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Improvement in HDL cholesterol in postmenopausal women supplemented with pumpkin seed oil: pilot study

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Key words: PUMPKIN SEED OIL, PHYTOESTROGENS, MENOPAUSAL WOMEN, ESTROGEN, PLASMA LIPIDS

ABSTRACT

Objective Pumpkin seed oil is rich in phytoestrogens and animal studies suggest that there is some benefit to supplementation in low estrogen conditions. This study is the first to evaluate the benefit of pumpkin seed oil in postmenopausal women.

Methods This pilot study was randomized, double-blinded and placebo-controlled. Study participants included 35 women who had undergone natural menopause or had iatrogenically entered the climacteric due to surgery for benign pathology. Wheat germ oil (placebo; $n = 14$) and pumpkin seed oil ($n = 21$) were administered to eligible participants over a 12-week period at a dose of 2 g per day. Serum lipids, fasting plasma glucose and blood pressure were measured and an 18-point questionnaire regarding menopausal symptoms was administered; the atherogenic index was also calculated. Differences between groups, as well as before and after the period of supplementation, were evaluated with Student's *t*-test, Wilcoxon matched-pair signed-ranked test and Mann–Whitney test, as appropriate (Stata version 10.1).

Results Women receiving pumpkin seed oil showed a significant increase in high density lipoprotein cholesterol concentrations (0.92 ± 0.23 mmol/l vs. 1.07 ± 0.27 mmol/l; $p = 0.029$) and decrease in diastolic blood pressure (81.10 ± 7.94 mmHg vs. 75.67 ± 11.93 mmHg; $p < 0.046$). There was also a significant improvement in the menopausal symptom scores (18.1 ± 9.0 vs. 13.2 ± 6.7; $p < 0.030$), with a decrease in severity of hot flushes, less headaches and less joint pains being the main contributors. Women in the group receiving wheat germ oil reported being more depressed and having more unloved feeling.

Conclusion This pilot study showed pumpkin seed oil had some benefits for postmenopausal women and provided strong evidence to support further studies.

INTRODUCTION

Menopause is defined as the absence of menstrual periods for 12 months due to the cessation of ovulation. There is decrease in estrogen secretion, and the low availability of estrogen has been associated with negative changes in lipids, as well as an increased risk of hypertension and changes in glucose homeostasis[1–3].

There has been an increased interest in the estrogenic potential of phytoestrogens[4–8]. Phytoestrogens are naturally occurring compounds found in plants, including foods, and are functionally and structurally similar to 17β-estradiol or synthetic estrogens, such as diethylstilbestrol. They are able to bind to estrogen receptors, but are 100–1000 times weaker than estradiol. Soy and flaxseed are examples of rich sources of phytoestrogens and epidemiological observations[5,9], as well as placebo-controlled studies, have shown that both are useful in the management of menopause[10,11]. In one placebo-controlled study involving flaxseed supplements for 1 year in menopausal women, there was improvement in blood pressure and severity of hot flushes[10].

The seeds of the pumpkin plant *(Cucurbita species)* contain approximately 265 mg of phytoestrogen/100 g of seeds and the main phytoestrogen identified is secoisolariciresinol[12,13].

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Secoisolariciresinol is metabolized by gut bacteria to produce enterodiol and enterolactone, which are absorbed from the gastrointestinal tract and exert both estrogenic and antiestrogenic activities[14,15]. Secoisolariciresinol has also been shown to decrease serum cholesterol concentrations and provide cardioprotection through increasing angiogenesis and reducing apoptosis[16,17]. Previous studies conducted in our laboratory have shown significant benefits of pumpkin seed oil supplementation in a low-estrogen rat model; these include prevention of decreases in high density lipoprotein (HDL) cholesterol concentration and elevations in blood pressure[18]. It is therefore proposed that these benefits will be seen from supplementation in postmenopausal women and this paper presents the findings of a pilot study.

