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Catabolism of leukotriene B_5 in humans

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Abstract Human neutrophils, enriched by dietary supplementation with eicosapentaenoic acid, form leukotriene (LT)B_5 in addition to LTB_4 upon stimulation. LTB_5 is one order of magnitude less biologically active than the potent chemokinetic and chemoattractant LTB_4. Catabolites of LTB_5 have not yet been characterized in vitro and ex vivo. It is unknown whether catabolism of LTB_5 interferes with catabolism of LTB_4. This report describes catabolism of LTB_5 to 20-OH-LTB_5, which in turn is catabolized to 20-COOH-LTB_5. The structures of the two catabolites were established by UV-absorbance, behavior on reverse-phase high-performance liquid chromatography, enzymatic analysis of human neutrophils, and gas chromatography–mass spectrometry. In vitro, formation of LTB_4 was delayed and formation of its catabolites was depressed by exogenous eicosapentaenoic acid. By supplementing the diet of six volunteers with 5 g eicosapentaenoic acid/day for 7 days, eicosapentaenoic acid quadrupled in neutrophil phospholipid fatty acids. Consequently, LTB_5, 20-OH-LTB_5, and 20-COOH-LTB_5 were detected ex vivo. In contrast to the findings in vitro, however, levels of LTB_4, 20-OH-LTB_4, and 20-COOH-LTB_4 were unaltered by the dietary intervention. Thus, in vitro, but not ex vivo, addition of eicosapentaenoic acid, and subsequent formation of LTB_5, impeded catabolism of proinflammatory LTB_4. — **von Schacky, C., C. Fahrer, and S. Fischer.** Catabolism of leukotriene B_5 in humans. *J. Lipid Res.* 1990. **31:** 1831–1838.

Supplementary key words eicosapentaenoic acid • n-3 fatty acids • fish oil • eicosanoids

Polymorphonuclear leukocytes isolated from volunteers who ingested eicosapentaenoic acid (EPA) form LTB_5 (1-3). LTB_5 is one order of magnitude less biologically active than LTB_4, the potent neutrophil chemotactic and chemokinetic compound, derived from arachidonic acid (4-9). Binding of LTB_5 to human neutrophil LTB_4 high affinity binding sites is lower than that of LTB_4 (8, 10). In vitro, with exogenous EPA or 12S-hydroxy-EPA, neutrophil formation of LTB_4 can readily be reduced dose dependently (1, 11-13). Thus, arachidonic acid, EPA, and its platelet-derived metabolite 12S-hydroxy-EPA compete for 5-lipoxygenation in stimulated neutrophils. Enrichment of human neutrophils with EPA, by dietary supplementation for at least 3 weeks, reduces their formation of LTB_4 ex vivo (14). By the mechanisms cited, and by suppression of monocyte cytokine formation (15), EPA is thought to mediate the antiinflammatory effects, observed in epidemiologic and some animal studies (14). In dietary intervention studies with fish oils in patients with chronic polyarthritis, asthma, or chronic inflammatory bowel disease, some benefit has been reported after 4-12 weeks of dietary n-3 fatty acid supplementation (14, 16-19).

In human polymorphonuclear neutrophils, LTB_4 is catabolized, and thereby largely inactivated, to 20-OH-LTB_4 by a cytochrome P-450 enzyme system (20-26). Arachidonic acid and a number of lipoxygenase products therefrom are also subject to 20-hydroxylation by cytochrome P-450 enzyme system(s) (26-28). 20-OH-LTB_4 is further catabolized to an even less biologically active 20-COOH-LTB_4 (20, 21, 24) via the aldehyde intermediate 20-CHO-LTB_4 (29). Preliminary evidence for formation of 20-OH-LTB_5 has recently been obtained in vitro (30). Further catabolism of 20-OH-LTB_5 is yet unknown. Qualitative differences between cellular metabolism of EPA in vitro and ex vivo have been described (31, 32). Catabolites of LTB_5, however, have not been demonstrated ex vivo.

LTB_5 seems to inhibit 20-hydroxylation of LTB_4 in a purified enzyme preparation (26). Competition for 20-carboxylation between 20-OH-LTB_4 and a 20-OH-LTB_5, if formed, is likely. These interactions at each catabolic step might very well affect levels of the biologically highly active LTB_4, and remain to be studied. Moreover, catabolism of LTB_4 during ingestion of n-3 fatty acids has not yet been investigated.

Therefore, we studied simultaneous formation and catabolism of LTB_4 and LTB_5 in human neutrophils in vitro and ex vivo. Neither LTB_4 nor LTB_5 has been quantified in this manner in the presence of exogenous or endogenous EPA.

Abbreviations: EPA, eicosapentaenoic acid; leukotriene (LT) B_4, 5S,12R-dihydroxy-6-*cis*-8,10-*trans*-14-*cis*-eicosatetraenoic acid; 20-OH-LTB_4, 5S,12R,20-trihydroxy-6-*cis*-8,10-*trans*-14-*cis*-eicosatetraenoic acid; 20-COOH-LTB_4, 5S,12R-dihydroxy-20-carboxy-6-*cis*-8,10-*trans*-14-*cis*-eicosatetraenoic acid; LTB_5, 5S,12R-dihydroxy-6-*cis*-8,10-*trans*-14,17-*cis*-eicosapentaenoic acid; 20-OH-LTB_5, 5S,12R,20-trihydroxy-6-*cis*-8,10-*trans*-14,17-*cis*-eicosapentaenoic acid; 20-COOH-LTB_5, 5S,12R-dihydroxy-20-carboxy-6-*cis*-8,10-*trans*-14,17-*cis*-eicosapentaenoic acid; HPLC, high performance liquid chromatography; RP-HPLC, reverse-phase HPLC.

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MATERIALS AND METHODS

Neutrophil preparations

After an overnight fast, venous blood was obtained from healthy male volunteers via a free flow technique through a 17-G needle. Neutrophils were prepared as described previously (13, 28, 33). The preparation resulted in platelet-free suspensions, in which 99% were polymorphonuclear neutrophils; 3 × 10^7 were used for each experiment. Neutrophils were stimulated with the model compound ionophore A23187 (10 μM) (Sigma, Munich, FRG) or carrier (13, 28, 33).

