Low Body Mass Index Is a Risk Factor for Impaired Endothelium-Dependent Vasodilation in Humans: Role of Nitric Oxide and Oxidative Stress

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OBJECTIVES
The purpose of this study was to evaluate the relationship between body mass index (BMI), including low BMIs, and endothelial function.

BACKGROUND
Epidemiologic study has demonstrated that not only obesity but also a low BMI may be a risk factor for cardiovascular disease.

METHODS
The forearm blood flow (FBF) response to acetylcholine (ACh) and isosorbide dinitrate (ISDN) was measured in 87 healthy young men (15 low BMI, 51 normal, 14 obese, and 7 extremely obese).

RESULTS
Plasma concentrations of 8-hydroxy-2'-deoxyguanosine and serum concentrations of malondialdehyde-modified low-density lipoprotein were higher in low BMI, obese, and extremely obese subjects than in normal subjects and were similar among the low BMI, obese, and extremely obese groups. The FBF response to ACh was greater in the normal group than in the other groups (p < 0.001), and was lower in the extremely obese group as compared with the other groups (p < 0.001). The ACh-stimulated vasodilation was similar between the low BMI group and the other groups (p = 0.001). The ISDN-stimulated vasodilation was similar in all four groups. There were no significant differences in ACh-stimulated vasodilation between the four groups after the nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine infusion. Co-infusion of vitamin C augmented the FBF response to ACh in low BMI, obese, and extremely obese groups—but not in normal BMI group.

CONCLUSIONS
These findings suggest that not only obesity but also a low BMI may be a risk factor for impaired endothelium-dependent vasodilation through the increased oxidative stress, leading to the reduced bioavailability of NO. (J Am Coll Cardiol 2003;42:256–63) © 2003 by the American College of Cardiology Foundation

Nitric oxide (NO) plays an important role in the regulation of vascular tone, inhibition of platelet aggregation and adhesion, and suppression of smooth muscle cell proliferation (1–3). Obesity is associated with the alteration of NO release from vascular endothelium (4,5). Several investigators, including ourselves, have shown that endothelium-dependent vasodilation of forearm and leg circulation is impaired in obese individuals compared with lean individuals (6,7). Large epidemiologic studies have shown that obesity is a risk factor for cardiovascular disease (8). Therefore, reduced NO bioavailability may, at least in part, contribute to the obesity-induced cardiovascular complications.

On the other hand, in studies that demonstrate endothelial dysfunction in obese individuals but not lean individuals, few or no low body mass index (BMI) subjects are included in the lean group. Epidemiologic study has demonstrated that subjects who have a BMI of 22.2 kg/m² have the lowest morbidity, suggesting that there is a J-curve phenomenon between prevalence of disease, including cardiovascular complications, and BMI (9). These findings suggest that not only obesity but also a low BMI may be a risk factor for cardiovascular disease. There is little information concerning the relationship between a low BMI and endothelial function.

Therefore, we evaluated the endothelium-dependent vasodilation induced by acetylcholine (ACh), and the endothelium-independent vasodilation induced by isosorbide dinitrate (ISDN), in low BMI individuals without a history of smoking, hypertension, hypercholesterolemia, diabetes mellitus, or renal dysfunction.

METHODS
Subjects. We studied 15 extremely low BMI healthy men (mean age, 29 ± 6 years), 51 normal BMI healthy men (mean age, 27 ± 5 years), 14 obese healthy men (mean age, 28 ± 4 years), and 7 extremely obese healthy men (mean age, 29 ± 7 years). Low BMI was defined as <18.5 kg/m², normal as ≥18.5 to <25 kg/m², obesity as ≥25 to <30
Pharmaceutical Co., Tokyo, Japan) were evaluated in all
forearm vascular responses to ACh alone and in
combination with vitamin C (24 mg/min) were evaluated
using a protocol identical to this study protocol.

Forearm blood flow was calculated from the linear
portions of plethysmographic recordings by two independent
observers blinded to the study protocol. The intraobserver
coefficient of variation was 3.4%. We confirmed the repro-
ducibility of FBF response to ACh and ISDN in all
healthy males (mean age, 25 ± 4 years) on two separate occasions. The coefficients of variation were 6.2% and 4.7%,
respectively.

