma, respectively, were assessed at preloading baseline (0 min). Arginine (30 g) was administered by intravenous infusion of a 10% L-arginine hydrochloride solution over 30 min. Blood was collected at seven time points: preloading (0 min) and 15, 30, 45, 60, 90, and 120 min after arginine loading. Circulating IRG and CPR were measured at each time point and were used to construct the arginine-stimulated time-response curves. The values of the area under the concentration-time curve for IRG (AUC_{IRG}) and CPR (AUC_{CPR}) between time 0 and 120 min were calculated by means of the trapezoidal rule and indicate the insulin- and glucagon-secreting responses to arginine. The homeostasis model assessment of insulin resistance (HOMA-IR) (15) and the quantitative insulin sensitivity index (QUICKI) (16) were used as conventional indexes for insulin resistance. The values for HOMA-IR and QUICKI were calculated using the following formulas: HOMA-IR = [fasting insulin (μU/ml) × fasting plasma glucose (mmol/l)]/22.5 and QUICKI = 1/{log[fasting plasma glucose (μU/ml)] + log[fasting insulin (mmol/l)]}.

The immunoenzymometric assays used for quantifying C-peptide and insulin were performed using kits purchased from Tosoh (Shunan, Japan). Plasma glucagon levels were measured using a radioimmunoassay kit (Daiichi; Daiichi Radioisotope Labs, Tokyo, Japan). The lower limits of quantification for CPR and IRG were 0.2 ng/ml and 15.6 pg/ml, respectively. The intra- and interassay coefficients of variation were both <6%. Glucose and A1C were measured by standard methods.

Statistical analysis

Data are expressed as means ± SD. Statistical analyses were performed with StatView software (SAS Institute, Cary, NC). Differences among groups were tested by ANOVA with a post hoc test of Fisher's protected least significant differences. Pearson's product moment correlation coefficients were obtained to estimate linear correlation among the variables. $P < 0.05$ was considered statistically significant.

RESULTS

Responses of insulin and glucagon to arginine challenge in patients with type 1 and type 2 diabetes and nondiabetic subjects

Responses of CPR and glucagon to arginine are shown in Fig. 1. Type 2 diabetic and nondiabetic subjects had relatively high levels of basal CPR and AUC_{CPR} compared with type 1 diabetic patients (Fig. 1). Interestingly, the mean glucagon response did not differ among the three treatment groups (i.e., type 1 and type 2 diabetes and control). However, some type 1 or type 2 diabetic patients exhibited an exaggerated glucagon response to arginine.

Distinct relationship between AUC_{IRG} and AUC_{CPR} in patients with type 1 versus type 2 diabetes

To address the pathophysiology underlying the exaggerated glucagon response to an arginine challenge, we examined the relationship between AUC_{IRG} and AUC_{CPR} in type 1 and type 2 diabetic patients and in nondiabetic control subjects (Fig. 2). In patients with type 1 diabetes, AUC_{IRG} was negatively correlated with AUC_{CPR} ($AUC_{IRG} = 32{,}462 - 17.8\ AUC_{CPR}$; $r = -0.388$, $P = 0.023$) (Fig. 2*A*). In contrast, AUC_{IRG} was positively correlated with AUC_{CPR} in type 2 diabetic patients ($AUC_{IRG} = 19{,}419 + 18.8\ AUC_{CPR}$; $r = 0.396$, $P < 0.0001$) (Fig. 2*B*). No correlation was found between AUC_{IRG} and AUC_{CPR} in nondiabetic subjects ($AUC_{IRG} = 30{,}803 - 4.7\ AUC_{CPR}$; $r = -0.079$, $P = 0.655$) (Fig. 2*C*). These results suggest that the mechanisms involved in the glucagon response to arginine challenge are distinctly different between type 1 and type 2 diabetic patients.

Relationship between AUC_{IRG} and AUC_{CPR} stratified by the class of diabetes duration in patients with type 1 diabetes

Intraislet insulin deficiency may determine the exaggerated glucagon response to arginine challenge (17). To test our hypothesis that type 1 diabetic patients with a longer diabetes duration are deficient in