Experiments with exogenous EPA

In vitro studies. EPA (Sigma, Munich, FRG) was freshly prepared as sodium salt in ethanol (13). Two sets of experiments were carried out four times with blood from different donors: *a*) time course: neutrophils or buffer were incubated for 0, 20, 40, 60, 80, 100, or 120 min in the presence or absence of 15 μM EPA; *b*) concentration curve: neutrophils or buffer were incubated for 80 min in the presence of 0, 0.6, 1.5, 3.0, 6.0, 15, 30, or 60 μM EPA.

HPLC analysis. Incubations were stopped by addition of 1.5 vol acetone, and 500 ng PGB_2 was added as internal standard. Samples were extracted as described previously (33). Analysis was carried out using a Waters 5μ Bondapak C18 RP-HPLC-column (30 cm, 3.9 mm i.d.). Samples dissolved in methanol–water 50:50 (by vol) were injected. As solvent a 45-min gradient, curve 6 on a Waters 660 solvent programmer, from 50:50:0.01 to 80:20:0.01 (by vol) methanol–water–acetic acid, pH adjusted to 5.7 with ammonia, was used at 1.4 ml/min. The eluate was monitored at 280 nm with a Waters 490 UV-detector, and the chromatogram was recorded on a Fresenius BD9 recorder. LTB_4, 20-OH-LTB_4, and 20-COOH-LTB_4 were identified by comparison with retention times of synthetic standards (Paesel, Frankfurt, FRG). The peaks were measured manually and quantified by peak area comparison with the internal standard, assuming identical absorbance at 280 nm for all compounds quantified.

Gas chromatography–mass spectrometry. HPLC-eluates believed to contain 20-OH-LTB_5 or 20-COOH-LTB_5 were collected, pooled, acidified to pH 3.5 with H_3PO_4, followed by extraction (34, 35). Gas chromatography–mass spectrometry was performed as described previously (13). Briefly, a Finnigan MAT44s equipped with a DB 1 fused silica capillary column (30 m, 0.25 mm internal diameter, J + W Scientific, Inc., distributed by ICT, Frankfurt, FRG) was used. A portion of 20-OH-LTB_5 was hydrogenated in methanol using PtO_2 as catalyst. All samples were esterified in methanol with ethereal diazomethane. Trimethylsilylethers were generated with BSTFA (1 h, 20°C) (13).

Experiments with endogenous EPA

Volunteer study. Six healthy male volunteers, as judged by a routine clinical examination and normal laboratory screening tests, gave informed consent, and supplemented their otherwise unaltered Western diet with 50 ml of cod liver oil (Peter Möller, Oslo, Norway), containing 11.3% 16:0, 22.8% 18:1, 9.4% EPA, 0.9% 22:5n–3, and 13.8% docosahexaenoic acid (36), per day for 7 days. Volunteers were questioned for side effects. Two hundred ml blood was taken, before and after the dietary intervention, as described. Routine laboratory tests were repeated, and neutrophils were prepared as described above.

Ex vivo studies. Neutrophils were stimulated with 10 μM ionophore A 23187 for 0, 10, 20, 40, 60, 80, 100, and 120 min. Samples were extracted and analyzed as described above; 3 × 10^7 unstimulated neutrophils were extracted (37) and subjected to fatty acid analysis as described previously (13).

The study was approved by the Ethics Committee of the faculty of Medicine of the Ludwig-Maximilians Universität, Munich.

Student's two-tailed paired *t*-test was used.

RESULTS

Experiments with exogenous EPA

In vitro studies. When 15 μM EPA was incubated with human neutrophils, LTB_5 was formed (**Fig. 1**). A new peak was observed on HPLC, eluting prior to 20-OH-LTB_4, representing a compound more polar than LTB_5. An even more polar peak was noted prior to 20-COOH-LTB_4. Retention times in min (± SD), n = 10, were: 20-COOH-LTB_4, 10.5 (± 0.4); 20-OH-LTB_4, 14.8 (± 0.4); PGB_2, 25.1 (± 0.4); LTB_5, 26.9 (± 0.5); LTB_4, 31.8 (± 0.4). In control incubations, without ionophore or in the absence of cells, only the internal standard was detected at 280 nm.

Gas chromatography–mass spectrometry. The less polar peak, with a retention time (± SD) of 12.3 (± 0.5) min was characterized by gas chromatography–mass spectrometry as 20-OH-LTB_5. The mass spectrum demonstrated interfering fragments from biological background not separated by gas–liquid chromatography. As a methylester-trimethylsilylether derivative 20-OH-LTB_5 displayed the following prominent ions: m/z 580 = $(M)^+$, the characteristic molecular ion of the derivative of 20-OH-LTB_5, m/z 490 = $(M-90(Me_3SiOH))^+$ had two mass units less than m/z 492, detected from 20-OH-LTB_4 (20, 23), consistent with the additional double bond at C17. $(M-(197+90$ $(\cdot CH_2\text{-}CH = CH\text{-}CH_2\text{-}CH = CH\text{-}CH_2\text{-}CH_2\text{-}OSi$ $(CH_3)_3))^+$ at m/z 293 was an ion also obtained from LTB_4, 20-OH-LTB_4, 20- COOH-LTB_4 and LTB_5 (1, 20, 24). When 20-OH-LTB_5 was hydrogenated, and the methylester-trimethylsilylether derivative was injected, prominent ions

