HYPERREACTIVE ONCHOCERCIASIS EXHIBITS REDUCED ARACHIDONATE AND LINOLEATE LEVELS IN SERUM TRIGLYCERIDES

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Abstract. The mechanism by which the minority of patients with onchocerciasis exhibiting the hyperreactive (sowda) form of the disease may be able to kill the microfilariae of *Onchocerca volvulus* is still poorly understood. In this study, the relative amounts of arachidonate and linoleate in serum phospholipids and triglycerides were investigated by gas chromatography both in patients infected with *O. volvulus* who exhibited either a hyperreactive or a generalized form of onchocerciasis and in persons with no filarial infections. Remarkable differences were observed in the serum triglycerides but not in the phospholipids. In comparison to persons without any filarial infection, significantly lower relative amounts of arachidonate—indicated by elevated triene-tetraene ratios—and of linoleate—indicated by lower diene + tetraene + triene values—were detected in patients with hyperreactive onchocerciasis, and less pronounced differences were found in persons with generalized onchocerciasis. The relationship between reduced amounts of arachidonate and linoleate in serum triglycerides and possible implications on the eicosanoid production in the host-parasite relationship leading to parasite elimination are discussed.

INTRODUCTION

Onchocerciasis, a disease caused by infection with the filarial nematode *Onchocerca volvulus,* is an important cause of blindness and severe dermatitis that affects about 20 million residents of Africa and Latin America.1 Stunted growth and epilepsy associated with *O. volvulus* infection have been found in some children.1–3 The majority of *O. volvulus* after DEC treatment.16,17 These observations have been attributed in part to the inhibitory action of DEC on filarial worms. Diethylcarbamazine (DEC) on filarial worms. Diethylcarbamazine, a drug used for the treatment of filarial infections, has no direct microfilaricidal activity.13 However, it stimulates the release of eicosanoids by the parasite and the host cells.14 The inhibition of eicosanoids’ release would withdraw from the MF a capacity to modulate the host’s humoral and cellular immune responses, which may render the MF prone to attacks.5,18

Onchocerciasis is a disease caused by infection with the filarial nematode *Onchocerca volvulus,* is an important cause of blindness and severe dermatitis that affects about 20 million residents of Africa and Latin America.1 Stunted growth and epilepsy associated with *O. volvulus* infection have been found in some children.1–3 The majority of *O. volvulus-*infected persons develop a generalized form of the disease, characterized by varying microfilariae (MF) densities and numbers of adult parasites, low degree of inflammatory processes in the skin, and the inability to eliminate the MF.4 In contrast, a small proportion of patients exhibit a hyperreactive form of onchocerciasis, characterized by very low MF densities, only a few nodules of adult worms, pronounced cellular and humoral immune responses, and the ability to eliminate the MF.4–6 The underlying mechanisms of the immunological differences between generalized and hyperreactive onchocerciasis are poorly understood.

The elaboration of eicosanoids—paracrine lipid mediators with high potency to regulate physiological and pathophysiological processes7,8—by the parasite may be crucial for the survival of the worms in the host.9–12 Evidence has come from experiments investigating the effects of the pharmacologic inhibitors of eicosanoid synthesis, in particular by diethylcarbamazin (DEC) on filarial worms. Diethylcarbamazin, a drug used for the treatment of filarial infections, has no direct microfilaricidal activity.13 However, it stimulates the adherence to and cytotoxicity of leucocytes for MF in vitro.14 Animal studies suggest that antigen-specific T-cells and antifilarial antibodies are necessary for DEC-mediated microfilaricidal activity in vivo.15 This agrees with in vitro and histological findings, in which the neutrophils and eosinophils were found to attack and damage the MF of *O. volvulus* after DEC treatment.16,17 These observations have been attributed in part to the inhibitory action of DEC on the release of eicosanoids by the parasite and the host cells.11 The inhibition of eicosanoids’ release would withdraw from the MF a capacity to modulate the host’s humoral and cellular immune responses, which may render the MF prone to attacks.5,18

The possible involvement of eicosanoids in the modulation of the host responses to the parasite indicates the importance of the fatty acids used in the synthesis of eicosanoids, namely arachidonic and linoleic acid, in the host-*O. volvulus* interrelationship. The inability of the filarial worms to synthesize arachidonate and linoleate de novo19 further stresses the significance of these fatty acids in the above relationship. In the present study, the amounts of arachidonate and linoleate in serum phospholipid—a lipid class reflecting most clearly the essential fatty acid status20—and serum triglycerides—the fatty acid pattern of which is similar to that of the adipose tissue21,22—were analyzed in patients with generalized onchocerciasis, unable to kill the MF and patients with hyperreactive onchocerciasis, able to kill the MF. A comparison was made with persons without any filarial infection.

