HHS Public Access

Author manuscript

Br J Nutr. Author manuscript; available in PMC 2018 October 01.

Published in final edited form as:

Br J Nutr. 2018 October ; 120(7): 787–796. doi:10.1017/S0007114518002039.

Serum fatty acids and progression from dengue fever to dengue hemorrhagic fever / dengue shock syndrome

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Abstract

Polyunsaturated fatty acids (PUFA) might modulate inflammatory responses involved in the development of severe dengue. We aimed to examine whether serum PUFA concentrations in patients diagnosed with dengue fever (DF) were related to the risk of progression to dengue hemorrhagic fever / dengue shock syndrome (DHF/DSS). A secondary aim was to assess correlations between fatty acids (FA) and inflammatory biomarkers in DF patients. We conducted a prospective case-control study nested within a cohort of DF patients who were followed during the acute episode. We compared the distribution of individual FA % of total FA concentration at onset of fever between 109 cases who progressed to DHF/DSS and 235 DF non progressing controls using unconditional logistic regression. We estimated correlations between baseline FA and cytokine concentrations, and compared FA concentrations between the acute episode and >1 y post-convalescence in a subgroup. Docosahexaenoic acid (DHA) was positively related to progression to DHF/DSS (multivariable adjusted odds ratio [AOR] for DHA in quintile 5 vs. 1 = 5.34, 95% confidence interval [CI]: 2.03, 14.1; *P*, trend = 0.007). Dihomo-γ-linolenic acid (DGLA) was inversely associated with progression (AOR for quintile 5 vs. 1 = 0.30, 95% CI: 0.13, 0.69; *P*, trend = 0.007). Pentadecanoic acid concentrations were inversely related to DHF/DSS. Correlations of PUFA with cytokines at baseline were low. PUFA were lower during the acute episode than in a disease-free period. In conclusion, serum DHA in DF patients predicts higher odds of progression to DHF/DSS whereas DGLA and pentadecanoic acid predict lower odds.

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CONFLICT OF INTEREST

None.

AUTHORSHIP

E.V. and L.A.V. designed the research. L.A.V., A.L., and O.F.H. conducted the research. V.M.H. conducted data management and quality assurance. E.V. performed the statistical analyses, wrote the paper, and had primary responsibility for the final content. All authors have read and approved the final version of the manuscript.

Keywords

fatty acids; polyunsaturated fatty acids; dengue; dengue hemorrhagic fever; dengue shock syndrome

INTRODUCTION

Dengue is the most common mosquito-borne viral infection worldwide, affecting as many as 100 million people annually[1,2]. The typical presentation of dengue is as a febrile illness with a variety of accompanying symptoms. In a few cases, the disease can progress to a life-threatening syndrome characterized by plasma leakage. The factors that induce progression from dengue fever (DF) to these potentially fatal forms of the disease, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), are uncertain. They may involve secondary infections by new viral serotypes, agent virulence, and host characteristics[3].

Among the host factors that could influence the risk of developing DHF/DSS, the nutritional status could play an important role because many nutrients serve key immunomodulatory functions. For example, vitamin E supplementation to DF patients resulted in increased platelet counts in India[4], 25-hydroxy vitamin D (25[OH]D) levels were positively related to severe dengue in Colombia[5] and India[6] whereas 1,25[OH]D was inversely associated with DHF/DSS in Nicaragua[7], and zinc and copper serostatus were related to dengue disease severity in cross-sectional studies in Indonesia[8] and India[9]. Fatty acids (FA) are salient amidst the nutrients that could affect pathophysiological pathways leading to severe forms of dengue virus (DENV) infection. FA influence the organization of cell membranes and the composition of lipid rafts through their incorporation as phospholipids and sphingolipids[10]. Lipid raft microdomains play an essential role in DENV protein synthesis and replication[11]. In addition, some n-3 polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) could reduce expression of pro-inflammatory cytokines through different mechanisms including downregulated gene expression in mononuclear cells, decreased synthesis of pro-inflammatory eicosanoids derived from n-6 PUFA, reduced chemotaxis and lymphocyte proliferation, enhanced apoptosis of Th-1 cells, and decreased endothelial activation and dysfunction[12]. Some n-6 PUFA, including arachidonic acid (AA, 20:4n-6), are considered pro-inflammatory while others such as dihomo-γ-linolenic acid (DGLA, 20:3n-6) exhibit anti-inflammatory properties[13]. Despite the potential of FA to regulate pathways involved in the etiology of severe dengue, they have not been systematically studied in the context of this infection. Some investigations indicate that FA concentrations are altered during the acute stages of dengue infection[14,15]. Because therapies to prevent progression of DF to DHF/DSS are virtually inexistent, identifying modifiable factors associated with risk of these severe forms of the disease is a high research priority.

We conducted a prospective case-control study nested in a cohort of patients who were diagnosed with DF and followed during the acute episode. The primary aim was to investigate the associations of FA concentrations in serum collected < 96 hours from the

onset of fever with progression to DHF/DSS. A secondary aim was to determine whether serum FA could be affected by acute inflammation at the early stages of DF. This was realized by assessing the correlations between inflammatory biomarkers and FA and by comparing FA concentrations during the acute episode and a disease-free period, after > 1 y post-convalescence from the disease.