METHODS

The methods used in this study were reviewed and approved by the Faculty of Medical Sciences, University Hospital of the West Indies Ethic Committee. Postmenopausal women were recruited using flyers and posters (displayed on notice boards), with the assistance of a gynecologist assigned to the study. Places involved in the recruitment included the University Hospital of the West Indies, the Health Center at the University of the West Indies Mona Campus and various meeting opportunities, such as health fairs, women expositions and the Menopause Society meetings. All interviews with potential recruits took place at the Diagnostic Unit and the Gynecology Clinic of the University Hospital of the West Indies, and at the Department of Community Health Clinic at the University of the West Indies, Mona Campus.

Inclusion and exclusion criteria

Women were eligible if they were postmenopausal, that is, being amenorrheic for at least 12 months or having had a bilateral oophorectomy at least 3 months prior to the recruitment. All participants were eligible if their systolic blood pressure was $\leq$140 mmHg and their diastolic blood pressure $\leq$90 mmHg (below the hypertensive level), their body mass index was in the range 18–35 kg/m^2 and they were not on any prescribed medication. After being deemed eligible to participate, the women were given a consent form containing information about the study. The details of the consent form were discussed with each potential participant and they were then given time to consider being part of the study. Willing volunteers were required to sign the consent form before joining the study.

Supplement source

Pumpkin seed oil (PSO) capsules and wheat germ oil (WGO) capsules containing 1 g of oil were purchased from Soft-gel Technologies (USA). Pumpkin seed oil from Soft-gel Technologies is extracted from *Cucurbita pepo* L. seeds. Capsules of PSO and WGO were similar in size but of different colors. For distribution to patients, capsules were packaged in white bottles and the bottles placed in sealed brown paper bags.

Randomization and blinding

Eligible, consented research subjects were randomly assigned to the groups receiving PSO or WGO using a computer-generated random table. Supplement distribution was made by the principal investigator, who had no contact with the participants. The study was double-blind as neither the subjects nor anyone having direct contact with them had knowledge of the group assignments. The capsules were distributed to each research subject (using a table of random numbers) during the first visit.

Procedure

Demographic data and information on any dietary supplements used were collected from each subject at the start of the study. A food frequency questionnaire (FFQ) was created using foods rich in phytoestrogens that are commonly consumed by Jamaicans. This FFQ was constructed using information gathered from various databases indicating the phytoestrogen content of foods. The food items on the FFQ were chosen because, after reviewing the information gathered from various journals, these food items were rich in phytoestrogens[19–21]. On the first visit, each research subject completed the FFQ using standard measuring cups as a guide to the quantities of the foods consumed over the past week. The approximate range of phytoestrogen content in each food item, along with the information gathered using the FFQ, were then used to calculate the approximate phytoestrogen content for each research subject. This information was then used to give an estimate of the dietary phytoestrogen content of each group.

Blood pressure was measured prior to supplementation and at the end of the supplementation period using a Datascope Accutor electronic sphygmomanometer. Body weight and height were measured using a physician beam scale with a stadiometer attached. Body weight was also measured at the end of the supplementation period. Each subject received a bottle containing 168 capsules and was asked to take two capsules (2 g or approximately 40 mg/kg body weight) daily for 12 weeks. All measurements were made by one physician and a research assistant assigned to the study.

Subjects were asked to fast overnight (12 h) for blood sample collection prior to supplementation and at the end of the supplementation period. A venous blood sample was taken from each research subject by the physician assigned to the study prior to starting oil consumption and at the end. The sample from each subject was collected in two BD® Vacutainer tubes, one potassium oxalate/sodium fluoride-coated and one silicone-coated. Each sample was sent to the

Chemical Pathology Laboratory at the University Hospital of the West Indies and centrifuged, using a Thermo Scientific Cl40 centrifuge at 3500 rpm. Serum was removed from the silicone-coated tubes and stored at −20°C for lipid assays. Plasma was removed from the sodium fluoride tubes and stored at 20°C for the glucose assay.

Supplementation compliance assessment was made at the final visit. Subjects returned the bottles containing the capsules and the remaining capsules were counted to measure compliance, as was done by Deutch and colleagues[22].

Unexpected or unsafe reactions to the use of the supplements were reported using an adverse event reporting form.

