Endothelial Dysfunction and Increased Arterial Intima-Media Thickness in Children With Type 1 Diabetes

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Background—Endothelial dysfunction may play a pathophysiological role in the development of atherosclerosis in subjects with type 1 diabetes. We examined whether alterations in vascular endothelial function exist in children with type 1 diabetes and tested the hypothesis that endothelial dysfunction is associated with early structural atherosclerotic vascular changes in these children.

Methods and Results—Noninvasive ultrasound was used to measure brachial artery flow-mediated dilation (FMD) responses and carotid artery intima-media thickness (IMT) in 75 children (mean age 11±2 years), 45 with type 1 diabetes (diabetes duration 4.4±2.9 years) and 30 healthy control children. Children with diabetes had lower peak FMD response (4.4±3.4% versus 8.7±3.6%, $P<0.001$) and increased IMT ($P<0.001$) compared with controls. Sixteen children with diabetes (36%) had endothelial dysfunction defined as total FMD response in the lowest decile for normal children. These children had increased carotid IMT (0.58±0.05 versus 0.54±0.04 mm, $P=0.01$) and higher LDL cholesterol concentration (2.63±0.76 versus 2.16±0.60 mmol/L, $P=0.03$) compared with diabetic children without endothelial dysfunction. Multivariate correlates of increased IMT included diabetes group ($P=0.03$), low FMD ($P=0.03$), and high LDL cholesterol ($P=0.08$).

Conclusions—Impaired FMD response is a common manifestation in children with type 1 diabetes and is associated with increased carotid artery IMT. These data suggest that endothelial dysfunction in children with type 1 diabetes may predispose them to the development of early atherosclerosis. **(*Circulation*. 2004;109:1750-1755.)**

Key Words: arteries ■ atherosclerosis ■ diabetes mellitus ■ ultrasonics

Type 1 diabetes is an important risk factor for cardiovascular events. Individuals with diabetes have 2-fold to 4-fold increased risk of developing atherosclerotic diseases, which is inadequately explained by differences in the levels of traditional vascular risk factors.[1] Observations from postmortem studies have indicated that atherosclerosis in young adults is associated with the prediabetic state.[2] Therefore, subjects who are affected by type 1 diabetes in childhood may be at especially high risk of developing subsequent cardiovascular disease. Thus, there is considerable interest in defining factors responsible for the accelerated development of atherosclerosis in individuals with diabetes.

High-resolution ultrasound is a reliable, noninvasive method for detecting early structural and functional atherosclerotic changes in the arterial wall. Increased carotid intima-media thickness (IMT) is a structural marker of early atherosclerosis that correlates with vascular risk factors, relates to the severity and extent of coronary artery disease, and predicts the likelihood of cardiovascular events in population groups. Flow-mediated dilation (FMD) of the brachial artery is a marker of endothelial function that can be assessed by measuring arterial diameter responses to increased flow.[3] Brachial artery FMD occurs mainly as the result of endothelial release of nitric oxide[4] and correlates with coronary endothelial function.[5] Both increased IMT and impaired FMD have been detected in young children with risk factors for atherosclerosis, such as familiar hypercholesterolemia,[3,6] and diabetes.[7,8]

Although the levels of serum LDL cholesterol are typically not increased in adults with type 1 diabetes, the diabetic state has been associated with alterations in the characteristics of LDL particles, such as increased oxidation and glycation.[9,10] It has been suggested that the increased risk of atherosclerosis in diabetic subjects might be due to enhanced foam cell formation after postsecretory LDL modification.[10] The association between LDL modification and early atherosclerosis has not been studied previously in children with diabetes in vivo.

Received October 14, 2003; revision received January 20, 2004; accepted January 22, 2004.

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Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000124725.46165.2C**

Endothelial dysfunction is thought to be an early event in the atherosclerotic process and has been implicated in the pathogenesis of diabetic atherosclerotic vascular disease.[11] Therefore, we hypothesized that diabetic children with endothelial dysfunction would be at especially high risk of having early structural atherosclerotic vascular changes. To test this, we studied whether impaired FMD responses in children with type 1 diabetes are associated with increased carotid artery IMT. To gain insight for the possible role of LDL modification in the pathogenesis of early vascular alterations in diabetes, we also measured markers of LDL oxidation[12] and glycation[13] in these children.