METHODS

Study design

This was a case-control study nested within a cohort of patients diagnosed with DF who were followed during the acute episode. Ambulatory patients with suspected dengue were recruited during non-epidemic (05/2003–09/2009) and epidemic (10/2009 to 12/2010) periods at health care centers in 5 areas of Bucaramanga, a city in northeast Colombia. Eligible participants had an acute febrile syndrome caused by DENV infection, had onset of symptoms < 96 hours before consultation, and were ≥ 5 years of age. Exclusion criteria were: history of diabetes, acquired immunodeficiency syndrome, hematologic disorders, cancer, or cardiovascular disease; or, at baseline, DHF or DSS (case definition below), major bleeding, hypoalbuminemia, effusions, or shock. At the time of recruitment, we elicited information on sociodemographic characteristics, medical history, and symptoms through a questionnaire. Height and weight were measured on calibrated instruments with the use of standardized techniques and a complete physical examination was carried out. Blood samples were obtained to determine hematocrit, platelet counts, and albumin concentration. To identify the occurrence of DHF/DSS, we followed participants during the acute episode daily at their homes until the 7^{th} day of disease or the day of hospital discharge if they were admitted. Data collected during this period included signs and symptoms of DHF/DSS, as well as daily hematocrit measures. To detect thrombocytopenia, a DHF/DSS diagnostic criterion, we started daily platelet count measurements when patients had spontaneous hemorrhage, signs of effusion, edema, a hematocrit change > 10%, or a platelet count < 120,000/mm^3. We saw the patients again in the convalescent period, 7–15 days after the onset of symptoms, and obtained a new blood sample. DENV infection was confirmed according to a diagnostic algorithm that included IgM seroconversion from the acute to the convalescent samples (shift from negative to positive or a titer increase ≥ 4) plus either a positive result for NS1 antigen or viral genome amplification per RT-PCR in acute serum (< 96 h from the onset of fever). IgM antibodies were determined with use of an enzyme-linked immunosorbent assay (Panbio Dengue IgM Capture ELISA, Alere, Australia), NS1 antigen was quantified with the ELISA NS1 Dengue kit (Panbio, Alere, Australia), and RT-PCR amplification of viral RNA was conducted with the QIAamp Viral RNA kit (QIAGEN). We determined whether DENV infection was secondary with use of the Panbio Dengue IgG Capture ELISA test (Alere, Australia).

Case definition.—Cases were patients who developed DHF or DSS during follow-up, according to the 1997 World Health Organization criteria, prevalent at the time[16]. DHF cases met all of the following criteria: a platelet count ≤ 100,000/mm^3, any spontaneous hemorrhage or ≥ 1 positive tourniquet test, and evidence of plasma leakage (i.e., pleural effusion, ascites, hypoalbuminemia < 3 g/dL, or an increase in hematocrit > 10%). A

hemoconcentration of 10%, instead of 20%, was chosen because this criterion has greater sensitivity in identifying dengue-related complications[17] and has been associated with severe morbidity in patients from areas endemic for dengue[18]. DSS cases had all criteria above plus any reading of mean arterial pressure < 70 mmHg or pulse pressure < 20 mmHg during follow-up.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki. All participants gave written informed consent before recruitment. Among children, written consent was sought from the primary care provider and assent from the children was confirmed before recruitment. The study protocol was approved by the Medical Ethics Committee of the Industrial University of Santander. The University of Michigan Health Sciences and Behavioral Sciences Institutional Review Board approved the use of data and samples from the study.

Study population

We recruited 820 participants into the cohort. Of them, 173 (21.1%) developed DHF/DSS during follow-up. For the case-control study we selected all 109 cases (63.0% of all cases in the cohort) whose first available serum sample (at the time of the initial consultation) had been collected within 96 hours from the onset of fever (“acute” serum sample) and had sufficient volume for FA quantification. Next, we randomly selected a group of controls among the patients who did not reach DHF/DSS criteria during follow-up, using a 2:1 ratio of controls per case plus an additional 10% in anticipation of losses due to lack of an acute serum sample. The final number of controls was 235. Selected controls were comparable to those not selected with respect to sex, age, socioeconomic status, hours with fever before consultation, and signs of severity. Nevertheless, selected controls were more likely to have become infected during the epidemic period and were taller and heavier compared with non-selected controls. Selected cases compared with non-selected cases in the same manner as controls.

A post-convalescence blood sample was obtained in a subgroup of 15 cases and 29 controls who were visited at home after a median 1.8 years (range 1.3 to 2.5) from the acute episode.

