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Test meals rich in marine long-chain n-3 polyunsaturated fatty acids increase postprandial chylomicron response

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ABSTRACT

Postprandial lipid abnormalities are considered an independent cardiovascular risk factor. Hence, it is important to find nutritional strategies that are able to positively influence these abnormalities. Since the effect of n-3 polyunsaturated fatty acids (PUFA) and polyphenols on postprandial lipids in humans is still under debate, we evaluated the acute response of triglyceride-rich lipoproteins to test meals that are naturally rich in polyphenols and/or marine long-chain (LC) n-3 PUFAs. We hypothesized that LC n-3 PUFA would have a different effect on chylomicron and very low density lipoproteins when compared with polyphenols or their combination. We randomly assigned 78 individuals who were at high cardiometabolic risk to 4 isoenergetic diets. These diets only differed in amount of LC n-3 PUFA and/or polyphenols. Prior to starting the intervention, each subject underwent a test meal similar to the type of diet assigned: low in LC n-3 PUFA and polyphenols (control), rich in LC n-3 PUFA and low in polyphenols, rich in polyphenols and low in LC n-3 PUFA, or rich in both. Blood samples were taken before and up to 6 hours after the test meal in order to evaluate cholesterol and triglycerides (plasma and triglyceride-rich lipoprotein), apolipoprotein B-48 (large very low density lipoprotein), glucagon-like peptide-1, and free fatty acid plasma levels. The levels of chylomicron cholesterol and triglyceride in response to the test meal rich in LC n-3 PUFA were significantly higher than after the control meal ($P = .037$ and $P = .018$); there was no difference in the other variables. In conclusion, this study indicates that acute administration of marine LC n-3 PUFA increases postprandial chylomicron response in contrast with their lowering chronic effects. These differences underline the importance of understanding the acute and chronic effects of nutritional, as well as of other types of, interventions.

1. Introduction

Postprandial abnormalities of lipid metabolism are actually considered an independent risk factor for cardiovascular diseases [1,2]. For this reason, it is important to identify possible strategies, based on nutrition factors, which are able to positively influence the postprandial state. Among dietary factors, n-3 fatty acids and polyphenols have been investigated

Abbreviations: BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acid; GLP-1, glucagon-like peptide-1; IAUC, incremental postprandial area under the curve; LC n-3 PUFA, long-chain n-3 polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; VLDL, very low density lipoprotein.

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in both studies of medium-term duration and in acute settings. Despite this research, many points remain controversial and unclear. In regard to n-3 fatty acids and lipid metabolism, medium- to long-term studies indicate a reduction in fasting triglyceride levels, especially when long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs) are given in high amounts. There is also some improvement in postprandial triglyceride metabolism, particularly for exogenous triglyceride-rich lipoproteins [3–6]. In contrast, in some acute studies, a higher chylomicron response was evident in the first part of the postprandial curve after a test meal rich in LC n-3 PUFA, when compared with test meals rich in saturated fatty acid (SFA), monosaturated fatty acid (MUFA), and n-6 PUFA [7].

Recently, there has also been attention paid to the possible effects of polyphenols on postprandial lipid metabolism [8]. The results from human studies are very heterogeneous, and investigations have only focused on the effects of single polyphenols and fasting lipid levels, especially in acute settings [9,10]. Furthermore, the acute effects of the combinations of these 2 dietary components are not known, whereas the chronic effects on postprandial lipid metabolism have been recently published by our group [4].

Therefore, we hypothesized that test meals rich in LC n-3 PUFA may have different effects on lipoprotein subfractions in the postprandial state when compared with test meals rich in polyphenols, which may have positive effects on postprandial lipid response in acute settings. To test our hypothesis, we evaluated the response of chylomicrons and large very low density lipoprotein (VLDL) in subjects given test meals naturally rich in different polyphenols and marine LC n-3 PUFA.

