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Determination of phospholipid fatty acid and triacylglycerol composition of rat caecal mucosa

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ABSTRACT

The lipid composition of rat caecal mucosa, including the fatty acid composition of phospholipids and triacylglycerols, has been examined by capillary gas chromatography. Thirty-seven peaks were resolved, ranging in chain length from 12 to 24 carbon atoms. Preliminary identification of fatty acids by comparison with authentic standards was confirmed by gas chromatography–mass spectrometry using electron-impact ionization. The neutral and polar components were examined. Fatty acid methyl esters were quantified in absolute amounts with respect to the percentage of total phospholipid and triacylglycerols. The results show significantly higher levels of 16:0, 18:0, 18:1(n − 9), 18:1(n − 7), 18:2(n − 6) and 20:4(n − 6) in phospholipids, and higher levels of 16:0, 18:1(n − 9) and 18:2(n − 6) in triacylglycerols. On the other hand, analysis of caecal triacylglycerols revealed *sn*-glycerol-palmitate-oleate-palmitate, *sn*-glycerol-palmitate-linoleate-palmitate and *sn*-glycerol-palmitate-linoleate-oleate as major components.

INTRODUCTION

The animal organism has to maintain a balance between the storage of fatty acids as triacylglycerols and their use in the biosynthesis of phospholipids, which are essential membrane components. Furthermore the lipids of the colonic mucosa, like those of all active organs, are in a dynamic state, susceptible to continual changes by both internal and external factors, such as dietary fat and the presence of hormones.

A major task in the biochemical study of mucosa is the compiling of information on the phospholipid fatty acyl chains that contribute significantly to membrane structure and function [1,2]. There is a lack of data on such triacylglycerol composition. Tissue lipid composition plays several important roles in the regulation of cell function. The first is structural: fatty acids, when incorporated into membrane phospholipids, can alter the physicochemical characteristics (microviscosity or fluidity) of the membrane lipid matrix. This in turn can influence the structure, mobility and function by the modification of intrinsic and extrinsic membrane-bound proteins, such as hormone receptors, ion gates and bound enzymes [3,4]. Secondly, the (n − 6) and (n − 3) fatty acid families, as integral components of cell membranes, are transformed by cyclooxygenase and lipoxygenase enzymes to prostaglandins, thromboxane and leukotrienes when released from their membrane phospholipid reservoirs [5].

The rat gastrointestinal tract caecum has been characterized as a reservoir for intestinal contents, where a high concentration of various microbial flora helps to digest carbohydrates, cellulose and pectides via microbial fermentation processes [6]. Unique lipids may be present in the caecal mucosa that could be involved in physicochemical, biochemical and transport functions. The present study reports the results from a detailed analysis of the polar and neutral lipids of rat caecum mucosa, including fatty acid and triacylglycerol composition.

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EXPERIMENTAL

Fatty acid methyl ester standards were obtained from Larodan Fine Chemicals (Malmo, Sweden). The internal standard solution was prepared by dissolving 200 mg of tricosanoic acid methyl ester ($C_{23:0}$) in 100 ml of hexane. The calibration solutions were prepared by dissolving known amounts of fatty acid methyl ester standards in hexane containing 2,6-di-*tert.*-butyl-*p*-cresol (butylated hydroxytoluene, BHT) obtained from Sigma (Poole, UK).

Apparatus

For fatty acid analysis, a Hewlett Packard Series 5890 gas chromatograph with flame ionization detector and heated injection ports was used. A Supelcowax 10 fused-silica capillary column (60 m × 0.25 mm I.D., film thickness 0.25 μm) was obtained from Supelco (Bellefonte, PA, USA).

Mass spectral data were obtained with an automated gas chromatographic–mass specrometric (GC–MS) system, composed of an HP-5890 gas chromatograph interfaced directly to an AEI MS-30 VG/70 updated mass spectrometer and a VG-11/250 data system (VG Analytical, Manchester, UK).