SUBJECTS AND METHODS

Study population. Sera were obtained during the 1980s in the areas in Liberia, West Africa, that were endemic for onchocerciasis but where no other human filarial infections occurred. Procedures and methods of examination were explained to the participants in the local language, and informed consent was obtained from adult participants and from parents or legal guardians of minors. The procedures had been approved by the Ethics Committee of the Medical Board in Hamburg. Because the hyperreactive onchocerciasis in West Africa is more frequent among younger females,23 in this study, only girls and women between 10 and 32 years of age were considered. Nineteen sera were from patients with severe hyperreactive onchocerciasis, with a median MF density of 0 MF/mg and a mean age of 18.5 ± 7.0 years. Clinically typical features of sowda24 as well as MF densities in the skin were used to distinguish patients with the hyperreactive from those with the generalized form of onchocerciasis. Twenty sera were from patients with a generalized form of onchocerciasis, with a median MF density of 25 MF/mg skin and a mean age of 25.4 ± 6.8 years.22,24 Sera from 22 persons without any filarial infection were collected in a hypoendemic *O. volvulus* area (Jung-
The methyl ester references were scraped off and resuspended in 1 mL methanol containing tert-butylhydroxytoluene. The unstable C22:3n6 fatty acid (1.41 μg/lipid probe) was added as an internal standard to follow the recovery of arachidonic and linoleic acid. The lipid classes were methylated using boron trifluoride-methanol as described.

Gas chromatography. Gas chromatography analysis—including gas chromatography instrumentation, fatty acid methyl esters identification, and quantification—were done as described previously. The methyl ester references were all purchased from Sigma (Deisenhofen, Germany). Excellent resolution of C20:3n9 from C20:2n6, as well as resolution of other methyl esters, was obtained under the above gas chromatography conditions. The fatty acid analysis included 21 methyl esters from C16 to C22. The proportions of individual fatty acids were expressed as percentages of total fatty acids, and formulas described by Holman were used to derive ratios indicating the extent of depletion of arachidonate and values showing total linoleate in each subject.

Statistics. Data are given as mean ± standard deviation (SD) in the tables and as median and quantiles (10, 25, 75, 90%) in the figures. The paired Wilcoxon signed-rank test was used to compare the 2 groups of persons infected with *O. volvulus* with the filaria-negative persons. A P value of less than 0.05 was taken as the lower limit of significance. Data analysis was done using Excel and Microcal Origin software.

### RESULTS

The composition of serum phospholipids and triglycerides in sera of the 3 groups of persons studied was analyzed by gas chromatography, and the individual fatty acids were expressed as percentages of the total fatty acids in each lipid class.

**Serum phospholipid class.** The proportions of polyunsaturated fatty acids are given in Table 1. In the 3 groups studied, the predominant fatty acids were linoleic acid (C18:2n6) and its derivatives, dihomo-γ-linolenic (C20:3n6), and arachidonic acid (C20:4n6). In animal tissues, an insufficient supply of arachidonic acid leads to an accumulation of eicosatrienoic acid (C20:3n9), a fatty acid type that is endogenously synthesized. Therefore, in lipid studies, the ratio of arachidonate and values showing total linoleate in each subject was used to derive ratios indicating the extent of depletion of arachidonate and values showing total linoleate in each subject.