2. Methods and materials

2.1. Subjects

Among patients referred to the obesity outpatient clinic at Federico II University Hospital in Naples, Italy, we recruited 78 individuals (male and female) between 35 and 70 years of age who had a body mass index (BMI) of 27 to 35 kg/m^2 and a waist circumference of more than 102 cm (men) or more than 88 cm (women). Health status and medical history were examined via interview, clinical examination, and routine laboratory tests, whereas glucose tolerance was evaluated by a 75-g oral glucose tolerance test. In addition to high BMI and waist circumference, subjects had to meet at least one more criterion that identified the metabolic syndrome (Adult Treatment Panel-III). Exclusion criteria included the following: cardiovascular events (myocardial infarction or stroke) in the 6 months prior to enrollment, diabetes mellitus, fasting plasma triglycerides ≥400 mg/dL or cholesterol >270 mg/dL, regular intensive physical activity, renal failure (serum creatinine >1.7 mg/dL), liver abnormalities (transaminases twice the normal values), anemia (hemoglobin level <12 g/dL), any other chronic disease, and/or use of drugs that could influence glucose and lipid metabolism. These patients also participated in an intervention study following a protocol approved by the Federico II University Ethics Committee and registered at ClinicalTrials.gov (#NCT01154478), whose details have been already published [4]. After giving their written informed consent, participants were randomly assigned to 1 of 4 nutritional interventions.

In this article, we reported the baseline data before starting the intervention. After a 12-hour overnight fast, the participants consumed a 1000-kcal test meal that was rich in fat and similar to the diets to which they were assigned: (*a*) control test meal low in LC n-3 PUFA (total LC n-3 PUFA = 0.94 g; linolenic acid = 0.89 g; eicosapentaenoic acid [EPA] = 0.05 g; docosahexaenoic acid [DHA] = 0 g) and low in polyphenols (total polyphenols = 50 mg), (*b*) test meal rich in LC n-3 PUFA (total LC n-3 PUFA = 2.31 g; linolenic acid = 0.84 g; EPA = 0.63 g; DHA = 0.83 g) and low in polyphenols (total polyphenols = 50 mg), (*c*) test meal rich in polyphenols (total polyphenols = 770 mg) and low in LC n-3 PUFA (total LC n-3 PUFA = 0.92 g; linolenic acid = 0.87 g; EPA = 0.05 g; DHA = 0 g), or (*d*) test meal rich in LC n-3 PUFA (total LC n-3 PUFA = 2.31 g; linolenic acid = 0.84 g; EPA = 0.63 g; DHA = 0.83 g) and rich in polyphenols (total polyphenols = 770 mg). All of the other components of the test meals were similar (Table 1). The test meal consisted of rice, butter, Parmesan cheese, bresaola, and white bread, with the addition of olive oil, extravirgin olive oil, salmon, and decaffeinated green tea in order to obtain a similar composition to the assigned diet. The test meal composition was taken from the tables of the Italian National Research Institute for Food and Nutrition [11], whereas polyphenol content was calculated according to US Department of Agriculture tables [12]. The polyphenol content in tea was measured directly [13]. Before the meal and then 2, 4, and 6 hours afterward, blood samples were taken to analyze cholesterol and triglyceride concentrations in plasma and triglyceride-rich lipoproteins (chylomicrons and large VLDL). Apolipoprotein B-48 was measured in large VLDL at fasting and at 4 and 6 hours after the test meal. Plasma glucagon-like peptide-1 (GLP-1) levels were measured at 0, 30, 60, 90, 120, and 180 minutes after the

Table 1 – Composition of the test meals given to participants

	Control	High LC n-3 PUFA	High polyphenols	High LC n-3 PUFA and high polyphenols
Energy (kcal)	998	994	998	999
Protein (%)	12	12	12	12
Fat (%)	57	57	57	57
SFA (%)	31	31	31	31
MUFA (%)	19	17	19	17
Cholesterol (mg)	201	193	199	193
Carbohydrates (%)	31	31	31	31
Sugars (%)	0.6	0.8	0.6	0.8
Vitamin E (mg)	274	286	297	286
Total LC n-3 PUFA (g)	0.94	2.31	0.92	2.31
Linolenic acid (g)	0.89	0.84	0.87	0.84
EPA (g)	0.05	0.63	0.05	0.63
DHA (g)	0	0.83	0	0.83
Polyphenols (mg)	50	50	770	770

test meal, and free fatty acid (FFA) levels were taken at 0, 60, 120, 240, and 360 minutes.