Methods

Subjects

We studied 45 children with type 1 diabetes and 30 healthy control children. The groups were matched in terms of age, gender, and body size. The children with diabetes were recruited consecutively from the outpatient clinic of the Department of Pediatrics, Turku University Central Hospital. The inclusion criteria for diabetic children were age 7 to 14 years, diabetes duration >6 months, normotensive, and no chronic diseases other than type 1 diabetes. The mean duration of diabetes was 4.4±2.9 years. None of the diabetic children were taking regular medications other than daily insulin. The mean daily insulin dose was 0.97±0.26 IU/kg (range 0.62 to 1.53 IU/kg). None of the diabetic patients had evidence of microvascular complications, such as diabetic retinopathy, neuropathy, or microalbuminuria. In the diabetic group, the mean glycosylated hemoglobin (HbA1c) level was 8.9±1.4% (range 6.2% to 12.8%; reference range 4.2% to 6.0%), and urinary albumin to urinary creatinine ratio was 0.80±0.53 mg/mmol. Participants did not differ in any clinical characteristics from the entire eligible diabetic clinic population of the same age.

The healthy control children included in the study were friends of the diabetic children studied and children of Turku University or Turku University Central Hospital staff members. Written informed consent was acquired from the legal guardians of the children. The study was conducted according to the Declaration of Helsinki, and the study protocol had been approved by the Joint Commission on Ethics of the University of Turku and Turku University Central Hospital.

Ultrasound Studies

All studies were performed with an Acuson Sequoia 512 mainframe (Acuson) with a 13.0-MHz linear-array transducer by previously described techniques.[14] All ultrasound scans were performed by a single experienced vascular sonographer. The studies were performed in the morning between 7 and 9 AM in a fasting subject. In the diabetic children, the ultrasound studies and blood sampling were done before administration of morning insulin.

Brachial Artery Physiology

Brachial artery diameter was measured from B-mode ultrasound images at rest, during reactive hyperemia, and after administration of sublingual nitroglycerin, as described previously.[14] Briefly, a resting scan was performed, and arterial flow velocity was measured with a Doppler signal. Increased flow was then induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mm Hg for 4.5 minutes, followed by release. A second scan was taken continuously 40 to 180 seconds after cuff deflation, including a repeat flow-velocity recording for the first 15 seconds after the cuff was released. Vessel diameter was measured at a fixed distance manually by an experienced blinded observer using ultrasonic calipers at end diastole, incident with the R wave on a continuously recorded ECG. The maximal percent dilation from baseline (peak FMD, %) and the total dilation response, defined as the area under the FMD versus time curve during 40 to 180 seconds after hyperemia (AUC, %×s), were assessed.[14] Nitrate-mediated (endothelium-independent) dilation capacity was tested by administration of a 200-μg sublingual dose of nitroglycerin. Maximum diameter 5 minutes after nitrate administration was used to calculate the maximal nitrate-mediated dilation (NMD, %). In our laboratory, the interobserver variation (coefficient of variation) of FMD measurements was 8.6%, and the between-study coefficient of variation (12 subjects studied twice with 2 hours between studies) in FMD measurements was 9.3%.[15]

Endothelial dysfunction was determined to be present if the value for total dilation response (AUC, between 40 and 180 seconds after cuff release) was below the 10th percentile cutpoint for healthy children. The distribution of normal values was derived from a population of 105 healthy children (mean age 10, range 9 to 16 years).[14] This cutoff point corresponds to a peak FMD of ≤3.3%. Some of the control children included in the present study were participants of the prior study. According to the aforementioned definition, none of the control children included in the study had endothelial dysfunction.