### Table 1

**Polyunsaturated fatty acid profile of serum phospholipids**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Trivial name</th>
<th>Filaria-negative (n = 22)</th>
<th>Generalized onchocerciasis (n = 20)</th>
<th>Hyperreactive onchocerciasis (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18:2n6</td>
<td>Linoleic</td>
<td>12.97 ± 3.11*</td>
<td>15.57 ± 3.86*</td>
<td>13.45 ± 4.71*</td>
</tr>
<tr>
<td>C18:3n6</td>
<td>γ-linolenic</td>
<td>0.18 ± 0.17</td>
<td>0.15 ± 0.14</td>
<td>0.14 ± 0.11</td>
</tr>
<tr>
<td>C18:3n3</td>
<td>–</td>
<td>0.27 ± 0.13</td>
<td>0.36 ± 0.14</td>
<td>0.32 ± 0.13</td>
</tr>
<tr>
<td>C20:3n9</td>
<td>–</td>
<td>0.79 ± 0.41</td>
<td>0.33 ± 0.31</td>
<td>0.67 ± 0.33</td>
</tr>
<tr>
<td>C20:3n6</td>
<td>Dihomo-γ-linolenic</td>
<td>2.60 ± 1.13</td>
<td>2.81 ± 1.11</td>
<td>2.47 ± 0.95</td>
</tr>
<tr>
<td>C20:3n3</td>
<td>–</td>
<td>0.06 ± 0.11</td>
<td>0.02 ± 0.07</td>
<td>0.00 ± 0.02</td>
</tr>
<tr>
<td>C20:4n6</td>
<td>Arachidonic</td>
<td>2.68 ± 1.41</td>
<td>5.52 ± 1.82</td>
<td>3.39 ± 1.66</td>
</tr>
<tr>
<td>C22:2n6</td>
<td>–</td>
<td>0.10 ± 0.17</td>
<td>0.14 ± 0.14</td>
<td>0.18 ± 0.25</td>
</tr>
<tr>
<td>C22:4n6</td>
<td>–</td>
<td>0.39 ± 0.61</td>
<td>0.51 ± 0.70</td>
<td>0.42 ± 0.53</td>
</tr>
<tr>
<td>C22:6n3</td>
<td>–</td>
<td>0.87 ± 0.63</td>
<td>1.84 ± 1.03</td>
<td>1.28 ± 0.70</td>
</tr>
</tbody>
</table>

*Fatty acids were named using the fatty acid nomenclature. In this nomenclature, the first two digits stand for the number of carbon atoms in the acyl chain, the third digit indicates the number of double bonds and the last digit gives the number of carbon atoms from the methyl end of the acyl chain to the terminal double bond.

* Columns of the amount (mean ± st) of individual fatty acids given as percentage of total fatty acids.

hanns K, unpublished data); the determination of absence of onchocerciasis in this group was based on negative skin snips and Mazzotti tests for microfilariae and on negative serological tests for antifilarial antibodies. The average age of the filaria-negative persons was 18.5 ± 6.0 years. People in the above 3 study groups exhibited no signs of other acute infections, and the intestinal worm infections (hookworm, *Trichuris trichiura*, *Ascaris* lumbricoides) were comparable in all groups.

**Serum lipid extraction.** All solvents used were of analytical grade and were purchased from Merck (Darmstadt, Germany). To prevent oxidation of fatty acids during the extraction process, 100 μg tert-butylhydroxytoluene was added to 500 μL of serum. This was followed by extraction 3 times with 1,500 μL Folch’s reagent (chloroform-methanol 2:1, v:v). The extracts were filtered through sodium sulfate, and the solvents were evaporated under a stream of nitrogen. The dry residue was dissolved in 100 μL N-hexane.

**Separation of lipid classes and transmethylation.** The lipid fractions were separated by spotting 100 μL of total lipid extract on a 2-cm-wide lane of the silica gel plates (Merck). The plates were developed in a thin-layer chromatography chamber containing hexane-diethyl ether-acetic acid (80:20:1, v:v:v) for 30 min. After visualization of lipid classes on the silica gel plate by iodine vapor, the classes were scraped off and resuspended in 1 mL methanol containing 50 μg tert-butylhydroxytoluene. The unstable C22:3n3 fatty acid (1.41 μg/lipid probe) was added as an internal standard to follow the recovery of arachidonic and linoleic acid. The lipid classes were methylated using boron trifluoride-methanol as described.

