# Blood Phospholipid Fatty Acid Analysis of Adults With and Without Attention Deficit/Hyperactivity Disorder

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**ABSTRACT:** Several psychiatric disorders, including juvenile Attention Deficit/Hyperactivity Disorder (ADHD), have been associated with abnormalities of certain long-chain PUFA (LCPUFA). Despite this reported association, the FA levels of patients with the adult form of ADHD have not previously been evaluated. In this study we measured the total blood phospholipid FA concentrations in 35 control subjects and 37 adults with ADHD symptoms to determine whether adults with ADHD symptoms would show abnormalities of FA relative to control subjects. In the serum phospholipids, adults with ADHD symptoms had significantly lower levels of total saturated, total polyunsaturated, and total omega-6 (n-6) FA, as well as the omega-3 (n-3) LCPUFA DHA (22:6n-3), and significantly higher levels of total monounsaturated FA and the n-3 LCPUFA docosapentaenoic acid (22:5n-3). In the erythrocyte membrane phospholipids, adults with ADHD symptoms had significantly lower levels of total PUFA, total n-3 FA, and DHA, and significantly higher levels of total saturated FA. Neither serum nor erythrocyte membrane phospholipid DHA was related to ADHD symptom severity (as assessed by the Amen questionnaire) in ADHD subjects. Although the exact cause of these variations is unknown, both environmental and genetic factors may be involved.

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Omega-3 and omega-6 (n-3 and n-6, respectively) FA are considered essential as they cannot be synthesized by mammalian cells and must be obtained from the diet. The essential n-3 FA α-linolenic acid (ALA; 18:3n-3) and the essential n-6 FA linoleic acid (LA; 18:2n-6) can undergo elongation, desaturation, and β-oxidation to form the n-3 long-chain PUFA (LCPUFA) EPA (20:5n-3), docosapentaenoic acid (DPA; 22:5n-3), and DHA (22:6n-3), and the n-6 LCPUFA γ-linolenic acid (GLA; 18:3n-6), dihomo-γ-linolenic acid (DGLA; 20:3n-6), and arachidonic acid (AA; 20:4n-6), respectively (1). As primary constituents of the cell membrane phospholipid bilayer, FA, particularly DHA and AA, make up a large proportion of the brain's lipids (2). The prominent structural role of FA in the brain translates into a functional role, since they affect membrane-associated proteins such as transporters, enzymes, and receptors (3,4).

Attention Deficit/Hyperactivity Disorder (ADHD) is a condition involving "a persistent pattern of inattention and/or hyperactivity-impulsivity that is more frequent and severe than is typically observed in individuals at a comparable level of development" (5). Although originally thought to occur only in children, it is now recognized that in up to 60% of sufferers ADHD persists into adulthood (6). In adults, ADHD is manifested by disorganization, impulsivity, and poor work skills, and sufferers tend to be impatient and easily bored (7). The *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn., requires evidence of hyperactive-impulsive or inattentive symptoms to be present before age 7, although individuals are often diagnosed after the symptoms have been present for several years (5). Since no specific test is diagnostic of ADHD, making an accurate diagnosis is difficult, especially in adults (7).

There is emerging evidence that LCPUFA abnormalities may play a role in a wide range of learning and mood disorders, including ADHD (8–26). Several FA, including AA (9), EPA (9), and DHA (8,10), are present at abnormal levels in children with ADHD. Despite this reported association, the same relationship has not been reported previously in adults. Based on this link, investigation to determine whether there is also an association in adults is warranted. The present study measured erythrocyte membrane and serum phospholipid FA concentrations in control subjects and in adults with ADHD symptoms, and the prospectively defined hypothesis was that adults with ADHD symptoms would show abnormalities of certain LCPUFA, particularly DHA, EPA and AA, relative to control subjects.

