

Original Articles

Acute exercise and impaired glucose tolerance in obese humans

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BACKGROUND: Individuals with impaired glucose tolerance (IGT) have a greater risk of developing diabetes and cardiovascular disease compared with those with normal glycemic control. The aim of this study was to examine the effects of acute aerobic exercise on glycemia, regional arterial stiffness, and oxidative stress in obese subjects with IGT.

DESIGN: Twelve obese subjects (7 men and 5 women; 48.0 ± 9.4 years; body mass index 32.4 ± 7.0 kg/m²) with IGT participated in a 30-minute bout of walking at 65% of maximum predicted heart rate. Pulse wave velocity (PWV, for determination of arterial stiffness) and blood pressure were examined before and after exercise, whereas venous blood samples were drawn for the determination of glucose, blood lipids, and indices of oxidative stress and inflammation (lipid hydroperoxides; superoxide dismutase; high-sensitivity C-reactive protein).

RESULTS: After exercise PWV (9.1 ± 1.2 m/s vs. 8.6 ± 1.0 m/s), glucose (5.7 ± 0.6 mmol·L⁻¹ vs. 5.4 ± 0.6 mmol·L⁻¹), and diastolic blood pressure (94 ± 14 mm Hg vs. 86 ± 13 mm Hg) decreased, respectively ($P < .05$). A correlation was observed between PWV and glucose ($r = 0.544$, $P < .05$). There were no changes in lipid hydroperoxides, superoxide dismutase, high-sensitivity C-reactive protein, or blood lipids ($P > .05$).

CONCLUSIONS: These findings suggest that acute aerobic exercise can reduce regional arterial stiffness in obese subjects with IGT by possibly improving glucose metabolism, independent of changes in oxidative stress.

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Vascular dysfunction has emerged as a seminal step in the development and progression of cardiovascular disease (CVD), specifically atherosclerosis.¹ In much of the current literature, vascular dysfunction refers to an impairment of

nitric oxide (NO)-mediated endothelium-dependent dilation, which is inversely related to an increase in vessel stiffness.² NO deficiency results from a decreased synthesis and/or release, in combination with exaggerated consumption by reactive oxygen species.³

Non-insulin dependent diabetes mellitus (NIDDM) is associated with a marked increase in cardiovascular mortality.⁴ Yet, cardiovascular complications are apparent in

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individuals who do not exhibit overt diabetes but are exposed to conditions of altered glucose homeostasis, such as impaired glucose tolerance (IGT).⁵ Subjects with IGT have an increased risk of progressing to NIDDM⁶ and have greater degrees of vascular dysfunction than normoglycemic individuals.⁷ Kidawa et al⁸ demonstrated a decrease in flow-mediated vasodilation with a concurrent increase in arterial stiffness (as measured by pulse wave velocity [PWV]) among patients with IGT compared with age- and sex-matched control patients. Oxidative stress produced during prolonged and exaggerated postprandial hyperglycemia and hypertriglyceridemia is postulated to be largely responsible for IGT and associated vascular dysfunction.⁹ High caloric diets and decreases in physical activity can lead to an increase in intracellular glucose and free fatty acids, which eventually leads to an increase in citric acid cycle activity, resulting in an excess production of mitochondrial NADPH. When excess NADPH cannot be dissipated by oxidative phosphorylation, the mitochondrial proton gradient increases, and electrons are transferred to oxygen, resulting in the formation of the superoxide radical.¹⁰ Increased superoxide can combine with NO,¹¹ creating large concentrations of peroxynitrite, a potent long-lived oxidant.¹² The subsequent decrease in NO bioavailability may have negative implications on the regulation of vascular tone.

