Dietary Diacylglycerol Suppresses Accumulation of Body Fat Compared to Triacylglycerol in Men in a Double-Blind Controlled Trial

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ABSTRACT We examined the effects of the long-term ingestion of dietary diacylglycerols (DG) in a double-blind controlled study of human lipid metabolism. Healthy men (n = 38; aged from 27 to 49 y, body mass index (BMI) ranging from 21.8 to 27.4 kg/m²) completed the study. To accustom the subjects to the test diets prior to the experiment, they were supplied with test diets of triacylglycerol (TG) oil for 4 wk (control period). The test oils (10 g/d) were included in bread, mayonnaise or shortbread and served for the breakfast. The target for total lipid intake was 50 g/d (15 g for breakfast, 15 g for lunch and 20 g for dinner) throughout the study. The subjects were then divided into two groups so that mean BMI and the hepatic fat content, determined by computed tomography, for each group were not different. One group (DG group) consumed test meals containing DG-rich oil (10 g/d) while the other group (TG group) consumed the same meal as during the control period. Ten grams of the DG-rich oil contained 5.5 g 1,3-DG, 2.5 g 1,2-DG and 2 g TG. The actual lipid intake during the study was 43 g/d. Body weight, BMI and waist circumference decreased in both groups at the end of the test period of 16 wk. However, the magnitudes of decreases in these variables were significantly greater in the DG group than in the TG group. Decreases of total fat, visceral fat area and subcutaneous fat area of the abdominal traverse images of computed tomography in the DG group were also significantly greater than those in the TG group. Hepatic fat content decreased significantly in the DG group while no change was observed in the TG group. Serum lipid concentrations (TG, total cholesterol, free fatty acid) and related metabolites (glucose, insulin, total ketone body) did not change significantly in either group. Thus, in contrast to TG, DG apparently suppressed accumulation of fat and possibly reduces the risk of diseases associated with visceral fat obesity. J. Nutr. 130: 792–797, 2000.

KEY WORDS: • diacylglycerol • triacylglycerol • humans • visceral fat

The risk of diseases such as diabetes and coronary artery disease as well as all-cause mortality increases in proportion to decreases in body adiposity above optimal (Lindsted et al. 1991, Manson et al. 1987, Pt-Sunyer 1991, Seidell et al. 1996), and intra-abdominal distribution of fat in the body is associated more closely to disease risks (Bouchard et al. 1990, Kannell et al. 1991, Matsuzawa et al. 1995). Obesity characterized by an excessive body fat accumulation is generally associated with enhanced lipid consumption (Fricker et al. 1989). Therefore, numerous studies on the bioavailability of various structured lipids have been conducted for the management of obesity.

In our laboratory, we have been studying the nutritional characteristics and dietary effects of diacylglycerol (DG)². DG has been recognized as an intermediate in the process of triacylglycerol (TG) digestion in the digestive tract. DG that occurs in the digestive process is 1,2- and 2,3-species since the lingual or pancreatic lipase cleaves fatty acids only at 1- or 3-position of the TG molecule (Murata et al. 1994). When 1,2- and 2,3-species of DG are heated, they are converted to 1,3-species by acyl migration to the extent intrinsic to the fatty acid composition in the molecule (Kodali et al. 1990). DG is a normally consumed dietary oil because it is a component (2–10%) of edible fats and lipids of various sources (Abdel-Nabey et al. 1992, D’alonzo et al. 1982). Although our daily-consumed oils contain 1,3-species of DG, nutritional characteristics of DG of 1,3-species evaluated in humans are seldom found in the literature.

We have previously shown that intragastric infusion of an emulsion containing DG mainly of 1,3-species, compared to that containing TG, significantly retarded the lymphatic transport of TG as chylomicrons in rats (Murata et al. 1994). We recently showed that the postprandial increase in serum TG and chylomicron concentration after a single dose of DG emulsion was less than after a TG emulsion in humans (Naito et al. 1997). Since the fatty acid composition in DG and TG

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2Abbreviations used: BMI, body mass index; CT, computed tomography; DG, diacylglycerol; FFA, free fatty acid; HU, Hounsfield unit; L-HU/S-HU, the liver-to-spleen ratio of the HU of CT; TG, triacylglycerol; V/S, visceral-to-subcutaneous fat area ratio.