Analytical methods. Samples of venous blood were placed in
tubes containing EDTA-Na (1 mg/ml) and in polysty-
rene tubes. The EDTA-containing tubes were chilled
promptly in an ice bath. Plasma was separated immediately
by centrifugation at 3,100 rpm at 4°C for 10 min, and serum
at 1,000 rpm (at room temperature) for 10 min. Samples
were stored at −80°C until assayed. Routine chemical
methods were used to determine serum concentrations of
total cholesterol, high-density lipoprotein cholesterol, low-
density lipoprotein cholesterol, triglycerides, creatinine,
glucose, insulin, and electrolytes. The 8-hydroxy-2′-
deoxyguanosine (8-OHdG), a marker of oxidative deoxyri-
onucleic acid (DNA) damage, were measured by enzyme-
linked immunosorbent assay using high-sensitive 8-OHdG
assay kits (Nihon Yushi Co., Tokyo, Japan). Plasma
concentrations of nitrate/nitrite (NOx) were assayed by colorimetric methods using NOx assay kits
(Cayman Chemical Co., Ann Arbor, Michigan). Plasma

After the subjects were placed in the supine position for
30 min, FBF and arterial BP were measured. Next, the
effects of ACh and ISDN on forearm hemodynamics were
studied; ACh (3.75, 7.5, and 15 μg/min) and ISDN (0.75,
1.5, and 3.0 μg/min) were infused intra-arterially for 5 min
each dose using a constant rate infusion pump (Terfusion
STG-523, Termo Co., Tokyo, Japan). The FBF was
measured during the last 2 min of the infusion. Infusions of
ACh and ISDN were carried out in a random order. Each
study proceeded after the FBF had returned to baseline.

Furthermore, after a 30-min rest period, NG^2^-monomethyl-
L-arginine (L-NMMA, CLINALFA Co., Läufelfingen,
Switzerland), an inhibitor of NO synthase, was infused
intra-arterially at a dose of 8 mol/min for 5 min while basal
FBF and arterial BP were recorded. After L-NMMA
infusion was complete, ACh (3.75, 7.5, and 15 μg/min) was
administered.

To assess the effect of oxidative stress on endothelium-
dependence, we infused ACh in the presence of antioxidant,
vitamin C (Fuso Pharmaceutical Co., Osaka, Japan) in 7 of
the 15 low BMI subjects (mean age, 29 ± 7 years), in 10 of
the 51 normal BMI subjects (mean age, 28 ± 6 years), in 7
of the 14 obese subjects (mean age, 29 ± 5 years), and in 5
of the 7 extremely obese subjects (mean age, 30 ± 8 years).
The forearm vascular responses to ACh alone and in
combination with vitamin C (24 mg/min) were evaluated
using a protocol identical to this study protocol.

Forearm blood flow was calculated from the linear
portions of plethysmographic recordings by two independent
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assay kits (Nihon Yushi Co., Tokyo, Japan). Plasma
concentrations of nitrate/nitrite (NOx) were assayed by colorimetric methods using NOx assay kits
(Cayman Chemical Co., Ann Arbor, Michigan). Plasma
concentrations of angiotensin II and endothelin-1 were assayed by radioimmunoassay. Plasma concentrations of norepinephrine were measured by high-performance liquid chromatography. Fasting concentrations of insulin and glucose were used to determine homeostatic model assessment (HOMA) parameters of insulin resistance using a program based on the HOMA algorithm (HOMA resistance = insulin/22.5e-2×glucose), as previously described (12).

Statistical analysis. Results are presented as mean ± SD. Values of p < 0.05 were considered statistically significant. Multigroup comparisons of variables were carried out by one-way analysis of variance (ANOVA) followed by the Bonferroni correction. Comparisons of dose-response curves of parameters during drug infusion were analyzed by ANOVA for repeated measures by the Bonferroni correction for multipaired comparisons after adjustment for body weight.