Triglyceride analysis was carried out on a Chrompack CP 9000 gas chromatograph (Chrompack International, Middelburg, Netherlands) fitted with a split injector and a flame ionization detector.

Methods

Male Wistar-strain rats, purchased from Iffa-Credo (Lyon, France) and weighing 250–300 g each, were used. The animals were given feed and tap water *ad libitum* and housed in a room maintained at 21°C with lights on from 08:00 to 20:00 h. The animals were killed by stunning and cervical dislocation prior to decapitation. The caecum was removed from each rat and immediately rinsed free of intestinal contents with ice-cold physiological saline solution and weighed. The mucosa was scraped off with a glass slide, weighed, immediately frozen in liquid nitrogen and stored at −70°C until the assay of lipids. Quantitative extraction of total lipid from caecum was carried out following the method of Folch *et al.* [7] in the presence of 0.01% (w/v) BHT as antioxidant. Tissue dissociation was achieved by homogenization in ice-cold chloroform–methanol (2:1, v/v) contaning 0.01% BHT, using an Ultra-Turrax Model Tip-TP-18-1.

Separation and quantification of lipids

Neutral lipids from the caecum were separated by thin-layer chromatography (TLC) on silica gel 60 plates using a solvent system of hexane–diethyl ether–acetic acid (80:20:1). After development of the plate, the solvent was allowed to evaporate. Lipid bands were visualized by exposing the edges of the plates to iodine vapour. Once the iodine had been eliminated, the plates were scraped. This system separates phospholipids, cholesterol, triglycerides and cholesterol esters in increasing order of R_F. Individual lipid zones were scraped from TLC plates and eluted from the silica gel with either diethyl ether or chloroform–methanol, according to the individual lipids. Phospholipids were estimated by measurement of total phosphate, essentially according to the procedure of Bartlett [8] as described by Christie [9]. Triglycerides were estimated by the Vioque and Holman method [10]. Cholesterol was determined as previously described by Allan *et al.*

Preparation of fatty acid methyl esters

Lipids were transmethylated according to a modified method of Drenthe and Daemen [12]. The lipid bands on silica gel 60 plates were sprayed lightly with a solution of 0.1% (w/v) BHT in methanol prior to visualization. The phospholipids were eluted from the silica gel with two 15-ml portions of chloroform–methanol–water (10:10:1, v/v/v). Neutral lipids were eluted with two 10-ml portions of chloroform–methanol (2:1, v/v). The solvent was evaporated in a stream of nitrogen, and 10 μg of tricosanoic acid (23:0, internal standard) were added prior to the addition of 40 μl of chloroform–methanol (1:1, v/v) and immediately, 200 μl of boron trifluoride–

methanol complex. The sample was flushed with nitrogen, sealed in a vial fitted with a PTFE-lined cap, and heated at 120°C for 1 h. After the sample was cooled, the fatty acid methyl esters were extracted with 500 μl of hexane.

Analysis of fatty acid methyl esters

The sample (a 1-μl injection of test material was made) was injected into the gas chromatograph. Following injection, the oven temperature was maintained at 200°C for 10 min, then programmed to increase at a rate of 2°C/min to a final temperature of 260°C for 30 min. The flow-rate of helium was 2 ml/min, the column head pressure was 250 kPa, the split ratio was 1:25, the detector and injector temperatures were 275°C, and the detector auxiliary flow-rate was 25 ml/min. For MS analysis, a Supelcowax-10 fused-silica column (60 m × 0.25 mm I.D.) of 0.25 μm film thickness was used; helium was used as carrier gas. The column temperature was programmed from 130°C (for 2 min) to 200°C (100 min) at 4°C/min; the injector temperature was 250°C. The MS conditions were as follows: ionization by electron impact, 70 eV; accelerating voltage, 4 kV; emission current, 100 μA; ion-source temperature, 200°C. The data were processed with a VG 11/250 data system. Each fatty acid methyl ester present in the extract was identified by comparison of its retention time and mass spectrum with those of the authentic compounds.