Laboratory methods

Serum fatty acids.—Samples were shipped frozen to the University of Michigan where total serum FA were quantified at the Regional Comprehensive Metabolomics Resource Core. Total lipids were extracted from 200 μL serum per the methods described by Bligh and Dyer[19]. 10 μL of 4 mM nonadecanoic acid (C19:0) was added as an internal standard. Boron trifluoride-methanol was used to derivatize the fatty acid portion of the total lipids into their methyl esters as previously described[20]. To extract the methyl esters, a 2:1 hexane-water mixture was added and the sample was centrifuged. The hexane layer containing the methyl esters was removed from the aqueous layer and dried, and the methyl esters were re-suspended in 100–200 μL of hexane, according to the volume of the original sample. 1–2 μL was injected via an autosampler and analyzed on a gas chromatographer (Agilent, Model 6890N, Santa Clara, CA, USA) equipped with a flame ionization detector and a 100 m × 0.25 mm × 0.2 μm SP-2560 column (Sigma-Aldrich, Bellefonte, PA, USA).

To quantify FA, known amounts of C19:0 and other authentic methyl esters were used to create a calibration curve. The authentic methyl esters were also used to identify FA in samples based on their retention times. Eluted peaks were analyzed with Chemstation software (Agilent). FA concentrations were expressed as the percentage of each FA relative to the total FA concentration (FA %). This FA % was estimated by dividing the area of each FA by the total area. The limit of detection for pentadecanoic acid (C15:0), a FA present at low concentrations, was 50 pmol.

The activity of key enzymes in FA metabolism was estimated from desaturation and elongation indices calculated as the product/substrate ratios: stearoyl-coA-desaturase (SCD) as 18:1n-9/18:0; elongase as 18:1n-7/16:1n-7; Δ6-desaturase (D6D) as 18:3n-6/18:2n-6; and Δ5-desaturase (D5D) as 20:4n-6/20:3n-6.

Cytokines.—We determined concentrations of pro-and anti-inflammatory cytokines that may be independent predictors of progression to DHF/DSS. Interferon (IFN)-y, interleukin (IL)-10, IL-6, and tumor necrosis factor (TNF)-a were measured at the University of Michigan Cancer Center Immunology Core with the use of Luminex assays (Thermo Fisher Scientific Inc. Waltham, MA) in all acute sera and in the samples collected post-convalescence.

DENV serotype.—DENV serotypes were identified in a subsample of 49 controls and 28 cases using conventional and real time RT-PCR assays. Viral RNA was isolated from serum using the commercial kit QIAamp® Viral RNA (QIAGEN). Conventional RT-PCR tests were performed following the Lanciotti protocol[21]. Real time RT-PCRs were conducted with the CDC Kit DENV-1–4 Real-Time RT-PCR[22].

Data analysis

Variables.—Case (DHF/DSS) status was the outcome of interest. FA % were the primary exposures and enzyme activity indices were secondary exposures. We considered as covariates baseline characteristics that could confound the associations between FA status and progression to DHF/DSS. These included sociodemographic (age, sex, socioeconomic status), anthropometric (height and body mass index [BMI] as nutritional status indicators), clinical (time since the beginning of fever, early signs of severity), virological (secondary infection, serotype), and immunological (cytokine concentrations) characteristics.

Comparison of controls and cases.—We first compared the distribution of baseline covariates between controls and DHF/DSS cases, categorized as presented in Table 1. Case-control differences were tested with the use of χ^2 and Wilcoxon Rank-Sum tests for categorical and continuous characteristics, respectively.

Fatty acids and progression to DHF/DSS.—We compared the distribution of FA between controls and cases using means ± SD and the Wilcoxon Rank Sum test. Next, we categorized each FA into quintiles according to the distribution among controls and estimated odds ratios (OR) with 95% confidence intervals (CI) with the use of unconditional logistic regression. Tests for linear trend were conducted by introducing into the models a variable representing the median values of each quintile as a continuous predictor.

Multivariable adjusted OR and 95% CI were estimated for each FA in models that included as covariates sex, age, hours with fever before consultation, and IL-10 and TNF-α concentrations at the time of consultation. Finally, we fitted a model with simultaneous adjustment for the FA that were statistically significant predictors of DHF/DSS progression in multivariable analyses and that remained significant in the final model at $P < 0.05$.

Acute inflammation andfatty acids.—To ascertain whether acute inflammation in the course of DENV infection was related to FA concentrations we estimated sex-and age-adjusted partial Spearman correlation coefficients between the cytokines (IFN-γ, IL-10, IL-6, and TNF-α) and each FA separately for controls and cases. Next, we estimated the correlations between the analytes measured in the samples collected after > 1 y post-convalescence. We also examined the prevalence of thrombocytopenia at baseline by FA quintiles since platelet count is a strong predictor of progression to severe dengue.

Change in fatty acid concentrations from the acute episode to post-convalescence.—In the subgroup with a post-convalescence blood sample, we estimated the difference in FA concentrations between after > 1 year of recovery and the acute episode with use of a repeated measures linear regression model with each FA as the continuous outcome and order of measurement as the predictor. An independent covariance matrix was specified to account for within-subject correlations. Participants with a post-convalescence sample were more likely to have been recruited during the epidemic period, were younger and of lower socioeconomic status, and consulted later after the onset of fever than those without these samples. They also differed with respect to some of the FA at baseline.

Associations of DENV serotype with fatty acids.—Because DENV serotype is a strong independent predictor of progression to severe dengue[23] and we lacked this information on the majority of participants, we examined the associations of serotype with serum FA in the subset with available data to explore whether serotype could have confounded the key associations between FA and case status. We compared the distribution of FA by serotype with use of Wilcoxon Rank-Sum tests.

