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Changes in the liver, kidney and heart fatty acid composition following administration of ibuprofen to mice

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Abstract

Crl:NMRI-BR male mice received 0.6 mg/day of ibuprofen (animal model for human dose 1200 mg/day) in the diet for a period of 6 weeks. This treatment resulted in increased body mass, liver mass, and total lipid content in the liver tissue. Changes in the fatty acid composition in the individual lipid classes were most important in kidney tissue; levels of polyunsaturated fatty acids were increased in phospholipids and decreased in neutral lipids. These changes were compensated for by opposite changes in the levels of saturated and monoenoic acids. Similar changes were also observed in liver and heart lipids. An increased level of an unusual component was observed in heart tissue, which was identified as isopropyl myristate by GC–MS and verified by comparing the mass spectra and retention times with those of synthetic standards.

1. Introduction

Side effects on lipid metabolism of various drugs, especially those which are used for a long period (order of years), are one of secondary risk factors of atherosclerosis together with coffee and alcohol intake, smoking and lifestyle in developed industrial countries. Detailed studies have been published concerning the side-effects of all types of antihypertensives [1–3] and contraceptives [4–6]. Much less is known about anti-inflammatory drugs, which are widely used in human medicine. A recent study with human bioptic material [7] described the effect of long-term treatment with aspirin, which resulted in significantly increased liver mass and also increased liver lipid content, both neutral and polar lipids. Prolonged treatment with these drugs can significantly influence both lipid levels and their molecular species and thus contribute to the early development of atherosclerosis.

The aim of this study was to analyse changes in tissue lipid composition after administration of ibuprofen, which is one of the most frequently used anti-inflammatory and antirheumatic drugs.

2. Experimental

2.1. Standards and reagents

Analytical-reagent grade solvents were purchased from Merck (Darmstadt, Germany) and Lachema (Brno, Czech Republic) and were

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further distilled, if necessary. Silica gel $HF_{254+366}$ for preparation of TLC plates was purchased from Merck. Boron trifluoride (14% in methanol) was obtained from Supelco (Gland, Switzerland). Standard fatty acid methyl esters (FAME), triacylglycerols (TG), cholesteryl esters (CE) and phospholipids (PL) were purchased from Sigma (St. Louis, MO, USA). Ibuprofen 200 was obtained from Spofa (Prague, Czech Republic).

2.2. Sample preparation

Crl:NMRI-BR male mice, initial body mass 30.5 ± 1.2 g, received 0.6 mg of ibuprofen daily for a period of 6 weeks in the standard Larsen diet for laboratory animals. The mice were then decapitated, pooled blood was collected from individual groups of mice in test-tubes containing 5% EDTA solution and individual organs were excised, washed with physiological saline, weighed and lyophilized.

Total lipid was extracted from blood plasma and homogenized organs by the method of Folch *et al.* [8]. Individual lipid classes were separated by thin-layer chromatography on silica gel with the mobile phases heptane–diethyl ether–acetic acid (85:15:1, v/v/v) for the separation of CE and TG and chloroform–methanol–water (60:30:5, v/v/v) for the separation of phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Separated lipid classes were isolated from silica gel by a "dry column technique" using chloroform–methanol (1:1, v/v) for elution of neutral lipids and chloroform–methanol–water–acetic acid (13:5:1:0.2, v/v) for PL. Dried samples were saponified with 1 *M* KOH in methanol and acidified, and fatty acids were extracted with hexane. After drying under nitrogen, samples were esterified with methanol–boron trifluoride for 3 min at 100°C. Methyl esters were extracted twice with hexane, dried under nitrogen and analysed by GC.

2.3. Gas chromatography

Gas chromatography was performed with a Model 9000 gas chromatograph (Chrompack, Middelburg, Netherlands) equipped with a capillary split–splitless injector and flame ionization detector. The chromatograph was interfaced with an IBM PS/2 Model 30 computer and Epson LQ 550 printer. Chrompack integration software was used for data acquisition and handling.

Determinations of FAME were performed on a fused-silica capillary column (25 m × 0.25 mm I.D.) coated with chemically bonded CP-WAX 52 CB stationary phase (0.2 μm layer thickness) (Chrompack). The oven temperature was programmed from 160 to 220°C at 2°C/min and then kept isothermal for 10 min. The injector and detector temperatures were 250 and 270°C, respectively. The carrier gas (hydrogen) was maintained at a head pressure of 80 kPa, with a splitting ratio of 1:20.