Analysis of triglycerides

Samples (1 μl) of triglycerides in hexane (0.1%) were injected into the gas chromatograph equipped with a 400 65 HT, 25 m × 0.25 mm I.D., aluminium-clad silica capillary column coated with 65% phenylmethylsilicone (Quadrex, New Haven, CT, USA) and operated under the following conditions: injector and detector temperatures, 360°C; initial temperature, 350°C for 1 min and then raised to 360°C (6 min) at 0.5°C/min. Helium was used as carrier gas at a column head pressure of 130 kPa; the split ratio was 60:1; the detector auxiliary flow-rate was 30 ml/min. The triacylglycerols were identified by their elution times, because the retention is affected not only by the number of carbon atoms but also by the number of double bonds [13].

RESULTS

Total lipid composition

The total lipid composition of rat caecal mucosa is given in Table I. Phospholipids (PL) accounted for 90% of the total lipid, with cholesterol, triglycerides (TG), diglycerides (DG), and fatty acids (FA) making up the remainder. Phosphatidylethanolamine (PE) was the predominant PL (36.08%), phosphatdylcholine (PC) the second most abundant (22.46%), and cardiolipin (CL), phosphatidylserine (PS) and phosphatidylinositol (PI) were present in lesser amounts.

Fatty acid composition

The fatty acid profile of polar and neutral lipids of rat caecal mucosa is given in Table II. The major fatty acids present in the total lipid classes were palmitic (16:0), stearic (18:0), oleic [18:1(n − 9)], linoleic [18:2(n − 6)] and arachidonic [20:4(n − 6)] acids. Neutral lipids contained the greatest amounts of palmitic, oleic and linoleic acids. However, stearic acid accounted for *ca.*

TABLE I

LIPID COMPOSITION OF RAT CAECAL MUCOSA

Results are given as mean ± S.D. for six separate determinations.

Lipid	Composition (%, w/w)
Neutral lipid	4.95 ± 0.45
Cholesterol	5.19 ± 0.32
Total phospholipid	88.97 ± 1.32
Phospholipid classes[a]	
PE	36.08 ± 2.83
PC	22.46 ± 1.58
CL	7.92 ± 0.18
PS	7.63 ± 0.24
PI	6.95 ± 0.27

[a] PE = phosphatidylethanolamine; PC = phosphatidylcholine; CL = cardiolipin; PS = phosphatidylserine; PI = phosphatidylinositol.

TABLE II

FATTY ACID COMPOSITION OF THE POLAR AND NEUTRAL LIPIDS OF RAT CAECAL MUCOSA

Results are given as mean ± S.D. for six separate determinations.

Fatty acid[a]	Composition (%, w/w)	
	Phospholipids	Triglycerides
12:0	0.12 ± 0.03	–
14:0	1.49 ± 0.23	1.84 ± 0.23
14:1(n − 7)	0.24 ± 0.04	–
15:0	0.81 ± 0.31	–
15:0-ai	0.23 ± 0.02	–
16:0-ai	–	2.62 ± 0.31
16:0-i	0.30 ± 0.02	0.57 ± 0.43
16:0	23.90 ± 1.07	29.41 ± 2.34
16:1(n − 7)	1.76 ± 0.32	0.68 ± 0.31
16:1(n − 9)	1.07 ± 0.21	5.90 ± 0.53
16:2(n − 7)	0.12 ± 0.07	–
17:0-i	2.07 ± 0.10	–
17:0	0.92 ± 0.21	–
17:1(n − 8)	0.89 ± 0.13	–
16:4(n − 3)	0.52 ± 0.02	–
17:1(n − 7)	0.21 ± 0.01	–
18:0	17.30 ± 2.32	5.46 ± 0.43
18:1(n − 9)	16.83 ± 1.22	30.28 ± 0.42
18:1(n − 7)	5.54 ± 0.92	4.40 ± 0.71
18:1(n − 5)	0.52 ± 0.11	–
18:2(n − 6)	9.45 ± 0.23	20.39 ± 1.73
18:3(n − 6)	1.40 ± 0.31	0.21 ± 0.20
19:0	0.04 ± 0.01	–
18:3(n − 3)	0.15 ± 0.08	–
20:1(n − 9)	0.11 ± 0.01	–
20:1(n − 7)	0.08 ± 0.02	–
20:3(n − 9)	0.50 ± 0.02	–
20:3(n − 6)	0.06 ± 0.01	0.82 ± 0.31
20:4(n − 6)	8.60 ± 0.32	–
20:3(n − 3)	0.12 ± 0.01	–
22:0	0.12 ± 0.02	–
22:4(n − 6)	0.15 ± 0.03	–
22:5(n − 6)	0.68 ± 0.3	–
22:5(n − 3)	0.98 ± 0.03	–
22:6(n − 3)	2.06 ± 0.21	–
24:0	0.19 ± 0.08	–
24:1	0.15 ± 0.06	–
Saturated	47.00 ± 5.37	27.23 ± 2.80
Mono	27.40 ± 2.54	41.80 ± 6.00
Poly	25.28 ± 3.00	30.60 ± 2.50
n − 6	20.34 ± 3.00	20.60 ± 2.40
n − 3	3.83 ± 0.3	–