Figure 1. The forearm blood flow (FBF) response to acetylcholine (ACh) in low body mass index (BMI), normal, obese, and extremely obese subjects after adjustment for body weight.

Figure 2. The forearm blood flow (FBF) response to isosorbide dinitrate (ISDN) in low body mass index (BMI), normal, obese, and extremely obese subjects after adjustment for body weight.
weight (Figs. 1 to 3). Relationship between variables were determined by a spline fit. The data were processed using either the software package StatView IV (SAS Institute, Cary, North Carolina), Super ANOVA (Abacus Concepts, Berkeley, California), or SAS version 8.02 (SAS Institute Inc.).

RESULTS

Clinical characteristics. The baseline clinical characteristics of the 15 low BMI, 51 normal, 14 obese, and 7 extremely obese individuals are summarized in Table 1. The BMI was significantly higher between the groups in the following order: extremely obese > obese > lean > low BMI. Plasma concentrations of 8-OHdG and serum concentrations of MDA-LDL, indexes of oxidative stress, were significantly higher in the low BMI, obese, and extremely obese subjects than in normal BMI subjects and were similar among the low BMI, obese, and extremely obese groups. Serum concentrations of insulin were significantly higher in the extremely obese group than in the other three groups. Homeostatic model assessment resistance was significantly higher in the extremely obese group than in the low BMI, normal, and obese groups (3.34 ± 0.56 vs. 1.78 ± 0.31, 1.89 ± 0.24, and 2.01 ± 0.37, respectively; p < 0.05, for all) and

Table 1. Clinical Characteristics of Low BMI, Normal, Obese, and Extremely Obese Subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Low BMI (n = 15)</th>
<th>Normal (n = 51)</th>
<th>Obese (n = 14)</th>
<th>Extremely Obese (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>17.9 ± 0.5</td>
<td>22.9 ± 1.8*</td>
<td>27.5 ± 1.4*</td>
<td>34.6 ± 3.7†‡</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>116.6 ± 8.2</td>
<td>114.1 ± 11.8</td>
<td>121.3 ± 6.5</td>
<td>118.4 ± 15.4</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>67.6 ± 6.7</td>
<td>65.8 ± 6.8</td>
<td>67.5 ± 4.2</td>
<td>66.1 ± 8.8</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>64.2 ± 7.3</td>
<td>65.6 ± 6.7</td>
<td>64.7 ± 6.4</td>
<td>70.4 ± 8.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.08 ± 0.69</td>
<td>5.16 ± 0.84</td>
<td>5.19 ± 0.81</td>
<td>5.27 ± 0.87</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.18 ± 0.56</td>
<td>1.28 ± 0.65</td>
<td>1.34 ± 0.57</td>
<td>1.46 ± 0.93</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.41 ± 0.40</td>
<td>1.33 ± 0.42</td>
<td>1.39 ± 0.43</td>
<td>1.36 ± 0.39</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.51 ± 0.55</td>
<td>3.63 ± 0.69</td>
<td>3.47 ± 0.58</td>
<td>3.77 ± 0.72</td>
</tr>
<tr>
<td>Serum glucose (mmol/dl)</td>
<td>4.9 ± 0.4</td>
<td>5.0 ± 0.6</td>
<td>5.2 ± 0.6</td>
<td>5.1 ± 0.7</td>
</tr>
<tr>
<td>Serum insulin (pmol/l)</td>
<td>55.3 ± 11.8</td>
<td>58.9 ± 11.7</td>
<td>60.5 ± 13.3</td>
<td>87.9 ± 20.3*</td>
</tr>
<tr>
<td>Plasma NOx (µmol/l)</td>
<td>24.3 ± 11.7</td>
<td>21.8 ± 13.5</td>
<td>30.2 ± 18.3</td>
<td>26.2 ± 17.8</td>
</tr>
<tr>
<td>Plasma norepinephrine (pmol/l)</td>
<td>1.44 ± 0.81</td>
<td>1.57 ± 0.97</td>
<td>1.52 ± 0.76</td>
<td>1.78 ± 0.89</td>
</tr>
<tr>
<td>Plasma endothelin-1 (pg/ml)</td>
<td>1.9 ± 0.3</td>
<td>1.8 ± 0.4</td>
<td>1.7 ± 0.2</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>Plasma angiotensin II (pg/ml)</td>
<td>14.3 ± 8.6</td>
<td>13.9 ± 6.8</td>
<td>15.3 ± 7.6</td>
<td>16.5 ± 9.1</td>
</tr>
<tr>
<td>Plasma 8-OHdG (ng/ml)</td>
<td>2.76 ± 1.42†</td>
<td>1.14 ± 0.80</td>
<td>2.44 ± 1.65†</td>
<td>3.91 ± 2.36†</td>
</tr>
<tr>
<td>Serum MDA-LDL</td>
<td>76.5 ± 30.2†‡</td>
<td>58.8 ± 21.2</td>
<td>79.6 ± 25.7†</td>
<td>83.4 ± 29.7†</td>
</tr>
<tr>
<td>FBF (ml/min/100 ml tissue)</td>
<td>4.2 ± 1.2</td>
<td>4.7 ± 1.3</td>
<td>4.6 ± 1.3</td>
<td>4.3 ± 1.3</td>
</tr>
<tr>
<td>FVR (mm Hg/ml/min/100 ml tissue)</td>
<td>19.5 ± 3.7</td>
<td>17.5 ± 4.1</td>
<td>18.6 ± 3.6</td>
<td>19.3 ± 4.3</td>
</tr>
</tbody>
</table>