Measurement of Carotid IMT

All studies were done according to a predetermined, standardized scanning protocol for the right and left carotid arteries, as described previously.[8] Briefly, the proximal part of the carotid bulb was identified on both sides, and the segments of the common carotid arteries 1 to 2 cm proximal to the bulb and the left bulb region were scanned. The image was focused on the posterior (far) wall, and the resolution box function was used to magnify the arterial far wall. Two angles were used in each case for common carotid IMT on both sides: anterior oblique and lateral. All scans were digitally stored for subsequent offline analysis. One end-diastolic frame (captured adjacent to the R wave on a continuously recorded ECG) for each interrogation angle was selected and analyzed for mean and maximum IMT. The images were analyzed by 2 independent readers who were blinded to the subject's clinical details. Six to eight measurements of far-wall IMT were taken manually, by both observers, for each image using ultrasound calipers, and the average values of these readings were used in the analyses. The mean and maximum IMTs from these 5 arterial wall segments were used in the analyses. In our laboratory, the between-study coefficient of variation (22 subjects studied twice 5 to 8 days apart) in IMT measurements was 3.9%.[16]

Serum Lipids, Apolipoprotein B, Blood HbA1c, and Plasma Glucose

Venous blood samples were taken in the morning, after an overnight fast (10 to 12 hours). Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured by standard enzymatic methods with the use of Boehringer Mannheim GmbH reagents, with a fully automated analyzer (Hitachi 917; Hitachi Ltd). LDL cholesterol concentration was calculated by Friedewald's equation.[17] Serum apolipoprotein B was measured by immunonephelometry (Behring Nephelometer II, Dade Behring Inc). HbA1c was measured with high-performance liquid chromatography (Variant Analyser, Bio-Rad). In the diabetic children, the fasting plasma glucose level was measured within 1 minute before the ultrasound test before morning insulin by a standard enzymatic method.

Determination of Oxidized LDL and Glycated LDL

EDTA plasma samples were stored frozen at −70°C until analyzed. Thawed samples were used for the determination of oxidized LDL by a capture ELISA according to the manufacturer's instructions (Mercodia AB). Shortly, microtiter plates were coated with the specific murine monoclonal antibody mAb-4E6,[12] followed by incubation of the sample. The amount of bound conjugate was detected spectrophotometrically at 450 nm. The detection limit of the method is <1 mU/L.[12] In the present study population, there was a significant correlation between oxidized LDL and apolipoprotein B (r=0.75, P<0.001).

The quantification of glycated LDL (GlycLDL) was performed with a commercial kit (Glycacor, Exocell Inc). The competitive

TABLE 1. Characteristics of Study Groups

	Control Children	Diabetic Children	*P*
No. of subjects (boys)	30 (18)	45 (30)	
Age, y	11±2	11±2	0.28
Height, m	1.51±0.09	1.52±0.12	0.86
Weight, kg	46.6±12.4	44.9±12.2	0.56
Body mass index, kg/m^2	20.1±3.8	19.1±2.5	0.22
Systolic blood pressure, mm Hg	114±8	111±9	0.14
Diastolic blood pressure, mm Hg	66±7	65±7	0.46
Total cholesterol, mmol/L	4.08±0.72	4.29±0.83	0.26
LDL cholesterol, mmol/L	2.31±0.63	2.33±0.69	0.89
HDL cholesterol, mmol/L	1.43±0.38	1.64±0.38	0.02
Triglycerides, mmol/L	0.75±0.37	0.71±0.52	0.71
Oxidized LDL, mU/L	47.6±17.4	47.6±15.3	0.99
Glycated LDL, mg/dL	1.43±0.40	1.61±0.55	0.14
Apolipoprotein B, mg/dL	71.4±15.7	72.5±16.2	0.85
HbA1c, %	5.3±0.3	8.9±1.4	0.001
Glucose, mmol/L	...	12.2±4.5	...

Values are mean±SD.

ELISA assay used a mouse monoclonal antibody that recognizes a specific epitope on glycated apolipoprotein B in the LDL complex.[13] GlycLDL in the solid phase was detected with horseradish peroxidase–conjugated goat anti-mouse antibody based on color reaction. The absorbance was determined in a microplate reader at 450 nm (Spectra II EIA Analyser, Wallac). The concentration in a patient sample was determined from a simultaneously run standard curve with glycated apolipoprotein B diluted serially from lyophilized preparation of human GlycLDL of a known concentration. The within-run and between-run coefficients of variation were 15.6% and 18.9%, respectively. The average coefficient of variation for sample replicates was 8.9%. In the present study population, GlycLDL correlated significantly with HbA1c (r=0.27, P=0.02) but not with apo B (r=−0.01, P=0.93).