All analyses were carried out with Statistical Analysis Software version 9.4 (SAS Institute Inc. Cary, NC).

RESULTS

Characteristics of controls and cases.

Fifty percent (n = 173) of participants were female. Mean ± SD age was 26.6 ± 15.2 years (range 5–86); 25% (n = 86) were < 15 years-old. Compared with controls, cases were older, consulted earlier after the onset of fever, had lower platelet counts, and had higher cytokine concentrations at baseline (Table 1).

Fatty acids and progression to DHF/DSS.

Mean pentadecanoic (15:0) and stearic (18:0) acid concentrations were significantly lower in cases compared with controls (Table 2, Supplemental Figure 1), whereas behenic acid (22:0) levels were higher. There were no significant differences in MUFA concentrations by case

status. Mean concentrations of the long chain n-3 PUFA DPA and DHA, and of the n-6 PUFA DGLA, AA, and adrenic acid were significantly higher in cases compared with controls (Table 2, Supplemental Figure 1). SCD and D5D activities were higher in cases than controls. When the fatty acid exposures were considered in quintiles of their distributions among controls (Supplemental Table 1), pentadecanoic acid and DGLA were inversely associated with case status whereas DHA, AA, SCD, and D5D were positively related to progression to DHF/DSS, after adjustment for sex, age, hours with fever before consultation, and IL-10 and TNF-α concentrations. In a multivariable model adjusted for these FA simultaneously pentadecanoic acid, DHA, and DGLA remained significantly associated with progression to DHF/DSS (Table 3) whereas the associations with AA and SCD were attenuated and became non-statistically significant. For pentadecanoic acid, compared with patients in the lowest quintile those in the highest had a 76% lower odds of progression ($P = 0.002$). DHA was positively related to case status in a non-linear manner; compared with patients in the lowest quintile, odds of progression were 5-times higher (OR: 4.96; 95% CI: 2.05, 11.99; $P = 0.0004$) for patients with concentrations in the second quintile or above. DGLA was inversely related to DHF/DSS progression; the OR (95% CI) between quintiles 5 and 1 was 0.30 (0.13, 0.69; $P = 0.005$). When D5D was substituted for DGLA in the model, D5D was positively associated with DHF/DSS progression (Q5 vs. Q1 OR: 3.28; 95% CI: 1.42, 7.58; $P = 0.006$).

Acute inflammation and fatty acids.

Correlations between FA and cytokine concentrations were generally low (Supplemental Table 2). DGLA was weakly, inversely correlated with IL-10 at baseline but not post-convalescence. DPA, DHA, and D5D were related to low platelet counts; whereas the n-6 PUFA γ-linolenic acid (GLA, 18:3n-6) and DGLA were associated with less thrombocytopenia (Supplemental Table 3). Pentadecanoic acid was not related to platelet counts at baseline.

Change in fatty acid concentrations from the acute episode to post-convalescence.

Among the 44 patients with post-convalescent samples, concentrations of most SFA, MUFA, and n-3 and n-6 PUFA changed from the time of the acute episode (Supplemental Table 4). Concentrations of SFA, except for palmitic acid, tended to increase from the acute episode to post-convalescence; whereas MUFA concentrations, except for palmitoleic acid, decreased. ALA, EPA, DPA, and all n-6 PUFA concentrations increased. DHA was higher during the acute episode than post-convalescence, but the change was not statistically significant. There were no changes in *trans* FA. D5D activity decreased. Changes did not differ by case status during the acute episode (Supplemental Table 5).

DENV serotype and fatty acids.

The distributions of some FA varied significantly by DENV serotype in the subset with available data (Supplemental Table 6). Of the FA significantly associated with progression to DHF/DSS, only DHA was related to DENV serotype. DHA of participants infected with serotypes 2 or 3 was lower than that of patients infected with DENV 1 or 4.

DISCUSSION

In this prospective investigation of patients diagnosed with DF, serum DHA at the time of diagnosis was related to increased odds of progression to DHF/DSS whereas the SFA pentadecanoic acid and the n-6 PUFA DGLA were associated with decreased odds. Also, increased D5D activity was related to higher odds of progression.