2.2. Biochemical measurements

Chylomicrons (Sveldberg flotation unit, Sf >400) and large VLDL (Sf 60-400) were isolated from plasma by discontinuous density gradient ultracentrifugation, as previously described [14]. Cholesterol, triglyceride (Roche Molecular Biochemicals, Mannheim, Germany), and FFA (Wako Chemicals GmbH, Neuss, Germany) concentrations were assayed by enzymatic-colorimetric methods on a Cobas Mira autoanalyzer (ABX Diagnostics, Montpellier, France). Apolipoprotein B-48 was determined by enzyme-linked immunosorbant assay methods (Shibayagi Co Ltd, Shibukawa, Gunma, Japan) on a TriturusAnalyzer (Grifols, SA, Barcelona, Spain) [15]. Active GLP-1 was assayed by a nonradioactive, highly specific sandwich enzyme-linked immunosorbant assay method (Merck-Millipore, Darmstadt, Germany) that had 100% cross-reactivity with active isoforms of GLP-1 (7-36 amide and 7-37 glycine extended) but without reactivity with inactive isoforms (9-36 amide and 9-37 glycine extended), GLP-2, or glucagon, as described by Di Marino et al [16].

2.3. Statistical analyses

Data are expressed as means ± SD, unless otherwise stated. Variables not normally distributed were analyzed after logarithmic transformation. Postprandial incremental areas under the curve (IAUCs) were calculated by the trapezoidal method. Differences between the different test meals and the control meal were evaluated by the Dunnett *t* test that adjusts for multiple comparisons. Time course effect on all variables was analyzed by general linear model for repeated-measures analysis. The level of statistical significance was $P < .05$ (2 tailed). Statistical analyses were performed according to standard methods, using the Statistical Package for Social Sciences software (SPSS/PC; SPSS, Chicago, IL, USA). In order to detect a 30% difference between test meals in the total AUCs of triglyceride concentrations in chylomicron and VLDL fractions after a fat-rich meal, with an 80% power at 5% significance level, 80 patients had to be studied. This degree of change after treatment is clinically based, corresponding to the differences observed between patients with type 2 diabetes and healthy controls in a previous study [17].

3. Results

The 4 groups were comparable for age, body weight, BMI, waist circumference, and fasting levels of plasma cholesterols and triglycerides (Table 2). Chylomicron cholesterol and triglyceride response to the test meal rich in LC n-3 PUFA was significantly higher compared with the control meal ($P = .037$ and $P = .018$, respectively, by repeated-measures analysis of variance) (Fig.1).

The increase in chylomicron lipid response after the LC n-3 PUFA meal was particularly evident in the second part of the curve. In fact, the chylomicron cholesterol and triglycerides IAUC was higher than the IAUC after the control meal, when calculated from 240 to 360 minutes (cholesterol IAUC 763 ± 136 vs 404 ± 70 mg/dL at 120 minutes, $P = .072$; triglycerides IAUC 20 376 ± 3581 vs 10 371 ± 1455 mg/dL at 120 minutes, $P = .036$, LC n-3 PUFA vs control) but was not different when determined at 360 minutes. No significant differences in lipids levels were observed after the other test meals. Cholesterol and triglycerides of large VLDL did not change after any of the meals (Fig. 1). Polyphenols seemed to counterbalance the effects of LC n-3 PUFA: in fact, the lipid response after the test meal rich in LC n-3 PUFA and polyphenols was superimposable to that obtained after the control meal (Fig. 1). Apolipoprotein B-48 showed the same trend (a greater increase after the test meal rich in LC n-3 PUFA), although the difference was not statistically significant (Fig. 2). The LC n-3 PUFA–rich meal also induced some differences in GLP-1 response; GLP-1 tended to decrease at 30 minutes after the test meal compared with the control meal (5.2 ± 2.6 vs 5.7 ± 2.1 pmol/L, LC n-3 PUFA vs control, $P = .581$) (Fig. 3). The IAUC of GLP-1 (303 ± 310 vs 391 ± 385 pmol/L · 180 minutes, LC n-3 PUFA vs control, $P = .408$) also tended to be lower after the LC n-3 PUFA–rich meal. No significant differences were observed for FFA response (Fig. 3).

