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INFLUENCE OF AGE AND GENDER ON HUMAN LYMPHATIC PUMPING PRESSURE IN THE LEG

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ABSTRACT

Lymph transportation is controlled, at least in part, by the intrinsic pumping of lymphatic vessels. The objectives of this study were to evaluate the influences of age and gender on leg lymphatic pumping pressure. A total of 399 subjects between the ages of 20 and 91 years (199 males and 200 females) volunteered to participate in this study. Lymphatic pumping was measured in 798 legs of the 399 participants. Indocyanine green (ICG) fluorescence lymphography was performed, and the real-time fluorescence images of lymph propulsion were obtained in a sitting position using an infrared-light camera system. A custom-made transparent sphygmomanometer cuff was wrapped around the lower leg and connected to a standard mercury sphygmomanometer. The cuff was inflated, and then gradually deflated until the fluorescent dye exceeded the upper border of the cuff. Lymph pumping pressure was defined as the value of the cuff pressure when the dye exceeded the upper border of the cuff. There was a significant correlation between the leg lymphatic pumping and age: r =-0.34 (p<0.0001). Comparison of lymphatic pumping between males and females indicated that the age-related decrease in lymphatic pumping pressure was more marked in females of postmenopausal age.

Keywords: lymphatics; contraction; aging; lymph; pressure

The lymphatic system plays an important role in maintaining balance of body fluids and macromolecules, lipid absorption, and immunity. Lymphatic vessels are responsible for transporting lymph from the tissues back to the bloodstream. The lymphatic system uses lymph pumps that depend on forces generated both extrinsically and intrinsically and on lymphatic valves to prevent regurgitation (1,2). The intrinsic lymph pump relies on the intrinsic contraction of lymphatic muscle to generate force. Decreases in lymphatic pumping may reduce the lymph velocity and cause lymph stasis or lymphedema (3-6). Most developed countries face aging populations, where elderly people tend to suffer from leg edema. Because aging is associated with diverse declines in body functions, the concept of reduced lymphatic pumping and increased susceptibility to edema formation in older adults is intuitively understandable. However, unlike measurements for blood pressure or muscular strength, there is no clinically available diagnostic test for measuring lymphatic function except lymphoscintigraphy (7).

We have recently developed a novel method of measuring lymph pumping pressure in human legs using a custom-made transparent sphygmomanometer cuff and indocyanine green (ICG) fluorescence lymphography (8). ICG fluorescence lymphography has allowed for real-time visualization of lymph flow safely and inexpensively (9,10). We took advantage of

TABLE 1
Clinical Characteristics

Age range (years)	n		Age (years) Mean (SD)		Weight (kg) Mean (SD)		Height (cm) Mean (SD)		BMI (kg/m2) Mean (SD)		SBP (mmHg) Mean (SD)		DBP (mmHg) Mean (SD)		P pump (mmHg) Mean (SD)	
	M	F	M	F	M	F	M	M	M	F	M	F	M	F	M	F
20 - 29	55	35	23.5 (1.9)	23.5 (2.8)	64.3 (7.8)	51.4** (7.5)	171.8 (5.8)	40.2 (14.9)	40.2 (14.9)	20.2** (2.5)	122.8 (10.8)	109.2** (11.8)	68.4 (7.7)	65.7 (10.2)	40.2 (14.9)	40.3 (17.3)
30 - 39	19	24	33.7 (3.2)	35.5 (2.8)	63.2 (10.9)	50.4** (5.7)	169.1 (5.8)	32.1 (14.9)	32.1 (14.9)	19.9* (2.0)	122.9 (11.0)	115.1* (11.9)	75.9 (5.9)	67.7* (9.4)	32.1 (14.9)	32.4 (14.5)
40– 49	10	19	45.1 (3.6)	44.2 (3.1)	74.8 (19.8)	50.6** (10.8)	174.7 (2.3)	31.3 (9.4)	31.3 (9.4)	20.5* (2.4)	128.7 (8.5)	121.6 (6.9)	78.5 (9.2)	77.8 (8.1)	31.3 (9.4)	30.3 (17.8)
50 - 59	15	39	55.4 (2.7)	55.4 (2.9)	65.5 (6.9)	53.3** (6.3)	167.8 (6.6)	32.0 (18.1)	32.0 (18.1)	21.1 (4.1)	136.4 (14.5)	130.6 (14.4)	87.1 (7.1)	78.7 (9.5)	32.0 (18.1)	24.6* (15.1)
60– 69	32	56	65.1 (2.9)	63.1* (2.8)	63.0 (9.1)	52.0** (7.4)	166.2 (5.0)	28.0 (18.0)	28.0 (18.0)	21.9 (4.2)	144.3 (12.9)	129.2* (19.9)	82.5 (11.5)	74.6 (10.5)	28.0 (18.0)	25.9 (15.3)
70– 79	41	19	74.0 (2.7)	72.4* (2.7)	57.8 (8.3)	52.5* (9.2)	162.1 (5.6)	25.5 (17.3)	25.5 (17.3)	23.2 (4.0)	144.5 (15.5)	135.1 (19.7)	80.4 (11.4)	75.4 (9.2)	25.5 (17.3)	16.6* (14.1)
≥ 80	27	7	83.5 (3.5)	84.0 (4.0)	53.5 (8.1)	49.9 (9.7)	161.8 (6.3)	25.7 (16.1)	25.7 (16.1)	22.9 (3.5)	141.9 (14.9)	143.5 (17.6)	74.2 (10.2)	76.5 (3.6)	25.7 (16.1)	13.6** (14.9)