Statistical Methods

Results are expressed as mean±SD, unless stated otherwise. Data on serum triglycerides were skewed toward high values and were included as their logarithms in the analyses. Comparisons between 2 groups (diabetics and controls) were conducted by Student's *t* test or nonparametric Mann Whitney *U* test, as appropriate. An ANOVA of 3 groups (controls and diabetics with and without endothelial dysfunction) was performed, followed by the Bonferroni method to allow pairwise significance testing. Multivariate correlation analyses were done by the linear regression technique. With the number of subjects enrolled, the present study had >80% power to detect a 2.5% unit difference in FMD between cases and controls at the P<0.05 level. All statistical analyses were performed with the statistical analysis system SAS version 8.01.[18]

Results

The characteristics of the study groups are shown in Table 1. There were no significant differences in age, gender distribution, body size, or blood pressure between the groups (Table 1). Mean plasma glucose in the diabetic children was 12.2±4.5 mmol/L (range 4.0 to 21.2 mmol/L). The results of ultrasound studies are shown in Table 2. There were no differences between the 2 groups in brachial artery baseline diameter or increase in blood flow during reactive hyperemia. But both peak FMD and AUC were reduced in children with diabetes. Peak FMD was similarly attenuated in diabetic girls and boys compared with controls (girls 3.9±3.1% versus 8.1±3.7%, P=0.003; boys 4.6±3.6% versus 9.1±3.5%, P=0.001). Figure 1 shows that the temporal development of FMD responses measured between 40 and 180 seconds after cuff release followed a similar pattern over time in the 2 groups, but the magnitude of the response was significantly blunted in children with diabetes. Maximal NMD did not differ between the 2 groups (Table 2). Diabetic children had increased carotid IMT compared with control children (Table 2).

TABLE 2. Results of Ultrasound Studies in Study Groups

	Control Children	Diabetic Children	*P*
Brachial artery baseline diameter, mm	3.2±0.3	3.2±0.3	0.99
Increase in blood flow during hyperemia, %	456±433	409±225	0.99
Peak FMD, %	8.7±3.3	4.4±3.4	0.001
Area under the FMD curve, %×s	687±359	251±443	0.001
Maximal nitrate-mediated dilation, %	11.5±4.5	9.7±5.0	0.35
Mean carotid IMT, mm	0.42±0.03	0.47±0.03	0.001
Maximum carotid IMT, mm	0.51±0.04	0.55±0.05	0.003

Sixteen children (36%) with diabetes had endothelial dysfunction (see Methods for definition). The diabetic children with endothelial dysfunction had higher LDL cholesterol (2.63±0.76 versus 2.16±0.60 mmol/L, P=0.03), increased IMT (mean IMT 0.49±0.04 versus 0.46±0.04 mm, P=0.049; maximum IMT 0.58±0.05 versus 0.54±0.04 mm, P=0.01), and lower peak FMD (2.0±1.3% versus 5.7±3.5%, P<0.001) but similar brachial artery baseline diameter (3.2±0.4 versus 3.2±0.3 mm, P=0.56) and NMD (8.5±2.6% versus 10.4±5.9%, P=0.18) compared with diabetics without endothelial dysfunction. No differences were observed in body size, blood pressure, duration of diabetes, HbA1c, oxidized LDL, GlycLDL, or serum lipoproteins other than LDL cholesterol (data not shown). Figure 2 shows the maximum IMT values across 3 groups (controls and diabetic children with and without endothelial dysfunction).

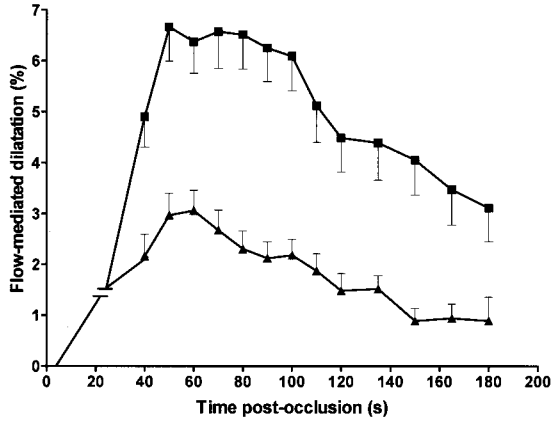

Figure 1. Temporal development of brachial artery FMD responses in diabetic children (▲) and healthy controls (■) (P=0.001 for AUC). Error bars are SEM.