The positive association of DHA levels with progression to DHF/DSS is consistent with results from a small untargeted metabolomics study[7]. Serum DHA within 4 days from the onset of fever was higher in 16 Nicaraguan children diagnosed with DF who progressed to DHF/DSS compared with 15 children who did not progress. This finding is unexpected because DHA contributes to the resolution of inflammation through different pathways[12] and effective resolution of inflammation should be related to less risk of progression. Nevertheless, the association should also be interpreted in light of the comparison of DHA levels during the acute episode and post-convalescence. In our study, DHA was higher during the acute episode than in a presumably disease-free period. Although this difference was not statistically significant, it is in line with findings from a metabolomics study of adults with DF in which DHA was higher during the febrile stage of infection than it was 3–4 weeks after resolution of the episode[14]. An increase in DHA levels during acute DF has been interpreted as an early attempt to initiate the resolution of inflammation, which could start concomitantly with the initial pro-inflammatory response[7,14]. A more severe inflammatory process during the early stages of DF predicts progression to DHF/DSS; thus, a correspondingly more aggressive anti-inflammatory response involving increased DHA levels could explain the association of DHA with progression even in the absence of an adverse causal effect of DHA. Of note, however, in our data DHA was generally not correlated with cytokine concentrations and the association of DHA with progression was observed even after controlling for these inflammation biomarkers. An adverse effect of DHA cannot be discarded. Rodent models indicate that n-3 long chain PUFA supplementation can be detrimental in infections by intracellular pathogens including influenza A and herpes simplex viruses by suppressing immune cell responses needed to eradicate infected cells[24], although a study of mice showed no effect on n-3 PUFA on responses to vaccinia virus infection[25]. Evidence on the effects of PUFA on acute febrile illnesses in humans is scant. Some studies suggest that n-3 PUFA may reduce the incidence of pneumococcal infection in the elderly and n-3 PUFA supplementation to infants or school-age children has resulted in decreased respiratory morbidity[26,31], possibly of viral etiology. We also noted that DHA was positively associated with thrombocytopenia at baseline. Whether this could be a mechanism to explain a potential effect on progression to severe disease is a matter of speculation. DHA might dampen the procoagulant function of platelets through altered synthesis of thrombin precursor proteins[32]; nevertheless, there is no evidence to suggest that it may affect platelet numbers. Additional *in vitro* and non-human experimentation is needed to clarify the role of n-3 PUFA in DENV infection before considering a potential therapeutic role for these nutrients.

The inverse associations of pentadecanoic acid and DGLA with progression to DHF/DSS are novel findings. Pentadecanoic acid is an odd-chain SFA that, together with heptadecanoic acid (C17:0), represents dairy fat intake[33]; its endogenous production is

negligible[34]. Epidemiological studies have consistently shown inverse associations of pentadecanoic and heptadecanoic acid biomarkers with long term cardiometabolic disorders[35], but the biological mechanisms underlying these relations are uncertain. Investigators have posited that one of the biological functions of pentadecanoic acid involves the regulation of circulating propionic acid[34], a short-chain SFA produced by intestinal bacteria through fermentation of dietary fiber. Propionic acid and other short-chain SFA may be potential mediators of the effects of microbiota on intestinal immunity and inflammation. These fatty acids regulate leukocytes' recruitment, migration, and activation through production of cytokines, chemokines, and eicosanoids[36]. Whether these effects extend systemically in the event of an acute infection such as dengue remains to be elucidated. We observed a decrease in palmitic acid, the most abundant SFA, with a concomitant increase in palmitoleic acid from the acute episode to post-convalescence. Palmitic acid can be synthesized endogenously or ingested from diet, whereas palmitoleic acid can be formed from palmitic acid by the stearoyl-coA-desaturase. If diet remained constant, one could speculate that stearoyl-coA-desaturase activity might be dampened during the acute dengue episode, but the clinical implications of this potential effect are uncertain.

DGLA is a long chain n-6 PUFA synthesized endogenously from linoleic acid through desaturation and elongation. D5D can convert DGLA into AA, a pro-inflammatory fatty acid, and other acute viral infections, including influenza, induce upregulation of the lipoxygenase pathway increasing the synthesis of pro-inflammatory oxylipins derived from AA[37]. Nevertheless, DGLA is generally considered anti-inflammatory because it can interfere with eicosanoid biosynthesis and can be converted to prostaglandin E_1, a suppressor of chronic inflammation[13]. An increase in DGLA relative to AA could acutely attenuate the synthesis of pro-inflammatory eicosanoids derived from AA, including 4-series leukotrienes, 2-series prostaglandins, and platelet-activating factor, which may be involved in the pathophysiology of severe dengue[7]. Consistent with this possibility, increased activity of D5D, the enzyme that converts DGLA into AA, was related to higher odds of progression to DHF/DSS. We found that DGLA was inversely correlated with IL-10 at baseline, especially among cases, and elevated IL-10 seemed to predict progression to DHF/DSS in this and other studies[38,39]. This could indicate that a potential protective effect of DGLA on progression to severe dengue might be mediated through reduced inflammation. Another intriguing potential mechanism could be related to virucidal activity against encapsulated viruses that some long chain n-6 PUFA have shown *in vitro*[40–43]. DGLA breastmilk concentrations were inversely related to cell-free and cell-associated human immunodeficiency virus (HIV) load in milk in a study of Tanzanian HIV-infected women[44]. Non-causal explanations are also plausible. We noted that DGLA concentrations were lower during the acute episode than in an apparently disease-free period in a subgroup of participants. Assuming there were no major changes in diet between the two measurements, this opens the possibility that acute inflammation during the early stages of the disease influences DGLA concentrations and that an inverse association of DGLA with progression to severe dengue be only a reflection of early inflammatory status. Notwithstanding this possibility, DGLA supplementation increases blood DGLA in a dose-response manner[45,46] and should not be discarded as a potential intervention that could be considered for testing in the treatment of acute DF.

