Declining β-Cell Function in Type 2 Diabetes: 5-Year Follow-up and Immunologic Studies of the Population of Wadena, MN

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The aim of the study was to describe 5-year changes in meal-stimulated pancreatic insulin reserve in adults with normal and impaired glucose tolerance (NGT, IGT) and diabetes, with or without islet-related antibodies. This was a 5-year follow-up of 270 residents of Wadena, MN, of northern European origin, with good kidney function, defined as creatinine clearance greater than 60 mL/min/1.73 m^2. The subjects comprised a population-based sample originally studied in 1986 to 1987. Urine C-peptide (CP), in a 260-minute collection, was the integrated measure of insulin secretion; Ensure-Plus (Ross, Columbus, OH) was the liquid meal. Islet cytoplasmic antibodies (ICA), insulin autoantibodies (IAA), and glutamate decarboxylase antibodies (GAD65ab) were measured. In 182 subjects with NGT, there was no mean within-subject change in urine CP over 5 years ($P = .34$). In 41 subjects with impaired GT (IGT), there was a moderate, but nonsignificant, increase in mean CP, and 6 (15%) subjects increased. In 37 type 2 diabetic subjects not taking insulin (type 2-No Ins), who had a mean diabetes duration at the 5-year examination of 9.6 ± 6.3 years, there was a 21% decrease in mean urine CP ($P = .012$), attributable mostly to a major drop in 8 of the 37 subjects (22%). Islet-related antibody tests were mostly negative; GAD65ab positivity was related to CP decline only among insulin-taking subjects. In summary, in Wadena adults, meal-stimulated urine CP was stable or increased over 5 years in subjects with NGT and IGT, but CP decreased significantly in about one fifth of type 2-No Ins subjects, with no relation to antibody test results.

IT IS CURRENTLY accepted that the common form of diabetes (type 2) involves both resistance to insulin and insulin deficiency.[1] It is sometimes further assumed that all type 2 individuals, once hyperglycemia is manifest, progress uniformly through a process of islet-cell failure leading inevitably to marked insulin deficit and the need for insulin treatment. However, the time scale for islet failure has not been extensively studied. The most pertinent prospective studies to date were published in the 1990s.[2-4] Our own preliminary experience had shown persisting insulin secretion after many years in some cases of documented overt hyperglycemia, using urine C-peptide (CP) excretion rate as a measure of insulin reserve.[5]

We began, in 1986, a population-based cross-sectional study of meal-stimulated urine CP in adult residents of the community of Wadena, MN, which is primarily made up of descendants of northern European immigrants.[6] We found that a majority of the type 2 subjects in the community were non–insulin-taking (type 2-No Ins) and showed no relation between meal-stimulated urine CP and self-reported duration of diabetes, with a range of 3 to 34 years. On the other hand, those type 2 subjects who were taking insulin (type 2-Ins) showed a trend toward an inverse relationship between CP and diabetes duration (-6%/yr for plasma CP, $P < .001$; -3%/yr for urine CP, $P = .7$).[6] About half of the type 2-Ins subjects and a few of the type 2-No Ins subjects had very low urinary CP levels. We concluded, tentatively, that loss of insulin reserve may be long delayed in type 2 diabetes, but may be profound when it does occur.

To help clarify these cross-sectional findings and to provide more epidemiologic evidence about the time scale and cause for islet failure, this report describes the extent to which urine CP declined in participants followed for 5 years, according to their glucose tolerance status at baseline. We also tested the specific hypothesis that autoimmunity to islet cell components might explain declining β-cell function in type 2 diabetes.[7-9] Finally, we searched for other variables that might predict this decline.