All results are presented as mean ± SD. *p < 0.05 vs low BMI; †p < 0.05 vs. normal; ‡p < 0.05 vs. obese.

BMI = body mass index; FBF = forearm blood flow; FVR = forearm vascular resistance; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MDA-LDL = malondialdehyde-modified low-density lipoprotein; NOx = nitrite/nitrate; 8-OHdG = 8-hydroxy-2’-deoxyguanosine.
were similar between the other three groups. The other parameters such as plasma NOx, angiotensin II, endothelin-1, norepinephrine, and serum glucose were similar in all four groups.

**ACh- and ISDN-induced vasodilation.** The intra-arterial infusion of ACh significantly increased FBF in a dose-dependent manner in all groups. The response of FBF to ACh was significantly greater in the normal group than in the other three groups (p < 0.001) and was lower in extremely obese subjects than in the other three groups (p < 0.001) (Fig. 1). The vasodilatory effect of ACh was similar in the low BMI and obese groups (Fig. 1). The intra-arterial infusion of ISDN significantly increased FBF in a dose-dependent manner in all groups. This response was similar in the four groups (Fig. 2). No significant change was observed in the arterial BP or heart rate with the intra-arterial infusion of either ACh or ISDN in any group.

**Effects of L-NMMA on the FBF response to ACh.** The intra-arterial infusion of the NO synthase inhibitor, L-NMMA, decreased basal FBF in all groups. Changes in the basal forearm vascular responses to L-NMMA infusion were similar in the four groups. The L-NMMA infusion also decreased the FBF response to ACh infusion in all groups. There was no significant difference in the change in FBF response to ACh after L-NMMA infusion between the groups (Fig. 3). No significant changes in arterial BP or heart rate were detected during the infusion of L-NMMA in any group.

**Effects of vitamin C on the FBF response to ACh.** Co-infusion of vitamin C augmented the FBF response to ACh in low BMI (maximal FBF, 24.2 ± 5.1 vs. 30.1 ± 4.9 ml/min per 100 ml; p < 0.01), obese (maximal FBF, 20.3 ± 3.6 vs. 29.7 ± 5.2 ml/min per 100 ml; p < 0.01), and extremely obese groups (maximal FBF, 12.6 ± 3.1 vs. 23.1 ± 4.8 ml/min per 100 ml; p < 0.01), but not in the normal BMI group (maximal FBF, 38.1 ± 7.3 vs. 41.2 ± 7.1 ml/min per 100 ml; p = 0.56; Fig. 4). No significant change was observed in the arterial BP or heart rate with the intra-arterial infusion of ACh in combination with vitamin C.

