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Anger induced by interferon-alpha is moderated by ratio of arachidonic acid to omega-3 fatty acids

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Abstract

Objective—Anger worsens in some patients during interferon-alpha (IFN- α) therapy. Elevated anger has also been associated with lower long-chain omega-3 (LCn-3) fatty acid levels. We examined whether fatty acids could influence vulnerability to anger during IFN- α exposure.

Methods—Plasma arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) levels were determined prior to IFN- α therapy by mass spectroscopy. Repeated-measure analyses examined the relationship between AA/EPA+DHA and the subsequent development of labile anger and irritability in 82 subjects who prospectively completed the Anger, Irritability, and Assault Questionnaire (AIAQ) during the first eight weeks of IFN- α therapy.

Results—Prior to IFN- α therapy, AA/EPA+DHA did not correlate with either labile anger or irritability. Pre-treatment AA/EPA+DHA did correlate with the subsequent maximal increase in labile anger during IFN- α therapy ($r=0.33$; $p=0.005$). Over time, labile anger increased more in subjects with above median AA/EPA+DHA ratios ($p<0.05$). Of the 17 subjects ultimately requiring psychiatric intervention for anger, 14/17 had above-median AA/EPA+DHA ratios ($p=0.009$). There was also an interaction with the tumor necrosis factor-alpha (TNF- α) promoter polymorphism (A-308G), such that only those with both elevated AA/EPA+DHA and the A allele had increased labile anger ($p=0.001$). In an additional 18 subjects, we conversely observed that selective serotonin reuptake inhibitor treatment was associated with increased irritability during IFN- α therapy.

Conclusion—LCn-3 fatty acid status may influence anger development during exposure to elevated inflammatory cytokines, and may interact with genetic risk for increased brain TNF- α . LCn-3 supplements may be one strategy for minimizing this adverse side effect of IFN- α .

Keywords

Anger; Aggression; Irritability; Cytokine; Fatty acid; Inflammation; Antidepressant; Tumor Necrosis Factor; Interferon

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Irritability, hostile aggression, and ‘anger attacks’ can occur in the context of many psychiatric disorders (1–7), contributing to work-place violence (8), domestic violence (9), and possibly road-rage (10). Aggression is one of the most common reasons for “off-label” use of psychiatric medications (11). Aggression, labile anger, irritability, hostility, and assaultiveness may be different but overlapping constructs – but they are each notably distinct from depression. The abnormal regulation of anger has several putative genetic and neurobiological influences (3, 12–15); and many studies implicate the serotonergic system (16–18). A second likely influence includes inflammatory cytokines (16). For example, tumor necrosis factor- α (TNF- α) levels have been associated with hostility (19–21). Interestingly, risk for atherosclerotic vascular disease co-occurs with hostility and may share similar pro-inflammatory influences (22, 23). In mice, an elevated pro-inflammatory response has been associated with aggression (24).

“Irritability” in questionnaires can be operationalized as chronic feelings of annoyance and hypersensitivity to provocation -- while “labile anger” is defined as abrupt transitions from being calm to feeling furious. Both irritability and labile anger are different from actual assaultiveness (the extent to which one behaviorally acts on irritability and anger). Although the role of inflammation in depression is becoming increasingly recognized, which aspect of anger is associated with inflammation has been less-well delineated.

Nonetheless, consistent with a role for inflammatory cytokines, anger and/or hostility can worsen during exogenous interferon-alpha (IFN- α) therapy (25–32). Moreover, this clinical phenomenon of increased anger during IFN- α therapy may be distinct from major depression (33, 34). For instance, we have observed that a promoter polymorphism in TNF- α is uniquely associated with risk for ‘labile anger’ but not for depression (35). These prospective observations support the hypothesis for a causal influence of inflammatory cytokines on labile anger. However, most people are not vulnerable to this effect, and associated risk and protective factors remain poorly understood.