*p<0.05, **p<0.01, Male versus female

this technique to trace the movement of lymph flow and succeeded in measuring lymph velocity (11,12). To measure lymphatic pumping pressure, we measured the cuff pressure of the lower leg with a sphygmomanometer; the measurement was taken when the lymphatic pumping pressure overcame the cuff pressure and moved toward the proximal region of the leg. This cuff pressure is being used to reflect the maximum contraction force of the lymphatics. Using this method, the present study investigates age-related differences in the maximum lymphatic pumping pressure in the lower legs of men and women of varying ages.

METHODS

Ethical Approval

This study was approved by the Ethics Committee of Hamamatsu University School of Medicine. Informed consent was obtained from all participants.

Subjects

Participants were recruited at the Hamamatsu University School of Medicine. Potential volunteers were found by advertising the project locally and with poster boards in the most frequented areas at the university. Advertising was also performed with the assistance of The Hamamatsu Chamber of Commerce and Industry. Individuals of all ages were invited to participate in the study. After preliminary screening, subjects with no serious allergies, and no history of leg injury or surgery, were included in the study. Those with iodine allergy were excluded, according to the industry's

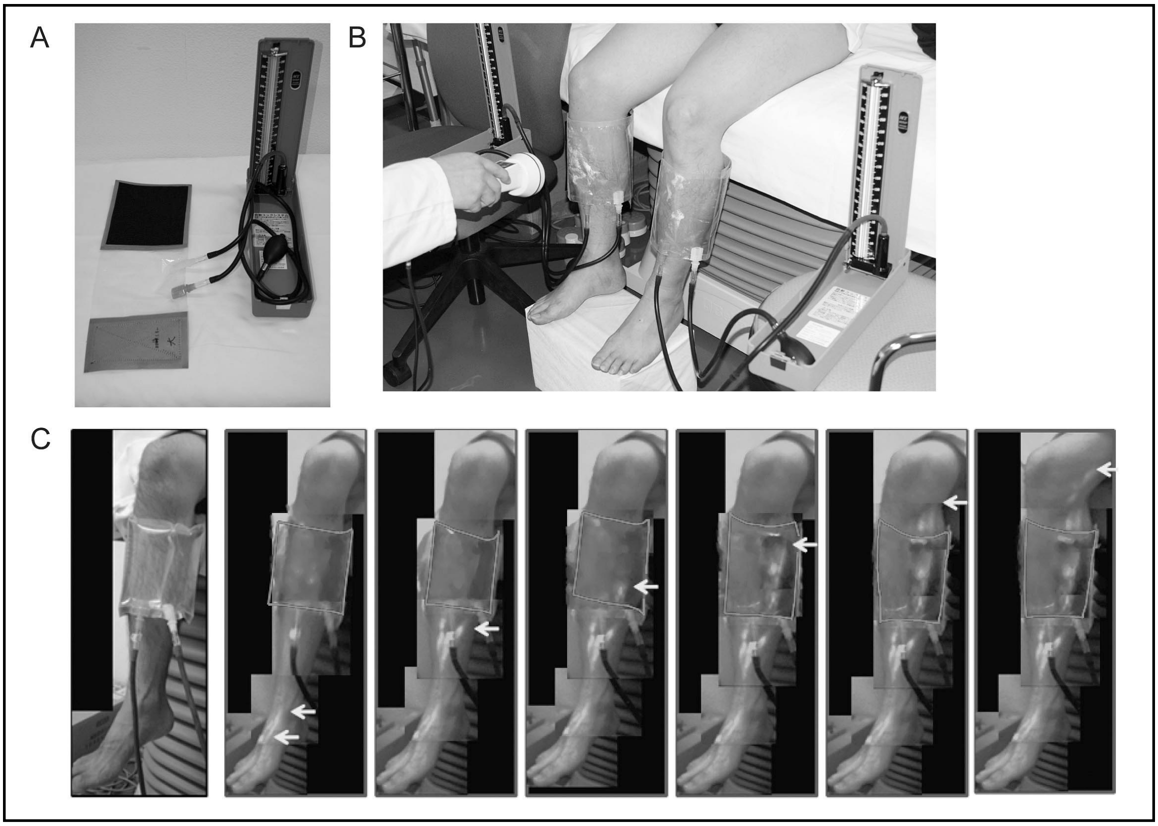

