Dietary n–3 and n–6 polyunsaturated fatty acid intake interacts with FADS1 genetic variation to affect total and HDL-cholesterol concentrations in the Doetinchem Cohort Study¹–³

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ABSTRACT

Background: The δ-5 and δ-6 desaturases, encoded by the FADS1 and FADS2 genes, are rate-limiting enzymes in polyunsaturated fatty acid (PUFA) biosynthesis. Single nucleotide polymorphisms in the FADS gene cluster region have been associated with both PUFA concentrations in plasma or erythrocyte membrane phospholipids and cholesterol concentrations in recent genome-wide association studies.

Objective: We examined whether genetic variations in the FADS gene cluster region interact with dietary intakes of n–3 (omega-3) and n–6 (omega-6) PUFAs to affect plasma total, HDL-, and non-HDL-cholesterol concentrations.

Design: Dietary intakes of n–3 and n–6 PUFAs, plasma concentrations of total and HDL cholesterol, and rs174546, rs482548, and rs174570 in the FADS gene cluster region were measured in 3575 subjects in the second survey of the Doetinchem Cohort Study.

Results: Significant associations between rs174546 genotypes and total and non-HDL-cholesterol concentrations were observed in the group with a high intake of n–3 PUFAs (≥0.51% of total energy; \( P = 0.006 \) and 0.047, respectively) but not in the low-intake group (\( P \) for interaction = 0.32 and 0.51, respectively). The C allele was associated with high total and non-HDL-cholesterol concentrations. Furthermore, the C allele was significantly associated with high HDL-cholesterol concentrations in the group with a high intake of n–6 PUFAs (≥5.26% of total energy, \( P = 0.004 \)) but not in the group with a low intake (\( P \) for interaction = 0.02).

Conclusion: Genetic variation in the FADS1 gene potentially interacts with dietary PUFA intakes to affect plasma cholesterol concentrations, which should be investigated further in other studies. Am J Clin Nutr 2010;92:258–65.

INTRODUCTION

The δ-5 and δ-6 desaturases, encoded by FADS1 and FADS2 genes, are rate-limiting enzymes in the biosynthesis of long-chain polyunsaturated n–3 and n–6 fatty acids (n–3 PUFAs and n–6 PUFAs). They introduce \( \text{cis} \) double bonds at specific positions in a fatty acid chain (1, 2). FADS1 shares 62% and 70% nucleotide sequence homology with FADS1 and FADS2, respectively, and encodes for an as-yet-uncharacterized protein (3, 4). These 3 genes are located on chromosome 11 (11q12-13.1) and form the FADS gene cluster (Figure 1) (3). Several single nucleotide polymorphisms (SNPs) and haplotypes in this region have been shown to be associated with blood or erythrocyte membrane phospholipid PUFA concentrations in either candidate or genome-wide association (GWA) studies (6–12). Some of these SNPs have also been associated with blood cholesterol concentrations in recent GWA studies (13–15). Recent emerging evidence suggests that the observed association between genetic variation in the FADS gene cluster region with blood cholesterol concentrations is functionally related to the availability of PUFAs with ≥4 double bonds and its effect on the homeostasis of different glycerophospholipids (11). It has long been known that blood cholesterol concentrations are influenced by dietary PUFA intakes (16–22); however, the underlying molecular mechanisms of these associations are still unclear. We want to investigate whether the SNPs in the FADS gene cluster region participate in the influence of dietary PUFAs on blood cholesterol concentrations (23, 24). In the present study, we evaluated the potential interaction between 3 candidate SNPs in the FADS gene cluster region and dietary n–3 and n–6 PUFA intakes on plasma total, HDL-, and non-HDL-cholesterol concentrations.