Gas chromatography. Gas chromatography analysis—including gas chromatography instrumentation, fatty acid methyl esters identification, and quantification—were done as described previously. The methyl ester references were all purchased from Sigma (Deisenhofen, Germany). Excellent resolution of C20:3n9 from C20:2n6, as well as resolution of other methyl esters, was obtained under the above gas chromatography conditions. The fatty acid analysis included 21 methyl esters from C16 to C22. The proportions of individual fatty acids were expressed as percentages of total fatty acids, and formulas described by Holman were used to derive ratios indicating the extent of depletion of arachidonate and values showing total linoleate in each subject.
2. The amounts of the essential fatty acids C18:2n6, C20:3n6, and C20:4n6 were less in the triglycerides compared to the phospholipid fraction, confirming a dilution of polyunsaturated fatty acids in this lipid class by the high proportions of 16- and 18-carbon acids, which are known to occur abundantly in the triglycerides. One of the remarkable observations was the several-fold increase of C18:3n6 and C20:3n6, which are the intermediate metabolites in the conversion of linoleic (C18:2n6) to arachidonic acid (C20:4n6). The amounts of C18:3n6 (γ-linolenic), a fatty acid rarely encountered in high levels in animal tissues because of its rapid conversion to the subsequent derivative, were about 4-fold higher in patients with generalized and 2-fold higher in patients with hyperreactive onchocerciasis as compared to the control group. Similarly, the quantities of C20:3n6, a derivative metabolite of C18:3n6, were 8-fold higher in patients with generalized and 4-fold higher in those with hyperreactive onchocerciasis as compared to the control group.

The ratios C20:3n9/C20:4n6, indicating the relative content of arachidonate in serum triglycerides, are presented in Figure 2. Patients with generalized onchocerciasis expressed significantly higher ratios than filaria-negative persons (P = 0.020), which were even more pronounced in patients with hyperreactive onchocerciasis (P = 0.003). These high ratios represent a strong depletion of arachidonate of the triglyceride lipid fraction, especially in the hyperreactive form of *Onchocerca volvulus* infection. Unlike in the phospholipid class, in the triglyceride fraction the maximum normal limit for the triene-tetraene ratios in humans is not well established. However, it was evident that several of the control subjects also exhibited high ratios, suggesting depletion of arachidonate. These differences in the triglyceride fraction were in marked contrast to those seen in the phospholipid class.

Total linoleate content in the triglyceride lipid fraction (Figure 3; calculated using the formula diene + tetraene –

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**Table 2**

Polyunsaturated fatty acid pattern of serum triglycerides

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Trivial name</th>
<th>Filaria-negative (n = 22)</th>
<th>Generalised onchocerciasis (n = 20)</th>
<th>Hyperreactive onchocerciasis (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18:2n6</td>
<td>Linoleic</td>
<td>7.42 ± 3.5*</td>
<td>7.32 ± 4.62*</td>
<td>5.23 ± 3.39*</td>
</tr>
<tr>
<td>C18:3n6</td>
<td>γ-linolenic</td>
<td>0.15 ± 0.20</td>
<td>0.56 ± 0.46</td>
<td>0.26 ± 0.28</td>
</tr>
<tr>
<td>C18:3n9</td>
<td>–</td>
<td>0.54 ± 0.20</td>
<td>0.50 ± 0.22</td>
<td>0.41 ± 0.52</td>
</tr>
<tr>
<td>C20:3n6</td>
<td>Dihomo-γ-linolenic</td>
<td>0.30 ± 0.20</td>
<td>2.42 ± 2.57</td>
<td>1.29 ± 1.10</td>
</tr>
<tr>
<td>C20:3n3</td>
<td>–</td>
<td>0.58 ± 0.80</td>
<td>0.73 ± 0.59</td>
<td>0.41 ± 0.24</td>
</tr>
<tr>
<td>C20:4n6</td>
<td>Arachidonic</td>
<td>0.74 ± 0.50</td>
<td>0.65 ± 0.29</td>
<td>0.42 ± 0.64</td>
</tr>
<tr>
<td>C22:2n6</td>
<td>–</td>
<td>0.33 ± 0.30</td>
<td>0.33 ± 0.46</td>
<td>0.28 ± 0.20</td>
</tr>
<tr>
<td>C22:4n6</td>
<td>–</td>
<td>0.34 ± 0.70</td>
<td>0.30 ± 0.15</td>
<td>0.30 ± 0.18</td>
</tr>
<tr>
<td>C22:6n3</td>
<td>–</td>
<td>0.43 ± 0.80</td>
<td>0.44 ± 0.34</td>
<td>0.61 ± 0.43</td>
</tr>
</tbody>
</table>