Fig. 1. Measurement of lymphatic pumping pressure in the lower leg wrapped with a custom-made transparent sphygmomanometer cuff. A) A custom-made transparent sphygmomanometer cuff and a standard mercury sphygmomanometer. B) Measurement of lymphatic pumping pressure in a sitting position. C) Real-time images of indocyanine green fluorescence lymphography after subcutaneous injection at the dorsum of the foot. The arrows indicate the most advanced indocyanine green contrast agent in the lymph vessel and the solid line outlines the transparent cuff.

recommendations, because ICG contains iodine. Subjects with varicose veins in the legs (C3,4,5: CEAP classification), lymphedema, or a medical history of deep vein thrombosis were also excluded from the test. The methods and all procedures used in this study were in accordance with current local guidelines and the Declaration of Helsinki. A total of 399 subjects between the ages of 20 and 91 years (199 males and 200 females) volunteered to participate in this study (*Table 1*). Prior to the study, all subjects were informed about the procedures as well as the study's purpose. Written informed consent was obtained from all participants.

Measuring Leg Lymphatic Pumping Pressure

Using a 27-gauge needle, we subcutaneously injected 0.3 ml of indocyanine green (ICG: Diagnogreen 0.5%; Daiichi-Sankyo Pharmaceutical, Tokyo, Japan) in the dorsum of each participant's foot. Immediately after the injection, fluorescence images of subcutaneous lymphatic drainage were obtained using an infrared camera system (PDE^{TM}; Hamamatsu Photonics K.K. Hamamatsu, Japan), which activates ICG with emitted light (wavelength: 760 nm) and filters out light with a wavelength below 820 nm. The light source for emission of ICG