2.4. Gas chromatography–mass spectrometry

GC–MS analyses were performed with an Incos 50 system (Finnigan MAT, San Jose, CA, USA). A fused-silica capillary column (30 m × 0.32 mm I.D.) coated with DB-5 chemically bonded stationary phase (0.3 μm layer thickness) (J&W, Folsom, CA, USA) was used. The oven temperature programme was isothermal at 80°C for 2 min, followed by a temperature gradient to 250°C at 3°C/min and a hold at 250°C for 10 min. Splitless injection was carried out at 250°C with a splitless time of 30 s. The carrier gas (helium) was maintained at a head pressure of 28 kPa. The mass spectrometer was operated in the electron impact mode with ionization energy 70 eV, emission current 0.8 mA and ion source temperature 150°C.

3. Results and discussion

The dose of ibuprofen was chosen to simulate the usual human daily dose of three tablets of ibuprofen 400 (according to body mass). It is clear that the results cannot be directly extrapolated to a human model; however, positive results should be a signal for more detailed human study.

Basic parameters are summarized in Table 1.

Table 1
Basic parameters of the individual groups of mice

Parameter	Control		Treated		
	Mean	S.D.[a]	Mean	S.D.[a]	*P*
Body mass increase (g)	4.9	3.4	7.9	1.7	NS[c]
Liver mass (g)	1.618	0.210	1.860	0.190	0.05
Kidney mass (g)	0.553	0.078	0.610	0.077	NS
Heart mass (g)	0.177	0.222	0.175	0.018	NS
Liver fat (mg/g)	52.8	15.4	91.7	10.9	0.001
Kidney fat (mg/g)	45.7	7.8	43.8	2.8	NS
Heart fat (mg/g)	51.0	3.9	46.9	4.8	NS
Plasma lipid (mg/ml)[b]	10.8	–	11.4	–	–

[a] $n = 7$.
[b] Pooled samples.
[c] NS = not significant.

Significant increases in liver mass and liver lipid content are important findings, as liver plays a very important role in lipid metabolism. Increased lipid levels were observed for all lipid classes, CE, TG and PL, which were determined gravimetrically. The relative increase in all these parameters is comparable to that observed in a human bioptic study after aspirin administration [7].

Comparison of the fatty acids in the individual

Table 2
Composition of fatty acids in liver neutral lipids (molar %)

Acid	CE control	CE treated	TG control	TG treated
14:0	1.08	1.73[a]	1.10 (0.34)	0.92 (0.30)
16:0	23.55	24.70	34.24 (1.96)	34.89 (1.66)
16:1*n*7	8.51	8.48	5.03 (1.85)	4.49 (0.73)
18:0	6.96	7.80	4.16 (1.85)	2.98 (0.81)
18:1*n*9	31.61	35.07	32.50 (1.65)	32.47 (2.58)
18:1*n*7	3.26	2.21[a]	3.82 (1.28)	2.86 (0.47)
18:2*n*6	16.64	15.91	14.04 (2.47)	17.18 (2.53)*
18:3*n*6	tr	tr	0.24 (0.07)	0.23 (0.07)
20:3*n*6	0.79	0.44[a]	0.74 (0.22)	0.61 (0.11)
20:4*n*6	5.59	2.95[a]	2.19 (1.03)	1.74 (0.56)
20:5*n*3	tr	tr	tr	tr
22:5*n*3	tr	tr	0.36 (0.08)	0.25 (0.06)
22:6*n*3	1.64	0.65	1.58 (0.42)	1.32 (0.51)
Satur.[b]	31.59	34.25	39.49 (2.65)	38.80 (2.05)
Mono.[c]	43.38	45.75	41.36 (4.53)	39.83 (2.80)
Sum *n*6[d]	23.02	19.34[a]	17.20 (3.53)	19.75 (2.64)
Sum *n*3[d]	1.99	0.65[a]	1.94 (0.47)	1.59 (0.53)

In Tables 2–7, each value represents the mean of seven measurements with S.D. in parentheses; tr, traces. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
[a] Expected significant differences between pooled samples.
[b] Satur. = molar % of all saturated fatty acids.
[c] Mono. = molar % of all monoenoic fatty acids.
[d] molar 0% of all polyenoic fatty acids *n*6 and *n*3, respectively.