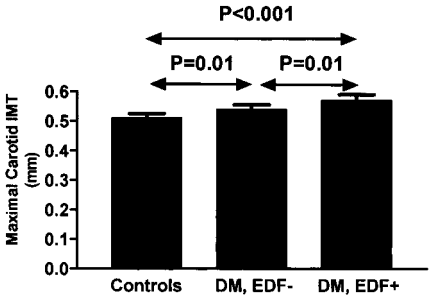

Figure 2. Carotid IMT in control children, diabetic children without endothelial dysfunction (DM, EDF-), and diabetic children with endothelial dysfunction (DM, EDF+). Error bars are SEM.

The correlates of increased IMT were studied by multivariate regression analysis (Table 3). In a multivariate model, diabetes (group variable) and peak FMD were independently associated with IMT. The effects of systolic blood pressure and LDL cholesterol were of borderline significance.

Discussion

The present study demonstrates that impaired brachial artery FMD response is a common vascular manifestation in young children with type 1 diabetes that may predispose to the development of increased carotid artery IMT. This result is consistent with the hypothesis that endothelial dysfunction is a risk factor for the development of atherosclerosis and important in the pathogenesis of premature macrovascular disease in individuals with diabetes.

Most but not all studies have demonstrated endothelial dysfunction adults with type 1 diabetes,[19] whereas the study of arterial function in diabetic children has until recently obtained relatively little attention.[7,20,21] Wiltshire et al[21] studied flow-mediated dilation in 36 diabetic children with a mean age of 14 years and a mean duration of diabetes of under 6 years and in 20 healthy controls. These diabetic children without diabetic complications had attenuated endothelial function compared with controls. Previously, Donaghue and coworkers[20] demonstrated in a study of 20 diabetic adolescents that young diabetics with clinical complications had decreased endothelial and smooth muscle function compared with healthy controls. The mean FMD and carotid IMT values in children with diabetes in the present study were similar to those observed in previous studies in diabetic and hypercholesterolemic children.[6,21,22] Compared with the diabetic children of these former studies, those included in the present study were younger, had a shorter duration of diabetes, and were free of diabetic complications.

TABLE 3. Multivariate Correlates for Maximum Carotid IMT

Variable	Regression Coefficient	Standard Error	*P*
Peak FMD, %	−0.003	0.002	0.03
Diabetes group variable	0.029	0.013	0.03
LDL cholesterol, mmol/L	0.014	0.008	0.08
HDL cholesterol, mmol/L	0.004	0.015	0.77
Systolic blood pressure, mm Hg	0.001	0.001	0.07
Age, y	0.001	0.004	0.84
Gender (1=male, 2=female)	−0.010	0.011	0.37

Only 1 previous study has examined both FMD responses and carotid IMT simultaneously in diabetic adolescents.[7] Singh et al[7] found no differences in carotid IMT between diabetic and healthy teenagers, although FMD was lower in the diabetic group. The authors concluded that although endothelial function is impaired within the first decade of the onset of type 1 diabetes, an increase in carotid IMT would only occur after a considerably longer exposure to the diabetic milieu. The results of the present study demonstrate that those diabetic children with impaired endothelial function also show increased carotid IMT. Differences in methodology and study populations may explain the discrepancy. Significant differences in lifestyle, diet, or other risk factors between Finnish and North American children are unlikely.[23] The potential influence of additional genetic risk factors on atherosclerosis burden has been suggested,[7] but no supporting data exist. In the present study, we used the latest digital ultrasound technology and a 15/13-MHz scanning frequency, which yields very reproducible and high-quality images from the common carotid artery far wall. The study by Singh et al[7] used a 10/5-Mhz scanning frequency and reported lower IMT values for both children with diabetes and controls compared with the present study and previous studies in children.[6,24,25]