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used in these studies had been adjusted to be approximately equal, we concluded that the difference was due to the different metabolic fates due to the different structures of the lipids. Since elevations of postprandial TG-rich plasma lipoproteins are closely associated with visceral obesity and diseases such as diabetes mellitus and coronary artery disease (Cohn 1998, Couillard et al. 1998, Donald 1995, Dubois et al. 1998), we speculated that the long-term ingestion of DG may improve lipid metabolism in humans.

In the present study, we assessed the potential health benefits of DG in comparison with TG using 38 healthy male volunteers. The study was carried out in a double-blind, controlled manner. Changes in anthropometric variables, body fat and serum lipid profiles before and after the 16 wk treatment were compared to those in subjects consuming the TG diet.

**MATERIALS AND METHODS**

**Subjects.** Subjects were 38 men aged from 27 to 49 y with body mass index (BMI) of 24.1 ± 0.4 kg/m². All subjects were generally healthy and had no history of diabetes or lipemia. Most of the subjects were classified as level 1 in daily activity (mild) according to the 5th Recommended Dietary Allowances for the Japanese (The Ministry of Health and Welfare 1994).

This study with human volunteers was carried out with sufficient respect for the spirit of the Helsinki Declaration of 1975 as revised in 1983. The procedures had been fully explained to the volunteers. All subjects gave their signed informed consent before admission.

**Test diets.** The 1,3-DG rich oil was prepared by esterifying glycerol with fatty acids from rapeseed oil with low erucic acid content by the method of Birgitte et al. (1988) using the reverse reaction of immobilized lipase. The TG oil was prepared by mixing the same rapeseed, soybean, and safflower oils so that the fatty acid composition is similar to that of the 1,3-DG-rich oil. All of these material oils were obtained from Nissin Oil Mills (Tokyo, Japan).

Table 1 shows the fatty acid compositions of the test oils. The 1,3-DG-rich oil contained 83 g/100 g of 1,3- and 1 (or 3),2-DG and 17 g/100 g of TG. The ratio of 1,3-DG to 1 (or 3),2-DG was 68:32. The combustion energy of both the 1,3-DG-rich and TG oil measured with a bomb calorimeter was about 38 kJ/g (analyzed by the Japan Food Analysis Center, Tokyo, Japan).

Bread, mayonnaise and shortbread containing 5 g of the 1,3-DG-rich or the TG oil were prepared and were designated as the DG diet and the TG diet, respectively. Subjects were allowed to choose two from these diet forms so that each test oil amounted to 10 g for breakfast.

**Protocol.** This study was carried out in a double-blind, controlled manner. Before the beginning of this study, all subjects were trained to estimate fat intake using the 4th Revision of the Standard Tables of Food Composition in Japan (The Ministry of Science and Technology 1982). For 4 wk before the test period (designated as control period), the subjects were asked to consume about 50 g of total fat daily. The daily total fat intake of 50 g is equal to that described in the 1994 Results of National Nutritional Survey of the Japanese (The Ministry of Health and Welfare 1996). The test diet was given only as breakfast, and the daily intake of the test oil was set at 10 g. The subjects were asked to consume the test diet every day throughout the study period. For breakfast, besides the test diet, they were asked to consume additional food containing about 5 g of fat of their own choice. For lunch from Monday to Friday, the subjects chose food with known fat contents (about 15 g in total, calculated by a nutritionist) in the cafeteria. For dinner from Monday to Friday, the subjects consumed packed meals with total fat of ~20 g prepared under the guidance of the nutritionist. For lunch and dinner on Saturday, Sunday and holidays, the subjects consumed self-selected meals with about 50 g/d of total fat including test oils. The fat contents in self-selected meals were calculated according to the 4th Revision of the Standard Tables of Food Composition in Japan and recorded by the subjects. Since no restriction was imposed as for the total energy intake, the subjects were free to have foods with low-fat content such as plain rice.