This study has several strengths. Reverse causation bias was minimized by the prospective nature of the design. Recall bias was avoided with the use of objective measures of exposure. We had an opportunity to control for important potential confounders, including inflammatory biomarkers that predict progression of the disease to severe forms. We compared exposure status between the acute episode and an apparently healthy period in a subsample of participants; this approach is stronger than using a different set of healthy controls. Some limitations are also worth noting. Selection bias could occur if the selection of controls is not independent of exposure status. We noted that selected controls differed from the cohort's noncases in some characteristics that could be related to FA status, including body weight; thus, it is not possible to completely rule out selection bias. Second, confounding could have occurred if an unmeasured predisposing factor for progression was related to FA concentrations at the onset of the febrile episode. Third, because FA concentrations are conventionally expressed as percent of total FA in the sample, associations observed with higher levels of a given FA could also represent those due to lower levels of another and vice versa. Fourth, pentadecanoic acid is present at low concentrations which are prone to measurement error. The confidence bounds around the OR were wide. Nevertheless, the mean serum C15:0 concentration in this population, 0.16%, was the same or very close as that reported in other settings including Costa Rica[47] and the United States[48–50], lending support to the external validity of this finding. Fifth, random error may have occurred due to differences in the timing of the last meal since the acute serum sample in this study was not always a fasting sample. Serum FA concentrations do not necessarily reflect long-term intake as some may change from day to day depending on the FA composition of recent meals. Correlations between long-term intake and serum concentrations vary for different FA. They are moderate to high for exogenous FA including odd chain saturated FA and essential PUFA (ALA and LA), or for endogenous FA that may nevertheless be abundant in diet such as oleic acid, EPA, and DHA[47]. By contrast, serum FA that are not common in the diet and mostly reflect endogenous hepatic metabolism, such as GLA and DGLA, may not represent long term intake[47]. Random error may occur due to differences in the timing of the last meal since the acute serum sample in this study was not always a fasting sample. Sixth, the subsample of patients with post-convalescence samples was not comparable with the rest of participants with regard to some characteristics. Finally, lack of sample volume and funding constraints prevented us from determining serotype in all study subjects. DENV serotype is a strong predictor of progression to severe disease and could have confounded the associations if it were also related to exposure status. Nonetheless, the data do not support this notion because only DHA was related to both progression and serotype in the subset with available information; and the directions of the serotype-DHA and serotype-DHF/DSS associations would have caused an attenuation rather than an exaggeration of a positive DHA-DHF/DSS relation.

In sum, serum DHA concentrations at the early stages of dengue fever are positively associated with progression to DHF/DSS whereas pentadecanoic acid and DGLA concentrations are inversely related to progression. Serum FA concentrations differ between an acute dengue episode and an apparently healthy period. These results should be confirmed in other populations as the next step in identifying FA as eventual therapeutic targets in patients with DF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FINANCIAL SUPPORT

This study was supported by the National Institute of Allergy and Infectious Diseases (R21AI103364) and the Colombian Administrative Department of Science, Technology, and Innovation - Colciencias (1102–04–12919, 1102–04–18205, 1102–459–21561, and BPIN 2013000100011).

The funding source had no role in the design, analysis, or writing of this article.

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Table 1.

Characteristics of dengue fever controls and dengue hemorrhagic fever/dengue shock syndrome cases at the time of uncomplicated dengue fever diagnosis (baseline)

Characteristics	Controls n = 235	Cases n = 109	*P*[1]
Sociodemographic and anthropometric, %			
Male sex	50.2	48.6	0.78
Age, y			
Mean ± SD	26.5 ± 16.1	26.8 ± 13.3	0.29
Median (range)	22.4 (5.0, 86.0)	23.8 (5.0, 72.0)	0.29
% <15 y	29.8	14.7	0.003
Low socioeconomic status[2]	33.2	33.3	0.98
Short stature[3]	12.6	15.2	0.51
Underweight[4]	6.6	7.6	0.73
Obesity[5]	8.5	9.5	0.75
Clinical, %			
Episode in the epidemic period[6]	38.3	23.9	0.008
72 to <96 hours with fever before consultation	65.5	55.1	0.06
Spontaneous hemorrhage	8.9	9.2	0.94
Orthostatic hypotension	12.3	15.6	0.41
Tourniquet test positive	39.5	49.1	0.10
Thrombocytopenia[7]	14.9	50.0	<0.0001
Virological, %			
Secondary infection	27.2	27.6	0.94
Serotype[8]			0.01
1	34.7	10.7	
2	8.2	32.1	
3	34.7	42.9	
4	22.5	14.3	
Immunological, mean ± SD			
Interferon-γ, pg/ml	41.8 ± 53.5	53.0 ± 65.0	0.17
Interleukin-10, pg/ml	138.4 ± 211.5	198.1 ± 242.5	0.0001
Interleukin-6, pg/ml	4.9 ± 9.8	5.1 ± 3.6	<0.0001
Tumor necrosis factor-α, pg/ml	14.7 ± 8.8	16.5 ± 10.6	0.17

[1] χ^2 and Wilcoxon Rank-Sum tests for dichotomous and continuous characteristics, respectively.

[2] Strata 1 and 2 (out of 5) of the local government's socioeconomic status classification of households for tax and planning purposes.