## MATERIALS AND METHODS

*Subjects.* Eighty-eight subjects aged 18 to 65 yr were recruited by advertisements and flyers in the local community. Inclusion criteria for adults with ADHD symptoms included a previous diagnosis of ADHD by a physician, based on a comprehensive psychological evaluation, and the ability to give informed consent. Because the physician evaluations were not performed using uniform criteria, these subjects were labeled "adults with ADHD symptoms," although all subjects had in fact been previously diagnosed with ADHD. Both control and ADHD subjects were excluded for one or more of the following

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Abbreviations: AA, arachidonic acid; ADHD, Attention Deficit/Hyperactivity Disorder; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; DPA, docosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid; LCPUFA, long-chain PUFA; LNA, linolenic acid; n-3, omega-3; n-6, omega-6; PLA2, phospholipase A2; RBC, red blood cell.

reasons: (i) diagnosis of another psychiatric disorder (as reported by the subject), (ii) use of dietary supplements (other than vitamins/minerals) within the past month, (iii) use of n-3 FA supplements within the past 6 mon, (iv) history of head injuries or seizures, (v) diagnosis of a lipid metabolism disorder or other serious chronic condition, and (vi) consumption of fish more than once per week. Of the 88 subjects screened, 14 were excluded due to a lack of formal diagnosis of ADHD by a physician, 1 was excluded owing to use of fish oil supplements within the last 6 mon, and 1 was excluded owing to use of flaxseed oil supplements within the last 6 mon. This left 72 subjects, 37 adults with ADHD symptoms and 35 control. ADHD subjects were not excluded on the basis of pharmacological treatment for their condition. ADHD subjects also were not screened for their use of alcohol, tobacco, or recreational drugs. This study was approved by the Research Ethics Board of the University of Guelph.

*Behavior assessment.* All subjects completed a questionnaire developed by Dr. D. Amen designed to identify and classify ADHD subtypes in adults (27). Questionnaires were graded, with 1 point given for each score of 3 (symptom is experienced frequently) or 4 (symptom is experienced very frequently). The questionnaire has a highest possible score of 71. The Amen questionnaire was not used to diagnose ADHD but rather served to highlight the differences in ADHD symptoms between adults with ADHD symptoms and control subjects. Quantitatively assessing ADHD symptoms also allowed investigation of a correlation between FA levels and symptom severity.

*Preparation of sample.* All subjects had nonfasting venous blood drawn into heparinized tubes. Whole blood was then centrifuged at room temperature for 10 min at $1000 \times g$, and red blood cells (RBC) were separated from serum. For 30 ADHD subjects, nonfasting blood was also obtained by the finger prick method for serum isolation. Whole blood was centrifuged at room temperature for 10 min at $1250 \times g$ and serum obtained. Serum was stored at –20°C until further analysis. Serum samples were stored for a period of 1–4 mon. Phospholipid FA in serum obtained by venipuncture were analyzed for 42 subjects (7 ADHD and 35 control) and by the finger prick method for the remaining 30 ADHD subjects.

For RBC membrane isolation, cells were washed in cold PBS (pH 7.4) and centrifuged at $1200 \times g$ for 10 min at 4°C (repeated twice). Cells were then lysed in PBS (pH 8) and centrifuged at $20{,}000 \times g$ for 10 min at 4°C (repeated four times). Isolated RBC membranes were stored at –20°C until further analysis. All samples were stored under nitrogen gas to reduce LCPUFA oxidation. RBC membrane samples of ADHD subjects were stored for 1–2 mon, whereas those of control subjects were stored for a period of 2 to 3 mon.

*Extraction of lipid.* RBC membrane lipids were extracted using chloroform/methanol (2:1), the volume of which was 20 times greater than that of the sample. Serum lipids were extracted using equal volumes of chloroform/methanol (2:1) in the presence of 17:0 internal standard. TLC was used to separate the lipid fraction from both the RBC membranes and serum using hexane/isopropyl ether/acetic acid (60:40:30, by vol) as the solvent system. The samples were spotted on silica gel TLC plates and allowed to develop within 2 cm of the top of the plate, after which the plates were removed and air-dried. The phospholipid bands were scraped, and a methylating agent (6% $H_2SO_4$ in methanol) was added. Five micrograms of 17:0 internal standard was added to the RBC solution. All samples were heated for 60 min at 80°C, and lipids were extracted with *n*-hexane. The FAME were analyzed by GC at the Lipid Analytical Laboratory (Guelph, Ontario, Canada) with a Varian 3800 gas chromatograph (Palo Alto, CA) equipped with a 30-m DB-23 capillary column (0.32 mm i.d., 0.1 μm film thickness; Varian). The sum of FA from 14:0 to 24:1 was taken as 100, and levels of individual FA were expressed as a percentage of this sum.