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**Figure 4.** The effect of concomitant administration of the antioxidant, vitamin C, on the forearm blood flow (FBF) response to acetylcholine (ACh) administration in low body mass index (BMI), normal, obese, and extremely obese subjects.
DISCUSSION

In the present study, we demonstrated that endothelium-dependent vasodilation of forearm resistance artery is impaired not only in obese individuals but also in individuals with a low BMI compared with normal BMI individuals. This impairment is due to reduced bioavailability of NO. The 8-OHdG and MDA-LDL concentrations, indexes of oxidative stress, significantly increased in the low BMI and obese groups compared with the normal BMI group. Vitamin C augmented the FBF response to ACh in low BMI, obese, and extremely obese groups, but not in the normal BMI group.

Endothelial function in subjects with a low BMI. In the present study, we selected healthy and relatively young men (mean age, 27 ± 5 years; range, 20 to 41 years) to avoid confounding factors such as atherosclerosis, hypertension, hypercholesterolemia, diabetes mellitus, aging, smoking, and menstrual cycle, which alter endothelium-dependent vasodilation. Endothelium-dependent vasodilation, but not endothelium-independent vasodilation, was impaired to a similar degree in low BMI and obese subjects and was impaired to a greater extent in extremely obese subjects through the reduced availability of NO. This indicates selective impairment of the L-arginine-NO pathway rather than a generalized reduction in vascular smooth muscle cell response to NO in subjects with a low BMI as well as in obese individuals. It is generally accepted that endothelium-dependent vasodilation in the forearm, renal, femoral, and coronary resistance arteries is selectively impaired as a characteristic of numerous vascular diseases (10,11,13–16).

Alterations in endothelial function comprise an initial step in the pathogenesis of arteriosclerosis, leading to an increased incidence of cardiovascular and cerebrovascular diseases in these subjects (17). Our results support the findings of epidemiologic studies that suggest a J-curve phenomenon, which peaks at a BMI of 22.2 kg/m², exists between disease morbidity, including cardiovascular disease, and BMI (9). Although the relationship between low BMI and cardiovascular morbidity and mortality is controversial, several studies have shown that not only overweight but also lean (low BMI) increases risk of cardiovascular mortality (18,19). Interestingly, lean hypertensive patients have a higher cardiovascular mortality rate than do other hypertensive patients (20). A low BMI was found to be an independent risk factor for death and stroke in the Systolic Hypertension in the Elderly Program (21).

Mechanisms of impaired endothelium-dependent vasodilation in subjects with a low BMI. In the present study, there were no significant differences in ACh-stimulated vasodilation between the four groups, including the low BMI group, after L-NMMA infusion, suggesting that a reduced bioavailability of NO may be involved in the impairment of endothelium-dependent vasodilation in low BMI subjects.

Interestingly, 8-OHdG, one of the most commonly used markers for evaluation of oxidative DNA damage, was significantly increased in the low BMI, obese, and extremely obese groups compared with the normal group. The 8-OHdG is a specific product formed by the attack of a hydroxy radical on DNA (22). Several studies suggest that the oxidative DNA damage is increased in non–insulin-dependent diabetes mellitus and aging (23,24). The MDA-LDL also is an index of oxidative stress (25–27). The measurement of MDA-LDL has been proposed as the biologic signature of clinical in vivo LDL oxidation (25,26). Maggi et al. (27) have recently reported that serum MDA-LDL concentration is higher in essential hypertensive patients than in normal controls. Other experimental and clinical studies have shown that oxidative stress is strongly implicated in endothelial dysfunction (28–31). Rubanyi et al. (30) demonstrated that superoxide radicals suppress endothelium-dependent vasodilation induced by ACh in the canine coronary artery. Mugg et al. (31) have shown that ACh-stimulated vasodilation is attenuated after the inhibition of antioxidant defense systems. Superoxide radicals, including hydroxy radicals, directly scavenge NO and produce toxic peroxynitrite (32). A balance between ambient levels of superoxide and released NO plays a crucial role in the maintenance of normal endothelial function. In the present study, endothelium-dependent vasodilation was attenuated despite unchanged NO production in low BMI, obese, and extremely obese individuals, suggesting that decreased availability of NO could be due to its degradation by superoxide radicals rather than decreased NO production. In addition, vitamin C, an antioxidant, augmented the FBF response to ACh in low BMI, obese, and extremely obese groups. These findings suggest that oxidative stress may be, at least in part, involved in impaired endothelium-dependent, NO-mediated vasodilation in subjects with a low BMI as well as obese individuals. However, the precise mechanism responsible for increased oxidative stress in low BMI and obese subjects remains unknown.