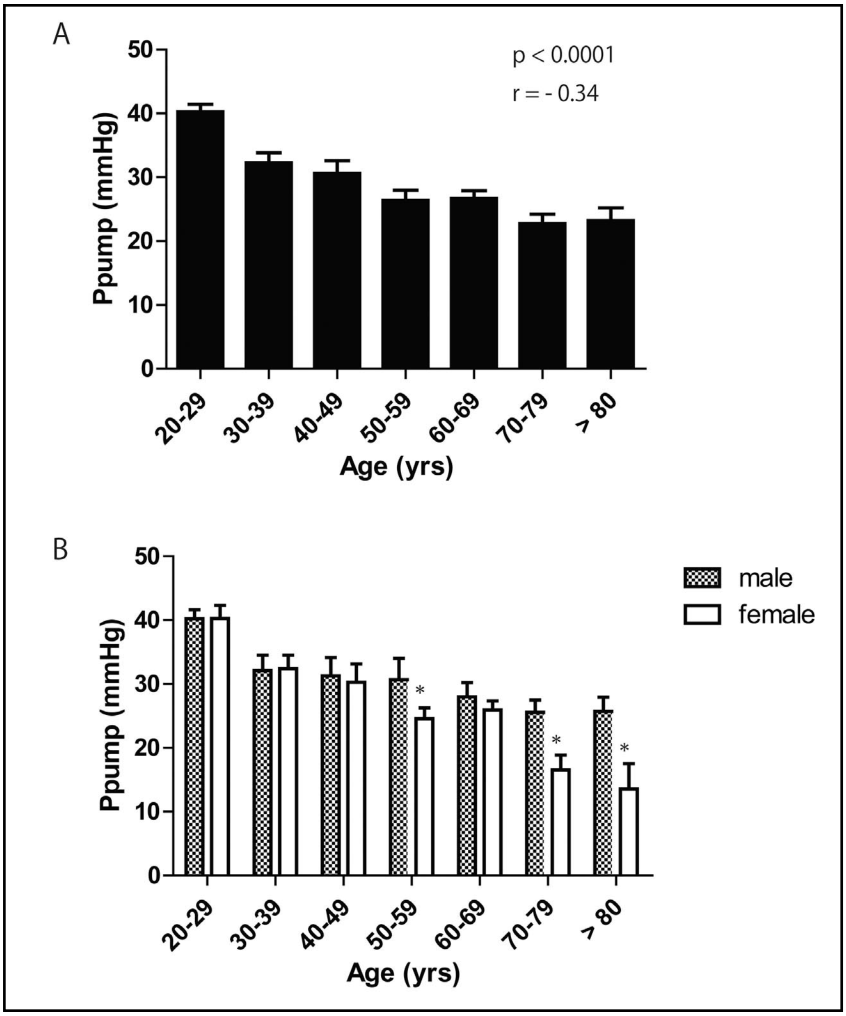

*Fig. 2. Influence of Age and gender on leg lymphatic pumping pressure (P_{pump}). A) Relationship between age and P_{pump} among all participants. B) Relationship between age and P_{pump} in males and females.*p<0.05 versus male at the same age group.*

consisted of 760-nm LEDs, and the detector was a charge-coupled device (CCD) camera. The fluorescence images were continuously observed on the monitor of a laptop computer (LaVie G, Type T; NEC Co., Tokyo, Japan).

Before injection of ICG, a custom-made transparent sphygmomanometer cuff was wrapped around the lower leg just below the popliteal fossa. The cuff was connected to a standard mercury sphygmomanometer. Measurements of lymphatic pumping were performed with the subject in a sitting position. Immediately after subcutaneous injection of ICG, the transparent cuff was inflated to 70 mmHg, then gradually deflated to lower the pressure by 5 mmHg increments every 2 min. This proceeded until the fluorescence dye exceeded the upper border of the cuff, indicating that lymphatic contraction had overcome the cuff pressure. The value of the cuff pressure at this point was used as a measure of lymph pumping pressure (P_{pump}) (*Fig. 1*). The subjects did not move their legs during the measurements to