Table 3
Composition of fatty acids in liver phospholipids (molar %)

Acid	PE control	PE treated	PC control	PC treated
14:0	0.53 (0.07)	0.69 (0.11)*	0.11 (0.03)	0.15 (0.03)*
16:0	26.98 (1.65)	26.64 (1.50)	31.99 (2.23)	30.98 (3.29)
16:1*n*7	1.02 (0.33)	0.88 (0.28)	1.40 (0.49)	1.24 (0.39)
18:0	19.32 (1.23)	19.36 (1.43)	15.82 (1.58)	16.49 (2.06)
18:1*n*9	7.24 (0.81)	7.01 (1.49)	11.21 (1.22)	9.70 (0.96)*
18:1*n*7	1.93 (0.49)	1.60 (0.33)	2.17 (0.65)	1.84 (0.36)
18:2*n*6	6.93 (0.65)	7.25 (1.27)	13.77 (1.59)	15.00 (1.71)
18:3*n*6	0.29 (0.10)	0.23 (0.04)	0.21 (0.05)	0.25 (0.05)
20:3*n*6	1.03 (0.12)	0.97 (0.16)	2.24 (0.49)	2.14 (0.55)
20:4*n*6	15.46 (1.19)	15.62 (1.42)	13.75 (2.23)	14.64 (1.82)
20:5*n*3	0.39 (0.20)	tr	0.22 (0.10)	0.17 (0.09)
22:5*n*3	0.93 (0.07)	0.73 (0.10)**	0.41 (0.07)	0.34 (0.06)
22:6*n*3	17.86 (2.47)	18.69 (1.95)	6.66 (1.21)	7.02 (1.16)
Satur.	46.85 (2.26)	46.55 (2.03)	47.93 (1.30)	47.63 (1.80)
Mono.	10.25 (1.27)	9.64 (1.72)	14.78 (2.18)	12.79 (1.48)
Sum *n*6	23.71 (0.68)	24.08 (1.06)	29.98 (1.88)	32.03 (1.65)
Sum *n*3	19.18 (2.51)	19.71 (1.06)	7.29 (1.22)	7.54 (1.25)

lipid classes in individual tissues is shown in Tables 2–7. In liver lipids, a slight decrease in unsaturated fatty acids was observed (except for TG), which was compensated for by a similar increase in saturated acids. The most significant effect of ibuprofen treatment was found in kidney lipids: decreased levels of polyunsaturated fatty acids in NL and increased levels in PL, again compensated for by opposite changes in saturated and monoenoic acids. In heart TG and PC, a similar effect as in kidney PL was observed.

The main role of ibuprofen, as a non-steroidal anti-inflammatory drug (NSAID), is to inhibit

Table 4
Composition of fatty acids in kidney neutral lipids (molar %)

Acid	CE control	CE treated	TG control	TG treated
14:0	2.75	3.95	3.75 (0.16)	3.29 (0.38)*
16:0	24.72	26.54	30.89 (1.49)	30.76 (1.80)
16:1*n*7	5.75	5.34	11.59 (4.63)	11.36 (3.77)
18:0	5.78	6.65	4.36 (1.04)	3.60 (0.72)
18:1*n*9	20.36	22.28	34.68 (2.49)	34.64 (2.17)
18:1*n*7	2.56	2.69	2.66 (0.11)	2.67 (0.13)
18:2*n*6	17.45	17.34	11.44 (1.80)	13.68 (2.68)
20:3*n*6	1.37	1.00		
20:4*n*6	12.18	9.46[a]		
20:5*n*3	0.36	0.21[a]		
22:5*n*3	0.75	0.40[a]		
22:6*n*3	5.95	4.16[a]		
Satur.	33.25	37.14	39.01 (1.75)	37.64 (1.92)
Mono.	28.68	30.31	48.91 (3.41)	48.67 (2.43)
Sum *n*6	31.00	27.80	11.75 (1.95)	13.68 (2.68)
Sum *n*3	7.06	4.76[a]		

Table 5
Composition of fatty acids in kidney phospholipids (molar %)