4. Discussion

In an acute setting, the main results of this study indicate that (1) the response to a high-fat test meal rich in LC n-3 PUFA is

Table 2 – Clinical characteristics and fasting biochemical parameters of the participants

	Control	High LC n-3 PUFA	High polyphenols	High LC n-3 PUFA and high polyphenols
Sex (male/female)	8/12	8/11	9/11	8/11
Age (y)	54 ± 9	56 ± 8	53 ± 9	55 ± 9
BMI (kg/m^2)	33 ± 3	32 ± 4	32 ± 3	30 ± 3
Waist circumference (cm)	104 ± 7	105 ± 10	104 ± 9	101 ± 8
Fasting plasma triglycerides (mg/dL)	120 ± 47	138 ± 68	120 ± 59	125 ± 78
Fasting plasma cholesterol (mg/dL)	194 ± 38	191 ± 26	194 ± 34	193 ± 27
Fasting plasma glucose (mg/dL)	106 ± 12	104 ± 14	100 ± 10	102 ± 14

Data are expressed as means ± SD.

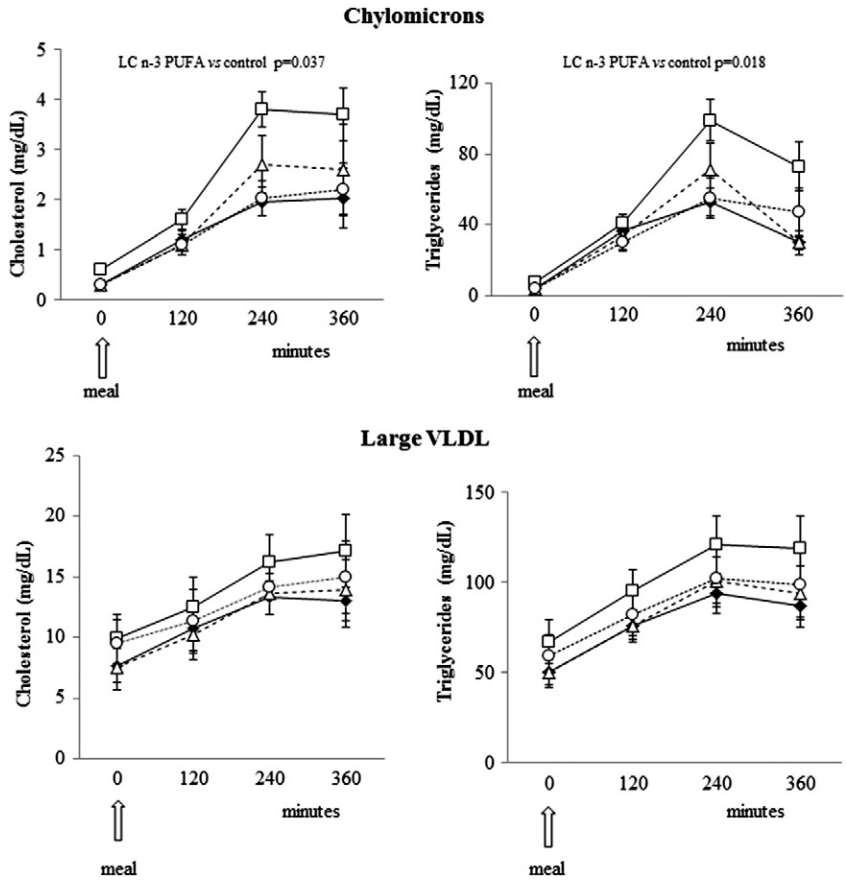

Fig. 1 – Chylomicrons and large VLDL at fasting and after the 4 test meals: (*a*) control (black diamond), (*b*) rich in LC n-3 PUFA (white square), (*c*) rich in polyphenols (white triangle), and (*d*) rich in LC n-3 PUFA and polyphenols (white circle). Data are expressed as mean ± SEM. General linear model for repeated-measures, post hoc analysis (Dunnett *t* test) showed a significant meal effect for cholesterol (*P* = .037) and for triglycerides (*P* = .018) of chylomicron, LC n-3 PUFA vs control meal.