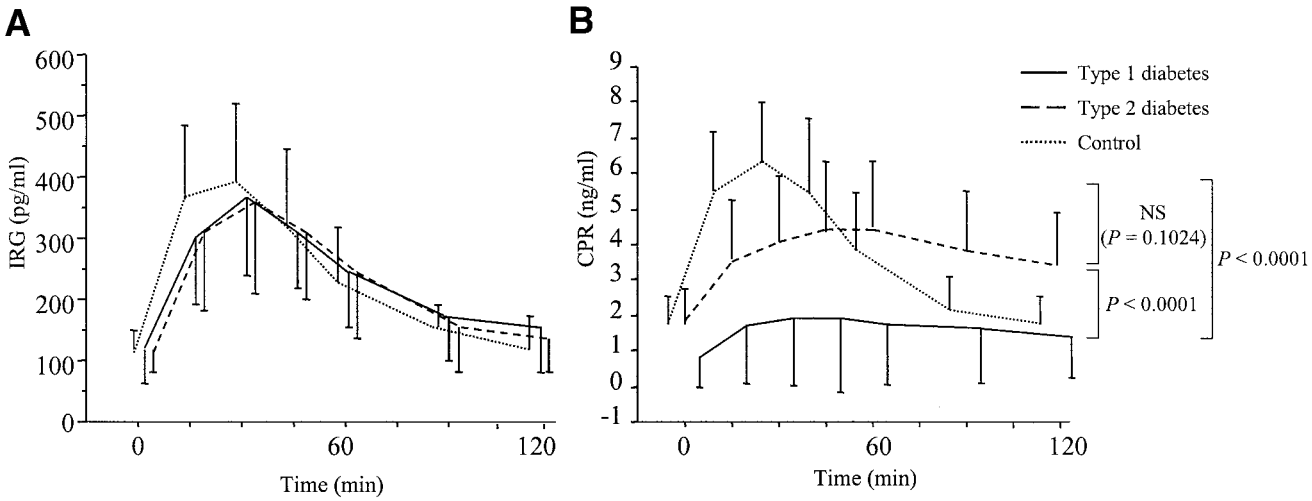

Figure 1—*IRG* (A) *and CPR* (B) *response curves to arginine. The glucagon responses were similar among type 1 and type 2 diabetic patients and nondiabetic control subjects, whereas the CPR responses of type 2 diabetic patients and control subjects were greater than those of type 1 diabetic patients. Data are shown as mean values; error bars denote SD. NS, not significant.*

insulin secretory capacity, we analyzed the relationship between the AUC_{IRG} or AUC_{CPR} and diabetes duration in type 1 diabetic patients (Table 2 and supplemental Fig. 2*A*) and further investigated the correlation between AUC_{IRG} and AUC_{CPR} stratified by classes of diabetes duration, i.e., 0–72 months and 96–348 months (supplemental Fig. 2*B*). The patients with slowly progressive type 1 diabetes were excluded from this analysis because the onset of diabetes seemed to be unclear. As the diabetes duration increased, AUC_{CPR} decreased and AUC_{IRG} increased in type 1 diabetic patients (Table 2 and supplemental Fig. 2). This relationship was not evident in patients with type 2 diabetes (data not shown). These findings suggest that intraislet insulin deficiency with long disease duration determines the exaggerated glucagon response to arginine challenge in patients with type 1 diabetes.

To rule out possible influences of insulin treatment on the glucagon response to arginine in type 1 diabetic patients, we analyzed the relationship of the AUC_{IRG} or AUC_{CPR} to insulin dose (supplemental Fig. 3) and glycemic control indexes such as A1C and fasting plasma glucose (Table 2 and supplemental Fig. 4). We found that neither the AUC_{IRG} nor the AUC_{CPR} was correlated with either insulin dose or glycemic control status; thus, exogenous insulin replacement therapy and glycemic control status were unlikely to have affected our results and conclusions in patients with type 1 diabetes.

Relationship between AUC_{IRG} and AUC_{CPR} stratified by class of BMI and insulin resistance indexes in patients with type 2 diabetes

Previous studies have suggested that the glucagon response to arginine is inversely related to insulin sensitivity (18–21). To test the hypothesis that obese type 2 diabetic patients have impaired insulin sensitivity, hyperinsulinemia, and hyperglucagonemia, we analyzed the relationship between the AUC_{IRG} or AUC_{CPR} and insulin resistance indexes such as BMI, basal CPR level, HOMA-IR, and QUICKI in patients with type 2 diabetes (Table 2 and supplemental Fig. 5*A*). Both the AUC_{IRG} and AUC_{CPR} were positively correlated with BMI, basal CPR level, and HOMA-IR, and both were negatively correlated with QUICKI. No significant cor-

