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# ORIGINAL ARTICLE

# The relationship between folate and docosahexaenoic acid in men

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**Objective:** Docosahexaenoic acid (DHA, 22:6*n*-3), an essential omega 3 fatty acid, may protect against disorders of emotional regulation as well as cardiovascular disease. Animal studies demonstrate that dietary folate can increase tissue concentrations of DHA, although the literature, to date, includes no human studies examining the possibility that folate status may affect plasma DHA concentrations. The objective of this study is to determine if the blood concentrations of folate and DHA are correlated in humans.
**Design:** Retrospective study.
**Setting:** An American research hospital.
**Subjects:** A total of 15 normal and 22 hostile and aggressive subjects, with a mean age of 38 years.
**Methods:** Concentrations of plasma polyunsaturated essential fatty acids and red blood cell folate (RBC folate) were obtained prior to 1996, before American flour was enriched with folate.
**Results:** RBC folate was significantly correlated with plasma DHA, $r = 0.57$, $P = 0.005$ in the aggressive group. Age, smoking and alcohol consumption did not alter the results. No other essential fatty acids were significantly associated with RBC folate in either group.
**Conclusions:** The positive relationship between plasma DHA and RBC folate concentrations suggests that these two nutrients should be examined together in order to make the most accurate inferences about their relative contributions to disease pathogenesis. Our findings present one explanation why some conditions associated with hostility and low DHA status, such as cardiovascular disease and emotional disorders, are also associated with low folate status.
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**Keywords:** RBC folate; docosahexaenoic acid; folic acid; *n*-3 fatty acids; phosphatidylethanolamine-*N*-methyltransferase

## Introduction

There is growing recognition that long-chain omega-3 polyunsaturated fatty acids (*n*-3 PUFA) reduce the risk of emotional dysregulation (Hibbeln and Makino, 2002) as well as cardiovascular disease (Kris-Etherton *et al*., 2002). The *n*-3 PUFA docosahexaenoic acid (DHA), found in fish oil, is selectively concentrated in nervous tissue, where it influences membrane bound receptor function and cell signaling (Salem *et al*., 2001). Animal studies suggest that an important modulator of tissue DHA status could be the intake of folic acid. For example, rats fed supplemental folate for 15 days had significantly increased concentrations of DHA in platelet, erythrocyte and intestinal phospholipids (Pita and Delgado, 2000). Conversely, rats fed 6 weeks on a low folate diet had decreased concentrations of DHA in plasma and platelets (Durand *et al*., 1996). Most importantly, dietary folate deficiency has been found to cause the depletion of DHA in rat nervous tissue (Hirono and Wada, 1978).

If folate status influences DHA concentrations in humans as it does in rats, then folate supplements could be a

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significant way to increase tissue DHA concentrations in humans. As a first step in exploring this possibility in humans, we sought to determine if DHA and folate concentrations are correlated. Such a relationship has been suggested by others (Quere *et al*., 2002), but has never been reported in humans. We studied a subject group in which DHA concentrations may be critical: individuals with a history of aggressive behavior. Low plasma concentrations of DHA are associated with aggressive behavior (Virkkunen *et al*., 1987; Iribarren *et al*., 2004), while supplementation with *n*-3 PUFA can improve emotional regulation. *N*-3 PUFA has been shown to reduce hostility in students (Hamazaki *et al*., 1996), aggressive outbursts in borderline personality disorder patients (Zanarini and Frankenburg, 2003), hospitalization rates in bipolar patients (Stoll *et al*., 1999a, b) and felony level violence in British prisoners when combined with multiple vitamins (Gesch *et al*., 2002).

We were particularly interested in studying hostile and aggressive males because of their increased risk for cardiovascular disease and depression, disorders which may benefit from increased DHA status (Williams, 1987; Hibbeln and Salem, 1995). We hypothesized that plasma DHA concentrations would be positively associated with the concentration of folate in red blood cells.