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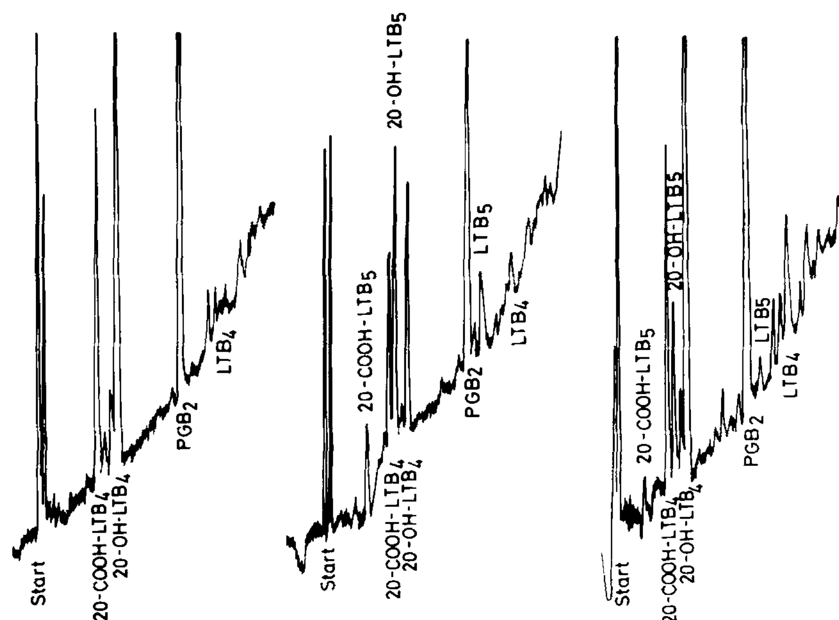

Fig. 1. Human polymorphonuclear neutrophils (3 × 10^7) were stimulated with the ionophore A23187 (10 μM, 80 min), in the absence (left), and in the presence of 15 μM eicosapentaenoic acid (middle), and after addition of 50 ml/day cod liver oil to an otherwise unchanged diet for 7 days (approximately 5 g eicosapentaenoic acid/day, right). The reaction was terminated by extraction and products were analyzed with a gradient system on RP- HPLC (Methods). Therefore, the baseline is ascending. Absorbance was monitored at 280 nm.

were *m/z* 575 = $(M-15(CH_3))^+$ and *m/z* 203 = $(M-(C_6-C_{20}))^+$. They were identical to those detected from hydrogenated 20-OH- LTB_4 (24) after the same derivatization procedure.

The other new peak on HPLC, now identified as 20-COOH-LTB_5, eluted with a shorter retention time (± SD) or 5.8 (± 0.4) min, and was therefore more polar. The mass spectrum obtained from this peak also contained interfering biological background material (**Fig. 2**). Prominent ions detected were: *m/z* 446 = $(M-90\ (Me_3SiOH))^+$, two mass units less than the corresponding fragment *m/z* 448 of *20-COOH-LTB₄* (20, 23), consistent with the additional double bond at C17. Fragments *m/z* 415 = $(M-(90+31)\ (OCH_3))^+$, *m/z* 383 = $(M-153\ (\cdot CH_2-CH=CH-CH_2-CH_2=CH-CH_2-COOCH_3))^+$, and *m/z* 293 = $(M-(153+90))^+$ were identical to fragments from LTB_4, 20-OH-LTB_4, 20-COOH-LTB_4, and LTB_5 (1, 20, 24). Fragment (*m/z* 255 = $(M-281(CH_3OOC-(CH_2)_3-CHOSi(CH_3)_3-(CH)_6\cdot))^+$ had two mass units less than the corresponding ion *m/z* 257 of 20-COOH-LTB_4 (20, 24), consistent with the additional double bond at C17.

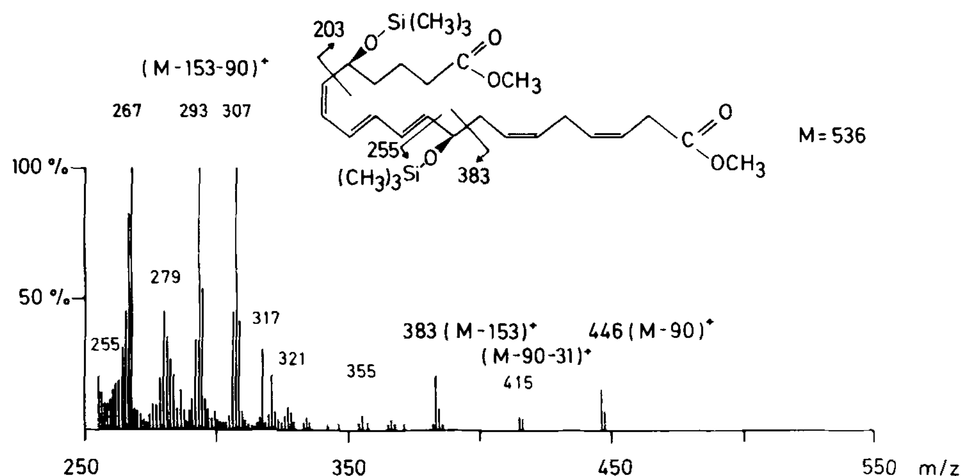

Fig. 2. Mass spectrum of the methylester-trimethylsilylether derivative of 20-carboxy-leukotriene B_5.

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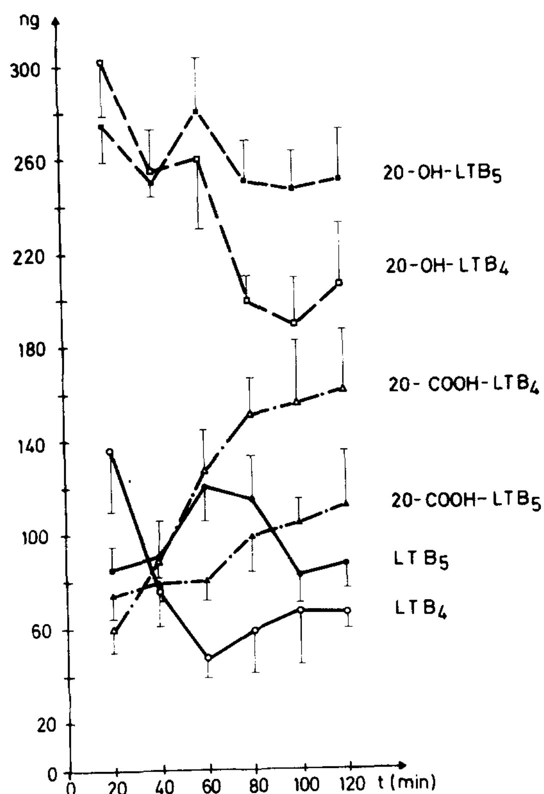

Fig. 3. Human neutrophils were stimulated with ionophore (10 μM) for 20–120 min in the presence of 15 μM EPA. Depicted is the mean amount of product formed (± SEM) in four sets of experiments with blood from different donors.