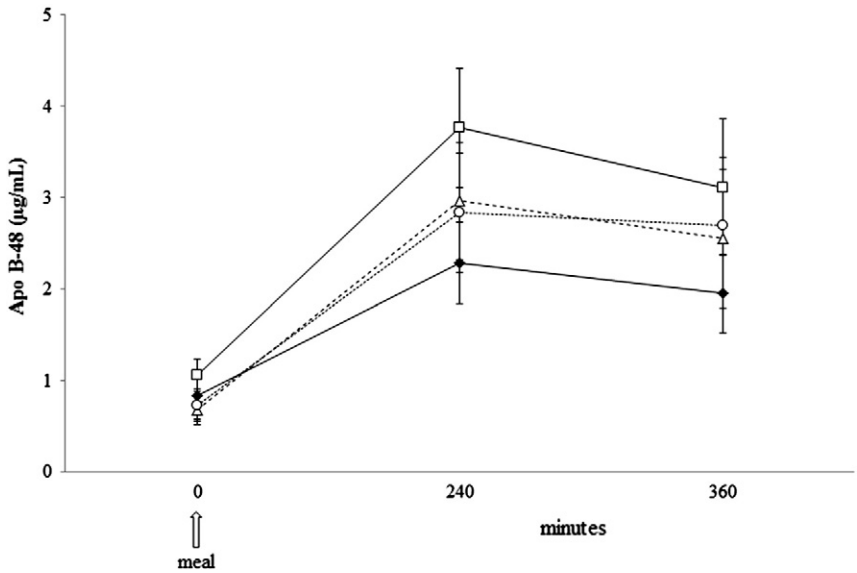

Fig. 2 – Apolipoprotein B-48 in large VLDL after the 4 test meals: (*a*) control (black diamond), (*b*) rich in LC n-3 PUFA (white square), (*c*) rich in polyphenols (white triangle), and (*d*) rich in LC n-3 PUFA and polyphenols (white circle). Data are expressed as mean ± SEM.