MATERIALS AND METHODS

Subjects and Kidney Function

Wadena, MN (population 4,699, 1980 US census) is a stable small city, 200 km from the metropolitan area of Minneapolis-St. Paul; over 85% of participants reported parents or grandparents from Germany, Scandinavia, or the British Isles, the remainder from other parts of Europe. Two cohorts of Wadena residents ≥ 20 years of age had been recruited at baseline: a sample randomly chosen from approximately 3,000 individuals not known to be diabetic, stratified for age and sex (n = 389), and a cohort including all persons known to care-givers in Wadena as diabetic (n = 87). A total of 476 individuals participated in most or all baseline studies in 1986 to 1987. The 2 cohorts were combined for further data analysis; details have been reported previously.[6,10-12]

Five-year follow-up testing was performed in 1991 to 1992; 317 subjects completed both CP and immunologic studies. Death was the

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most frequent reason for drop out (31% of the drop-outs); other reasons included major illness and moving outside the state of Minnesota. Subjects taking insulin or with systolic blood pressure ≥ 160 mm Hg were significantly less likely to return for testing at 5 years.[12]

We report here 5-year follow-up urine CP measurements and antibody studies in 270 participants in the Wadena City Health Study for whom complete data were available and who had relatively good kidney function, defined as creatinine clearance (Cl_{Cr}) ≥ 60 mL/min/1.73 m^2. They constituted 85% of the 317 subjects who returned for follow-up and 57% of the 476 tested at baseline. The rationale for the restriction on kidney function was based on concern that altered kidney function (that is, reduced glomerular filtration rate) might be accompanied by reduced renal ultrafiltration of CP[13] and thereby confound the interpretation of urine CP as a measure of pancreatic insulin secretion. Accordingly, correlation between urine CP and Cl_{cr} was examined for subjects with Cl_{cr} below 60 mL/min at either time point and for subjects with normal or near-normal Cl_{cr} (above 60 mL/min at both time points). For subjects with reduced clearance, there was significant correlation between CP excretion and Cl_{Cr}: $r = .41$, $P = .004$ at baseline and $r = .37$, $P = .010$ at 5 years. In contrast, for the near-normal to normal subjects ($Cl_{cr} \geq 60$ mL/min), there was no correlation ($r = .09$, $P = .14$ at baseline and $r = -.01$, $P = .85$ at 5 years). Further data analysis was therefore confined to the 270 subjects with $Cl_{cr} \geq 60$ mL.

The study was reviewed and approved by the Committee on the Use of Human Subjects in Research of the University of Minnesota. Written informed consent was secured from all participants.

Glucose Tolerance Classification and Demographic Variables

The subjects had been classified at baseline using a standard oral glucose tolerance test, performed with the procedure recommended by the National Diabetes Data Group (NDDG)[14] and the World Health Organization (WHO).[15] WHO criteria for venous plasma glucose were used to define diabetes status at baseline: normal glucose tolerance (NGT) (fasting and 2-hour < 7.8 mmol/L); impaired GT (IGT) (fasting < 7.8 mmol/L, but 2-hour value between 7.8 and 11.1 mmol/L) or diabetes (fasting ≥ 7.8 mmol/L and/or 2-hour ≥ 11.1 mmol/L). Insulin and oral antidiabetic medication were deferred on the morning of testing.

Three subjects were classified as type 1 diabetics on the basis of medical record review, requiring a physician's statement of continuous insulin treatment from the time of diagnosis and a documented history of ketosis. All other subjects with a baseline diabetic GT test (including members of the original randomly chosen sample of Wadena adults) or with a documented history of prescription of hypoglycemic agents were considered to be type 2 diabetic regardless of current insulin treatment.

The type 2-subjects were further classified as type 2-No Insulin (type 2-No Ins) or type 2-Insulin (type 2-Ins) according to use of insulin at baseline. The numbers of subjects reported (after removing those with reduced creatinine clearance) are as follows, by class: NGT, 182 (instead of 204); IGT, 41 (instead of 50); type 2-No Ins, 37 (instead of 50); type 2-Ins, 7 (instead of 10); type 1, 3 (no change); all participants, 270 (instead of 317).

Medians of selected baseline variables are provided in Table 1. Time in years since diagnosis of diabetes, by medical record review and self-report, was used as a conservative measure of diabetes duration. Five years later, there was no significant change in median values for plasma glucose, glycosylated hemoglobin, or body mass index (BMI). The subjects remained under the care of their primary physicians in the community, including all aspects of management of diabetes.

Other Procedures and Laboratory Methods

As at baseline, a liquid mixed meal (Ensure-Plus, Ross, Columbus, OH) was used as the stimulus to insulin/CP secretion; this contained 95 g carbohydrate, 26 g protein, and 25 g of fat, providing 710 kcal, which were consumed within 20 minutes. Urine was freely voided and was collected under supervision for 260 minutes, during the meal and for 4 hours afterward.