After the control period, all subjects underwent baseline measurements and were divided into two groups of 19. The grouping was random except that two variables, BMI and hepatic fat, were not significantly different between the groups. One group consumed the DG diet and the other group consumed the TG diet for breakfast. The subjects in both groups consumed meals or snacks similarly to the control period. The test diets were supplied for 16 wk every day (designated as test period). During the control and test period, the daily alcoholic beverage intake was restricted to the amount equivalent to about 30 mL of alcohol. The subjects were also asked to maintain the daily exercise at a fixed level throughout the study.

**Diet diary.** The subjects were instructed to record the contents of daily meals and snacks in the diet diary for the entire control period and the last 4 wk of the test period. Daily energy intake, fat intake and percentage of fat energy to the total energy intake were calculated from the diary record by a nutritionist on the basis of the 4th Revision of the Standard Tables of Food Composition in Japan.

**Anthropometric measurements.** All measurements were performed by investigators trained in anthropometric measurements. Participants wore light clothing with footwear removed. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Minimum waist circumference was taken halfway between the coastal bone and the umbilicus, and maximum hip circumference was obtained at the level of the greatest posterior protuberance. Both waist and hip circumferences were measured to the nearest 0.1 cm in a standing position. These measurements were performed on wk 0 and 16.

**Blood sampling and clinical analysis.** On wk 0, 16, blood samples were collected from fasting subjects. Blood sampling and anthropometric measurements were performed in the same day. Alcoholic beverage was prohibited for 1 wk before blood sampling. The subjects were deprived of food overnight from 2100 h; a venous blood sample was obtained between 0900–1000 the next day. All the analyses except for glucose were performed with serum samples. When plasma was prepared for glucose determination, blood samples were collected into tubes containing EDTA. Plasma and serum were obtained by centrifugation at 1,500 × g for 15 min at 4°C. Plasma and serum were analyzed by Mitsubishi Chemical BCL (Tokyo, Japan). TG concentration was measured using an LPL-CK-GPO-POD assay kit (Triglyzim-600° “EIKEN”; Eiken Chemical, Tokyo, Japan). Free fatty acids (FFA) concentration was measured using an enzymatic assay kit (Determiner® NEFA; Kyowa Medex, Tokyo, Japan). Total cholesterol concentration was measured using an enzymatic assay kit (Daiya auto T-cho, Daiya Chemical, Tokyo, Japan). Insulin concentration was measured using a solid-phase radio immunoassay kit (INSULINRIAPEAT® II; Dainabot, Tokyo, Japan). Glucose concentration was measured using a glucose-dehydrogenase assay kit (Merck Liquid GLU; Kanto Chemicals, Tokyo, Japan). Total ketone body was measured using direct enzymatic assay kit (Ketone test A “Sawana” liquid & Ketone test B “Sawana” liquid; Sawana Kagaku Kenkyusho, Nagoya, Japan).

**Measurement of fat by computed tomography (CT).** The subjects underwent CT scanning within 3 d before or after each blood sampling at either of the following two facilities and instru-
measurement: Yabuki Clinic, (Tochigi, Japan, TCT-300, Toshiba Medical); Hakujuji General Hospital (Ibaraki, Japan, W950SR, Hitachi Medico). Transverse tomograms at the umbilical level and those at fixed intervals in which the spleen and liver were observed in the same plane were taken. Total fat area, visceral fat area and subcutaneous fat area were determined from the CT images at the umbilical level by the method of Tokunaga et al. (1983). Hepatic fat content was estimated by the method of Kato et al. (1984). The Hounsfield unit of the liver and that of the spleen in a CT image were determined, and the liver-to-spleen ratio of the values (L-HU/S-HU) was calculated as an index of the hepatic fat content.

**Measurement of body fat.** Body fat before and after the test period was measured by the air-replacement method (Dempster and Aitkens 1995) using a body densitometer MAB-1000 (Nihon Kohden, Tokyo, Japan).

