Regular Aerobic Exercise Prevents and Restores Age-Related Declines in Endothelium-Dependent Vasodilation in Healthy Men

Christopher A. DeSouza, PhD; Linda F. Shapiro, MD; Christopher M. Clevenger, PhD; Frank A. Dinenna, PhD; Kevin D. Monahan, MS; Hirofumi Tanaka, PhD; Douglas R. Seals, PhD

Background—In sedentary humans endothelium-dependent vasodilation is impaired with advancing age contributing to their increased cardiovascular risk, whereas endurance-trained adults demonstrate lower age-related risk. We determined the influence of regular aerobic exercise on the age-related decline in endothelium-dependent vasodilation.

Methods and Results—In a cross-sectional study, 68 healthy men 22 to 35 or 50 to 76 years of age who were either sedentary or endurance exercise–trained were studied. Forearm blood flow (FBF) responses to intra-arterial infusions of acetylcholine and sodium nitroprusside were measured by strain-gauge plethysmography. Among the sedentary men, the maximum FBF response to acetylcholine was 25% lower in the middle aged and older compared with the young group ($P<0.01$). In contrast, there was no age-related difference in the vasodilatory response to acetylcholine among the endurance-trained men. FBF at the highest acetylcholine dose was almost identical in the middle aged and older (17.3±1.3 mL/100 mL tissue per minute) and young (17.7±1.4 mL/100 mL tissue per minute) endurance-trained groups. There were no differences in the FBF responses to sodium nitroprusside among the sedentary and endurance-trained groups. In an exercise intervention study, 13 previously sedentary middle aged and older healthy men completed a 3-month, home-based aerobic exercise intervention (primarily walking). After the exercise intervention, acetylcholine-mediated vasodilation increased $30\%$ ($P<0.01$) to levels similar to those in young adults and middle aged and older endurance-trained men.

Conclusions—Our results indicate that regular aerobic exercise can prevent the age-associated loss in endothelium-dependent vasodilation and restore levels in previously sedentary middle aged and older healthy men. This may represent an important mechanism by which regular aerobic exercise lowers the risk of cardiovascular disease in this population. (Circulation. 2000;102:1351-1357.)

Key Words: exercise ■ endothelium ■ vasodilation ■ nitric oxide ■ blood flow

The vascular endothelium plays an important role in the regulation of vascular tone and the maintenance of cardiovascular homeostasis. Importantly, endothelial dysfunction, particularly impaired endothelium-dependent vasodilation, has been linked to the pathogenesis of atherosclerotic vascular disease and acute cardiovascular events. Indeed, reduced endothelial vasodilatory function occurs early in atherogenesis before histological and angiographic evidence of atherosclerosis. In addition, impaired endothelium-dependent vasodilation is a common characteristic of several atherosclerosis risk factors including diabetes, hypertension, dyslipidemia, and aging.

In humans, advancing age is associated with a progressive impairment in endothelium-dependent vasodilation. Age-related reductions in endothelium-dependent vasodilation have been observed in both the brachial and coronary arteries. The progressive loss in endothelial vasodilatory function is thought to contribute to the increased risk of atherosclerosis and thrombosis with adult aging. Regular aerobic exercise is associated with a reduced risk of atherosclerotic vascular disease and acute cardiovascular events, particularly in middle-aged and older adults. In addition to favorably modifying traditional risk factors such as blood pressure, a novel mechanism by which regular exercise may confer this protection is through improved vascular endothelial function. Indeed, recent evidence suggests that regular aerobic exercise is an effective intervention strategy for improving endothelium-dependent vasodilation in disease states such as chronic heart failure and hypertension. However, whether regular exercise can prevent the age-related loss in endothelial
vasodilator function and/or restore lost function in previously sedentary middle aged and older adults is unknown.