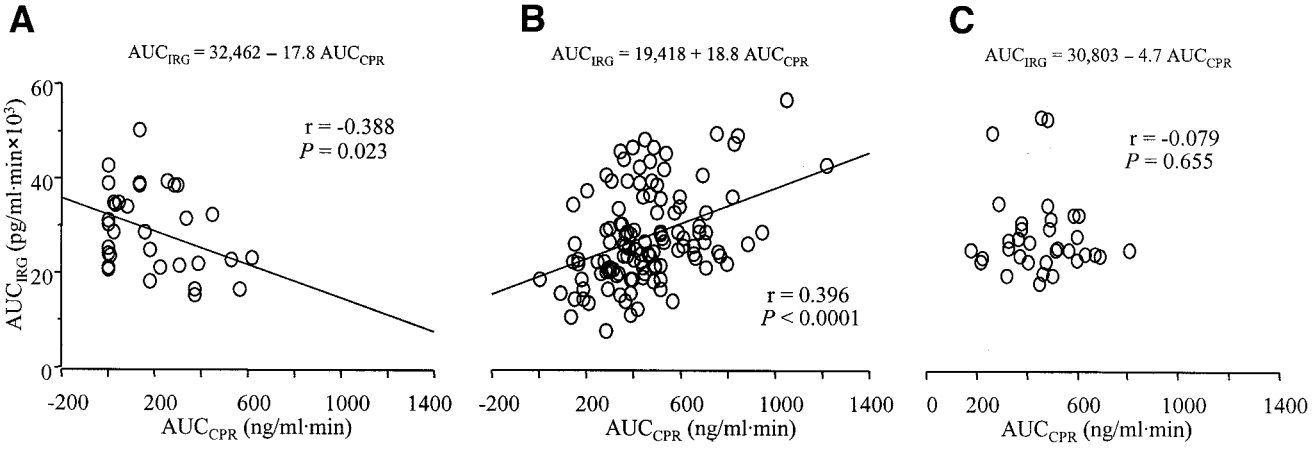

Figure 2—*Relationship between AUC_{IRG} and AUC_{CPR} in type 1* (A) *and type 2* (B) *diabetic patients and nondiabetic control subjects* (C). *AUC_{IRG} was negatively correlated with AUC_{CPR} in type 1 diabetic patients* ($r = -0.388$, $P = 0.023$), *whereas AUC_{IRG} was positively correlated with AUC_{CPR} in type 2 diabetic patients* ($r = 0.396$, $P < 0.0001$). *In the control group, AUC_{IRG} was not correlated with AUC_{CPR}* ($r = -0.079$, $P = 0.655$).

Table 2—*The relationship between AUC_{IRG} or AUC_{CPR} and clinical parameters in type 1 and type 2 diabetic patients and nondiabetic control subjects*

	Diabetes duration	BMI	Basal CPR	HOMA-IR	QUICKI	A1C	FPG
AUC_{IRG}							
Type 1 diabetic patients							
r	0.445	−0.233	−0.391	ND	ND	0.022	0.009
P	0.033	0.208	0.024	ND	ND	0.910	0.961
Type 2 diabetic patients							
r	−0.174	0.467	0.269	0.361	−0.319	0.013	0.040
P	0.057	<0.001	0.003	0.001	0.005	0.889	0.721
Nondiabetic subjects							
r	ND	0.094	−0.042	0.036	−0.127	ND	ND
P	ND	0.592	0.813	0.851	0.502	ND	ND
AUC_{CPR}							
Type 1 diabetic patients							
r	−0.477	0.359	0.938	ND	ND	−0.194	−0.137
P	0.021	0.047	<0.001	ND	ND	0.323	0.447
Type 2 diabetic patients							
r	−0.260	0.455	0.844	0.343	−0.385	−0.204	−0.155
P	0.005	<0.001	<0.001	0.002	0.001	0.089	0.173
Nondiabetic subjects							
r	ND	0.348	0.695	0.511	−0.557	ND	ND
P	ND	0.043	<0.001	0.004	0.001	ND	ND

FPG, fasting plasma glucose; ND, not determined.

relation was found between the AUC_{IRG} and any of the insulin resistance indexes in either patients with type 1 diabetes (Table 2 and supplemental Fig. 6) or nondiabetic subjects (Table 2 and supplemental Fig. 7). We further analyzed the relationship between the AUC_{IRG} and AUC_{CPR} stratified by BMI class, basal CPR level, HOMA-IR, and QUICKI (supplemental Fig. 5*B*). Both the AUC_{IRG} and AUC_{CPR} significantly increased in relation to increases in BMI, basal CPR level, and HOMA-IR in patients with type 2 diabetes, whereas both significantly decreased with increases in QUICKI. In contrast, neither glycemic control status (Table 2 and supplemental Fig. 8) nor treatment (supplemental Fig. 9) affected the AUC_{IRG} in type 2 diabetic patients. These results suggest that insulin resistance contributes to the exaggerated glucagon response to arginine in patients with type 2 diabetes but not in those with type 1 diabetes or in nondiabetic subjects.