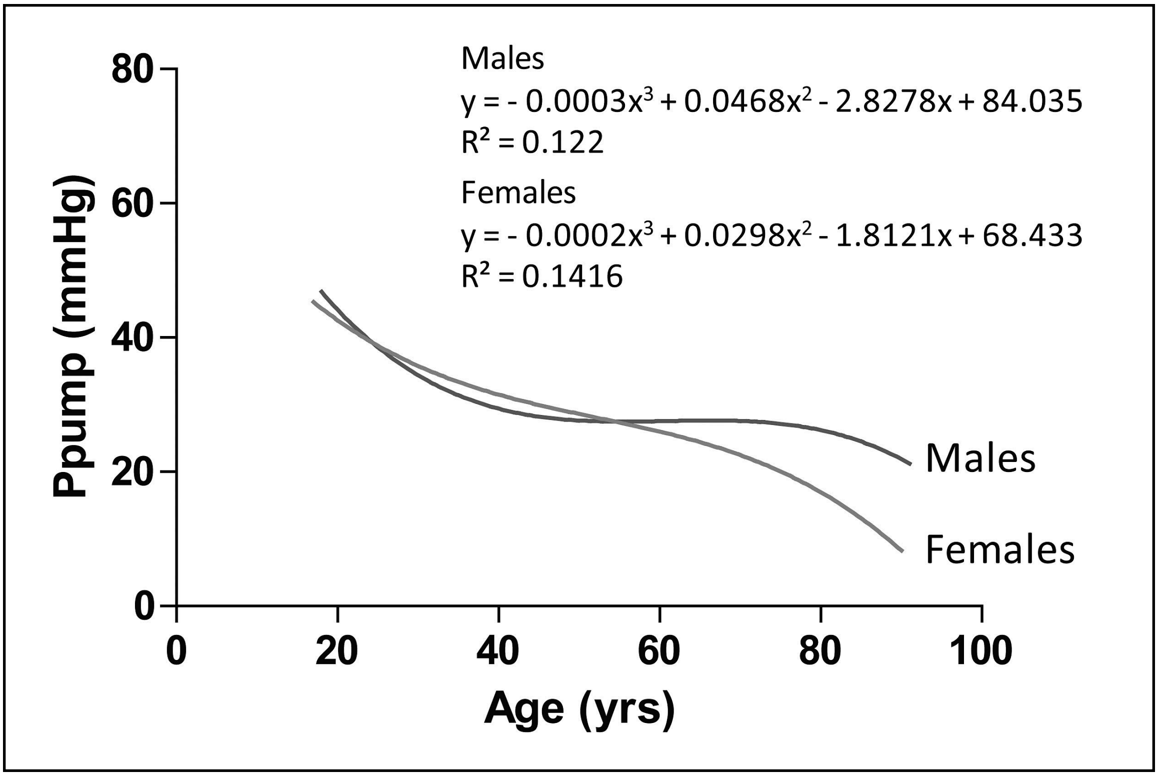

Fig. 3. Association between age and leg lymphatic pumping pressure (P_{pump}).

prevent any movement of lymph via extrinsic compression forces.

Statistical Analysis

Data are expressed as the mean ± (SD) and differences in the means of the 2 comparison groups were assessed using paired t-tests. GraphPad Prism (v.5; GraphPad Software, CA, USA) was used to conduct all statistical analyses, including regressions and correlations. Regression curve analysis (cubic curve relationship) was performed to estimate the relationship between age and P_{pump}. A p value of <0.05 was considered statistically significant.

RESULTS

Table 1 shows the physical characteristics of subjects in each age group. The screening was successfully performed in each participant. Lymphatic pumping pressure was measured in both legs of 399 participants (199 men and 200 women). No side effects or complications occurred during measurements of lymphatic pumping pressure with ICG fluorescence lymphography. *Fig 2* shows that there was a significant correlation between the leg lymphatic pumping and age: r=-0.34 (p<0.0001). Both males and females showed a significant correlation between the leg lymphatic pumping pressure and age (r=-0.32 (p<0.0001), r=-0.37 (p<0.0001), males and females, respectively) (*Fig. 3*). Comparison of lymphatic pumping pressure between males and females in the same age groups indicated that the lymphatic pumping pressure in females is lower than that of males' in the age groups 50-59, 70-79, and >80 years old (*Fig. 3*). In estimating the regression curve, the relation between age (x) and lymphatic

pumping pressure (y) demonstrated a cubic curve in both genders as follows (*Fig. 3*).
Male: $y=-0.0003x^3 + 0.0468x^2 - 2.8278x + 84.035$ ($R^2=0.122$, $p<0.05$)
Female: $y = -0.0002x3 + 0.0298x2 - 1.8121x + 68.433$ ($R^2=0.142$, $p<0.05$)

DISCUSSION

This study explored age-related differences in lymphatic pumping pressure using a novel measurement method. Direct measurements of intraluminal pressure, first performed in human legs (13), use a pressure-transducer probe inserted by cutting down the lymphatic vessels. In contrast, our method is minimally invasive and takes a relatively short time (20-30 minutes per participant to measure bilateral leg lymphatic pressure simultaneously). Previously, dynamic lymphoscintigraphy was also applied to measure lymphatic pumping with a sphygmomanometer cuff similar to our method (6). However, the radioisotope is expensive and potentially teratogenic, making lymphoscintigraphy unsuitable for the screening of younger women, who may be pregnant. Consequently, there have been no human studies performed to determine the relationship between aging and lymphatic pumping pressure. On the other hand, ICG has been daily used in worldwide hospitals by intravenous injection to assess hepatic function with safe and low cost, so that there is no ethical problem to use ICG for a surveillance study. The sphygmomanometer cuff technique after subcutaneously injection of ICG was simple and thus, easy to understand for every participants in this study.