[a] ai = anti-iso; i = iso; Mono = monounsaturated; Poly = polyunsaturated.

5% of the total fatty acids present in this lipid fraction, and arachidonic acid was not present. In contrast, PL showed smaller amounts of palmitic, oleic and linoleic acids, but considerable amounts of stearic and arachidonic acid were also present. Other fatty acids of chain length greater than C_{20} were detected only in the PL fraction and accounted for less than 5% of the total fatty acids present in this fraction. The (n − 3) fatty acids were found in PL and DG fractions in very small amounts.

In this study we examined the composition of rat caecal mucosa including the fatty acid composition. As far as we are aware, the lipids of caecal mucosa have not previously been determined. However, there are reports on the lipid composition of the small intestine [13–16] and the colon [17–19]. The caecum is a part of the gastrointestinal tract. Previous studies have shown that the caecum plays a significant role in gastrointestinal physiology, modifying digestive function in pathological processes [20,21]. The triggering mechanism for modifying transport properties may be alterations in the membrane proteins due to changes in the lipid composition.

The fluidity and permeability of biological membranes may be determined by the fatty acid composition of their phospholipids [2]. A decrease in the chain length or an increase in the degree of unsaturation leads to an increase in fluidity. The high ratio of both saturated and unsaturated fatty acids and cholesterol-phospholipids in the small intestine during postnatal maturation of rat brush border membranes between birth and suckling age coincides with a decrease in the fluidity of the brush border membrane [14]. Rat caecal mucosa has a high unsaturated phospholipid content and a marked increase in the phospholipid/cholesterol ratio. Assuming these molar ratios are indices of membrane fluidity, the rat caecal mucosa seems to have a high fluidity. However, although the fatty acid composition provides some indication of membrane fluidity, verification awaits direct measurement of the permeability of the membrane of rat caecal mucosa.

Arachidonic acid esterified at the C-2 position of PL is the precursor of eicosanoids, such as

prostaglandins and leukotrienes, which are important as second messengers in signal transport [5]. Arachidonic acid may play an important role as the precursor of eicosanoids in the caecal mucosa. There are several reports of increased phospholipid arachidonic acid in colonic mucosa from patients with inflammatory bowel disease, such as ulcerative colitis and Chrohn's disease [17,22]. Patients with the latter syndrome synthesize larger amounts of protaglandins and thromboxanes than normal. Thus eicosanoids are thought to be an important mediator of inflammation in these diseases.