SUBJECTS AND METHODS

Study population

This study was performed within the framework of the Doetinchem Cohort Study—a regional survey aimed at monitoring risk factors for chronic diseases and conducted in a rural area in the eastern Netherlands. Institutional review boards approved the Doetinchem Cohort Study. A detailed description of the design and methods was published elsewhere (25). Briefly, subjects were surveyed between 1987 and 1991 for baseline...
FIGURE 1. Genomic context of rs174546, rs174570, and rs482548 and surrounding single nucleotide polymorphisms (SNPs) in the FADS gene cluster region. The genomic locations of genes in the FADS gene cluster region on chromosome 11 (61.32–61.42 Mb) are shown. Arrows indicate rs174546, rs174570, and rs482548 according to their position on the genomic sequence. Data are from the UCSC (University of California, Santa Cruz) Genome Browser (http://genome.ucsc.edu/) “RefSeq Gene” track (I). Pairwise disequilibrium coefficients for SNPs with minor allele frequencies >0.1% from HapMap CEU (CEPH; http://hapmap.ncbi.nlm.nih.gov/) data (phase II, release 22) in a 6.5-kb interval (between rs4246215 and rs174550) in the 3’ untranslated region (UTR) of FADS1 in which rs174546 is located are generated by using Haploview software (http://www.broadinstitute.org/haploview/). The strength of the linkage disequilibrium between SNPs increases from white to black ($r^2 = 1$; black diamonds) or from purple to red ($D^2 = 1$; red diamonds) by using Haploview’s standard color scheme (II). The predicted or experimentally verified miRNA binding sites relative to rs174546 from “PicTar miRNA and T-Scan3 miRNA” tracks at UCSC Genome Browser are shown as small vertical lines, pink and green, respectively. MULTIZ vertebrate alignment of 11 mammal species indicates evolutionary conservation (5). The lowest row shows the position of known SNPs according to dbSNP build 125 (http://www.ncbi.nlm.nih.gov/snp/) (III).
information, and a subsample of the subjects was followed up about every 5 or 6 y. The second survey of the Doetinchem Cohort Study was approved by the ethical review board of TNO (Netherlands Organization for Applied Scientific Research), and all participants provided their written informed consent. The subjects were surveyed for demographic, anthropometric, and lifestyle information (smoking, alcohol use, physical activity, and dietary habits); disease history; and medication use by questionnaires. A nonfasting blood sample was collected from all participants; fractionated into plasma, white blood cells, and erythrocytes; and subsequently stored. A validated semiquantitative food-frequency questionnaire was used in the second and third surveys to assess the habitual consumption of 178 food items during the previous year (26). Nutrient and energy intakes were quantified for each individual by using the updated computerized Dutch food-composition table (27). Detailed dietary n-3 and n-6 PUFA intakes were only available in the second survey; therefore, the data from the second survey were used in the present study. Characteristics of the study population were reported previously (28, 29). In brief, 48% of the subjects were men. The mean (± SD) age at the second survey was 46.7 ± 9.8 y. They had consistent smoking habits, and none of the women were pregnant at any of the 3 surveys. Intakes of n-3 PUFAs [α-linolenic acid (ALA; 18:3n-3), eicosapentaenoic acid (EPA; 20:5n-3), all-cis-7,10,13,16,19-docosapentaenoic acid (DPA; 22:5n-3), and docosahexaenoic acid (DHA; 22:6n-3)] and n-6 PUFAs [linoleic acid (LA; 18:2n-6), eicosadienoic acid (EDA; 20:2n-6), dihomo-γ-linolenic acid (20:3n-6), arachidonic acid (AA; 20:4n-6), adrenic acid (22:4n-6), and all-cis-4,7,10,13,16-docosapentaenoic acid (22:5n-6) were calculated in grams per day. n-3 PUFAs and n-6 PUFAs were summed separately and converted to percentage of the energy.

**Laboratory assessment of total and HDL-cholesterol concentrations**

Plasma total and HDL-cholesterol concentrations were assayed in the Lipid Reference Laboratory of the University Hospital Dijkzigt in Rotterdam by using standardized enzymatic methods within 3 wk after storage. Total cholesterol was measured by using a CHOD-PAP method (Boehringer, Mannheim, Germany) (30). HDL cholesterol was measured in the supernatant fluid after precipitation of apolipoprotein (apo) B–containing lipoproteins with phosphotungstic acid/MgCl2 (Boehringer) (31). Non-HDL cholesterol includes all lipoproteins that contain apo B and was calculated as total cholesterol minus HDL cholesterol (32). The Lipid Reference Laboratory Rotterdam is a permanent member of the International Cholesterol Reference Method Laboratory Network (33). It has been standardized to the Centers for Diseases Control and Prevention (CDC) through participating in the CDC/National Heart Lung and Blood Institute Lipid Standardization Program. The accuracy of total and HDL-cholesterol measurements fulfilled National Cholesterol Education Program recommendations throughout the entire period (33–35).