For CP assay, urine samples were stored at -20°C for an average period of 6 months before assay by the radioimmunoassay method of Heding,[16] using materials from Novo-Nordisk (Wilton, CT). The stability of urine CP during storage was demonstrated by serial assay of separately-frozen aliquots from the same control urine sample(s) over more than 5 years; there was no upward or downward trend. Urine CP excretion rate was calculated as nmol/260 minutes. Urine CP of 4 nmol/260 minutes was the lowest 5th percentile for the 270 subjects at baseline and was used as the cut point defining a low value. Further details of the urine CP assay have been previously published.[6]

Venous plasma glucose was measured in triplicate using the YSI glucose analyzer (glucose oxidase methodology) (Yellow Springs, OH). Glycosylated hemoglobin was measured by affinity chromatography as described by Klenk et al[17] with columns supplied by Pierce (Rockford, IL). Urinary and serum creatinine were measured with the Jaffe reaction; CP and creatinine were measured on the same 260-minute urine specimen.

Immunologic Studies

Antibody tests were performed on sera collected halfway between baseline and 5-year followup testing. Pancreatic islet cell cytoplasmic antibodies (ICA) were measured according to methodology shared by the Immunology of Diabetes Workshop (IDW) Group[18] using frozen blood group O human pancreas and monitored by periodic exchange of reference samples. Sera were diluted 1:10 before assay. The lower limit of sensitivity of the assay in our laboratory was 10 JDF units. Specificity was 100%, sensitivity about 50%. Competitive insulin autoantibodies (IAA) were measured by a radiobinding assay using acid-

Table 1. Wadena Study Participants: Selected Variables at Baseline, by GT Classification

			Median (range)					
	No.	Sex	Age (yr)	Fasting Plasma Glucose (mmol/L)	2-Hour Plasma Glucose (mmol/L)	Glyco hgb (%)	BMI (kg/m^2)	Duration DM*
NGT	182	94/88	46 (21-78)	5.4 (4.2-7.3)	5.6 (2.6-7.7)	5.1 (3.1-7.5)	25.5 (18.5-50.8)	—
IGT	41	18/23	58 (24-81)	5.7 (4.6-7.6)	8.7 (7.8-10.8)	5.4 (3.0-8.9)	27.0 (19.6-38.7)	—
Type 2 DM-No Ins	37	20/17	63 (35-77)	7.6 (5.2-14.2)	14.5 (7.6-23.2)	6.1 (4.6-11.3)	31.6 (21.0-48.7)	3.0 (0-33)
Type 2 DM-Ins	7	4/3	61 (36-69)	7.9 (6.5-12.4)	19.4 (16.8-33.6)	8.1 (5.7-12.8)	30.2 (21.1-34.8)	10 (3-16)
Type 1 DM	3	2/1	64 (43-66)	6.6 (5.6-8.1)	20.6 (—)	9.8 (6.5-10.4)	22.9 (22.3-28.3)	30 (16-45)
All participants	270	138/132	51 (21-81)	5.6 (4.2-14.2)	6.5 (2.6-33.6)	5.2 (3.0-12.8)	26.3 (18.5-50.8)	—

Abbreviations: DM, diabetes mellitus; glyco hgb, glycosylated hemoglobin; NGT, normal glucose tolerance; IGT, impaired glucose tolerance.
*Years since diagnosis.

charcoal extraction and cold insulin displacement (delta % >0.79).[19] Specificity was 98%, sensitivity 31%. Autoantibodies to glutamate decarboxylase (GAD65ab) were measured in duplicate by immunoprecipitation assay as previously described.[20,21] Briefly, radiolabeled human islet GAD65 was extracted from hamster fibroblasts stably transfected with the appropriate human cDNA and radiolabeled with ^{35}S-methionine. Sera precipitating radioactivity greater than 3 SD above the mean of that of 100 controls were considered positive. This assay achieved 100% sensitivity and 100% specificity in the first GAD Antibody Workshop.[21]

Statistical Methods

Statistical significance was set at $P = .05$; *t* tests were 2-tailed and paired or unpaired as appropriate. Because CP data were not normally distributed, geometric mean values are presented for this variable; regressions, 95% confidence intervals, *t* tests, and analyses of variance were calculated after logarithmic transformation.