Taken together, these results show that analysis of the lipid composition of rat caecal mucosa did not reveal the presence of any unusual lipids. However, several small peaks, corresponding to 16:1(n − 7), 18:1(n − 7) and 20:1(n − 7), were noted on chromatograms obtained by GC on different capillary columns. Thus, the PL fraction contained a relative high proportion of 5% 18:1(n − 7). In addition, iso- and anti-iso-fatty acids were detected in trace amounts. Thus, it is not possible to conclude from this study whether the plasma membrane composition of mucosal cells was altered or the PL changes reflect differences in the amount of certain organelles in the caecum cell [23]. Another limitation of this study is that although the fecal material was removed and the caecum was rinsed very thoroughly, it is possible that bacteria remained adhered to the mucosa, contaminating the lipid sample. Thus the presence of 15:0 and 17:0 may be due to microbial flora in the fecal material.

Polar capillary GC provides new insights into the molecular association of the fatty chains in the relatively saturated animal tissue triacylglycerols, which are not readily resolved by $AgNO_3$ TLC or by GC on apolar columns. Although not all molecular species were fully characterized, on the basis of their relative retention times it is possible to recognize the prominent presence of species containing C_{16} and C_{18} saturated acids, in

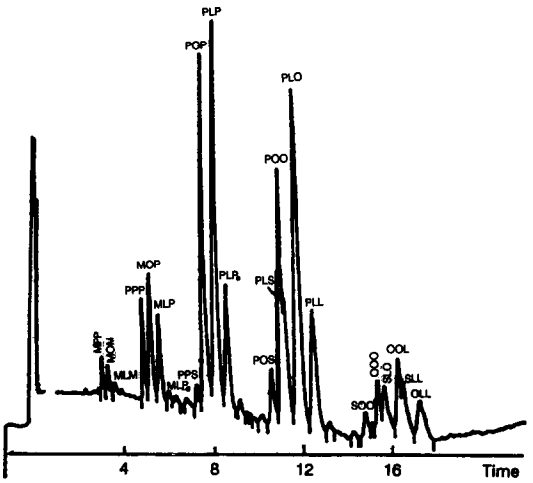

Fig. 1. Polar capillary GC of rat caecal mucosa triacylglycerols. The triacylglycerols are identified by the combination of the component fatty acids with regard to positional location. GC conditions and instrumentation as in the text. Peak identification as in Table III.

TABLE III

AVERAGE TRIACYLGLYCEROL COMPOSITION OF RAT CAECAL MUCOSA LIPID

Fatty acids: M = myristic acid, tetradecenoic acid, 14:0; P = palmitic acid, hexadecenoic acid, 16:0; S = stearic acid, octadecenoic acid, 18:0; Po = palmitoleic acid, hexadecenoic acid, 16:1; O = oleic acid, *cis*-9-octadecenoic acid, 18:1; L = linoleic acid, *cis,cis*-9,12-octadecenoic acid, 18:2. Triglycerides: PPP = *sn*-glycerol-tripalmitate; MLP = *sn*-glycerol-myristate-linoleate-palmitate; PLO = *sn*-glycerol-palmitate-linoleate-oleate; etc.

Triacylglycerol	Composition (%, w/w)
MMP	0.55 ± 0.01
MOM	0.40 ± 0.01
MLM	0.13 ± 0.01
PPP	2.30 ± 0.02
MOP	4.32 ± 0.01
MLP	2.59 ± 0.02
PPS	0.72 ± 0.01
POP	11.88 ± 1.11
PLP	15.28 ± 1.32
PLPo	4.85 ± 0.45
POS	2.28 ± 0.23
POO	9.36 ± 1.13
PLS	4.87 ± 0.31
PLO	17.26 ± 2.56
PLL	6.39 ± 0.36
SOO	1.00 ± 0.10
OOO	2.36 ± 0.13
SLO	3.38 ± 0.23
OOL	3.58 ± 0.31
SLL	3.25 ± 0.27
OLL	3.27 ± 0.16

combination with the C_{18} mono- and di-unsaturated fatty acids [13].

Triglyceride composition

The caecal triacylglycerols (Fig. 1 and Table III) contain POP, PLP, and PLO as major components, as anticipated from the fatty acid composition, assuming a random association. In addition, they contain MMP, MOM, and MLM in small amounts. However, comparison of our results with others is difficult because of the absence of qualitative and quantitative studies on triglycerides in caecal mucosa.

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