Biochemical analysis

Biochemical assays on the serum were performed with a multichannel Abbott Spectrum autoanalyzer (Abbott Laboratories, Abbott Park, Chicago, IL, USA). All assays were performed in the Chemical Pathology Laboratory at the University Hospital of the West Indies with the assistance of a medical technologist. Parameters that were determined included: fasting plasma glucose, total cholesterol, triglycerides, HDL cholesterol and low density lipoprotein (LDL) cholesterol. Glucose concentration was measured using the glucose oxidase colorimetric test. Glucose oxidase catalyzes the oxidation of glucose to gluconic acid. The generation of hydrogen peroxide is indirectly measured by oxidation of *o*-dianisidine in the presence of peroxidase[23]. Total cholesterol was determined by an enzymatic method. The cholesterol esters are hydrolyzed to free cholesterol by cholesterol esterase. The free cholesterol is then oxidized by cholesterol oxidase to cholesten-3-one, with the simultaneous production of hydrogen peroxide. The hydrogen peroxide produced couples with 4-aminoantipyrine and phenol, in the presence of peroxidase, to yield a chromogen with maximum absorbance at 505 nm[24]. HDL cholesterol was measured by an enzymatic method on the supernatant obtained after selective precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid, in the presence of magnesium ions and centrifugation[25]. Serum LDL cholesterol was calculated according to the procedures of Friedewald and colleagues [LDL cholesterol = (total cholesterol – HDL cholesterol – triglycerides)/2.2 (mmol/l)][26]. Triglycerides were determined by an analytical method based on the sequence of reaction described by Fossati and Prencipe[27]. In this direct colorimetric procedure, serum triglycerides are hydrolyzed by lipase, and the released glycerol is assayed in a reaction catalyzed by glycerol kinase and L-alpha-glycerol-phosphate oxidase in a system that generates hydrogen peroxide. The hydrogen peroxide is monitored in the presence of horseradish peroxidase with 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone as the chromogenic system. The absorbance of this chromogen system is measured at 510 nm[27,28]. The methods adopted by the automated instrument for the determinations of the above parameters are according to the manufacturer's instruction (Abbott Laboratories).

Atherogenic index

The atherogenic index was calculated by using equations previously described[29–31] [(total cholesterol – HDL cholesterol)/HDL cholesterol].

Menopausal symptom scores

Menopausal symptom scores were adapted from those of Jones and colleagues[32] and have been used in other studies[33,34]. This score sheet involved an 18-point menopausal symptom assessment: hot flushes, light headaches, headaches, depression, irritability, unloved feelings, anxious feelings, anxious sleeplessness, unusual tiredness, backache, joint pains, muscle pains, new facial hairs, usual dry skin, less sex drive, less sexual feelings, dry vagina and dyspareunia (painful intercourse), using a four-point scale (0 = absent; 1 = mild; 2 = moderate; 3 = severe) for each item. Administration of the assessment was made by a physician conducting the interview, who was blinded to the supplement given to each subject.

Data analysis

Except for the individual components of menopausal symptom scores, where median and interquartile range were used, all other data were expressed as mean ± standard deviation (SD). Differences in serum lipids, atherogenic index, fasting glucose, blood pressure, body mass index and phytoestrogen intake were compared using Student's *t*-test. The total for the menopausal symptom scores was obtained by adding each component. Student's *t*-test was also used to compare baseline menopausal symptom scores with the 12-week score, and further comparison of the individual components was made using the Wilcoxon matched-pair signed-ranked test. The Mann–Whitney test was used for between-group comparisons of individual scores. Compliance data were compared using Fisher's exact test. A value of $p < 0.05$ was considered significant. All statistical analysis was performed using Stata version 10.1.

RESULTS

This study was conducted over a 14-month period, December 2008–February 2010, and involved screening approximately 100 participants; 65 postmenopausal women expressed interest in the study. After exclusion, based on elevated blood pressure (systolic > 140 mmHg or diastolic > 90 mmHg), body mass index range requirement (>18 kg/m^2 <35 kg/m^2) and not on any prescribed medication, only 35 women were eligible for the study. On randomization, 21 women were assigned to the group receiving PSO and 14 to the group receiving WGO. The ages of the participants ranged between 46 and 75 years (mean age ± SD: PSO group 54.78 ± 4.44 years, WGO group 59.67 ± 10.77 years), with no significant difference between the groups. Most of the subjects were

of African descent (20 in the PSO group and 11 in the WGO group), with the rest being Indian (two in the WGO group), Caucasian (one in the PSO group) and Chinese (one in the WGO group). These women had been menopausal for at least 1 year or had surgically induced climacteric symptoms at least 3 months prior (ten women).