**SNP selection and genotype determination**

SNPs were determined in 3575 subjects who participated in 3 surveys. Two SNPs (rs174546 in FADS1 3′ untranslated region and rs482548 in FADS2 3′ untranslated region) in the FADS gene cluster region [targets genes (FADS1 and FADS2) regulated by transcription factor SREBP1c (36)] were selected by using the Web-based program SNPSelector, which prioritizes the SNPs on the basis of their potential functional effect in each gene, minor allele frequency, and haplotype block information (37). At the time of SNP selection, no study of their associations with metabolic endpoints was available. They were included in a high throughput SNP genotyping platform-Illumina Golden Gate assay (Illumina Inc, San Diego, CA). Illumina GenCall software (version 6.1.3.28) was used for automated genotype clustering and calling. The detailed quality control protocol was described previously (28). Another SNP (rs174570 in FADS2 intron_1) in the FADS gene cluster region was selected later on the basis of its latest published associations with blood cholesterol concentrations (13) and was genotyped by K Bioscience (Hoddesdon, Hertfordshire, United Kingdom) by using the KASPar chemistry, which is a competitive allele-specific polymerase chain reaction SNP genotyping system that uses FRET quencher cassette oligonucleotides (http://www.kbioscience.co.uk).

**Statistical analysis**

Deviation from Hardy-Weinberg equilibrium and pairwise linkage disequilibrium (LD) were tested by using the R genetics package (R Foundation for Statistical Computing, Vienna, Austria). The relations between SNPs and plasma total, HDL-, and non-HDL-cholesterol concentrations were explored by using General Linear Models by using SAS version 9.1 software (SAS Institute, Cary, NC). To adjust for potential confounding effects and to improve model fitting, age, sex, current smoking (yes or no), alcohol use (glasses of alcohol/d), body mass index (BMI; in kg/m2), fasting status (blood collected after ≥8 h fasting: yes or no), physical activity (meeting the guideline of physical activity: yes or no), and use of cholesterol-lowering medication were added to the models as covariates. Multiple linear regression models were used to assess the relation between n-3 PUFAs or n-6 PUFAs intakes as a percentage of energy and plasma HDL-cholesterol concentrations with additional adjustment for n-6 PUFA or n-3 PUFA intakes, respectively, as a percentage of energy if necessary. Dietary n-3 and n-6 PUFA intakes were also classified into 2 groups according to the PUFA-specific median percentage of energy: n-3 PUFAs (low: ≤0.51% of energy; high: ≥0.51% of energy) and n-6 PUFAs (low: ≤5.26% of energy; high: ≥5.26% of energy). The interactions between genotypes and dichotomized n-3 PUFA (n-6 PUFA) intakes on total, HDL-, and non-HDL-cholesterol concentrations were explored by using General Linear Models by including additional interaction terms into the models. Distributions of continuous variables in groups were expressed as means ± SEMs. All reported P values were 2-tailed, and statistical significance was defined at the α = 0.05 level based on our candidate SNP approach.