Lifestyle interventions, such as the prescription of exercise, have been shown to stabilize glycemic control in individuals with IGT and prevent the onset of NIDDM.⁶ Proposed adaptive responses from such interventions include enhanced insulin action on the skeletal muscle glucose transport system, a reduced hormonal stimulation of hepatic glucose production, improved blood flow to skeletal muscle, and modulation of the lipid profile.¹³ However, there have been few studies examining the impact of acute exercise on subjects with IGT. Given that a single bout of acute exercise can induce a glucose-lowering response in normal and diabetic individuals,^{13,14} the influence of acute exercise in subjects with IGT warrants investigation. Interestingly, as individuals are recommended to engage in at least 30 minutes of moderate physical activity per day,¹⁵ any observed change(s) after acute or "daily" exercise may be important in highlighting to the public the importance of physical activity for the promotion of health. It is thus proposed that acute exercise will reduce PWV and measures of oxidative stress and improve glycemic control in obese patients with IGT. Therefore, the aim of this study was to examine the effects of a single bout of moderate intensity aerobic exercise on PWV, glycemic control, and oxidative stress markers in obese subjects with IGT.

Methods

Subject characteristics

After approval from the Office for Research Ethics Committees Northern Ireland (ORECNI), and in accordance

with the Declaration of Helsinki (2000), 12 (7 men and 5 women) obese (body mass index ≥ 30) subjects who were diagnosed with IGT were recruited from the Diabetic Clinic at the Ulster Hospital, Dundonald. Before participation, all subjects completed a health history questionnaire to ensure they had no medical conditions that would compromise their involvement. Complete study details, including potential risks, were fully explained to participants before written informed consent was obtained. Those subjects undergoing lipid-lowering therapy, hypertensive treatment, antioxidant supplementation, or those taking any medication that may have potentially interfered with glycemic control were excluded from participation. All participants were non-smokers and free from cardiovascular disease at time of recruitment.

Experimental design

Subjects participated in a 2-tier study. The first component involved confirmation of glucose tolerance (after an oral glucose tolerance test [OGTT]), which was subsequently followed on a separate day (no more than 7 days) by an acute bout of exercise. Subjects were instructed to maintain a 12-hour overnight fast before each of the respective trial components. Subjects were also asked to refrain from exercise and alcohol consumption for 48 hours before each test. Subjects were instructed to consume 500 mL of water on the morning of the exercise test. All tests were conducted between 8:00 and 10:00 AM.

Anthropometric measures

Upon arrival at the laboratory, measurements of body mass, stature, and body fat percentage (%; without footwear and in light clothing) were obtained from each subject. Body mass in kilograms (kg) was recorded to the nearest 0.1 kg by the use of standard laboratory scales (Seca delta, Germany). Stature was recorded to the nearest 0.1 cm by the use of a freestanding stadiometer (Holtain Limited, Crosswell, Crymmych, Dyfed, UK). Body fat was measured as a percentage of overall body mass by an automated bioelectrical impedance automated device (Bodystat, Havant, Hampshire, UK) with the positioning of electrodes at the third and fourth metacarpals of the right hand and the third and fourth metatarsals of the right foot. Measurements of body fat percentage were taken in a supine position on a non-conductive surface. See Table 1 for subject characteristics.

OGTT

The OGTT was used to diagnose potential subjects for IGT. A fasting blood sample (approximately 2 mL) was drawn from a prominent forearm vein for determination of plasma glucose concentration. All subjects were then requested to ingest an oral load of 75 g of glucose, which corresponded to 394 mL of a commercially available energy drink (Lucozade Energy, GlaxoSmithKline, Brentford, Middlesex, UK). After a 2-hour period, a further venous

Table 1 Age and physical characteristics

Dependent Variable	Mean \pm SD
Age (years)*	48.0 \pm 9.4
Height (cm)	168.0 \pm 7.0
Body mass (kg)	90.7 \pm 16.8
BMI (kg/m ²) [†]	32.4 \pm 7.0
Body fat (%)	45.6 \pm 10.4

Participants were screened for IGT between April and June 2007.

All data are mean (\pm SD).

BMI, body mass index.

*Range = 32-63 years.

[†]Range = 28.2-45.9 kg/m².

blood sample was drawn for determination of plasma glucose. IGT was diagnosed by plasma glucose concentration of ≥ 7.8 and < 11.1 mmol/L according to World Health Organization criteria.¹⁶

Exercise protocol

For the acute exercise trials, subjects exercised at 65% of their age-predicted maximum heart rate (HR)¹⁷ for 30 minutes on a motorized treadmill (Power, H-P Cosmos, Essen, Germany). Speed was adjusted to ensure HR was kept constant (ie, 65% max HR). HR was continuously measured by the use of an electrocardiograph short-range telemetry system (Polar Electro, Oulu, Finland). Given the sedentary nature and poor aerobic capacity of obese subjects with IGT, it was thought that the exercise intensity chosen was sufficient to induce cardiorespiratory and metabolic responses without causing any adverse effects, often associated with higher exercise intensities.