## Subjects

This study was conducted by reviewing data from a larger study of domestic violence which was conducted at the National Institutes of Health Clinical Center in Bethesda, Maryland. The study took place prior to 1996, before the government mandated fortification of enriched flour with folic acid. All participants were healthy males, as confirmed by a normal physical exam, clinical laboratory studies and electrocardiogram. Subjects were on no medications or dietary supplements. The aggressive subjects were selected based on their history of inflicting repeated acts of physical violence towards a significant other while control subjects had no such behavior. Aggressive subjects were recruited with newspaper advertisements seeking people who 'lose control and are violent towards their spouse or significant other'. All subjects were admitted to the hospital for study, and signed an informed consent as approved by the National Institute on Alcohol Abuse and Alcoholism Institutional Review Board.

## Methods

We measured folic acid bound within red blood cells (RBC folate) to avoid variations in plasma folate due to transient dietary changes. At the time of admission, blood was obtained for measurement of RBC folate after overnight fasting in the hospital. RBC folate was measured by radioassay using the Ciba Corning Diagnostic Corp 'Magic' competitive protein-binding assay in which folate from the patients sample is mixed with a constant amount of [$^{125}$I]folate. Fasting plasma fatty acid composition was determined from plasma stored at $-70^{\circ}$C for batch analysis. Specific fatty acids were quantified using gas chromatography and internal standards (Hibbeln *et al*., 1998). To evaluate possible confounding effects of social status and behavior on DHA and folate intake (Singhal *et al*., 1998), we used the Hollingshead Socioeconomic Index (Hollingshead and Redlich, 1958) to quantify socioeconomic status (SES), the Hamilton Depression Scale (Hamilton, 1967) to quantify depression and the Buss Durkee Hostility Index (Buss and Durkee, 1957) to quantify hostility. Alcohol and cigarette consumption can potentially affect both DHA and folate (Simon *et al*., 1996; Durand *et al*., 1998) and consumption of both were carefully quantified from interviews with the subjects. To minimize the possible confounding effect of alcohol consumption, individuals who reported regular consumption of alcohol were abstinent for 3 weeks prior to the fatty acid determination. Other potentially confounding variables, including age, BMI and liver function, as measured by alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were recorded.

Statistical analyses were computed using STATISTICA for Windows 6.0 (Statsoft, Tulsa, OK, USA). Correlations between RBC folate and each polyunsaturated fatty acids as well as the other factors (age, BMI, liver function, hostility, depression, SES, cigarette consumption and days of alcohol use) were tested. Correlations with $P<0.01$ were considered significant.

## Results

Our sample consisted of 15 male healthy volunteers and 22 aggressive males (Table 1). The only variables that differed significantly between groups were the Buss Durkee Hostility Index and the Hamilton Depression Scale, which were both greater in the aggressive group. Correlation matrices were examined to determine if RBC folate concentrations were related to the concentration of DHA or other variables (Table 2). Among the control group, there were no significant correlations. Among the aggressive group RBC folate concentrations were positively correlated with DHA concentrations ($r=0.57$) (Figure 1). No other *n*-3 or *n*-6 polyunsaturated fatty acids were significantly correlated with RBC folate concentrations in either group. We also examined the relationship between folate and DHA when DHA was expressed as a percentage of the weight of total fatty acids and found this to be significant only in the aggressive group ($r=0.58$, $P=0.005$). The potentially confounding factors of age, BMI, smoking, ALT, AST, Holingshead Index, Buss Durkee Hostility Index, Hamilton Depression Index, cigarettes per day and frequency of alcohol consumption over the past 180 days were not correlated with either the concentration of DHA or RBC folate in either group.