*Statistical analysis.* Statistical analysis was performed using SPSS version 11.5 (SPSS, Inc., Chicago, IL) statistical software. Data were examined for normality of distribution by using frequency distribution plots. Levels of the individual and groups of FA were compared between ADHD and control groups using Mann–Whitney nonparametric tests since the data were not normally distributed. A total of 26 comparisons were made. The Amen questionnaire scores and demographic data also were analyzed using Mann–Whitney tests, again owing to a lack of normal data distribution. Linear regression was used to investigate the relationship between both serum and erythrocyte DHA levels and Amen questionnaire scores. The $P$ value was set at 0.03 because of the increased likelihood of significant results appearing by chance due to the number of comparisons performed.

## RESULTS

Thirty-five control and 37 ADHD subjects completed the study. The two groups did not differ with respect to age or sex, as shown in Table 1. There was a statistically significant difference between the scores of the two groups on the Amen

**TABLE 1**
**Subject Characteristics of Control Adults and Adults with ADHD Symptoms[a]**

| | Control | ADHD | *P* value | *Z* value |
|---|---|---|---|---|
| Age | 29.97 ± 12.99 | 30.59 ± 13.57 | NS | –0.21 |
| Sex (males/females) | 16/19 | 19/18 | NA | NA |
| Amen questionnaire results[b] | 4.23 ± 4.24 | 32.65 ±16.54 | <0.001 | –7.14 |

[a]Values are reported as mean ± SD for $n = 35$ (control) and 37 (ADHD).
[b]Score out of a possible score of 71. ADHD, Attention Deficit/Hyperactivity Disorder; NA, not applicable; NS, not significant.

**TABLE 2**
**Total FA Analysis of Serum Phospholipids in Control Adults and Adults with ADHD Symptoms (wt%)[a]**

| FA | Control | ADHD | *P* value | *Z* value |
|---|---|---|---|---|
| Saturated | 46.24 ± 1.12 | 43.53 ± 2.03 | <0.001 | –4.44 |
| Monounsaturated | 10.55 ± 1.20 | 13.33 ± 2.17 | <0.001 | –5.48 |
| Polyunsaturated | 43.21 ± 1.52 | 40.37 ± 2.43 | 0.001 | –3.47 |
| n-6 | 38.15 ± 1.83 | 35.48 ± 2.61 | 0.007 | –2.71 |
| LA | 23.30 ± 2.81 | 21.30 ± 2.98 | NS | –1.39 |
| GLA | 0.08 ± 0.05 | 0.11 ± 0.07 | NS | –1.94 |
| DGLA | 2.29 ± 0.62 | 2.82 ± 0.69 | NS | –0.55 |
| AA | 10.79 ± 2.11 | 10.06 ± 1.51 | NS | –1.17 |
| n-3 | 5.06 ± 1.08 | 4.89 ± 1.36 | NS | –0.83 |
| ALA | 0.23 ± 0.09 | 0.28 ± 0.16 | NS | –1.60 |
| EPA | 0.66 ± 0.24 | 0.72 ± 0.33 | NS | –0.53 |
| DPA | 0.83 ± 0.21 | 0.99 ± 0.22 | 0.002 | –3.08 |
| DHA | 3.23 ± 0.98 | 2.69 ± 1.10 | 0.009 | –2.62 |

[a]Values are reported as mean ± SD for $n = 35$ (control) and 36 (ADHD) individuals. LA, linoleic acid; GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; ALA, α-linolenic acid; DPA, docosapentaenoic acid; for other abbreviations see Table 1.

questionnaire, with adults with ADHD symptoms scoring much higher than control subjects.

Table 2 shows the total serum phospholipid FA concentrations of ADHD and control subjects as a percentage of total FA. Serum FA values were not obtained for one subject due to technical difficulties. In the serum phospholipids, adults with ADHD symptoms had significantly lower levels of total saturated, total polyunsaturated, and total n-6 FA, as well as the n-3 LCPUFA DHA, and significantly higher levels of total monounsaturated FA and the n-3 LCPUFA DPA.