IFN- α based therapies are often clinically employed for treating chronic hepatitis C (HCV) (32, 36), where depression occurs in about patients (37–41). One risk factor for depression may be lower levels of long-chain omega-3 (LC- n -3) polyunsaturated fatty acids (42, 43). Specifically, depression risk has been associated with the ratio of the omega-6 fatty acid arachidonic acid (AA; 20:4 n -6) to two LC- n -3 fatty acids, eicosapentaenoic acid (EPA; 20:5 n -3) and docosahexaenoic acid (DHA; 22:6 n -3) – congruent with a range of other findings where the AA/EPA+DHA ratio correlates with depression (44–48), though not in all studies (49).

There are also multiple lines of evidence to support the hypothesis that vulnerability to anger is influenced by AA/EPA+DHA. Homicide rates amongst countries correlate with lower seafood intake (50); low dietary intake of LC- n -3 correlates with increased hostility in young urban adults (51); lower levels of LC- n -3 correlates with elevated hostility in drug-free schizophrenia subjects (52); and trans fatty acids, which are associated with lower LC- n -3 levels, correlate with increased irritability and aggression in adults (53). Lower LC- n -3 levels are associated with aggression, (54) with poor affective regulation and impulse control (55), and with increased rejection of unfair offers in a neuro-economic task (56). Rodent models implicate low LC- n -3 levels in increased aggression, particular in settings of increased stress (57, 58); and even aggression in German Shepherd dogs is associated with low DHA levels (59).

In further support for a causal role, supplementation of LC- n -3 fatty acids may reverse increased anger and aggression. LC- n -3 supplementation decreased anger in substance users (60), where increased plasma DHA levels correlated with lower anger (61). Similarly, EPA

supplementation reduced aggression in patients with borderline personality (62). *LCn-3* supplements have likewise improved irritability in patients with bipolar disorder (63), hyperactive behaviors in autistic children (64), aggression in female Japanese school children (65), aggressive incidents in prisoners (66), and aggression in settings of high stress (67). There are only a few counter examples. For instance, while DHA supplementation could prevent increase stress-induced aggression, there was no benefit for aggression in non-stressful situations (68). Another study observed that EPA reduced suicidality but had no benefit for hostility (69).

It is plausible that these anti-irritability/anger/hostility/aggression effects of EPA and DHA may be related to their anti-inflammatory properties. They, as well as their lipoxygenase and cyclo-oxygenase metabolites (known as “resolvins” and “protectins”), can influence cytokine synthesis (70) and resolve inflammation (71, 72). As one example, DHA can be converted to ‘neuroprotectin,’ which has potent inflammatory resolving activities in the brain (73). At least one receptor for D-series resolvins exists in the brain, formyl peptide receptor 2 (FPR2) (74). Conversely, AA is a substrate for the synthesis of prostacyclins, thromboxanes, and prostaglandins such as PGE₂, which stimulate the synthesis of inflammatory cytokines (75, 76). Consistent with these observations, elevated AA/EPA +DHA ratios are associated with greater lipopolysaccharide-induced elevations in IL-6 (77), while *LCn-3* fatty acids can reverse inflammatory cytokine-induced behaviors (78).

Thus our primary hypothesis, guided by this literature, was that pre-treatment measures of AA/EPA+DHA would prospectively and specifically predict subsequent vulnerability to inflammatory cytokine-associated anger in human patients treated with IFN- γ . Also, DHA and/or its D-series resolvins can diminish TNF- α production by macrophages (79, 80), and experimental *LCn-3* fatty acid deficiency is associated with elevated TNF- α levels in rat plasma (81). Because we have previously found that a promoter polymorphism in the gene encoding for TNF- α is associated with risk for labile anger (35), we additionally explored whether there might be an interaction with AA/EPA+DHA.