At baseline (recruitment), there were no significant differences in the body mass index, blood pressure, and concentrations of fasting blood glucose, total cholesterol and triglyceride between the groups. Further analysis of the lipids also showed no differences in the HDL cholesterol or LDL cholesterol levels at baseline. There was no significant difference in the total menopausal symptom score between groups (Table 1).

At the end of the supplementation period, women in the PSO group showed a 16% significant increase in HDL cholesterol ($p = 0.029$), while the WGO group had a borderline increase of 12% ($p = 0.077$). There was no significant change in concentrations of total cholesterol, LDL cholesterol or triglycerides for either the PSO or the WGO group; however, as components of the atherogenic index, over the 12 weeks a significant improvement in the atherogenic index for both groups ($p = 0.019$ for the PSO group and 0.039 for the WGO group) was observed.

For the blood pressure at the end of the supplementation period, there was a significant decrease (7%) in diastolic blood pressure in the PSO group ($p = 0.046$), but there was no significant change in systolic blood pressure; both components remained unchanged in the WGO group.

On assessment of the total menopausal symptom scores, the PSO group ($n = 18$) showed a significantly less total severity of symptom score ($p = 0.038$), but this was not apparent for the WGO group ($n = 13$) (Table 2).

Between-group comparison of the individual items of the menopausal symptom score at baseline showed no difference for any of the individual scores. Further analysis showed that, for 13 of the 18 items, there was no significant difference when comparison was made within groups. Of the five other symptoms evaluated (Table 3), between baseline and 12-week supplementation, the women in the PSO group reported significantly less severe hot flushes ($p = 0.049$), headaches ($p = 0.026$) and joint pains ($p = 0.030$), while those in the WGO group reported being significantly more depressed ($p = 0.009$) and having more unloved feelings ($p = 0.026$).

A food frequency questionnaire was administered to each research subject and the phytoestrogen content of each food was found by looking at various literature sources. The weekly phytoestrogen intake for each research subject was estimated at baseline. For the PSO group, the mean phytoestrogen consumed was 9.38 mg/week (95% confidence interval (CI) 0.86–17.90 mg/week) and for the WGO group it was 4.00 mg/week (95% CI 0.54–7.47 mg/week). There was no significant difference in mean estimated phytoestrogen intake when the PSO group was compared with the WGO group.

Using the pill count method, there was 88% compliance in the PSO group and 89% compliance in the WGO group, with no significant difference in compliance between the groups.

DISCUSSION

With advancing age, women become more predisposed to developing negative physiological and biochemical changes which have been associated with the reduced availability of estrogens, including changes in glucose homeostasis, plasma lipids and blood pressure[1]. Current trends to prevent and relieve these complications related to estrogen deficiency have included the evaluation of phytoestrogen-rich dietary supplements[6]. In a review by Adolphe and colleagues[35], the benefits of the flaxseed, a dietary supplement rich in secosolariciresinol, were identified to include lowering fasting glucose, improving plasma cholesterol and lowering blood pressure. This is the first study to report the possible benefits of PSO supplementation, another

Table 1 Comparison of anthropometric measurements, serum lipids and blood pressure for 35 women at baseline and between arms at baseline

	Baseline				
	WGO group		*PSO group*		
Measures	*Mean ± SD*	*n*	*Mean ± SD*	*n*	*p Value (Student's t-test)*
Weight (kg)	71.28 ± 11.64	14	72.87 ± 11.36	21	0.691
Body mass index (kg/m^2)	27.59 ± 3.15	14	28.74 ± 3.16	21	0.707
Systolic blood pressure (mmHg)	127.29 ± 11.80	14	125.52 ± 10.90	21	0.653
Diastolic blood pressure (mmHg)	77.21 ± 7.64	14	80.48 ± 7.01	21	0.202
Fasting glucose (mmol/l)†	5.28 ± 0.58	10	4.79 ± 0.87	17	0.130
Total cholesterol (mmol/l)†	5.68 ± 1.24	9	5.65 ± 0.84	15	0.954
Triglycerides (mmol/l)†	1.31 ± 0.43	9	1.35 ± 0.65	15	0.976
HDL cholesterol (mmol/l)†	1.06 ± 0.17	9	0.91 ± 0.23	14	0.114
LDL cholesterol (mmol/l)†	4.02 ± 1.04	9	4.12 ± 0.66	14	0.764
Atherogenic index†	4.44 ± 1.33	9	5.56 ± 1.85	14	0.132
Total menopausal scale score	14.9 ± 6.7	13	18.1 ± 9.0	18	0.282