Besides considering change in urine CP as a continuous variable, we also studied the pattern of individual changes in urine CP, with the assumption that some participants declined equal to or more than a cut point X, to be determined; some remained stable within ± X; and some increased ≥ X. We examined the distribution of urine CP among the type 2-No Ins participants and found that the cut point X = 8 nmol/260 minutes maximized the number of decliners, while minimizing the number of increasers. We then used this same cut point to define decliners and increasers in the groups with NGT and IGT. Within each of the 3 major groups, we compared the percent decliners with the percent increasers (a test of symmetry around 0 change), using a *z* test for the difference in proportions, modified to account for the correlation in the proportion of decliners (p1) with the proportion of increasers (p3). In this *z* test, the variance of the difference was [p1 * (1 - p1) + p3 * (1 - p3) + 2p1p3]/n.

SAS software (version 6.12; SAS Institute, Cary, NC) was used with a VAX computer (Digital Equipment Corporation, Boston, MA).

RESULTS

Changes in GT Classification Over 5 Years

Changes in classification over 5 years were as follows: of 182 NGT subjects, 162 remained unchanged after 5 years; 18 were classed as IGT and 2 as type 2-No Ins (1.1% incidence in 5 years). Of 41 IGT cases, 15 were classed as NGT after 5 years, 21 were unchanged, and 5 moved to type 2 diabetes-No Ins (12.2% incidence in 5 years). Of 37 type 2-No Ins subjects, 1 was classed as NGT after 5 years, and 3 as IGT; 29 remained type 2-No Ins and 4 had moved to type 2-Ins. Three of the last-named showed gross hyperglycemia at year 5. Subjects who were taking insulin at baseline (7 type 2-Ins, and 3 type 1) did not change class.

Changes in Geometric Mean Meal-Stimulated CP Response Over 5 Years in NGT, IGT, and Type 2-No Ins Baseline Groups

The range of 5-year urine CP values in NGT subjects was very broad (1.7 to 43.0 nmol/260 minutes), and there was no significant change after 5 years in mean urine CP in subjects with NGT (baseline, 9.6 nmol/260 minutes; 5 years, 9.2 nmol/260 minutes, for a change of −4%; $P = .34$, n = 182). The mean value for IGT subjects was slightly, but not significantly, higher (baseline, 10.8 nmol/260 minutes; 5 years, 11.2 nmol/260 minutes, for a change of 4%; $P = .67$, n = 41). In contrast, there was a significant mean decrease of 21% in the 37 type 2-No Ins subjects (baseline, 12.7 nmol/260 minutes; 5 years, 10.0 nmol/260 minutes; $P = .012$).

Number and Percent of Subjects Whose CP Declined, Increased, or Stayed the Same

Change in CP was also examined as a discrete variable, using a change of = 8 nmol/260 minutes to define decline or increase. Among 182 NGT participants, there was a slight tendency towards increase: 5 (3%) declined, 165 (90%) were stable, and 12 (7%) increased ($z = -1.7$, $P = .09$, comparing percent who declined to percent who increased). Furthermore, among 41 IGT cases, 1 (2%) declined, 34 (83%) were stable, and 6 (15%) increased, from average values of 14 nmol/260 minutes at baseline to 24 nmol/260 minutes after 5 years ($z = -2.0$, $P = .05$).

In contrast, and despite the "normality" of insulin production in absolute terms in most type 2-No Ins subjects, a number in this group showed a major decline in CP over 5 years (see Fig 1). Among the 37 type 2-No Ins subjects, 8 (22%) decreased more than 8 nmol/260 minutes (from average values of 24 nmol/260 minutes at baseline to 10 nmol/260 minutes after 5 years), while 28 (76%) changed less than 8 nmol/260 minutes between examinations, and 1 (2%) increased more than 8 nmol/260 minutes ($z = 2.5$, $P = .01$). Among 4 type 2-No Ins subjects who were started on insulin after baseline, 1 showed a decrease in CP > 8 nmol/260 minutes; the other 3 remained stable.