**RESULTS**

The genotype distributions of the 3 SNPs studied were consistent with Hardy-Weinberg equilibrium expectations (see Table S1 under “Supplemental data” in the online issue). The 3 SNPs were not in strong pairwise LD (r^2 = 0.01–0.36). The mean n-3 PUFA intake was 0.53% of the total energy intake, whereas the
mean n−6 PUFA intake was 5.47% of the total energy intake. PUFA intake did not significantly differ according to FADS genotype \( (P = 0.12 \pm 0.99); \text{see Table S2 under “Supplemental data” in the online issue).}

Subjects with high n−3 PUFA intakes had significantly higher HDL-cholesterol concentrations than did subjects with low n−3 PUFA intakes \( (P = 0.02); \text{see Table S3 under “Supplemental data” in the online issue}.\) No significant associations between n−6 PUFA intake and any of the lipid variables were observed. Mean total, HDL-, and non-HDL-cholesterol concentrations according to the genotypes of 3 SNPs are presented in Table 1. No associations were found between any of the SNPs and HDL- or non-HDL-cholesterol concentrations, but total cholesterol concentrations differed significantly according to the rs174546 genotype \( (P = 0.02).\) The C allele was associated with higher total cholesterol concentrations. However, this association was more pronounced and only statistically significant \( (P = 0.006)\) in subjects with a high n−3 PUFA intake (Table 2). In the high n−3 PUFA intake group, the C allele was also associated with higher non-HDL-cholesterol concentrations \( (P = 0.047).\) However, the \( P\) values for interaction between rs174546 genotypes and n−3 PUFA intake on total and non-HDL-cholesterol concentrations were not statistically significant \( (P = 0.32\) and 0.51, respectively). Also, the n−6 PUFA intake modified the association of the rs174546 genotype with lipid concentrations (Table 3). The C allele was associated with a statistically significant higher HDL-cholesterol concentrations in subjects with a high n−6 PUFA intake \( (P = 0.004),\) whereas no difference was observed in subjects with a low n−6 PUFA intake \( (P = 0.59; \text{for interaction} = 0.024).\) PUFA intake did not modify the associations between the other 2 SNPs (rs174570 and rs482548) and total, HDL-, or non-HDL-cholesterol concentrations. A modest correlation between n−3 and n−6 PUFA intakes was observed in this population \( (\rho = 0.23, P < 0.0001).\) Further adjustments of the above associations between PUFA intake or SNPs and plasma total, HDL-, and non-HDL-cholesterol concentrations with corresponding PUFA intake did not substantially change the results (data not shown). Results from the haplotype analysis, either including all 3 SNPs or only rs174546 and rs174570, showed that the haplotype associations seemed to be mainly derived from and were similar to those observed with rs174546 alone (data not shown).

**DISCUSSION**

In the present study, the previously reported association between rs174546 in FADS1 and cholesterol concentrations was confirmed, whereas the association between rs174570 in FADS2 and cholesterol concentrations was not replicated. We observed that rs174546 genotypes potentially interacted with dietary n−3 and n−6 PUFA intakes to affect total, HDL-, and non-HDL-cholesterol concentrations.

The association between genetic variants in the FADS gene cluster region and blood cholesterol concentrations was highlighted in 3 recent GWA studies (13–15). The minor \( T\) allele of rs174546 was associated with decreased LDL- (11, 12, 15), HDL- (14, 38), and total cholesterol concentrations (12) on the basis of high LD among SNPs in the FADS1 3’ untranslated region (Figure 1). Several miRNA target sites have been predicted in this region (Figure 1) (39, 40). On the basis of PolymiRTS and Patrocles databases, rs174546 is within one of the miRNA target sites and is also in complete LD with another miRNA target site polymorphism (rs174545) in this region, which results in disruption of one of the conserved miRNA target sites. The potential effect on FADS1 transcript abundance of rs174546 (or other SNPs in high LD with rs174546 in this region) is further corroborated by recently published human transcription data. The major \( C\) allele of rs174546 was positively associated with FADS1 expression in lymphoblastoid cells (41), in the liver (14, 42), and in adipose tissue (43) on the basis of complete LD between rs174546 and rs174547. rs174546 is in high LD with recently reported SNPs that are associated with plasma or erythrocyte membrane PUFA concentrations (6–12). It has been observed that the haplotype including the major \( C\) allele is associated with an increase in the concentrations of desaturase products, whereas it is associated with a decrease in its substrates (6–12). Despite the lack of a study that has directly assayed desaturase activity so far, all the abovementioned evidence indicates that the major \( C\) allele of rs174546 may be associated with an increased efficiency of the fatty acid \( \delta-5\) desaturase reaction. The potential mechanism underlying the association between high desaturase activity and high total or LDL-cholesterol concentrations (11, 12, 15) and high HDL-cholesterol concentrations (14, 38) is still unclear. Because the expression of \( \delta-5\) and \( \delta-6\) desaturases is highest in the liver (1, 2, 4), a major contribution of the liver desaturase–elongase enzyme system to the observed association between genetic variation in the FADS1 and FADS2 genes and plasma cholesterol metabolism seems likely.