To avoid confounding by antidepressant use, these evaluations were performed in patients not taking antidepressants. A previous report found that paroxetine treatment of depression could minimize hostility during IFN- γ therapy in 11 subjects (82). Conversely, SSRI use may increase risk for irritability in patients with family histories of bipolar disorder (83). We therefore additionally explored whether open-label prophylaxis with a selective serotonin reuptake inhibitor (SSRI) might affect subsequent irritability and anger during IFN- γ therapy.

METHODS

Participants

Subjects (n=82) who were prospectively assessed for anger are a subset of a cohort previously described in a study of depression development (43). All initiated weekly injections of pegylated (PEG) IFN- γ 2 (PEG-IFN- γ 2a: 135 μ g/week or PEG-IFN- γ 2b: 120 or 150 μ g/week) augmented with oral ribavirin at the University of Pittsburgh Center for Liver Disease as treatment for HCV; all (age 18–80) had blood samples available at baseline for fatty acid assessment (though plasma samples were not available after starting IFN- γ therapy); all were without active mood, anxiety, psychotic, or drug/alcohol use disorders for at least 6 months prior to starting IFN- γ therapy; none were taking corticosteroids, antidepressants, anticonvulsants, antipsychotics, and/or *LCn-3* dietary supplements; and all provided consent as approved by the University of Pittsburgh Institutional Review Board.

Anger and Irritability Assessment

Subjects in this study completed the Anger Irritability and Assault Questionnaire (AIAQ), which is a 42 item self-report (84) with subscales that include labile anger, irritability, direct assault, indirect assault, and verbal assault. Individual items were scored 0 to 3 and are based on a self-assessment of the prior two weeks. “Labile anger” is defined by questions related to sudden transitioning from a calm state to feeling furious with the urge to yell or hit with minimal provocation. “Irritability” is related more to chronic feelings of annoyance, lack of patience, and hypersensitivity to provocation. Assaults include slamming doors, yelling angrily, saying nasty things, hitting, or actual fighting. The AIAQ was administered prior to starting IFN- γ , and then at weeks 2, 4, and 8. Completion and scoring was done blinded to phospholipid fatty acid assessments.

Because many subjects develop major depression (diagnosed using a mood disorder focused Structured Clinical Interview for DSM-IV Axis I Disorders) (SCID-IV) (85) and were started on antidepressants (which may affect AIAQ scores), we only compared scores during the first 8 weeks of treatment. The AIAQ could be completed during scheduled office visits or could be returned by mail (86). The hepatology teams treating these patients were also encouraged to contact the research team any time psychiatric symptoms were concerning (including development of suicidal thoughts, assaultive behaviors, or worsening psychosocial function) -- which would prompt a mood-disorder focused SCID-IV interview and treatment if necessary.

Phospholipid Fatty Acid Assessment

Pre-treatment plasma fatty acid levels were determined as previously described (43) using an HP 6890/5973 gas chromatograph/mass selective (GC/MS) detector (Agilent Technologies, Santa Clara, CA), and identified based on retention times of authenticated fatty acid methyl ester standards (GLC 473B) and controls (GLC 462 and GLC 463) (NuCheck Prep, Elysian, MN). Prior to GC/MS analysis, total lipids were extracted from plasma (obtained between 10AM and 4PM, non-fasting and random with respect to prior food consumption, and initially stored at -80°C) using Folch reagent and reconstituted with chloroform. These were washed with chloroform and then acetone using a reversed-phase packed SPE column (Alltech, Nicholasville, KY). This phospholipid sample was then eluted with methanol and subsequently methylated using NaOH/MeOH, followed by BF_3/MeOH followed by heating for 15 minutes at 85°C . Hexanewas used to extract the methylated fatty acids for GC/MS analysis. Levels of AA, DLGA, EPA and DHA were quantified and are expressed as weight percent of the total fatty acid pool. Our primary focus was on the AA/EPA+DHA ratio based on previous findings regarding depression (43).