CONCLUSIONS — The arginine stimulation test has been demonstrated to be a valid method for evaluating residual β-cell function, even during periods of hyperglycemia (3,4,22–24). However, the relevance of the glucagon response to arginine remains uncertain and has not been comprehensively analyzed in patients with type 1 and type 2 diabetes. In the present study, we revealed the pathophysiology of the glucagon secretion response to an arginine challenge in patients with diabetes. Although no difference was observed in the glucagon response between patients with type 1 and type 2 diabetes, the glucagon response reflected a distinct pathophysiology in each type of diabetes.

In patients with type 1 diabetes, AUC_{IRG} was inversely correlated with AUC_{CPR} in the arginine challenge test and the insulin response to arginine inversely correlated with diabetes duration, suggesting that intraislet insulin deficiency determines the exaggerated glucagon response to arginine. Autoimmune type 1 diabetes is caused by a β-cell–targeted immune reaction and destruction with relatively conserved α-cell mass (25). Therefore, the glucagon response to arginine cannot be suppressed by intrinsic insulin in hypoinsulinemic diabetic patients (12). Conversely, in patients with type 2 diabetes, the AUC_{CPR}, but not the AUC_{IRG}, was negatively correlated with diabetes duration (data not shown). In addition, patients with insulinopenic type 2 diabetes did not exhibit an exaggerated glucagon response to arginine (Fig. 2*B*). Based on these findings, we hypothesize that only absolute insulin deficiency, as observed in type 1 diabetes, is associated with an exaggerated glucagon response. These results are in agreement with a previous study that suggested that absolute deficiencies in insulin secretion are the cause of an exaggerated glucagon response to arginine in patients with diabetes (11,12). In addition, insulin replacement therapy can correct these deficiencies in patients with type 1 diabetes (11,12). The molecular mechanism underlying this observation may involve the suppression of glucagon release by intraislet insulin via the GABA (γ-aminobutyric acid)-$GABA_A$ receptor system (26). Given that some patients displayed no C-peptide response and a relatively conserved glucagon response to arginine in the present study, it is possible that other currently unknown factors may also regulate glucagon and CPR responses to arginine.

In hyperinsulinemic patients with impaired glucose tolerance or type 2 diabetes, the glucagon response to arginine was reported to be inversely related to insulin sensitivity (19–21). In these studies, however, it remained unclear whether the exaggerated glucagon response to arginine was caused by intraislet insulin deficiency or α-cell insulin resistance. In the present study, we demonstrated a positive correlation between AUC_{IRG} and AUC_{CPR} in type 2 diabetes, which was opposite the

relationship in type 1 diabetes. The exaggerated glucagon response to arginine was also related to insulin resistance. The mean AUC_{IRG} and AUC_{CPR} values increased with increases in BMI and insulin resistance indexes, suggesting that β-cell hypertrophy associated with obesity might determine the exaggerated glucagon secretion in response to arginine in patients with type 2 diabetes. This pathology was not evident in patients with type 1 diabetes. Thus, we speculate that α-cell insulin resistance may be a key pathology causing the exaggerated glucagon response to arginine in patients with type 2 diabetes. In this regard, Hamaguchi et al. (18) previously reported that an exaggerated α-cell response to arginine infusion in obese hyperinsulinemic patients with glucose intolerance was secondary to a reduction in insulin action on the pancreatic α-cell. In addition, they observed that exogenous insulin replacement normalized these abnormalities (18), suggesting that compensatory hyperinsulinemia can overcome α-cell insulin resistance. However, we did not observe such compensation in type 2 diabetic patients with severe hyperinsulinemia (Fig. 2*B*). Our results are in agreement with a previous report (11) that insulin replacement cannot correct an exaggerated glucagon response in patients with type 2 diabetes. These findings further support the hypothesis that α-cell insulin resistance occurs with increasing systemic insulin resistance in patients with type 2 diabetes.

In summary, intraislet insulin deficiency and α-cell insulin resistance may cause exaggerated glucagon secretion in response to arginine, which might in turn contribute to impaired suppression of hepatic glucose output in both type 1 and type 2 diabetes. Collectively, our data support the idea that exaggerated secretion of glucagon may be a therapeutic target in both insulinopenic diabetes and type 2 diabetes with insulin resistance. Therefore, the arginine challenge test could be useful for assessing the heterogenous nature of diabetes and may be a valid method for identifying responders to therapy targeted at glucagon and its receptor, such as glucagon-like peptide 1 analogs. Large-scale clinical studies are needed to test this hypothesis.

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