Accordingly, the aims of the present investigation were to determine (1) whether the decline in endothelium-dependent vasodilation observed with sedentary aging is absent in men who regularly perform aerobic-endurance exercise and (2) if a program of aerobic exercise training improves endotheli um-dependent vasodilation in previously sedentary middle aged and older men. We hypothesized that the normal decline in endothelium-dependent vasodilation with age would not occur in habitually endurance-trained men and that regular aerobic exercise would restore the age-associated loss of endothelium-dependent vasodilation in previously sedentary middle aged and older men.

To systematically address these aims, we used 2 experimental approaches. First, we used a cross-sectional model to examine the influence of habitual aerobic exercise on the age-associated reduction in endothelium-dependent vasodilation. We then performed an intervention study to determine the effects of aerobic exercise training on endothelium-dependent vasodilation in sedentary middle aged and older men.

**Methods**

**Subjects**

**Cross-Sectional Study**

We studied 68 healthy men 22 to 35 or 50 to 76 years of age: 12 young and 24 middle aged and older sedentary men and 12 young and 20 middle aged and older endurance-trained (runners) men. The endurance-trained subjects were matched for age-adjusted running performance as described previously by our laboratory and had been running for 7±2 and 20±2 years, respectively. The endurance-trained men were recruited from various running clubs throughout the Boulder area and from participants in the Boulder Transportation System, the second largest 10-km road race in the United States. The sedentary subjects were recruited through various forms of advertisement and had not participated in a regular aerobic exercise program for ≥2 years before the start of the study. All subjects were free of overt disease as assessed by medical history and fasting blood chemistries. Subjects were excluded from the study if they presented a history or evidence of hepatic, renal, or hematological disease; peripheral vascular disease; stroke; diabetes (fasting plasma glucose >7.0 mmol/L);20 dyslipoproteinemia (cholesterol ≥6.2 mmol/L, triglycerides ≥4.5 mmol/L);21 hypertension (blood pressure ≥140/90 mm Hg);22 or body mass index (BMI) >35 kg/m². The middle aged and older sedentary and endurance-trained men were further evaluated for clinical evidence of cardiovascular disease with a focused physical examination and resting and maximal exercise ECGs and blood pressure. No subjects were taking medication, and all subjects were nonsmokers. Before participation, all of the subjects had the research study and its potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Colorado at Boulder.

**Intervention Study**

Thirteen of the 24 middle aged and older sedentary men who participated in the cross-sectional study went on to complete a 3-month aerobic exercise training program. All baseline measures were subsequently repeated in these subjects after the exercise intervention.

**Measurements**

Endurance-trained subjects and subjects who completed the 3-month exercise intervention were studied 20 to 24 hours after their last exercise training session to avoid the immediate (acute) effects of exercise while still representing their normal physiological state (ie, habitually exercising).

**Body Composition**

Body mass was measured to the nearest 0.1 kg with a medical beam balance. Percentage of body fat was determined by dual-energy x-ray absorptiometry (DXA, model DPX-IQ, Lunar Radiation Corp).23 BMI was calculated as weight (kilograms) divided by height (meters) squared. Minimal waist circumference was measured according to previously published guidelines.24

**Treadmill Exercise Test**

For assessment of aerobic fitness, subjects performed incremental treadmill exercise with a modified Balke protocol as previously described.25 Maximal oxygen consumption (V̇O₂max) was measured with on-line, computer-assisted, open-circuit spirometry. In addition, heart rate and rating of perceived exertion (RPE)25 were measured throughout exercise, and total exercise time to exhaustion was recorded.

**Metabolic Measurements**

Fasting plasma lipid and lipoprotein, glucose, and insulin concentrations were determined with conventional methods by the clinical laboratory affiliated with the General Clinical Research Center as previously described.26

**Plasma Homocysteine**

Fasting plasma concentrations of homocysteine were measured in duplicate with a commercially available enzyme immunoassay (Bio-Rad).27

**Arterial Catheterization**

Under strict aseptic conditions, a 5-cm, 20-gauge catheter was inserted into the brachial artery of the nondominant arm under local anesthesia (2% lidocaine). The catheter was connected to a pressure transducer and continuously flushed with 3 mL/h with heparinized saline (2 U/mL). Heart rate and arterial pressure were continuously measured throughout. Mean arterial pressure (MAP) was calculated as one-third pulse pressure plus diastolic pressure.