Time course in vitro. **Fig. 3** depicts the results of neutrophil stimulations for 20–120 min in the presence of 15 μM EPA. Maximum amounts of LTB_5 can be detected at 60 min, when levels of LTB_4 decrease to a nadir. Large and equivalent amounts of 20-OH-LTB_5 and 20-OH-LTB_4 were detectable at 20 min, with 20-OH-LTB_5 remaining quantitatively unaltered, whereas 20-OH-LTB_4 decreased with time. Amounts of 20-COOH-LTB_5 increased more slowly than quantities of 20-COOH-LTB_4.

Concentration curve in vitro. In **Fig. 4**, results of neutrophil stimulation for 80 min in the presence of 0–60 μM EPA are shown. Dose-dependently, addition of EPA increased formation of LTB_5 from undetectable levels in controls and at 0.6 μM EPA to 350 ng/3 × 10^7 cells. Interestingly, levels of LTB_4 increased moderately up to 6 μM exogenous EPA, whereas at 15 μM, LTB_4 started to decline. 20-OH-LTB_5 was detectable in small amounts in control incubations, and increased up to 15 μM EPA, and declined at 30 and 60 μM. Levels of 20-OH-LTB_4 were depressed depending on the dose of exogenous EPA. 20-COOH-LTB_5 was undetectable in controls and at 0.6 μM exogenous EPA, then increased up to 15 μM, and declined to low levels at 30 and 60 μM. 20-COOH-LTB_4 roughly parallelled 20-OH-LTB_4.

Effect of endogenous EPA

Dietary intervention. The addition of 50 ml/day of cod liver oil to an otherwise unaltered diet was well tolerated. No side effects, except "fishy hiccups," were reported. A detailed routine laboratory check showed no alterations, except a decrease of triacylglycerols (± SD) from 131.17 ± 65 to 77.17 ± 24.3 mg/dl ($2P < 0.05$), which is consistent with previous reports (14). EPA, 22:5n–3, and docosahexaenoic acid increased in neutrophil phospholipid fatty acids at the expense of linoleic and arachidonic acids (**Table 1**). Interestingly, 16:0, which is contained in cod liver oil at 11.3%, increased, whereas 18:0 decreased in equivalent amounts.

Ex vivo studies. Whereas LTB_5 and 20-COOH-LTB_5 were not observed prior to dietary intervention, 20-OH-LTB_5 was detectable in small amounts, comparable to small amounts of EPA in neutrophil phospholipid fatty acids. 20-OH-LTB_5 increased, and LTB_5 and 20-COOH-LTB_5 became visible upon addition of EPA to the volunteers' diet. A representative HPLC tracing, demonstrating 20-OH-LTB_5 and 20-COOH-LTB_5 ex vivo, is shown in Fig. 1, right.

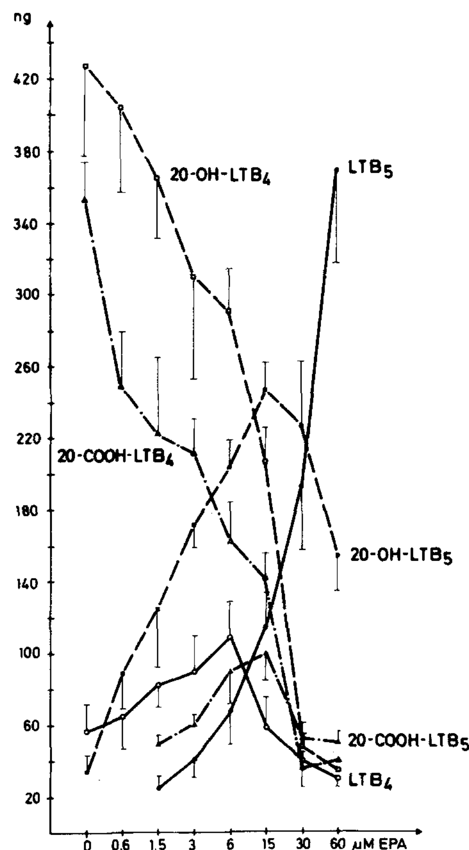

Fig. 4. Human neutrophils were stimulated with ionophore for 80 min in the presence of 0–60 μM eicosapentaenoic acid. Depicted is the mean amount of product formed (± SEM) in four sets of experiments with blood from different donors.

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TABLE 1. Neutrophil phospholipid fatty acid composition before and after 7 days of 50 ml/day cod liver oil, containing 9.4% eicosapentaenoic acid (C20:5n-3) and 13.8% docosahexaenoic acid (C22:6n-3), in healthy human volunteers

Fatty Acid	Control	Cod Liver Oil	*P* Value
16:0	20.6 (± 1.4)	22.6 (± 0.5)	0.032
18:0	17.3 (± 0.7)	15.0 (± 1.5)	0.028
18:1n-9	27.1 (± 1.8)	24.7 (± 1.7)	0.557
18:2n-6	10.3 (± 1.8)	7.6 (± 1.1)	0.0001
20:4n-6	14.9 (± 1.8)	12.3 (± 1.0)	0.006
20:5n-3	0.9 (± 0.4)	3.3 (± 0.4)	0.0001
22:5n-3	2.0 (± 0.5)	3.4 (± 0.5)	0.0001
22:6n-3	1.3 (± 0.5)	2.6 (± 0.4)	0.0001

Values are relative % mean (± SD), n = 6, of total fatty acids set as 100%. Student's two-tailed paired *t*-test was applied. Remaining fatty acids were not significantly altered.