Despite the decline in urine CP among type 2-No Ins subjects, low urine CP (≤4 nmol/260 minutes) was a rare occurrence (3% to 10% among NGT, IGT, and type 2-No Ins). In contrast, 4 of 7 (57%) of the type 2-Ins subjects showed ≤ 4 nmol/260 minutes at either time point, and all 3 type 1 subjects had unmeasurable urine CP (<0.2 nmol/260 minutes) at both baseline and 5 years.

Immunologic Studies and CP

No ICA were detected in any subject. Apart from samples from subjects known to have been treated with insulin, IAA were detected only in low titer in 1 subject with NGT; this

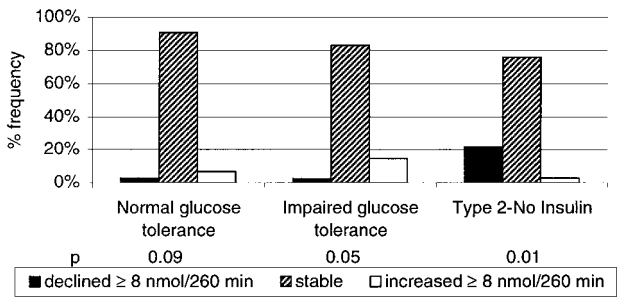

Fig 1. Summary of within-individual changes in urine CP over 5 years in adult residents of Wadena according to glucose tolerance class at baseline. NGT, normal glucose tolerance; IGT, impaired glucose tolerance; type 2-No Ins, type 2 diabetes not taking insulin. Bars indicate percentage of subjects within the class whose CP declined more than 8 nmol/260 minutes, remained stable, or increased more than 8 nmol/260 minutes. *P* values from *z* test for symmetry, namely that the % decliners = % increasers.

subject was negative for GAD65ab. No type 2-No Ins or IGT subjects showed IAA.

Twelve of 270 subjects were positive for GAD65ab. Of these, 6 came from 182 subjects with NGT at baseline (3% positive). This may be compared with 0% (0 of 41) with IGT, 3% (1 of 37) with type 2-No Ins diabetes, 43% (3 of 7) with type 2-Ins, and 67% (2 of 3) with type 1.

When all cases were analyzed together (n = 270), there was a significant association between GADab positivity and having a low CP (≤4 nmol/260 minutes) at baseline: 4 of 32 or 13% of subjects with a low CP value were GAD65ab positive compared with 8 or 3% GAD65ab positive of 238 subjects who did not show a low CP ($P = .04$, Fisher's exact test). The association was entirely due, however, to the presence of insulin-taking type 1 and type 2 subjects; when these were left out, the association disappeared ($P = .49$). Furthermore, over 5 years, there were no decliners of 8 nmol/260 minutes or more among any GAD65ab-positive individuals, and none of the decliners among type 2-No Ins subjects was GAD65ab-positive. The single GAD65ab-positive subject among the type 2-No Ins participants showed an increase in CP over 5 years, from 27 to 31 nmol/260 minutes.

Overall, GAD65ab positivity appeared to play no role in CP decline in subjects who were not taking insulin. None of the 6 GAD65ab-positive NGT subjects showed low urine CP. None of these 6 moved from NGT to type 2 No Ins over 5 years compared with 2 of 176 GAD65ab-negatives.

Other Possible Predictors of Urine CP Decline in Type 2-No Ins Subjects

In an effort to characterize the decliners further, mean values were calculated for a number of variables for decliners versus nondecliners using the cut point of 8 nmol/260 minutes. There were significantly higher values for decliners with respect to BMI (decliners, 35.6 kg/m ^{2} at baseline and 35.3 at 5 years, compared with nondecliners, 30.5 and 29.8 kg/m^2) and lower values for age at baseline (48 years, decliners, compared with 64 years, nondecliners). Creatinine clearance was higher for decliners at both time points (129 mL at baseline and 107 mL at 5 years for decliners compared with 90 mL and 87 mL for nondecliners). For other variables, including fasting and 2-hour plasma glucose, glycosylated hemoglobin, blood lipids, blood pressure, duration of diabetes, and smoking history, there were no apparent differences ($P > .10$) between decliners and nondecliners. The known duration of diabetes among type 2-No Ins subjects was similar at 7.9 and 10.0 years in decliners and nondecliners, respectively, at 5 year follow-up (overall mean, 9.6 ± 6.3 years).