PWV measurement

Arterial stiffness was measured (approximately 2 minutes) before and (within 5 minutes) after completion of exercise by the use of the PWV device (Sensor Technology and Devices Ltd, Northern Ireland).¹⁸ Before PWV measurements, each subject was instructed to rest in a seated position for approximately 5 minutes. The pressure pulse detection was achieved by using two polyvinylidene fluoride piezoelectric conformal and flexible sensor strips of dimension 2 cm by 5 cm, at 2 separate points along the arterial tree of the arm. The sensors generate a measurable voltage at the output contacts if they are mechanically deformed. The 2 points used for this study were each subject's brachial and radial arterial pulses of the left arm, identified via palpation. A reference electrode, linked to the device, was also attached to electrocardiographic skin tacts along the bicep of the left arm. The distance between the 2 sites was then measured (cm) for each PWV measurement through the use of a measuring tape. For each trial, the sites of measurement were marked after palpation to ensure continuity. PWV was calculated (PWV (m/s) = $l/\Delta t$) during a 10-second measuring period, which was initiated

upon the appearance of consistent waveforms. Pulse wave traces were calculated by use of the Labview program (Version 7.0). The PWV value was based upon the time delay in the mean foot-to-foot (based on the velocity of the "foot" or leading edge of the pressure pulse wave), peak-to-peak, and cross-correlational values recorded between the two marked sites in the 10-second measuring period. Coefficient of variation (CV) was 2.8%.

Blood pressure (BP)

Systemic arterial BP was measured in the brachial artery by use of an Omron M5-I fully automatic BP monitor (Surrey, UK). Subjects were requested to rest in a supine position for 5 minutes before each BP measurement. Three measurements were taken at each interval and the mean was recorded.

Blood biochemistry

Blood sampling

Blood samples were taken (with minimal stasis) immediately before and after exercise from a prominent forearm vein while subjects rested in a supine position. After immediate blood collection, blood plasma was obtained by the use of tubes containing potassium ethylenediaminetetraacetic acid. These were placed on ice while whole blood in serum separating clot activator tubes was allowed to clot at room temperature (for 15 minutes) before centrifugation at 3500 rpm (1500 g) for 5 minutes at 4 °C. Plasma and serum were removed and transferred to 1.5-mL plastic vials and stored at -70 °C before subsequent analysis. Post exercise blood samples were corrected for any shift in plasma volume using the method of Dill and Costill.¹⁹

Superoxide dismutase (SOD)

Serum samples were spectrophotometrically assayed at 490 nm on a microplate reader for SOD levels by use of a Superoxide Dismutase Activity Assay Kit (Chemicon International, Inc., Billerica, MA). The test involves generating O₂⁻ anions via the addition of xanthine and xanthine oxidase solution to the samples. These are detected by a chromagen solution. When SOD is present, superoxide concentrations are lowered, thereby lowering the colorimetric signals. The CV for this assay was 9.6%.

Serum high-sensitivity C-reactive protein (hs-CRP)

Hs-CRP samples were assayed on an automated analyzer (Aeroset, Abbott Labs, Abbott Park, IL) by the use of a CRP high-sensitivity assay kit (Randox Laboratories Ltd, Northern Ireland). Samples were combined with a buffer and an anti-CRP-coated latex. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 550 nm. CVs for hs-CRP were 1.82% at 2.18 mg/L and 1.85% at 4.93 mg/L.

Plasma glucose

Glucose levels were determined by an immobilized enzyme membrane method in conjunction with a Clark electrode on a YSI 2300 analyzer (Yellow Springs, OH). The principle of the test involves measuring electrode flow from a steady state H_2O_2 concentration, which is proportional to the concentration of glucose. CV for glucose was 2.1%.