[3] For participants < 18 years of age, height-for-age < –1 *Z* according to the World Health Organization sex-specific growth reference for school-aged children and adolescents. For participants ≥ 18 years of age, height < –1 *Z* of the sex-specific distributions of controls (< 151 cm for women and < 167 cm for men).

[4] For participants < 18 years of age, body mass index (BMI)-for-age < –2 *Z* according to the World Health Organization sex-specific growth reference for school-aged children and adolescents.(51) For participants ≥ 18 years of age, BMI < 18.5 kg/m^2.

[5] For participants < 18 years of age, BMI-for-age ≥ 2 *Z* according to the World Health Organization sex-specific growth reference for school-aged children and adolescents. For participants ≥ 18 years of age, BMI ≥ 30 kg/m.

[6] The epidemic period occurred between 10/2009 and 12/2010.

[7] Platelet count < 100,000/mm^3.

[8] DENV serotyping was conducted in a subsample of 49 controls (20.9%) and 28 cases (25.7%).

Table 2.

Distribution of serum fatty acids (FA) % in dengue fever controls and dengue hemorrhagic fever/dengue shock syndrome cases[1]

Fatty acid[2]	Controls n = 235	Cases n = 109	*P*[3]
SFAs (%)			
14:0 myristic acid	0.35 ± 0.22	0.30 ± 0.16	0.12
15:0 pentadecanoic acid	0.16 ± 0.06	0.15 ± 0.06	0.02
16:0 palmitic acid	23.43 ± 2.31	23.15 ± 2.06	0.17
17:0 margaric acid	0.37 ± 0.08	0.36 ± 0.11	0.08
18:0 stearic acid	8.14 ± 1.10	7.70 ± 1.04	0.005
20:0 arachidic acid	0.16 ± 0.06	0.16 ± 0.04	0.24
22:0 behenic acid	0.33 ± 0.11	0.36 ± 0.13	0.02
24:0 lignoceric acid	0.28 ± 0.14	0.30 ± 0.14	0.11
Total SFAs	33.21 ± 2.80	32.47 ± 2.48	0.02
MUFAs (%)			
16:1n-7 palmitoleic acid	1.82 ± 0.61	1.77 ± 0.54	0.79
18:1n-9 oleic acid	23.30 ± 2.81	23.62 ± 2.98	0.54
18:1n-7 cis-vaccenic acid	2.28 ± 0.56	2.36 ± 0.59	0.08
20:1n-9 gondoic acid	0.19 ± 0.07	0.20 ± 0.06	0.30
24:1n-9 nervonic acid	0.43 ± 0.25	0.45 ± 0.23	0.14
Total MUFAs	28.13 ± 3.20	28.48 ± 3.31	0.63
n-3 PUFA (%)			
18:3n-3 α-linolenic acid	0.29 ± 0.18	0.25 ± 0.13	0.09
20:5n-3 EPA	0.27 ± 0.12	0.30 ± 0.14	0.22
22:5n-3 DPA	0.39 ± 0.15	0.43 ± 0.17	0.03
22:6n-3 DHA	1.50 ± 0.72	1.72 ± 1.11	0.01
Total long chain n-3 PUFA[4]	2.16 ± 0.80	2.45 ± 1.15	0.005
Total n-3 PUFA	2.53 ± 0.92	2.76 ± 1.18	0.05
n-6 PUFA (%)			
18:2n-6 linoleic acid	26.42 ± 3.82	25.97 ± 3.34	0.45
18:3n-6 γ-linolenic acid	0.24 ± 0.14	0.26 ± 0.13	0.19
20:2n-6 EDA	0.23 ± 0.12	0.24 ± 0.09	0.41
20:3n-6 dihomo-γ-linolenic acid	1.38 ± 0.42	1.30 ± 0.44	0.03
20:4n-6 arachidonic acid	6.08 ± 1.47	6.62 ± 1.48	0.006
22:4n-6 adrenic acid	0.48 ± 0.39	0.73 ± 0.97	0.006
Total long chain n-6 PUFA[5]	8.17 ± 1.70	8.89 ± 2.01	0.006
Total n-6 PUFA	34.83 ± 4.51	35.12 ± 4.11	0.39
18:2n-7ct CLA (%)	0.06 ± 0.11	0.05 ± 0.10	0.13
trans-fatty acids (%)			
16:1n-7 *trans*	0.23 ± 0.15	0.22 ± 0.09	0.92
18:1 *trans*	0.62 ± 0.50	0.56 ± 0.46	0.12

Fatty acid[2]	**Controls n = 235**	**Cases n = 109**	***P***[3]
18:2 *trans*	0.38 ± 0.25	0.35 ± 0.17	0.28
Total *trans*-fatty acids	1.23 ± 0.75	1.13 ± 0.60	0.14
Enzyme activity indices			
Stearoyl-coA-desaturase 18:1 n-9/18:0	2.91 ± 0.51	3.14 ± 0.68	0.008
Elongase 18:1n-7/16:1n-7	1.39 ± 0.65	1.48 ± 0.71	0.11
Δ6-desaturase 18:3n-6/18:2n-6	0.009 ± 0.006	0.100 ± 0.005	0.20
Δ5-desaturase 20:4n-6/20:3n-6	4.79 ± 1.98	5.58 ± 2.17	0.0001

[1] Mean ± SD.