**Forearm Blood Flow**

Forearm blood flow (FBF) was measured in both the experimental (nondominant) and contralateral (dominant) forearm with strain-gauge venous occlusion plethysmography (D.E. Hokanson), with both forearms positioned above heart level.28 One minute before the measurement of FBF, circulation to the hand was arrested by inflation of wrist cuffs to suprasystolic levels. Thereafter, a cuff placed around each upper arm was inflated to 50 mm Hg to occlude venous outflow. Flow was recorded 4 times each minute at rest and throughout each drug infusion protocol. Flows during the last minute of rest and each drug dose were measured and the mean value reported. All FBF values are presented in milliliters per 100 milliliters of forearm volume per minute. Forearm volume was determined by the water displacement method.

**Intra-Arterial Infusion Protocol**

All studies were performed between 7 and 10 AM after a 12-hour overnight fast in a temperature-controlled room. Drug infusion rates were normalized per 100 mL tissue and infused at 5 mL/min by a syringe pump. After the measurement of resting blood flow for 5 minutes, endothelium-dependent and endothelium-independent vasodilation was assessed by the FBF responses to incremental doses of acetylcholine (IOLAB Pharmaceuticals) and sodium nitroprusside (Elkins-Sinn), respectively. Acetylcholine was infused at rates of 1.0, 2.0, 4.0, 8.0, and 16.0 μg/100 mL forearm tissue per minute and sodium nitroprusside at 0.25, 1.0, 2.0, and 4.0 μg/100 mL forearm tissue per minute. Each dose was infused for 5 minutes, and sufficient time (~20 minutes) was provided to allow FBF to return to resting levels between drug infusions. To avoid an order effect, the sequence of administration of acetylcholine and sodium nitroprusside was randomized.
Exercise Intervention
To initiate the 3-month aerobic exercise training program, the subjects underwent a supervised orientation, after which they exercised on their own. For the first 2 to 3 weeks of the exercise program subjects walked for 30 minutes per day, 3 to 4 days per week, at an intensity of ~60% of their individually determined maximal heart rate. As their tolerance for exercise improved, subjects were asked to increase the duration of exercise to 40 to 45 minutes per day and the intensity of their exercise to 70% to 75% of their maximal heart rate for 5 to 6 days per week. Compliance was documented with the use of heart rate monitors and personal activity logs. The subjects were asked to record their prescribed exercise activity as well as any other additional physical activity on a daily basis. Both the heart rate monitors and physical activity logs were returned to the laboratory every 2 weeks and analyzed.

Statistical Analysis
Data for the cross-sectional study were analyzed by multifactor ANOVA (age times training status). When indicated by a significant F value, specific mean comparisons were performed to identify significant group differences. Because MAP did not change throughout the infusion protocol, forearm vascular conductance (FVC) was calculated as FBF divided by MAP and expressed as arbitrary units (AU) × 10^2. Group differences in the FBF and FVC responses to acetylcholine and sodium nitroprusside were determined by repeated-measures ANOVA. Relations between variables of interest were assessed by means of Pearson’s correlation coefficient and linear regression analysis. Changes in the dependent variables resulting from the exercise intervention were assessed by repeated-measures ANOVA. All data are expressed as mean ± SEM. Statistical significance was set at P < 0.05.

Results
Cross-Sectional Study
Table 1 presents selected subject characteristics. Body mass, BMI, and waist circumference were higher (P < 0.01) in the sedentary men compared with their age-matched, endurance-trained counterparts. VO2 max was higher in the endurance-trained men than in the sedentary men at both ages (P < 0.01). There were no differences in resting MAP, FBF, or FVC among the 4 groups. Although all metabolic factors were well within clinically normal levels, in general, the middle aged and older sedentary men demonstrated the highest plasma triglyceride, homocysteine, glucose, and insulin concentrations of all groups (P < 0.05).