Acid	PE control	PE treated	PC control	PC treated
14:0	0.92 (0.28)	0.84 (0.10)	0.21 (0.06)	0.31 (0.06)*
16:0	16.46 (3.30)	12.16 (1.19)**	38.64 (4.32)	43.96 (2.64)*
16:1*n*7	0.76 (0.18)	0.58 (0.14)	1.02 (0.37)	0.89 (0.23)
18:0	32.34 (5.18)	28.11 (0.88)	21.67 (2.93)	12.39 (1.35)***
18:1*n*9	11.52 (1.33)	8.47 (0.64)***	12.12 (1.75)	10.48 (1.68)
18:1*n*7	1.72 (0.53)	1.31 (0.28)	3.35 (0.77)	3.06 (0.24)
18:2*n*6	4.10 (0.60)	3.77 (0.50)	6.31 (1.03)	8.00 (1.24)*
20:3*n*6	0.61 (0.12)	0.56 (0.08)	1.50 (0.21)	1.26 (0.16)*
20:4*n*6	22:83 (5.62)	29.54 (0.70)*	7.37 (1.67)	7.90 (0.54)
20:5*n*3	0.49 (0.15)	0.46 (0.15)	0.08 (0.05)	0.12 (0.02)
22:5*n*3	0.42 (0.13)	0.65 (0.10)**	0.51 (0.19)	0.56 (0.15)
22:6*n*3	7.80 (1.92)	13.59 (0.99)***	7.22 (0.65)	11.07 (2.05)*
Satur.	49.72 (7.01)	41.06 (0.72)*	60.51 (3.54)	56.67 (1.65)*
Mono.	14.01 (2.00)	10.37 (0.86)**	16.49 (2.55)	14.43 (1.81)
Sum *n*6	27.55 (6.07)	33.87 (0.61)*	15.18 (2.19)	17.15 (1.56)
Sum *n*3	8.72 (2.12)	14.69 (107)***	7.81 (3.90)	11.75 (2.20)

Table 6
Composition of fatty acids in heart triacylglycerol (molar %)

Acid	TG control	TG treated
14:0	5.36 (1.20)	5.80 (1.60)
16:0	37.81 (2.86)	39.71 (2.96)
16:1*n*7	9.44 (2.08)	7.11 (1.28)*
18:0	7.48 (1.56)	8.62 (1.75)
18:1*n*9	33.76 (2.65)	31.40 (1.98)
18:1*n*7	2.71 (0.40)	2.47 (0.12)
18:2*n*6	3.42 (0.51)	4.85 (1.23)*
Satur.	50.67 (4.52)	54.13 (2.18)
Mono.	45.91 (4.61)	40.99 (2.41)*
Sum *n*6	3.42 (0.51)	4.85 (1.23)*

cyclooxygenase and lipoxygenase pathways of polyunsaturated fatty acids. Thus, an increase in these acids in PL can be explained by this inhibitive reaction, resulting in an increased substrate concentration. However, a more complex influence on the other enzymes of lipid metabolism (elongation and desaturation system, lipolytic enzymes) should also be considered.

It is known that ibuprofen, as a xenobiotic fatty acid, is incorporated into TG of adipose tissue *in vivo* [9]. Incorporation of ibuprofen into the TG fraction was observed also *in vitro* with

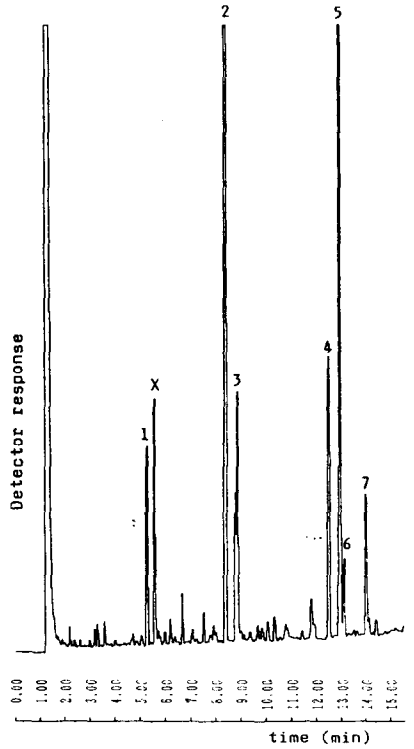

Fig. 1. Part of the gas chromatograms of heart lipid FAME on a CP-WAX 52 CB column. Peaks: 1 = 14:0; X = isopropyl myristate; 2 = 16:0; 3 = 16:1*n*7; 4 = 18:0; 5 = 18:1*n*9; 6 = 18:1*n*7; 7 = 18:2*n*6.