[2] SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; EDA, eicosadienoic acid; CLA, conjugated linoleic acid.

[3] Wilcoxon rank-sum test.

[4] Sum of 20:5n-3 EPA, 22:5n-3 DPA, and 22:6n-3 DHA.

[5] Sum of 20:2n-6 EDA, 20:3n-6 dihomo-γ-linolenic acid, 20:4n-6 arachidonic acid, and 22:4n-6 adrenic acid.

Table 3.

Adjusted odds ratios for progression to dengue hemorrhagic fever/dengue shock syndrome according to baseline characteristics and serum fatty acids %

Characteristic	Controls: Cases	Unadjusted OR (95% CI)[1]	Adjusted OR (95% CI)[2]	Adjusted *P*[3]
Sex				0.85
Female	117:56	1.00	1.00	
Male	118:53	0.94 (0.60, 1.48)	0.95 (0.57, 1.58)	
Age, y				
<15	165:93	1.00	1.00	0.003
≥15	70:16	2.47 (1.35, 4.49)	2.75 (1.41, 5.34)	
Hours with fever before consultation				0.02
<72	81:49	1.00	1.00	
72 to <96	154:60	0.64 (0.41, 1.02)	0.52 (0.31, 0.89)	
Interleukin-10, per 200 pg/ml	-	1.25 (1.03, 1.53)	1.23 (0.97, 1.57)	0.09
Tumor necrosis factor-α, per 10 pg/ml	-	1.22 (0.97, 1.55)	1.31 (0.98, 1.74)	0.07
15:0 pentadecanoic acid quintile[4], % (median)				0.002
Q1 (0.11)	46:27	1.00	1.00	
Q2 (0.14)	47:27	0.98 (0.50, 1.91)	0.67 (0.32, 1.40)	
Q3 (0.16)	47:23	0.83 (0.42, 1.66)	0.55 (0.26, 1.19)	
Q4 (0.19)	47:22	0.80 (0.40, 1.60)	0.53 (0.24, 1.15)	
Q5 (0.24)	47:9	0.33 (0.14, 0.77)	0.24 (0.09, 0.60)	
22:6n-3 DHA quintile[4], % (median)				0.007
Q1 (0.81)	47:7	1.00	1.00	
Q2 (1.11)	47:26	3.71 (1.47, 9.39)	4.44 (1.67, 11.8)	
Q3 (1.37)	47:20	2.86 (1.10, 7.39)	4.57 (1.64, 12.7)	
Q4 (1.68)	47:24	3.43 (1.35, 8.72)	5.58 (2.01, 15.4)	
Q5 (2.26)	47:32	4.57 (1.84, 11.4)	5.34 (2.03, 14.1)	
20:3n-6 dihomo-γ-linolenic acid quintile[4], % (median)				0.007
Q1 (0.91)	47:31	1.00	1.00	
Q2 (1.14)	47:22	0.71 (0.36, 1.40)	0.51 (0.24, 1.09)	
Q3 (1.34)	47:23	0.74 (0.38, 1.46)	0.58 (0.27, 1.24)	
Q4 (1.55)	47:17	0.55 (0.27, 1.12)	0.43 (0.19, 0.96)	
Q5 (1.98)	47:16	0.52 (0.25, 1.07)	0.30 (0.13, 0.69)	
Δ5-desaturase[5] quintile (median)				0.0004
Q1 (2.89)	47:15	1.00	1.00	
Q2 (3.80)	47:12	0.80 (0.34, 1.89)	0.97 (0.38, 2.50)	
Q3 (4.50)	47:14	0.93 (0.41, 2.15)	0.85 (0.34, 2.12)	
Q4 (5.16)	47:22	1.47 (0.68, 3.17)	1.40 (0.60, 3.28)	
Q5 (6.77)	47:46	3.07 (1.51, 6.23)	3.28 (1.42, 7.58)	

[1] From unconditional logistic regression models with case status (dengue hemorrhagic fever/dengue shock syndrome) as the outcome and each covariate presented as predictor.

[2] From an unconditional logistic regression model with case status as the outcome and predictors that included sex (1 indicator for male), age (1 indicator for < 15 y), hours with fever before consultation (1 indicator for ≥ 72 h), interleukin-10 (continuous), tumor necrosis factor-α (continuous), 15:0 pentadecanoic acid quintile (4 indicators), 22:6n-3 DHA quintile (4 indicators), and 20:3n-6 dihomo-γ-linolenic acid quintile (4 indicators). Estimates for Δ5-desaturase were obtained from a model that excluded 20:3n-6 dihomo-γ-linolenic acid due to collinearity.

[3] For sex, age, hours with fever before consultation and cytokines, Wald test. For FA, test for linear trend when a variable representing the median of each quartile was introduced into the logistic regression model as a continuous predictor.

[4] % of total serum FA. DHA, docosahexaenoic acid.

[5] Ratio of 20:4n-6 / 20:3n-6.