**Table 1** Subject characteristics[a]

| | *Control group (n = 15)* | *Aggressive group (n = 22)* |
|---|---|---|
| Age (years) | 37.5 ± 8.4 | 38.5 ± 7.2 |
| BMI (kg/m$^2$) | 25.7 ± 4.4 | 26.9 ± 3.0 |
| Hostility[b,c] | 15.6 ± 7.8 | 42.5 ± 13.9 |
| Depression[b,d] | 1.7 ± 2.3 | 8.4 ± 6.6 |
| S.E.S[e] | 4.3 ± 0.6 | 3.6 ± 1.4 |
| Cigarettes/day | 1 ± 3.9 | 11.6 ± 14.0 |
| Days alcohol used in past 180 | 6.1 ± 8.1 | 47.5 ± 66.6 |
| ALT (U/l) | 24.6 ± 11.8 | 25.6 ± 20.7 |
| AST (U/l) | 20.3 ± 6.1 | 20.5 ± 10.5 |
| 18:2 *n*-6, LA (mmol/l) | 1.896 ± 0.527 | 1.936 ± 0.302 |
| 18:3 *n*-6, GLA (mmol/l) | 0.036 ± 0.027 | 0.035 ± 0.014 |
| 20:3 *n*-6, DGLA (mmol/l) | 0.083 ± 0.033 | 0.086 ± 0.023 |
| 20:4 *n*-6, AA (mmol/l) | 0.414 ± 0.093 | 0.359 ± 0.104 |
| 22:4 *n*-6, AdrA (mmol/l) | 0.014 ± 0.006 | 0.014 ± 0.0047 |
| 22:5 *n*-6, ObA (mmol/l) | 0.011 ± 0.004 | 0.011 ± 0.003 |
| 18:3 *n*-3, ALA (mmol/l) | 0.044 ± 0.027 | 0.035 ± 0.013 |
| 20:5 *n*-3, EPA (mmol/l) | 0.035 ± 0.026 | 0.026 ± 0.015 |
| 22:5 *n*-3, DPA (mmol/l) | 0.029 ± 0.011 | 0.024 ± 0.007 |
| 22:6 *n*-3, DHA (mmol/l) | 0.097 ± 0.049 | 0.078 ± 0.034 |
| RBC folate (ngl/l) | 1050 ± 370 | 886 ± 252 |

[a]Mean ± s.d. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LA, linoleic acid; GLA, $\gamma$-linolenic acid; DGLA, dihomo-$\gamma$-linolenic acid; AA, arachidonic acid; AdrA, adrenic acid; ObA, Osbond acid; ALA, $\alpha$-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
[b]Significant Mann–Whitney *U* comparisons between groups $P < 0.01$.
[c]Hostility was measured by the Buss Durkee Hostility Index (Buss and Perry, 1992).
[d]Depression was measured by the Hamilton Depression Scale (Hamilton, 1967).
[e]Socioeconomic status (SES) was measured by the Hollingshead Socioeconomic Index (Hollingshead and Redlich, 1958).

**Table 2** Correlations of red blood cell folate with plasma polyunsaturated fatty acids

| *Polyunsaturated fatty acid concentration* | *Control group (n = 15)* | | *Aggressive group (n = 22)* | |
|---|---|---|---|---|
| | r-*value* | P | r-*value* | P |
| 18:2 *n*-6, LA (mmol/l) | 0.06 | NS | −0.41 | NS |
| 18:3 *n*-6, GLA (mmol/l) | 0.26 | NS | 0.04 | NS |
| 20:3 *n*-6, DGLA (mmol/l) | −0.07 | NS | 0.02 | NS |
| 20:4 *n*-6, AA (mmol/l) | −0.04 | NS | 0.05 | NS |
| 22:4 *n*-6, AdrA (mmol/l) | 0.08 | NS | 0.15 | NS |
| 22:5 *n*-6, ObA (mmol/l) | −0.12 | NS | 0.09 | NS |
| 18:3 *n*-3, ALA (mmol/l) | 0.09 | NS | −0.17 | NS |
| 20:5 *n*-3, EPA (mmol/l) | 0.19 | NS | 0.19 | NS |
| 22:5 *n*-3, DPA (mmol/l) | 0.10 | NS | 0.26 | NS |
| 22:6 *n*-3, DHA (mmol/l) | 0.27 | NS | 0.57 | 0.005 |

LA, linoleic acid; GLA, $\gamma$-linolenic acid; DGLA, dihomo-$\gamma$-linolenic acid; AA, arachidonic acid; AdrA, adrenic acid; ObA, Osbond acid; ALA, $\alpha$-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; NS, not significant at $P < 0.01$.