**Statistical analyses.** Values were expressed as means \( \pm \) SEM. The differences in the raw data between the groups were examined by Student’s \( t \) test (two-tailed). The changes from the baselines in each group were examined by Student’s \( t \) test for paired values (two-tailed). A simple linear regression equation was calculated by the least-squares method. Correlation was tested for equality using the Fisher’s Z transformation (Kleinbaum and Kupper 1978). The significance of the treatment effect on the variables that showed significant differences between the groups at baseline, as examined by \( t \) test, was analyzed by analysis of covariance. The extent of change from the baseline for each group was analyzed using the baseline value as the covariate. The significance levels were set at \( P < 0.05 \) for all tests. These statistical calculations were performed with Stat View for windows version 4.58 (SAS Institute, Cary, NC).

### RESULTS

**Characteristics of subject’s energy consumption.** Daily energy intakes, fat intakes and percent of fat energy intakes did not differ significantly between the groups. Although the subjects were asked to consume 50 g/d fat including test oils, the actual fat intake was calculated to be 43 g/d. The daily energy intake was 7950 kJ, and the percent fat energy was about 21%. All these values agreed with the appropriate intakes defined by the guidelines of the Ministry of Health and Welfare in Japan.

**Effect on body size.** Body weight, BMI and waist circumference decreased significantly in both groups after the test period. The extent of decrease in each variable, however, was significantly greater in the DG group than that in the TG group (Table 2).

Hip circumference did not change significantly in either group (Table 2). The waist-to-hip circumference ratio and body fat decreased significantly and similarly in the two groups (Table 2).

### Abdominal fat analysis. In both groups, total fat area determined by the abdominal CT images decreased significantly after the test period (Table 2). The extent of decrease in total fat was significantly greater in the DG group than that in the TG group (Table 2). Visceral fat and subcutaneous fat areas decreased significantly from baseline only in the DG group. Hence, changes over the experimental periods differed between the groups (Table 2). In the DG group, the visceral-to-subcutaneous fat area ratio (V/S) decreased significantly.

### TABLE 2

**Changes of anthropometric values and body composition of the men fed either diacylglycerol or triacylglycerol diet for 16 wk**

<table>
<thead>
<tr>
<th>wk</th>
<th>Diacylglycerol diet group</th>
<th>Triacylglycerol diet group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Change (^2)</td>
<td>Change</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>0</td>
<td>72.1 ± 1.8</td>
</tr>
<tr>
<td>16</td>
<td>69.5 ± 1.7(^*)</td>
<td>67.0 ± 1.5(#)</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>0</td>
<td>24.1 ± 0.4</td>
</tr>
<tr>
<td>16</td>
<td>23.2 ± 0.4(#)</td>
<td>23.2 ± 0.4</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>0</td>
<td>85.0 ± 1.1</td>
</tr>
<tr>
<td>16</td>
<td>80.6 ± 1.3(#)</td>
<td>79.5 ± 1.2(#)</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>0</td>
<td>97.1 ± 0.9</td>
</tr>
<tr>
<td>16</td>
<td>96.1 ± 0.9</td>
<td>95.4 ± 0.8</td>
</tr>
<tr>
<td>W/H(^3)</td>
<td>0</td>
<td>0.87 ± 0.01</td>
</tr>
<tr>
<td>16</td>
<td>0.84 ± 0.01(#)</td>
<td>0.83 ± 0.01(#)</td>
</tr>
<tr>
<td>Body fat, g/100 g</td>
<td>0</td>
<td>21.6 ± 1.2</td>
</tr>
<tr>
<td>16</td>
<td>20.6 ± 1.3(#)</td>
<td>18.6 ± 1.3(#)</td>
</tr>
<tr>
<td>Total fat area, cm(^2)</td>
<td>0</td>
<td>227 ± 16(\ast)</td>
</tr>
<tr>
<td>16</td>
<td>189 ± 16(#)</td>
<td>164 ± 19(#)</td>
</tr>
<tr>
<td>Visceral fat area, cm(^2)</td>
<td>0</td>
<td>79 ± 7(\ast)</td>
</tr>
<tr>
<td>16</td>
<td>63 ± 7(#)</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>Subcutaneous fat area, cm(^2)</td>
<td>0</td>
<td>148 ± 11</td>
</tr>
<tr>
<td>16</td>
<td>126 ± 10(#)</td>
<td>118 ± 13</td>
</tr>
<tr>
<td>V/S(^4)</td>
<td>0</td>
<td>0.55 ± 0.04</td>
</tr>
<tr>
<td>16</td>
<td>0.50 ± 0.04(#)</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>L-HU/S-HU(^5)</td>
<td>0</td>
<td>1.24 ± 0.04</td>
</tr>
<tr>
<td>16</td>
<td>1.30 ± 0.03(#)</td>
<td>1.24 ± 0.03</td>
</tr>
</tbody>
</table>