The mechanisms of endothelial dysfunction and accelerated atherosclerosis in diabetes are multifactorial and have not been fully characterized. Postsecretory modifications of LDL particles, including LDL oxidation, and glycation have been proposed as potential causative agents. Former studies have associated oxidized LDL with arterial dysfunction,[26–28] increased carotid artery IMT,[29] decreased arterial elasticity,[30] and severity of acute coronary syndromes.[31] Glycosylation of LDL apolipoprotein B through nonenzymatic linkages and production of advanced glycation end products are characteristic to diabetic hyperglycemia.[32] Recent experimental studies have shown GlycLDL to induce various atherogenic events, including endothelial dysfunction.[33] Thus, the modification of native LDL particles is currently considered important for development of atherosclerosis.[34] In the present study, diabetic children with endothelial dysfunction had higher LDL cholesterol concentration than diabetic children with normal endothelial function. Levels of oxidized LDL and GlycLDL, however, were comparable between the groups. This result is partly contradictory to our previous observations, which have suggested that increased in vitro oxidizability of LDL particles in children with diabetes contributes to the development of increased carotid artery IMT.[16] Differences in the methods of measuring LDL oxidation may explain this disparity. The method used in the present study employs anti-apolipoprotein B antibodies to recognize oxidized LDL in the serum samples. In our study population, this measurement of oxidized LDL closely paralleled serum total apolipoprotein B concentration. The significant correlation between GlycLDL and HbA1c, together with the lack of correlation between GlycLDL and apolipoprotein B, on the other hand, offers some reassurance that this variable is a marker of LDL glycation and not merely apolipoprotein B concentration. Thus, although we observed increased serum LDL cholesterol con-

centration in diabetic children with endothelial dysfunction, we were unable to show any associations between vascular changes and the markers of LDL oxidation and glycation. This may either be due to methodological inaccuracies or may reflect a lack of a pathophysiological role of LDL oxidation and glycation in the development of early vascular changes in children with diabetes.

The present study has limitations. We included a relatively small number of participants, and therefore it is not clear whether the study was adequately powered for some of the subgroup analyses. Nevertheless, the findings were rather distinct, and the limited sample size is not likely to detract from the validity of our main findings. Serum insulin levels were not measured in the present study, although insulin is known to induce vasodilation. The children were, however, studied in a fasting state, before administration of morning insulin dose. We also measured plasma glucose in the diabetic children at the time of the ultrasound study. Endothelial dysfunction was arbitrarily defined as AUC below the 10th percentile cutpoint for healthy children.[14] The biological validity of this definition is not clear. However, this value corresponds to an FMD of less than 3%, which in practice is interpreted as an impaired endothelium-dependent vasodilatory response.[35] A stricter way to define abnormal endothelial function was not possible because of the limited sample size.

Our present findings may have implications in the study of the origins of vascular disease in type 1 diabetes, as well as in the management of pediatric patients with diabetes. Our results emphasize the importance of early detection and control of vascular risk factors in these children. For example, the target level for serum LDL cholesterol concentration may be different in diabetic than in healthy children, because diabetes may render the arterial wall more susceptible to harmful influences of circulating LDL cholesterol.[36] Because diabetics with endothelial dysfunction appear to be at particular risk for developing early structural atherosclerotic changes, the ultrasound assessment of arterial FMD responses might provide a valuable tool for risk stratification of pediatric patients with type 1 diabetes. Several interventions to improve endothelial dysfunction, including antioxidant vitamins and statins, have been tested in adults with diabetes and in children with familial hypercholesterolemia.[22,37–43] Children with type 1 diabetes do not routinely receive treatment other than insulin to reduce their risk for vascular atherosclerotic complications. Studies in children with type 1 diabetes would therefore be needed to examine whether an improvement in arterial endothelial function in these children would translate into a decreased risk of developing atherosclerotic vascular complications.

Acknowledgments

This study was supported financially by the Finnish Medical Foundation, the research foundation of Orion Corporation, the Turku University Foundation, the Academy of Finland (grants 201 888 and 74 092), a government grant for Turku University Hospital, the Juho Vainio Foundation, the Emil Aaltonen Foundation, the Päivikki and Sakari Sohlberg Foundation, the Finnish Cultural Foundation, the Research Foundation of Instrumentarium, Elli and Elvi Oksanen Fund, the Pirkanmaa Fund under the auspices of Finnish Cultural Foundation, and the Medical Research Fund of Tampere University Hospital.

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