## Discussion

As predicted, we found a positive correlation between the concentrations of DHA and folate in a group of hostile and aggressive men. Potentially confounding factors could not account for this association. Since we did not evaluate dietary intake, we cannot rule out the possibility that individuals who consumed foods with higher concentrations of folate (e.g., green leafy vegetables) also consumed foods with higher concentrations of DHA (e.g., seafood). However, if this were the case, we would expect to find a correlation of RBC folate with eicosapentaenoic acid, a marker of fish consumption, but we did not. A previous study found that DHA supplementation had no effect on human folate status (Grundt *et al*., 2003), suggesting that our results are most consistent with the hypothesis that in some individuals, plasma DHA concentrations are influenced by variations in folate status.

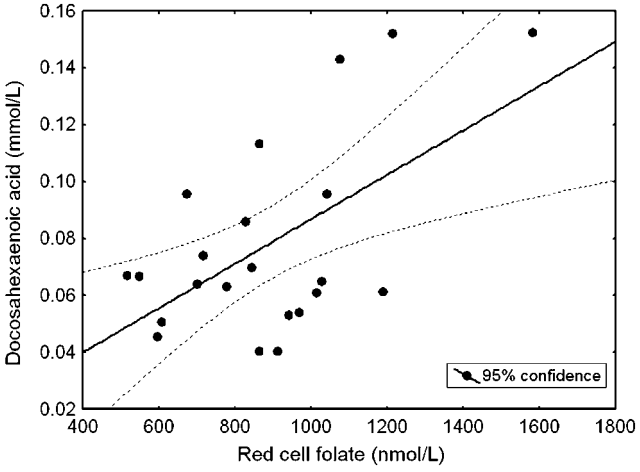


**Figure 1** Correlation of plasma RBC folate and docosahexaenoic acid concentrations in hostile men.

Durand *et al*. (1996) have suggested a mechanism by which folate may affect DHA. Folate can reduce the generation of reactive oxygen species by decreasing homocysteine and subsequent homocysteine auto-oxidation. Since highly allylic polyunsaturated fatty acids such as DHA have a greater propensity for lipid peroxidation (Cosgrove *et al*., 1987), the reduced oxidative stress promoted by folate may spare DHA, resulting in DHA accumulation. This mechanism is supported by a recent study suggesting that the reduced concentrations of PUFA in children with attention-deficit/hyperactivity disorder is due to the selective oxidation of *n*-3 PUFA (Ross *et al*., 2003). However, this mechanism does not explain why DHA is selectively spared.

An alternate mechanism by which plasma DHA concentrations are selectively increased by folate may involve the ability of dietary folate to provide methyl groups to the liver. Methyl groups may be critical for the release into the plasma of DHA stored in the liver. *S*-adenosyl-L-methionine (SAMe) is an important methyl donor formed when the folate derivative 5-methyltetrahydrafolate transfers a methyl group to homocysteine. SAMe can transfer methyl groups to phosphatidylethanolamine (PE) yielding