Figure 1 shows the FBF and FVC responses to acetylcholine in the sedentary and endurance-trained groups. As expected, the FBF and FVC responses to acetylcholine were attenuated with age in the sedentary men (P < 0.01). At the highest acetylcholine dose (16 μg/100 mL tissue per minute), FBF was 25% lower in the middle aged and older (12.1 ± 0.9 mL/100 mL tissue per minute) compared with young (16.1 ± 1.7 mL/100 mL tissue per minute) sedentary men. In contrast, the endurance-trained men did not demonstrate an age-associated decline in endothelium-dependent vasodilation; FBF at the highest dose of acetylcholine was almost identical in the middle aged and older (17.3 ± 1.3 mL/100 mL tissue per minute) and young (17.7 ± 1.4 mL/100 mL tissue per minute) groups. As such, the FBF and FVC responses to acetylcholine in the middle aged and older endurance-trained subjects were markedly greater (P < 0.01) than those in the middle aged and older sedentary men. The vasodilatory responses to acetylcholine were similar between the young sedentary and endurance-trained men. There were no significant differ-

### TABLE 1. Selected Subject Characteristics of the Cross-Sectional Study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sedentary</th>
<th>Endurance-Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young (n=12)</td>
<td>Older (n=24)</td>
</tr>
<tr>
<td>Age, y</td>
<td>27±1</td>
<td>58±2*</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>82.5±4.6</td>
<td>83.3±2.6</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>19.6±2.6</td>
<td>26.3±1.2*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>86.7±3.8</td>
<td>96.5±1.8*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.5±1.3</td>
<td>26.2±0.7</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>79±3</td>
<td>86±2</td>
</tr>
<tr>
<td>VO₂max, mL · kg⁻¹ · min⁻¹</td>
<td>41.8±2.0</td>
<td>31.8±1.1*</td>
</tr>
<tr>
<td>FBF, mL/100 mL tissue per minute</td>
<td>3.7±0.3</td>
<td>3.7±0.2</td>
</tr>
<tr>
<td>FVC, AU</td>
<td>4.7±0.5</td>
<td>4.4±0.3</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.1±0.2</td>
<td>4.5±0.1</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.3±0.1</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.5±0.3</td>
<td>2.7±0.1</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.9±0.1</td>
<td>1.6±0.2*</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.9±0.1</td>
<td>5.4±0.2*</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>31.5±4.5</td>
<td>43.2±3.9*</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>4.6±0.2</td>
<td>6.7±1.0*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.05 vs young of same training status. †P < 0.05 vs age-matched sedentary.
ences among the groups in the forearm vasodilatory responses to sodium nitroprusside (Figure 2).

In the overall study population, FBF at the highest dose of acetylcholine was related to waist circumference ($r=0.32$; $P<0.01$) and $\dot{V}O_2$ max ($r=0.25$; $P<0.05$). No other significant relations were observed.

### Exercise Intervention Study

All 13 middle aged and older men (age $56\pm2$ years) completed the 3-month exercise intervention study. Subjects exercised an average of $5.5\pm0.3$ days per week for $42\pm1$ minutes per day at $72\pm1\%$ of maximal heart rate. There were no significant changes in body mass, adiposity, heart rate at rest, arterial blood pressure, or plasma cholesterol, glucose, or insulin concentrations (Table 2). Aerobic exercise training increased exercise time by $20\%$ ($P<0.01$), and decreased heart rate and RPE at the same absolute submaximal level of exercise ($\sim70\%$ of baseline maximal oxygen consumption; $P<0.05$); $\dot{V}O_2$ max was not significantly changed. There were no significant changes in resting FBF or FVC after exercise training.

Aerobic exercise training significantly increased the FBF and FVC responses to acetylcholine (Figure 3). FBF at the highest dose of acetylcholine was $\sim30\%$ higher after $15.9\pm1.5$ mL/100 mL tissue per minute) versus before ($12.3\pm1.3$ mL/100 mL tissue per minute) exercise training ($P<0.01$). The FBF responses to acetylcholine after exercise training were not significantly different from those observed in the young adults and middle aged and older endurance-trained men. There were no differences in the vasodilatory responses to sodium nitroprusside after exercise training (Figure 3). There were no significant correlates of the improvement in endothelium-dependent vasodilation.