When we recruited the volunteers, we excluded the people with possible chronic venous insufficiency (CVI) such as varicose vein and deep vein thrombosis. Because venodynamics and lymphodynamics may interact as an inseparable and mutually dependent dual outflow system, CVI causes an increase in venous pressure and subcutaneous capillary network, thus may perturb lymphatic network (12,14). People with past medical history of leg injury were also excluded due to the possible damages to the lymphatic systems. Moreover, as we and others reported that patients with secondary lymphedema were affected their lymphatic pumping at their very early stages, people with the medical history of pelvic surgery such as uterus, prostate, and rectal surgery were also excluded from this study (8,15). However, there are likely many other unknown pathophysiologies in which lymphatic pumping is modulated.

The main result of the present study was the finding that lymphatic pumping pressure significantly decreases with increasing age. Because aging has adverse effects on many body functions, the results are not surprising. However, the mechanism of this observed lymphatic system remains to be determined. The larger collecting lymphatics, as in the leg, possess layers of smooth muscle cells in their walls and generate contractile force to propel lymph upstream. Almost a half century ago, Rabinovitz et al reported on anatomical changes in the human thoracic duct in older adults. They reported that increased fibrosis of the thoracic duct with alterations in the internal elastic plate occurred with age. The distribution of smooth muscle cells in the duct wall also changed. They called the characteristic changes as 'lymphatic sclerosis' as compared to arteriosclerosis or phlebosclerosis (16). Such age-related anatomical changes might be associated with loss of lymphatic contractility. With aging, structural changes in the skin result in lack of elasticity, reduced blood flow, and alteration of its fat composition; these characteristics are associated with the reduction of tissue turgor (17), which may alter the extrinsic force that propels the lymph.

On the other hand, the intrinsic force, which is generated by the active spontaneous contractions of lymphangions, might also be affected in the elderly. The contraction/relaxation mechanisms of the lymphatic muscle is complex and not well understood.

Lymphatic and arterial endothelial cells have the ability to produce nitric oxide (NO) (18,19). The importance of NO in the endothelium-dependent modulation of the lymphatic contractile cycle was demonstrated in animal experiments both *in vitro* and *in vivo* (20,21). NO regulates flow-mediated lymphatic pumping and in turn its production is regulated by the endothelium during contraction (22,23). In old rats, endothelial NO synthase (eNOS) expression in the thoracic duct was markedly decreased and the flow-dependent modulation of pumping was completely abolished, suggesting that aging may disturb NO-dependent regulatory mechanisms in lymphatic vessels (24).

In peripheral arteries, endothelial-dependent dilatation is impaired in postmenopausal women in part as a result of reduced eNOS expression and activation (25). In this study, the comparison between males and females suggested that aging appears to be a more important determinant of lymphatic pumping in females than in males. The chronological decrease of lymphatic pumping was more marked in females at postmenopausal ages suggesting that regulation of lymphatic pumping may also involve estrogen-NO dependent mechanisms. Lymphatic pumping failure may delay clearance of excessive fluid and perturb the balance of fluids/macromolecules. Thus, pumping failure may be associated with leg oedema in older adults, thereby affecting their life style. Moreover, leg lymphatic dysfunction may accompany other lymphatic pumping failure such as mesenteric lymphatics, thoracic ducts, and whole body lymphatics. In 1898, Sir William Osler, a legendary physician, stated that 'A man is as old as his arteries' (26). About 100 years have passed since he made this remark and considering the significance of increase in cardiovascular diseases in Western culture, we now really understand the significance of this concept. Considering the important functions of the lymphatics, such as body fluid regulation, macromolecular homeostasis, lipid absorption and immune function, we may safely say that a man is as old as his lymphatics.

In conclusion, using ICG fluorescence lymphography with a transparent sphygmomanometer cuff, we measured leg lymphatic pumping pressure in adult Japanese men and women. The results demonstrated a progressive decrease in the lymphatic pumping pressure with advancing age. The magnitude of this age-associated lymphatic pumping decline was most significant in women of postmenopausal age.

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