In our population, significant associations between rs174546 and total and non-HDL-cholesterol concentrations were found only in the group with a high n−3 PUFA intake, not in the group with a low intake. Non-HDL cholesterol is highly correlated with total apo B concentrations (32, 44, 45) and could indirectly indicate the total number of apo B–containing particles (VLDL, IDL, and LDL) (44–46). The major \( C\) allele of rs174546 is

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**TABLE 1**

Plasma total cholesterol, HDL-cholesterol, and non-HDL-cholesterol concentrations according to genotypes rs174546, rs174570, and rs482548 in the FADS gene cluster region

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol</th>
<th>HDL-cholesterol</th>
<th>non-HDL-cholesterol</th>
<th>( P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs174546</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( TT ) (n = 362)</td>
<td>5.48 ± 0.09</td>
<td>1.27 ± 0.03</td>
<td>4.21 ± 0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>( TC ) (n = 1503)</td>
<td>5.56 ± 0.07</td>
<td>1.30 ± 0.03</td>
<td>4.26 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>( CC ) (n = 1505)</td>
<td>5.62 ± 0.08</td>
<td>1.31 ± 0.03</td>
<td>4.31 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>rs174570</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( TT ) (n = 95)</td>
<td>5.46 ± 0.12</td>
<td>1.28 ± 0.04</td>
<td>4.18 ± 0.12</td>
<td>0.22</td>
</tr>
<tr>
<td>( TC ) (n = 874)</td>
<td>5.52 ± 0.08</td>
<td>1.31 ± 0.03</td>
<td>4.21 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>( CC ) (n = 2539)</td>
<td>5.57 ± 0.07</td>
<td>1.30 ± 0.02</td>
<td>4.27 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>rs482548</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( TT ) (n = 40)</td>
<td>5.67 ± 0.16</td>
<td>1.30 ± 0.06</td>
<td>4.37 ± 0.17</td>
<td>0.75</td>
</tr>
<tr>
<td>( TC ) (n = 590)</td>
<td>5.56 ± 0.08</td>
<td>1.31 ± 0.03</td>
<td>4.26 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>( CC ) (n = 2914)</td>
<td>5.55 ± 0.07</td>
<td>1.30 ± 0.02</td>
<td>4.26 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

\( 1\) All values are adjusted means ± SEMs. \( P\) values for differences between genotypes were obtained by using general linear models (ANOVA) adjusted for age, sex, BMI, fasting state, current smoking status, alcohol consumption, physical activity, and use of cholesterol-lowering medication.
associated with increased concentrations of n–3 PUFA with ≥4 double bonds (6–12), whereas n–3 PUFA intervention decreases hepatic triglyceride synthesis and VLDL secretion (16, 19, 22). We would therefore expect that C allele–associated high desaturase activity would be associated with low non-HDL-cholesterol concentrations in a population with a high dietary n–3 PUFA intake. However, we observed a non-HDL-cholesterol–increasing effect. Considering the kinetics of the whole apo B pool, this may suggest that with a high dietary n–3 PUFA intake, C allele–associated high desaturase activity suppresses the uptake of apo B–containing particles, such as VLDL remnants or LDL. This hypothesis is supported by both positive associations between serum n–3 PUFA concentrations and LDL-cholesterol concentrations in a Japanese observational study (47) and increased LDL-cholesterol concentrations in EPA and DHA or fish oil–intervention studies (16–19, 21). It is also mechanistically supported by in vitro and in vivo observations of reduced rat hepatic precursor and nuclear (much stronger) forms of SREBP-1 with fish oil (or DHA) treatment (48–50). One of the isoforms encoded by SREBP-1 (SREBP-1z) is a potent transcription factor that regulates LDLR gene expression (36, 51). Our laboratory recently showed that feeding fish oil to mice down-regulated hepatic LDLR gene expression (1.9-fold; Q value = 4.23E-6; M Boekshoten, personal communication, 2009), consistent with a previous observation in rabbits (52).