### Discussion

The primary new findings of the present study are that (1) endurance-trained men do not demonstrate an age-related decline in endothelium-dependent vasodilation;
between the young trained and sedentary men. This finding
ences in the forearm vascular responses to acetylcholine
ary men of similar age.
endurance-trained men demonstrated significantly greater
endurance-trained men. As such, the middle aged and older
responses to each dose of acetylcholine were almost
ably well preserved with age in men who regularly
peers, endothelium-dependent vasodilation was remark-
study, however, was that in stark contrast to their sedentary
findings. Specifically, we observed a 25% age-related
function lost as a result of sedentary aging.
Our cross-sectional findings of greater endothelial va-
sodilatory capacity in trained compared with sedentary
older men suggested that regular aerobic exercise may be
effective lifestyle intervention strategy for improving
endothelial vasodilatory function in middle aged and older
sedentary men. The results of our exercise intervention
study support this postulate. Specifically, 3 months of
regular aerobic exercise (primarily walking) resulted in a
30% increase in endothelium-dependent vasodilation in
previously sedentary middle aged and older men. More-
over, this improvement occurred without concomitant
changes in body mass, adiposity, arterial blood pressure,
total cholesterol, or \( V_{\text{O}}_{\text{max}} \) (a measure of habitual physical
activity/aerobic fitness) and waist circumference were the
only physiological correlates of the maximal vasodilation
to acetylcholine.
Collectively, the results of our cross-sectional study
suggest that impaired endothelium-dependent vasodilation
may not be an inevitable (intrinsic) consequence of bi-
ological aging. Rather, this dysfunction may be due, at least
part, to age-related reductions in physical activity/
aerobic fitness and associated increases in body fatness. In
the present study, \( V_{\text{O}}_{\text{max}} \) (a measure of habitual physical
activity/aerobic fitness) and waist circumference were the
only physiological correlates of the maximal vasodilation
to acetylcholine.
In men, endothelium-dependent vasodilation has been
shown to decline progressively with advancing age, starting as early as 20 years of age. The results of our
cross-sectional study are in line with these previous
findings. Specifically, we observed a 25% age-related
reduction in acetylcholine-mediated vasodilation in seden-
tary men, whereas endothelium-independent vasodilation
was unaltered. The key finding of our cross-sectional
study, however, was that in stark contrast to their sedentary
peers, endothelium-dependent vasodilation was remark-
ably well preserved with age in men who regularly
performed aerobic endurance exercise. In fact, the FBF
responses to each dose of acetylcholine were almost
identical between the young and middle aged and older
endurance-trained men. As such, the middle aged and older
endurance-trained men demonstrated significantly greater
acetylcholine-mediated vasodilation compared with seden-
tary men of similar age.
In the present study, we observed no significant differ-
ences in the forearm vascular responses to acetylcholine
between the young trained and sedentary men. This finding
is at odds with those of Kingwell et al, who reported enhanced vascular reactivity to acetylcholine in young
endurance-trained athletes compared with age-matched
sedentary control subjects. This discrepancy is likely due
to the differences in plasma cholesterol concentrations
among subjects in each study. Elevations in plasma cho-
lesterol levels, at any age, are associated with impaired
endothelium-dependent vasodilation. In the study by
Kingwell and coworkers, plasma cholesterol levels were
lower in the endurance-trained athletes compared with the
sedentary control subjects and correlated with the en-
hanced vascular responsiveness to acetylcholine. In contrast,
there were no differences in plasma cholesterol concentrations between the young sedentary and
endurance-trained men in the present study. Thus, regular
aerobic exercise, independent of changes in total chole-
sterol, does not appear to improve acetylcholine-mediated
brachial vasodilation in young healthy adult males. It is
important to recognize the context of this finding (ie, young healthy adults) and that regular aerobic exercise
may be beneficial in young adults who have depressed
endothelial vasodilatory function such as young patients
with essential hypertension.
(2) middle aged and older men who regularly perform
aerobic exercise exhibit greater acetylcholine-mediated
vasodilation compared with their sedentary peers; and
(3) regular aerobic exercise can restore the loss of endo-
thelium-dependent vasodilation in previously sedentary
middle aged and older men. To the best of our knowledge,
this is the first study to demonstrate that regular aerobic
exercise not only can prevent the age-associated loss in
endothelium-dependent vasodilation but can restore the
function lost as a result of sedentary aging.
Figure 3. FBF and FVC responses to acetylcholine and sodium
citroprusside before and after 3 months of aerobic exercise
training. Values are mean±SEM.
(walking) and intensity (moderate) of exercise that can be safely performed by most if not all sedentary healthy older men.