Genotyping

The G-308A polymorphism for TNF- α was assessed as previously described (35), comparing those with the A allele (A/A and A/G) and those with the G/G genotype.

Statistical Analyses

All statistics employed SPSS 20.0. Results are reported as mean $\pm$ standard deviation, and in graphs are presented as mean $\pm$ standard error of the mean. To compare changes in AIAQ subscales over time, we divided the subjects by a median split into those with “high” and “low” AA/EPA+DHA. Repeated-measure mixed-effect analyses, robust to randomly missing data, were used to compare those with high vs. low AA/EPA+DHA. We selected covariance structures that provided the smallest Aikake Information Criteria (AIC), which was typically unstructured unless otherwise indicated.

Seventeen subjects developed clinical problems with irritability and anger that did not meet criteria for major depression, but which did result in either stopping IFN- γ therapy or initiating psychiatric medication. We explored the time until this non-MDD “irritable anger” disorder using Cox regression. To examine whether selective serotonin reuptake inhibitor (SSRI) treatment might influence AIAQ scores, we next compared irritability in 18 euthymic adults who were taking open-label SSRIs prophylactically for depression prevention with the above 82 subjects.

RESULTS

Average AA/EPA+DHA ratios were 21.6 ± 16.3 , and ranged from 4.8 to 121.6. As seen in Table 1, most of this variability arose from differences in DHA – which ranged from 0.05% to 1.88% of the total fatty acid pool. Older subjects and those who self-report African-American ancestry tended to have lower AA/EPA+DHA ratios (Table 1), and both these variables were therefore included as co-variates in subsequent analyses. The subjects were generally healthy with few medical illnesses other than HCV, as reflected in their Cumulative Illness Rating Scale- Geriatric (CIRS-G) scores, although we did not assess dietary influences on fatty acid levels. All had similarly low Montgomery-Asperg Depression Rating Scale (MADRS) scores at baseline. Prior to administration with the exogenous cytokine, AA/EPA+DHA levels were neither associated with irritability nor with labile anger scores (Table 1).

Baseline AA/EPA+DHA correlated with the maximal increase (above pre-treatment baseline) in labile anger during subsequent treatment ($r=0.33$; $B=0.08 \pm 0.03$; $p=0.005$) and trended towards a correlation with maximal increase in irritability ($r=0.2$; $p=0.09$). The association with maximal increase in labile anger continued even when controlling for MADRS scores ($B=0.1 \pm 0.03$; $p=0.002$), while the association with irritability remained a trend ($B=0.05 \pm 0.03$; $p=0.07$). Thus, depression does not appear to mediate these associations. In repeated-measure analyses, those with high (above median) AA/EPA+DHA had both a greater increase in irritability and labile anger during IFN- γ treatment than those with low (below median) AA/EPA+DHA ($F_{3,55.6} = 4.0$; $p=0.01$ and $F_{3,40.3} = 3.2$; $p=0.03$, respectively) (Figure 1). These results were best fit using a first-order ante-dependence covariance structure, but were robustly consistent when using unstructured, Toeplitz, and heterogeneous compound symmetry covariance structures. When including race and age as covariates, the relationship between AA/EPA+DHA and irritability was more pronounced ($F_{3,44.0} = 5.4$; $p=0.003$). We next also included MADRS scores as a covariate to determine if depression could explain the findings. The association of higher AA/EPA+DHA and worsening irritability during IFN- γ treatment continued to be present ($F_{3,45.3} = 2.9$; $p=0.04$), although the relationship with labile anger lost statistical significance ($F_{3,16.6} = 1.5$; $p=0.2$).