1 Values are means \( \pm \) SEM, \( n = 19 \).
2 16-wk value minus 0-wk value.
3 Waist-to-hip circumference ratio.
4 Visceral-to-subcutaneous fat area ratio.
5 Liver-to-spleen ratio of Hounsfield unit of computed tomography.
6 Significantly different from the initial value by Student’s \( t \) test: \#P < 0.05, \#\#P < 0.01. Significantly different from triacylglycerol diet group by Student’s \( t \) test for paired value: \( * P < 0.05, ** P < 0.01 \). Significantly different from triacylglycerol diet group by analysis of covariance: \( \ast P < 0.05 \).
after the test period, while no change was observed in the TG group (Table 2).

Since we assigned the subjects into two groups randomly except that the means of BMI and L-HU/S-HU were not different, the initial values for some other variables such as total fat area and visceral fat area incidentally differed between the groups. The initial value of visceral fat in the DG group was significantly higher than that in the TG group. Initial abundance of visceral fat area is significantly related to a larger loss of visceral fat (Leenen et al. 1992) after body weight loss. In fact, a weak but significant correlation between the initial visceral fat area and the loss of visceral fat (Leenen et al. 1992) after body weight loss.

The initial values for total fat area and visceral fat area differed significantly between the groups. The correlation between the initial visceral fat area and the loss of visceral fat area after the treatment was consistent with the results of Leenen et al. (1992). The analysis of covariance, however, clearly demonstrated that in contrast to ordinary TG oil, 1,3-DG-rich oil had beneficial effects on abdominal obesity.

The present study was initially designed for the participants to ingest 50 g/d of total fat, which is approximately equal to the average daily fat intake for Japanese. However, the actual total fat intake was 43 g/d (accounting for 21% of daily energy) in both DG and TG groups. According to the 5th Recommended Dietary Allowances for the Japanese (The Ministry of Health and Welfare 1994), the fat requirement for those

#### Discussion

This study was designed to evaluate the nutritional characteristics of DG in comparison with TG. The participants were not obese on average (BMI = 23.8 kg/m²) but included some near-obese individuals (ranging from 21.6 to 27.4 kg/m²). After the test period of 16 wk, decreases in body weight, BMI, waist circumference, total fat area, visceral fat area, subcutaneous fat area and V/S in the DG group were significantly greater than those in the TG group. Moreover, hepatic fat decreased significantly in the DG group while no change was detected in the TG group as determined by L-HU/S-HU.

The initial values for total fat area and visceral fat area differed significantly between the groups. The correlation between the initial visceral fat area and the loss of visceral fat area after the treatment was consistent with the results of Leenen et al. (1992). The analysis of covariance, however, clearly demonstrated that in contrast to ordinary TG oil, 1,3-DG-rich oil had beneficial effects on abdominal obesity.