Table 3 shows the total RBC membrane phospholipid FA concentrations of ADHD and control subjects as a percentage of total FA. RBC FA values were not obtained from one subject due to difficulty drawing blood by venipuncture. Adults with ADHD symptoms had significantly lower levels of total PUFA, total n-3 FA, and DHA, and significantly higher levels of total saturated FA.

Figure 1 illustrates the relationship between RBC membrane phospholipid DHA and Amen questionnaire scores. There was no significant association between these variables ($r = 0.271$). There was also no significant association between serum phospholipid DHA and Amen questionnaire scores ($r = 0.150$).

## DISCUSSION

The objective of this study was to determine whether abnormalities of LCPUFA are present in adults with ADHD symptoms. Our primary finding was a decrease of DHA in both erythrocyte membrane and serum phospholipids. Since FA levels are expressed as a percentage of total weight, the decrease in DHA resulted in decreased levels of total PUFA in both blood fractions, as well as total n-3 FA in erythrocyte membranes. As well, adults with ADHD symptoms showed

**TABLE 3**
**Total FA Analysis of RBC Membrane Phospholipids in Control Adults and Adults with ADHD Symptoms (wt%)[a]**

| FA | Control | ADHD | *P* value | *Z* value |
|---|---|---|---|---|
| Saturated | 44.59 ± 6.61 | 44.84 ± 3.32 | 0.004 | –2.87 |
| Monounsaturated | 17.74 ± 2.06 | 18.70 ± 1.99 | NS | –0.82 |
| Polyunsaturated | 37.67 ± 5.44 | 36.47 ± 3.91 | 0.02 | –2.29 |
| n-6 | 30.53 ± 4.47 | 29.95 ± 3.76 | NS | –1.34 |
| LA | 10.33 ± 1.58 | 9.96 ± 1.15 | NS | –1.60 |
| GLA | 0.07 ± 0.12 | 0.05 ± 0.06 | NS | –1.14 |
| DGLA | 1.69 ± 0.65 | 1.65 ± 0.46 | NS | –0.68 |
| AA | 13.96 ± 2.52 | 13.70 ± 2.30 | NS | –1.04 |
| n-3 | 7.14 ± 1.57 | 6.52 ± 1.29 | 0.01 | –2.49 |
| ALA | 0.09 ± 0.07 | 0.10 ± 0.09 | NS | –0.43 |
| EPA | 0.46 ± 0.25 | 0.45 ± 0.21 | NS | –0.20 |
| DPA | 2.57 ± 0.65 | 2.64 ± 0.61 | NS | –0.09 |
| DHA | 3.92 ± 1.03 | 3.19 ± 0.94 | <0.001 | –3.49 |

[a]Values are reported as mean ± SD for $n = 35$ (control) and 36 (ADHD) individuals. RBC, red blood cell; for other abbreviations see Tables 1 and 2.

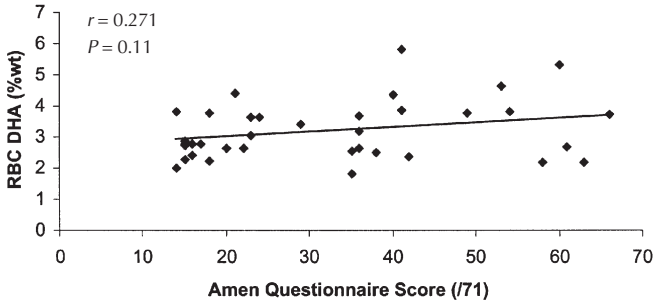


**FIG. 1.** Correlation between red blood cell (RBC) DHA and Amen questionnaire scores.

decreased levels of total n-6 FA in serum phospholipids, although individual n-6 FA were not significantly affected. Total monounsaturated FA and the n-3 FA DPA were increased in serum phospholipids. Levels of DHA in blood phospholipids were not correlated with symptom severity.