WGO, wheat germ oil; PSO, pumpkin seed oil; HDL, high density lipoprotein; LDL, low density lipoprotein
†, Data missing for some women because of hemolysis of blood samples

Table 2 Comparison of anthropometric measurements, serum lipids level and blood pressure (BP) from baseline to week 12 by arm for 35 women and between arms. Data are given as mean ± standard deviation

					p Values	
	n	*Baseline*	*12 weeks*	*Differences*	*Within groups*	*Between groups (at 12 weeks)*
Weight (kg)						
WGO	14	71.28 ± 11.64	71.76 ± 12.29	−0.48 ± 2.45	0.477	0.919
PSO	21	72.87 ± 11.36	72.96 ± 11.71	−0.09 ± 3.12	0.568	
Body mass index (kg/m^2)						
WGO	14	27.59 ± 3.15	27.74 ± 3.16	−0.15 ± 0.95	0.574	0.483
PSO	21	28.05 ± 3.62	28.23 ± 4.31	−0.18 ± 1.37	0.555	
Systolic BP (mmHg)						
WGO	14	127.29 ± 11.80	126.29 ± 15.64	1.00 ± 15.15	0.809	0.476
PSO	21	126.62 ± 9.95	122.57 ± 13.61	2.05 ± 11.67	0.431	
Diastolic BP (mmHg)						
WGO	14	77.21 ± 7.64	74.00 ± 7.95	3.21 ± 9.76	0.240	0.623
PSO	21	81.10 ± 7.94	75.67 ± 11.93	5.43 ± 11.71	0.046*	
Fasting glucose (mmol/l)†						
WGO	8	5.25 ± 0.65	5.48 ± 1.04	−0.23 ± 1.06	0.567	0.094
PSO	16	4.90 ± 0.83	4.73 ± 0.57	0.17 ± 0.91	0.471	
Total cholesterol (mmol/l)†						
WGO	9	5.68 ± 1.24	5.56 ± 1.15	0.12 ± 0.81	0.662	0.992
PSO	17	5.59 ± 0.86	5.55 ± 1.06	0.04 ± 1.03	0.871	
Triglycerides (mmol/l)†						
WGO	9	1.31 ± 0.43	1.44 ± 0.44	−0.13 ± 0.29	0.215	0.515
PSO	16	1.37 ± 0.64	1.35 ± 0.48	0.02 ± 0.54	0.881	
HDL cholesterol (mmol/l)†						
WGO	9	1.0 ± 0.17	1.19 ± 0.19	−0.13 ± 0.19	0.077	0.361
PSO	15	0.92 ± 0.23	1.07 ± 0.27	−0.15 ± 0.23	0.029*	
LDL cholesterol (mmol/l)†						
WGO	9	4.02 ± 1.04	3.70 ± 1.04	0.31 ± 0.77	0.257	0.609
PSO	14	4.12 ± 0.66	3.88 ± 1.00	0.25 ± 0.88	0.312	
Atherogenic index†						
WGO	9	4.44 ± 1.33	3.79 ± 1.33	0.65 ± 0.79	0.039*	0.417
PSO	14	5.56 ± 1.85	4.47 ± 1.99	1.09 ± 1.51	0.019*	
Total severity of menopausal symptom score (18 items)						
WGO	13	14.9 ± 6.7	17.1 ± 7.4	−2.2 ± 6.0	0.217	0.142
PSO	18	18.1 ± 9.0	13.2 ± 6.7	4.9 ± 9.3	0.038	

WGO, wheat germ oil; PSO, pumpkin seed oil; HDL, high density lipoprotein; LDL, low density lipoprotein
†, Data missing for some women because of hemolysis of blood samples; *, significant $p < 0.05$ when compared to baseline

dietary supplement rich in secosolariciresinol, in postmenopausal women. The study supplemented postmenopausal women with PSO for 12 weeks and assessed changes in concentrations of fasting plasma glucose and serum lipids, as well as blood pressure changes and menopausal symptom scores.