We next assessed whether self-reported changes in actual assaultive behaviors during IFN- γ treatment were associated with pre-treatment AA/EPA+DHA (Figure 2). Interestingly, both verbal and direct assaults (e.g. yelling, slamming doors and banging tables) decreased during IFN- γ therapy ($F_{3,41.4} = 4.0$; $p=0.01$; $F_{3,40.1} = 4.1$; $p=0.01$, respectively), possibly a result of subjects becoming more fatigued. Thus the total AIAQ score (summing labile anger, irritability, and total assaults) also went down over time ($F_{3,20.2} = 4.3$; $p=0.01$). Nonetheless, those with higher AA/EPA+DHA ratios had more self-reported verbal and direct assault behaviors throughout treatment ($F_{1,71.2} = 5.4$; $p=0.02$; $F_{1,71.9} = 6.6$; $p=0.01$), consistent with their elevated anger and irritability scores. Total assaults scores in the high AA/EPA+DHA group decreased during IFN treatment from 22.8 ± 1.6 to 19.4 ± 1.8 by week eight, compared to going from 18.4 ± 1.6 to 15.5 ± 1.7 in the low AA/EPA+DHA group; and the overall change in self-reported assaults over time was similar in both ‘high’ and ‘low’ fatty acid groups ($F_{3,40.7} = 0.6$; $p=0.6$). We next individually explored DHA, EPA, or AA in

similar analyses of labile anger and irritability. There was no consistent relationship of any of these fatty acids and anger -- when assessed singly.

As a measure of anger and irritability, the AIAQ is limited by being a self-report. However, 17 of these subjects had IFN- γ treatment interrupted by their hepatologist and/or were started on psychiatric medications for “irritable anger” -- and not for SCID-IV diagnosed depression, and blind to AIAQ results. The most common concerns were severe spousal or vocational discord related to “irritable anger.” We therefore examined time until treatment interruption/psychiatric intervention for “irritable anger” for these 17 subjects. These subjects did not meet criteria for a major depressive episode (assessed using an abbreviated SCID-IV interview), nor for a hypomanic/manic episode, nor for generalized anxiety disorder, nor was treatment interrupted for medical reasons. Using Cox Regression analysis, the time until clinically significant “irritable anger” was strongly associated with baseline AA/EPA+DHA ($B=0.02 \pm 0.008$; $p=0.009$). The area-under-the-curve (AUC) for the relationship between AA/EPA+DHA and this “irritable anger disorder” was 0.67 ± 0.07 , and is evident in a Kaplan Meier plot (Figure 3), whereby only 3/17 subjects with low pre-treatment AA/EPA+DHA developed this behavioral complication (Mantel-Cox log rank $\chi^2=7.9$; $p=0.005$).

We have previously observed that labile anger during IFN- γ treatment is also associated with a TNF- α promoter polymorphism (35). As seen in Figure 4, there is a three-way interaction between time, the TNF- α polymorphism, and AA/EPA+DHA ($F_{3,26,6}=6.9$; $p=0.001$). Those with both the A (higher expressing) allele and elevated AA/EPA+DHA were most at risk for worsening labile anger during IFN- γ therapy. The other three groups were much less vulnerable to this side effect.

We also followed an additional 18 subjects who were taking an SSRI antidepressant on a stable dose for at least three months prior to starting IFN- γ therapy (Figure 5). Each of these 18 patients was euthymic at baseline but had histories of major depression and had elected to start or remain on an antidepressant prior to IFN- γ treatment. Comparing these 18 subjects already on SSRIs with the 82 subjects who were not taking any antidepressants, we observing that SSRIs were associated with *worse* irritability scores during IFN- γ treatment ($F_{1,164.5}=6.3$; $p=0.01$), despite no differences in baseline fatty acid levels.

DISCUSSION

During IFN- γ therapy, the AA/EPA+DHA ratio was positively associated with worsening labile anger and irritability, which is consistent with prior studies of fatty acids and anger (52). Depression was not a likely mediator, as the association with anger was not influenced when co-varying for depression. Also, there was minimal association of fatty acids with irritability and anger at pre-treatment baseline. The associations only became manifest during the administration of high doses of the exogenous cytokine, PEG-IFN- γ . This would be in line with a role for the resolvin and protectin metabolites of LC $n-3$ fatty acids in resolving inflammation-related processes (87, 88). Similarly, DHA supplements may have no effect on aggression in non-stressful situations, but have been effective for alleviating stress-induced aggression (68).