The mechanisms by which regular aerobic exercise may prevent and/or reverse the age-associated decline in endothelium-dependent vasodilation are not clear. One potential mechanism for the exercise-induced increase in endothelium-dependent vasodilation is increased nitric oxide (NO) production and/or release. Sessa et al. demonstrated enhanced endothelial NO synthase (eNOS) gene expression and NO production in dogs after 10 days of exercise training. Exercise-induced increases in eNOS gene expression may account, at least in part, for the adaptation in vasodilatory function observed in the forearm in response to exercise involving primarily the legs. It is plausible that mechanical alteration/deformation of the endothelium during exercise as a result of increased systemic arterial pressure and pulsatile flow contributes to eNOS upregulation. The prominent leftward shift in the dose-response curve to acetylcholine that we observed after compared with before exercise training suggests that regular exercise may improve endothelial cell muscarinic receptor sensitivity, number, and/or the intracellular signal transduction pathway that links receptor activation to NO synthesis and release. Cheng et al. have demonstrated an upregulation of endothelial muscarinic receptors after acute exercise in rats. Other potential mechanisms that may contribute to the favorable effect of exercise on endothelial vasodilatory function include increased prostaglandin release, reduced free radical–mediated NO degradation, and lower sympathetic vasoconstrictor tone.

Considering that many of the cardiovascular complications associated with sedentary aging such as hypertension, coronary artery disease, and thrombosis are pathogenetically linked to endothelial dysfunction, our findings may have important implications regarding both primary and secondary prevention of cardiovascular disease. From a primary prevention perspective, our results suggest that habitual aerobic exercise may prevent the age-related reduction in endothelium-dependent vasodilation observed in sedentary adults. Preserved endothelium-mediated vasodilation may contribute to the lower incidence of cardiovascular events observed in middle aged and older men who exercise regularly. With regard to secondary prevention, the present findings indicate that regular aerobic exercise is an effective lifestyle intervention for reversing the loss in endothelium-dependent vasodilation in middle aged and older sedentary men. Improved endothelial function, if sustained, should reduce the risk of cardiovascular disease and related thrombotic events in this population.

Conclusions

The results of the present study provide experimental support for the hypothesis that regular aerobic exercise can prevent the age-related decline in endothelium-dependent vasodilation and restore the loss in vasodilatory function in previously sedentary middle aged and older men. Given the clinical importance of endothelial function to cardiovascular health, regular aerobic exercise represents an important therapeutic strategy for countering the deleterious effects of sedentary aging on endothelial function.

Acknowledgments

This study was supported by National Institutes of Health awards HL-03840 (Dr DeSouza), AG00847 (Dr Tanaka), and AG13038, AG03765, and AG16071 (Dr Seals); by the General Clinical Research Center (5-01-RR-00051); and by American Heart Association awards CWFW-02-98 (Dr Shapiro) and 996023AZ (Dr Tanaka). We would like to thank all of the subjects who participated in the study as well as Yoli Casas, Jayne Semmler, Jill Tanaka, and Teresa Wilson for technical assistance.

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Circulation. 2000;102:1351-1357
doi: 10.1161/01.CIR.102.12.1351

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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