In the present study, endothelium-dependent vasodilation was impaired in obese individuals, and impaired to the greater degree in extremely obese individuals. These results are consistent with previous studies that demonstrate that obesity is associated with endothelial dysfunction (6,7,33). The degree of HOMA impairment, an index of insulin resistance, was greater in the extremely obese group than in the other three groups. Obesity is a major cause of insulin resistance in humans. Recently, Steinberg et al. (6) demonstrated that methacholine-induced increases in leg blood flow were approximately 40% lower in obese insulin resistance subjects (BMI ≥28 kg/m²) than normal BMI insulin-sensitive controls (BMI <28 kg/m²). These findings suggest that obesity–related insulin resistance is associated with endothelium-dependent vasodilation. In the present study, 8-OHdG tended to be higher, although not significantly, in the extremely obese group than in the low BMI and obese groups. Therefore, oxidative stress, in combination with an increase in insulin resistance, may contribute to the greater
attenuation of endothelium-dependent vasodilation in extremely obese individuals.

A balance of vasodilators and vasoconstrictors plays an important role in the physiologic regulation of vascular tone (1–3). It is well known that various vasoconstricting factors released from endothelium, such as angiotensin II and endothelin-1, affect endothelium-dependent vasodilation in humans. We have confirmed that circulating levels of both angiotensin II and endothelin-1 are similar between the low BMI, normal, obese, and extremely obese groups. However, we cannot deny the possibility that other vasoconstrictors contribute to differences in the degree of ACh-stimulated vasodilation.

Previous studies have shown that autonomic dysfunction, especially sympathetic dysfunction, occurs in obese individuals (34,35). Norepinephrine, which acts as a potent vasoconstrictor, attenuates endothelium-dependent vasodilation. However, plasma norepinephrine concentrations were almost the same because most of the subjects were students. Lifestyle characteristics of all the subjects in our study were compared. Therefore, the differences in FBF response to ACh between the four groups cannot be explained by differences in sympathetic nervous system activity.

**Study limitations.** In the present study, we focused on the relationship between BMI, especially low BMI, and endothelial function and showed that endothelium-dependent vasodilation is distinguished in low BMI individuals through increases in oxidative stress. However, the mechanisms responsible for alterations in endothelial function are varied and multifactorial. Further studies are needed to address the other mechanisms of endothelial dysfunction. In the present study, the number of subjects is relatively small compared with large epidemiologic trials. A larger sample size of subjects may show more specific conclusions.

It is well known that lifestyle characteristics, including aerobic exercise, diet, smoking, sodium intake, and alcohol intake influence endothelial function. We consider that the lifestyle characteristics of all the subjects in our study were almost the same because most of the subjects were students of the Hiroshima University. However, the possibility that differences in lifestyle characteristics affected the FBF response to ACh cannot be ruled out.

**Conclusions.** Our results suggest that not only obesity, but also a low BMI, is a risk factor for impaired endothelium-dependent vasodilation in humans through increased oxidative stress, leading to the reduced bioavailability of NO. Endothelial dysfunction is an early finding in the pathogenesis of arteriosclerosis, leading to increases in the incidence of cardiovascular complications. A low BMI may be an additional risk factor for cardiovascular disease. Studies defining the relationship between BMI and endothelial function in the setting of arteriosclerosis, hypercholesterolemia, hypertension, diabetes mellitus, and heart failure in which endothelium-dependent vasodilation is impaired would be of great interest.

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