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#### Table 3

<table>
<thead>
<tr>
<th>Serum lipid concentrations and other metabolites in men fed either diacylglycerol or triacylglycerol diet for 16 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diacylglycerol diet group</strong></td>
</tr>
<tr>
<td><strong>Change</strong></td>
</tr>
<tr>
<td><strong>Triacylglycerol, mmol/L</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Free fatty acids, mmol/L</strong></td>
</tr>
<tr>
<td><strong>Total cholesterol, mmol/L</strong></td>
</tr>
<tr>
<td><strong>Glucose, mmol/L</strong></td>
</tr>
<tr>
<td><strong>Insulin, pmol/L</strong></td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (n = 19).
2 16-wk value minus 0-wk value.
engaged in level 1 (mild) activity is from 20 to 25% of daily energy. Although the total energy intake was not restricted, body weight, body fat and total fat area in the umbilical CT image decreased in both groups. It seemed that total fat and total energy intakes of the subjects during the study period were less than their habitual intakes before the study. Nevertheless, the reduction of body weight, BMI, waist circumference, total fat area, visceral fat area, subcutaneous fat area and V/S was significantly greater in the DG group than in the TG group.

When the 1,3-DG-rich and TG oils are given in equal amounts by weight, fatty acid content of the DG diet theoretically is slightly lower than that of the TG diet. The energy of the 1,3-DG-rich and TG oils calculated by USDA Agriculture Handbook (Merrill and Watt 1955, Reeves and Weihrauch 1979) was 38.7 kJ/g and 39.6 kJ/g, respectively. Since the amount of test oil consumed was 10 g/d, the difference of 0.9 kJ/g will produce a 9 kJ difference per day. This corresponds to < 0.11% of the total energy intake (7950 kJ/d). Thus, the effect of long-term DG ingestion is not due to the reduced amount of the fatty acid in the test meals. Moreover, the effect of DG cannot be attributed to the altered digestibility of DG since there was no difference in the absorption between the two oils as determined by the faecal excretion of the undigested lipids in rats (Watanabe et al. 1997). The significant decreases in body weight, BMI, waist circumference and visceral fat in the DG group are, rather, caused by the different metabolic features of DG and TG in the small intestine as we described previously (Hara et al. 1993, Murata et al. 1994, Naito et al. 1997).

We previously showed that the extent of postprandial serum TG increase, especially chylomicron TG, after a single dose of DG emulsion was less than that observed after a TG emulsion in humans (Naito et al. 1997). Although the mechanism has not been fully elucidated, impaired postprandial TG clearance was shown to be associated with visceral obesity (Couillard et al. 1998, Mekki et al. 1999). Suppression of the extent of the postprandial serum TG increase after a single dose of the DG emulsion, as compared to the TG emulsion, may therefore at least partly explain the reduction of visceral fat after repeated ingestion of the DG-rich oil. We reported that 17 or 34 d ingestion of the DG diet caused a reduction of serum TG concentration in rats compared to the TG diet (Hara et al. 1993). In addition, reduction of the enzyme activities of fatty acid synthesis and concomitant increases of the enzyme activities involved in the beta-oxidation were observed in liver homogenates of rats fed DG as compared to those fed TG (Murata et al. 1997). Although the mechanism of DG function in rats was discussed to some extent in our previous paper (Hara et al. 1993, Murata et al. 1997), it is necessary to carry out clinical trials using human subjects in various clinical conditions to assess the field of application such as diabetes mellitus and hyperlipidemia.

Recently, Nelson et al. (1996) reported that body fat increases as intake of dietary lipids increases. Among various types of fat deposition, visceral fat-type obesity is one of the risk factors for diseases such as diabetes mellitus, hyperlipidemia, hypertension and atherosclerotic diseases (Fujikura et al. 1987). Therefore, reduction of such risk factors has become a matter of interest for researchers and for obese and near-obese individuals. The amount of test oil ingested in this study (10 g/d) was set to meet the average of daily cooking oil consumption in Japan, which has the daily fat consumption of 50 g. The effects of the DG-rich oil described in the present study therefore can be achieved in daily life if the cooking oil is simply replaced with the DG-rich oil. Efficacy of the DG-rich oil for those consuming higher levels of fat therefore needs to be evaluated in a different experiment since we have not yet tested the dose-response relationship in humans.

In conclusion, we showed that DG, in contrast to TG, suppresses both body weight and regional fat deposition including visceral and hepatic fat in healthy men. DG, if used in place of the ordinary cooking oil, may be beneficial to health by suppressing visceral fat deposition.

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LITERATURE CITED


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