* Columns of the amount (mean ± s.e) of individual fatty acids given as percentage of total fatty acids.
In this study, the proportions of arachidonate and linoleate were compared between persons with no filarial infections and patients infected with *O. volvulus* expressing either a generalized or a hyperreactive form of onchocerciasis. These persons were living in the same geographical region and belonged to the same social class; therefore, similar diets were expected. The relative amounts of arachidonate and total linoleate were calculated by applying the established formulas that yield values of physiological importance and that also minimize differences due to biological variations of a fatty acid.

In the phospholipid fraction, no significant reduction in the relative amounts of arachidonate was observed in persons with *O. volvulus* infection. Applying the criteria of Holman et al. to our phospholipid results, we found that infection with *O. volvulus* may not lead to an essential fatty acid deficiency status. The suggestive lipid-deficient status in the 6 persons in the control group and in the 1 patient with hyperreactive onchocerciasis may have been due to individual diet or may have resulted from an abnormality in lipid metabolism. Because the hyperreactive type of onchocerciasis is more frequent among younger females in the area studied, our investigations, as noted in the Method section, involved only girls and women of younger ages, between 10 and 32 years. This meant that our control group represented a restricted sample as well, and this may possibly account for the high frequency (27%) of persons with a suggestive deficient lipid status. That similar frequencies were not observed in patients with *O. volvulus* infection may be either coincidental or indicative of an interrelationship between a normal phospholipid status and the development of the disease onchocerciasis, especially of the generalized form.

In contrast, the fatty acid pattern of the serum triglycerides may arise from a tissue-specific competitive metabolism. The fatty acid pattern of the serum triglycerides of patients with generalized onchocerciasis, especially of the generalized form. These alterations remained significant even when the 6 persons with a suggestive lipid-deficient status were included in the control sample; hence, their eventual exclusion cannot account for the observed differences. These differences cannot be a result of a marginal essential fatty acid deficiency related to illness, as proposed by Love and others, because otherwise, similar and possibly more pronounced differences would have been observed in the phospholipid class, as discussed by Holman and others. The observed differences, which are restricted to the triglyceride fraction, are suggestive of local or microenvironnent lipid alterations, which may arise from a tissue-specific competitive metabolism.

The fatty acid composition of serum triglycerides, but not of serum phospholipids, is similar to the fatty acid composition of adipose lipids. Therefore, the alterations in the fatty acid composition of serum triglycerides presented in this study suggest similar changes in the adipose tissue. Interestingly, we also found that the fatty acid pattern of serum-free fatty acids (data not shown), which are released...
into plasma by lipolysis of adipose triglycerides,24 show a similar though less pronounced trend, with the dilution effect in this particular lipid class by fatty acids directly derived from meals25 probably accounting for the less pronounced differences. Irvine25 pointed out that the triglycerides of the adipose tissue significantly contribute to the arachidonic pool of the subcutaneous tissue, where MF and the adults of *O. volvulus* predominantly reside.1

In humans, the enzymes desaturases and elongases act along the lipid metabolic pathway (linoleic [C18:2n6] → γ-linolenic [C18:3n6] → dihomo-γ-linolenic [C20:3n6]) to yield arachidonate [C20:4n6]. The several-fold increase in the intermediate fatty acids, C18:3n6 and C20:3n6, may indicate an attempt by the adipose tissue of the patients to yeild more arachidonate. Remarkably, the elevation in the intermediate metabolites was less pronounced in patients with hyperreactive onchocerciasis, probably supporting our findings in this group on the reduced total linoleate (Figure 3), an essential fatty acid that cannot be synthesized in animal tissues but must instead be obtained from diet.20,27