Blood lipids

Total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) were measured by enzyme assay kits by the use of an automated analyzer (Aeroset, Abbott Labs). For total cholesterol, samples were centrifuged for 10 minutes at 3000 rpm (1000 g) at room temperature. Samples were allowed to react with cholesterol esterase and then cholesterol oxidase, resulting in the formation of a chromophore, which was quantitated at 500 nm. For HDL-C, samples were centrifuged at 3600 rpm (1500 g) for 10 minutes to separate. A polyanion was added to assist with complexing cholesterol subfractions. The second reagent released only HDL-C, allowing it to react with cholesterol esterase and cholesterol oxidase, in the presence of chromagen to produce color. Estimates of low-density lipoprotein cholesterol concentration were calculated with the Friedewald formula.²⁰ The CV was <1.6% for all blood lipids.

Serum lipid hydroperoxides (LOOH)

Serum LOOHs were measured with the use of the FOX-1 assay.²¹ Standard solutions were incubated for 30 minutes with the FOX-1 reagent. Preparation of the FOX-1 reagent involves the addition of the following ingredients: 100 $\mu\text{M.L}^{-1}$ Xylenol orange, 250 $\mu\text{M.L}^{-1}$ ammonium ferrous sulfate, 100 mM.L^{-1} sorbitol, and 25 mM.L^{-1} of sulfuric acid (H_2SO_4). After thawing, 50 μL of each serum aliquot was added to 950 μL of FOX-1 reagent. Samples were then vortexed and left to incubate for 30 minutes at room temperature in a dark room. The absorbance of the supernatant was then read at 560 nm against the standard curve for the concentration range 0–5 $\mu\text{M.L}^{-1}$. Intra- and inter-assay of CV at 0.57 $\mu\text{M.L}^{-1}$ were 4.6% and 6%, respectively.

Statistical analysis

Statistical analysis was performed by the use of the SPSS social statistics package- version 11.0 (Surrey, UK). Prospective calculations of power were performed according to the Altman method.²² Data were analyzed by parametric statistics following mathematical confirmation of a normal distribution using repeated Shapiro Wilks W tests. The data were analyzed by the use of paired sample t tests. The linear relationship between 2 continuous variables was established with a Pearson's correlation. The alpha level was set at $P < .05$. Data are expressed as mean \pm standard deviation (SD) unless otherwise stated.

Results

Vascular function

Figure 1 indicates that PWV decreased by 5% after an acute bout of moderate-intensity exercise when compared to pre-exercise values ($P < .05$). Δ Change for PWV = -0.47 .

CVD risk indices

Table 2 demonstrates no changes in total cholesterol, HDL-C, estimated serum low-density lipoprotein cholesterol, triglycerides, or hs-CRP after exercise when compared to pre-exercise values ($P > .05$).

Oxidative stress indices

There was a trend (-7.5%) but no statistically significant change in LOOHs after exercise (Table 2). There were no changes in SOD immediately following the exercise in relation to pre-exercise concentrations ($P > .05$).

Glucose data

As observed in Table 2, glucose decreased by more than 5% after exercise, when compared with pre exercise glucose ($P < .05$).

Correlations

There were no correlations observed between PWV and LOOH concentrations, or between LOOH and glucose ($P > 0.05$, respectively). However, there was a positive correlation between brachial-radial PWV and mean glucose (Fig. 2; $r = 0.544$, $P < .05$).

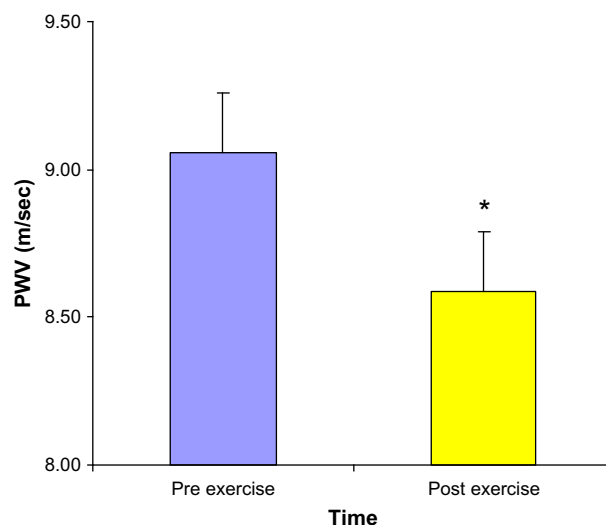


Figure 1 PWV values before and after moderate-intensity exercise ($n = 12$). * $P < .05$ before vs. after exercise. PWV, pulse wave velocity.