The time course of ex vivo formation of LTB_4, 20-OH-LTB_4, 20-COOH-LTB_4 and LTB_5, 20-OH-LTB_5, and 20-COOH-LTB_5 before (left) and after (right) the dietary intervention is depicted in **Fig. 5**. LTB_4 rapidly decreased from its 10 min value to a nadir at 40 min, followed by a small increase at 60 min, and a slow decrease to the 120 min value. 20-OH-LTB_4 remained in the vicinity of 440 ng/3 × 10^7 neutrophils throughout the incubation period. From 10 to 60 min 20-COOH-LTB_4 increased time-dependently from 157 to a plateau around 350 ng/3 × 10^7 cells. The kinetics of 20-COOH-LTB_4 remained unaltered by the addition of cod liver oil to the volunteers' diet. Levels of LTB_5 were maximal with 33 ng at 10 min and decreased slowly to 17 ng/3 × 10^7 cells. Before dietary cod liver oil, 20-OH-LTB_5 was in the vicinity of 40 ng/3 × 10^7 cells; it increased about twofold after the addition of cod liver oil to the diet. 20-COOH-LTB_5 increased slowly from 16 to 42 ng/3 × 10^7 cells.

DISCUSSION

We studied human catabolism of LTB_5, a compound that is formed by stimulated neutrophils after enrichment of the diet with EPA (1-3). For the first time, we present definite evidence for formation of 20-OH-LTB_5 and 20-COOH-LTB_5 in vitro and ex vivo.

Proofs of structures of 20-OH-LTB_5 and 20-COOH-LTB_5 were based on four independent lines of evidence. *i*) 20-OH-LTB_5 and 20-COOH-LTB_5 absorb UV light at 280 nm, as does LTB_5, indicating that the intrinsic triene structure remained intact. *ii*) Our HPLC system separated compounds according to polarity and their number of double bonds. More polar compounds eluted first, as evidenced by retention of 20-COOH-LTB_4 < 20-OH-LTB_4 < LTB_4. This was paralleled by retention of 20-COOH-LTB_5 < 20-OH-LTB_5 < LTB_5 (Fig. 1). Moreover, retention times of these EPA derivatives were consistently shorter than those of the respective arachidonic acid derivatives. Interestingly, 20-OH-LTB_5 and 20-COOH-LTB_5 appeared at retention times as anticipated in comparison with retention times of LTB_4, LTB_5, and 20-OH-LTB_4 or LTB_4, LTB_5, and 20-COOH-LTB_4, respectively. *iii*) Human neutrophils possess enzyme systems necessary for the metabolic conversion: neutrophils 20-hydroxylate LTB_4 (20, 24), 12-hydroxyeicosatetraenoic acid (38), 12-hydroxy-EPA (13), and a number of mono- and di-hydroxyeicosapolyenoic acids (26). LTB_5 seems to competitively inhibit 20-hydroxylation of LTB_4 in a neutrophil enzyme preparation (26). Neutrophils also have been shown to 20-carboxylate 20-OH-LTB_4 (20, 24), and 12,20-dihydroxyeicosatetraenoic acid (33). The metabolic conversions mentioned have largely been found to be catalyzed by cytochrome P-450 enzyme system(s) (22, 23, 25-28). Therefore, in the formation of 20-OH-LTB_5 and 20-COOH-LTB_5, cyto-

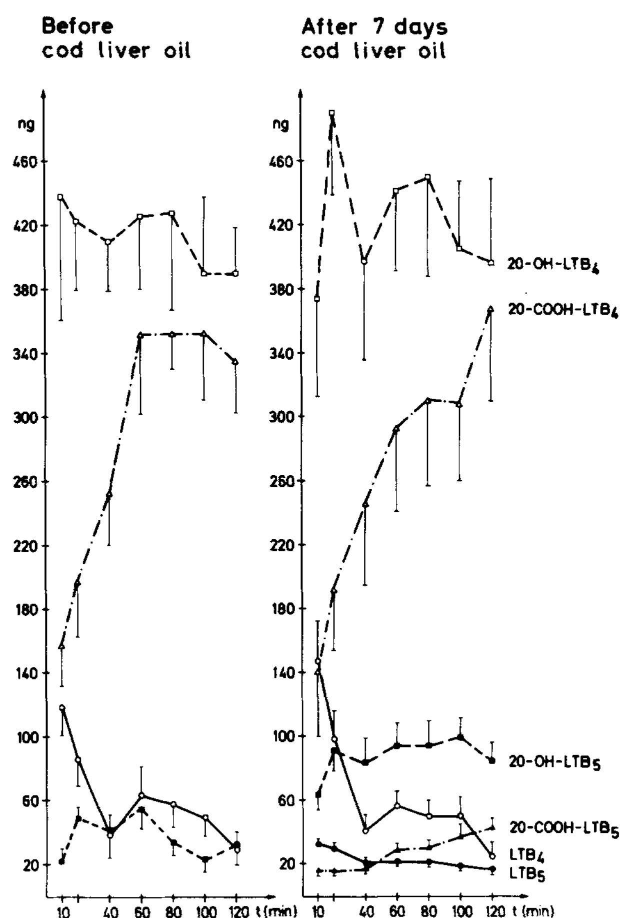

Fig. 5. Formation of leukotrienes B during stimulation for 10-120 min in human neutrophils from six volunteers (mean ± SEM). Neutrophils were prepared from the same volunteers before (left) and after (right) ingestion of 50 ml/day cod liver oil for 7 days in addition to a regular diet. 20-COOH-LTB_5 and LTB_5 were not detectable before cod liver oil ingestion. 20-OH-LTB_5 was significantly elevated after cod liver oil, $P <$ or $<< 0.05$, two-tailed paired *t*-test.

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chrome P-450 enzyme system(s) may also be involved. *iv*) Final evidence is derived from the fragmentation pattern of a mass spectrum obtained by gas chromatography-mass spectrometry of 20-COOH-LTB_5 purified by HPLC and derivatized as methylester trimethylsilylether (Fig. 2). Due to interfering biologic background material, not resolvable by gas chromatography, characteristic fragments, but no mass spectrum, of 20-OH-LTB_5 were obtained. In conjunction with the mass spectrum of its derivative 20-COOH-LTB_5, we consider the structure of 20-OH-LTB_5 established.