Previous studies have suggested a relationship between LCPUFA abnormalities and ADHD in children. In 1981, it was observed that hyperactive children showed increased thirst relative to children without hyperactivity (28). Increased thirst may be a symptom of EFA deficiency (29). In 1987, Mitchell *et al.* (8) showed that hyperactive children reporting significantly greater thirst did in fact have lower plasma levels of the n-6 FA DGLA and AA and the n-3 FA DHA. Since then, low levels of various LCPUFA have been found in children with ADHD including AA (9), EPA (9), and most commonly, DHA (8,10). Stevens *et al.* (9) also found that a greater number of behavior, learning, and health problems were reported in subjects with lower concentrations of total n-3 FA.

As a result of the observed LCPUFA abnormalities in childhood ADHD, clinical trials have been conducted using a variety of FA to treat the disorder. These trials have yielded mixed results. In 1987, children with marked inattention and hyperactivity were supplemented for a period of 4 wk with a combination of LA and GLA. There was minimal or no improvement in symptoms, depending on the outcome measure used (30). In 2001, Voigt *et al.* (11) treated ADHD children with DHA from an algal source for a period of 4 mon and observed no decrease in symptoms between treated and control subjects, although they did observe an increase in plasma DHA of 260%. And in 2002, Richardson and Puri (31) found that 12 wk of treatment with DHA and evening primrose oil, a source of GLA, improved mean scores for cognitive and general behavior problems on 7 out of 14 scales, vs. no improvement with placebo. Because different treatments, procedures, measures, and selection criteria were used in these trials, it is difficult to make comparisons between them. At this time, there is no clear evidence to suggest that supplementation with any particular LCPUFA or combination thereof is an effective treatment for ADHD in children. There have been no trials investigating the effect of LCPUFA supplementation in adults with ADHD.

ADHD in both children and adults is characterized by difficulties regulating attention and/or monitoring their motor behavior or impulses (32). Dopamine, which acts as a modulator of attention, motivation, and emotion (33), may serve as a link between abnormalities of LCPUFA, particularly DHA, and ADHD. Although very little research has been done on humans, single-emission computed tomography has found that adults with ADHD exhibit increased striatal availability of a dopamine transporter (32), and medications used to treat ADHD commonly exert their effect *via* inhibition of this transporter (34). Animal studies have demonstrated that several aspects of dopamine physiology are affected by levels of FA intake. It has been observed that when piglets are fed a diet deficient in AA and DHA, but adequate in levels of ALA and LA, there is a decrease in dopamine concentration in the frontal cortex (35). Furthermore, when rats are fed a diet deficient in both short- and long-chain n-3 FA, there is inadequate storage of newly synthesized dopamine (36) and an overall reduction in the dopaminergic vesicle pool (37). Alternatively, when rats are fed fish oil containing EPA and DHA, there is a 40% increase in frontal cortex dopamine concentrations as well as a greater binding to dopamine $D_2$ receptors (38). The low levels of DHA observed in this study could therefore theoretically affect the availability of dopamine, which could subsequently result in impaired attention, motivation, and emotion, which are classical symptoms of ADHD.

As the basic structural components of phospholipids, FA can be found in different locations in the body, including serum and RBC membranes. The levels of both serum (39,40) and RBC (41) phospholipid FA that we observed are similar to those observed in other studies. Phospholipids in different locations contain different proportions of each FA, as is demonstrated in Tables 2 and 3. Phospholipids in different locations also respond differently to dietary FA manipulation. Serum phospholipid FA reflect the dietary intakes of the past few days (42), whereas erythrocyte membrane phospholipid FA reflect the dietary intakes of the past month (43). As compared with the response of serum phospholipids, erythrocytes show less response to dietary change, but their phospholipid FA composition is more stable than that of serum owing to the high turnover rate of FA in the former (44). This likely accounts for the differences that we observed in serum and erythrocyte FA. It has been demonstrated that both serum (45,46) and erythrocyte (47) phospholipid EPA and DHA are correlated with fish consumption, so dietary intake of the n-3 LCPUFA may be accurately reflected in both of these pools of lipids. In this study, we attempted to control for intake of flaxseed and fish oils, which are major contributors of short- and long-chain n-3 FA to the diet, but in the absence of dietary record analysis it is not possible to determine whether differences in dietary intake existed between the two groups of subjects. Therefore, diet may be a contributing cause to the FA abnormalities that we observed.