Table 2 Biochemical and hemodynamic measures before and after exercise

Variable	Before Exercise	After Exercise	P Value
Total cholesterol (mmol·L ⁻¹)	5.06 ± 0.96	5.08 ± 0.93	>.05
LDL cholesterol (mmol·L ⁻¹)	3.14 ± 0.86	3.15 ± 0.85	>.05
HDL cholesterol (mmol·L ⁻¹)	1.22 ± 0.24	1.23 ± 0.24	>.05
TGs (mmol·L ⁻¹)	1.53 ± 0.51	1.53 ± 0.40	>.05
Glucose (mmol·L ⁻¹)*	5.68 ± 0.58	5.43 ± 0.60	<.05†
hs-CRP (mg/L)	2.74 ± 2.73	2.73 ± 2.62	>.05
LOOHs (μM·L ⁻¹)	1.20 ± 0.47	1.11 ± 0.49	>.05
Systolic BP (mmHg)	145 ± 14	137 ± 17	>.05
Diastolic BP (mmHg)	94 ± 14	86 ± 13	<.05†

LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; CRP, high-sensitivity C-reactive protein; LOOH, serum lipid hydroperoxides; BP, blood pressure.

*Range at baseline 4.9 and 6.5 mmol·L⁻¹.

†Denotes difference between resting conditions and immediately post exercise conditions ($P < 0.05$). Data are mean (\pm SD).

Discussion

This study demonstrates a decrease in upper limb arterial stiffness after a single bout of moderate intensity aerobic exercise (in line with daily physical activity recommendations [15]) in obese subjects with IGT. This is the first study examining the effects of acute exercise in such a population—much of the related research has sought to examine the effects of chronic exercise on arterial stiffness. An earlier study reported that a 30-minute bout of moderate intensity cycling reduces PWV in central and peripheral arteries of healthy participants.²³ The increased shear stress associated with aerobic exercise is postulated to stimulate the c-Src, phosphatidylinositol-3-kinase, and Akt/serine-dependent phosphorylation of endothelial nitric oxide synthase.^{24,25} Endothelial nitric oxide synthase acts to potentiate NO availability and activity, ultimately promoting flow-dependent dilation of vascular beds.²⁶ It is conceivable that this proposed increase in NO bioavailability may account for the reduction in upper-limb stiffness in the current study given that augmented flow-mediated dilation and stimulation of NO have been reported to reduce PWV and improve vascular function.^{2,27}

Another finding of this study was that plasma glucose decreased immediately after exercise. The effects of acute exercise on glucose uptake are caused by increases in the translocation to the plasma membrane and intrinsic activity of the main skeletal muscle glucose transporter protein, GLUT-4.²⁸ It is well established that acute exercise can lead to enhancements in glucose transport with increases in plasma membrane GLUT-4 protein in both diabetic and healthy subjects,^{13,29} but the effects in subjects with IGT have been less rigorously investigated. Exercise increases the sensitivity of skeletal muscle to the action of insulin,³⁰ which may serve to increase GLUT-4 trafficking via increases in insulin receptor substrate proteins and phosphatidylinositol-3-kinase signalling³¹ although, again, this finding has yet to be fully investigated in individuals with altered glucose homeostasis.

Contractions of skeletal muscle are another potential mechanism to explain the increased glucose transport

associated with exercise in the present study. The increases in intracellular Ca²⁺ levels from muscular contractions are a major stimulus for an increase in calmodulin-dependent kinase II and protein kinase C³² and AMP-activated protein kinase (AMPK) activity (activated by alterations in the ATP/AMP ratio), initiating a signaling process that results in GLUT-4 translocation to the plasma membrane.³³ It should be noted that, unlike calmodulin-dependent kinase II, the AMPK pathway was initially proposed to only function in fast-twitch muscle fibers.³⁴ Yet, emerging evidence indicates that AMPK activation is essential for submaximal contraction-induced glucose transport in the skeletal muscle of mice.³⁵