A 20-hydroxylated metabolite of the EPA-derivative PGE_3 cannot be detected in urine after supplementation of the human diet with fish oil (39, 40). In the same samples, however, PGE_3 and the arachidonic acid derivative PGE_2, as well as its 20-hydroxylated catabolite, can be detected (39, 40). Thus, 20-hydroxylation does not appear to be a universal form of catabolism in the human eicosanoid system.

20-OH-LTB_4 is catabolized to 20-COOH-LTB_4 via the aldehyde intermediate 20-CHO-LTB_4 (29). We did not detect an aldehyde intermediate that can be postulated for conversion of 20-OH-LTB_5 to 20-COOH-LTB_5. When investigating neutrophil 20-carboxylation of 12,20-dihydroxyeicosatetraenoic acid to 12S-hydroxyeicosatetraen-1,20-dioic acid, no evidence for an aldehyde intermediate was obtained (33). The assumed aldehyde intermediate, 20-CHO-LTB_5, may undergo rapid conversion to 20-COOH-LTB_5.

We did not attempt to investigate the enzyme systems involved in great detail. However, some alterations in metabolism of LTB_4 are interesting to note. Exogenous EPA (15 μM) "shifted" levels of LTB_4, lowered levels of 20-OH-LTB_4, and slowed formation of 20-COOH-LTB_4, as compared to control (Fig. 3 and Fig. 5 left). Exogenous EPA (0.6–6 μM) increased levels of LTB_4 at 80 min, whereas levels of 20-OH-LTB_4 decreased (Fig. 4). These findings are in accord with delayed catabolism of LTB_4 being caused by competition for neutrophil 20-hydroxylase (26), as an explanation for increased levels of LTB_4 in the presence of exogenous EPA in vitro. The sum of 20-OH-LTB_4 and 20-OH-LTB_5 remained in the vicinity of 500 ng/3 × 10^7 cells at all time points in vitro and ex vivo (Figs. 3, 5), and at 0–15 μM exogenous EPA (Fig. 4). Ex vivo, levels of LTB_4 were not increased by the administration of n–3 fatty acids. Thus, ex vivo, neutrophil 20-hydroxylase seems to have a limited, but sufficient, capacity to inactivate the amounts formed of both LTB_4 and LTB_5. Competition for 20-carboxylation between 20-OH-LTB_4 and 20-OH-LTB_5 is also likely to occur, and merits further study.

20-OH-LTB_5 and 20-COOH-LTB_5 possess the same rigid structure in the C17–C18 region of the molecule as does LTB_5. This rigidity, due to the n–3 double bond, has been thought to interfere with the active site specificity of LTB_5 to a substantial degree (41). Moreover, LTB_4 is sequentially inactivated by transformation to its 20-OH and 20-COOH derivatives (20, 24). LTB_5, which has little proinflammatory activity (8, 9), might very well be inactivated further along the same route. Although biological properties not as yet characterized may be attributable to LTB_5, we speculate that 20-OH-LTB_5 and 20-COOH-LTB_5 may not be biologically active.

Assuming that catabolism of leukotrienes B occurred after formation, and formation was completed within a limited time, we used a 2-h time-frame for neutrophil stimulations. Unexpectedly, both leukotrienes B and all their respective catabolites were detectable in considerable amounts at all timepoints studied. Therefore, rather than peak levels, overall amounts of leukotrienes B formed may be of biologic significance.

Neutrophil phospholipid fatty acid composition was altered dramatically by the dietary intervention. EPA increased fourfold, and docosahexaenoic acid doubled in the neutrophil membrane. Importantly, incorporation of n–3 fatty acids occurred at the sole expense of n–6 fatty acids (Table 1). 22:5n–3 is present only in trace amounts in the cod liver oil used. 22:5n–3 increased in phospholipid neutrophil fatty acids. Presumably 22:5n–3 is derived from EPA by endogenous chain-elongation (42); its biological properties however, remain to be elucidated in humans (43, 44).

Our 1 week supplementation of cod liver oil reduced the amount of arachidonic acid in neutrophil membranes to the same degree as was observed in much longer dietary intervention studies (14). In our study, the amount

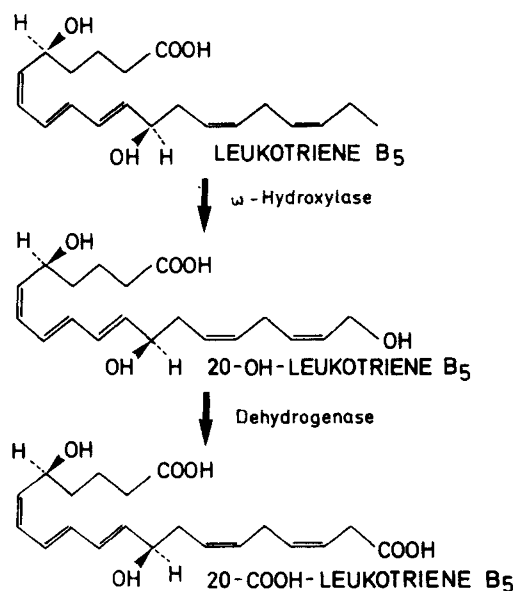

Fig. 6. Catabolism of leukotriene B_5 in human polymorphonuclear neutrophils.

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LTB_4 formed ex vivo was unaltered (Fig. 5). In 4-6 week studies, formation of LTB_4 was reduced under comparable assay conditions (2, 3, 14, 16-19). In vitro, inhibition of LTB_4 formation can only be induced by large amounts of exogenous EPA leading to ratios of LTB_4 to LTB_5 not attained ex vivo (Figs. 4 and 5, refs. 1, 2, 11, 12). Labeled human neutrophils (2) or platelets (32) release a lower percentage of radioactive arachidonic acid upon stimulation during long-term dietary n-3 fatty acids than in control periods. Thus, reduced production of LTB_4 by human neutrophils during long-term ingestion of n-3 fatty acids cannot simply be explained by reduced arachidonic acid content or competition for 5-lipoxygenase.