Better defining what this “irritable anger” syndrome represents will require future employment of more than a single self-report questionnaire – a limitation of this study. There could be multiple overlapping facets to hostility, irritability, aggression, and anger. In the AIAQ, labile anger is defined by questions related to sudden transition from a calm state to feeling furious with the urge to yell or hit with minimal provocation (84). Conversely, irritability is related more to chronic feelings of annoyance, lack of patience,

and hypersensitivity to provocation. Some have suggested that anger could represent a depression subtype (2, 33). However, it doesn't always occur in the present of depression symptoms (34). Moreover, both irritability and labile anger can occur across a variety of diagnoses including depression, mania, anxiety disorder, intermittent explosive disorder, personality disorders, and various childhood disorders (1). Although we rarely observed co-occurring grandiosity or increased goal-directed activities during IFN- treatment, some clinicians have also referred to a mixed-mood disorder with hostility (89, 90) during IFN-therapy. Congruent with this possibility, labile mood and irritability may be related to risk for bipolar disorder in other populations (91–97), which is consistent with our open-label observations that SSRIs could possibly exacerbate this side effect. That is, SSRIs can potentially trigger agitation in those at risk for bipolar disorder (98, 99). Regardless of its exact phenomenology however, this “irritable anger” syndrome became clinically significant in a subset of subjects, resulting in discontinuation of IFN- therapy and/or starting a psychiatric medication (typically an antidepressant or a mood stabilizer).

We initially excluded subjects taking SSRIs from these analyses because we hypothesized that SSRIs might prevent the labile anger and irritability (82), and thus confound our ability to observe a relationship with AA/EPA+DHA. However, when we explored whether this was true or not, we found the opposite. That is, SSRI use was associated with worsening irritability during IFN- therapy. This is congruent with observations of elevated irritability following antidepressant use in the children of parents with bipolar disorder (83). If our open-label observation of increased irritability is replicated in a controlled, randomized and blinded manner, then this may be one contra-indication to SSRI use in this population. It is important to note that we cannot conclude that there is a causal link between SSRIs and increased irritability. Ours was an unexpected observation of only 18 patients, and people prescribed SSRIs could be uniquely predisposed to develop irritability -- whether on SSRIs or not. Nonetheless, there is support for a trial of LCn-3 fatty acid supplementation to address irritability and labile anger in these patients (100) – which would be an alternative to otherwise effective SSRI depression prophylaxis (101–104). Recently, supplementation with EPA was associated with improved anti-viral outcome during IFN- therapy (105).

Similar to elevated AA/EPA+DHA, the A allele in the TNF- A-308G polymorphism has previously been associated with increased risk for labile anger (35). TNF- levels in the brain can be influenced by stress or peripheral inflammation (106–108), and both DHA and its metabolite 17-hydroxy-DHA are known to diminish induced TNF- production in macrophages (79, 80). The A-308G polymorphism may affect the binding of some transcriptional regulators (109–113). Congruent with an effect of IFN- on local cytokines within the hippocampus (114), the A allele has been associated with increased cognitive problems even without evidence for differences in peripheral TNF- levels (115). Here we found novel evidence that only those with both elevated AA/EPA+DHA and the TNF- A allele are at risk for increased irritability.