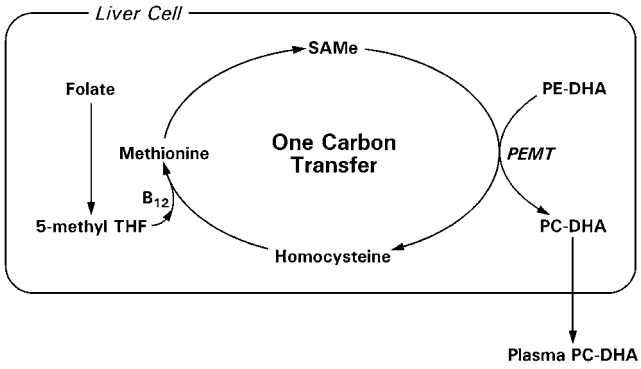


**Figure 2** Methyl transfer in the liver producing phosphatidylcholine-DHA is critical for mobilization of DHA into the blood. Dietary folate is converted in the body to 5-methyl tetrahydrofolate (5-methyl THF). Methyl transfer from 5-methyl THF to homocysteine requires vitamin $B_{12}$ and results in the synthesis of methionine. Methionine is converted into *S*-adenosyl-L-methionine (SAMe). Methyl groups from SAMe are transferred by phosphatidylethanolamine-*N*-methyltransferase (PEMT) to ethanolamine in a series of steps that convert it to choline and produce homocysteine. In this way, liver DHA incorporated into phosphatidylethanolamine is transformed into the nonpolar phosphatidylcholine-DHA. DHA which undergoes this process can be released from the liver into the plasma.

phosphatidylcholine (PC), a reaction catalyzed by phosphatidylethanolamine-*N*-methyltransferase (PEMT). This reaction increases the ratio of PC to PE in biological membranes, an important change which can make membranes more fluid and modify receptor function (Hirata and Axelrod, 1980). In the liver, the conversion of phospholipid from PE to PC (via SAMe and PEMT) is critical for DHA mobilization from the liver into the plasma (Figure 2). When this reaction does not occur normally, as seen in PEMT knockout mice (and possibly in folate deficiency), plasma concentrations of DHA are selectively decreased (Watkins *et al*., 2003). Since heavy alcohol intake may cause SAMe depletion in the liver (Lieber, 2000), folate availability may be especially critical for the creation of PC from PE in subjects who drink alcohol. This could reduce DHA mobilization from the liver into the plasma, causing tissue depletion of DHA. This effect may explain why the correlation was noted in our aggressive group and not the control group, and should be explored in future studies.

It is interesting to note that many disorders which have been associated with low levels of DHA (or low fish consumption) have also been associated with low folate status, including cardiovascular disease (Loria *et al*., 2000; Kris-Etherton *et al*., 2002), depression (Hibbeln and Salem, 1995; Alpert *et al*., 2000), Alzheimer disease (Wang *et al*., 2001; Morris *et al*., 2003), stroke (Bazzano *et al*., 2002; Skerrett and Hennekens, 2003) and breast cancer (Terry *et al*., 2003; Zhang *et al*., 2003). In addition, a number of disorders suggested to benefit from treatment with fish oil are associated with low folate or with a marker of low folate status, homocysteine (Jacob *et al*., 1994). These disorders include Crohn's disease (Belluzzi *et al*., 1996; Chowers *et al*., 2000), rheumatoid arthritis (Kremer *et al*., 1987; Schroecksnadel *et al*., 2003), mania (Hasanah *et al*., 1997; Stoll *et al*., 1999a, b) and hostility (Stoney and Engebretson, 2000; Iribarren *et al*., 2004). One study suggests that folate, like fish oil, can lower triglycerides, LDL and cholesterol (Connor *et al*., 1993; McGregor *et al*., 2000). It should be noted that some of these disorders exist in comorbid association with each other (Severus *et al*., 2001; Honig *et al*., 2003). It is intriguing to consider whether some of the health benefits associated with national policies to enrich grain with folate may be mediated, in part, by improvements in DHA status. Alternatively, DHA and folate might act synergistically to benefit a number of these conditions (de Bree *et al*., 2004). It would be interesting to investigate the possibility that DHA levels are low in babies born with neural tube defects, a developmental malformation associated with folic acid deficiency.

In conclusion, we demonstrated an association between the concentrations of DHA and RBC folate in aggressive men. Further studies investigating the interactions of DHA and folate are needed. For example, cell culture and animal studies should consider the effects of folate on DHA syntheses and catabolism as well as on tissue and cellular membrane distributions. Human studies could evaluate the effect of supplemental folate on DHA status, and consider other factors which may effect this interaction such as vitamin $B_{12}$. A larger study could also evaluate the importance of aggressive and hostile traits for this association. Our results suggest that by considering the possibility of an interaction between DHA and folate, it may be possible to make more accurate inferences about the respective roles of each in human pathology. The many disorders in which folate and DHA have both been found to be abnormally low suggest that we have much to learn about the relationship between these two essential nutrients.

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