In addition to differences in dietary intake, it has been suggested that LCPUFA abnormalities may be due to differences in metabolism. As previously mentioned, the EFA must undergo complex biotransformation in order to be converted into their long-chain derivatives, and individuals vary in the efficiency of their conversion mechanisms (1). The hypothe-

sis that the LCPUFA abnormalities observed in ADHD patients may be associated with inefficient conversion of short-chain FA is supported by the fact that serum zinc levels have been found to be significantly associated with low EFA levels in children with ADHD (48), and zinc deficiency inhibits the Δ6-desaturase enzyme (8). Both Δ6-desaturase (49) and Δ5-desaturase (50) enzymes are highly active in the brain, making them candidates for involvement in this disorder. Phospholipase A2 (PLA2), an enzyme that acts to remove highly unsaturated FA from the *sn*-2 position of membrane phospholipids (51), is another possible candidate. PLA2 has been implicated in several neurodevelopmental and psychiatric conditions, including dyslexia (52), depression and bipolar disease (53), schizophrenia (16,17), and Alzheimer's disease (54). ADHD is commonly co-morbid with dyslexia (55), depression (56), and bipolar disease (23).

In fact, it is estimated that 75% of adults with ADHD suffer from other psychiatric disorders (57), and LCPUFA abnormalities have been implicated in many such conditions, including dyslexia (57), depression (12–15), schizophrenia (16–18), autism (58,59), and bipolar disorder (20). In dyslexia, the severity of the disorder has been shown to be related to the degree of clinical signs of deficiency (60). Although one of the exclusion criteria in this study was a diagnosis of another psychiatric condition, since this information was self-reported, it is possible that our ADHD subjects suffered from other psychiatric disorders. Furthermore, we did not control for the use of substances such as alcohol or tobacco. Adults with ADHD show a high frequency of substance abuse disorders (56), and both alcohol (61) and tobacco (62,63) use have been shown to affect levels of LCPUFA adversely. Together, these factors make it difficult to conclude that the observed correlation in this study between LCPUFA abnormalities and ADHD symptoms has not been confounded by other conditions also known to involve variations in FA levels.

LCPUFA in the RBC membranes of autistic subjects exhibit an increased level of degradation relative to control subjects when stored at –20°C (58), and it has been shown that there is a high degree of clinical overlap between autism and ADHD (57). Schizophrenic patients also show an increased rate of LCPUFA degradation at –20°C relative to control subjects, although it may be that this increased degradation is limited to a subset of patients with schizophrenia (64). Conversely, it has been shown that patients with Asperger's syndrome, a condition that shows clinical overlap with autism (65) and is considered by some to be a condition of high-functioning autism (66), show unusually stable RBC membrane LCPUFA when stored at –20°C (58). This variation in stability of membrane LCPUFA at low temperatures has been attributed to variations in phospholipase activity, with low activity increasing the stability at low temperatures and high activity decreasing it (58). Although the degradation of LCPUFA in RBC membranes of ADHD subjects at –20°C has not previously been evaluated, it is possible that they might also show an increased rate relative to control subjects. This is an issue that should be investigated in the future and may be a contributing factor to the low level of DHA observed in this study. However, the similarity between levels of serum and erythrocyte membrane DHA, and the short storage period of all samples, suggests that degradation due to storage temperature was minimal.

It is unclear whether peripheral LCPUFA composition reflects the phospholipid composition of neuronal membranes. Preliminary data in schizophrenic patients do suggest a correlation between RBC membrane phospholipid composition and that of the brain, although such a correlation does not necessarily indicate a causal relationship (67). In this study, we observed low levels of the LCPUFA DHA in both the serum and RBC membrane phospholipids of adults with ADHD symptoms, and although the exact cause of this abnormality is unknown, both environmental and genetic factors may be involved. This observation is complicated by the fact that adult ADHD often co-exists with other psychiatric conditions known to involve alterations of LCPUFA, and by the possibility of enhanced degradation of these FA following storage at –20°C. Regardless, the body of evidence does appear to suggest that abnormalities of LCPUFA can contribute to deficits in concentration, and understanding the precise role of these FA in cognitive processes, as well as the etiology of LCPUFA abnormalities in conditions such as adult ADHD, may prove useful in their management and treatment.

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