Which neurochemical pathway mediates fatty acid influences on irritability and anger remains speculative. Consistent with a relationship between the serotonin (5-HT) system and aggression, lower LCn-3 levels also correlate with lower CSF 5-HT metabolites (116), and administration of LCn-3 can influence 5-HT levels in the brain (117). There are also potential developmental effects of LCn-3 on 5-HT systems (118, 119). Deficiencies in LCn-3 fatty acids in rats and subsequent elevations in the AA/EPA+DHA ratio may not only increase inflammatory cytokine production (77, 120, 121) but also result in changes in central serotonin turnover (81, 122). Supporting a relationship between inflammation and 5-HT, inhibiting cyclooxygenase-2 (COX-2) decreases inflammation-induced increases in extra-cellular hippocampal 5-HT (123). A second possibility for both LCn-3 and TNF- involves glutamate signaling. Enhanced production of quinolinic acid and kynurenate may

mediate some psychiatric effects of IFN- γ (124, 125) via their effects on glutamate transmission. LCn-3 supplementation can reverse age-related changes in glutamate receptor subunits (126). LCn-3 deficiency can affect receptor subunit expression (127). DHA can be converted in the brain to docosahexaenoyl ethanolamide, which has effects on synaptogenesis and glutamate receptor subunit expression (128). DHA can prevent the effect of APOe4 on presynaptic glutamate transporters (129). And LCn-3 fatty acids may slow down the rate of glutamate reuptake by astrocytes (130). A third possibility involves the regulation of brain-derived neurotrophic factor (BDNF) by LCn-3 fatty acids through a mitogen associated protein-kinase mechanism (131, 132), which is potentially relevant given the plausible role of BDNF in IFN- γ -associated depression (133) and bipolar symptoms (134).

Regardless of mechanism, DHA was less than 2% of the total fatty acids in all 82 subjects, as compared to countries such as Spain where median levels are about 6% (135) and unhealthy levels are likely below 4% (136). Although the average AA levels in these subjects were not grossly abnormal (137), diet is likely a critical reason for differences in AA/EPA+DHA (138). Western diets often have a ratio of LCn-6 to LCn-3 fatty acids of about 10:1 to 20:1, compared to an estimated historical diet of 1:1 (139). In addition, low levels of DHA have also been associated with low folate (54) and alcohol use (140). However, three limitations of this study are that (i) we did not assess diet or other contributors (e.g., genetic polymorphism and exercise) to fatty acid levels, (ii) we did not obtain prospective measures of fatty acids during IFN- γ treatment, (iii) and the levels reported are percentages of total fatty acids. Thus we cannot definitely state why the DHA percentages were so low, nor whether they further changed during IFN- γ therapy. Also, both AA and the LCn-3 fatty acid 'levels' are defined as a percentage of the total fatty acid pool. If other fatty acids (including medium chain and other long chain fatty acids) increase, then the percentage of DHA and AA goes down. When assessing the role of individual fatty acids, we did not consistently find a relationship with anger. As with depression (44–48), it may be the AA/EPA+DHA ratio that is therefore important.

Additional strengths of the study include prospective assessments of the change in irritability and labile anger over time, and accounting for potentially confounding variables such as race, age, depression symptoms, and antidepressant use. However, whether these findings can be generalized to other inflammatory conditions and increased anger/irritability/hostility, for example vascular disease (22, 23), remains unknown. And several questions remain to be addressed. (i) Could LCn-3 supplementation prevent irritability and labile anger, specifically in the setting of increased inflammatory cytokines? More specifically, might LCn-3 supplementation prevent both depression and irritable labile anger during IFN- γ therapy – a potential advantage over SSRIs? (ii) What are the influential relationships between AA/LCn-3 ratios and pro-inflammatory cytokines such as TNF- α and IL-1 in the brain? The exploratory finding of an interaction between the TNF- α promoter polymorphism and AA/EPA+DHA suggests this as a possibility. (iii) Are associations with anger and irritability mediated by glutamatergic and/or serotonergic mechanisms?

A number of other polymorphisms may have relationships to depression and fatigue during settings of increased IFN- γ and inflammation (141–146). However, genetic influences on irritability/anger have not been as well characterized. Nonetheless, these results add to a growing literature that indicates a robust relationship between low LCn-3 levels and irritability/anger. Specifically, we find that the association between fatty acids (AA/EPA+DHA) and vulnerability for irritability/anger may be critically important in the setting of increased inflammation. Although publication bias may be a reason, there are few published examples that contradict this association. Additionally, our findings indicate that this role may be most important in those with the higher expressing A allele in the TNF- α gene.