Although an exercise-induced increase in NO has hitherto been identified as a potential modulator for PWV, the possibility exists that NO may also confer an intrinsic effect in the regulation of glucose transport. An up-regulation in skeletal muscle GLUT-4 mRNA and protein expression has been observed after the administration of *S*-nitroso-N-

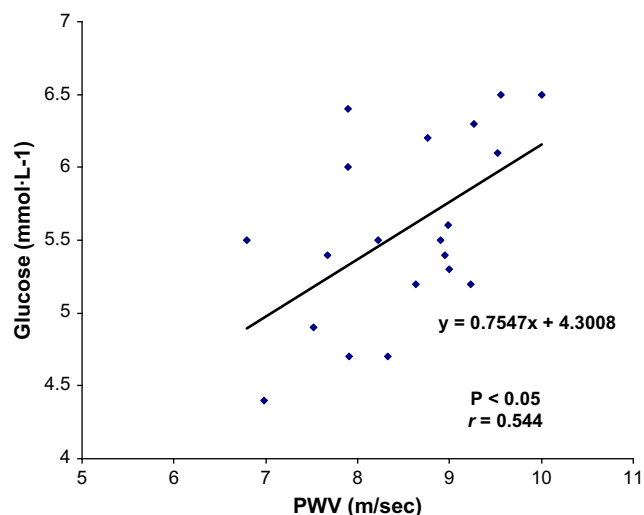


Figure 2 Correlation of PWV with Glucose concentration in obese subjects with IGT. y = equation of the line; r = correlation coefficient.

penicillamine (ie, SNAP, an NO donor). Moreover, when the cultured cells were exposed to N^G -nitro-L-arginine methyl ester, the response in GLUT-4 mRNA was diminished considerably.³⁶ It is further postulated that NO may elicit such effects via an AMPK-dependent mechanism because the addition of AMPK inhibitor compound C ablates the up-regulation in GLUT-4.³⁶

In the context of the current study, it is plausible to suggest that an increase in flow-mediated NO, to some degree, may have the effect of potentiating GLUT-4 activity during the exercise bout to account for the increase in glucose clearance. Research assessing this putative mechanism in humans is accordingly required. A positive correlation was also reported between glucose and PWV (both decreased after exercise) that, given the increase in shear stress associated with exercise, could further support a dual role for NO in the modulation of vessel stiffness and glucose clearance. However, this correlation is concurrently in line with other research, intimating a link between hyperglycemia and vascular dysfunction.^{9,37} This link is based on the notion that a single hyperglycemic event can generate oxidative stress, resulting in a reduction of NO-dependent vascular function.^{32,38} Yet, the observed postexercise improvements in PWV appear to be independent of any changes in oxidative stress in this instance because there were no changes in either LOOH or SOD concentrations.

Considering the trend for a 7.5% post exercise reduction in LOOHs, it can be speculated that a larger sample size may be required to detect any interaction effects in markers of oxidative stress. Moreover, the effects of exercise on blood flow persist for a number of hours after exercise cessation; it has been reported that blood flow to peripheral tissues is vastly increased the day after a prolonged bout of moderate exercise.³⁹ Thus, it is plausible that changes in SOD and LOOHs, owing largely to the antioxidant effects of increased shear stress, may still occur throughout a longer postexercise period. Further parallel research is vital before firm conclusions can be drawn on the role of exercise in this cohort.

Conclusion

Diabetes is one of biggest challenges facing health professionals. Aside from the deleterious effects on morbidity and mortality, it is estimated that £1 million per hour is spent on diabetes in the United Kingdom.⁴⁰ The results of this study indicate that an acute bout of moderate intensity aerobic exercise can improve upper-limb arterial stiffness and plasma glucose transport in obese subjects with IGT, independent of changes in oxidative stress. Considering that obese subjects with IGT are at a greater risk for developing NIDDM and CVD, this study demonstrates that a single bout of exercise, matching current daily physical activity recommendations, can elicit desirable physiological responses that may be beneficial in the protection against these conditions. We believe these data could be

valuable in considering further studies to delineate the protective mechanisms associated with exercise training. Further evaluation of variables such as intensity and duration of exercise may provide crucial insights.

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