We investigated human neutrophil catabolism of LTB_5, as it occurs in vitro, and ex vivo after a diet rich in EPA. LTB_5 is catabolized to 20-OH-LTB_5, which in turn is metabolized to 20-COOH-LTB_5 (**Fig. 6**). Presumably the same enzyme systems are involved in the catabolism of LTB_5 that are responsible for catabolism of LTB_4. Although we present evidence for competition of LTB_4 and LTB_5 for catabolism to their respective 20-OH-derivatives, and of these to their respective 20-COOH-derivatives in vitro, ex vivo catabolism of proinflammatory LTB_4 was not impeded.

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REFERENCES

1. Strasser, T., S. Fischer, and P. C. Weber. 1985. Leukotriene B_5 is formed in human neutrophils after dietary supplementation with icosapentaenoic acid. *Proc. Natl. Acad. Sci. USA.* **82:** 1540-1543.
2. Lee, T. H., R. L. Hoover, J. D. Williams, R. I. Sperling, J. Ravalese, B. W. Spur, D. W. Robinson, E. J. Corey, R. A. Lewis, and K. F. Austen. 1985. Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acid on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N. Engl. J. Med.* **312:** 1217-1224.
3. Prescott, S. M., G. A. Zimmerman, and A. R. Morrison. 1985. The effects of a diet rich in fish oil on human neutrophils: identification of LTB_5 as a metabolite. *Prostaglandins.* **30:** 209-227.
4. Borgeat, P., and B. Samuelsson. 1979. Arachidonic acid metabolism in polymorphonuclear leukocytes: effect of ionophore A23187. *Proc. Natl. Acad. Sci. USA.* **76:** 2148-2152.
5. Goetzl, E. J., and W. C. Pickett. 1980. The human PMN leukocyte chemotactic activity of complex hydroxy-eicosatetraenoic acids (HETEs). *J. Immunol.* **125:** 1789-1791.
6. Ford-Hutchinson, A. W., M. A. Bray, M. V. Doig, M. E. Shipley, and M. J. H. Smith. 1980. Leukotriene B_4, a potent chemokinetic and aggregating substance released from polymorphonuclear neutrophils. *Nature. (London)* **286:** 264-265.
7. Palmblad, J., C. L. Malmsten, A. M. Udén, O. Rådmark, L. Engstedt, and B. Samuelsson. 1981. Leukotriene B_4 is a potent and stereospecific stimulator of neutrophil chemotaxis and adherence. *Blood.* **58:** 658-661.
8. Goldman, D. W., W. C. Pickett, and E. J. Goetzl. 1983. Human neutrophil chemotactic and degranulating activities of leukotriene B_5 (LTB_5) derived from eicosapentaenoic acid. *Biochem. Biophys. Res. Commun.* **117:** 282-288.
9. Lee, T. H., J. M. Mencia-Huerta, C. Shih, E. J. Corey, R. A. Lewis, and K. F. Austen. 1984. Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B_5 and the double lipoxygenase product. *J. Biol. Chem.* **259:** 2383-2389.
10. Charleson, S., J. F. Evans, R. J. Zamboni, Y. Leblanc, B. J. Fitzsimmons, C. Leveille, P. Dupuis, and A. Ford-Hutchinson. 1986. Leukotriene B_3, leukotriene B_4, and leukotriene B_5: binding to leukotriene B_4 receptors on rat and human leukocyte membranes. *Prostaglandins.* **32:** 503-516.
11. Lee, T. H., J. M. Mencia-Huerta, S. Shih, E. J. Corey, R. A. Lewis, and K. F. Austen. 1984. Effects of exogenous arachidonic, eicosapentaenoic, and docosahexaenoic acids on the generation of 5-lipoxygenase pathway products by ionophore-activated human neutrophils. *J. Clin. Invest.* **74:** 1922-1933.
12. Prescott, S. M. 1984. The effect of eicosapentaenoic acid on leukotriene B production by human neutrophils. *J. Biol. Chem.* **259:** 7615-7621.
13. von Schacky, C., A. J. Marcus, L. B. Safier, H. L. Ullman, N. Islam, M. J. Broekman, and S. Fischer. 1990. Platelet-neutrophil interactions. 12S,20- and 5S,12S-dihydroxyeicosapentaenoic acids: two novel neutrophil metabolites from platelet-derived 12S-hydroxyeicosapentaenoic acid. *J. Lipid Res.* **31:** 801-810.
14. von Schacky, C. 1987. Prophylaxis of atherosclerosis with marine omega-3 fatty acids—a comprehensive strategy. *Ann. Int. Med.* **107:** 890-899.
15. Endres, S., R. Ghorbani, V. E. Kelley, K. Georgilis, G. Lonneman, J. W. M. van der Meer, J. G. Cannon, T. S. Rogers, M. S. Klempner, P. C. Weber, E. J. Schaefer, S. M. Wolff, and C. A. Dinarello. 1989. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by monoculear cells. *N. Engl. J. Med.* **320:** 265-271.
16. Payan, D. G., M. Y. S. Wong, T. Chernov-Rogan, F. H. Valone, W. C. Pickett, V. A. Blake, W. M. Gold, and E. J. Goetzl. 1986. Alterations in human leukocyte function by ingestion of eicosapentaenoic acid. *J. Clin. Immun.* **6:** 402-410.
17. Sperling, R. I., M. Weinblatt, J-L. Robin, J. Ravalese, R. L. Hoover, F. House, J. S. Coblyn, P. A. Fraser, B. W. Spur, D. W. Robinson, R. A. Lewis, and K. F. Austen. 1987. Effects of dietary supplementation with marine fish oil on leukocyte lipid mediator generation and function in rheumatoid arthritis. *Arthritis Rheum.* **30:** 988-997.
18. Arm, J. P., C. C. Horton, J. M. Mencia-Huerta, F. House, N. M. Eiser, T. J. H. Clark, B. W. Spur, and T. H. Lee. 1988. Effect of dietary supplementation with fish oil lipids on mild asthma. *Thorax.* **43:** 84-92.
19. Lorenz, R., P. C. Weber, P. Szimnau, H. Heldwein, T. Strasser, and K. Loeschke. 1989. Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease—a randomised, placebo-controlled, double-blind cross-over trial. *J. Int. Med.* **225, Suppl. 1:** 225-232.
20. Hansson, G., J. Å. Lindgren, S-E. Dahlén, P. Hedquist, and B. Samuelsson. 1981. Identification and biological ac-