Results of the triglyceride fraction showed similar amounts of total linoleate between the control group and patients with generalized onchocerciasis. However, there was a significant reduction in the relative amounts of arachidionate, although the pronounced intermediate fatty acids, C18:3n6 and C20:3n6, suggested attempts for a high turnover of arachidonate in the generalized group. These two observations may imply a high consumption of arachidonate attributable to the presence of *O. volvulus*, which would lend support to the *in vitro* observations of Longworth and others,29 who found that MF of another filarial parasite, *B. malayi*, rapidly took up arachidonate without reaching saturation levels even at very high concentrations of the fatty acid. A reduction in the host arachidonate resulting from *O. volvulus* infection is conceivable, given that a person with just 5 palpable nodules may have a median total load of 40 million MFs.30

On the other hand, significant reductions in both total linoleate and its derivative, arachidonate, were observed in the patients with hyperreactive onchocerciasis. This observation suggests consequences in the host-parasite relationship. Lands and others37 asserted that when adequate levels of arachidonate in a tissue are not maintained, adequate physiology may not be maintained as well. Indeed, it is known that an insufficient supply of arachidonate in tissues inhibits the production of eicosanoids.37 Therefore, our results suggest that in patients with hyperreactive onchocerciasis, the parasite *O. volvulus* is living in a microenvironment in which it may be unable to sufficiently elaborate eicosanoids or induce the host cells’ production.9,13 This would in turn make the parasite prone to attacks by cellular and humoral host responses that are not down-regulated by the parasite-associated eicosanoids,17 resulting in MF killing. Some individuals without any filarial infection also exhibited relatively low amounts of arachidonate and linoleate in serum triglycerides, possibly a genetic disposition attribute or the result of other factors unknown to us. The question arises whether individuals with such lipid status living in endemic areas of onchocerciasis might not express predisposition to the development of hyperreactive onchocerciasis.

Elaboration of eicosanoid-like products by *O. volvulus*, leading to the suppression of interleukin 2 (IL-2) production of the host cells, have been implicated in the cellular hyporesponsiveness observed in persons with onchocerciasis.5 Prostaglandin E2, an eicosanoid produced or induced by filarial worms,11 was reported to suppress the production of immunoglobulin E (IgE) antibodies38 and type 1 T-helper cell (Th1) cytokines, particularly IL-2 and interferon gamma.8,30 Interestingly, our previous observations showed significantly elevated total24 and specific IgE antibodies40 and elevated IL-2 levels in patients with hyperreactive onchocerciasis when compared to patients with the generalized form. Our earlier findings, mentioned previously, with profound implications on the host’s antibody-dependent cytotoxicity mechanism, appear to support the hypothesis presented in this study that inadequate elaboration of eicosanoids by *O. volvulus* may prevent down-regulation of cellular and humoral responses to the parasite in persons with hyperreactive onchocerciasis. This study has shown that the fatty acid pattern of the triglyceride fraction exhibited significant differences between patients infected with *O. volvulus* and persons with no filarial infection. The association of *O. volvulus* infection with local alterations in the host lipid patterns may have important medical implications. The World Health Organization’s Expert Committee on Ochocerciasis1 has pointed out that the possibility of *O. volvulus* infection leading to cases of epilepsy and stunted growth in Africa has to be considered. It is known that an insufficient supply to the brain of the essential fatty acids, especially in children, can affect not only the proper formation of the nerve cells but the integrity and functioning of the pituitary gland, a center for the regulation of hormones, including growth hormones, in humans.7,42,43 An altered fatty acid pattern in the cerebrospinal fluid caused by MF of *O. volvulus* in children with early indications of stunted growth may be worth studying. This proposition is based on 3 observations: that storage lipids in the brain are comparatively scarce,37 that MF of *O. volvulus* can migrate into the cerebrospinal fluid of the host,44 and that infection caused by *O. volvulus* can, as shown in this study, be associated with local or microenvironmental restricted lipid pattern alterations.

In conclusion, this study shows that persons infected with the parasite *O. volvulus* exhibit reduced arachidonate in the triglyceride lipid fraction. Furthermore, the report shows that the parasite *O. volvulus* fails to successfully establish itself in those patients exhibiting significant reductions in both arachidonate and linoleate amounts in the triglycerides.

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