Table 7
Composition of fatty acids in heart phospholipids (molar %)

Acid	PE control	PE treated	PC control	PC treated
14:0	3.60 (2.44)	4.08 (1.31)	0.44 (0.09)	0.44 (0.07)
16:0	28.94 (2.04)	30.47 (2.60)	35.45 (2.66)	33.36 (3.10)
16:1*n*7	5.15 (2.54)	5.02 (0.88)	2.31 (0.64)	1.89 (0.66)
18:0	29.64 (2.80)	29.93 (3.67)	26.46 (1.73)	28.07 (2.22)
18:1*n*9	18.46 (3.46)	16.00 (2.00)	21.98 (1.91)	20.57 (3.00)
18:1*n*7	3.86 (0.38)	4.39 (0.51)	7.90 (0.52)	7.07 (0.57)*
18:2*n*6	4.34 (2.08)	3.64 (0.93)	3.08 (1.04)	3.50 (1.32)
20:3*n*6			0.21 (0.10)	0.34 (0.13)
20:4*n*6	0.89 (0.48)	0.98 (0.53)	0.82 (0.33)	1.99 (1.10)*
20:5*n*3			0.21 (0.18)	0.18 (0.08)
22:5*n*3	1.07 (0.31)	0.67 (0.32)*	0.33 (0.10)	0.57 (0.17)*
22:6*n*3	3.06 (0.67)	4.56 (3.84)	0.81 (0.32)	2.01 (1.18)*
Satur.	62.17 (2.64)	64.48 (4.84)	62.35 (1.50)	61.87 (2.06)
Mono.	30.49 (6.90)	25.41 (2.52)	32.18 (1.50)	29.53 (3.56)
Sum *n*6	5.55 (3.02)	4.79 (1.36)	4.11 (1.35)	5.84 (2.44)
Sum *n*3	4.13 (0.78)	5.31 (3.61)	1.36 (0.49)	2.76 (1.37)*

liver slices and hepatocytes [10,11]. The aim of this study was not to search for ibuprofen and its metabolites in the tissues under investigation. However, a relatively high content of an unusual component was observed in heart tissue TG with an elution time close to that of methyl myristoleate (Fig. 1). This component was identified as isopropyl myristate by GC–MS and verified