We examined the possible relation between fasting glucose and simultaneous CP collection, but the results were negative: baseline, $r = .008$, $P = .96$; 5 years, $r = .117$, $P = .49$.

DISCUSSION

The purpose of the present study was to describe and compare pancreatic insulin secretion reserve over 5 years in adult subjects with varying degrees of glucose intolerance or diabetes in a community of mostly northern European origin. The study provides epidemiologic evidence about the time scale for the decline in islet β-cell function, which may accompany type 2 diabetes.

Urinary excretion of CP was chosen as a suitable measure of insulin response to a meal stimulus in our study based on the report of Horwitz et al.[22] They had found no relationship between creatinine clearance and urinary CP excretion or urinary CP clearance in nondiabetic subjects with a wide range of normal and abnormal kidney function. In our study population, however, we found a significant direct correlation between urine CP and creatinine clearance, but only in subjects with Cl_{cr} below 60 mL/min/1.73 m^2. There is no obvious reason for the discrepancy between our results and those of Horwitz et al[22]; possibly diabetic nephropathy in Wadena differs from other renal diseases with respect to its effects on renal CP metabolism. Empirically, restriction of our data analysis to the 85% of subjects with normal or near-normal kidney function removed the confounding of pancreatic function with renal function.

Our findings are consistent with the idea that the transition of type 2 diabetic persons not taking insulin to an insulin-deficient state may be long delayed in some subjects. Nevertheless, about one fifth of our type 2-No Ins subjects did appear to be progressing towards insulin deficiency within the 5-year follow-up period, on average about 10 years since diagnosis of diabetes. The percentage of type 2 subjects who experienced a major decline in urine CP may be underestimated in this follow-up study, because some of the sicker individuals may have dropped out. In contrast, a subset of IGT subjects showed an increased insulin production over 5 years. Warram et al[23] reached a similar conclusion from family studies. The stability of the NGT subjects supports a previous cross-sectional report from the Wadena study, which found no decline and a possible increase in CP with increasing age in NGT subjects.[11]

With 22% of type 2-No Ins subjects showing declining CP, progressive islet failure was more frequent in the Wadena diabetic population than the 3% to 10% noted in clinic-based prospective studies.[2,3,24-26] Closer to our results are the population-based data from another Minnesota community (Rochester), which estimated at 14% the proportion of type 2 subjects who showed maximum plasma CP decline.[4] Population-based sampling may provide a more complete picture of progressive islet failure.

There is a hypothetical possibility that insulin treatment might inhibit insulin secretion. Our limited experience with 4 subjects starting insulin treatment after baseline suggests that it did not have a major suppressive effect, because 3 of the 4 remained stable.

Our results gave no evidence for an immunologic mechanism at work, as had been observed by others,[7-9] except in insulin-taking subjects. We had expected to find some subjects positive for IAA or ICA. With a lower limit of detection of 10 JDF units, our ICA assay was specific, but insensitive. We may have missed transient seropositivity because of the nature of our study sample, which included older, long-duration, as well as short-duration, cases.[27]

The Wadena results agree in many respects with a recent report from a population-based Swedish study,[28] which found GAD65ab to be positive in 7% (2 of 27) of non–insulin-taking type 2 subjects versus 2% (1 of 37) in Wadena. Note that the Swedish diabetic subjects were young adults (mean age, 28.5

years) versus a mean baseline age of 60.4 years in Wadena. The older subjects would be expected to be more representative of the general population of type 2 subjects. Note also that in Wadena, type 2-No Ins CP decliners averaged 16 years younger than nondecliners, suggesting an age effect not related to GAD65ab level.

The significance of a 3% positivity rate for GAD65ab in the normoglycemic subjects in our population sample remains to be determined; it was not related to CP levels.

In summary, in an American population of northern European origin, we found that 41 subjects with IGT at baseline showed little change over 5 years in geometric mean urine CP response to a mixed meal; note however that 6 (15%) of them showed an increase of at least 8 nmol/260 minutes. In contrast, 37 type 2 diabetic subjects not taking insulin showed a significant decrease of 21% of baseline over 5 years in their geometric mean urine CP response, with 8 (22%) subjects showing a 5-year decline of at least 8 nmol/260 minutes. Among those who did show a decline in response over this period, none showed evidence for autoimmune involvement in the form of a positive antibody test against GAD65.

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