A statistically significant interaction between dietary n–6 PUFA intakes and rs174546 genotypes on HDL-cholesterol concentrations was observed in our study. There was a significant difference in HDL-cholesterol concentrations among rs174546 genotypes in the high n–6 PUFA intake group. In controlled trials, substituting carbohydrates isoenergetically with PUFAs (largely n–6) is associated with increased HDL-cholesterol concentrations (20). In addition, serum n–6 PUFA concentrations were positively associated with HDL-cholesterol concentrations in both white and Japanese middle-aged men (47). The more efficient desaturase reaction associated with the major C allele (see above), together with a high dietary intake of n–6 PUFA, could lead to much larger differences in the availability of n–6 PUFAs with ≥4 double bonds between C allele carriers and TT homozygotes of rs174546 (11) as compared with a situation when n–6 PUFA intake is low. These PUFAs, especially their metabolic derivatives, are potent activators of peroxisome proliferator–activated receptors (53–56), which results in the increased expression of genes directly involved in HDL production (57, 58). Interestingly, however, in TT homozygotes, HDL-cholesterol concentrations were even lower in the high n–6 PUFA intake group than in the low intake group (P = 0.014). From a quantitative point of view, linoleic acid (precursor of n–6 PUFAs) is 10–100 times more abundant in Western diets than is α-linolenic acid (precursor of n–3 PUFAs) (9). The n–3 and n–6 PUFAs competitively use the same desaturase–elongase enzyme system (59), and n–3 PUFAs are relatively potent peroxisome proliferator–activated receptor ligands compared with n–6 PUFAs (54–56, 59). With a high n–6 PUFA intake, linoleic acid accumulates, especially in TT homozygotes. This potentially poses a strong inhibiting effect on the metabolism of n–3 PUFAs (59–62) and their associated HDL-cholesterol–increasing effects (18, 29, 22, 47, 63). This hypothesis is supported by our own observation that the association between dietary n–3 PUFA intake and HDL-cholesterol
concentrations became stronger after further adjustment for dietary n–6 PUFA intakes (see Table S4 under “Supplemental data” in the on-line issue).

The present study had some limitations. First, we did not measure the internal concentrations (plasma or tissue membrane) of different PUFA s that are directly involved in the underlying biological processes. However, relatively strong correlations between dietary intakes of PUFA s and internal PUFA concentrations have been observed (16, 18, 60–62). Furthermore, the detailed questionnaire used in this study covered all food items habitually consumed in the Netherlands and was well validated for micronutrient intake (26). Second, despite the relatively large sample size, the interactions between n–3 PUFA intakes and rs174546 genotypes on total and non-HDL-cholesterol concentrations were not statistically significant ($P > 0.05$), because a larger sample size is needed to obtain statistical significance. Third, considering our candidate SNP approach, the associations between dietary n–6 PUFA intake (low and high intakes) were obtained by including the interaction terms in the general linear models and were 0.13, 0.16, and 0.21 for total cholesterol concentrations; 0.02, 0.42, and 0.47 for HDL-cholesterol concentrations; and 0.49, 0.31, and 0.21 for non-HDL-cholesterol concentrations, respectively. $P$ values for differences between genotypes were obtained by using general linear models (ANOVA) adjusted for age, sex, BMI, fasting status, current smoking status, alcohol consumption, physical activity, and use of cholesterol-lowering medication.

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The authors’ responsibilities were as follows—YL, JMAB, and EJM F conceived and designed the study; METD, SI, JMAB, EJM F, WWMV, and MM acquired the data; YL, JMAB, EJM F, and MM analyzed and interpreted the data; YL wrote the manuscript; and JMAB, EJM F, MM, METD, SI, and WMMV critically revised the manuscript. None of the authors had any conflicts of interest.

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