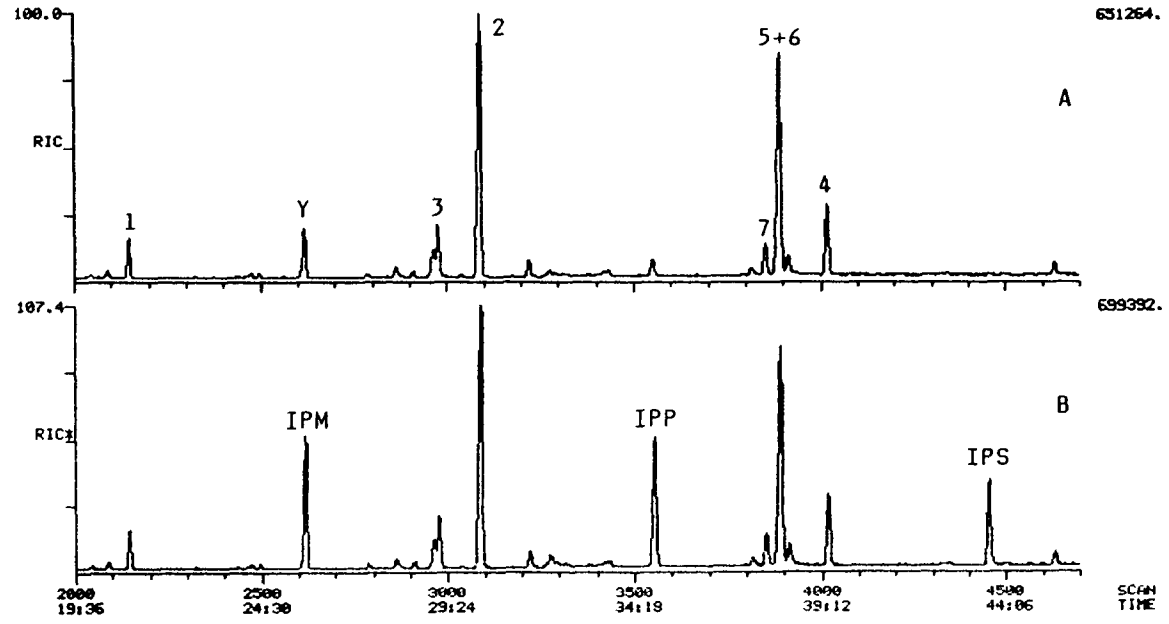

Fig. 2. Total ion chromatograms of heart lipid FAME on a DB-5 column. (A) Original biological sample (Y = unresolved peak of isopropyl myristate and methyl pentadecanoate); (B) biological sample with addition of isopropyl myristate (IPM), isopropyl palmitate (IPP) and isopropyl stearate (IPS). Other peaks as in Fig. 1.

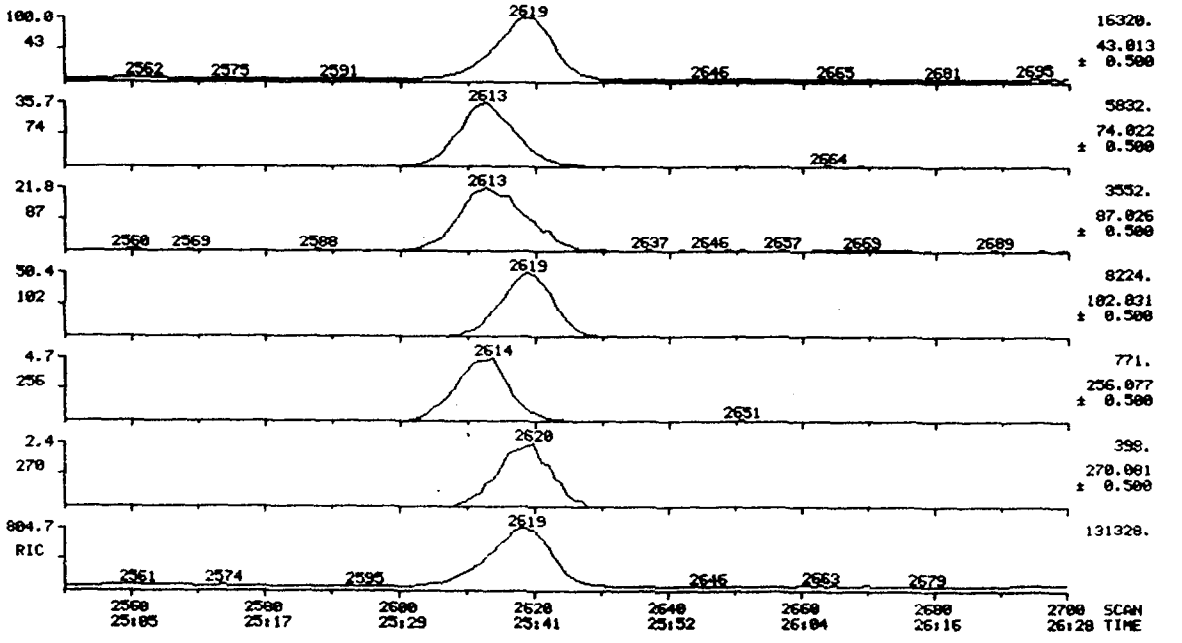

Fig. 3. Detail of total ion chromatogram from Fig. 2A and ion chromatograms of characteristic ions of isopropyl myristate (m/z 270, 102, 43) and methyl pentadecanoate (m/z 256, 87, 74).

by comparing the mass spectra and retention times with those of synthetic standards. Reconstructed ion chromatograms are shown in Figs. 2 and 3. The relative retention times of the observed metabolic product, synthetic isopropyl myristate and the methyl ester of ibuprofen related to methyl myristate were 1.063, 1.061 and 1.038, respectively. The isopropyl group esterified with myristic acid, and to a much lower extent with palmitic acid, can theoretically originate from cleaved ibuprofen, which is hydroxylated on the isobutyl chain [12,13]. This hypothesis should be confirmed by further experiments with isotopically labelled ibuprofen. Possible relationships between changes in molecular species of kidney lipids observed with animal models and renal impairment in man [14] should also be confirmed by a more detailed human study.

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