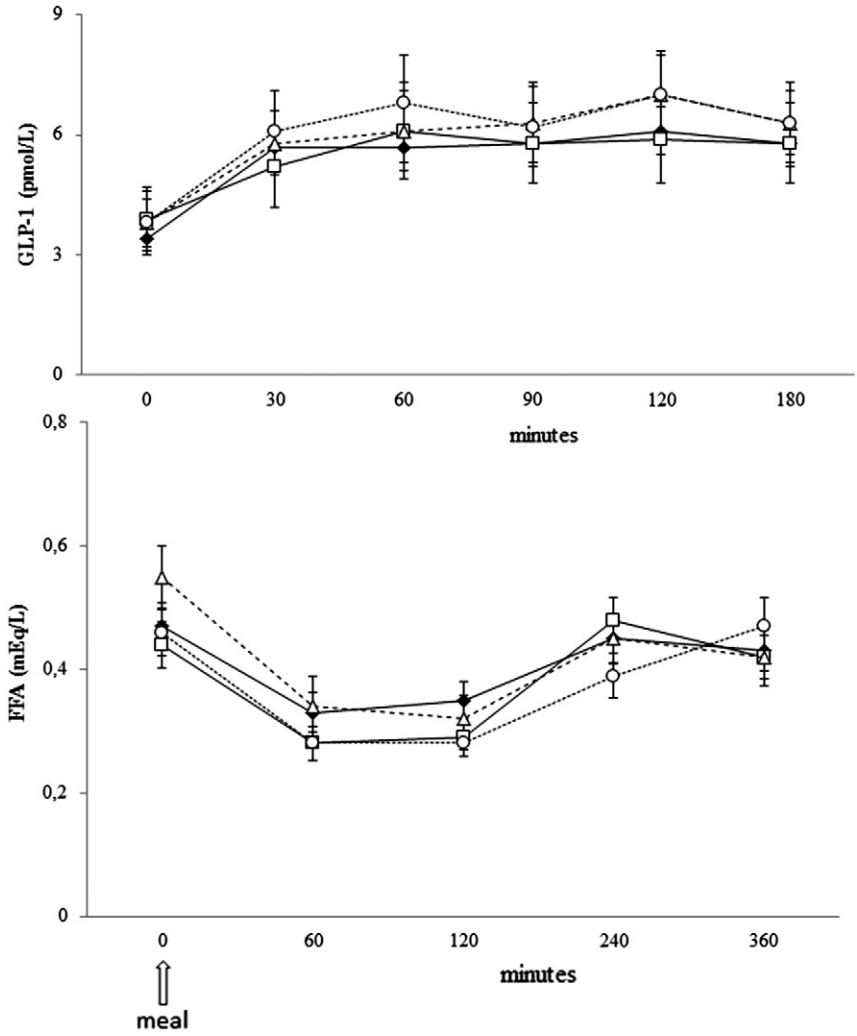

Fig. 3 – Plasma GLP-1 and FFA concentrations after the 4 test meals: (*a*) control (black diamond), (*b*) rich in LC n-3 PUFA (white square), (*c*) rich in polyphenols (white triangle), and (*d*) rich in LC n-3 PUFA and polyphenols (white circle). Data are expressed as mean ± SEM.

characterized by an increase in the cholesterol and triglyceride content of chylomicrons compared with a control test meal; (2) a test meal rich in polyphenols does not influence the lipid response; (3) polyphenols seem to counterbalance the acute effects of LC n-3 PUFA on chylomicron response; and (4) after the test meal with LC n-3 PUFA, there is a tendency toward a lower GLP-1 response in the earlier phase of the postprandial period. The results of this study confirm our hypothesis regarding LC n-3 PUFA but not for polyphenols.

The effects of LC n-3 PUFA on chylomicrons are somewhat surprising, especially in relation to the results achieved after medium-term interventions with LC n-3 PUFA that specifically show a decrease in postprandial chylomicron response through both functional foods enriched with n-3 and foods naturally rich in marine LC n-3 PUFA [4,5]. However, considering the acute experiments, our results are in line with those obtained by Robertson and colleagues [7], which showed that a test meal rich in LC n-3 PUFA increases chylomicron response compared with a test meal rich in other types of fat, such as SFA, MUFA, and n-6 PUFA. In addition, our results show that the increase in the chylomicron response is also detectable in comparison with other types of nutrients and with lower amounts of LC n-3 PUFA.

As to the possible explanations, in the study by Robertson and colleagues, PUFA n-3 induced a lower response in cholecystokinin and GLP-1, which is associated with a more rapid gastric emptying of the fat load. Therefore, it was hypothesized that the more rapid gastric emptying linked to LC n-3 PUFA would mean a more rapid fat absorption, with a subsequent increase in chylomicron production. The tendency to a lower GLP-1 response in the first part of the postprandial curve in our experiment is in line with this hypothesis. However, it is also possible that a test meal with LC n-3 PUFA induces the secretion of larger chylomicrons, which may undergo slower hydrolysis by lipoprotein lipase and/or reduced uptake by liver receptor [18]. At odds with the study by Robertson and colleagues [7], the increase in chylomicron response is not limited to the first part of the postprandial curve, but it is particularly evident in the second part of the curve. Taking into

account that the physical structure of fat in a meal may modulate intestinal lipid absorption [19], our results may be due to the fact that solid foods and not oils were used. Either way, it is important to underline that the acute effects of LC n-3 PUFA on postprandial chylomicrons are not maintained in the long term, thus implying that compensatory mechanisms acting at the gut level and/or at the catabolic site may come into play in the long term.

Another interesting result of our study is that test meals rich in polyphenols did not have an acute effect on lipid response, which is at odds with what happens chronically [4]. However, the addition of LC n-3 PUFA with polyphenols was not effective, at least in acute conditions, compared with the effect of LC n-3 PUFA on chylomicron response. This may be due to a reduction in the absorption of LC n-3 PUFA by polyphenols.

Of course, our study has some limitations. First, it was carried out in a particular population, such as individuals with high cardiometabolic risk, and the result cannot be extrapolated to other populations. Second, our data reflect an acute postprandial response which may not predict what happens chronically.

In conclusion, this study indicates that marine LC n-3 PUFA and polyphenols, in acute experiments, affect postprandial lipid response differently from that after medium-term interventions. These differences underline the importance of having the right perspective when attempting to interpret the acute and chronic effects of nutritional and other types of interventions.

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