The most significant finding was an increase in HDL cholesterol, a benefit that is well established to lower the risk of cardiovascular complications[36]. While other lipid components did not change significantly, there was an overall improvement in the atherogenic index in these women. The atherogenic index is positively correlated to risk of atheroma development[29–31], thus providing further support to the cardiovascular benefits of supplementation with PSO.Therefore, the overall benefit of the changes seen with PSO supplementation would suggest that cardiovascular benefits can be obtained. The women receiving PSO also showed a significant decrease in diastolic blood pressure, thus further supporting the cardiovascular benefits of this rich phytoestrogen source.

Assessment of the menopausal symptom scores also supported the potential benefit of PSO supplementation, as there was a significant reduction in the total scores. This improvement was associated with reduced severity of vasomotor disturbances (hot flushes and headaches) and joint pains.

Our study did not find any effect of PSO supplementation on fasting glucose concentrations in the postmenopausal state; however, all the women in the study had normal fasting blood glucose concentration and therefore investigations in women with an elevated fasting glucose state require further study.

Table 3 Median (interquartile range, IQR) for severity of menopausal symptom score (range 0–3). Score for each item ranged from 0 for absent to 3 for severe. Of the 18 items, 13 showed no change from baseline

	Median (IQR)		*p Value*	
	Baseline	*12 weeks*	*Within group (Wilcoxon signed rank)*	*Between group at 12 weeks (Mann–Whitney)*
Hot flushes				
WGO	1.5 (1–2)	2 (1–2)	0.822	0.470
PSO	2 (1–2)	1 (1–2)	0.049*	
Headaches				
WGO	0 (0–2)	0 (0–1)	0.511	0.685
PSO	1 (0–2)	0 (0–1)	0.026*	
Depression				
WGO	0 (0–0)	1 (0–2)	0.009*	0.046†
PSO	0 (0–1)	0 (0–1)	0.364	
Unloved feelings				
WGO	0 (0–0)	1 (0–2)	0.026*	0.042†
PSO	0 (0–1)	0 (0–0)	0.089	
Joint pain				
WGO	1.5 (0–2)	1 (0–2)	0.801	0.663
PSO	2 (1–2)	1 (1–2)	0.030*	

WGO, wheat germ oil; PSO, pumpkin seed oil
†, Significant $p < 0.05$ between groups at 12 weeks; *, significant $p < 0.05$ when compared to baseline

Although wheat germ has constituents which can also provide cardiovascular benefits, it has been used as a suitable placebo in other studies involving phytoestrogen supplementation in postmenopausal women[10,37]. In the study by Dodin and colleagues[10] with wheat germ as placebo at 40 g/day for 1 year, no benefit was seen on serum lipids, as the HDL cholesterol concentration remained the same and there was some indication of increased total cholesterol and LDL cholesterol. It was on this basis that WGO was accepted as a suitable placebo for this study. The borderline increase in HDL cholesterol for the placebo group, as well as the significant decrease in the atherogenic index were unexpected and require further investigation, especially given the limited number of subjects. While there was this trend with serum lipids, the women receiving WGO supplement did not report any improvement in menopausal symptom scores and actually reported more emotional disturbances when compared with their score at baseline.

The limitations of this study included the small number of subjects involved and the inability to assay a number of blood samples for lipids and glucose because of hemolysis. The inability to assay blood levels of estrogen and other relevant hormonal factors, as well as the expected metabolites from phytoestrogen intake, such as enterodiol and enterolactone, prevented any mechanistic discussion. Additionally, the ability of WGO to influence some of the factors mentioned was a limitation.

The data from this pilot study do, however, suggest that pumpkin seed oil given to menopausal women has some benefit in reducing the cardiovascular complications associated with lack of estrogen and do warrant the need for further studies with a larger numbers of subjects and attempts to evaluate the mechanisms.

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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