Because anger and irritability appear to be clinically important adverse effects of IFN-therapy, preliminary exploratory findings suggest that there could be an advantage of LC α -3-based therapies over SSRIs in treating subjects who are on IFN-therapy.

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Figure 1.

Mean scores from the Anger Irritability and Assault Questionnaire (AIAQ), during treatment with interferon- α . Scores worsened more in those with above median ratios (squares) of arachidonic acid (AA) to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) compared to those with below median ratios (circles) for both irritability (Panel A; $F_{3,55.6} = 4.0$; $p=0.01$) and labile anger (Panel B; $F_{3,40.3} = 3.2$; $p=0.03$).

Figure 2.

Mean scores from the Anger Irritability and Assault Questionnaire (AIAQ), during treatment with interferon- α . Scores were higher during treatment in those with above median ratios (squares) of arachidonic acid (AA) to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) compared to those with below median ratios (circles) for both verbal assaults (Panel A; $F_{1,71.2} = 5.4$; $p = 0.02$) and direct assaults (Panel B; $F_{1,71.9} = 6.6$; $p = 0.01$).

Figure 3.

The development of irritable anger resulted in either discontinuing treatment and/or initiating psychiatric medication in 17 subjects. 14/17 of those subjects had above median (high) ratios of arachidonic acid (AA) to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), compared to the 3/17 with below median ratios (Mantel-Cox log rank $\chi^2=7.9$; $p=0.005$).

Figure 4.

Increases in labile anger during interferon- therapy are most evident in those with the A allele in the -308 promoter region of the tumor necrosis factor- gene who also had above median ratios (squares) for arachidonic acid (AA) to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), compared to those with below median ratios (circles). There is a three-way interaction between time, the TNF- polymorphism, and AA/EPA+DHA ($F_{3,26.6} = 6.9$; $p = 0.001$).

Figure 5. Individuals who were already taking selective serotonin reuptake inhibitors (n=18, squares) had greater irritability during interferon- therapy than those not taking antidepressants (n=82, circles) ($F_{1,164.5} = 6.3$; $p = 0.01$).

Table I

	<u>Low</u> <u>AA/EPA+DHA</u>	<u>High</u> <u>AA/EPA+DHA</u>	
Female	33%	32%	N.S.
African-American	29%	9%	P=0.004
Age	52.2 +/- 9.0	44.6+/- 10.9	P<0.001
Weight (Kg)	85.8 +/- 18.8	84.5 +/- 14.8	N.S.
MADRS	2.8 +/- 3.8	3.1 +/- 3.6	N.S.
History of depression	21%	22%	N.S.
CIRS-G total	4.3 +/- 1.9	3.6 +/- 1.7	N.S.
AIAQ total	31.8 +/- 18.3	37.4 +/- 16.7	N.S.
Irritability score	8.8 +/- 5.1	10.5 +/- 5.2	N.S.
Labile Anger score	4.4 +/- 3.8	4.1 +/- 3.8	N.S.
AA	10.1 +/- 2.2	9.7 +/- 2.6	N.S.
EPA	0.12 +/- 0.09	0.08 +/- 0.04	P=0.02
DHA	0.83 +/- 0.34	0.32 +/- 0.16	P<0.001
AA/EPA+DHA ratio	12.1 +/- 3.4	30.3 +/- 18.5	P<0.001

Prior to starting IFN- γ , subject demographics, Montgomery-Asperg Depression Rating Scale (MADRS), Cumulative Illness Rating Scale-Geriatric (CIRS-G), Anger, Irritability, and Assault Questionnaire (AIAQ), and the ratio of arachidonic acid (AA) to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).