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tivity of novel ω-oxidized metabolites of leukotriene B_4 from human leukocytes. *FEBS Lett.* **130:** 107-112.
21. Jubiz, W., O. Rådmark, C. Malmsten, G. Hansson, J. Å. Lindgren, J. Palmblad, A-M. Udén, and B. Samuelsson. 1982. A novel leukotriene produced by stimulation of leukocytes with formylmethionylleucyl-phenylalanine. *J. Biol. Chem.* **257:** 6106-6110.
22. Shak, S., and I. M. Goldstein. 1984. Carbon monoxide inhibits ω-oxidation of leukotriene B_4 by human polymorphonuclear leukocytes: evidence that catabolism of leukotriene B_4 is mediated by a cytochrome P-450 enzyme. *Biochem. Biophys. Res. Commun.* **123:** 475-481.
23. Powell, W. S. 1984. Properties of leukotriene B_4 20-hydroxylase from polymorphonuclear leukocytes. *J. Biol. Chem.* **259:** 3082-3089.
24. Shak, S., and I. M. Goldstein. 1984. ω-Oxydation is the major pathway for the catabolism of leukotriene B_4 in human polymorphonuclear leukocytes. *J. Biol. Chem.* **259:** 10181-10187.
25. Shak, S., and I. M. Goldstein. 1985. Leukotriene B_4 ω-hydroxylase in human polymorphonuclear leukocytes. Partial purification and identification as a cytochrome P-450. *J. Clin. Invest.* **76:** 1218-1228.
26. Soberman, R. J., R. T. Okita, B. Fitzsimmons, J. Rokach, B. Spur, and K. F. Austen. 1987. Stereochemical requirements for substrate specificity of LTB_4 20-hydroxylase. *J. Biol. Chem.* **262:** 12421-12427.
27. Hatzelmann, A., and V. Ullrich. 1985. The ω-hydroxylation of arachidonic acid by human polymorphonuclear leukocytes. *Eur. J. Biochem.* **173:** 445-452.
28. Marcus, A. J., L. B. Safier, H. L. Ullman, N. Islam, M. J. Broekman, and C. von Schacky. 1987. Studies on the mechanism of ω-hydroxylation of platelet 12-hydroxyeicosatetraenoic acid (12-HETE) by unstimulated neutrophils. *J. Clin. Invest.* **79:** 179-187.
29. Soberman, R. J., J. P. Sutyak, R. T. Okita, D. E. Wendelborn, L. J. Roberts, and K. F. Austen. 1988. The identification and formation of 20-aldehyde leukotriene B_4. *J. Biol. Chem.* **263:** 7996-8002.
30. Mahadevappa, V. G., and W. S. Powell. 1989. The metabolism of arachidonic and eicsoapentaenoic acids in human neutrophils stimulated by A23187 and FMLP. *J. Cell. Biochem.* **40:** 341-352.
31. Ahmed, A. A., and B. J. Holub. 1984. Alteration and recovery of bleeding times, platelet aggregation and fatty acid composition of individual phospholipids in platelets of human subjects receiving a supplement of cod liver oil. *Lipids.* **19:** 617-624.
32. von Schacky, C., W. Siess, S. Fischer, and P. C. Weber. 1985. A comparative study of eicosapentaenoic acid metabolism by human platelets in vivo and in vitro. *J. Lipid Res.* **26:** 457-464.
33. Marcus, A. J., L. B. Safier, H. L. Ullman, N. Islam, M. J. Broekman, J. R. Falck, S. Fischer, and C. von Schacky. 1988. Platelet-neutrophil interactions. 12S-Hydroxeicosatetraen-1,20-dioic acid: a new eicosanoid synthesized by unstimulated neutrophils from 12S,20-dihydroxyeicosatetraenoic acid. *J. Biol. Chem.* **263:** 2223-2229.
34. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911-917.
35. Shak, S. 1987. Leukotriene B_4 catabolism: quantitation of leukotriene B_4 and its ω-oxidation products by reversed-phase high-performance liquid chromatography. *Methods Enzymol.* **141:** 355-371.
36. von Schacky, C., S. Fischer, and P. C. Weber. 1985. Long term effects of dietary marine n-3 fatty acids upon plasma- and cellular-lipids, platelet function and eicosanoid formation in humans. *J. Clin. Invest.* **76:** 1626-1631.
37. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497-509.
38. Marcus, A. J., L. B. Safier, H. L. Ullman, N. Islam, M. J. Broekman, J. R. Falck, S. Fischer and C. von Schacky. 1987. Cell-cell interactions in the eicosanoid pathway. *Ann. N.Y. Acad. Sci.* **516:** 407-411.
39. Fischer, S., C. von Schacky, and H. Schweer. 1988. Prostaglandins E_3 and $F_{3\alpha}$ are excreted in human urine after ingestion of n-3 polyunsaturated fatty acids. *Biochim. Biophys. Acta.* **963:** 501-508.
40. Knapp, H. R., and G. A. FitzGerald. 1989. The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. *N. Engl. J. Med.* **320:** 1037-1043.
41. Lee, T. H., T. Sethi, A. E. G. Crea, W. Peters, J. P. Art, C. E. Horton, M. J. Walport, and B. W. Spur. 1988. Characterization of leukotriene B_3: comparison of its biological activities with leukotriene B_4 and leukotriene B_5 in complement receptor enhancement, lysozyme release and chemotaxis of human neutrophils. *Clin. Sci.* **74:** 467-475.
42. von Schacky, C., and P. C. Weber. 1985. Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. *J. Clin. Invest.* **76:** 2446-2450.
43. Careaga, M. M., and H. Sprecher. 1984. Synthesis of two hydroxy fatty acids from 7,10,13,16,19-docosapentaenoic acid by human platelets. *J. Biol. Chem.* **259:** 14413-14417.
44. Ackman, R. G., and C. A. Eaton. 1988. N-3 docosapentaenoic acid in blubber of dam and pup grey seals (*Halichoerus gypus*): implications in the Inuit diet and for human health. *Can. J. Zool.* **66:** 2428-2431.

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